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## Original Paper

# Efficacy of MGI 114 (6-hydroxymethylacylfulvene, HMAF) Against the mdr1/gp170 Metastatic MV522 Lung Carcinoma Xenograft

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**Illudins are a novel class of agents with a chemical structure entirely different from current chemotherapeutic agents. A new semisynthetic derivative, MGI 114 (NSC 683863, 6-hydroxymethylacylfulvene, HMAF), is markedly effective in a variety of lung, breast and colon carcinoma xenograft models. This analogue, MGI 114, is currently in phase I human clinical trials, and is scheduled for two different phase II trials. To determine if MGI 114 could be effective *in vivo* against mdr tumour cells, we generated an mdr1/gp170-positive clone of the metastatic MV522 human lung carcinoma line by transfecting a eukaryotic expression vector containing the cDNA encoding for the human gp170 protein. This MV522/mdr1 daughter line retained the metastatic ability of parental cells. The parental MV522 xenograft is mildly responsive *in vivo* to mitomycin C and paclitaxel, as evidenced by partial tumour growth inhibition and a small increase in life span, whereas MV522/mdr1 xenografts were resistant to these agents. In contrast to mitomycin C and paclitaxel, MGI 114 produced xenograft tumour regressions in 32 of 32 animals and completely eliminated tumours in more than 30% of MV522/mdr1 tumour-bearing mice. Thus, MGI 114 should be effective *in vivo* against mdr1/gp170-positive tumours.** 

**Key words: 6-hydroxymethylacylfulvene, HMAF, xenograft, mdr1, MGI 114, gp170, antitumour activity, non-small cell lung cancer, carcinoma, MV522, NSC 683863**
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## INTRODUCTION

CARCINOMA OF the lung is the leading cause of death from cancer in the U.S.A. [1]. The proportion of lung cancers classified as adenocarcinoma of the lung (ACL) is increasing and now outnumbers other histological types [1]. Systemic chemotherapy for ACL has limited efficacy [2, 3], in contrast to therapy for small cell lung cancer which can be effective [4]. Many ACL patients have metastases at presentation and patients with localised disease often relapse with systemic disease after initial treatment [5, 6].

We previously identified a class of compounds, called illudins, which are preferentially cytotoxic *in vitro* with short exposure times to a variety of carcinomas, including ACL [7–13]. Preferential cytotoxicity toward carcinoma cells appears to derive from a rapid intracellular, energy-dependent accumulation of the drug [8]. In contrast to other known chemotherapeutic agents (such as BCNU, (1,3-*bis*(2-chloroethyl)-1-nitrosourea), mitomycin or cisplatin), repair of illudin-induced DNA damage requires the action of ERCC2 and ERCC3 DNA repair enzymes [14]. We previously demonstrated that illudin derivatives are effective against various carcinoma xenografts [9–13]. One new semisynthetic analogue, MGI 114 (HMAF, 6-hydroxymethylacylfulvene, NSC 683863)* induced tumour regression in MV522 lung, MX-1 breast and HT-29 (CX-1) colon carcinoma xenograft models [12, 13] and markedly increased the life span of MV522 tumour-bearing animals [12]. This new analogue,

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*The acylfulvene class of analogues are covered by U.S. Patents 5,439,936 and 5,523,490, pending U.S. patent application claims, European Common Patent 0565511 and pending European Common and foreign patent application claims.

MGI 114, is currently in phase I human clinical trials with promising initial results at non-toxic dosages and scheduled for two different phase II trials.

Multidrug resistance is a term that refers to cross-resistance to multiple agents first observed in cell culture tumour models (reviewed in [15, 16]). Although the term 'MDR' was initially applied to overexpression of the *mdr1* gene located on human chromosome 7, which encodes for a 170 KDa glycoprotein (gp170), it is clear overexpression of mdr1 explains only a subset of multidrug resistance. Recent work identified resistance related to other abnormalities, such as topoisomerase I and II alterations, glutathione S-transferase Π overexpression, altered thiol content, DNA repair and overexpression of another glycoprotein called gp180/MRP [15, 16]. The role of mdr1 as a factor in determining therapeutic outcome in human malignancies is controversial [17–19]. However, recent evidence in haematological malignancies suggests that the *mdr1* phenotype plays a role in the development of resistance of these human tumours to chemotherapeutic agents [20]. Tumour resistance in ACL patients has been attributed, in part, to mdr phenotypes [21].

