and DNA-PK and act as a radiosensitizer, potentiates the cytotoxicity of etoposide, a topoisomerase II poison.
Materials and methods: Cell lines competent or defective in non-homologous end-joining (ATM, Ku86, DNA-PKcs) or in the zeta isoform of protein kinase C (PKCzeta), were exposed to graded concentrations of etoposide without or with pre-treatment with wortmannin. Cell survival, topoisomerase II decatenation activity, DNA double-strand break rejoining and phosphorylation of effectors downstream, on the one hand from ATM or ATR (Chk2, Chk1), on the other hand from PI3K (Akt/PKB, PKCzeta) were taken as an endpoint.
Results: Wortmannin stimulated the decatenating activity of topoisomerase II, promoted accumulation of DNA double-strand breaks and potentiated the lethal effect of etoposide through two pathways. Sensitization to high, micromolar amounts of etoposide required DNA-PK integrity. In contrast, wortmannin dramatically enhanced the susceptibility to low, nanomolar amounts of etoposide in a large fraction of the cell population irrespective of the status of ATM, Ku86 and DNA-PKcs, and shifted the specificity for cell killing by etoposide from S to G1 phase of the cell cycle. To determine whether PKCzeta was involved in this process, U937 cells bearing stable expression of a dominant-negative, kinase dead mutant of PKCzeta were exposed to submicromolar amounts of etoposide. PKCzeta-defective cells actually reproduced the hypersensitivity pattern induced by wortmannin. In addition, transient hyperphosphorylation of PKCzeta was observed in PKCzeta-competent cells at 2 h interval from contact with etoposide and was abolished by wortmannin.
Conclusions: It is proposed that wortmannin acts through disruption of a phosphorylation cascade involving PI3K, type I phosphoinositide-dependent protein kinase (PDK1) and PKCzeta in sequence, resulting in loss of topoisomerase II Ser phosphorylation. After Plo et al. (J Biol Chem 277: 31407–31415, 2002) it is suggested that potentiation of the cytotoxic response to submicromolar concentrations of etoposide induced by wortmannin or PKCzeta knockout, is due to increased activity of the beta isoform of topoisomerase II.

517 POSTER

AQ4N mediated potentiation of chemoradiotherapy in human lung tumour xenografts

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Background: AQ4N (1,4-bis[[2-(dimethylamino)ethyl] amino]-5,8-dihydroxy-anthracene-9,10-dione bis-N-oxide) is a bioreductive topoisomerase II inhibitor that is currently in a Phase I clinical trial in combination with radiation. The purpose of this study was to provide data to support the use of AQ4N in chemo (cisplatin)-radiation protocols in carcinoma of the lung.
Materials and Methods: Calu-6 xenografts (established from the ID implant of 2×10^6 cells in 0.1ml of a 1:1 matrigel:serum free RPMI mix) were treated at a size of 240–280mm^3. Radiotherapy was given as 2 Gy fractions (five days on, two days off) to maximum doses of 20 or 30 Gy. AQ4N (60mg/kg) was given weekly 30 minutes prior to the first radiation dose. Cisplatin (2mg/kg) was given once 6 hours after the first fraction. The growth delay for tumours to quadruple in size was taken as the experimental endpoint. To disclose the hypoxic fraction of Calu-6 xenografts, pimonidazole was administered at 60mg/kg. AQ4N metabolism was evaluated using HPLC.
Results: Pimonidazole binding revealed that Calu-6 xenografts have a clinically relevant hypoxic fraction of 1–10%. AQ4N potentiated the response of Calu-6 xenografts to chemoradiotherapy *in vivo*. When combined with 10×2 Gy and cisplatin, AQ4N afforded an additional 7-day growth delay compared with cisplatin-radiotherapy alone (44 ± 1 versus 37 ± 4 days). This enhancement equated to that which would be achieved using an additional 4 fractions of radiation. In support of previous data AQ4N significantly enhanced the outcome of radiotherapy alone yielding a growth delay of 37 ± 2 days compared with 26 ± 5 days ($p=0.05$). Metabolism studies revealed that nitric oxide synthase (NOS), an enzyme that is commonly over-expressed in human tumours, can reduce AQ4N to form the $2e^-$ intermediate AQ4M whereas cytochrome p450s may have a preferential role in the conversion of AQ4M to the cytotoxic AQ4 moiety.
Conclusions: These data support the future clinical evaluation of AQ4N in cisplatin based chemoradiotherapy protocols in carcinoma of the lung and indicate that tumour specific expression of NOS may facilitate reductive metabolism of this agent.

