Cancer Chemother Pharmacol (2005) 55: 307–317
DOI 10.1007/s00280-004-0913-z

ORIGINAL ARTICLE

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Patupilone (epothilone B, EPO906) and imatinib (STI571, Glivec) in combination display enhanced antitumour activity in vivo against experimental rat C6 glioma

Received: 30 April 2004 / Accepted: 24 August 2004 / Published online: 26 October 2004

Abstract *Purpose*: The microtubule-stabilizing agent patupilone (epothilone B, EPO906) and the tyrosine kinase inhibitor imatinib (STI571, Glivec) which primarily inhibits Bcr-Abl, PDGF and c-Kit tyrosine kinase receptors, were combined in vivo to determine if any interaction would occur with respect to antitumour effect and tolerability using rat C6 glioma xenografted into nude mice. *Methods*: Patupilone and imatinib were administered alone or in combination at suboptimal doses. Imatinib treatment (orally once daily) was initiated 4 days after s.c. injection of rat C6 glioma cells into athymic nude mice and patupilone administration (i.v. once per week) was started 3 or 4 days after imatinib treatment. *Results*: As a single agent, imatinib was inactive in the regimens selected (100 mg/kg: T/C 86% and 116%; 200 mg/kg: T/C 68% and 84%; two independent experiments), but well tolerated (gain in body weight and no mortalities). Patupilone weekly monotherapy demonstrated dose-dependent antitumour effects (1 mg/kg: T/C 67% and 70%; 2 mg/kg: T/C 32% and 63%; 4 mg/kg: T/C 3% and 46%). As expected, dose-dependent body weight losses occurred (final body weight changes at 1 mg/kg were −7% and −3%; at 2 mg/kg were −23% and −13%; and at 4 mg/kg were −33% and −15%). Combining 2 mg/kg patupilone and 200 mg/kg per day imatinib in one experiment produced a non-statistically significant trend for an improved antitumour effect over patupilone alone (combination, T/C 9%), while in the second experiment, enhancement was seen with the combination and reached statistical significance versus patupilone alone (combination, T/C 22%; $P = 0.008$). Reduction of the imatinib dose to 100 mg/kg per day resulted in no enhancement of antitumour activity in combination with 2 mg/kg patupilone. Reduction of the patupilone dose to 1 mg/kg resulted in a reduced antitumour effect, and only a trend for synergy with either imatinib dose (combination, T/C 46% and 40%). Pooling the data from the two experiments confirmed a significant synergy for the combination of 2 mg/kg patupilone and 200 mg/kg per day imatinib ($P = 0.032$), and a trend for synergy at the 1 mg/kg patupilone dose. Reduction in the imatinib dose to 100 mg/kg per day resulted only in additivity with either dose of patupilone. Body weight losses were dominated by the effect of patupilone, since no greater body weight loss was observed in the combination groups. *Conclusion*: Combining patupilone with high-dose imatinib produced an increased antitumour effect without affecting the tolerability of treatment in a relatively chemoresistant rat C6 glioma model. Such results indicate that further evaluation is warranted, in particular to elucidate possible mechanisms of combined action.

Keywords Patupilone (epothilone B, EPO906) · Imatinib (STI571, Glivec) · Glioma · Mouse xenograft · Combination chemotherapy

Abbreviations Abl: Abelson kinase · ANOVA: Analysis of variance · Bcr: Break-point cluster region · c-Kit: Stem cell factor receptor · Clarke CI: Clarke combination index · IFP: Interstitial fluid pressure · i.v.: Intravenous · PDGF: Platelet-derived growth factor · PDGFR: Platelet-derived growth factor receptor · SCF: Stem cell factor · SEM: Standard error of the mean

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Introduction

Imatinib (STI571, Glivec) is a potent and selective inhibitor of the protein tyrosine kinase activity of Abl/Bcr-Abl and the receptors for platelet-derived growth factor (PDGF) and stem cell factor (SCF), c-Kit [13, 15]. In vivo, as a single orally administered agent imatinib shows antitumour activity against human and murine tumours in athymic nude mice [19] and is a clinically active monotherapy against chronic myelogenous leukemia (CML) and gastrointestinal stromal tumours (GIST) [15]. In order to improve its antitumour effects and combat the emergence of drug resistance, imatinib is being evaluated in combination with conventional anticancer agents [36, 38, 56]. The interaction between imatinib and conventional (largely antileukaemia) agents indicates that for a given combination, positive or negative interactions can occur dependent upon the cellular system, the ratio of imatinib and the partner drug, and the absolute concentrations of each partner [50]. Adding various conventional agents to imatinib may lead to an increase in apoptotic events in CML cells [20, 40].

Less information is available regarding drug combinations containing imatinib against the growth of solid tumours. Small-cell lung cancers frequently overexpress c-Kit and blocking of SCF activity by imatinib can induce apoptotic events, but fails to enhance the activity of carboplatin or etoposide in vitro [37]. Imatinib inhibits the phosphorylation of PDGFR in experimental peritoneally located ovarian tumours, but is without significant effect on the growth of the tumour mass. Imatinib does, however, improve the antitumour effect of paclitaxel [6]. Imatinib has a slight antitumour effect against orthotopic L3.6pl pancreatic tumours concordant with reduction in the level of phosphorylated PDGFR, and it potentiates the effect of gemcitabine [29]. In both the ovarian and pancreatic tumour models, inhibition of PDGFR on both tumour and endothelial cells by imatinib has been observed.

In a model of prostate cancer bone metastases, paclitaxel and imatinib in combination have shown superior antitumour activity compared to monotherapies [58]. Zhang et al. have demonstrated synergistic interaction of imatinib and cisplatin against A549 lung carcinoma cells [59]. Imatinib enhances the activity of 5-fluorouracil against syngeneic PROb colon tumours in BDIX rats and of paclitaxel against experimental KAT-4 tumours in SCID mice [44]. These effects have been observed only in vivo and have been shown to be due to enhanced uptake of the conventional agent following reduction of tumour interstitial fluid pressure (IFP) through modulation of PDGF signaling by imatinib [43, 44]. A similar effect has been observed with combinations involving imatinib and the epothilone patupilone [45].

