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Enhanced antitumour activity of 6-hydroxymethylacylfulvene in combination with topotecan or paclitaxel in the MV522 lung carcinoma xenograft model

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Abstract

6-hydroxymethylacylfulvene (HMAF; MGI 114; Irofulven) is a semisynthetic analogue of the toxin illudin S, which is a product of the *Omphalotus* mushroom. MGI 114 induces cytotoxicity against a broad range of solid tumours *in vivo*, including the drug-refractory MV522 human lung cancer xenograft. In this study, the potential application of MGI 114 in the treatment of lung cancer was explored by evaluating the activity of MGI 114 in combination with either topotecan (TPT) or paclitaxel. Groups of eight nude mice bearing MV522 xenografts were treated with MGI 114, TPT or paclitaxel as single agents and with MGI 114 in combination with TPT or paclitaxel. MGI 114 was administered at doses of 2.5 and 5.0 mg/kg intraperitoneally (i.p.) daily on days 1–5, while TPT and paclitaxel were administered at doses of 0.5 or 1.0 mg/kg and 20 mg/kg, respectively, i.p. on days 1–5. In the single-agent studies, MGI 114, TPT and paclitaxel all resulted in decreased final tumour weights compared with vehicle-treated controls. As single agents, TPT, at the 0.5 mg/kg dose level, and paclitaxel, at the 20 mg/kg dose level, produced partial shrinkages (PSs). All combinations of MGI 114, and either TPT or paclitaxel, produced decrements in final tumour weights compared with monotherapy with the same doses of MGI 114, TPT and paclitaxel. Although all animals treated with the combination of MGI 114 and paclitaxel experienced PSs or complete shrinkages (CSs) (or died), analysis of the time to tumour doubling revealed that the combination of MGI 114 and TPT at 2.5 and 0.5 mg/kg, respectively, was synergistic. These results suggest that cytotoxic activity is enhanced when MGI 114 is combined with either TPT or paclitaxel, and clinical trials to further evaluate these combination regimens are warranted.

Keywords: 6-hydroxymethylacylfulvene (MGI 114; Irofulven); Topotecan; Paclitaxel; Lung cancer; Illudins

1. Introduction

6-hydroxymethylacylfulvene (HMAF, MGI 114 or Irofulven) is a semi-synthetic analogue of the sesquiterpene toxin illudin S, which is a natural product of the *Omphalotus* mushroom [1]. The mechanism by which illudins damage DNA is unique and appears to require functional DNA helicases for DNA repair to proceed [2,3]. Although the precise mechanism of MGI 114 cytotoxicity has not been entirely elucidated, recent studies have suggested that MGI 114 inhibits DNA synthesis, arrests the cell cycle in S phase, and induces apoptosis [4].

The illudins are cytotoxic to a variety of leukaemias and solid tumour cell lines, including those with multidrug-resistant phenotypes [5,6]. Human MCF7 mammary carcinoma, HT29 colon carcinoma and MV522 lung carcinoma cells are all sensitive to MGI 114 at nanomolar concentrations [7,8]. In addition, partial and complete tumour shrinkages have been seen following the administration of MGI 114 to nude mice bearing human MX1 breast, HT29 colon and MV522 lung carcinoma xenografts [7,8]. This broad antitumour activity

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against a broad range of human tumours *in vitro* and *in vivo* reflects the ability of MGI 114 to inhibit cell growth regardless of p53 status and WAF-1 status [9].

The activity of MGI 114 in the MV522 lung carcinoma xenograft model is striking compared with the activity of conventional cytotoxic agents, including cisplatin, doxorubicin, methotrexate, carmustine and 5-fluorouracil. Mitomycin C, when administered at a toxic dose (LD_{20}), caused a mild, but variable, increase in median lifespan (41–61%) [10,11]. However, toxic doses of paclitaxel inhibited primary tumour growth in animals surviving drug treatment, while failing to significantly increase animal lifespan [10]. Subsequent clinical studies confirmed the activity of paclitaxel (two phase II trials with a 21% and 24% response rate) [12,13], and mitomycin C (prolongation of survival in combination studies) [14,15] in non-small cell lung cancer as predicted by the initial MV522 lung carcinoma xenograft data.

