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Preclinical report

Efficacy of MGI 114 (HMAF) against the MRP$^+$ metastatic MV522 lung carcinoma xenograft

Michael J Kelner,[1] Trevor C McMorris,[2] Leita A Estes,[1] Michelle Y Oval,[1] Rafael J Rojas,[1] Joshua R Lynn,[1] Kevin A Lanham[1] and Kyra M Samson[1]

Departments of [1]Pathology and [2]Department of Chemistry & Biochemistry, University of California, San Diego, La Jolla, CA 92093, USA.

This study is part of an effort to evaluate efficacy of the novel agent MGI 114 (HMAF) against tumors resistant to conventional chemotherapeutic agents. MGI 114 is a novel semisynthetic anticancer agent currently in chemotherapeutic phase II trials to evaluate activity against various solid tumors. Previous studies indicate MGI 114 was active against human MDR1/gp170$^+$ solid tumor xenografts. Recent evidence suggests overexpression of the MRP protein may also be clinically relevant to development of drug resistance in solid tumors. We evaluated the efficacy of MGI 114 against a human MRP$^+$ lung carcinoma xenograft. Parent MV522 lung carcinoma cells were transfected with a MRP cDNA expression vector and resistant cells selected by exposure to vinblastine (30-fold resistance). Analysis of resistant clones indicated 20- to 40-fold increases in expression of both MRP mRNA and MRP protein. Administration of MGI 114 at the maximum tolerated dose (7 mg/kg, 5 $\times$/week for 3 weeks) to MRP tumor-bearing mice demonstrated this novel agent was active against MRP$^+$ tumors and significantly extended their lifespan ($p < 0.001$). In contrast, other cytotoxic agents had minimal activity against this MRP$^+$ xenograft. These results indicate MGI 114 should retain activity *in vivo* against MRP$^+$ tumor types. The development of this MRP$^+$ xenograft model, in conjunction with the parent MV522 and MDR1/gp170$^+$ xenograft models, will be useful for screening new classes of agents for activity against multidrug-resistant tumors.

Key words: gp180, HMAF, MGI 114, multidrug resistance, MRP, xenograft.

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Correspondence to MJ Kelner, Pathology 8320, UCSD, 200 West Arbor Drive, San Diego, CA 92103-8320, USA.
Tel: (+1) 619 543-5976; Fax: (+1) 619 543-3730;
E-mail: mkelner@ucsd.edu

Introduction

Illudins are sesquiterpenoid natural products isolated from mushrooms of the genus *Omphalotus (O. illudens*).[1–3] Although illudins displayed dose-limiting toxicity in early studies,[4] they possess several unique properties desirable in a chemotherapeutic agent. Illudin S is a potent inhibitor of DNA synthesis and caused cell cycle arrest in the S phase at nano or picomolar concentrations.[4,5] Although the exact mechanism of illudin cytotoxicity remains unknown, the repair of illudin-induced DNA damage, in contrast to other agents, appears to require functional DNA helicase activity for the process to occur.[6] In addition, multidrug-resistant MDR1/gp170$^+$ cells did not display cross-resistance to illudins.[7,8] Therefore, an effort was made to synthesize chemical derivatives of illudins with a better therapeutic index. Earlier classes of illudin derivatives included the dehydroilludin and acylfulvene classes.[9–11] The acylfulvene derivative MGI 114 (HMAF, 6-hydroxymethylacylfulvene, NSC683863) displayed excellent activity in several human solid tumor xenografts,[12,13] and is currently in various phase II clinical chemotherapeutic trials (further information can be obtained from the NCI Clinical Trial Site at URL http://cancernet.nci.nih.gov/trialsrch.shtml).