Paclitaxel is active in the nanomolar range against glioma cell lines in vitro [14, 53], inhibiting both cell growth and motility. Paclitaxel treatment also inhibits the invasive capacity of gliomas in vitro [55] and sensitizes gliomas to radiation-induced cytotoxicity [24]. Paclitaxel is active in subcutaneous glioma xenograft models, slowing tumour growth but without producing regression [26, 49]. However, paclitaxel penetrates poorly brain tissue of normal rats or rats bearing orthotopic C6 gliomas [23]. Clinically, although paclitaxel penetrates the blood-brain barrier poorly of patients without brain malignancies, conflicting data exist regarding brain tissue penetration in glioma patients. Thus, Heimans et al. have found that paclitaxel is able to penetrate to therapeutic levels [25], while Glantz et al. have reported poor penetration [23]. However, both Taxol [46] and Taxotere [21] have been evaluated in phase II trials in glioma, without conclusive evidence of activity. This suggests that although microtubules appear to be a valid target for the treatment of glioma, the current microtubule inhibitors of the taxane class may be insufficient for effective treatment.

The epothilones, secondary metabolite macrolides produced by the cellulose-degrading myxobacterium *Sorangium cellulosum* [27] represent a novel class of non-taxane microtubule-stabilizing natural products. Preclinical work has shown that patupilone (epothilone B, EPO906) is the most active variant among the natural epothilones. Like paclitaxel, patupilone promotes the polymerization of tubulin heterodimers into microtubule polymers, and stabilizes microtubules against depolymerization, resulting in mitotic cell cycle arrest and eventually cell death via apoptosis [2, 4, 5, 10]. However, albeit supported only by limited data, Broker et al. have suggested that cell death due to epothilone exposure may occur without induction of apoptosis [11]. Patupilone has been found to be more potent than the taxanes, and, importantly, to retain activity in vitro and in vivo against taxane-resistant cancer cells overexpressing P-gp or bearing β-tubulin mutations [5, 10, 22, 57]. Additionally, patupilone may possesses antiangiogenic activity through cytotoxicity against endothelial cells [8]. Thus, both this compound class as well as the target are considered to have great potential for the treatment of cancer [2, 3].

Imatinib has shown single-agent activity in preclinical models of glioma [35], and since imatinib produces positive interactions with microtubule-active agents such as paclitaxel, docetaxel and vincristine [38, 56], the purpose of the present study was to evaluate the tolerability and antitumour effectiveness of imatinib in combination with patupilone in rat C6 glioma tumour xenografts in BALB/c nude mice.

Methods

Materials

Patupilone and imatinib were obtained from Chemical Development, Novartis Pharma (Basel, Switzerland). Healthy pathogen-free female BALB/c *nu/nu* (athymic

nude) mice were obtained from Iffa Credo (L'Arbresle, France). Liquid media, fetal bovine serum (FBS) and medium additives were from Life Technologies (Basel, Switzerland).

Cells and cell culture conditions

The rat C6 cell line was obtained from the American Type Culture Collection (ATCC, Rockville, Md.). Rat C6 cells possess functional PDGFRs, proliferate in response to PDGF, are growth-inhibited by PDGFR inhibitors [1, 39, 52] and grow rapidly as subcutaneous (s.c.) tumours in mice. The cells were cultured in DMEM (high glucose), 10% v/v FBS, 1% w/v glutamine 1% v/v penicillin-streptomycin and incubated at 37°C under an atmosphere containing 5% v/v CO_2 and 80% relative humidity. Cell monolayers (60–80% confluent) were used to establish subcutaneous tumours in BALB/c *nu/nu* athymic nude mice as described below.

Preparation, administration and scheduling of compounds

Patupilone was dissolved in PEG300 and then diluted with physiological saline (0.9% w/v NaCl) to obtain a mixture of 30% v/v PEG300 and 70% v/v physiological saline for i.v. administration to mice. The injection volume was 10 ml/kg i.v. Imatinib was dissolved in Ca^{2+}-free and Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS). The solution was administered by gavage (oral administration) at 10 ml/kg. Formulated compounds were prepared just prior to administration to the mice.

Patupilone and imatinib were administered alone or in combination in suboptimal regimens starting 4 days after s.c. injection of rat C6 glioma cells into athymic nude mice. Two independent experiments were performed. When the tumours reached about 75 mm^3 (day 4), imatinib treatment was begun at 100 or 200 mg/kg orally every 24 h. In experiment 1, on days 7 and 15 after cell injection, patupilone was administered i.v. at 1, 2 or 4 mg/kg. In experiment 2, on days 8 and 15 after cell injection, patupilone was administered i.v. at 1 and 2 mg/kg, and on day 8 only at 4 mg/kg. Thus, the first treatment with patupilone was always 3 days after the initiation of treatment with imatinib. This schedule was used since experiments in another nude mouse tumour model had shown it to be the most favourable schedule for increasing the efficacy of this combination [45].

Assessment of in vivo antitumour activity against s.c. transplanted tumours and host toxicity

Female BALB/c athymic nude mice were kept under pathogen-controlled optimal hygienic conditions with free access to food and water. Tumours were initiated by the s.c. injection of 1×10^6 rat C6 cells. Tumour growth and body weights were monitored twice weekly. All treatments were initiated when the mean tumour volume reached approximately 75 mm^3. Tumour volumes were determined according to the formula length $\times$ diameter^2 $\times$ $\pi/6$, where "length" is the longest dimension and "diameter" the shortest. In addition to presenting changes in tumour volumes over the course of treatment, antitumour activity is expressed as %T/C (mean increase in tumour volumes of treated animals divided by the mean increase in tumour volumes of control animals multiplied by 100). Note that for the %T/C parameter standard deviations cannot be calculated. Tumour regression (%) was calculated as [(mean tumour volume at the end of treatment minus mean tumour volume at the start of treatment)/mean tumour volume at the start of treatment]×100.