A previous study examining MGI 114 in different chemotherapy combinations revealed synergistic activity *in vitro* in various paediatric cell lines between both topotecan (TPT) and MGI 114, and paclitaxel and MGI 114 [16]. Statistically significant antitumour synergy was observed with the combinations of MGI 114 and TPT and MGI 114 and paclitaxel in 10/12 (83%) and 2/5 (40%) of cell lines, respectively. Further evaluation of a topoisomerase I inhibitor, CPT-11, in combination with MGI 114 in the HT29 human colon cancer xenograft model confirmed enhanced activity [17]. Paclitaxel was also chosen for further investigation as it has activity against the MV522 xenograft model, has a different mechanism of action, and is additive in *in vitro* combination testing in paediatric cell lines.

In the current study, MGI 114 was administered as a single agent and in combination with either TPT or paclitaxel to nude mice bearing MV522 xenograft tumours to explore the potential application of MGI 114 in the treatment of lung cancer.

2. Materials and methods

2.1. Cytotoxic drugs

MGI 114 was synthesised using illudin S from still cultures of *O. illudens* [1]. MGI 114 at 7.0 mg/kg was previously determined to be the maximum tolerated dose (MTD) on a daily for five consecutive days dosing regimen [6]. The doses of MGI 114 administered in this present study were 2.5 and 5.0 mg/kg. The vehicle for MGI 114 was 1% ethanol in 5% dextrose in water. TPT and paclitaxel were obtained from SmithKline Beecham (Philadelphia, PA, USA) and Bristol-Myers Squibb (Princeton, NJ, USA), respectively, and diluted in 5% dextrose in water.

2.2. In vivo *evaluation in human tumour xenograft models*

MV522 human lung carcinoma cells were obtained as previously described [7,8]. Nude mice (Harlan Sprague–Dawley, Inc., IN, USA) were implanted subcutaneously (s.c.) by trocar with fragments of MV522 human lung tumours harvested from s.c. growing tumours in nude mice. When tumours were approximately 5 mm×5 mm in size (usually approximately 12 days after inoculation), the animals were pair-matched into treatment and control groups (day 1). The day 1 tumour weights ranged from 65.2 to 66.9 mg for all groups. Each group consisted of eight tumour-bearing mice that were ear-tagged and followed individually throughout the study. The i.p. administration of drugs or vehicle began on day 1 and continued for a total of five consecutive days. MGI 114, whose MTD was previously established as 7.0 mg/kg, was administered at doses of 2.5 and 5.0 mg/kg daily on days 1–5. TPT was administered daily for five consecutive days at doses of 0.5 or 1.0 mg/kg which were one-third and two-thirds, respectively, of the MTD. Paclitaxel was administered at its MTD of 20 mg/kg. The control group received the vehicle for MGI 114 (1% ethanol in 5% dextrose in water). Following tumour implantation, mice were weighed and tumour measurements were performed twice weekly, starting on day 1. These tumour measurements were converted to tumour weight (mg) using the established formula:

$$\text{Weight (mg)} = \frac{\text{Width (mm)}^2 \times \text{Length (mm)}}{2}$$

Experiments were terminated when tumours in the control animals reached a size of approximately 1 g. At termination, all mice were sacrificed and their tumours were excised. Tumours were then weighed, and the mean tumour weights per group were calculated. The tumour growth inhibition (TGI) was calculated for each group as:

$$\text{TGI} = 100\% - \left(\frac{\Delta\text{Mean treated tumour weight}}{\Delta\text{Mean control tumour weight}} \times 100\%\right)$$

The percentage of tumour reduction was calculated for individual animals using calculated day 1 weights and actual final tumour weights as follows:

$$\%\ \text{Tumour reduction} = \frac{\text{day 1 tumour weight} - \text{final tumour weight}}{\text{day 1 tumour weight}} \times 100\%$$

A partial shrinkage (PS) was defined as any partial reduction in the actual final tumour weight compared