Multidrug resistance (MDR), whether inherited (intrinsic to tumor type) or acquired, is a major barrier to chemotherapeutic success. The classical form of MDR is due to increased activity of a P-glycoprotein (gp170) encoded by the MDR1 gene on chromosome 7. Cole *et al.*[14] have identified a second gene, designated MRP (multidrug-resistance-associated protein), which was overexpressed in a gp170$^-$ MDR clone isolated from the human small lung carcinoma line H69.[14] Recent evidence suggests that overexpression of MRP, which encodes for another glycoprotein

(gp180), is involved in development of drug resistance in a variety of solid tumor types.[15,16]

We previously determined MGI 114 was highly active against a metastatic lung carcinoma xenograft (MDR1/gp170$^-$ and MRP/gp180$^-$) which was resistant to a variety of conventional and experimental chemotherapeutic agents.[17] Recent studies have attributed clinical resistance in non-small cell lung cancer patients, in part, to expression of the MRP phenotype.[18–22] To determine whether MGI 114 retained activity against MRP-expressing tumor cells, we developed a MRP$^+$ xenograft model. We used this MRP$^+$ lung carcinoma xenograft to demonstrate efficacy of MGI 114 against MRP-expressing cells.

Materials and methods

Athymic mice

BALB/c *nu/nu* 4-week-old female mice weighing 18–22 g were obtained from Simonsen (Gilroy, CA) and maintained in the Athymic Mouse Colony of the University of California, San Diego, under pathogen-free conditions using HEPA filter hoods. The animals were provided with sterilized food and water *ad libitum* in groups of four in plastic cages vented with polyester fiber filter covers. Clean, sterilized gowns, gloves, masks, shoe and hood covers were worn by all personnel handling the animals. All studies were conducted in accordance with the guidelines of the NIH *Guide for Care and Use of Animals*, the University of California, San Diego Guidelines for assessing illness and morbidity in rodents used in studies involving experimental neoplasia, and approved by the University Institutional Animal Care and Use Committee (Protocol 3-006-3).

Cell lines, culture conditions and cytotoxicity assays

The MV522 lung carcinoma line used for xenograft studies[17] was maintained in antibiotic-free RPMI 1640 media (Mediatech, Herndon, VA) supplemented with 10% fetal bovine serum plus 2 mM glutamine in an humidified 5% CO_2 incubator at 37°C. The MV522 MRP/gp180$^+$ clones were maintained under the same conditions as parent cells except that vinblastine was added to the cultures at a final concentration of 50 ng/ml. MV522/MRP cells were maintained continuously in vinblastine-containing media until injected s.c. into mice. The HL60 human promyelocytic and doxorubicin-resistant daughter line HL60/adr, in which resistance is also mediated by expression of MRP, was obtained from Dr M Center.[23,24]

The *in vitro* toxicity of MGI 114, vinblastine, mitomycin C, cisplatin and paclitaxel to parent MV522 cells and the MV522/MRP clones was determined by comparing IC_{50} values derived from continuous 48 h exposure studies with cell viability assessed by Trypan blue exclusion. The use of a 48 h exposure period allows comparison to results obtained for other illudin analogs.[4,5]

Drugs

Anticancer drugs were obtained from the UCSD Medical Center Pharmacy and if necessary were formulated with the supplied diluent according to directions provided by the manufacturer. Drugs included: paclitaxel (Taxol; Bristol-Myers Squibb, Princeton, NJ) in Cremophor EL, mitomycin C (Mutamycin; Bristol-Myers Squibb) reconstituted in sterile water, vinblastine sulfate (Velban; Lilly Research, Indianapolis, IN) reconstituted in normal saline, topotecan (Hycamtin; Smith-Kline Beecham, Philadelphia, PA) reconstituted in sterile saline and cisplatin (Platinol; Bristol-Myers Squibb) in sterile water. Pharmaceutical grade MGI 114 (MGI Pharma, Minneapolis, MN) was prepared by reconstituting in a sterile 40% DMSO/normal saline mixture and diluted to appropriate concentrations with sterile 10% DMSO/normal saline.[12] Structures of the semisynthetic agent MGI 114 and the parent compound illudin S are provided (Figure 1).