Toxicity was determined by changes in body weight versus those recorded in the vehicle-treated controls. No other obvious toxicity was observed in the host nude mice, e.g., diarrhoea (the dose-limiting toxicity observed in clinical trials), although we have observed that some, but not all, rat strains show delayed diarrhoea 3–5 days after injection of patupilone.

Measurement of tumour interstitial fluid pressure (IFP)

The IFP of s.c. C6 tumours was measured using the wick-in-needle method as described previously [45]. Briefly, mice were divided into 3 groups of 6 mice and anesthetised using 2.5% isoflurane delivered at 2 L/min. A standard 23-gauge needle connected to a pressure transducer, was inserted into the central part of s.c. grown C6 tumours (mean volume of 500 mm^3) and the pressure monitored for a period of 10 min. Mice were then treated daily for 3 days using vehicle, or 100 or 200 mg/kg imatinib (p.o.) before a repeat measurement of IFP.

Statistics

The results are presented as mean ± SEM. The differences in tumour volumes or delta tumour volumes were statistically analysed using a one-way ANOVA with post hoc Tukey test to compare all groups. In some cases, the data were normalized by taking $\log_{10}$ prior to statistical analyses. Within-group differences in body weights were determined using paired *t*-tests, and between-group differences used a one-way ANOVA with Tukey tests post hoc. For all tests, the level of significance was set at $P < 0.05$. Statistical calculations were performed using SigmaStat 2.0 (Jandel Scientific). In addition, the significance of combination data was assessed using the method of Clarke's interaction (synergy, additivity or antagonism) from the T/C values as defined above [17]. For compound A or B or the combination AB, the T/C values for the individual treatments are multiplied together; if the result (the calculated Clarke combination index) is greater than the T/C for the actual

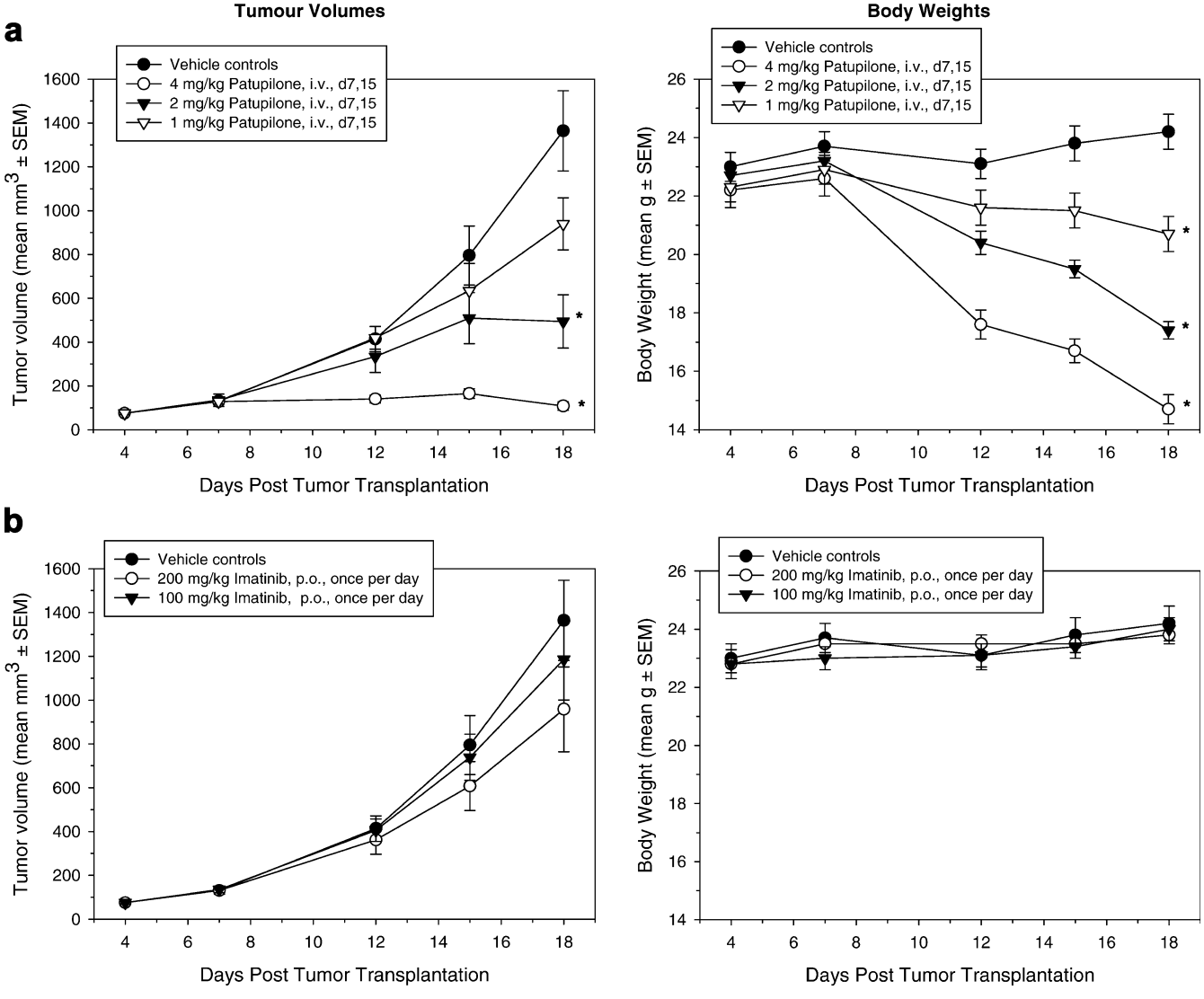
combination (experimental), then synergy is assumed, if it is very similar, additivity is assumed, and if it is lower, then antagonism is assumed.