Table 1
MGI 114 and topotecan (TPT) in the MV522 human lung tumour xenograft

Group	*n*	i.p. Dose (mg/kg)	Schedule	Final tumour weight in mg (mean±S.D.)	Mean % TGI	Mice with tumour shrinkage		Maximal % tumour weight loss (day 5)	Mean % tumour reduction	No. of toxic deaths
						Partial shrinkage (PS) *n*	Complete shrinkage (CS) *n*			
Control	8	Vehicle	Daily×5	925±152.1	0	0	0	+2.1	–	0
MGI 114	8	2.5	Daily×5	630±53.7	34.4	0	0	−1.5	–	0
MGI 114	8	5.0	Daily×5	506±83.9	48.7	0	0	−7.4	–	0
TPT	8	0.5	Daily×5	579±98.7	31.8	1	0	−1.0	13.6	0
TPT	8	1.0	Daily×5	648±60.4	32.4	0	0	+2.4	–	0
MGI 114/TPT	8	2.5/0.5	Daily×5	156±32.5	87.7	1	0	−9.7	34.4	0
MGI 114/TPT	8	2.5/1.0	Daily×5	172±43.7	85.9	1	0	−14.8	43.8	0
MGI 114/TPT	8	5.0/0.5	Daily×5	59.8±15.2	97.8	3	1	−17.4	65.0	0
MGI 114/TPT	8	5.0/1.0	Daily×5	38.0±0.0	–	1	0	−21.0	49.3	7

i.p., intraperitoneal; S.D., standard deviation; TGI, tumour growth inhibition.

with the calculated day 1 tumour weight (% tumour reduction $<100\%$), whereas a complete shrinkage (CS) required the disappearance of the tumour (% tumour reduction $=100\%$). The maximum percentage of animal weight loss, as an indication of toxicity was calculated for individual animals as:

$$\%\ \text{Animal weight loss} = \frac{\text{daily weight} - \text{minimum weight on study}}{\text{day 1 weight}} \times 100\%$$

2.3. Statistics

Statistical analysis examined time to tumour doubling. Time to tumour doubling was defined as the difference in days from initiation of treatment to the day that tumour size was double that of baseline, with intermediate days being estimated by linear interpolation. Groups with toxic deaths were omitted from the analysis. Survival analysis was used to summarise and compare the groups. Survival curves were computed using the Kaplan–Meier product-limit method and compared using the generalized Wilcoxon test [18].

3. Results

3.1. Single-agent studies

Tables 1 and 2 summarise the efficacy studies of MGI 114, TPT and paclitaxel in MV522 xenografts. There were no spontaneous tumour regressions observed in the control group. Single-agent MGI 114 administered intraperitoneally (i.p.) daily for five consecutive days produced modest activity against the MV522 human lung tumour model with a mean TGI of 34.4% and 48.7% at doses of 2.5 and 5.0 mg/kg, respectively. MGI 114 doses used in this study were approximately one-third and two-thirds of the previously identified MTD of 7.0 mg/kg i.p. on a daily consecutive schedule for five days. Fig. 1 illustrates that MGI 114-treated tumours continued to grow at a somewhat slower rate than controls. No partial shrinkages (PSs) or complete shrinkages

Table 2
MGI 114 and paclitaxel in the MV522 human lung tumour xenograft

Group	*n*	i.p. Dose (mg/kg)	Schedule	Final tumour weight in mg (mean+S.D.)	Mean % TGI	Mice with tumour shrinkage		Maximal % tumour weight loss (day 5)	Mean % tumour reduction	No. of toxic deaths
						Partial shrinkage (PS) *n*	Complete shinkage (CS) *n*			
Control	8	Vehicle	Daily×5	925±152.1	0	0	0	+2.1	–	0
MGI 114	8	2.5	Daily×5	630±53.7	34.4	0	0	−1.5	–	0
MGI 114	8	5.0	Daily×5	506±83.9	48.7	0	0	−7.4	–	0
Paclitaxel	8	20	Daily×5	181±46.1	84.0	1	0	+0.3	78.4	0
MGI 114/Paclitaxel	8	2.5/20	Daily×5	7.4±4.1	–[a]	3	5	−12.8	87.1	0
MGI 114/Paclitaxel	8	5.0/20	Daily×5	13.6±8.9	–[b]	5	2	−20.5	82.0	1

i.p., intraperitoneal; S.D., standard deviation; TGI, tumour growth inhibition.
[a] The term TGI does not apply here, as all animals in this group developed CS or PS.
[b] The term TGI does not apply here, as all animals in this group developed CS or PS or died and were not evaluable for tumour reduction.

