Table 1 presents the models' predictions from the relationship between SB-715992 dose and ANC for a selected subset of doses.

Table 1. Predicted response as a function of dose

Dose (mg/m^2)	Decrease ANC (%) (Emax model) with 90% CI	Probability (%) of Gr 4 neutropenia (ordinal model) with 90% CI
10	63.4 (54.4, 72.4)	1.9 (0.3, 12.5)
12.5	73.2 (64.8, 81.6)	7.9 (2.0, 26.2)
18	85.2 (77.2, 93.1)	68.7 (49.1, 83.3)
21	88.7 (81.3, 96.1)	92.8 (78.4, 97.9)

Conclusion: Exploratory PK/PD analysis suggests that dose (mg/m^2), total dose (mg), and AUC (log transformed) or Cmax (log transformed) are important independent predictors of a decline in ANC when evaluated separately. Dose is the most predictive of ANC decrease after SB-715992 administration. The Emax model and ordinal models are useful to predict ANC response after SB-715992 doses are administered once every 21 days.

56 POSTER

Hsp90-targeted therapy for small cell lung cancer

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Nearly all small cell lung cancer (SCLC) cell lines and tumors demonstrate functional inactivation of the retinoblastoma gene (RB). As all normal cells in the body express functional Rb, a drug that specifically targets cells with mutationally inactive or deleted Rb would represent a potential targeted approach for patients with SCLC. Cells with defective Rb treated with an Hsp90 inhibitor progress normally though G1 and arrest in mitosis. The mitotic block is unstable and leads to massive apoptosis. While these data suggest that Hsp90 inhibitors may be of clinical utility in patients with SCLC, 17AAG is relatively inactive in these cells. As the doses required for anti-tumor effects in SCLC cell lines appear greater that those achievable without toxicity in patients in the ongoing phase I studies, these data suggest that while Hsp90 may be an appropriate target in patients with SCLC, 17AAG is a poor choice for use in these patients. In contrast, the novel Hsp90 inhibitor PU24FCl retains activity in SCLC cells. PU24FCl binds tightly to Hsp90 found in SCLC cells, while its affinity for normal cell-Hsp90 is at least 10 – (brain, pancreas and lung) to 50 – (heart, kidney and liver) fold lower. We evaluated the in vitro growth inhibitory properties of PU24FCl against two SCLC cell lines, NCI-H69 and NCI-N417. PU24FCl inhibits cell proliferation and appears to be cytotoxic in these cells. By contrast to transformed cells, normal prostate epithelial cells (PrEC) and human renal proximal tubular epithelial (RPTEC) are 1-log more resistant to the effects of PU24FCl on growth. The effects of PU24FCl on growth correlate with its effects on Hsp90-client proteins (i.e. cMet, Raf-1, Akt) thought to be involved in the dysregulated growth, survival and metastatic potential of SCLC cells. SCLC cells are blocked in mitosis by PU24FCl; the mitotic block is unstable and leads to apoptosis with a significant increase in the number of apoptotic nuclei observed (i.e. 35% in NCI-N417, 65% in NCI-H526 at 10 uM, 72 hr post-treatment). PU24FCl maintains its activity in vivo as it is demonstrated by increased apoptosis and reduced proliferative potential of NCI-N417 xenografted tumors treated with the agent. In conclusion, our results define a novel strategy for the treatment of SCLC patients by specific inhibition of tumor Hsp90.

57 POSTER

The facilitative glucose transporter Glut-1 as a target for novel anti-cancer agents

