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Prednisolone abrogates patupilone (EPO906)-induced diarrhoea in rats without impacting on patupilone PK or efficacy

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Patupilone (PAT) is a non-taxane microtubule stabilizer binding β-tubulin with a higher affinity than taxanes and potently inhibiting growth of a broad range of human tumor cell lines *in vitro* and *in vivo*, including multi-drug resistant cells over-expressing the P-gp drug efflux pump. PAT is currently in phase-II/III clinical development where the dose-limiting toxicity (DLT) is diarrhoea; reduction of which would aid clinical development. Here we study the effect of the corticosteroid (CCS) prednisolone (Predn) on patupilone-induced diarrhoea in a rat model.

BDix rats with/out s.c. A15 gliomas were treated once with PAT (1.5 mg/kg i.v.) and with different schedules of Predn at various doses (1–10 mg/kg p.o.). Diarrhoea was graded from 0 in steps of 0.5 up to a maximum of 3 (liquid stool) each day after PAT treatment and quantified as the AUC and the number of days of diarrhoea greater than grade-1 (DD>G1). Toxicity was also assessed as the change in body-weight (delta%BW) and anti-tumour efficacy as the T/C_{TVol}. The PK of PAT was explored in various tissues to determine exposure over 2 weeks. Data are summarised as mean T/C (ratio of treated divided by control).

PAT caused a delayed-onset diarrhoea which peaked at 3−4 days post-treatment with little or no detectable diarrhoea at days 6–7 (<G1). Predn reproducibly reduced this diarrhoea in a dose-dependent manner with maximal effects at 7–10 mg/kg: T/C = 0–0.1. The effects of Predn were the same independent of whether the CCS was given 24 hr before or 24–72 hr after PAT, and even just 3-days treatment was sufficient to block diarrhoea. Importantly, Predn did not interfere with patupilone efficacy: T/C_{TVol} (day-14) was 0.26–0.41 for all Predn schedules in comparison to 0.35 for patupilone alone. Patupilone exposure in various tissues: brain, gut, liver and tumour was unaltered with a slight increase in the blood AUC of 30% ($P = 0.06$). Histopathological studies on the caecum and jejunum showed that patupilone caused the expected increase in mitotic-index, necrosis and inflammation, but there was no evidence that Predn reduced these effects. Predn consistently but only slightly increased the BW-loss associated with PAT-treatment.

These data show Predn significantly reduces PAT-induced diarrhoea without markedly adding to other toxicities and without affecting patupilone tissue exposure or impacting negatively on anti-tumour efficacy. The combination is being investigated clinically.

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Tubulin acetylation and the antimotility effects of tubulin-targeting agents

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Background: The microtubule stabilizing taxanes have been reported to possess potent anti-motility activity. We previously reported that the ability of paclitaxel to inhibit cell motility is unrelated to its anti-proliferative activity. The aim of this study was to investigate the molecular mechanisms of the anti-motility activity of taxanes, with particular interest for the induction of tubulin acetylation, a post-translational modification involved in microtubule stability and cell motility.

Material and Methods: Endothelial and tumor cells were exposed to taxanes at low concentrations and short exposure times, know to inhibit motility (Boyden chamber assay). Tubulin acetylation was analyzed by Western blot and by immunofluorescence. The role of histone deacetylases was investigated by using inhibitors and by overexpressing enzymes in tumor cells.

Results: Paclitaxel, at concentrations (10^{-8}–10^{-9} M, for 4 h) that affect cell motility but not proliferation, induced tubulin acetylation in endothelial and tumor cells. Comparative analysis of taxanes with different cytotoxic activity (paclitaxel and IDN 5390) and the use of tumor cell lines resistant to the antiproliferative activity of paclitaxel (1A9 and 1A9-PTX22) pointed to an association between inhibition of motility and induction of tubulin acetylation. The level of acetylation of tubulin is controlled by enzymatic activities of histone deacetylases. Inhibitors of histone deacetylases reproduced the effects of taxanes on cell motility and tubulin acetylation. Moreover the treatment of cell with inhibitors of histone deacetylases increased their sensitivity to the antimotility effects of paclitaxel. Conversely a reduction of tubulin acetylation, obtained by overexpressing histone deacetylases, increased cell motility and decreased the sensitivity to the antimotility effects of taxanes.