Results

Dose response of patupilone or imatinib monotherapy against C6 glioma tumours

Figure 1a presents the dose-dependent antitumour response following two administrations of patupilone (days 7 and 15 after tumour cell injection). Whereas 1 mg/kg patupilone produced a non-significant trend for antitumour activity (T/C 67%), profound antitumour effects were seen with 2 mg/kg (T/C 32%) and 4 mg/kg, the latter dose producing essentially stable disease (T/C 3%). All patupilone regimens also produced dose-dependent body weight losses (1 mg/kg −7%; 2 mg/kg −23%; 4 mg/kg −33%); in our experience with patupilone these body weight losses are transient and mortalities rare. However, there was one death at the 4 mg/kg dose occurring 3 days after the second patupilone treatment. At the doses chosen for this study, once-daily imatinib (beginning 4 days after tumour cell injection) produced only minor antitumour responses, but still retained a trend for a dose-response (100 mg/kg per day T/C 86%; 200 mg/kg per day T/C 68%). Imatinib treatments were extremely well tolerated with no significant body weight changes as compared with vehicle-treated controls (Fig. 1b). Imatinib is more active in this tumour model using a twice-daily regimen: 50 mg/kg T/C 80%; 100 mg/kg T/C 59%; 200 mg/kg T/C 44% (data not shown).

Fig. 1 Dose dependence of patupilone and imatinib monotherapy against rat C6 glioma tumour xenografts in female BALB/c athymic nude mice. Tumours were initiated by s.c. injection of 1×10^6 rat C6 cells ($n=8$/group). When the tumours reached about 75 mm^3 (day 4), imatinib treatment was begun at 100 or 200 mg/kg orally every 24 h (day 4). Patupilone was administered i.v. on days 7 and 15 after cell injection at 1, 2 or 4 mg/kg. There was one death in the 4 mg/kg patupilone group on day 18 (3 days after the second patupilone administration). *$P<0.05$ versus vehicle-treated controls (one-way ANOVA, Tukey analysis post hoc)



Antitumour effects of combined patupilone and imatinib (first experiment)

Based upon the previous data, 2 mg/kg patupilone and 200 mg/kg per day imatinib were combined (Fig. 2 and Table 1). A clear trend for improved antitumour effect of the combination compared to the monotherapies was observed: patupilone alone T/C 32%; imatinib alone T/C 68%; combination T/C 9% (similar to 4 mg/kg patupilone alone), although the combination was not significantly different from the individual treatments. There was no exacerbation of body weight loss in the combination treatment group, although there was one death, which occurred 5 days after the first patupilone treatment. Considering delta tumour volumes as the endpoint measure, Clarke's CI showed that patupilone and imatinib interacted synergistically in terms of antitumour effect at 2 and 200 mg/kg, respectively (see Table 1). However, reduction of the imatinib dose to 100 mg/kg per day abrogated the synergistic interaction (imatinib alone T/C 86%; patupilone T/C 32%; combination T/C 31%). Again, there was no exacerbation of body weight loss in the combination treatment group.

Fig. 2 Antitumour activity of patupilone and imatinib alone or in combination against rat C6 glioma tumour xenografts in female BALB/c athymic nude mice (first experiment). Tumours were initiated by s.c. injection of 1×10^6 rat C6 cells ($n=8$ per group). When the tumours reached about 75 mm^3, imatinib treatment was started at 200 mg/kg orally every 24 h (day 4). On days 7 and 15 after cell injection, patupilone was administered i.v. at 2 mg/kg. Delta values represent the difference between the initial values (day 4) and values on the final day of the experiment (day 18) (*IMA* imatinib, *PAT* patupilone). There was one death in the 200 mg/kg imatinib plus 2 mg/kg patupilone group (on day 12, 5 days after the first patupilone administration). $^*P<0.05$ versus vehicle-treated controls (one-way ANOVA, Tukey analysis post hoc)

