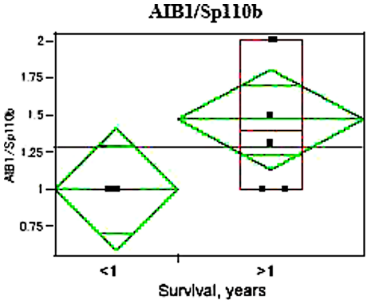
hybridization (ISH) was performed for AIB1, TIF2 and Sp110b. mRNA expression was graded on a 4-point scale, − through +++, based on the proportion of cells staining positive and the intensity of staining.
Results: Preclinically, coactivator/corepressor ratios correlated with TAC-101-induced RAR transcriptional activity: AIB1/Sp110b ($p = 0.009$); TIF2/Sp110b ($p = 0.048$); SRC1/Sp110b ($p = 0.0050$); correlations between individual cofactors and RAR response were not significant. MS for all pts treated was 12.8 months; 13.2 months (range 4.1−23.4) for the 10 pts analyzed here; 4 pts survived <1 yr and 6 pts >1 yr. Coactivator expression alone did not correlate with survival. For Sp110b, there was a trend toward greater expression for pts with survival <1 yr than for >1 yr ($p = 0.118$; Wilcoxon Rank Sum test). The ratio of AIB1/Sp110b correlated more closely with survival ($p = 0.070$; see figure); all patients with a ratio >1 survived >1 year.



Conclusions: Preclinically coactivator/corepressor ratios correlated with RAR response. In the pilot clinical study, coactivator/corepressor ratio correlated with survival. Validation of this observation and determination of whether this is prognostic for survival or predictive for response to TAC-101 therapy will be performed in a prospective, randomized clinical trial.

616 POSTER

Antitumor activity of Enzastaurin (LY317615) in human tumor xenografts in vitro

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Background: Protein kinase C beta (PKC β) is one of the most prominently overexpressed genes in fatal/refractory diffuse large B-cell lymphoma. The alternatively-spliced PKC β1 and β2 isoforms are the major PKC expressed by B lymphocytes. Activation of PKC β has been repeatedly implicated in tumor-induced angiogenesis, tumor cell proliferation, tumor invasiveness, and apoptosis. Enzastaurin (LY317615), an acyclic bisindolylmaleimide developed by Eli Lilly, is a potent, selective inhibitor of PKC β, with antiangiogenic activity and is now in clinical development. Data have been published that support the notion that Enzastaurin suppresses tumor growth through multiple mechanisms: direct suppression of tumor cell proliferation and the induction of tumor cell death coupled to the indirect effect of suppressing tumor-induced angiogenesis.
Materials and Methods: We have investigated the antitumor efficacy of Enzastaurin *in vitro* using a clonogenic assay in a panel of 51 human tumor xenograft models, which have been established in serial passage on nude mice in order to investigate tumor type selectivity. In addition, the effect on 3 preparations of hematopoietic stem cells was investigated. The tumor panel represented 13 different tumor types.
Results: Enzastaurin applied in continuous exposure at dosages ranging between 0.001 μM and 100.0 μM demonstrated both antitumor activity in a dose dependent manner and antitumor selectivity. Selectivity was observed particularly against tumor models of leukemia (2/3), lymphoma/myeloma (3/3), small cell lung cancer (2/2), and melanoma (2/5). Sensitive tumor models were in average about 9-fold more sensitive than the mean IC_{70}-value, and than hematopoietic stem cells as representatives for normal tissue, indicating a favourable therapeutic index.
Conclusions: Enzastaurin has shown antitumor effects *in vitro* without considering effects on angiogenesis, that cannot be measured in the clonogenic assay. *In vivo* studies in tumor-bearing nude mice, using the most sensitive *in vitro* tumor models, will be performed in order to confirm the observed antitumor activity of Enzastaurin, and to identify target tumor types for further clinical studies.

