Rhabdastrellic acid-A in HL-60 cells. Activation of downstream molecule of PI3K pathway such as AKT also was inhibited following Rhabdastrellic acid-A treatment.
Conclusion: It concludes from these results that Rhabdastrellic acid-A inhibits PI3K/Akt survival pathway and induces caspase-3-dependent apoptosis in leukemia cells.

375 POSTER

HIV-1 protease inhibitor induces growth arrest and apoptosis of human prostate cancer cells in conjunction with blockade of androgen receptor, STAT3, and AKT signaling

T. Ikezoe[1], Y. Yang[1], K. Bandobashi[1], H.P. Koeffler[2], H. Taguchi[1]. [1]*Kochi University, Hematology and Respiratory Medicine, Nankoku, Japan;* [2]*Cedars-Sinai Medical Center, Hematology and Oncology, Los Angeles, USA*

This study found that HIV-1 protease inhibitors (PIs), including nelfinavir, ritonavir, and saquinavir induced growth arrest and apoptosis of human prostate cancer cells (LNCaP, DU145 and PC-3 cells), as measured by MTT and TUNEL assay, respectively on the third day of culture. In addition, PIs blocked androgen receptor (AR) signaling in association with down-regulation of nuclear levels of AR in LNCaP cells as measured by reporter assay and Western blot analysis. As expected, PIs down-regulated the level of the AR target molecule prostate specific antigen in these cells. Moreover, PIs disrupted STAT3 signaling; PIs blocked IL-6-induced phosphorylation of STAT3 and inhibited STAT3 DNA binding activity in LNCaP and DU145 cells, as measured by Western blot analysis and ELISA-based assay, respectively. Furthermore, PIs blocked AKT signaling in prostate cancer cells as measured by kinase assay with GSK-3α /β as a substrate. Taken together, PIs inhibited proliferation of prostate cancer cells in conjunction with blockade of signaling by AR, STAT3, and AKT suggesting that this family of compounds might be useful for the treatment of individuals with prostate cancer.

376 POSTER

Tolerability results with the novel oral prenyl transferase inhibitor AZD3409 following single and multiple doses in volunteer studies

D. Smethurst, D. Sandall, A. Holt, A. Lindsay, D. Wilson, A. Hughes. *AstraZeneca, Macclesfield, UK*

Background: AZD3409 is a novel, oral, antitumour agent that acts as a prenyl transferase inhibitor. Here we report preliminary results of the tolerability assessments conducted during two studies of AZD3409 in healthy human volunteers.
Methods: In the single ascending dose study, a maximum of eight healthy male volunteers were dosed at each dose level (6 active, 2 placebo) in a randomised, double-blind, alternating panel design with doses escalated from 20 mg to 2500 mg. In the multiple dose study, a maximum of 16 volunteers (12 active, 4 placebo) were administered the same once-daily dose for 7 consecutive days at the following ascending doses for three consecutive cohorts: 500 mg, 1000 mg, and 1750 mg. The following were monitored: vital signs, ECG, clinical chemistry, haematology and urinalysis, and adverse events for 21 days after each dose. Data remain blinded and the analysis is ongoing.
Results: The maximum tolerated single dose of AZD3409 was 1750 mg, which was also tolerated on multiple dosing. In the multiple dose study, there have been no significant safety or tolerability issues identified with 500 mg, 1000 mg, or 1750 mg multiple doses. Possible drug-related adverse events include loose stools, abdominal discomfort, light-headedness, nausea, and transient rash. These adverse events were generally mild (CTC grade 1) and resolved without treatment. One subject in each of the 1000 mg and 1750 mg cohorts had loose stools graded as moderate (CTC grade 2) in the first half of the dosing week. The 'moderate severity' grading lasted no more than 1 day in both cases and both volunteers completed the full 7-day treatment schedule. The incidence, but not the severity, of gastrointestinal adverse events appears to correlate with increasing dose. No clinically important changes in clinical assessment, ECG, or routine laboratory safety data have been detected.
Conclusions: Based upon these results, 1750 mg of AZD3409 once daily for 7 days is well tolerated in healthy volunteers.

377 POSTER

Tolerability and limited activity of perifosine in patients with advanced soft tissue sarcoma (STS): a multi-center phase 2 consortium (P2C) study

H. Bailey[1], M. Mahoney[2], S. Okuno[2], D. Ettinger[3], W. Maples[4], P. Fracasso[5], C. Erlichman[2]. [1]*University of Wisconsin Comprehensive Cancer Center, Medicine, Madison, Wisconsin, USA;* [2]*Mayo Clinic, Rochester, MN, USA;* [3]*Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD, USA;* [4]*Mayo Clinic, Jacksonville, FL, USA;* [5]*Washington University School of Medicine, St. Louis, MO, USA*