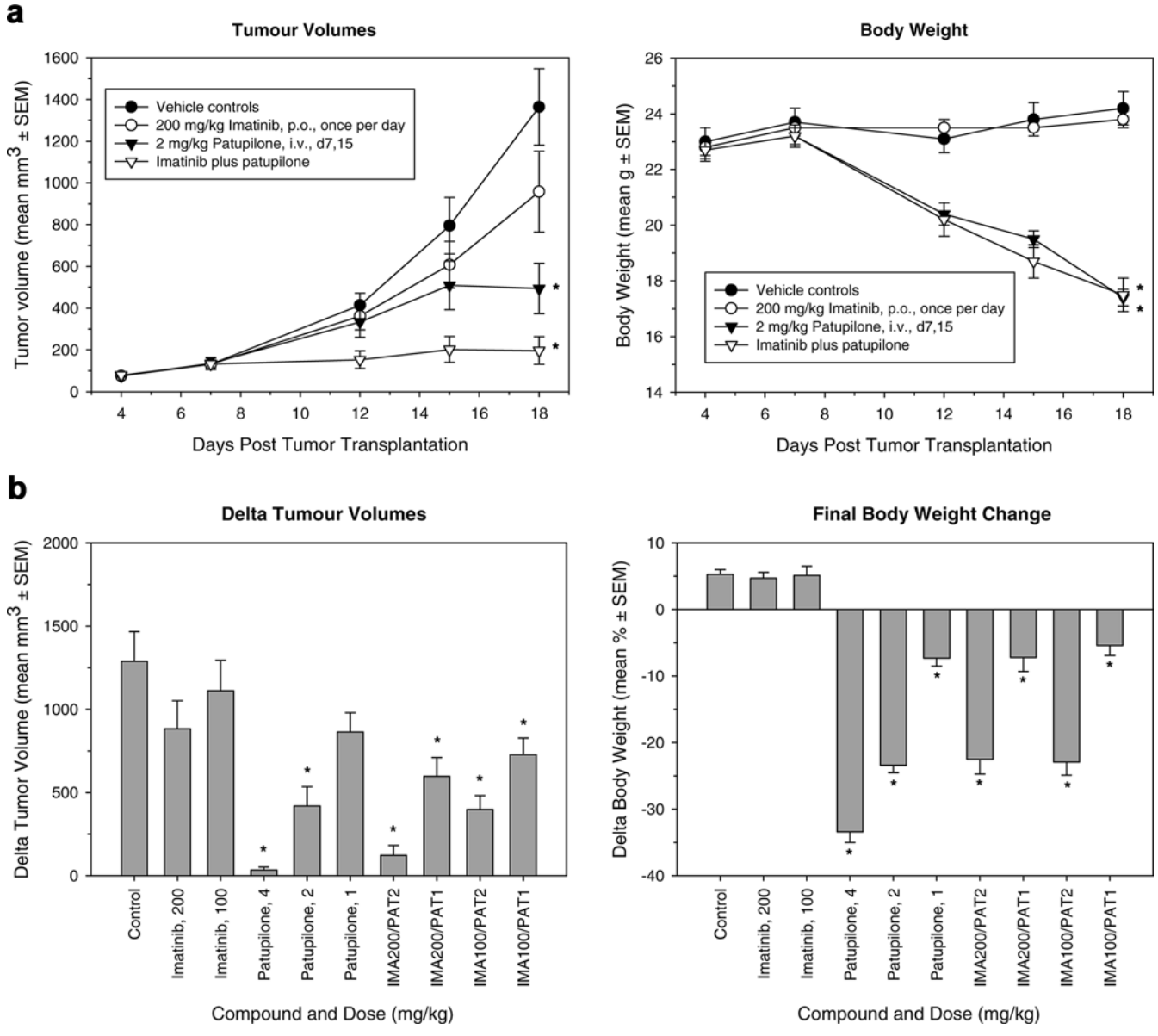


Table 1 Efficacy of patupilone and imatinib alone or in combination against rat C6 glioma tumour xenografts in female BALB/c athymic nude mice (both individual experiments). Tumours were initiated by s.c. injection of 1×10^6 rat C6 cells ($n=8$ per group). When the tumours reached about 75 mm^3 treatment began as described in "Methods". Delta values are the difference between the initial values (day 4) and the values on the final day of the experiment (day 18 in experiment 1, day 19 in experiment 2, respectively). The Clarke CI (calculated or experimentally determined) is defined in "Methods". The body weight changes seen with patupilone plus imatinib were not significantly greater than those seen with the corresponding patupilone dose alone (at any dose)

Treatment	Delta tumour volume (mm^3), mean ± SEM		%T/C		Clarke CI (calculated; experimental)		Body weight change (%), mean ± SEM	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
Vehicle	1289 ± 178	1561 ± 225	–	–	–	–	5.3 ± 0.7	6.9 ± 0.7
Imatinib, 200 mg/kg orally every 24 h	883 ± 169	1312 ± 104	68	84	–	–	4.7 ± 0.9	8.1 ± 0.9
Imatinib, 100 mg/kg orally every 24 h	1111 ± 184	1804 ± 99	86	116	–	–	5.1 ± 1.4	6.9 ± 1.9
Patupilone, 4 mg/kg i.v. every 7 days	34 ± 18*	718 ± 149*	3	46	–	–	−33.4 ± 1.6*	−15.2 ± 1.9*
Patupilone, 2 mg/kg i.v. every 7 days	419 ± 116*	983 ± 62*, **	32	63	–	–	−23.4 ± 1.1*	−12.7 ± 1.6*
Patupilone, 1 mg/kg i.v. every 7 days	864 ± 115*	1063 ± 232*	67	70	–	–	−7.3 ± 1.2*	−3.1 ± 1.7*
Imatinib 200 mg/kg + patupilone 2 mg/kg	122 ± 61*	347 ± 74*, **	9	22	0.22; 0.10	0.53; 0.22	−22.5 ± 2.2*	−16.7 ± 1.8*
Imatinib 200 mg/kg + patupilone 1 mg/kg	598 ± 112*	627 ± 43*	46	40	0.46; 0.46	0.57; 0.40	−7.2 ± 2.1*	−12.5 ± 2.7*
Imatinib 100 mg/kg + patupilone 2 mg/kg	399 ± 83*	903 ± 127*	31	58	0.28; 0.31	0.72; 0.58	−22.9 ± 2.0*	−1.5 ± 1.0*
Imatinib 100 mg/kg + patupilone 1 mg/kg	728 ± 99*	1104 ± 179*	56	71	0.58; 0.57	0.78; 0.71	−5.4 ± 1.5*	−0.8 ± 0.7*

* $P<0.05$ versus vehicle-treated controls, ** $P=0.008$ versus one another; one-way ANOVA with Tukey analysis post hoc for pairwise comparisons

Similarly, when the patupilone dose was reduced to 1 mg/kg, the Clarke CI showed only additivity at both doses of imatinib (Table 1). Body weight losses were apparently again dominated by the patupilone effect, as no increase in body weight loss was observed in the combination groups.