We previously developed a metastatic xenograft model in athymic nude mice that utilises human lung carcinoma MV522 cells. This xenograft model, whole refractory to conventional chemotherapeutic agents as defined by failure to increase life span by > 50%, is not mdr1/gp170-positive [22]. To aid in determining if MGI 114 is effective *in vivo* against mdr1 cells, we generated for this study an mdr1/gp170-positive MV522 clone that retained the metastatic abilities of the parent cells. We used this mdr1 xenograft model to demonstrate *in vivo* efficacy of the new illudin analogue, MGI 114, against mdr1/gp170 expressing cells.

## MATERIALS AND METHODS

*Athymic mice*

Balb/c nu/nu 4 week old female mice weighing 18–22 g were obtained from Simonsen Inc. (Gilroy, California, U.S.A.) 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 sterilised food and water *ad libitum* in groups of four in plastic cages vented with polyester fibre filter covers. Clean, sterilised 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 was derived as described previously [11, 12] and maintained in antibiotic-free RPMI 1640 media (Mediatech, Herndon, Viginia, U.S.A.) supplemented with 10% fetal bovine serum plus 2 mM glutamine in a humidified 5% carbon dioxide incubator at 37°C. The MV522/mdr1 gp170-positive clones were maintained under the same conditions as parent cells, except that vinblastine was added to the cultures at a final concentration of 50 mg/ml. MV522/mdr1 cells were maintained continuously in vinblastine-containing media until injected subcutaneously (s.c.) into mice.

The *in vitro* toxicity of various illudin analogues, mitomycin C, doxorubicin and paclitaxel, to parent MV522 cells and the MV522/mdr1 clones was determined by comparing $IC_{50}$ values derived from continuous 7 day exposure studies, with cell viability assessed by Trypan Blue exclusion. The use of a 7-day exposure period allows comparison with results obtained for other illudin analogues and experimental agents by the National Cancer Institute (Bethesda, Maryland, U.S.A.) in their human tumour cell line screening panel [7–12, 23].

The following mdr and parent cell lines were obtained from other investigators, as previously noted [8, 9]: CEM human T-cell parent and CEM/VM1 (topoisomerase II mutant) from Dr W. Beck; MDA-231 human breast carcinoma and doxorubicin-resistant daughter line MDA3-1 gp170+ from Dr W. McGuire; MCF7/wt breast carcinoma and doxorubicin-resistant daughter MCF7/Adr (expresses both gp170 and the embryonic glutathione transferase π isoform) from Dr C. E. Myers; HL60 human promyelocytic and doxorubicin daughter line HL60/Adr (resistance is mediated by a glycoprotein termed gp180/MRP that is immunologically distinct from the more commonly gp170 protein) from Dr M. Center; DC3F Chinese hamster line and DC3F/C10 (topoisomerase I mutant) from Dr Y. Pommier; L1210 murine leukaemia and daughter lines L1210/DDTP, L1210/BCNU, L1210/PAM and L1210/CPA from Dr DP. Griswold Jr; the 8226 human myeloma and daughter lines 8226/DO (gp170 positive) and 8226/LR5 from Dr W. Dalton. These mdr lines and parental lines were maintained at RPMI 1640, Dulbecco's Modified Eagle Medium (DMEM), or HITES media supplemented with 10% fetal bovine serum and 2 mM glutamine, in accordance with instructions provided by other investigators. The cytotoxicity of MGI 114 and several conventional anticancer agents, to parental and mdr daughter lines was assessed using continuous 48 h exposure and with cell viability assessed by Trypan Blue exclusion as previously described [7]

