resulted in a significant tumor growth retardation in vivo. Moreover, when combined with radiation, a 25 mg/kg dose of OXI 4503 reduced tumor cell survival 20–50-fold lower than that seen with radiation alone.
In summary, the present findings suggest that OXI 4503 bears certain similarities to the parent compound CA4DP. However OXI 4503 demonstrated greater antiumor efficacy as noted by its reduction of the rim of viable tumor cells at the periphery as well as its ability to induce significant tumor growth delays. These data suggest that OXI 4503 may hold greater therapeutic significance.

520 POSTER

Modulation of N,N-dimethylamino-benzoylphenylurea (BPU) absorption by the CYP3A and ABCG2 inhibitor ritonavir

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Background: BPU is a poorly water-soluble benzoylphenylurea derivative with significant cytotoxic activity that acts through inhibition of tubulin polymerization. A previous study has indicated that BPU is metabolized *in vitro* to the cytotoxic compounds desmethylBPU (mBPU) and aminoBPU (aBPU) and to several minor metabolites. The successive demethylation is mediated by cytochrome P450 (CYP) 3A4>CYP3A5>CYP3A7=CYP2D6 (Rudek et al, Clin Cancer Res 2003;9:6197s). A preliminary report also suggested that BPU is a substrate for the transporter protein ABCG2 (BCRP), but not for P-glycoprotein, which combined with CYP3A-mediated metabolism, may explain why the oral bioavailability of BPU in animals is low and highly variable (4.4 to 29%). Oral BPU is currently being evaluated in phase I clinical trials, and pharmacokinetic data have revealed that BPU is very extensively metabolized to mBPU and aBPU. The unpredictable extent of metabolic conversion has been linked to drug-induced neutropenia in patients and presents a major obstacle to further development of this agent. It was hypothesized that temporary, simultaneous inhibition of intestinal and hepatic activity of total CYP3A and ABCG2 would improve the low and variable oral absorption characteristics of BPU.
Materials and methods: To test this hypothesis, female C57BL/6 mice were treated with oral BPU at a dose of 10 mg/kg in the presence and absence of the HIV protease inhibitor ritonavir, a potent inhibitor of both CYP3A and ABCG2, administered orally 30 min prior to BPU at a dose of 12.5 mg/kg. Samples for pharmacokinetic studies were drawn from 3 animals per time point at 5, 15, and 30 min, and at 1, 2, 4, 6, and 24 h following administration of BPU. Samples were analyzed for the parent drug and its metabolites using solvent extraction followed by liquid chromatography with tandem mass spectrometric detection.
Results: Ritonavir co-treatment resulted in an approximately 10-fold increase in BPU area under the curve (AUC) [180 (BPU) vs 1744 nM.h (BPU+ritonavir); $P<0.05$] and a simultaneous decrease in aBPU AUC (5347 vs 2477 nM.h; $P<0.05$) and increase in time to peak concentration (2 vs 24 h). Surprisingly, there was no significant difference in exposure to mBPU (2436 vs 2217 nM.h), although the mBPU peak concentration was decreased by 1.7-fold. The combined exposure to BPU and the metabolites was affected to a lesser extent by ritonavir (8263 vs 6438 nM.h) than each of the compounds individually, suggesting that metabolism rather than transport is the major factor involved in the observed interaction.
Conclusions: These data show that oral BPU pharmacokinetics are significantly influenced by ritonavir. Based on these encouraging findings, a clinical trial is currently being planned to study the concept of intentional pharmacokinetic biomodulation in cancer patients to better control the extensive and variable first-pass metabolism of BPU.

521 POSTER

New synthetic Epothilone Derivative ZKEPO inhibits the proliferation of a human glioma implanted orthotopically in nude mice