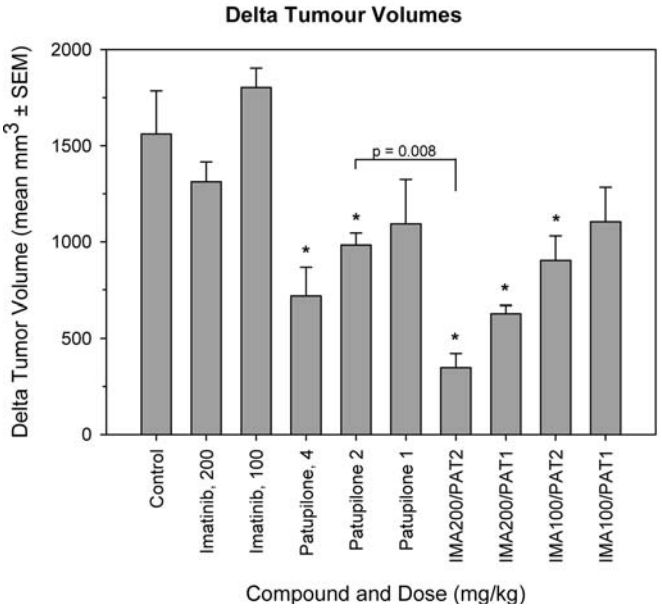
Antitumour effects of combined patupilone and imatinib (second experiment)

Figure 3 summarizes the data obtained in the repeat experiment (see Table 1). As in the first experiment, the combination of 2 mg/kg patupilone and 200 mg/kg per day imatinib resulted in an improved antitumour effect compared to monotherapy: patupilone alone T/C 63%; imatinib alone T/C 84%; combination T/C 22%. The T/C of the combination was significantly better than 2 mg/kg patupilone alone ($P=0.008$) and better than 4 mg/kg patupilone alone (T/C = 46%). Body weight losses were again apparently dominated by the patupilone effect, as no increase in body weight loss was observed in the combination group over 2 mg/kg patupilone alone. Reduction in the imatinib dose to 100 mg/kg per day also enhanced the activity of 2 mg/kg patupilone alone, although to a lesser extent than that seen with the 200 mg/kg per day dose (imatinib alone T/C 116%; combination T/C 58%), but this did not reach significance. The Clarke CI showed that patupilone and imatinib interacted synergistically in terms of antitumour effect at this high dose of imatinib (Table 1).

Reduction of the patupilone dose to 1 mg/kg reduced the antitumour effect, but the trend for potentiation in combination with 200 mg/kg per day imatinib remained: patupilone alone T/C 70%; imatinib alone T/C 84%; combination T/C 40%, not statistically significant versus 1 mg/kg patupilone alone, but equivalent to 4 mg/kg patupilone alone (T/C 46%). The Clarke CI showed synergy. Again, body weight losses were dominated by the patupilone effect, and no exacerbation of body weight loss was observed in the combination group. Reduction of the imatinib dose to 100 mg/kg per day abrogated the enhancement of antitumour activity (imatinib alone T/C 116%; patupilone alone T/C 70%; combination T/C 71%) and the Clarke CI showed additivity (Table 1).

Antitumour effects of combined patupilone and imatinib (meta analysis of data from pooled experiments)

The meta analysis confirmed a dose-response for patupilone antitumour activity (T/C values of 28, 50 and 68% for 4, 2 and 1 mg/kg, respectively) and that imatinib was not significantly active at either dose (Fig. 4, Table 2). The Clarke CI showed that high-dose imatinib (200 mg/kg per day) synergistically enhanced patupilone antitumour activity versus monotherapy to give T/C values of 17% (2 mg/kg) and 36% (1 mg/kg),



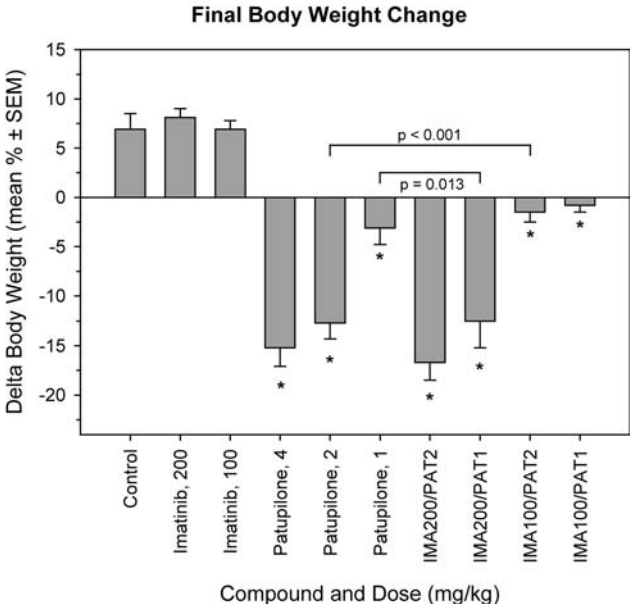
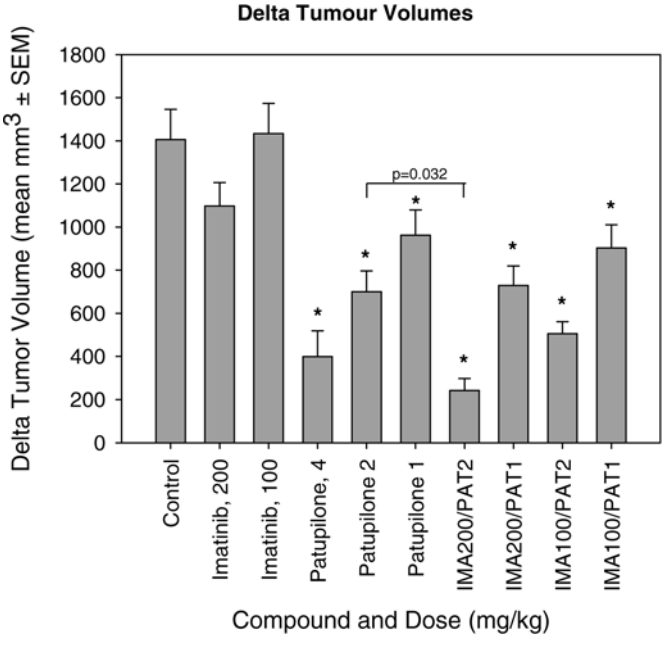