*Drugs*

Anticancer drugs were obtained from the UCSD Medical Center Pharmacy and formulated with the supplied diluent according to directions provided by the manufacturer. Drugs included: paclitaxel (Taxol; Bristol-Myers Squibb, Princeton, New Jersey, U.S.A.) reconstituted in Cremophor EL; mitomycin C (Mutamycin; Bristol-Myers Squibb) reconstituted in sterile water; vinblastine sulphate (Velban; Lilly Research, Indianapolis, Indiana, U.S.A.) reconstituted in normal saline; and doxorubicin (Adriamycin-PFS; Adria Laboratories, Dublin, Ohio, U.S.A.), already in sterile solution for injection. Vehicle controls were performed where appropriate. Illudin analogues were prepared for *in vivo* testing by dissolving at maximum solubility in a sterile 40% dimethylsulphoxide (DMSO) normal saline mixture and diluted to appropriate concentrations with sterile 20% DMSO/normal saline [12].

*Illudin isolation and analogue synthesis*

Illudin S was isolated from corn-steeped broth as described previously [24]. The novel MGI 114 analogue was synthesised from the acylfulvene analogue [11] using formaldehyde. Spectroscopic data and synthesis for MGI 114 are described in detail elsewhere [25]. The structures of the illudin analogues are provided (Figure 1).

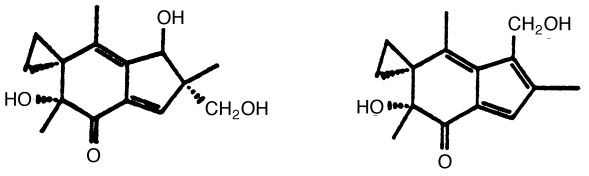


**Figure 1. Structures of the parent compound illudin S and the illudin-derived analogue MGI 114 tested in this study.**

*Vector preparation, transfection and isolation of resistant cells*

The mdr1/gp170 eukaryotic expression plasmid was obtained from Dr Michael M. Gottesman and propagated in DH5α *Escherichia coli* cells [26]. The plasmid was recovered and transfected into MV522 cells using lipofectin (Gibco-BRL, Gaithersburg, Maryland U.S.A.). Transfected cells were exposed to 50 ng/ml of vinblastine for 3 weeks and drug-resistant colonies isolated using cloning cylinders. Control MV522 cells were transfected with pBR322 plasmid and exposed to either 30 or 50 ng/ml vinblastine, but no control cells survived vinblastine selection.

In vivo *evaluation using the mdr/gp*170 + *MV*522 *xenograft model*

Mice were randomised into treatment groups of 12–16 animals each. Each animal was earmarked and followed individually throughout the experiment. The mice received s.c. injections of 10 million MV522 cells over the shoulder. Ten days after implantation of the MV522 cells, the animals received the desired drug and dosage.

Although MV522 cells kill by metastases [12], we also monitored primary s.c. tumour growth starting on the first day of treatment. Tumour size was measured in two perpendicular diameters and tumour weight estimated according to the formula $w = [(\text{width})^2 \times \text{length}/2]$ [27]. Relative weights were calculated to standardise variability in tumour size amongst test groups at initiation of the treatment by using the formula relative weight = $Wt/Wi$, where $Wi$ is the tumour weight for a given animal at the beginning of drug treatment and $Wt$ is tumour weight at a subsequent time $t$ [27]. Selected animals were autopsied and organs examined for metastases.