Background: Current treatments in STS are largely palliative and novel agents need exploration to improve patient (pt) outcome. A prolonged (>12 mos) objective response in a refractory STS pt during a phase I trial prompted our phase II evaluation of the 6-month progression-free rate of perifosine in pts with advanced STS. The study design required 4 of 15 evaluable pts to be progression-free at 6 mos. to enroll 27 additional pts.
Methods: Pts received a perifosine loading dose of 150 mg p.o. every 6 hours $\times$ 4 for day 1, followed by 100 mg once daily for d2–28. Subsequent 28 day cycles were the same, excluding the loading dose. Eligible pts had measurable disease and adequate organ function (total bilirubin and creatinine $\leqslant$ UNL, PLT > 100,000 μL, ANC > 1,500 μL). Serum was collected for PK analyses.
Results: 23 pts were enrolled. A majority had prior treatment: 1–2 chemotherapy regimens (87%), surgery (96%), and radiotherapy (52%). 22% presented with liver metastasis. Pts are aged 24–77 yrs (median 53); 65% are female; and a majority (56%) ECOG PS 1 (vs 0). 19 of 23 pts received at least 2 cycles of therapy (range 2–8). All pts are evaluable for toxicity. One pt had Gr. 4 ileus. 6 pts (26%) had Gr. 3 toxicity, including fatigue (2 pts) and 1 patient each of anemia, infection, muscle weakness, pain, rash, anorexia, dehydration, and diarrhea. 6 pts (26%) have died, all of which are non-treatment related. A pt having myxosarcoma had a partial response lasting 5+ mos. Two (1-myxosarcoma, 1-desmoid) pts are progression-free at 6 months [15%, 95% CI (2–41%)].
Conclusions: Although this study failed to satisfy the criteria to proceed with accrual, the regimen was tolerable. The preliminary observation of another potential prolonged responder raises the question of whether specific histologies or tumor characteristics might predict a more sensitive sub-population of STS pts. PK analyses are underway. Supported by N01-CM-17104.

378 POSTER

In vivo and in vitro enhanced antitumor activity of Oxaliplatin in combination with cetuximab (C225), a chimeric monoclonal antibody anti-epidermal growth factor receptor on a panel of human colorectal tumor xenografts

D. Balin, J.P. Delord, P. Rochaix, V. Mallard, F. Thomas, I. Hennebelle, R. Bugat, P. Canal, C.B. Allal. *Institut Claudius Regaud, EA 30 35, Toulouse, France*

Advanced colorectal carcinoma is a major cause of morbidity and mortality in the developed countries. Colorectal cancers frequently express the epidermal growth factor receptor (EGFR), which has been correlated with more aggressive disease and poor prognosis. Several EGFR inhibitors such as C225 (cetuximab), a chimeric anti-EGFR monoclonal antibody, are being developed in various indications. Oxaliplatin (L-OHP), is a major compound in the treatment of colorectal carcinoma but has not been yet evaluated in preclinical studies in association with C225.These two drugs have demonstrated efficacy as single agent in the inhibition of tumor growth and apoptosis induction in colon cancer *in vivo* and *in vitro*.
The aim of our study was to evaluate the effect of the combination of C225 and L-OHP on a panel of L-OHP-insensitive colon cancer cell lines.
These studies were performed both *in vivo* and *in vitro* on 4 colon cancer cell lines HCT-8; HT-29, SW620, HCT-116 showing different levels of EGF-R expression in WB analysis. We first assessed the cell growth and IC_{50} of L-OHP, C225 monotherapy or combination of both. The combination of L-OHP and C225 led us to observe an inhibition of tumor growth and a decrease in IC_{50} of L-OHP in HCT-8, and HT-29. On the other hand, the combination of C225 and L-OHP did not show any major modification in IC50 of L-OHP in SW 620 (EGF-R negative) or in HCT-116 (EGF-R positive).
Xenografts in nude mice were established by subcutaneous injection of 10 $X10^6$ human colon cancer cells in both flanks. Mice were then randomized into four treatment groups: control, anti-EGFR (C225), L-OHP or C225 plus L-OHP. C225 was administered i.p. at the dose of 0.5 mg. three times a week. L-OHP was infused at the dose of 10 mg/kg by i.v. route 7 days after implantation.The combination of C225 (0.5 mg) and L-OHP (10 mg/kg) strongly inhibited the growth of HCT-8 and had a slight effect on HT-29 established tumors. In a refractory tumor model SW620 and HCT-116, the

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

S. Guillard, P.A. Clarke, R. te Poele, F. Di Stefano, F. Raynaud, P. Workman. *Institute of Cancer Research, Cancer Research UK Centre for Cancer Therapeutics, Sutton, Surrey, UK*

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* α (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 μM range by contrast with 10–15 μM 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 p110α and p110β. 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

V. Jendrossek[1], M. Henkel[2], J. Hennenlotter[3], U. Vogel[3], I. Mueller[1], M. Kuczyk[2], A. Stenzl[2], C. Belka[1]. [1]*University Hospital, Radiation Oncology, Tuebingen, Germany;* [2]*University Hospital, Urology, Tuebingen, Germany;* [3]*University Hospital, Pathology, Tuebingen, Germany*

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

A. Awada[1], A. Hendlisz[1], T. Gil[1], B. Schwartz[2], S. Bartholomeus[1], E. Brendel[3], D. de Valeriola[1], C. Haase[3], T. Delaunoit[1], M. Piccart[1]. [1]*Institut Jules Bordet, Medical Oncology, Brussels, Belgium;* [2]*Bayer Pharmaceuticals Corporation, New Haven, USA;* [3]*Bayer Healthcare AG, Wuperthal, Germany*

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

S.C. Pacey[1], M.J. Ratain[2], P.O. O'Dwyer[3], H. Xiong[4], A. Patnaik[5], T. Eisen[7], D. Eaton[1], R.J. Lee[6], B. Schwartz[6], I. Judson[1]. [1]*The Royal Marsden Hospital, Clinical Pharmacology Department, Sutton, UK;* [2]*University of Chicago, USA;* [3]*University of Pennsylvania, Philadelphia, USA;* [4]*MD Anderson Cancer Center, Houston, USA;* [5]*Cancer Therapy and Research Center, San Antonio, USA;* [6]*Bayer Pharmaceuticals Corporation, USA;* [7]*The Royal Marsden Hospital, Medicine, London, UK*

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.