Vector preparation, transfection and isolation of resistant cells

The MRP eukaryotic cDNA expression plasmid P.C./RSV-MRP[25] was obtained from Dr Guido Zaman (Netherlands Cancer Institute) and propagated in DH5α *Escherichia coli* cells. The plasmid was recov-

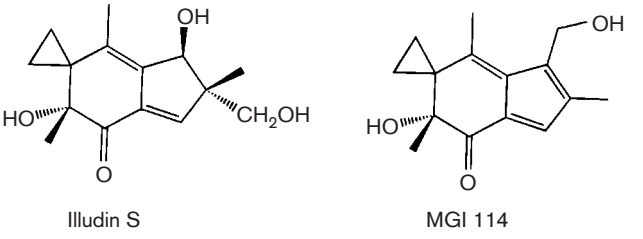

Figure 1. Structures of the parent compound illudin S and the illudin-derived analog MGI 114 (HMAF) tested in this study.

ered and then transfected into MV522 cells using the calcium phosphate technique. Transfected cells were initially exposed to 30 ng/ml of vinblastine, then 50 ng/ml, and drug-resistant colonies isolated using cloning cylinders. Control MV522 cells were transfected with pBR322 plasmid and exposed to 30 ng/ml vinblastine, but no control cells survived vinblastine selection.

In vivo evaluation using the MRP MV522 xenograft model

Mice were randomized into treatment groups of eight to 10 animals each. Each animal was earmarked and followed individually throughout the experiment. The mice received s.c. injections of 10×10^6 MV522 cells over the ribs. Ten days after implantation of the MV522 cells the animals received the desired drug and dosage. The primary s.c. tumor growth was monitored starting on the first day of treatment. Tumor size was measured in two perpendicular diameters and tumor weight estimated according to the formula $w=[(\text{width})^2 \times \text{length}/2]$.[26] Relative weights (RW) were calculated to standardized variability in tumor size amongst test groups at initiation of the treatment by using the formula $RW=W_t/W_i$, where W_i is the tumor weight for a given animal at the beginning of drug treatment and W_t is tumor weight at a subsequent time t.[26]

Detection of MRP/gp180 mRNA

For detection of MRP mRNA expression the MV522 cells were lysed directly in a culture flask by adding 1 ml of Trizol reagent (Gibco/BRL, Grand Island, NJ) per 10 cm^2 area of the culture flask. The HL60 and HL60/adr cells were pelleted by centrifugation and lysed with 1 ml Trizol reagent per 5×10^6 cells. Chloroform was added to the Trizol/cellular suspension at 0.2 ml chloroform per 1 ml Trizol and shaken vigorously for 30 s. Samples were centrifuged for 15 min at 12 000 *g* at 4°C. The colorless upper phase containing total RNA was re-extracted with chloroform. The aqueous phase was transferred to a new tube and total RNA precipitated by mixing with 0.5 original volume of isopropyl alcohol. Following a 10 min room temperature incubation, the samples were centrifuged for 15 min at 12 000 *g* at 4°C. The total RNA pellet was washed once with 70% ethanol and dissolved in RNAse-free water. The mRNA was isolated using a commercial oligo(dT)–cellulose spin column according to the manufacturer's instructions (Pharmacia Biotech, Piscataway, NJ).

Nylon membranes (Boehringer Mannheim, Indianapolis, IN) were presoaked in 10× sodium citrate/sodium chloride (SSC) and placed in a slot manifold (Gibco/BRL). The mRNA was denatured in 5 mM Tris at 65°C for 15 min. Each well was loaded with 250 ng of mRNA and wells were washed with 5 × SSC. Filters and mRNA were cross-linked in a UV Stratalinker 2400 oven (Stratagene, La Jolla, CA). Prehybridization and hybridization were performed in an Autoblot hybridization oven (Bellco Glass, Vineland, NJ) at 45°C in a hybridization solution containing 50 mM sodium phosphate (pH 6.5), Denhardts solution, SSC, SDS, salmon sperm DNA, dextran sulfate and formamide. The MRP probe was a 5.5 kb *Sal*I–*Not*I fragment recovered from the pJ3Ω-MRP expression vector[25] (kindly provided by Dr Guido Zaman). Probes were ^{32}P labeled by the random prime method according to manufacturer's instructions (Prime-it Rmt; Stratagene, La Jolla, CA). The filters were washed in 1.5 mmol/l Na citrate, pH 7, 15 mmol/l NaCl and 0.1% SDS at 50°C. For visual examination the filter was autoradiographed to X-ray film and for quantitation to a phosphor screen (Molecular Dynamics, Sunnyvale, CA). The X-ray film was developed using standard procedures. The phosphor screen was quantified on a Storm 800 phosphoimager (Molecular Dynamics) and the densities of each slot determined. To correct for variation in loading, the same membrane was stripped and then reprobed with cGAP probe as described above. Expression of MRP/gp180 was then calculated as a percent relative to cGAP expression to correct for loading differences.