(CSs) were noted when MGI 114 was administered as a single agent at these doses. TPT administered as a single agent also had modest activity against the MV522 xenograft at doses of 0.5 (mean TGI = 31.8%) and 1.0 (mean TGI = 32.4%) mg/kg/i.p. daily for five consecutive days. These doses of TPT represent approximately one-third and two-thirds the previously established MTD of TPT on this schedule [6]. There was one observed PS when TPT was administered at the 0.5 mg/kg dose level. Fig. 1 illustrates that TPT-treated tumours continued to grow at a rate slower than controls. No CSs were observed with TPT administration. Paclitaxel was administered at its previously determined MTD of 20 mg/kg daily for five consecutive days i.p. [6]. Substantial decreases in the actual final tumour weight were observed when single-agent paclitaxel was administered at the 20 mg/kg dose level as reflected by a mean TGI of 84.0%. There was one PS in the single-agent paclitaxel group with an observed 78.4% decrease in tumour size. Fig. 2 illustrates the mean tumour weight approaching zero particularly at the earlier time-points following treatment with paclitaxel 20 mg/kg.

3.2. Combination studies

The combination of MGI 114 and TPT was highly efficacious against the MV522 human lung tumour model (Table 1 and Fig. 1). MGI 114 and TPT at doses of 2.5 mg/kg and 0.5 or 1.0 mg/kg, respectively, resulted in mean TGI values averaging 87.7% and 85.9%, respectively. When the combination of MGI 114 and TPT was administered at doses of 5.0 mg/kg and 0.5 mg/kg, respectively, a mean TGI of 97.8% was obtained. In addition, the administration of MGI 114 and TPT at 2.5 mg/kg and 0.5 or 1.0 mg/kg respectively, resulted in one PS at each TPT dose level with a 34.4% and a 43.8% reduction in tumour size at the 0.5 and 1.0 mg/kg doses, respectively. The combination of MGI 114 and TPT at 5.0 mg/kg and 0.5 mg/kg, respectively, yielded three PSs with a mean reduction in tumour of 65% and one CS. The maximal antitumour response was observed 19 days after the administration of the agents (Fig. 1). The antitumour effect of MGI 114 together with TPT is further demonstrated in Fig. 3, which depicts the time to tumour doubling. Statistical analysis revealed that the combination of MGI 114 and TPT at 2.5 mg and 0.5 mg/kg, respectively, is synergistic ($P < 0.0001$).

The combination of MGI 114 (2.5 and 5.0 mg/kg) and paclitaxel (20 mg/kg) was also active against the MV522 human lung tumour model (Table 2 and Fig. 2). All animals treated with this combination experienced either a PS or a CS (or died). At the MGI 114 2.5 mg/kg and paclitaxel 20 mg/kg dose level, there were three PSs with a mean tumour reduction of 87.1%. At the next dose level, with administration of MGI 114 5.0 mg/kg and paclitaxel 20 mg/kg, five PSs (mean tumour reduction of 82%) and two CSs were observed. Synergy

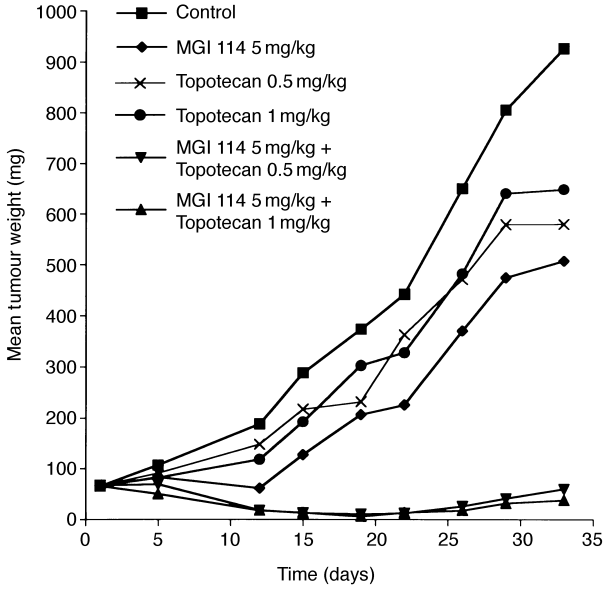

Fig. 1. MGI 114±topotecan against MV522 human lung tumour xenografts. MV522 human lung carcinoma fragments were implanted subcutaneously (s.c.) by trocar in nude mice. Treatment was initiated with MGI 114 and/or topotecan (TPT) as previously described. Data reflecting the results of MGI 114 at 2.5 mg/kg were omitted from this graph for clarity. Tumours were measured using calipers twice weekly, and the tumour weight calculated. Mean tumour weight for each treatment group is plotted ($n = 8$).