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Drugs interfering with cellular microtubules, i.e. paclitaxel and vinca alkaloids are one mainstay of anti-tumor chemotherapy. Human Gliomas, however, have been rather resistant to a treatment with paclitaxel by two reasons, limited delivery of paclitaxel to the glioma cells due to the existence of the blood-brain-barrier (although in tumors often leaky) and because of the development of multidrug resistance.
Epothilones represent a novel class of natural products which also stabilize microtubules. Based on a broad fully synthetic drug optimization program with more than 350 synthesized analogs, we have developed ZK-EPO, a new derivative with outstanding preclinical efficacy.
ZK-EPO is taken up rapidly by tumor cells, preferentially accumulates in the cell nucleus, is not recognized by cellular efflux mechanisms which lead to the development of multidrug resistance, and it diffuses into the brain.
The ability of ZK-EPO to cross the blood-brain-barrier was shown after i.v. application to scid mice. Similar concentrations of ZK-EPO in the brain (0.9 μg/g) and in the plasma (1.2 μg/ml) were detected 10 min after i.v. application. When comparing the partial areas under the plasma level/brain level time curves (0–40 min), a ratio AUC_{brain}/AUC_{plasma} of approx. 0.8 was found, indicating a free access to the brain.
The paclitaxel concentration was below the limit of quantitation in all brain samples (ratio AUC_{brain}/AUC_{plasma} of zero).
Based on these characteristics, we concluded that ZK-EPO should be effective in gliomas and tested ZK-EPO in an orthotopic human glioma model to proof this hypothesis.
In vivo, ZK-EPO produced strong antiproliferative activity in the human glioma model U373 in nude mice. These results suggest that ZK-EPO might also be suited for the treatment of human brain tumors.

522 POSTER

Oral taxane BMS-275183 demonstrates therapeutic synergy in human tumor xenografts when combined with cetuximab

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Combination therapy consisting of an oral taxane, BMS-275183, and the anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody, cetuximab, was assessed for enhanced therapeutic benefit in preclinical tumor models. Athymic mice bearing human tumor xenografts, either L2987 lung or GEO colon carcinoma, were administered the aforementioned treatments singly or in combination regimens. Delays in tumor growth, and tumor-free status, were evaluated and combination treatments were assessed relative to optimal solo treatments. Combinations of cetuximab plus BMS-275183 were tolerated, and synergistic outcomes were obtained at doses ranging from half to full solo maximum tolerated dose (MTD) levels of the oral taxane. The extent of the therapeutic enhancement was reproducibly more than one log cell kill greater than the antitumor effect caused by either solo agent applied optimally. For example, at the MTD of BMS-275183, 60 mg/kg/administration, given orally (po) once every three days for a total of six administrations (q3dx6), 1.0 gross log cell kill (LCK) was achieved in mice bearing well established (100–200 mg) L2987 tumors. Cetuximab, at an optimal dose of 1 mg/mouse, given intraperitoneally (ip) q3dx6, produced 1.3 LCK. When cetuximab, 1 mg/mouse, ip, plus BMS-275183, 25 mg/kg/administration, po, were both given q3dx6, the result was 2.6 LCK with 3 of 8 mice cured. Similar efficacy benefits were obtained in the GEO tumor model. In summary, the combination of oral taxane, BMS-275183, plus anti-EGFR monoclonal antibody, cetuximab, provided therapeutically synergistic antitumor activity in two different human tumor xenograft models. Synergies were observed at doses below MTD levels, but the combination was tolerated even at doses combining solo drug MTD or optimal dose levels. Clinical evaluation of this combination is recommended.

523 POSTER

Selective targeting of cancer cell tubulin with anti-tumor drugs

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Neuronal tubulin, isolated from bovine or porcine brain tissue, is the standard in the field for performing microtubule polymerization assays. One use of neuronal tubulin concerns screening for tubulin ligands which have anti-tumor activity. Neuronal tubulin is ideal for preliminary screens where a large number of compounds have to be screened for initial tubulin binding activity. However there has been poor correlation between IC50 values determined from dose response curves on neuronal tubulin versus tissue culture or patient studies. This is due to several reasons including blood brain barrier diffusion, neurotoxicity, resistant phenotypes and possibly differential tubulin isotype expression. Here we explore the latter by polymerizing neuronal and cancer cell tubulins in the prescence of paclitaxel, vinblastine and their derivatives and also compounds that failed drug approval via the FDA process. Bovine neuronal tubulin has mainly beta II (58%) and beta III (25%) tubulins (Banjeree and Luduena, 1992) in combination with alpha I to make the typical heterodimer, this is in contrast to HeLa cells which have mainly beta I (90%) and beta IV