*Table* 1. *Cytotoxicity of MGI* 114 *against mdr cell lines following a* 48 *h exposure*

| Cell lines | Mechanism* | 48 h $IC_{50}$ (nM)† |
|---|---|---|
| HL60 | Parent | 1050 ± 200 |
| HL60/Adr | gp180/MRP | 970 ± 100 |
| MDA 231 | Parent | 840 ± 90 |
| MDA 3-1 | gp170 | 420 ± 10 ($P < 0.01$)‡ |
| MCF7 | Parent | 1570 ± 400 |
| MCF7/Adr | gp170/GSHTR-π | 590 ± 100 |
| L1210/wt | Parent | 930 ± 80 |
| L1210/CPA | | 500 ± 90 |
| L1210/BCNU | | 530 ± 150 |
| L1210/PAM | | 810 ± 190 |
| L1210/DDPT | | 770 ± 40 |
| 8226/wt | Parent | 13 600 ± 3300 |
| 8226/DO | gp170 | 8800 ± 400 |
| 8226/LR5 | Thiol content | 5800 ± 600 |
| CEM | Parent | 1400 ± 250 |
| CEM/VM-1 | Topoisomerase II | 430 ± 150 ($P < 0.01$)‡ |
| DC3F | Parent | 1600 ± 80 |
| DC3F/C10 | Topoisomerase I | 1510 ± 240 |

*Mechanisms of mdr phenotype (if known). A description of the cell lines and the mdr mechanisms is provided in Materials and Methods. †Results are expressed as mean ± standard deviation ($n = 3$). ‡$P$ value indicates that these mdr daughter lines are actually significantly sensitive, not resistant, to MGI 114 as compared with the parental line (Student's $t$ test).

*Flow cytometry detection of mdr*1/*gp*170 *protein*

The parent MV522 and mdr1/gp170 Q6 daughter cells were assessed for mdr1 expression by immunofluorescence staining as previously described [28] using monoclonal antibody (MAb) MRK16 (kindly provided by Dr Catherine Spier, Department of Pathology, University of Arizona, Arizona, U.S.A.). Staining with MRK16 was compared with an isotype control.

*Statistical analysis*

To compare the relative tumour 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 [29]. To compare the relative cytotoxicity of a given drug between a parental cell line and its mdr daughter line, the $IC_{50}$ values were analysed using Student's $t$ test. Probability values less than 0.05 were considered statistically significant. Relative tumour weight and $IC_{50}$ values were analysed using Instat (version 2.02), and life span data were analysed using Prism (version 2.0) software package (Graph Pad Inc., La Jolla, California, U.S.A.).

## RESULTS

In vitro *efficacy of MGI* 114 *against mdr lines*

We studied the efficacy of MGI 114 against various mdr phenotypes to determine if structural alterations made to produce this analogue altered efficacy against mdr cells. None of the mdr lines tested were resistant *in vitro* to MGI 114 when compared with their respective parent cells (Table 1).

*Development of MV*522 *mdr*1 *cells*

A metastatic MV522/mdr1 xenograft model was created by transfecting parent MV522 cells with a eukaryotic expression vector containing the cDNA encoding for human mdr1/gp170 (Figure 2). MV522 clones were selected for mdr1

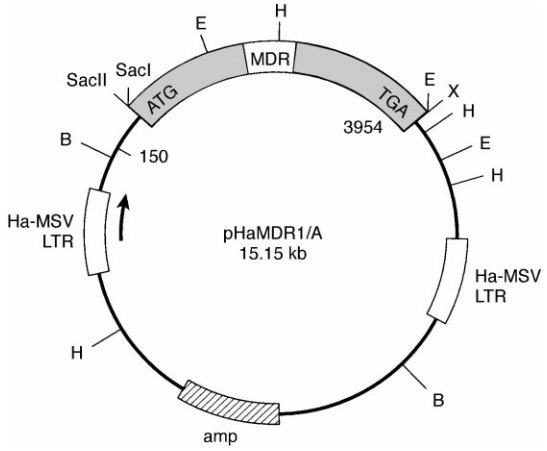


**Figure 2. The mdr1/gp170 eukaryotic expression vector used to generate the Q6 MV522 mdr clone used in this study [26].**