Conclusions: These results show that paclitaxel promote tubulin acetylation suggesting this as a possible mechanism of the antimotility, but not antiproliferative, activity of taxanes. Moreover these data show that combination of paclitaxel with histone deacetilases inhibitors might represent a novel antimotility therapeutic strategy.

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Antitumor efficacy of a new taxane, nanoparticle albumin bound ABI-013

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Background: Taxanes are potent cytotoxic agents used against a wide range of tumors. These chemotherapeutic agents function by stabilizing microtubules and blocking mitosis. *Nab* technology exploits the natural properties of albumin to enhance tumor targeting and antitumor efficacy of hydrophobic drugs through gp60 and caveolae-mediated transcytosis across tumor blood vessel endothelium and increased tumor accumulation by association with SPARC. Here, we report a novel microtubule-stabilizing taxane ABI-139, the preparation of its nanoparticle albumin-bound form *nab*-ABI-139 (ABI-013), and the comparison of its antitumor activity against docetaxel in multiple tumor xenograft models.

Material and Methods: Repeated-dose toxicity of ABI-013 was determined in mice with dose levels of 0 (saline), 15, 30, 60, 90, 113, and 150 mg/kg on a q4dx3 schedule. The antitumor efficacy of ABI-013 and polysorbate-based docetaxel (Taxotere®) was compared in HT-29 colon and MDA-MB-231 breast tumor xenograft models at their respective MTDs of 90 mg/kg and 15 mg/kg on a q4dx3 schedule.

Results: ABI-013, the nanoparticle albumin-bound form of ABI-139, was prepared with mean nanoparticle size of 70 nm. LD_{50} of ABI-013 was determined to be 113 mg/kg on a q4dx3 schedule in a dose-ranging study. In HT-29 and MDA-MB-231 xenografts, ABI-013 at 60 and 90 mg/kg displayed superior tumor growth inhibition as compared to 15 mg/kg polysorbate-based docetaxel on a schedule of q4dx3.

Conclusions: ABI-013 was superior to polysorbate-based docetaxel in multiple xenograft studies. ABI-013 is a promising, next generation antimicrotubule chemotherapeutic agent that utilizes *nab* technology to take advantage of natural albumin transport pathways through gp60 and SPARC.

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Combretastatin-induced changes in normal vascular physiology and the therapeutic implications

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Background: The tumour vascular disrupting agent combretastatin A-4 disodium phosphate (CA4DP) can also affect normal vascular physiology, leading to unwanted side effects. This study was to see if this latter effect could be prevented and whether it influenced the combination of CA4DP with other therapies.

Material and Methods: Restrained non-anaesthetised CDF1 mice, either non-tumor bearing or with a 200 mm^3 C3H mammary carcinoma in the right rear foot, were used. Drugs were injected intraperitoneally (i.p.) at 0.02 ml/g body weight. Haematocrit (Hct) was determined on a Hct centrifuge from blood samples taken from the sub-orbital sinus. Mean arterial blood pressure (MABP) was measured following cannulation of the carotid artery. Tumors were locally irradiated (240 kV x-rays) with single graded radiation doses or 1-hour later locally heated (41.5ºC; 60 min) by immersing the tumour bearing foot in a water bath. The percentage of mice in each treatment group showing local control at 90 days was recorded and the TCD50 values (radiation dose to control 50% of tumours) estimated from radiation dose response curves. A Chi-squared test (TCD50) or Student's t-test (Hct and MABP) were used for statistical analysis (significance level of $p < 0.05$).