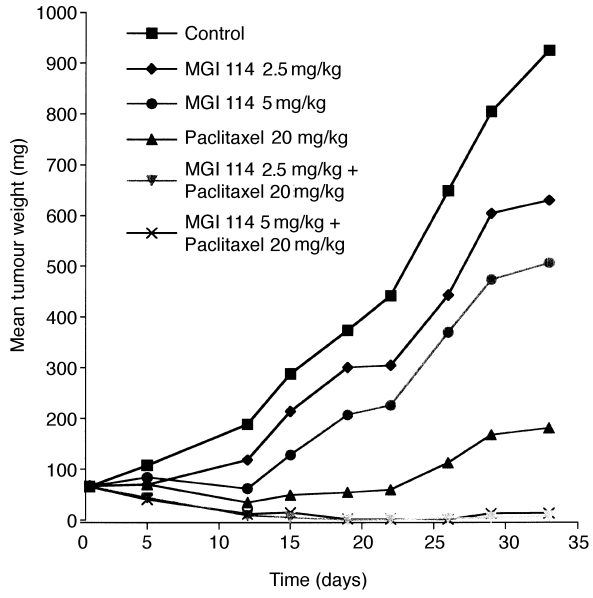

Fig. 2. MGI 114±paclitaxel against MV522 human lung tumour xenografts. MV522 human lung carcinoma fragments were implanted subcutaneously (s.c.) by trocar in nude mice. Treatment was initiated with MGI 114 and/or paclitaxel as previously described. Tumours were measured using calipers twice weekly, and the tumour weight calculated. Mean tumour weight for each treatment group is plotted ($n = 8$).

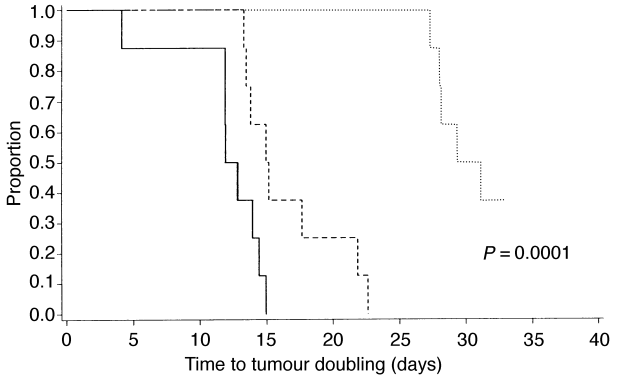

Fig. 3. Kaplan–Meier curves showing the time to tumour doubling. MGI 114 was administered as a single agent at 5.0 mg/kg (dashed line – – –). Topotecan (TPT) was administered as a single agent at 1.0 mg/kg (solid line —). MGI 114 was administered in combination with TPT at doses of 2.5 and 0.5 mg/kg (dotted line .....), respectively. Time to tumour doubling was significantly ($P=0.0001$) prolonged in animals treated with MGI 114 and TPT compared with animals treated with single-agent MGI 114.

between MGI and paclitaxel could not be formally examined.

3.3. Toxicity

There were no toxic deaths in the single-agent studies. The combination of MGI 114 at 5.0 mg/kg and TPT at 1 mg/kg resulted in seven out of eight toxic deaths. There were no observed toxic deaths at the other dose levels for the combination of MGI 114 and TPT. However, substantial weight loss was observed by day 5 with these MGI 114 and TPT combinations, especially at the MGI 114 and TPT doses of 5.0 and 1 mg/kg, respectively, where a mean weight reduction of 21% was observed. By the end of the study, animals in all treatment groups were gaining weight.

In the MGI 114 and paclitaxel combination studies, there was one toxic death in an animal treated with MGI 114 and paclitaxel at 5.0 mg/kg and 20 mg/kg, respectively. The administration of this drug combination resulted in a 20.5% weight loss on day 5, with subsequent weight gain in all animals.