*Table 2. Toxicity of agents against mdr1/gp170 + Q6 MV522 clone versus parental MV522 cells after a 7-day exposure*

| Agent | $IC_{50}$ (nM)* Parent | Q6 Clone | *P* value† |
|---|---|---|---|
| Vinblastine | 9 ± 4 | 86 ± 9 | < 0.001 |
| Mitomycin C | 27 ± 6 | 45 ± 3 | < 0.05 |
| Doxorubicin | 14 ± 6 | 152 ± 80 | < 0.001 |
| MGI 114 | 514 ± 101 | 527 ± 146 | |
| Paclitaxel | 15 ± 4 | 358 ± 58 | < 0.001 |

*Results expressed as mean ± standard deviation ($n = 3$). †*P* values indicate whether the Q6 clone is significantly resistant to that agent as compared with the parental line.

expression by continuous exposure to 50 nM vinblastine for 21 days. Twelve clones were selected at random and injected s.c. into athymic Balb/c nu/nu mice to determine ability to metastasise, as previously described [12]. All clones metastasised to the lungs. One clone (Q6) was highly metastatic as compared with the others and was chosen for further *in vitro* and xenograft studies. The MV522/mdr1 Q6 clone was markedly resistant to paclitaxel, vinblastine and doxorubicin (Table 2), and mildly resistant to mitomycin C. The resistance of the Q6 clone to these agents was stable for at least 6 weeks in culture in the absence of a selection agent, which exceeds the duration of drug administration for the *in vivo* experiments. The Q6 mdr1/gp170 clone was not resistant *in vitro* to MGI 114 (Table 2).

Analysis of gp170 expression by FACS was performed using MAb MRK16. Relative expression between parent and mdr1/gp170 expressing cells was compared by staining with MAb MRK16 and the non-binding IgG2a–FITC conjugate. The use of these two antibodies allowed a direct comparison of mdr1/gp170 expression with that in previous reports utilising mdr1 vectors [30, 31]. The Q6 MV522 daughter line expressed mdr1/gp170 protein (Figure 3). The relative increase in fluorescence attributed to gp170 expression (compared with IgG2a) was similar to the relative increase noted with the colchicine-selected mdr KB-8-5 gp170-positive line as compared with its parental line KB-3-1 [30, 31].

*Xenograft studies*

The efficacy of MGI 114, mitomycin C and paclitaxel against the MV522 mdr1/gp170 xenograft was determined.

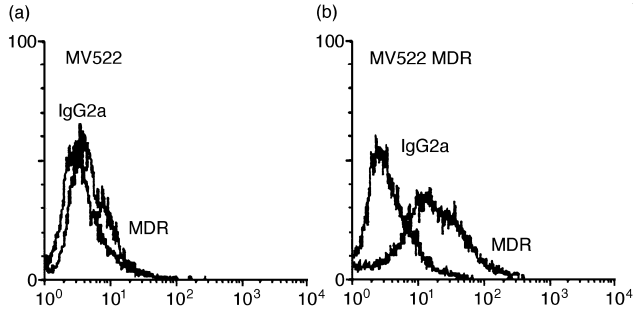


**Figure 3. Expression of the mdr1/gp170 glycoprotein in the Q6 MV522/mdr1 clone versus parent MV522 cells. The cells were stained with anti-gp170 MRK16 antibody in comparison with the isotype IgG 2A antibody. Relative fluorescence intensities: (a) parental MV522 cells; (b) Q6 mdr1/gp170 clone selected after exposure to vinblastine at 50 ng/ml. The *x* axis indicates relative cell count and the *y* axis indicates relative fluorescence.**

