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.

Materials and Methods: The tumour was a C3H mammary carcinoma implanted in the right rear foot of female CDF1 mice and treated when at 200mm^3 in size. Oxi 4503 was dissolved in saline immediately prior to each experiment and injected intraperitoneally at doses of 50–250 mg/kg either as single or multiple (once weekly for upto 4 weeks) treatments. Radiation (240 kV xrays) was given as single doses locally to tumours. Tumour response was estimated using either tumour growth time (TGT; time for tumours to grow from 200 to 1000 mm^3), or local tumour control (LTC; percentage of mice showing tumour control 90 days after treatment) and following logit analysis of the radiation dose response curves the TCD50 value (radiation dose producing 50% tumour control) calculated. Statistical analysis was performed using either a Student's t-test (TGT) or Chi-squared test (LTC), with the significance level being $p<0.05$ in both cases.
Results: The mean ($\pm$1 S.E.) TGT for control tumours was 6.3 (6.1–6.5) days. This was significantly increased following injection of 50, 100 or 250 mg/kg Oxi 4503. The respective TGTs being 8.8 (8.4–9.3), 9.1 (8.7–9.5) and 10.3 (10.0–10.6) days with single treatments, and 13.5 (13.2–13.8), 19.5 (17.6–21.4) and 28.1 (25.8–30.3) days with multiple treatments. The TCD50 value ($\pm$95% confidence limits) for radiation alone was 53 Gy (51–55). A single treatment with 50 mg/kg Oxi 4503 administered 30 minutes after irradiating significantly reduced this to 41 Gy (38–45). This was further significantly decreased to 34 Gy (31–38) when the same Oxi 4503 dose was given in a multiple schedule.
Conclusions: Unlike other tubulin-binding drugs Oxi 4503 has substantial anti-tumour activity when given as a single agent, this effect being dependent on the total drug-dose administered and how often it was given. Oxi 4503 also enhanced the tumour response to radiation when given either as a single drug treatment or in a repeated multiple-drug dosing schedule. The enhancement seen with the latter approach was greater than that seen with any other clinically relevant treatment combined with radiation in this tumour model.
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531 POSTER

Signaling dependent phosphorylation of tubulin folding factor controls microtubule biogenesis and functions: a new potential target in cancer cells

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Background: Microtubules (MT) are critical regulatory components of normal cellular processes such as chromosomal segregation, morphogenesis, organelle positioning and cell migration. MTs are also involved in pathological conditions such as the formation of multiple microtubule-organizing centers (MTOC), an accepted indicator of aneuploidy in cancer cells. We investigated that Pak1 may influence MT dynamics and functions through regulatory targeting of critical tubulin cofactor phosphorylation and function. Material and Methods: A yeast two-hybrid screening of human mammary gland cDNA expression library using Pak1 as bait identified tubulin cofactor (TCoF) as a Pak1-interacting protein. Results: Here we identified TCoF, an essential component of a-tubulin folding and MT biogenesis, as a substrate of Pak1. Pak1 but not Pak2 directly phosphorylated TCoF both in vitro and in vivo, and co-localized with the endogenous TCoF on newly synthesized microtubules as well as on centrosomes and mitotic spindles. TCoF interacted with the GTPase binding domain of Pak1 and activated Pak1 in both in vitro and in vivo assays. Pak1 and its upstream activators phosphorylated endogenous TCoF at two sites, serine 65 and serine 128. Transfection of a Ser65, 128Ala TCoF mutant protein as well as siRNA-mediated knock down of either endogenous TCoF or Pak1 inhibited microtubule biogenesis, suggesting that Pak1 phosphorylation is necessary for normal TCoF function and that TCoF is critical for microtubule assembly. Deregulation of TCoF in human epithelial cells expressing functional endogenous Pak1 resulted in a dramatic increase in the number of g-tubulin-containing MTOC. Further, Pak1 and TCoF were co-deregulated in human breast tumors. Conclusion: Our results show that Pak1 phosphorylates and regulates the functions of the microtubule assembly protein TCoF. We have discovered an unrecognized role of Pak1-dependent signaling pathways in microtubule biogenesis and functional dynamics. This regulatory interaction directly influences the number and function of MTOC of the cell, which determines the number, polarity, and organization of interphase and mitotic microtubules. Since TCoF deregulation in mammalian cells with functional Pak1 increased the number of MTOC, and since TCoF and Pak1 are deregulated in human breast tumors, these findings implicate a mechanistic role of Pak1-TCoF in the development of aneuploidy and associated genomic instability in human cancers.