Results: Mean (±1 S.E.) Hct and MABP in control mice were 48.3% (48.1–48.5) and 110 mmHg (109–111), respectively. These were significantly increased to 54.7% (54.5–54.9) and 127 mmHg (121–132) within 1-hour after injecting 100 mg/kg CA4DP. Similar peak increases in Hct and MABP were seen with a range of CA4DP doses (10–250 mg/kg). The CA4DP-induced increase in MABP, but not Hct, could be prevented using hydralazine (HDZ; 0.2 mg/kg). The TCD50 value (with 95% confidence interval) for radiation alone was 53 Gy (51–55). Irradiating tumours and 30 min later injecting either CA4DP (100 mg/kg), or CA4DP+HDZ, reduced the TCD50 values to 50 Gy (46–54) and 48 Gy (45–52), respectively. Heating tumours after irradiating further decreased the TCD50 value to

46 Gy (43–48). When radiation, drugs and heat were combined the TCD50 was 35 Gy (32−38), regardless of whether the drugs were CA4DP or CA4DP+HDZ.
Conclusions: CA4DP significantly increased Hct and MABP. This MABP increase, but not Hct, could be reversed with the antihypertensive drug HDZ. CA4DP also significantly improved tumour response to radiation or thermoradiation, neither of which was influenced by the addition of HDZ.

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Comparison of the effect of patupilone (EPO906) and other cytotoxic drugs on interstitial fluid pressure (IFP) and growth of human ovarian (1A9 and 1A9PTX10) and lung (A549 and A549.B40) xenografts in athymic mice

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Patupilone (PAT) is a natural, non-taxane microtubule stabilizing agent. PAT binds beta-tubulin with a higher affinity than taxanes and potently inhibits growth of a broad range of human tumor cell lines *in vitro* and *in vivo*, including multi-drug resistant cells over-expressing the P-gp drug efflux pump. In this study, we compared the efficacy of PAT with the drugs paclitaxel (PTX), docetaxel (DOC) and pegylated liposomal doxorubicin (Doxil) on human tumor xenografts with β-tubulin mutations: ovarian xenografts 1A9 (PTX-sensitive) and 1A9PTX10 (PTX-resistant) and on NSCLC xenografts A549 (PAT-sensitive) and A549.B40 (PAT-resistant). We also measured their effects on the IFP of 1A9 and 1A9PTX10 tumors. Xenograft tumors were grown ectopically in nude mice. Efficacy (TVol) and IFP (mm Hg) were measured in reference to vehicle-treated controls to give a T/C_{TVol} and T/C_{IFP} respectively. IFP was measured by insertion of a needle (WIN method). Data are summarised as mean T/C (ratio of treated divided by vehicle-control) with significance set at $p < 0.05$.
PAT treatment (2−4 mg/kg qw iv) dose-dependently inhibited 1A9 (max T/C_{TVol}=0.01) and 1A9PTX10 (max T/C_{TVol}=0.22) tumor growth and significantly decreased the IFP (max T/C_{IFP} = 0.04 and 0.15 for 1A9 and 1A9PTX10 respectively). Doxil (12 mg/kg qw iv) affected significantly the growth of 1A9 and 1A9PTX10 tumors (max T/C_{TVol}=0.06 and 0.13 respectively) and IFP of 1A9PTX10 tumor (max T/C_{IFP} = 0.21). PTX treatment (15−20 mg/kg iv q3w) abrogated 1A9 tumor growth (max T/C_{TVol}=0.05), and significantly decreased the tumor IFP of 1A9PTX10 tumors (max T/C_{IFP} = 0.42) but had no effect on their growth (max T/C_{TVol}=0.96).
PAT Treatment (2−4 mg/kg qw iv) dose-dependently inhibited A549 tumor growth (max T/C_{TVol} = −0.05) and had a weak effect on A549.B40 tumors (max T/C_{TVol}=0.61). Similarly, PTX and DOC (25 mg/kg qw iv) induced regression of A549 tumors (max T/C_{TVol} = −0.23 and −0.27 respectively) but had no significant effect on A549.B40 tumors (max T/C_{TVol}=0.73 and 0.65 respectively).
These data confirm that PAT retains activity against PTX-resistant ovarian tumors; while showing similar activity to Doxil. Interestingly, PAT showed similar activity to PTX and DOC in tumors selected for PAT-resistance. The PTX-driven decrease in IFP of PTX-resistant tumors suggests that IFP, and by inference tumor blood volume (Ferretti et al, Clin Cancer Res, 2005), is not causally related to efficacy, but is a biomarker of tumor drug exposure.