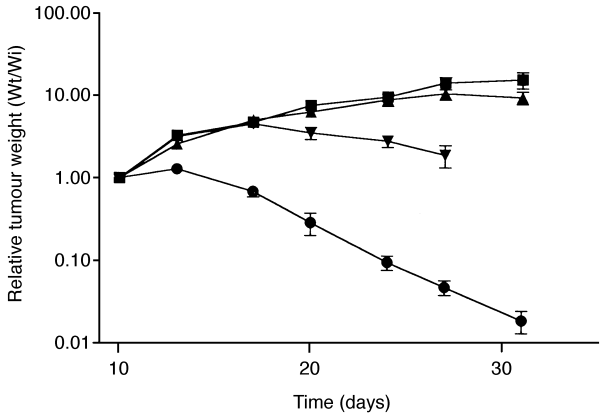


**Figure 4. Efficacy of MGI 114 versus acylfulvene, mitomycin C and paclitaxel at inhibiting the tumour growth of the primary Q6 mdr1/gp170 MV522. Control animals (■) ($n = 16$) received 20% dimethylsulphoxide/saline; mitomycin C (▼) ($n = 16$) at 2.4 mg/kg; paclitaxel (▲) ($n = 12$) at 12 mg/kg; and MGI 114 (●) ($n = 16$) at maximum tolerated dose of 10 mg/kg. Drugs were administered intraperitoneally three times per week for 3 weeks except for paclitaxel which was administered five times per week for 3 weeks. Data points indicate means for each group; bars standard error.**

Other anticancer agents that would normally be used, such as cisplatin, vinblastine, cytoxan, 5-fluorouracil, etc. were not chosen because the parental non-gp170 model is already resistant to treatment with these agents at a variety of doses, schedules and administration routes [13, 22]. Doses and schedules for mitomycin C and paclitaxel were chosen on the basis of previous studies which determined the maximum tolerated dose and the most efficacious schedule for these drugs [7–13]. In the first experiment, mitomycin C and paclitaxel had no effect on primary tumour growth, whereas MGI 114 induced primary tumour regressions in 16 of 16 animals ($P < 0.0001$) (Figure 4) and produced complete remissions in 6 of 16 animals ($P < 0.0001$) (Figure 5). Mitomycin C and paclitaxel were toxic as evidenced by weight loss of greater than 15% and a decrease in life span (Figure 5). Because of the development of this drug toxicity, the doses of mitomycin C and paclitaxel were reduced by

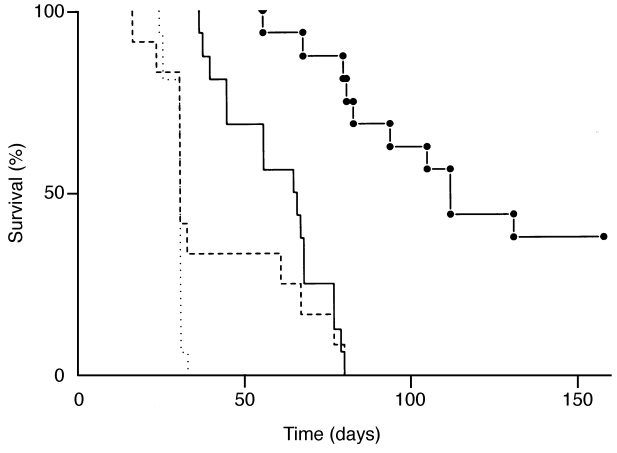


**Figure 5. Efficacy of MGI 114 versus acylfulvene, mitomycin C and paclitaxel in prolonging life span in mdr1/gp170 model. Control animals (——) ($n = 16$) received 20% dimethylsulphoxide/saline MGI 114 (●–●) ($n = 16$) at maximum tolerated dose of 10 mg/kg); mitomycin C (···) ($n = 16$) at 2.4 mg/kg; paclitaxel (– – –) ($n = 12$) at 12 mg/kg.**

15% in a second experiment. At these doses, there was no weight loss or toxic deaths. However, neither agent prolonged the life span (Figure 6) nor inhibited tumour growth (data not shown). MGI 114 again induced primary tumour regressions in 16 of 16 animals ($P < 0.0001$) (data not shown) and complete remissions in 5 of 16 animals ($P < 0.0001$ for MGI 114 life span versus control life span) (Figure 6).