Detection of MRP/gp180 protein

The parent MV522 and MRP-transfected daughter cells were assessed for MRP protein using the monoclonal antibody (mAb) MAB4100 (Chemicon, Temecula, CA) which does not cross-react with either human mdr1 or mdr3, mouse mdr1 or mdr3, or with mouse MRP gene products. Cellular extracts were prepared by exposing cells while on ice to an extraction buffer (1% NP-40, 150 mM NaCl and 50 mM Tris, pH 8.0) for 30 min with gentle rocking. The solution was transferred to microfuge tubes and centrifuged at 4°C for 10 min (10 000 *g*). The cellular extract was applied to nylon membranes (Boehringer-Mannheim) using a slot-blot apparatus. The membrane was incubated in blocking buffer (100 mM Tris, pH 7.5, 150 mM 0.9% NaCl 0.01% Tween and 1% bovine serum albumin) for 1 h at room temperature. The MAB4100 antibody was added to the blocking buffer and the membrane was incubated

overnight at 4°C. The membrane was washed 3 times with TBS-T buffer (100 mM Tris, pH 7.5, 150 mM 0.9% NaCl and 0.01% Tween), incubated with a 1:10 000 dilution of goat anti-mouse secondary antibody in blocking buffer for 1 h at room temperature, then rewashed 3 times with blocking buffer. The membrane was washed once with developing buffer (100 mM Tris, pH 9.5, 100 mM NaCl and 50 mM $MgCl_2$), and the nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate substrates added.

Statistical analysis

To compare the relative tumor weights between the groups of animals, ANOVA followed by Tukey–Kramer multiple comparison post-ANOVA analysis was performed. Comparison of survival curves between groups of animals was performed using the method of Kaplan and Meier.[27] To compare the relative cytotoxicity of a given drug between a parental cell line and its mdr daughter line, the IC_{50} values were analyzed using Student's *t*-test. Probability values less than 0.05 were considered statistically significant. Relative tumor weight and IC_{50} values were analyzed using Instat (version 2.02) and lifespan data was analyzed using the Prism (version 2.0) software package (Graph Pad, La Jolla, CA).

Results

Development of MV522 MRP cells

MRP-expressing cells were created by transfecting parent MV522 cells with the eukaryotic expression vector P.C./RSV-MRP containing the cDNA encoding for human MRP.[25] MV522 clones were selected for MRP expression by continuous exposure to 50 ng/ml vinblastine for 30 days. Five clones were selected at random and injected s.c. into athymic BALB/c *nu/nu* mice to determine their ability to grow as a xenograft as previously described.[17] One of the five clones, MRP clone #3, failed to survive expansion at high-dose vinblastine exposure (70 ng/ml). The ability of the remaining four clones to express MRP mRNA, as compared to the parental MV522 line, was determined. The HL60/adr cell line, previously demonstrated to be multidrug resistant on the basis of MRP expression,[23,24] was included as a positive control. The drug-resistant MV522 Q6 clone[28] was included as a negative control. This MV522/Q6 clone is drug resistant due to expression of mdr1/gp170,[28] which is distinct from mrp1/gp180. Analysis of vinblastine-resistant MV522 clones indicated 20- to 40-fold increases in expression of MRP mRNA (Figure 2) as compared to the parental MV522 cells. The Q6 clone, which is mdr1/gp170$^+$, did not demonstrate any relative increase in MRP mRNA expression. The HL60/adr clone, which is MRP/gp180$^+$,[23,24] did demonstrate a marked increase in MRP mRNA expression as compared to the parental HL60 line. The ability of the MRP clones to produce MRP protein was also confirmed (Figure 3). The sensitivity of the parental MV522 cells and the daughter MRP clones to MGI 114 was determined using both a 2 and 48 h exposure period (Table 1). There was no indication of resistance of the MRP clones to MGI 114 with a 48 h exposure period. All four of the MRP$^+$ clones demonstrated a mild resistance (about 2-fold) to MGI 114 with the 2 h exposure period.