4. Discussion

Interest in the illudins as antineoplastic agents stems from the discovery that they exhibit unique mechanisms with activity against a variety of refractory xenograft models [2,19]. The parent compound of this unique class of agents produces significant toxicity, while the derivative MGI 114 has a favourable therapeutic index and, therefore, has entered clinical trials for further evaluation [1,11]. MGI 114 demonstrated significant antitumour activity against MV522 human lung carcinoma in a previous study at its MTD of 7.0 mg/kg [7]. In addition, *in vitro* data looking at MGI 114 combinations also suggest that there is synergy with TPT and possibly with paclitaxel. Consequently, this present study was initiated to explore the interaction of MGI 114 with both TPT and paclitaxel in the MV522 xenograft model.

In this study, MGI 114 was administered at doses of 2.5 and 5.0 mg/kg, which is lower than the MTD of 7.0 mg/kg determined in previous MGI 114 studies. At these doses, there was a demonstrated reduction in the mean TGI (34.4% and 48.7%). The combination of MGI 114 and TPT was synergistic at doses of 2.5 and 0.5 mg/kg, respectively ($P<0.0001$). Increases in TGI were demonstrated for all combinations of MGI 114 and TPT. This increase in TGI with the combination of MGI 114 and TPT translated into increases in the number of PSs. This augmented activity *in vivo* is consistent with the synergy demonstrated *in vitro* in paediatric tumour cell lines with this same MGI 114 and TPT combination [16]. The combination of MGI 114 and paclitaxel, while not synergistic, did result in a tumour reduction at all doses. At the highest dose level of MGI 114 and paclitaxel, 5.0 mg/kg and 20 mg/kg, respectively, all animals developed PSs or CSs (and one died).

One possible explanation for the enhanced activity of MGI 114 when combined with paclitaxel and TPT is enhanced apoptosis. Paclitaxel stabilises microtubules [20]; continuous treatment prevents completion of mitosis with a cell cycle blockage in mitosis [21] and induces p53-independent apoptosis [22]. MGI 114 may thus augment the activity of paclitaxel through its ability to induce apoptosis [4]. TPT inhibits topoisomerase I by trapping both the enzyme and DNA, forming a ternary complex [23,24]. During DNA synthesis the cleavable complex collides with the advancing replication fork, resulting in a double-stranded break and subsequent apoptosis [25,26]. Thus, MGI 114 may also augment the activity of TPT through its ability to induce apoptosis [4].

The enhanced cytotoxic activity seen in this study with combinations of MGI 114, TPT and paclitaxel may be attributed to additive effects on cell cycle control. Cytokinetic studies show that MGI 114 blocks the G1/S phase interface indicating selective death of cells synthesising DNA or complete inhibition of new DNA synthesis [21]. Camptothecin-induced DNA strand breaks activate wild-type p53, leading to either apoptosis or G1 arrest [25]. Furthermore, camptothecins not only delay cell cycle progression through S phase [26], but also arrest cells in G2 — an effect attributed to cyclin B/cdc 2 kinase inactivation [27]. Crossin and Carney demonstrated that paclitaxel blocks another point of the cell cycle, the G0 to G1 transition [28].

A third mechanism leading to enhanced activity of the combination of MGI 114 and TPT may be decreased DNA repair. Functional DNA helicase is required for

the repair of illudin-induced DNA damage [2,3]. Helicase disrupts the hydrogen bonds between the two strands of the DNA double helix, creating torsional tension. Topoisomerase produces transient DNA strand breaks, resulting in relaxation of helicase-induced supercoiling [29]. Topoisomerase and helicase may work in tandem during DNA repair and replication, therefore direct inhibition of one enzyme could decrease the activity of the other enzyme and lead to diminished repair of illudin-induced DNA damage.

Further investigation into the mechanism of action of MGI 114 will be required to further clarify the nature of the interaction between MGI 114 and both TPT and paclitaxel. The synergistic activity between MGI 114 at 2.5 mg/kg and TPT at 0.5 mg/kg observed in the MV522 model awaits confirmation in clinical trials. The results of these studies with MGI 114, TPT and paclitaxel suggest that lung cancer should be considered a potential target in the clinical development of MGI 114, as other active agents against this malignancy failed to produce complete tumour regression in MV522 lung carcinoma xenografts [7]. The results of this study confirm the previous results that MGI 114 has broad spectrum activity in refractory xenografts. MGI 114 is currently in phase II clinical evaluations in a number of solid tumour types including non-small cell lung, breast, colon, ovarian, prostate, pancreas and renal. The synergistic activity of the combination of MGI 114 and TPT seen in this study suggests that this combination deserves further evaluation in the clinical setting.

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