upregulation of PKCδ, Raf1, p42/44 MAPK, JNK, p38 MAPK and PTEN and downregulation of PKCα, and AKT/PKB in sensitive Colo205 cells. This study aimed to describe changes in proliferation, adhesion, and invasiveness in cells with acquired resistance to PEP005.

Methods: A resistant colon cancer cell line was established by continuous exposure of Colo205 to PEP005. Parent sensitive (Colo205S) and resistant (Colo205R) cells were compared for proliferation (MTT), adhesion, invasion (Matrigel assay), and gene expression profile using a selected panel of genes (quantitative RT-PCR).

Results: Colo205R displayed a 300-fold resistance to PEP005 in comparison to Colo205S (IC_{50}s >100 μM *versus* 0.001 μM respectively). The resistant phenotype was not reversible in Colo205R cells ⩾6 passages in the absence of PEP005. Proliferation rate of both Colo205R and Colo205S cell lines were similar with doubling times of 48 hours, with no significant cell cycle modification. As compared to Colo205S, gene expression profiling showed a decreased expression of PKCα in Colo205R with an overexpression of antiapoptotic genes such as Bcl2 suggesting an increased cell survival potential in resistant cells.

In addition, phenotypical changes were observed in Colo205R with loss of round shape, cellular spreading, filopodia formation and increased adhesion properties (Fig. 1). These results correlate with an overexpression of genes implicated in cell adhesion and cell-cell contacts such as ICAM, β1 integrin, E-cadherin and Connexin 32 in Colo205R. Furthermore, Colo205R was highly more invasive than Colo205S: 0.65% cells entered into Matrigel *versus* 0.19% cells per insert, respectively. This increased invasiveness was associated with an overexpression of MMP9 and other genes involved in tumor angiogenesis such as Cox2.

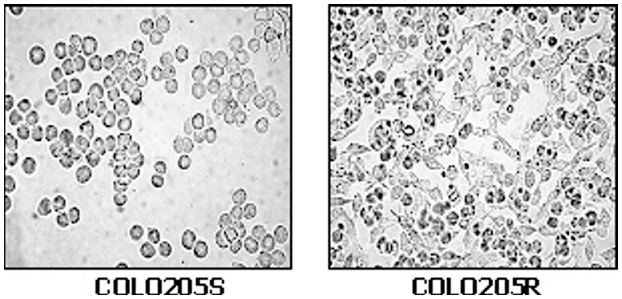

Fig. 1. Morphological changes induced by a continuous exposure to PEP005 in parental (Colo205S) and resistant (Colo205R) human colon cancer cells.

Conclusion: Acquired resistance to PEP005, a novel PKC modulator, was associated with no significant modification in proliferation but increased adhesion and invasion capacities in Colo205 colon cancer cells. Our results suggest that PKC isoforms are critical in the acquisition of a more invasive phenotype in malignant cells.

613 POSTER

GX15–070, a small molecule Bcl-2 family inhibitor, induces apoptosis and enhances cisplatin-induced apoptosis in non-small cell lung cancer cells

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Background: Overexpression of Bcl-2 family members as well as deregulated pathways that regulate apoptosis is a hallmark of lung cancer. Non-small cell lung cancer cells are typically resistant to cytotoxic chemotherapy and approaches that alter the balance between pro-survival and pro-death Bcl-2 family members have shown promise in preclinical models of lung cancer. GX15–070 (obatoclax) is a small molecule agent that can bind anti-apoptotic Bcl-2 proteins and interfere with their ability to interact with pro-apoptotic proteins.

Materials and Methods: Using NSCLC cell lines we evaluated the effects of a novel Bcl-2 inhibitor GX15–070 on lung cancer survival and its effect in combination with epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) as well as traditional cytotoxic agents. We evaluate the effect of GX15–070 and correlated the effect on EGFR status as well as Bcl-2 family protein expression.

Results: We identified differentially sensitivity of a panel of lung cancer cells to GX15–070 and no clear relationship existed between EGFR status or Bcl-2 family protein expression and sensitivity to GX15–070. GX15–070 was able to induce apoptosis in a subset of lung cancer cell lines and this correlated with the effects on cell viability. GX15–070 in combination with gefitinib was synergistic in a cell line dependent on EGFR for survival but GX15–070 could not reverse resistance to gefitinib in cell lines not dependent on EGFR for survival. Finally, we observed synergy between GX15–070 and cisplatin in lung cancer cells.

Conclusions: Based on these results, GX15–070 can trigger apoptosis in lung cancer cells and can enhance chemotherapy-induced death. These data suggest that clinical trials with GX15–070 in combination with cytotoxic chemotherapy are indicated.