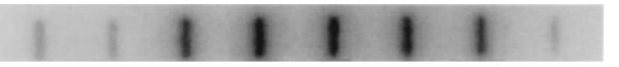

Figure 2. Expression of MRP mRNA in the resistant clones versus the parent MV522 cells. The harvested cellular mRNA was probed with a full-length MRP cDNA clone.[25] Lane A, parental MV522 cells; lane B, mdr1/gp170-expressing MV522 cells;[28] lane C, MRP clone #1; lane D, MRP clone #2; lane E, MRP clone #4; lane F, MRP clone #5; lane G, HL60/adr MRP-expressing cell line;[23,24] lane H, HL60 parent line.

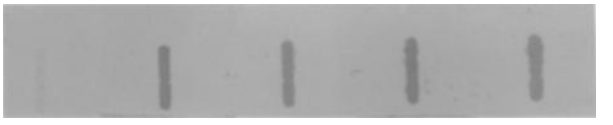

Figure 3. Expression of the MRP/gp180 protein in resistant clones versus the parent MV522 cells. Cellular extracts were probed with a monoclonal anti-MRP antibody that does not cross-react with either human mdr1 or mdr3, mouse mdr1 or mdr3, or mouse MRP gene products. Lane A, parental MV522 cells; lane B, MRP clone #1; lane C, MRP clone #2; lane D, MRP clone #4; lane E, MRP clone #5.

Table 1. Sensitivity of MRP-expressing clones to MGI 114

	IC_{50} value (nM)	
	2 h	48 h
Parent MV522	760 ± 50	330 ± 100
MRP clone 1	1350 ± 110	270 ± 50
MRP clone 2	1560 ± 230	430 ± 110
MRP clone 4	1690 ± 310	420 ± 240
MRP clone 5	1630 ± 410	540 ± 230

The results are expressed as mean ± SD (*n*=3).

One MRP+ clone, MRP1, displayed growth in athymic mice similar to the parental cells, and was chosen for further *in vitro* and *in vivo* studies. In agreement with previous results, the MRP1 clone was markedly resistant to vinblastine (greater than 30-fold) and to a lesser extent to doxorubicin (about 8-fold) (Table 2). There was no detectable resistance to cisplatin, mitomycin C, topotecan or paclitaxel. This pattern of resistance is in agreement with previous reports.[14–16] The MRP1 phenotype was stable for at least 6 weeks in culture in the absence of a selection agent as determined by stable resistance to vinblastine (data not shown), which exceeds the duration of drug administration for the *in vivo* experiments.

Xenograft studies

The efficacy of MGI 114 and mitomycin C against both the parent MV522 and the daughter MV522/MRP xenografts was determined. Other anticancer agents that one would normally use (vinblastine, cytoxan, 5-fluorouracil, etc.) were not chosen as the parental MV522 xenograft is already resistant to treatment with these agents at a variety of doses, schedules and administration routes.[8,9,12,13,17,28] Only mitomycin C, paclitaxel and to a lesser extent cisplatin demonstrate any ability to inhibit primary tumor growth or extend lifespan in the parent non-MDR xenograft.[17,28] The doses and schedules for MGI 114 and other agents were chosen on the basis of studies which previously determined MTD and the most efficacious schedule for these drugs.[12,13,28] These previous studies indicated that animals developed a weight loss above 15% when treated 5 ×/week for 3 weeks with MGI 114 at 8 mg/kg, mitomycin C at 2.0 mg/kg, Taxol at 12 mg/kg and cisplatin at 2.6 mg/kg. Thus, the MTDs in this study are about 90% of the dose that induces a body weight loss above 15% in this strain of mice. In the first experiment MGI 114 at MTD (7.0 mg/kg, 5 ×/week for 3 weeks) and mitomycin C (1.8 mg/kg, 5 ×/week for 3 weeks) displayed activity against the primary tumor in both the parent (non-MRP) MV522 xenograft, in agreement with previous results, and against the MRP+ xenograft (Figure 4). Mitomycin C, however, did not produce an increase in lifespan (ILS), whereas MGI 114 was capable of extending the lifespan in the MV522/MRP tumor-bearing animals ($p < 0.01$) (Table 3). MV522 cells harvested from control animals at the end of the experiment retained their ability to grow in culture in the presence of vinblastine (data not shown).

