signaling pathway include rapamycin and related molecules, which inhibit the mTOR kinase. However, inhibition of mTOR and its downstream effector p70S6K can lead to upregulation of PI-3 kinase signaling, including activation of Akt and downstream survival pathways.
EXEL-9418 (XL418) is a potent inhibitor of Akt and p70S6K, two important kinases that mediate PI-3 kinase pathway signaling. In biochemical assays, EXEL-9418 inhibits Akt and p70S6K with IC_{50} values in the low nanomolar range. In cellular assays, EXEL-9418 inhibits phosphorylation of ribosomal S6 protein (a substrate of p70S6K) and the Akt substrates GSK3β and PRAS40, and induces translocation of the FKHR transcription factor in tumor cells. Oral administration of EXEL-9418 in the A549 lung adenocarcinoma xenograft model inhibited p70S6K and Akt signaling, and these effects were correlated with inhibition of tumor cell proliferation and induction of apoptosis, respectively. In contrast, rapamycin inhibited proliferation but caused little or no apoptosis in this model. EXEL-9418 causes significant tumor growth inhibition in nude mouse xenograft models, with little effect on hematology and clinical chemistry parameters or body weight at efficacious doses. Moreover, combining EXEL-9418 with epidermal growth factor receptor (EGFR) inhibitors in an EGFR inhibitor-resistant cell line (MDA-MB-468) leads to downregulation of Akt and p70S6K signaling and a substantial increase in apoptosis compared to either agent alone, both in vitro and in vivo.
These data suggest that a dual inhibitor strategy, targeting both cell growth and cell survival pathways, may offer significant advantages over targeting growth signaling alone. Furthermore, such an inhibitor may have broad utility in potentiating the effects of EGFR inhibitors.

609 POSTER

Selective inhibition of Raf results in down regulation of the Ras/Raf/MEK/ERK pathway and inhibition of tumor growth in vivo

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The Ras/Raf/MEK/ERK signaling pathway is upregulated in approximately 30% of all human cancers, with activating Ras mutations evident in 15−30% of these cancers. Activating mutations of B-Raf, primarily B-RafV600E, have been identified in approximately 7% of human cancers, including 70% of malignant melanomas.
We have identified a highly potent and selective Raf kinase inhibitor, EXEL-2819 (XL281), which modulates MEK/ERK phosphorylation and tumor cell proliferation in vitro and in vivo. EXEL-2819 exhibits potent activity against c-Raf, B-Raf, and the activated mutant B-RafV600E in enzyme assays, with IC50 values in the low nanomolar range. EXEL-2819 is a highly selective inhibitor of Raf, with potency at least 250-fold greater for Raf compared to 100 other kinases. EXEL-2819 modulates the Raf/MEK/ERK pathway in a number of tumor cell lines harboring activating Ras and B-Raf mutations and potently inhibits the phosphorylation of MEK and ERK in these cells.
In pharmacokinetic studies, EXEL-2819 displays high oral bioavailability in mice, rats, dogs, and monkeys. In pharmacodynamic studies, administration of a single oral dose of EXEL-2819 results in decreased phosphorylation of MEK ($\leqslant$98% inhibition) and ERK ($\leqslant$78% inhibition) in xenograft tumors. In repeat-dose efficacy studies, EXEL-2819 inhibits tumor growth in a range of xenograft models including A375, MDA-MB-231, HCT116, and A431. Immunohistochemical analyses of tumors collected at the end of these studies reveal significant inhibition of phosphorylation of MEK and ERK, decreased cell proliferation (Ki67), and decreased tumor vascularization (CD31).
In summary, these data indicate that selectively targeting Raf kinases with EXEL-2819 results in substantial inhibition of the Raf/MEK/ERK pathway and of tumor growth in preclinical xenograft models, and provide a rational basis for the clinical development of this inhibitor for the treatment of solid tumors that rely on the Ras/Raf/MEK/ERK pathway.

610 POSTER

Synergistic cytotoxicity, inhibition of Akt and c-Kit phosphorylation and modulation of gene expression by sorafenib and gemcitabine in human pancreatic cancer cells