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Rapidly proliferating tumour cells outgrow their blood supply, which results in hypoxia. Hypoxia is a problem for the treatment of cancer because it is associated with chemo- and radioresistance, increased malignancy and poor prognosis. Tumour cells exposed to hypoxia survive by switching to anaerobic glycolysis. Expression of the facilitative glucose transporter Glut-1 is induced in response to hypoxia to satisfy the increased cellular demand for glucose. Glut-1 has been reported to be over-expressed in virtually all solid tumours, and has been shown to correlate with hypoxia in cancers of the head and neck and cervix (Airley *et al*., 2001; Oliver *et al*., 2004) and with prognosis in a wide variety of solid tumours. *In vitro* and *in vivo* studies using antisense down regulated Glut-1 have also shown the importance of Glut-1 overexpression to tumour growth. Therefore Glut-1 may prove to be an excellent therapeutic target for potential anticancer agents against chemo- and radioresistant cells within solid tumours.
In an effort to identify prospective drugs that may mediate toxicity through interaction with the Glut-1 transporter, we have recently carried out a COMPARE analysis of the correlation between Glut-1 expression in the NCI 60 cell line panel and the toxicity caused by standard agents and those agents from the BEC database of NCI compounds. To confirm that the action of agents that show a statistically significant positive correlation with Glut-1 expression, i.e. COMPARE "hits" is Glut-1-dependent, we are carrying out toxicity studies using stable clones that constituently over-express Glut-1, which we have derived from PC-3 (human prostate adenocarcinoma) and HT1080 (human fibrosarcoma) cell lines. To identify a possible relationship between the subcellular location of Glut-1 and its effect on tumour growth or Glut-1-mediated toxicity in hypoxic conditions, we have also transfected the HT1080 cell line with a vector carrying the cDNA for a Glut-1/EGFP fusion protein and have generated stable clones that constitutively over-express this gene product. Using fluorescence microscopy, we have established that like Glut-1, this fusion protein is located in discrete compartments in the cytoplasm and within the cell membrane.

58 POSTER

Pharmacodynamic responses to a novel histone deacetylase inhibitor, PXD101, in mice and humans

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PXD101 is a novel hydroxamate type inhibitor of histone deacetylase (HDAC) activity. Previously, we have shown that treatment of nude mice bearing human ovarian and colon tumour xenografts with PXD101 (10–40mg/kg/day i.p.) daily for 7 days causes a significant dose-dependent growth delay with no obvious signs of toxicity to the mice (Plumb et al, 2003, Mol Cancer Ther 2; 721). This evidence of efficacy without apparent toxicity suggests that pharmacodynamic assessment of drug activity will be important in determining the optimal dose and drug schedule in patients. A marked increase in acetylation of histone H4 was detected in mouse blood 1 and 2 hours after i.p. treatment with PXD101. Levels of acetylation in both blood and tumour increased with dose (10–40mg/kg). As part of an ongoing Phase I trial of PXD101 we have determined levels of histone acetylation in peripheral blood mononuclear cells (PBMCs) in blood taken from patients treated with PXD101. Patients received PXD101 (150–600mg/m^2) as a 30 minute intravenous infusion on days 1–5 and blood samples were taken on day 1 before the infusion and at various times from the end of the infusion (0–6hours). Histones were extracted from PBMCs and acetylated histones detected by Western blotting with antibodies specific for the acetylated form. All samples from an individual patient were run on the same gel and the level of acetylation was quantified by densitometry. To allow comparison of acetylation levels between patients each blot contained an internal standard of histones prepared from cell line A2780 exposed to PXD101 (0.2 μM) for 1 hour. For all patients acetylation of histone H4 was low in the pre-treatment sample but was markedly increased at the end of the infusion to levels comparable to that observed for the internal control. At the lowest dose (150mg/m^2) levels showed a clear decrease by 30 minutes and had returned to basal by 2 hours post-infusion. The rate of decrease of acetylation levels was slower at the higher doses. At the highest dose studied so far (600mg/m^2) levels remained elevated after 2 hours and then showed variable rates of decrease such that in some patients levels were still elevated after 6 hours. Although this is an ongoing Phase I trial we have shown that the HDAC inhibitor PXD101 at these starting doses can induce histone acetylation in PBMCs in patients. Our results show that these effects are transient but are more sustained with increasing dose of PXD101.

59 POSTER

Comparison of the efficacy of MS-275, CI-994 and SAHA in vivo in various experimental tumor models after oral application

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Background: Histone deacetylases (HDACs) are a family of enzymes that are involved in the epigenetic regulation of gene expression. The inhibition of HDACs is a new potential therapeutic option in cancer treatment