The study was repeated comparing topotecan to MGI 114. Topotecan was studied as its efficacy had never been determined in the MV522 xenograft, but synergistic action with MGI 114 was previously reported.[29,30] Mitomycin C was again included but at a lower dose as some mitomycin-treated animals in the first experiment displayed a weight loss above 15%. The MGI 114 (7.0 mg/kg, 5 ×/week for 3 weeks) and the mitomycin C (1.6 mg/kg, 5 ×/week for 3 weeks) again inhibited primary tumor growth in MV522/MRP-

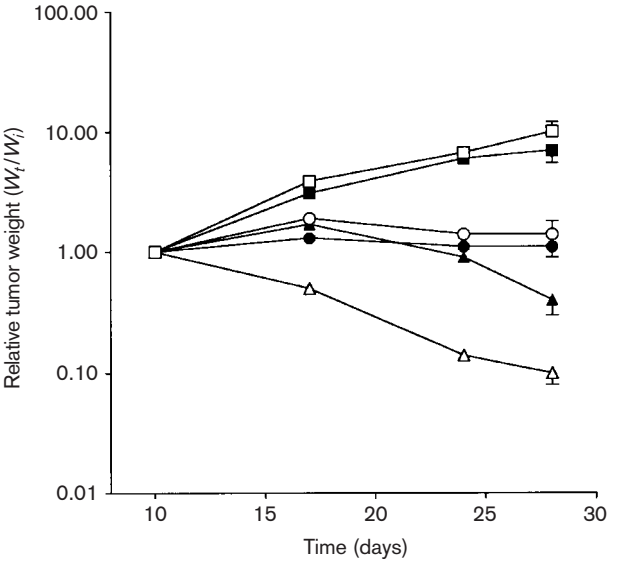

Figure 4. Efficacy of MGI 114 versus mitomycin C at inhibiting the MRP+ clone #1 as compared to the parent MV522 cells. MV522-bearing animals received 20% DMSO/saline as a control (□), mitomycin C at the MTD of 1.8 mg/kg (○) or MGI 114 at the MTD of 7.0 mg/kg (△). The MRP-bearing animals received 20% DMSO/saline as a control (■), mitomycin C at the MTD of 1.8 mg/kg (●) or MGI 114 at the MTD of 7.0 mg/kg (▲). Both drugs were administered i.p. 5 times a week for 3 weeks. There were 10 animals per group. The data points indicate means for each group and the bars represent SE.

Table 2. Toxicity of agents against MRP+ clone #1 versus parental MV522 cells (48 h exposure)

Agent	48 h IC_{50} (nM)	
	Parent MV522 cells	Daughter MRP1 cells
Mitomycin C	61 ± 13	51 ± 8
Vinblastine	3 ± 1	105 ± 12 ($p < 0.001$)
Taxol	2 ± 1	2 ± 1
Cisplatin	810 ± 130	700 ± 120
Doxorubicin	61 ± 5	440 ± 30 ($p < 0.001$)
Topotecan	18 ± 2	25 ± 4

The results are expressed as mean ± SD ($n = 3$). The p values indicate whether the MRP clone is significantly resistant to an agent as compared to the parental MV522 line.