(9%) or MCF7 cells which have 55% beta I, 6% beta III and 39% beta IV. We developed a micro-tubulin polymerization assay that is suitable for economically measuring the IC50's of compounds on cancer cell tubulin (and other low abundance tubulins, patent pending). Also we standardized the polymerization process such that these IC50's will be directly comparable for years to come, the standardized system creates a value called the Tubulin Ligand Index (TLI). The TLI is a ratio of neuronal IC50 divided by the cancer IC50, so a higher value indicates a more specific interaction with cancer cell tubulin. Surprisingly paclitaxel and its analogs have TLIs of 0.25 to 0.10 i.e. these compounds interact 4 to 10 fold less effectively with cancer tubulin compared to neuronal tubulin. Similarly vinblastine is less effective (TLIs 0.8 to 0.5) except less significant than paclitaxel and its analogs. We believe there is room for improving current anti-cancer compounds using this assay so that the difference between cancer cell and neuronal tubulin specificity is closer to 100 fold. Hopefully in the future this will result in greater anti-tumor specificity and lower neurotoxicity.

524 POSTER

MST-997: A novel taxane with superior efficacy that overcomes paclitaxel and docetaxel resistance in vitro and in vivo

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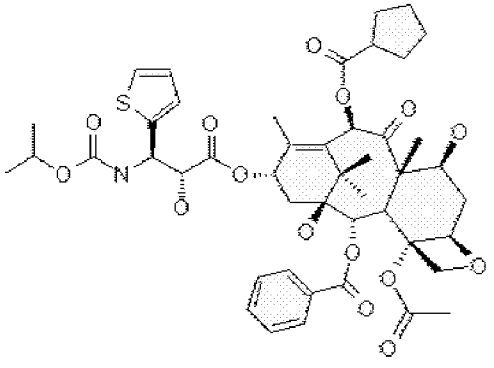
The anti-microtubule agents, paclitaxel (PTX) and docetaxel (DTX), are two approved taxanes that have been used to treat a wide variety of solid tumors. Since resistance to these taxanes is frequently observed, new anti-microtubule agents, in particular stabilizing agents, have been sought. We have previously identified a novel taxane, known as MAC-321, that that overcomes PTX-and DTX-resistance *in vitro* and *in vivo*. We now report a structurally distinct taxane compared with MAC-321 or marketed taxanes, designated as MST-997 [5β,20-epoxy 1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate-ester with (2R,3S)-*N*-isopropoxycarbonyl-3-(2-thienyl) isoserine], that has similar properties as MAC-321. MST-997 was a potent microtubule polymerizing agent (EC_{50} =0.9 μM) that induced the bundling of microtubules and induced G_2/M arrest in cells. The average IC_{50} of MST-997 in PTX and DTX-sensitive tumor cell lines that did not have detectable P-glycoprotein was 2.8±1.5 nM. In addition, minimal (1- to 3-fold) resistance to MST-997 was found in cell lines in which acquired (KB-8–5 and KB-P-15) and inherited (DLD-1 and HCT-15) resistance to PTX and DTX associated with overexpression of P-glycoprotein (MDR1). Moreover, in a cell line that had very high level of MDR1 over-expression, much less cross-resistance to MST-997 (44-fold) was detected whereas >425 or 821-fold resistant to DTX and PTX, respectively, was observed. Less or no resistance to MST-997 was also observed in two cell lines that were resistant to PTX, had no P-glycoprotein overexpression, and contained point mutations in β-tubulin. Most notable, MST-997 displayed superior in vivo efficacy since: 1) a single 70 mg/kg IV dose eliminated the detection of tumors that were partially responsive to a single dose of PTX, 2) MST-997 either partially or completely inhibited tumor growth in 3 models that overexpressed P-glycoprotein and were resistant to PTX and 3) unlike PTX or DTX, MST-997 was highly effective when given orally. Taken together, MST-997 represents a novel and potent microtubule-stabilizing agent that has greater pharmacological efficacy *in vitro* and *in vivo* than the currently approved taxanes. Our findings suggest that MST-997, which will soon begin clinical evaluation, may have broad therapeutic value.



Chemical Structure of MST-997.

