(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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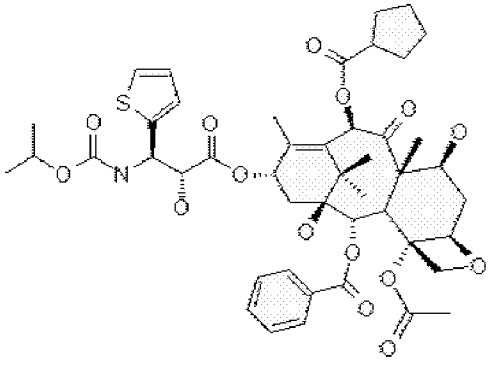
The anti-microtubule agents, paclitaxel (PTX) and docetaxel (DTX), are two approved taxanes that have been used to treat a wide variety of solid tumors. Since resistance to these taxanes is frequently observed, new anti-microtubule agents, in particular stabilizing agents, have been sought. We have previously 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

growth inhibition occurred on the schedule of 0.15 mg/kg followed by 0.3 (T/C of 30%), with optimal tolerance achieved using a 7-day gap between treatments.
These results suggest that tumour IFP reduction may be harnessed to guide clinical dosing regimens of ABJ879 or other epothilones aimed at optimizing their therapeutic index.

527 POSTER

ILX651 inhibits polymerization of alpha beta III tubulin and is cytotoxic to beta tubulin mutant tumor cell lines that overexpress beta III tubulin

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Background: ILX651 is a synthetic dolastatin 15 analog with a unique mechanism of action that potentially differs from that of other tubulin interacting agents. ILX651 has been chemically modified to provide improved pharmacological properties and is orally bioavailable with a potentially enhanced therapeutic window over previous generations of dolastatins. Based on preclinical and Phase I data, ILX651 has potential activity across a wide number of solid tumors. ILX651 inhibits the extent of microtubule assembly and induces a long lag time which is a unique finding for anti-tubulin drugs. It is possible that ILX651 acts by slowing down the rate of microtubule nucleation or elongation which may disrupt mitotic spindle function by this unique mechanism of action.
Methods: As the expression of specific β tubulin isotypes may play a significant role in cellular sensitivity or resistance to tubulin interacting agents, the effects of ILX651 on microtubule assembly of purified bovine brain tubulin isotypes (αβII, αβIII and αβIV) were examined. In addition, ILX651 response in drug-resistant cell lines with tubulin mutations was investigated. β tubulin isotypes were purified by immunodepletion chromatography and microtubule assembly was assessed by turbidimetry. Growth inhibition was evaluated by the alamar blue dye assay.
Results: ILX651 strongly inhibited microtubule assembly at concentrations as low as 1 μM in the presence of purified αβIII tubulin. Epothilone-resistant human acute lymphoblastic CCRF-CEM cell lines, CEM/dEpoB140 and CEM/dEpoB300, which overexpress βIII tubulin and harbor mutations in β tubulin, were exquisitely sensitive to ILX651 treatment with IC50 values of 0.9 nM and 0.4 nM, respectively, compared to an IC50 value of 11.4 nM for parental CCRF-CEM cells.
Conclusions: These results indicate that ILX651 has a profound inhibitory effect on polymerization of αβIII that may correlate, in part, to the cytotoxicity observed in β tubulin mutant cell lines that also overexpress βIII tubulin isotype. Tubulin mutations in the dEPO-resistant cells also affect microtubule stability and therefore may contribute to the hypersensitivity to ILX651. Because aberrant or modulated expression of class III β tubulin is associated with paclitaxel resistance, ILX651 may be active against paclitaxel-refractory tumors that overexpress βIII tubulin. Further studies are currently underway to elucidate ILX651 interactions with tubulin isotypes and MAP's.

528 POSTER

The orally effective taxane DJ-927 has little ability to induce drug resistance in human non-small cell lung cancer cells

