combination of C225 and L-OHP did not induced significant inhibition of tumor growth compared to single agent L-OHP or C225.
These results suggest that EGF-R blockade by C225 combined with L-OHP may be an effective therapy against some chemo refractory colorectal carcinoma tumors. In our models, the response is strictly dependent on the cell type and not correlated to the level of EGF-R expression suggesting ongoing experiments to characterize EGF-R dependant-pathway.

**379** POSTER

**Characterisation of a novel class I isoform selective phosphatidylinositol 3-kinase inhibitor in glioma**

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The phosphatidylinositol 3-kinase (PI3K) signalling pathway regulates multiple cellular processes often deregulated in cancer, including survival, proliferation, motility and cell cycle progression. Consequently, the PI3K pathway is highly attractive for therapeutic intervention. Glioma (primary brain tumour) cell lines represent a relevant model for the investigation of PI3K inhibitors as both cell lines and patient tumours often exhibit aberrant upregulation of the pathway, with a high frequency of *PTEN* (the negative regulator of downstream PI3K signalling) and *p110* $\alpha$ (a class IA isoform of the PI3K catalytic subunit) mutations. In addition, there is a pressing need for new glioma therapies as current treatments only have limited success. Here we describe the effects of PI-103, a novel PI3K inhibitor with potent activity against the PI3K class I isoforms (previously described by *Patel et al.*, Proc. Am. Assoc. Can. Res. 45: supp. p111) in a panel of six high-grade human glioma cell lines with defined molecular characteristics (LN229, U87MG, U138MG, U118MG, A172 and SF268). PI-103 demonstrates potent anti-proliferative effects throughout the cell line panel, with cellular $IC_{50}$ values at 96 hours in the 0.13–0.53 $\mu$M range by contrast with 10–15 $\mu$M for the broad spectrum PI3K inhibitor, LY294002. The sensitivity of glioma cells within the panel to PI-103 and LY294002 is independent of the *PTEN* status of the lines. However, all the lines have high constitutive levels of phospho-Akt ($Ser^{473}$) compared to most non-glioma derived cancer cell lines suggesting potential activation of the PI3K pathway by varied mechanisms. Treatment of the glioma lines with PI-103 for 24 hours causes inhibition of downstream signalling as demonstrated by decreased phospho-Akt ($Ser^{473}$) levels and Akt kinase activity. Interestingly, growth inhibition caused by PI-103 occurs by a cytostatic (G1 cell cycle block and growth arrest) mechanism whereas LY294002 tends to be both cytostatic and cytotoxic as demonstrated by flow cytometry and the cleavage of PARP by apoptotic proteases. The role of specific PI3K isoforms in glioma is currently being explored using siRNA knockdown of p110$\alpha$ and p110$\beta$. In summary, these results show PI-103 is a potent anti-proliferative compound in a glioma cell line panel, highlighting the promising therapeutic potential of targeting class I PI3K isoforms for the treatment of glioma.

**380** POSTER

**Analysis of complex PKB/Akt signaling pathways in human prostate cancer samples**

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**Background:** The protein kinase B/Akt (PKB/Akt) system is of key importance for cell survival and proliferation. Due to its crucial role for survival PKB/Akt is also of major relevance for the pathogenesis and modulation of treatment response of malignant tumors. The PKB/Akt system is found to be dysregulated in several tumors in vivo and in vitro. In order to more precisely define the role of the PKB/Akt system in prostate cancer we determined the expression level and the putative activation status of PKB/Akt and downstream targets in prostatectomy specimens from 22 patients with prostate cancer (PKB/Akt; phos-PKB/Akt, FKHR-L1; phos-FKHR, mTOR; phos-mTOR; GSK3b, phos-GSK3a/b; 4E-BP1, phos-4E-BP1, p27-kip1, phos-eIF4G).
**Material and Methods:** Tissue samples were initially scored regarding the pathological grade (Gleason) and subsequently analyzed by immunohistochemistry using specific antibodies directed against all of the proteins and the respective specifically phosphorylated forms as listed above. The expression pattern was examined regarding any putative correlation with the Gleason grade. In addition the hierarchical composition of the assumed signaling cascade was analyzed.
**Results:** All tissue samples with a Gleason 5–10 displayed a significant expression and strong phosphorylation level of PKB/Akt. In some cases of Gleason 5–6 a consecutive phosphorylation of downstream targets was detectable. For this subgroup a notable overexpression but not phosphorylation of the eucaryotic initiation factor 4E binding protein was found. In the majority of specimens with more aggressive Gleason grades (7–10) the consecutive activation of most downstream targets was seen. Similarly to the low grade Gleason tumors overexpression of eucaryotic initiation factor 4E binding protein was detectable. Analysis of the surrounding normal tissue revealed a highly reproducible loss of a strongly phos-FKHR expressing basal cell layer in the malignant compared to the normal glandular structures.
**Conclusions:** The data prove that a dysregulation of the PKB/Akt system is a common finding in patients with prostate cancer. However, we found a substantial heterogeneity in the expression and phosphorylation levels of the upstream molecule (PKB/Akt) and even more of the putative downstream targets of the kinase. The most common denominator of the malignant gland is the loss of the phos-FKHR expressing basal cell layer and the overexpression of 4E-BP1 in malignant glandular structures.