532 POSTER

The seco-taxane idn5390 is able to target class III beta-tubulin and to overcome paclitaxel resistance

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A prominent mechanism of drug resistance to taxanes is the overexpression of class III beta-tubulin. Levels of beta-tubulin isotypes by RT-PCR were assessed in cells treated with paclitaxel, IDN5390, a new taxane-derivative, and with their combination. In wt cells, paclitaxel was able to increase class III beta-tubulin levels, whereas IDN5390 induced the opposite effect, and the combination paclitaxel/IDN5390 prevented class III overexpression. In paclitaxel-resistant cells, which express a high level of class III beta tubulin, IDN5390 alone and in combination diminished the expression of the class III. Moreover, the combined treatment paclitaxel/IDN5390 yielded a strong synergism. The strong synergism paclitaxel/IDN5390 was also evident in cell-free tubulin polymerisation assays. In the presence of an anti class III beta-tubulin as blocking antibody, tubulin polymerisation induced by paclitaxel and IDN5390 was enhanced and not affected, respectively, whereas synergism was abolished, thereby indicating IDN5390 activity is not modulated by class III beta-tubulin levels. Such properties can be explained taking into consideration structure of class III beta-tubulin paclitaxel binding site: in fact, Serine 276 interacting with C group of paclitaxel in class I is replaced by an Arginine in class III. IDN5390 that has an open and flexible C ring with an extra negative charge better fits than paclitaxel with class III beta tubulin at the paclitaxel binding site. Taking altogether, these findings indicate that the concomitant treatment IDN5390/paclitaxel is able to target successfully class I and III beta-tubulin and could represent a novel approach to overcome paclitaxel-resistance.

533 POSTER

ABT-751 enhances the efficacy of 5-FU, cisplatin and gemcitabine in preclinical xenograft models

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ABT-751 is an orally active antimitotic agent that is currently in Phase II clinical trials. This agent binds to the colchicine site on β-tubulin and inhibits polymerization of microtubules. This disruption of microtubule dynamics leads to a block in the cell cycle at the G_2/M phase resulting in the induction of cellular apoptosis. The oral bioavailability of ABT-751 in mouse, rat, dog and monkey ranged from 33.0 to 87.7% with bioavailability highest in the mouse. ABT-751, as a single agent demonstrated antitumor activity against a series of xenograft models including colon, NSCLC, breast, and pancreas. The current studies were conducted to determine whether ABT-751 enhances antitumor activity of standard cytotoxic therapies currently in clinical use. Efficacy of ABT-751 in combination with 5-FU, cisplatin and gemcitabine was evaluated in the HT-29 colon, Calu 6 NSCLC and MiaPaCa-2 pancreatic xenograft models, respectively. HT-29 tumor-bearing *nude* mice were treated with ABT-751 orally once-a-day at 100 mg/kg/day on a 5 days on, 5 days off schedule for 2 cycles, in combination with maximally tolerated dose (MTD) 5-FU, 30 mg/kg/day, i.p., q.d.x5. The %T/C ratios on day 38 were 22, 28, and 5 and the %ILS values were 75, 75, and 150 for 5-FU, ABT-751, and 5-FU + ABT-751 treated groups, respectively. Efficacy of ABT-751 at the same dose and schedule was tested in combination with MTD of cisplatin (10 mg/kg/day, i.p., $\times$1) in Calu-6 tumor-bearing *nude* mice. The %T/C ratios on day 38 were 56, 37 and 6 and the %ILS values were 58, 65 and 188 for cisplatin, ABT-751 and the combination groups, respectively. ABT-751 at 100 mg/kg/day (p.o., q.d., for 2 cycles) was evaluated in combination with gemcitabine administered at 20 mg/kg/day on a q3dx4 schedule (1/6th of MTD) against MiaPaCa-2 human pancreatic cancer xenografts grown in the orthotopic site. ABT-751 enhanced efficacy of gemcitabine. The %T/C ratios on day 29 were 56, 82 and 33 for ABT-751, gemcitabine and the combination groups, respectively. Phase I pharmacokinetic data indicate that ABT-751 is well-absorbed and achieved plasma concentrations that were efficacious in preclinical models. Collectively, these studies demonstrate that ABT-751 enhanced efficacy of standard cytotoxic therapies at their MTDs with no overlapping toxicities.