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Background: pancreatic cancer is one of the most lethal tumours and, although gemcitabine produces a clinical meaningful response, there has been little improvement in prognosis. Therefore, research effort has focused on target-specific agents, such as sorafenib, which blocks both the RAF/MEK/ERK signaling pathway and receptors involved in neovascularization and tumour progression, including VEGFR-2 and c-Kit. We investigate whether sorafenib would be synergistic with gemcitabine against pancreatic cancer cell lines.
Material and Methods: cells were treated with sorafenib and gemcitabine, alone or in combination. Pharmacologic interaction was studied using the combination index (CI) method, while cell cycle was investigated with flow cytometry. Moreover, the effects of drugs on Akt and c-Kit phosphorylation, and on apoptosis induction were studied with ELISA and fluorescence microscopy, respectively. Finally, quantitative PCR analysis was performed to assess whether sorafenib modulated the expression of the gemcitabine activating enzyme deoxycytidine kinase (dCK) and the drug target ribonucleotide reductase (RR).
Results: sorafenib was cytotoxic against MIA PaCa-2, Capan-1, PANC-1 cells with IC50s of 3.48 ± 0.27, 0.61 ± 0.16, $4.56\pm1.32\,\mu$M, respectively. A dose dependent inhibition of cell growth was also observed after gemcitabine treatment with IC50s of 0.08 ± 0.01 (MIA PaCa-2), 0.10 ± 0.02 (Capan-1), 0.178 ± 0.039 (PANC-1) μM. The CI analysis showed synergism for both sequences. Flow cytometry demonstrated that gemcitabine enhanced cellular population in the S phase. Cell exposure to gemcitabine resulted in a significant Akt phosphorylation inhibition, whereas sorafenib exposure reduced c-Kit phosphorylation. Fluorescence microscopy demonstrated that cells treated with drugs and their combinations presented typical apoptotic morphology; in particular, drug combinations significantly increased apoptotic index with respect to single agents in Capan-1 and MIA PaCa-2 cells. PCR showed that sorafenib reduced the expression of RRM1 and RRM2 in MIA PaCa-2 and Capan-1 cells, enhancing the dCK/(RRM1$\times$RRM2) ratio ($p < 0.05$).
Conclusions: these data demonstrate that sorafenib and gemcitabine synergistically interact against pancreatic tumour cells, through suppression of Akt and c-Kit phosphorylation, induction of apoptosis and reduction of RRM1 and RRM2 gene expression, thus providing the experimental basis for developing this combination for the treatment of pancreas cancer.

611 POSTER

Sensitization of human prostate cancer cells to TRAIL/Apo2L by curcumin through inhibition of pro-survival Akt/NF-kB signaling pathways

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Materials and Methods: The expression levels of constitutively active Akt, NF-κB and NF-κB-dependent antiapoptotic proteins in LNCaP, PC3, and DU145 prostate cancer cells were determined by Western blotting. Curcumin, Akt inhibitor SH-6, and siRNA-Akt were used to sensitize cancer cells to TRAIL and to understand cross-talk between Akt and NF-κB signaling pathways and their role in resistance of prostate cancer cells to TRAIL-induced apoptosis.
Results: Each cancer cell line studied expressed transcriptionally active NF-κB which was inhibited by curcumin at concentration range of 10 to 30 μM. LNCaP and PC3 cells but not DU145 cells expressed p-Akt kinase which was also inhibited by curcumin. Inhibition of the NF-κB and p-Akt by curcumin sensitized cancer cells to TRAIL-induced cytotoxicity. Since NF-κB is a downstream target of p-Akt, we investigated whether inhibition of NF-κB by curcumin is mediated through suppression of Akt activation. Treatment of PC3 cells with SH-6, a specific inhibitor of Akt, or transfection with siRNA-Akt, not only inhibited p-Akt but also abrogated the nuclear expression of NF-κB. Furthermore, treatment with SH-6 or selective inhibition of Akt through siRNA-Akt inhibited NF-κB and sensitized cells to TRAIL. In contrast, SH-6 failed to inhibit NF-κB or sensitize DU145 prostate cancer cells to TRAIL as these cells do not express p-Akt. Because expression of antiapoptotic Bcl-2, Bcl-xL and XIAP is regulated by NF-κB, both curcumin and SH-6 decreased the levels of these proteins in PC3 cells through inhibition of NF-κB. Further, gene silencing of Bcl-2 with siRNA-Bcl-2 sensitized PC3 cells to TRAIL.
Conclusions: These data define a molecular pathway wherein curcumin sensitizes prostate cancer cells to TRAIL by inhibiting Akt-regulated NF-κB and NF-κB-dependent antiapoptotic Bcl-2, Bcl-xL and XIAP.

612 POSTER

Acquired resistance to drugs that yield PKCδ activation and PKCα inhibition modify adhesion and invasion in human cancer cells

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Background: PKCs are serine/threonine kinases modulating proliferation, apoptosis and invasion in cancer cells. Among PKC modulators used in clinical trials the new agent PEP005 was shown to induce apoptosis by

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.

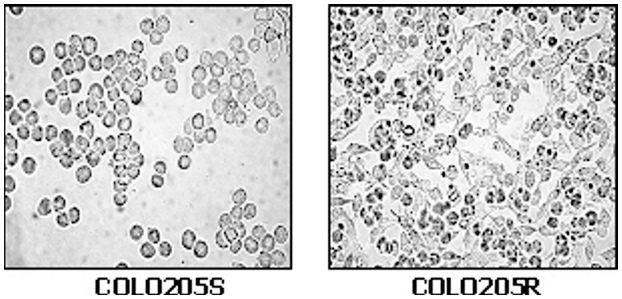

Fig. 1. Morphological changes induced by a continuous exposure to PEP005 in parental (Colo205S) and resistant (Colo205R) human colon cancer cells.

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*