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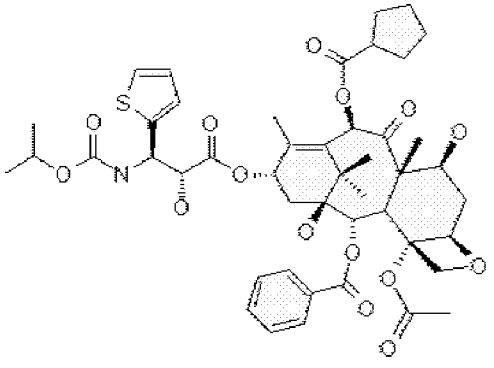
The anti-microtubule agents, paclitaxel (PTX) and docetaxel (DTX), are two approved taxanes that have been used to treat a wide variety of solid tumors. Since resistance to these taxanes is frequently observed, new anti-microtubule agents, in particular stabilizing agents, have been sought. We have previously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