## DISCUSSION

The chemical alterations made to the illudin structure, required to produce MGI 114, did not result in loss of efficacy against various mdr tumour lines. None of the mdr tumour lines, regardless of mechanisms of resistance, were resistant *in vitro* to MGI 114 (Table 1). Indeed, several mdr lines, such as the gp170-positive MDA 3-1 and CEM/VM-1 topoisomerase II mutant, were relatively more sensitive to MGI 114 than their respective parental cell lines. The basis for this sensitivity is not clear, but may be the result of the method utilised to produce drug resistance. The sudden exposure of cells to a toxin can result in resistance by more than one mechanism (e.g. the MCF7/adr line expresses both gp170 and the embryonic glutathione S-transferase π isoform), including mechanisms which may currently not be known. In contrast, transfectants, such as the Q6 line, would be expected to develop resistance primarily by the mechanism conferred by the expression vector utilised (i.e. gp170 in this study). Thus, it is plausible that the non-transfectant gp170 expressing lines sensitive to MGI 114 have other unknown alterations responsible for the sensitivity.

Mitomycin C was previously noted to have some activity in the original non-mdr MV522 xenograft model evidenced as partial tumour regressions and an occasional small increase in life span [7–13]. Paclitaxel at a high-dose of 15 mg/kg induces partial tumour regression, but has no effect on lifespan [22]. The gp170-positive phenotype resulted in a complete loss of *in vivo* efficacy by mitomycin C or paclitaxel, indicating that the Q6 clone maintained the mdr1/gp170 phenotype during the course of the xenograft studies. The MGI 114, in contrast, was effective in the MV522/mdr1 model by inducing tumour regression and producing long-term survivors (34%, $P < 0.001$). The activity of MGI 114 against the parental or non-gp170 xenograft model has been extensively studied [12, 13]. The 34% survivor rate noted here in the gp170 model is similar to that previously noted with the parental non-mdr1 model [12, 13]. It is noteworthy that with 12 other standard chemotherapeutic agents, at various dosages and administration schedules, there were no long-term survivors in this xenograft model [7–13].

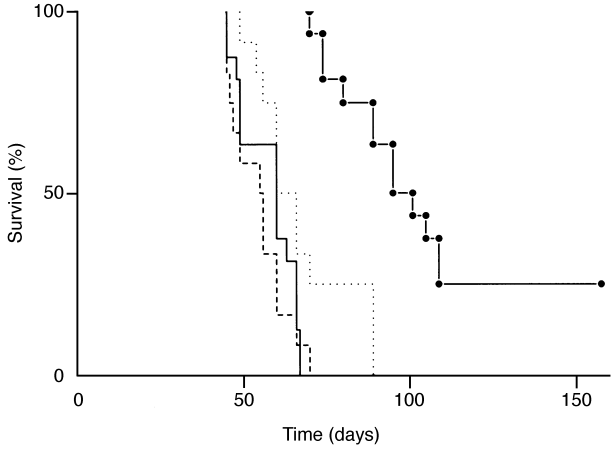


**Figure 6. Efficacy of MGI 114 versus acylfulvene, mitomycin C and paclitaxel on prolonging life span in mdr1/gp170 model. Control animals: (——) ($n = 16$) received 20% dimethylsulphoxide/saline; MGI 114 (●–●) ($n = 16$) at maximum tolerated dose of 10 mg/kg; mitomycin C (· · ·) ($n = 12$) at maximum tolerated dose of 2.0 mg/kg; paclitaxel (– – –) ($n = 12$) at maximum tolerated dose of 10 mg/kg.**

Previous studies in non-drug resistant human tumour xenograft models have suggested that illudin analogues are interesting and potentially useful agents for the treatment of solid tumours. The present study extends previous observations of MGI 114 efficacy by demonstrating activity of the analogue against a broad range of drug resistance mechanisms *in vitro*, as well as activity against the mdr1 drug resistant phenotype in a difficult to treat lung tumour xenograft. These results suggest that MGI 114 and other illudin analogues may prove to have broad utility in the treatment of refractory drug resistant human malignancies.

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