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.

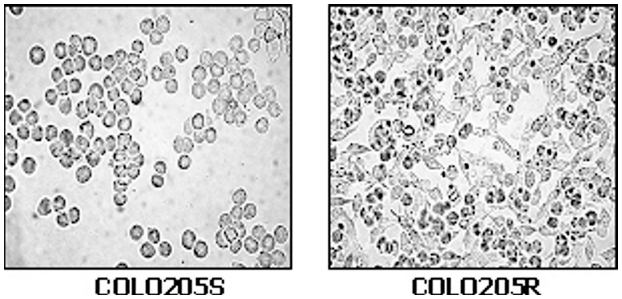

Fig. 1. Morphological changes induced by a continuous exposure to PEP005 in parental (Colo205S) and resistant (Colo205R) human colon cancer cells.

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

E. Haura[1], J. Li[1], J. Viallet[2]. *[1]H. Lee Moffitt Cancer Center, Thoracic Oncology, Tampa, USA; [2]GeminiX, Inc., Melvern, USA*

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

G.J. Peters[1], E.K. Hoebe[1], C.M. Huijts[1], A.D. Adema[1], K. Wosikowski[2]. *[1]VU University Medical Center, Department of Medical Oncology, Amsterdam, The Netherlands; [2]GPC Biotech, Munich, Germany*

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

K. Murakami[1], M. Thomas[2], T. Arima[3], K. Yonekura[1], Y. Yamamoto[1], T. Ueda[1], F. Hosei[1], Y. Yamada[1], Y. Lassere[2], J. Abbruzzese[2]. *[1]Taiho Pharmaceutical Co., Ltd., Advanced Research Laboratory (Oncology), Saitama, Japan; [2]M.D. Anderson Cancer Center, Gastrointestinal Medical Oncology, Houston, USA; [3]Taiho Pharmaceutical Co., Ltd., Strategic R&D Planning, Tokyo, Japan*

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*