of Kit, 50% inhibition of Kit phosphorylation is associated with a plasma concentration approximately equal to the IC50 for Kit inhibition in vitro, when measured in the presence of physiological concentrations of the plasma proteins albumin and alpha1-acid glycoprotein. Using the wild-type Kit-expressing H526 SCLC model we have found that higher plasma levels of OSI-930 are required to achieve a comparable level of inhibition of Kit phosphorylation. However, in both HMC-1 and H526 xenograft models, maximal tumor growth inhibition was observed at oral dose levels that maintained a high degree of inhibition of Kit phosphorylation (>90%) for the majority of the 24h dosing period, i.e. 50mg/kg q.d. in the HMC-1 model and 200mg/kg q.d. in the H526 model. The potential involvement of KDR inhibition in the anti-tumor activity of OSI-930 has also been investigated. Thus, OSI-930 potently inhibited VEGF-induced KDR phosphorylation in endothelial cells at concentrations that also inhibited angiogenic sprout formation from aortic ring explants. In addition, we have used the KDR-dependent mouse uterine edema model system to demonstrate that oral dosing of OSI-930 at 50mg/kg or above results in potent inhibition of KDR function in vivo, supporting a potential role for inhibition of KDR in the anti-tumor effects of OSI-930. The data suggest that anti-tumor activity of OSI-930 in mouse xenograft models is observed at dose levels that maintain a significant level of inhibition of Kit and KDR for a prolonged period. Therefore, prolonged inhibition of the molecular targets of OSI-930 in vivo may prove to be of therapeutic benefit in future clinical investigations of OSI-930 as a novel therapeutic agent.

414 POSTER

Correlation of mutations in EGFR with clinical outcomes in NSCLC patients treated with erlotinib

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Background: Erlotinib is a small molecule inhibitor of epidermal growth factor receptor (EGFR) that has demonstrated a statistically significant survival benefit in single agent treatment of unselected relapsed/refractory non-small cell small cancer (NSCLC) patients (ASCO 2004). *EGFR* mutations identified in a subset of NSCLC have been associated with sensitivity to gefitinib (Lynch et al. *NEJM* 2004; Paez et al. *Science* 2004). The prevalence of somatic mutations has been reported to be between 2% and 8% in unselected patients; mutations in exons 18 through 21 of *EGFR* were observed in 13 of 14 patients who responded to gefitinib and in none of the 11 patients who were treated and did not respond. The RAS pathway is also a target for somatic mutations in NSCLC. Mutations in *BRAF* and *KRAS* have been reported in 3% and 10–30% of NSCLCs, respectively, and have been associated with poor prognosis (Brose et al., *Cancer Res.* 2002;62:6997–7000; Silini et al. *Virchows Arch.* 1994;424:367–73).
In order to assess more accurately the prevalence of somatic mutations in NSCLC tumors and the influence of these mutations on patient outcome to erlotinib treatment, formalin-fixed paraffin-embedded tumor samples (FFPE) from patients treated with erlotinib in a phase **III** study were analyzed. Tribute, a phase **III** randomized trial conducted in the U.S, enrolled 1079 patients with previously untreated, advanced NSCLC to compare the survival of patients who received erlotinib administered concurrently with a regimen of carboplatin and paclitaxel (CP) (n=539) to patients who received CP alone (n=540). The erlotinib arm did not demonstrate a survival advantage (primary endpoint) over CP alone (ASCO 2004). Sample collection was optional in this study, and archival tumor tissue was available from a subset of the patients enrolled in the trial.
Methods: Mutational analysis of *EGFR*, *KRAS*, and *BRAF* in a subset of tumors from patients in Tribute was performed by DNA sequencing using fluorescent dye-terminator chemistry (Applied Biosystems, Foster City CA) of tumor cells isolated by laser capture microdissection (PixCell **II**, Arcturus). Descriptive summaries of duration of survival and objective response were produced for each of the categorical variables listed above for each treatment arm. These descriptive summaries consisted of the hazard ratio from unstratified Cox regression and Kaplan-Meier estimates of median time to the event.
Results and Conclusions: Baseline covariates were compared between the subsets of patients with and without tissue and no differences were observed. We have assessed the frequency and nature of mutations in this population, and have explored the effects of mutation status on survival, time to progression, and objective response rate survival. These data will be presented.