614 POSTER

Synergistic interaction between erlotinib and JM-118, the active metabolite of satraplatin

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Satraplatin (JM216) is a novel oral platinum analog. It is currently being evaluated for its efficacy in various Phase II studies. A pivotal Phase III trial evaluating satraplatin as 2nd-line therapy for hormone refractory prostate cancer completed accrual of >950 patients in 2005. JM-118, an active metabolite of satraplatin was shown to have anticancer activity in cells resistant to cisplatin, carboplatin and oxaliplatin, which are platinum analogs with activity in non-small cell lung cancer (NSCLC), ovarian and colon cancer. Erlotinib (Tarceva) is a potent inhibitor of the tyrosine kinase domain of the epidermal growth factor receptor (EGFR), which has shown activity in NSCLC (particularly in patients with mutations in EGFR) and colon cancer. Since upregulation of the AKT/ERK pathway, which is downstream of EGFR, may play a role in the resistance to platinum analogs, we evaluated whether inhibition of this pathway by erlotinib would enhance the sensitivity of NSCLC (A549, SW1573), colon (Lovo, WiDr) and ovarian (A2780, 2008) cancer cell lines to JM-118. A431 cells, which overexpress EGFR, were included as a positive control for erlotinib (IC_{50} 0.9 μM), while the other cell lines had IC_{50} values for erlotinib of between 4.6–6.4 μM. These cell lines have a wild type EGFR expression or K-ras mutations (A549, SW1573, Lovo), are mismatch repair deficient (MLH1 absent in Lovo) or have a low or absent excision repair (ERCC1 low in A2780, SW1573, Lovo). In all cell lines, except SW1573, JM-118 (IC_{50} values 0.3–2.2 μM) was more active than satraplatin (IC_{50} values 0.9–3.5 μM); JM-118 was also more active than cisplatin (IC_{50} values 0.5–6.9) and similarly active to oxaliplatin (IC_{50} values 0.2–2.2 μM). The interaction between JM-118 and erlotinib was evaluated with the median drug effect analysis, in which a combination index (CI) <0.9 is considered synergistic, $0.9 \leqslant CI \leqslant 1.1$ additive and >1.1 antagonistic. Cells were exposed to a fixed ratio of the drugs, based on the respective IC_{50} values. At simultaneous exposure, JM-118 and erlotinib were synergistic in A431, A549 and Lovo cells (CI: 0.5–0.8), and additive in the other cell lines. Pre-treatment of the cells with erlotinib for 24 hr resulted in a similar synergism in the same cell lines, as well as in 2008 and WiDR cells. Mechanistic studies were initiated focusing on platinum-DNA adduct formation and changes in the phosphorylation of AKT and ERK. In A549 cells, exposure to JM-118 for 24 hr at its IC_{50} increased the presence of p-AKT, whereas erlotinib prevented this increase. Presence of p-ERK was decreased by JM-118 and the combination. These data indicate that JM-118 and erlotinib differentially interfere with signalling downstream of EGFR.

In conclusion, the combination of JM-118, the active metabolite of the novel oral platinum drug satraplatin, and erlotinib has synergistic/additive activity in all of the cell lines tested, which may be related to changes in signalling. These data support clinical evaluation of the combination of satraplatin and erlotinib.

615 POSTER

Nuclear coactivator/corepressor expression ratio predicts survival in hepatocellular carcinoma patients treated with TAC-101, a synthetic retinoid

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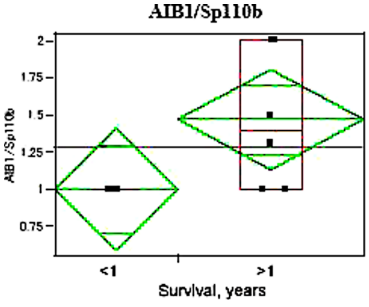
Materials and Methods: Cofactor expression in HCC cell lines was analyzed by RT-PCR. RAR transcriptional activity was evaluated by luciferase-reporter assay. Relationship between the survival of TAC-101 treated patients and the coactivator and corepressor levels in tumor prior to treatment was explored retrospectively in the pilot clinical study. Pts with HCC not amenable to treatment by surgery or ablative therapies were treated with TAC-101 20 mg daily for 14 days followed by 7 day rest periods; treatment continued until disease progression or unacceptable toxicity occurred. Paraffin block specimens were obtained from 15 of 28 pts in the study; 10 samples were evaluable for cofactor expression. *In situ*

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