**381** POSTER

**A phase I study of BAY 43-9006, a novel Raf kinase and VEGFR inhibitor, in combination with taxotere in patients with advanced solid tumors**

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**Background:** The objective of this study was to determine the safety profile and pharmacokinetics (PK) of BAY 43–9006 (BAY), a novel dual action Raf kinase and VEGFR inhibitor, in combination with capecitabine (CAP).
**Materials and Methods:** This was a single-center, dose-escalation study. BAY was given orally bid from Day 8 until Day 21 in Cycle 1, and continuously thereafter in three doses: 200 mg bid (cohort 1), 400 mg bid (cohort 2) and 200 mg bid for the first two cycles, then 400 mg bid for subsequent cycles (cohort 3, ongoing). CAP was given orally bid (2100 mg/m* per day) from Day 1 in a 2 weeks on/1 week off schedule. PK parameters were determined on Day 21 of Cycle 1 and on Day 7 of Cycle 2 for BAY, and on Day 7 of Cycles 1 and 2. for CAP.
**Results:** Twenty patients were enrolled, 19 of whom were evaluable (cohort 1: n=12; cohort 2: n=4; cohort 3: n=3). Common tumor types were renal cell carcinoma (RCC; n=6) and colorectal cancer (CRC; n=4). The median number of treatment cycles for all cohorts was 5.5 (range 0–22), including one patient with RCC (22 cycles) and one patient with CRC (21 cycles). The most frequent drug-related toxicities were hand-foot syndrome (HFS), diarrhea, fatigue, mucositis and nausea. Dose-limiting toxicities included HFS grade 3 and diarrhea grade 3 (cohort 1), HFS grade 3 and mucositis grade 3 (cohort 2). All four patients in cohort 2 discontinued the planned regimen after the first or second cycle due to anorexia grade 2 and weight loss grade 2 (1 patient), HFS grade 3 and mucositis grade 3 (1 patient), epigastric pain grade 2 and HFS (1 patient), and dyspnea grade 2 (1 patient). Treatment is ongoing in all patients in cohort 3. One heavily pretreated patient from cohort 1 with breast cancer and skin lymphangitis showed tumor regression. The plasma PK of BAY were not influenced to a clinically relevant degree by concomitant administration of CAP. Multiple dosing with BAY 43–9006 200 mg bid had no relevant effect on the PK profile of CAP.
**Conclusions:** BAY in combination with CAP resulted in a safety profile consistent with that of the individual agents. However, CAP 2100 mg/m* per day combined with BAY 400 mg bid led to a significant rate of patient discontinuations. Therefore, two further cohorts are ongoing: CAP 2100 mg/m* per day plus BAY 200 mg bid for two cycles then 400 mg bid thereafter, and CAP 1700 mg/m* per day plus BAY 400 mg bid. Final data on four cohorts will be presented.

**382** POSTER

**Phase II antitumor activity of BAY 43-9006, a novel Raf kinase and VEGFR inhibitor, in patients with sarcoma enrolled in a randomized discontinuation study**

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**Introduction:** BAY 43–9006 (BAY) is a novel, orally active Raf kinase and VEGFR inhibitor with broad-spectrum anti-tumor efficacy in multiple human tumor xenografts.