518 POSTER

Alterations of topoisomerase IIalpha, associated with in vivo resistance to tafluposide, are partially reversible

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Earlier studies identified tafluposide as a novel dual inhibitor of topoisomerases I and II, with different mechanistic properties from most specific inhibitors of topoisomerases. Indeed, tafluposide appears to act by preventing the binding of topoisomerase I or II to DNA through a preferential interaction with the enzyme, but it is also capable of trapping topoisomerase II in an ATP-independent noncovalent salt-stable complex. Subsequent studies provided evidence that tafluposide is also a potent inhibitor of nucleotide excision repair. This compound has marked antitumor activity in a series of experimental tumor models and is now ongoing Phase I clinical trials. In the attempt to identify *in vivo* the targets involved in the antitumor activity of tafluposide, a P388 leukemia subline resistant to tafluposide was established *in vivo*. The results showed that resistance to tafluposide was mainly associated with alterations of topoisomerase IIα and the endonuclease XPG, involved in NER, whilst only minor modifications of topoisomerases I and IIβ were recorded. P388/tafluposide resistant cells exhibited a marked reduction in topoisomerase IIα protein level (87%), and a K155N mutation of this enzyme. Nucleotide excision repair activity was decreased, which was more specifically associated with a decreased level of XPG. To further study the implication of topoisomerase IIα in tafluposide antitumor activity, the stability of the resistance to tafluposide was investigated. P388/tafluposide tumor cells were maintained in mice without treatment with tafluposide for 6 months. The resulting P388/tafluposide-6m cells did not exhibit further K155N mutation, whilst they still exhibited a reduction of the level of topoisomerase II, although it was less important (75%). These P388/tafluposide-6m cells partially recovered sensitivity to tafluposide treatment *in vivo*, as reflected by an optimal increase of lifespan of 100%, as compared to 0% for the P388/tafluposide resistant subline and 300% for the parental sensitive P388 cells. These data suggest that resistance to tafluposide is partially reversible and further support the implication of topoisomerase IIα in tafluposide antitumor activity.

Tubulin-interacting agents

519 POSTER

Preclinical evaluation of the second generation vascular disrupting agent OXI 4503

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Vascular disrupting agents (VDAs) for the treatment of cancer are designed to cause a rapid and selective shutdown of tumor vasculature, which leads to secondary ischemic tumor cell death. The lead drug combretastatin A4 disodium phosphate (CA4DP) are currently being examined in various clinical trials with encouraging results. Efforts are ongoing to develop new agents with improved activity and therapeutic windows. In the present studies, we studied the efficacy of a novel analog of combretastatin, OXI 4503.
The vascular and antitumor effects of OXI 4503 were assessed in the KHT murine sarcoma model. Tumor blood perfusion was estimated by Hoechst 33342 fluorescent labeling. Significant reduction in functional vessel was observed as early as * hr after a dose of 10 mg/kg or 25 mg/kg of OXI 4503. Histologic and morphometric assessments carried out using image analysis system on tumor sections showed the viable tissue remaining in these tumors 24 hr after treatment with a 25 mg/kg dose of OXI 4503 to be less than 6%. There was extensive necrosis induced within the tumor. This pattern extended to the very edge to the tumor, stopping at the surrounding soft tissue at many locations; while at other sites there remained a rim that was few cells thick. Tumor vascular status prior to and post treatment with OXI 4503 was also assess with non-invasive magnetic resonance imaging (MRI). The results of MRI GdDTPA inflow measurement revealed that OXI 4503 treatments significantly reduced perfusion in KHT sarcomas. The proportion of perfused tumor tissue was notably less after OXI 4503 treatment compared to CA4DP.
Administration of OXI 4503 to tumor bearing mice resulted in a dose-dependent increase in tumor cell killing. OXI 4503 was more potent than CA4P on a per dose basis. For example, compared to CA4DP, 4-fold lower doses of OXI 4503 resulted in equivalent reduction in KHT sarcoma clonogenic cell survival. Administration of OXI 4503 to the animals also

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