Table 3. Activity of MGI 114 against the parental MV522 and the MRP1$^+$ daughter xenograft

Drug	Dose	Xenograft	ILS		Mice with complete shrinkage
MGI 114	7.0	MV522	+158%	($p<0.001$)	3/10
Mitomycin C	1.8	MV522	−12%		0/10
MGI 114	7.0	MRP1	+45%	($p<0.01$)	0/10
Mitomycin C	1.8	MRP1	−36%		0/10
MGI 114	7.0	MV522	+163%	($p<0.001$)	3/8
Mitomycin C	1.6	MV522	+3%		0/8
Topotecan	1.5	MV522	+3%		0/8
MGI 114	7.0	MRP1	+93%	($p<0.001$)	3/8
Mitomycin C	1.6	MRP1	−10%		0/8
Topotecan	1.5	MRP1	−5%		0/8
MGI 114	7.0	MV522	+193%	($p<0.001$)	5/8
Taxol	10.0	MV522	+36%	($p<0.01$)	0/8
Cisplatin	2.3	MV522	+36%	($p<0.01$)	0/8
MGI 114	7.0	MRP1	+166%	($p<0.001$)	3/8
Taxol	10.0	MRP1	+77%	($p<0.01$)	0/8
Cisplatin	2.3	MRP1	+50%	($p<0.01$)	0/8

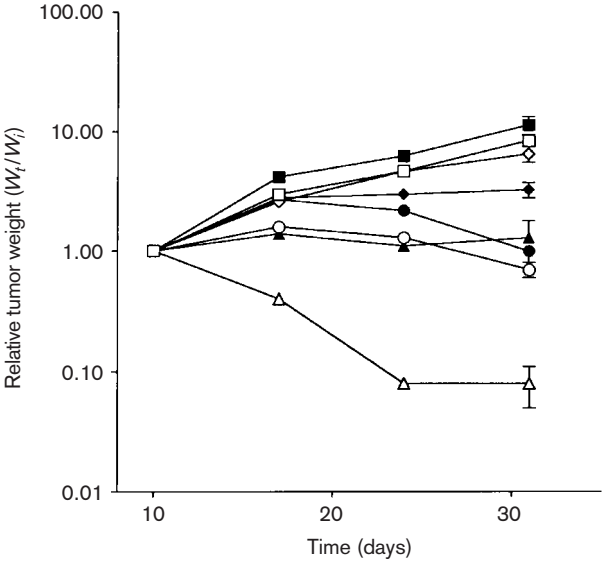

Figure 5. Efficacy of MGI 114 versus mitomycin C and topotecan at inhibiting the MRP$^+$ clone. MV522-bearing animals received 20% DMSO/saline as a control (□), MGI 114 at the MTD of 7.0 mg/kg (△), mitomycin C at the MTD of 1.6 mg/kg (○) or topotecan at the MTD of 1.5 mg/kg (◇). The MRP-bearing animals received 20% DMSO/saline as a control (■), MGI 114 at the MTD of 7.0 mg/kg (▲), mitomycin C at the MTD of 1.6 mg/kg (●) or topotecan at the MTD of 1.5 mg/kg (◆). All drugs were administered i.p. 5 times a week for 3 weeks. There were eight animals per group. The data points indicate means for each group and the bars represent SE.