Fig. 3 Antitumour activity of patupilone and imatinib alone or in combination against rat C6 glioma tumour xenografts in female BALB/c athymic nude mice expressed as delta changes (second experiment). Tumours were initiated by the s.c. injection of 1×10^6 rat C6 cells ($n=8$ per group). When the tumours reached about 75 mm^3, imatinib treatment was begun at 100 mg/kg or 200 mg/kg orally every 24 h (day 4). On days 8 and 15 after cell injection, Patupilone was administered i.v. at 1 and 2 mg/kg and on day 8 only at 4 mg/kg. Delta values are the difference between the initial values (day 4) and the values on the final day of the experiment (day 19) (*IMA* imatinib, *PAT* patupilone). There were no deaths. $^*P<0.05$ versus vehicle-treated controls, and as shown in the figure for other comparisons; one-way ANOVA with Tukey analysis post hoc for pairwise comparisons

similar or better than high-dose patupilone (4 mg/kg) alone, and this was statistically significant for the 2 mg/kg dose ($P=0.032$). However, low-dose imatinib showed

Fig. 4 Antitumour activity of patupilone and imatinib alone or in combination against rat C6 glioma tumour xenografts in female BALB/c athymic nude mice expressed as delta changes (meta analysis of pooled data). Tumours were initiated by the s.c. injection of 1×10^6 rat C6 cells ($n=8$ per group). When the tumours reached about 75 mm^3, imatinib treatment was begun at 100 or 200 mg/kg orally every 24 h. Patupilone was administered i.v. at 1, 2 and 4 mg/kg. The data presented are pooled data from experiments 1 and 2 presented in Table 2. Delta values are the difference between the initial values and the values on the final day of the experiment (*IMA* imatinib, *PAT* patupilone). $^*P<0.05$ versus vehicle-treated controls, $P=0.032$ for patupilone alone (2 mg/kg) versus imatinib (200 mg/kg) + patupilone (2 mg/kg); one-way ANOVA with Tukey analysis post hoc for pairwise comparisons



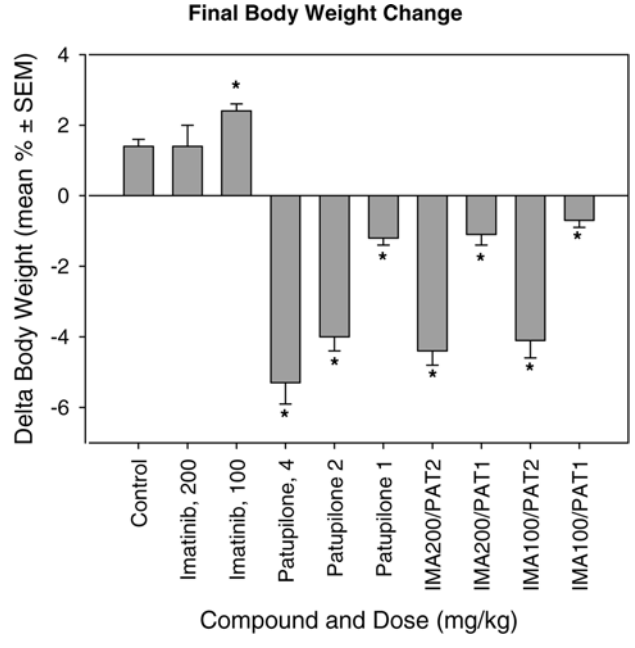


Table 2 Efficacy of patupilone and imatinib alone or in combination against rat C6 glioma tumour xenografts in female BALB/c athymic nude mice (meta analysis of pooled data). Tumours were initiated by s.c. injection of 1×10^6 rat C6 cells ($n=8$ per group). When the tumours reached about 75 mm^3, imatinib treatment was started at 100 or 200 mg/kg orally every 24 h (day 4). The data shown are pooled data from experiments 1 and 2 presented in Table 1, thus $n=16$. The Clarke CI (calculated or experimentally determined) is defined in "Methods". The body weight changes seen with patupilone plus imatinib were not significantly greater than those seen with the corresponding patupilone dose alone (at any dose)

Treatment	Delta tumour volume (mm^3), mean ± SEM	%T/C	Clarke CI (calculated; experimental)	Body weight change (%), mean ± SEM
Vehicle	1406 ± 139	–	–	1.4 ± 0.2
Imatinib, 200 mg/kg orally every 24 h	1097 ± 110	78	–	1.4 ± 0.6
Imatinib, 100 mg/kg orally every 24 h	1434 ± 139	102	–	2.4 ± 0.2
Patupilone, 4 mg/kg i.v. every 7 days	399 ± 120*	28	–	−5.3 ± 0.6*
Patupilone, 2 mg/kg i.v. every 7 days	700 ± 97*,**	50	–	−4.0 ± 0.4*
Patupilone, 1 mg/kg i.v. every 7 days	962 ± 118*	68	–	−1.2 ± 0.2*
Imatinib 200 mg/kg + patupilone 2 mg/kg	242 ± 56*,**	17	0.39; 0.17	−4.4 ± 0.4*
Imatinib 200 mg/kg + patupilone 1 mg/kg	506 ± 56*	36	0.53; 0.36	−4.1 ± 0.5*
Imatinib 100 mg/kg + patupilone 2 mg/kg	729 ± 91*	52	0.51; 0.52	−1.1 ± 0.3*
Imatinib 100 mg/kg + patupilone 1 mg/kg	903 ± 107*	64	0.70; 0.64	−0.7 ± 0.2*

*$P<0.05$ versus vehicle-treated controls, **$P=0.032$ (Tukey) versus one another; one-way ANOVA with Tukey analysis post hoc for pairwise comparisons

only additivity: T/C values of 52% and 64% for 2 and 1 mg/kg patupilone, respectively (Table 2). Imatinib did not impact negatively on body weights, while patupilone showed a dose-response for body weight loss (−5.3, −4.0, and −1.2% for 4, 2 and 1 mg/kg, respectively). This pattern of body weight loss at 1 and 2 mg/kg was not altered at all in combination with either dose of imatinib, suggesting no increased animal toxicity from combining these two agents compared to patupilone monotherapy (Fig. 4, Table 2).