414A POSTER

Pharmacological properties and in vitro and in vivo antitumour activity of the potent and selective PI3 kinase inhibitor PI103

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PI3 kinase is strongly validated as an important therapeutic target, eg by loss of the *PTEN* tumour suppressor gene and by overexpression and mutation of the *PI3KCA* gene that encodes the p110α catalytic subunit. We previously reported the identification of the pyridofuropyrimidine PI103 as a nanomolar potent and selective inhibitor of the class IA p110α and β isoforms of PI3 kinase (Patel et al Proc AACR LB-247 2003). Here we describe the pharmacological properties and *in vitro* and *in vivo* antitumour activity of PI103. This agent exhibited an IC_{50} of around 1.5–3nM against p110α and p110β. PI103 exhibited growth inhibitory activity against a panel of human cancer cell lines and was more potent against the *PTEN* negative PC3 prostate tumour (IC_{50} 88nM) than against the *PTEN* positive HCT116 colon cancer line (IC_{50} 1 μM). The predominant effect on cells was a G1 cell cycle arrest. Rapid and extensive inhibition of Ser473 Akt phosphorylation was seen at concentrations around the IC_{50} for growth inhibition. Following 30mg/kg ip, PI103 was well distributed (Vz=150mL) but cleared relatively rapidly from the general circulation (Cl=120mL/hr). Tumour levels were above IC_{50} levels for 3hrs. Significant growth inhibitory activity of PI103 was seen in both PC3M and HCT116 human tumour xenografts using well tolerated doses of 30mg/kg ip twice daily. In addition to a direct antiproliferative effect on cancer cells, we have obtained data that suggest that the activity of PI103 may also be due in part to its inhibitory effects on invasion and angiogenesis. Thus PI103 was shown to inhibit the invasion of PC3M tumour cells and to block motility, invasion and proteolysis by human endothelial cells *in vitro*. PI103 showed significant inhibition of the OVCAR3 human ovarian cancer xenograft in vivo with a corresponding decrease in Akt phosphorylation, consistent with inhibition of PI3 kinase in the tumour. Additional potential pharmacodynamic and prognostic biomarkers have been identified by gene expression microarray analysis. In conclusion, PI103 is a potent and selective class 1A PI3 kinase inhibitor with promising pharmacological properties, including *in vitro* and *in vivo* antitumour activity consistent with the mechanism of action. Thus it exemplifies the considerable potential of agents derived from this series to be developed for clinical evaluation. Optimisation is now underway in collaboration with PIramed.

Cyclins and CDKs

415 POSTER

Discovery and evaluation of inhibitors of cyclin E2–CDK2 and cyclin B1–CDK1

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Cyclin-dependent kinase (CDK) complexes regulate the temporal progression of cells through the cell cycle. Accumulating evidence demonstrates that the deregulation of cyclin E expression and subsequent activation of the CDK2 catalytic subunit plays a critical role in the progression of multiple tumor types. To further understand the role of CDK complexes in tumor formation novel small molecule inhibitors that inhibited cyclin B1-CDK1 and cyclin E2-CDK2 complexes at low nM potency were evaluated in cell based assays and *in vivo* tumor xenograft models. A novel cell based assay that measures the intracellular level of Rb phosphorylation by flow cytometry was developed and used to screen CDK inhibitors. These compounds arrested cells in both G1 and G2, induced apoptosis and inhibited the phosphorylation of Rb at low sub-micromolar concentrations. CDK inhibitors were tested in *in vivo* tumor models and demonstrated potent activity against both colon and prostate tumor xenograft models. In addition, we demonstrate that the CDK inhibitors prevented Rb phosphorylation *in vivo* and induced tumor cell death in *in vivo* tumors models. The discovery and evaluation of novel CDK2 and CDK1 inhibitors may aid in delineating the potential role that these CDK complexes play in regulating tumor formation and progression.