and a number of HDAC inhibitors are currently in development. Here, we summarize the results obtained with three different HDAC inhibitors (MS-275, SAHA and CI-994) directly compared in four tumor models.
Material and Methods: Four different cell lines (A375, melanoma; A549 and H460, NSCLC; MaTu, breast carcinoma) were grown as xenografts in nude mice. Tumors were treated after establishment, with all agents given daily p.o. Parameters determined were tumor area, tumor weight and body weight. Tumor weights were used for the calculation of a tumor/control ratio (T/C). Oral bioavailability of the compounds was determined by calculating the area under the curve from 0–4h (AUC_{0-4h}). The compounds were given at 50 and/or 100 mg/kg in 30% HP-ß-CD, pH 5.0 to nude mice. Blood samples were taken and analyzed by LC-MS/MS analysis.
Results: The oral bioavailability of MS-275 revealed an AUC of 45.7 μM*h after oral dosing of 50 mg/kg. CI-994 showed a very high bioavailability of 92.4 μM*h and 185.4 μM*h after 50 and 100 mg/kg, respectively. SAHA showed a very low bioavailability with 1.34 and 2.3 μM*h after application of 50 and 100 mg/kg, respectively. In the A375 melanoma model MS-275 revealed a significant dose-dependent efficacy at lower doses (T/Cs 0.18, 0.36, 0.51 for 50, 25 and 10 mg/kg, respectively) whereas SAHA was only effective at higher doses of 50 and 100 mg/kg (T/Cs 0.48 and 0.52). Similarly, in the MaTu breast carcinoma model, MS-275 showed significant efficacy at all three doses used (10, 25 and 50 mg/kg), whereas SAHA showed a statistically significant effect only at higher doses of 50 and 100 mg/kg. In the A549 NSCLC model only MS-275 50 mg/kg showed a statistically significant effect, all other doses of MS-275 and SAHA revealed either no effect or no statistically significant effect. MS-275 was found to generally exhibit greater efficacy than CI-994, although the compounds are structurally very similar. In the A549 model a 2-fold higher dose of CI-994 was needed to achieve the same efficacy as for MS-275 (T/Cs for MS-275 0.24, 0.42, 0.61 for 50, 25 and 10 mg/kg, respectively; T/Cs for CI-994 0.21, 0.37, 0.51 for 100, 50 and 25 mg/kg, respectively).
Conclusion: Although SAHA is described as a highly potent inhibitor of HDACs *in vitro* (IC_{50} 10nM for HDAC1) the head-to-head experiments revealed a lower efficacy *in vivo* than MS-275 in various tumor models after oral application. This observation can be linked to its low bioavailability, as shown for the AUC determination. CI-994 is structurally very similar to MS-275, but was also found to be less effective *in vivo* than MS-275. Currently, we are performing additional experiments to further evaluate the comparative therapeutic potential of these compounds.

60 POSTER

Geldanamycin combined with bortezomib interferes with the ER-associated protein degradation function of valosin-containing protein

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Inhibition of Hsp90 activity with geldanamycin (GA) and blocking the proteasome pathway with bortezomib (BZ) causes a massive accumulation of misfolded and ubiquitinated cellular proteins. The combination of GA plus BZ also initiates synergistic cytosolic and ER stress responses and promotes the formation of conspicuous, ER-derived cytoplasmic vacuoles. The Hsp90-dependence of the GA-induced vacuolization phenotype was verified by results showing that 17-AAG, radicicol, and analogs of both classes of Hsp90 targeting agents, but not geldampicin, were also capable of inducing cellular vacuolization, provided proteasome activity was partially inhibited by BZ. Additional results now implicate valosine-containing protein (VCP) in the vacuolization phenomenon induced by GA plus BZ. As a crucial participant in ER associated protein degradation (ERAD), VCP, in cooperation with its effector proteins, Ufd1 and Npl4, is responsible for the ATP-dependent retrograde transport of misfolded proteins from the ER prior to their degradation by cytosolic proteasomes. Mutational inactivation of the ATPase domain of VCP, or inhibition of VCP by over-expression of a small VCP-interacting protein (SVIP), promotes cytoplasmic vacuolization of cells that is virtually identical to that caused by GA plus BZ. Cells transiently transfected with flag-tagged SVIP plasmid developed numerous vacuoles that were visualized by anti-flag immunofluorescence, thus localizing VCP to the vacuole membrane. Interestingly, the incidence of vacuolated SVIP-transfected cells was increased several fold by GA. Following exposure to GA plus BZ, but not the individual drugs, a significant quantity of VCP, as well as Hsp90 and Hsp70, was relocalized into the detergent-insoluble pellet fraction of cell lysates, where presumably all three chaperones were associated with aggregated misfolded proteins. Although VCP co-immunoprecipitated with Hsp90 from tumor cell lysates, the overall cellular level of VCP, as well as its association with Hsp90, was GA-insensitive, making it unlikely that VCP is an Hsp90 client protein. We propose that the drug-induced vacuolization is a mechanism cells use to clear misfolded, GA destabilized Hsp90 client proteins from the ER secretory pathway when the ERAD function of VCP is compromised by a backup of proteins resulting from proteasome inhibition. The accumulation of misfolded proteins would eventually become cytotoxic and probably contributes to the demise of tumor cells.