Effect of imatinib on the IFP of C6 tumours

The IFP (in mm Hg) of untreated C6 tumours was 7.7 ± 1.8, 11.6 ± 2.6 and 14.6 ± 4.4 for three different groups of 6 mice and following daily treatment for 3 days, the IFP was significantly reduced (ca. 50%) by imatinib to 6.6 ± 1.9, 5.1 ± 2.0 and 5.4 ± 3.9 for vehicle, 100 mg/kg and 200 mg/kg respectively (p = 0.046, 1-way ANOVA).

Discussion

The novel microtubule stabilizer patupilone (epothilone B, EPO906) [5, 10] and the tyrosine kinase inhibitor imatinib (selective for Bcr-Abl, PDGFR and c-Kit tyrosine receptors) [15] were combined in vivo to determine if any interaction would occur with respect to antitumour effect and tolerability. Rat C6 glioma is a rapidly growing difficult-to-treat experimental tumour model, in which several chemotherapeutic agents, given at optimally tolerated doses, proved to be poorly active, including paclitaxel (15 mg/kg i.v. every 48 h, five administrations, T/C 106%), cisplatin (5 mg/kg i.v. every 7 days, two administrations, T/C 72%), and doxorubicin (9 mg/kg i.v. every 7 days, two administrations, T/C 60%) (unpublished). With the doses and regimens selected in this series of experiments, patupilone was a potent antitumour agent, markedly inhibiting tumour growth, while only a trend for antitumour effect was seen with the selected imatinib doses. The combination of patupilone and imatinib (2 mg/kg patupilone plus 200 mg/kg imatinib) resulted in a significantly increased antitumour effect that was not associated with a concomitant reduction in tolerability, and the antitumour activity was at least equivalent to high-dose (4 mg/kg) patupilone monotherapy. Thus the combination of 2 mg/kg patupilone and 200 mg/kg imatinib constituted a regimen with an improved therapeutic index over monotherapy. Reduction of the imatinib dose to 100 mg/kg resulted only in additivity for the antitumour effect, while reduction of the patupilone dose to 1 mg/kg showed a weaker trend to synergy. Thus, this is consistent with combination studies in vitro of imatinib with other chemotherapeutic agents where positive interactions have been shown to be dose-dependent [50].

Although the mechanisms of action of patupilone and imatinib have been well characterized, the mechanism of this interaction is uncertain, and the synergistic combination effects of patupilone and imatinib may not be solely related to direct effects of either partner on the cancer cells, but may also involve alterations of the tumour environment. Several mechanisms can be envisioned. Inhibition of PDGF signaling while directly affecting tumour cell growth is also known to be involved in the regulation of the cytoskeletal architecture [9], and thus could indirectly affect the sensitivity to microtubule-stabilizing agents. The PDGFR signaling cascade has also been reported to affect cytoskeletal dynamics through the Rho family of small GTPases [54]. Active Rho-dependent pathways appear to be involved in many cell responses to external stimuli, including PDGF [47], and have been implicated in

oncogenic transformation, metastasis [30] and apoptosis [16]. PDGF can induce actin polymerization through the GTPase Rac1 [41, 48]. Furthermore, the F-actin binding protein Abp1 is responsive to PDGFR signaling; Abp1 functionally links the actin cytoskeleton to the GTPase dynamin [34]. Although normally affecting the actin cytoskeleton, evidence indicates that Rho also regulates microtubules [28]. Lastly, internalization of PDGFR-PI3 kinase complexes involves microtubule binding [32] and the p85 subunit of PI3 kinase (one of the effector proteins of PDGF) has been shown to bind to α/β tubulin and γ tubulin [33]. Taken together, there appear to be multiple levels at which PDGFR signaling and the cellular cytoskeleton intersect. Although apparently not specifically studied, the interference with PDGFR signaling in a responsive cell such as C6 [1, 39, 52] may affect cytoskeletal dynamics and predispose the cell to the deleterious effects of microtubule stabilization. In addition, PDGFR is expressed on non-tumour cells within the solid tumour mass, i.e., epithelial cells and/or pericytes of the tumour vasculature, and thus targeting activated PDGFR on any solid tumour may improve the efficacy of cytotoxic agents [6, 7, 29, 58].

Lastly, as tumour IFP apparently impedes tumour uptake of anticancer drugs [31] and inhibition of PDGFR on tumour-associated stromal cells has been shown to reduce tumour IFP, it has been proposed that inhibitors of PDGF signaling, such as imatinib, may facilitate increased drug uptake and antitumour activity of cytotoxics [43, 44]. In two different tumour models, inhibition of PDGF receptor signaling in tumour stroma has been shown to enhance the antitumour effects of paclitaxel and 5-fluorouracil [44]. Increased tumour drug uptake has been shown to be the likely major mechanism of PDGF antagonists, and antiangiogenic effects of the combination treatment were excluded [44]. A similar effect has been observed with patupilone in the rat colon ProB tumour model [45], and in the present study we have shown that imatinib can decrease the IFP of s.c. rat C6 gliomas by 50%. Together, these observations have led to the proposal that inhibition of PDGF receptor signaling in tumour stroma might represent a novel, possibly general, strategy for enhancement of the therapeutic effects of chemotherapy. Therefore, the positive combination effects exerted by imatinib may be, at least in part, attributable to improved tumour uptake of patupilone. Selectivity of action to the tumour microenvironment may account for the lack of decreased tolerability of the combination observed in the current study.

In summary, the promising observation that the combination of patupilone and high-dose imatinib produced synergistic antitumour effects against an experimental glioma model, without negative effects on tolerability, warrants further investigations. Unlike patupilone, which demonstrates excellent brain penetration [12], imatinib penetrates brain poorly [42, 51] because it is a substrate for Pgp-170 [18]. Thus, penetration of tumour tissue by imatinib may be a limiting factor in the clinical application of this combination for treating glioma.

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