617 POSTER

In vivo evaluation of efficacy and pharmacodynamic biomarkers of AZD0530, a dual-specific Src/Abl kinase inhibitor, in preclinical, subcutaneous xenograft models

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c-Src kinase (Src) is a non-receptor tyrosine kinase ubiquitously expressed but highly regulated and inactive in most normal mammalian cells. There is significant evidence demonstrating deregulated, increased Src kinase activity in several types of human tumours. AZD0530 is a novel, orally potent, once-daily, highly selective and dual-specific Src/Abl kinase inhibitor that is currently being evaluated in the clinic. Preclinically, AZD0530 potently reverses Src-driven invasion phenotypes in cancer cells *in vitro* and can inhibit invasion/metastasis *in vivo* (Green T, *et al*. oral communication, AACR 2005; Serrels B, *et al.* Abstract 3774, AACR 2006).
AZD0530 was dosed once daily by oral gavage at 50 mg/kg/day and was evaluated in nude mice for anti-tumour efficacy in a panel of human colorectal (LoVo, HT29, Colo205), pancreatic (HPAC, AsPC1), breast (MDA-MB-231, BT474c, ZR-75–1) and lung (PC9, Calu-6) tumour xenografts. Pharmacodynamic (PD) analysis of Src kinase substrates pPaxillin (pPax) and pFocal adhesion kinase (pFAK) by immunocytochemistry and Luminex was conducted on *ex vivo* tumour tissues. Reduced phosphorylation of paxillin and FAK, consistent with inhibition of Src kinase activity, was observed in both responsive and non-responsive xenografts. Using these preclinical data, a PK–PD model was constructed linking AZD0530 plasma and tumour concentrations to pharmacodynamic effects (pPax and pFAK suppression). In addition to its effects on invasion and metastasis reported elsewhere, AZD0530 induces anti-tumour effects in some subcutaneous xenografts. These preclinical data support the use of pPaxillin and pFAK biomarkers to demonstrate inhibition of Src target mechanism and establishment of PK/PD relationships in AZD0530 clinical studies.

618 POSTER

A novel tyrosine kinase inhibitor exhibits significant anti-proliferative/pro-apoptotic effects in non-small cell lung cancer models

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Background: BMS-690514 is a panHER/VEGFR2 inhibitor targeting two pathways (HER-mediated signaling and angiogenesis). Here, the anti-proliferative/pro-apoptotic effects of BMS-690514 ± cisplatin, were evaluated *in vitro* in different NSCLC cell lines harboring mutated ("activating" L858R or "gatekeeper" T790M) or wild-type EGFR. To characterize BMS-690514-induced death, siRNAs targeting proteins with known roles in apoptosis/survival and DNA repair were employed.
Materials and Methods: NSCLC cell lines with different EGFR and p53 mutations were treated with BMS-690514 ± cisplatin, to induce death or growth arrest. Cells were transfected with siRNAs for 48 h prior to BMS-690514 ± cisplatin administration, then proliferation was assessed. Apoptosis-associated changes were evaluated by means of FACS analysis with $DiOC_{(6)}3$ for the loss of mitochondrial transmembrane potential ($\Delta\psi_m$) and PI for the loss of viability.
Results: BMS-690514 induced anti-proliferative/pro-apoptotic effects in NSCLC cells (including those carrying wild-type EGFR, L858R mutations, and those encoding both the L858R and the T790M mutations), in the following order of sensitivity: H1975 $\gg$ H1650 = H1299 > A549. BMS-690514 induced loss of $\Delta\psi_m$ and PI incorporation (associated with early and late apoptosis, respectively). Caspase inhibition had minor protective effects on the reduction of $\Delta\psi_m$ and no effect on loss of viability. Combined treatment with BMS-690514 + cisplatin resulted in synergic growth inhibition, while either drugs alone had small effects. Synergy occurred when BMS-690514 was given 24 h later than cisplatin and not when drugs were added in reverse order. Caspase-2 down-regulation provided partial protection against BMS-690514-induced death at 24 h, but not at 48 h. Bcl-2 down-regulation sensitized cells to BMS-690514, at 24 and 48 h.
Conclusion: BMS-690514 reduced growth and induced apoptosis in NSCLC cell lines, including cells harboring the EGFR T790M mutation that are insensitive to inhibitors like erlotinib and gefitinib. Its pro-apoptotic effects involved both caspase-dependent and caspase-independent routes. BMS-690514 sensitized NSCLC cells to cisplatin, in a sequence-dependent manner, suggesting that cycle arrest may enhance sensitivity to BMS-690514. siRNAs demonstrated a minor involvement of caspase-2 in BMS-690514 activity. In conclusion, BMS-690514 may become a valuable