61 POSTER

Mutant-PTEN leads to constitutive integrin-linked kinase (ILK) activity that regulates PKB/Akt activity in glioblastoma cancer cells and targeting ILK results in tumor growth-delay in vivo

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Purpose: The tumor suppressor gene PTEN, regulates the phosphatidyl-inositol-3′-kinase (PI3K) signaling pathway and has been shown to correlate with poor prognosis in high-grade astrocytomas when mutational inactivation or loss of the PTEN gene occurs. PTEN mutation leads to constitutive activation of protein kinase B (PKB/Akt) with phosphorylation at the PKB/Akt sites Thr-308 and Ser-473. Integrin-linked kinase (ILK) has been shown to regulate PKB/Akt activity with the loss of PTEN in prostate cancer. Data summarized in this report demonstrates that ILK activity regulates PKB/Akt activity in glioblastoma cells.
Methods: Three human glioblastoma cancer cell lines were used in this study: SF-188, U87MG and U251. U87MG cells were transiently transfected with ILK antisense (ILKAS) using Lipofectamine 2000. Retroviral constructs with either inactive PTEN (U87G129E, U87GR), or empty vector (U87EV) or with an inducible PTEN construct (U87.23) were generously provided by Dr. Michael Wigler. Muristirone A was added to these transfected cells for induction of PTEN expression. Antisense oligonucleotides against ILK (ILKAS) were derived from a patent from ISIS Pharmaceuticals Inc. in which antisense sequence ID no 37 (5′-GAGATTCTGGCCCATCTTCT-3′) was used. ILKAS is a 20mer antisense oligonucleotide (ODN) with a phosphothioate backbone. ILK kinase activity was determined in cell extracts by immunoprecipitation followed by *in vitro* kinase assays. *In vitro* analysis of ILKAS effects included assessments of P-Akt-Ser-473, P-Akt-Thr-308, apoptosis and nuclear morphology. Efficacy experiments were conducted in male SCID/Rag-2M mice bearing U87MG tumors (6 mice per group). Treatments were initiated on day 22-post inoculation. Saline control, ILKAS or antisense controls were administered using a treatment schedule of i.p. injections given once a day for 5 days with two days off, for a 3 week period at a dose of 5 or 10 mg/kg.
Results: The activity of ILK is constitutively elevated in a serum independent manner in PTEN mutant cells, and transfection of wild-type PTEN under the control of an inducible promoter into mutant PTEN cells inhibits ILK activity. Transfection of ILK antisense or exposure to a small molecule ILK inhibitor, suppresses the constitutive phosphorylation of PKB/Akt on Ser-473 in PTEN-mutant glioblastoma cell lines. In addition, the delivery of ILK antisense to PTEN negative glioblastoma cells results in apoptosis. Finally, glioblastomas generated in Rag-2M mice treated with ILK antisense shows tumor growth delay *in vivo*.
Conclusion: Our initial results indicate that therapeutic strategies targeting ILK may be beneficial in the treatment of glioblastomas.

62 POSTER

Isolation of pericytes from vasculature of human lung tumors

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Pericytes, also known as mural cells or myofibroblasts, are a key component of human vasculature. Pericytes wrap around the abluminal surface of blood vessels and interact directly with endothelial cells. Pericytes are also associated with tumor vasculature and are an attractive target for anti-angiogenic therapy. We have isolated pericytes from clinical lung samples of patients presenting an adenocarcinoma, a squamous cell carcinoma, or a tumor of neuroendocrine origin. Following surgical excision, tumors were digested with collagenase and elastase. Magnetic beads coupled with cell-specific antibodies were used to deplete blood cells (anti-CD14, -CD45, -CD64), epithelial cells (anti-BerEP4), and endothelial cells (anti-CD31). Remaining cells were placed in culture on poly-L-lysine coated flasks with media that supports pericyte growth and includes FGF, EGF, and IGF-1. Following expansion in culture for 1–2 weeks, pericytes were positively selected using magnetic beads coupled to an antibody against the proteoglycan NG2. These methods resulted in the isolation of a pericyte population with over 90% of the cells expressing NG2. The distinct morphology of the pericytes isolated is consistent with previous reports: elongated cytoplasmic extensions, ruffled membranes, and an