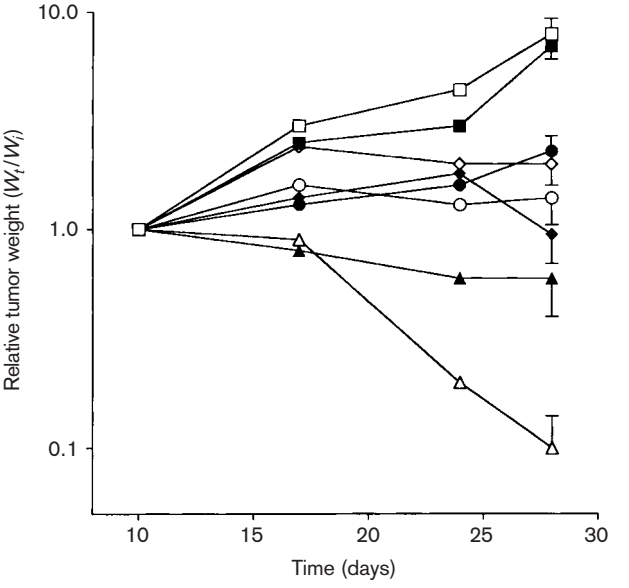

Figure 6. Efficacy of MGI 114 versus Taxol and cisplatin at inhibiting the MRP$^+$ clone. The MV522-bearing animals received 20% DMSO/saline as a control (□), MGI 114 at the MTD of 7.0 mg/kg (△), cisplatin at the MTD of 2.3 mg/kg (◇) or Taxol at the MTD of 10 m/kg (○). The MRP-bearing animals received 20% DMSO/saline as a control (■), MGI 114 at the MTD of 7.0 mg/kg (▲), cisplatin treated at the MTD of 2.3 mg/kg (◆) or Taxol at the MTD of 10 m/kg (●). All drugs were administered i.p. 5 times a week for 3 weeks. There were eight animals per group. The data points indicate means for each group and the bars represent SE.

bearing animals, whereas topotecan (1.5 mk/kg, 5 × / week for 3 weeks) was less effective (Figure 5). MGI 114 again prolonged the lifespan of MV522 or MRP1 tumor-bearing animals ($p \times 0.001$), whereas mitomycin C and topotecan failed to prolong the lifespan of either MV522 or MRP1 tumor-bearing animals (Table 3). In

this study, MGI 114 produced complete tumor regression (no evidence of tumor regrowth after 4 months) in three of eight animals in both the parent MV522 and the MRP1 groups. In contrast, neither mitomycin C or topotecan induced complete tumor regression in any tumor-bearing animal.

In a third study the antitumor ability of MGI 114 was compared to Taxol and cisplatin. Both Taxol (10 mg/kg, 5×/week for 3 weeks) and cisplatin (2.3 mg/kg, 5×/week for 3 weeks) demonstrated some ability to inhibit tumor growth (Figure 6) and prolong the lifespan (Table 3) in both the parental MV522 and the daughter MRP1 xenograft. Neither agent, however, produced complete tumor regression in any animal. MGI 114, in contrast, was markedly effective at inhibiting tumor growth (Figure 6) and prolonging the lifespan (Table 3) in both the parental MV522 and the daughter MRP1 xenograft ($p < 0.001$ versus control animals and $p < 0.05$ versus either Taxol or cisplatin). Complete remission of tumor was noted in some animals from both the parent MV522 and the daughter MRP1 group.

Discussion

Topotecan and mitomycin C demonstrated some ability to hinder primary tumor growth in both the parent (non-MRP) and the MRP+ xenograft. Neither agent was capable of extending the lifespan in either xenograft model. Cisplatin and Taxol, however, did demonstrate moderate ability to hinder primary tumor growth, and also extend the lifespan in both the parent and the MRP+ xenograft model. Neither cisplatin or Taxol was capable of inducing tumor remission in any animal from either xenograft model. The novel agent MGI 114, in contrast, was effective at inhibiting tumor growth and significantly prolonging the lifespan ($p < 0.001$) in both the parent and the MRP+ xenograft. In addition, MGI 114 was capable of producing complete tumor remission (defined as no tumor regrowth for greater than 4 months) in tumor-bearing animals from both groups. It is noteworthy that 14 standard chemotherapeutic agents, at various dosages and administration schedules, fail to extend the lifespan in the parent MV522 xenograft or produce any evidence of complete tumor remission.[9,12,13,17,28] Thus, the ability of MGI 114 to extend the lifespan in this MRP+ xenograft is unique and remarkable.

Previous studies in non-drug-resistant human tumor xenograft models have suggested that illudin analogs are potentially useful agents for the treatment of solid tumors. The present study extends previous observations of MGI 114 efficacy by demonstrating activity of the drug against a MRP+ drug-resistant xenograft model which is resistant to conventional chemotherapeutic agents. These results suggest MGI 114 and other illudin-derived analogs may prove to be efficacious in the treatment of refractory drug-resistant human malignancies. In addition, the development of this MRP+ metastatic xenograft complements the parent MV522 and the MDR1+ daughter xenografts previously described.[17,28] These three xenograft models will be useful for screening new classes of anticancer agents that display activity against MDR tumors.

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