**Methods:** This study is a multi-center, placebo-controlled, Phase II randomized discontinuation study in patients with advanced refractory progressive solid tumors which included advanced sarcoma patients. Initially, all patients received oral BAY at 400 mg twice-daily for12-weeks. At the end of this 12-week induction phase, antitumor responses were assessed. Patients whose target lesion tumor burden showed growth greater than 25%(progressive disease, PD) during the induction phase were discontinued from the study. Patients whose tumor demonstrated target lesion tumor burden shrinkage greater than 25% (responders) were not randomized and continued BAY in an open label phase, until disease progression or toxicity. Patients with tumor target lesion measurements that remained within 25% of the baseline pretreatment measurements (stable disease, SD) were randomized to receive either BAY, 400 mg every 12 hours, or matching placebo.
**Results:** To date 27 advanced sarcoma patients with different histologies have been enrolled of which 23 pts were evaluable for response. The median age was 56 years (range of 24y to 79y), ECOG performance status 0 (38%) and 1 (63%) and all (100%) had at least one prior systemic therapy. Seven pts discontinued study drug earlier than the 12-week assessment and 16 pts have been treated with BAY up to the 12-week assessment point. Investigator's assessment of response at the 12 week assessment point demonstrated 3 responders (continued on BAY), 5 SD (randomized to either BAY versus placebo) and 8 PD (off study) at the 12 week assessment point. Responder's histologies were 2 gastrointestinal stromal tumors (refractory to imatinib mesylate) and 1 synovial sarcoma. The most frequent drug-related toxicities included hand-foot skin reaction, rash/desquamation, anorexia, diarrhea, hypertension and fatigue.
**Conclusion:** While the study is still ongoing and the randomized portion of the study is yet to be analyzed, these preliminary data suggest that BAY may have potential anti-tumor activity in advanced sarcoma. Further clinical study in this setting is warranted.

**383** POSTER

**Preclinical antitumor activity of second generation analogs of SDX-101**

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**Introduction:** SDX-101 is an anti-neoplastic drug with a novel mechanism of action currently in Phase II clinical trials in leukemia. SDX-101 exerts its anti-neoplastic activity by inhibiting the activity of the beta-catenin pathway, via its interaction with the PPAR-RXR/beta-catenin nuclear complex.
**Aim:** The aim of this project was to synthesize and assess the cytotoxic activity and the mechanism of action of a series of SDX-101 analogs created by structural modification at various positions on the parent molecule.
**Results:** The SDX-101 analogs were screened in cell-based cytotoxicity assays and functional assays for beta-catenin inhibition. Three lead compounds have been identified: compound # 2, compound #5 and compound #8. As is the case with SDX-101, these compounds displayed selective cytotoxic activity for malignant cells when compared to the normal cells. The IC50 observed in LNCaP prostate cancer cell line ranged from 13±3 μM (#5) to 39±13 μM (#2). Compound #5 displayed an IC50 of 48±1 μM and 28±3 μM in the prostate cancer cell lines DU-145 and PC-3 and an IC50 of 16±2 μM and 8±3 μM in the colon cancer cell lines SW-480 and HCT-116. The IC50 values obtained with each of the analogs in these prostate cancer cells were markedly lower than those of SDX-101 (range 122–505 μM). Beta-catenin inhibitory activity of these leads was confirmed by reporter-promoter assays as well as by measuring mRNA and protein levels of beta-catenin-regulated genes such as cyclin D1 in cancer cell lines. The analogs were also potent in inhibiting tumor growth of Daudi xenografts in SCID mice. Following treatment with 125–250 mg/kg/d oral dose for four weeks, the mean tumor volumes for vehicle, #5, #8, and #2, were 1543 mm3, 946 mm3, 1078 mm3 and 825 mm3, respectively ($p<0.07$ each analog vs vehicle). The tumor volumes of SDX-101(400 mg/kd/d) and chlorambucil (2 mg/kg/d) treated mice were 1157 mm3 and 864 mm3 respectively. Time for the tumors to reach eight times (8X) the initial volume (100 mm3) was markedly delayed with all SDX-101 analogs: the controls reached 8X volume in approximately 9 days compared to approximately 21 days for the analogs. The treatment was well tolerated with no mortalities and no significant body weight loss.
**Conclusions:** Orally effective and well tolerated SDX-101 analogs have been identified with potent anti-neoplastic activity and similar mechanism of action.