525 POSTER

Functional characterisation of beta-tubulin mutations: Insights into paclitaxel/tubulin interactions

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Epothilones were the first novel structural class of compounds to be described since the discovery of paclitaxel, which bind to β-tubulin and stabilise microtubules. We have recently described the selection and chacterisation of a series of leukaemia sub-lines (CEM/dEpoB30–300) that display various levels of resistance to dEpoB (21–307-fold) (Chem Biol 10:597–607, 2003). While the dEpoB 30, 60 and 140 cells were similarly cross-resistant to paclitaxel (~15-fold), the dEpoB300 cells had a dramatic increase in resistance to paclitaxel (467-fold) that exceeded that of the selecting agent. A number of microtubule alterations were identified, including mutations in class I β-tubulin, A231T (located on helix 7 and resides within the paclitaxel binding site) and Q292E (located near the M-loop of β-tubulin). Since drug resistance is often multifactorial, we wanted to identify the contribution of the tubulin mutations to drug binding and chemosensitivity. Using a myc-tagged mammalian expression vector, pcDNA3.1/myc-His(−), full-length wild-type (Wt) and mutant Class I β-tubulin plasmids were stably transfected into mouse fibroblast NIH3T3 cells. Clones expressing the respective proteins were selected and expression confirmed by western blotting. The ability of the mutant β-tubulin protein to incorporate and assemble into microtubules was verified using an anti-myc antibody and immunofluorescence microscopy. NIH3T3 cells expressing the Q292E β-tubulin mutation had significantly diminished capacity to undergo paclitaxel-induced tubulin polymerisation compared to the empty vector controls and A231T β-tubulin mutant expressing cells. Clonogenicity assays revealed that both the A231T and Q292E β-tubulin mutant NIH3T3 expressing clones were resistant to paclitaxel. Paclitaxel binding assays are currently underway to determine if reduced drug binding is contributing to the resistance phenotype observed in the β-tubulin mutant expressing clones. Although both the A231T and Q292E β-tubulin mutations are capable of conferring resistance to paclitaxel, the mechanism of paclitaxel-induced microtubule disruption differs. This study provides the first direct functional evidence that β-tubulin mutations, A231T and Q292E, are involved in resistance to anti-microtubule drugs. The β-tubulin mutant expressing cells also provide valuable models to investigate microtubule-related drug-target interactions and dynamics.

526 POSTER

Optimisation of a pre-clinical dosing schedule for the novel epothilone analogue ABJ879 based on tumour interstitial fluid pressure modulation in rat mammary tumour models

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The epothilones comprise a novel class of non-taxane, microtubule stabilizing macrolides. ABJ879, a semi-synthetic derivative of the bacterially produced epothilone B (EPO906), is a potent growth inhibitor of a wide range of human tumour cell lines *in vitro* and *in vivo* and retains activity against P-gp overexpressing multi-drug resistant cells. ABJ879 is currently in Novartis-sponsored phase-I clinical development.
Interstitial fluid pressure (IFP) is elevated in many solid tumours and is considered to reduce uptake of drugs by tumours. We hypothesised that the reduction of tumour IFP observed following ABJ879 administration in pilot experiments may be harnessed to selectively increase uptake of subsequent doses of the drug. The IFP of BN472 rat mammary carcinomas grown orthotopically in syngeneic rats was studied by insertion of a needle (WIN method) before, 2 and 6–7 days after i.v. administration of vehicle or ABJ879. Single injections of ABJ879 (0.1–0.5 mg/kg) caused a significant ($p<0.05$) decrease in tumour IFP (30% compared to baseline) after 2 days, and this effect tended to increase with post-treatment time. In a separate set of cohorts, rats were treated with vehicle or ABJ879, followed 1, 2 or 7 days later by a second administration using ^{14}C-ABJ879. Whole-body distribution of ^{14}C-ABJ879 was measured using quantitative autoradiography of sagittal 40 μm sections. ABJ879 (0.3 mg/kg) decreased the IFP by 16±8%, 30±3% and 51±0% (mean±SEM) at days 1, 2 and 7, respectively. This was paralleled by a 45±5% and 98±29% increase in ^{14}C-ABJ879 in tumours at days 2 and 7, respectively, compared to day 1, while no significant change was observed in normal tissues (gut, liver, bone-marrow, kidney, lung and spleen). In the vehicle-treated arm, there was no increased uptake of ^{14} C-ABJ879 compared to normal tissues. In a third cohort, growth inhibition was studied over 4 weeks using 2 cycles of fortnightly treatment. Efficacy and tolerability of fortnightly injections of 0.15, 0.3 or 0.45 mg/kg ABJ879 was compared with administration of 0.15 followed by 0.3 mg/kg at day 2 or day 7, or its reverse schedule. Significant