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DJ-927 is an orally active taxane with higher solubility, lower neurotoxicity, and superior preclinical efficacy than the clinical taxanes docetaxel (DTX) and paclitaxel (PTX). In particular, DJ-927 shows marked efficacy in vitro and in vivo against intrinsic or acquired multidrug-resistant tumor cells that express P-glycoprotein (P-gp). In the present study, we established sublines resistant to DJ-927, DTX, or PTX from the human non-small cell lung cancer (NSCLC) cell line NCI-H460, and investigated their characteristics and mechanisms of resistance. Additionally, the antitumor effect of DJ-927 against a PTX-resistant clone was confirmed in vivo.
Drug-resistant sublines were selected by stepwise exposure of NCI-H460 cells to DJ-927, DTX, or PTX. Acquisition of 10-fold resistance against DTX required 58 days and against PTX required 86 days, while acquisition of DJ-927 resistance required more than 200 days. Both DTX- and PTX-resistant cell lines exhibited multidrug-resistant phenotypes and overexpressed P-gp. In contrast, the DJ-927-resistant cell line exhibited not only cross-resistance to DTX and PTX, but also increased sensitivity to tubulin-interacting agents such as navelbine and vincristine. Additionally, the amount of P-gp and α-, β-, and acetylated α-tubulin proteins in DJ-927-resistant cells were the same as the amount of control cells. Single clones were successfully derived from DTX- and PTX-resistant sublines (yields: >30%), but not from the DJ-927-resistant line (yield: <0.1%). In vivo antitumor effects of DJ-927, DTX, and PTX were examined using NCI-H460/PTX13 (PTX13), one of the PTX-resistant clones with confirmed tumorigenicity in nude mice. In this system, DJ-927 treatment at a total dose of 19.6 mg/kg exhibited significant antitumor activity (inhibition rate [IR] = 74.9%) even though one mouse died of toxicity. In contrast, neither DTX at toxic doses that caused severe body weight loss (a total dose of 75 mg/kg) nor PTX at its MTD (a total dose of 180 mg/kg) exhibited antitumor effects against PTX13 tumors (IR = 31.2% for DTX and 34.7% for PTX).
These results indicate that DJ-927 has little ability to induce P-gp-mediated multidrug-resistance, and that DJ-927 inhibits growth of human NSCLC cells that are resistant to current clinically available taxanes. Studies to elucidate the mechanisms of DJ-927-induced resistance are in progress.

529 POSTER

Nonlinear pharmacokinetic modeling of XAA296 administered to patients with advanced solid tumors once every 3 weeks (q3w) intravenously (IV) in a phase I clinical trial

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Background: XAA296, a natural product isolated from the marine sponge *Discoderma dissoluta*, stabilizes microtubules more potently than paclitaxel and demonstrates activity against paclitaxel-refractory xenografts.
Methods: In a phase I dose-escalation study, we investigated the MTD, safety, and PK profiles of XAA296. Patients (pts) with advanced solid tumors received XAA296 via IV q3w at a fixed rate of 0.77 mg/mL/min. Blood samples were drawn at various time points in a 3-week interval. Blood XAA296 levels were determined by an LC/MS/MS method with a quantification limit of 0.5 ng/mL using 0.25 mL of blood.
Results: Twenty-five pts (15 m/10 f; ages 19–79 yrs) provided complete blood samples after the first dose. The dose escalation was 0.6, 1.2, 2.4, 4.8, 9.6, 14.4, 19.2, and 25 mg/m^2. After a short infusion, XAA296 levels declined rapidly followed by a prolonged terminal phase. Nonlinear pharmacokinetics, evidenced by a secondary peak and a convexity on a semi-log scale of the terminal phase, was observed in all patients. The disposition of XAA296 was characterized by a 2-compartment model and an additional drug repository compartment. Following the short infusion, the drug was distributed to the peripheral compartment (Vp) and eliminated from the central compartment (Vc) by a first-order process ($\leqslant$2.4 mg/m^2) or by a Michaelis-Menten process (>2.4 mg/m^2). When recirculation took place, a fraction of the drug stored in the repository compartment was released back to the central compartment. The model was parameterized with volumes of Vc and Vp, inter-compartment diffusion parameters (Q2 and Q3), K_m, V_{max}, K_{10}, and a lag time (t_{lag}) for delayed recirculation. Saturable elimination was evident in pts receiving >2.4 mg/m^2 of XAA296 whereas pseudo-linear disposition profiles were observed in pts receiving $\leqslant$2.4 mg/m^2. Drug clearance rate in the central compartment and $t_{1/2}$ are concentration-variant parameters. PK parameters by model fitting are summarized in the table.

Dose	Vc (L)	Vp (L)	Q2 (L/h)	Q3 (L/h)	K_m (ng/mL)	V_{max} (ng/mL/h)	K_{10}	t_{lag} (h)
$\leqslant$2.4 mg/m^2, n=9	8.3 ±3.9	543 ±341	92 ±53	5 ±1.9	–	–	2.2 ±2.0	28 ±16
>2.4 mg/m^2, n=16	11.1 ±5.2	755 ±231	198 ±206	59 ±187	34 ±61	21±19	–	18 ±20

Conclusion: XAA296 administered to pts q3w has demonstrated non-linear pharmacokinetics at >2.4 mg/m^2, which is well described by a 2-compartment model and an additional drug repository compartment.

530 POSTER

Oxi 4503: a novel combretastatin analog with both single agent activity and the abilty to enhance radiation response

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Background: The aim of this study was to test the anti-tumour activity of the novel tubulin-binding agent, Oxi 4503, when used alone or in combination with radiation therapy, in a murine tumour model that generally shows a limited response to such agents.