**384** POSTER

**Sulindac sulfide modulates beta-catenin dependent expression of the metastasis-associated gene S100A4/mts1**

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**Background:** This study was designed to identify the impact of pathway modulators on the newly identified target gene of the beta-catenin/TCF pathway, the metastasis-associated gene S100A4/mts-1/metastasin.
**Material and Methods:** Gene knock-out technology was coupled to cDNA array analysis of the colon carcinoma cell line HCT116 (heterozygous for D45; wt/m) and a derived wt/-knock-out strain. Target gene confirmation was pursued using additional knock-out cell strains (wt/−; −/m) and a naturally nullosomic tumor cell line NCI-H28 investigating both mRNA and protein levels. Wild type and/or D45-beta-catenin-transduced clones of knock-out strains and NCI-H28 were created to prove the impact of D45-mutation on target gene expression and migration. The beta-catenin/TCF-pathway was analyzed by gel shift and reporter assays with several target gene promoter variants. In order to analyze the dependency of S100A4 expression on the beta-catenin/TCF-pathway, the modulators LiCl, known as inhibitor of the glycogen synthase kinase 3b, and sulindac sulfide, known to target the nuclear accumulation of beta-catenin, were employed.
**Results:** S100A4, which is associated with the metastatic phenotype, was dramatically down-regulated in wt/-knock-out strains compared with HCT116 cells and −/m knock-out strains. S100A4 expression positively correlated with the in vitro invasive phenotype. In NCI-H28/D45 cells, S100A4 levels were increased up to 70-fold, correlating with enhanced migration behavior. S100A4 promoter activity of deletion variants was dependent on beta-catenin availability and TCF-4 binding site presence. Treatment with the pathway activator LiCl led to induction of S100A4 expression in HCT116 cells and in the knock out strains analyzed. Treatment with the pathway inhibitor sulindac, however, clearly reduced the expression levels of S100A4 in these cell lines. Moreover, sulindac-treated cells showed reduced migration behavior compared with the non-treated controls.
**Conclusion:** S100A4 is a target gene of the beta-catenin/TCF-pathway. Mutant D45-beta-catenin allele acts in a dominant fashion to activate S100A4 expression. Modulators of beta-catenin signaling may offer potential as antimetastatic agents by interdicting S100A4 expression.

**385** POSTER

**A phase II trial to assess the efficacy and safety of Gefitinib (IressaTM) in patients with metastatic hormone refractory prostate cancer (HRPC) who progressed on treatment with a luteinising hormone releasing hormone analogue (or post orchiectomy) plus an antiandrogen**

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EGFR is overexpressed in HRPC. Gefitinib enhances antiproliferative effect of antiandrogen bicalutamide when coadministered to moderately androgen-independent prostate tumour xenografts. This is a phase II trial aimed to assess activity and safety of gefitinib in patients with metastatic HRPC who progressed to an LHRH analogue plus antiandrogen. Patients received gefitinib 250 mg daily and antiandrogen plus LHRH analogue for 2 months or until disease progression (PD). Patients with PD stopped antiandrogen therapy and continued gefitinib with LHRH analogue. Thirty-four patients have been planned for the study. Global health status, pain score and quality of life (QoL) have been assessed at baseline and every 2 months by visual analogue scale, McGill-Melzack and EORTC QLC-30 questionnaires. Patients who had no surgery underwent a prostate biopsy to study EGFR and HER2 expression. Serum HER2 and EGFR extracellular domain (ECD) were evaluated every 2 months. EGFR and HER2 ECD were assayed by ELISA method. A cut-off of 15 ng/ml was used for HER2 ECD. A reference range for HER1 was determined on 30 healthy subjects sera (45.7–71.3 ng/ml). From April 2003 to May 2004 18 patients have been enrolled. Baseline data are available for all cases, 16 cases are evaluable for safety and 11 for efficacy. The median age was 65 years (range 56–78). WHO performance status was 0 in 13 and 1 in 3 patients. Seven patients received no prior surgery. Median basal PSA was 35.9 ng/ml (8.2–463.0). Median duration of treatment with gefitinib was 98 days (5–369). A PSA levels drop (>25%) respect to baseline was observed in 2 patients, and PSA stabilization in 1 case. Median time to progression