Antiangiogenic/Antivascular Agents

B1 Results from phase 1 dose escalation study of the proteasome inhibitor NPI-0052 in patients with solid tumors and lymphomas

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Background: NPI-0052 is a novel proteasome inhibitor that produces prolonged inhibition of all three catalytic activities (C-L, T-L, CT-L) of the 20S proteasome. Preclinical data suggest NPI-0052 may demonstrate an improved therapeutic ratio, with activity in myeloma, lymphoma and solid tumor models, and has led to the initiation of clinical trials in these indications. The results of this study evaluate the endpoints of proteasome inhibition, pharmacokinetics and safety in patients with lymphomas and solid tumors at doses below the MTD.

Materials and Methods: Patients with advanced solid tumors and lymphomas without other treatment options were enrolled into this 3+3 design dose escalation study. Patients were treated with NPI-0052 administered as a weekly IV bolus for 3 weeks in 4-week cycles. The dose of NPI-0052 was escalated in cohorts of 3 patients dependent on observed adverse events. In addition to regular safety monitoring, proteasome inhibition (baseline, D1-2, D8, D15-17, D22 and D29) and pharmacokinetics (D1 and D15) were assessed in blood.

Results: Sixteen patients have been treated at doses ranging from 0.0125 mg/m² to 0.112 mg/m² for up to 6 cycles without reaching an MTD. A SAE of MRSA sepsis and renal failure occurred in one patient treated at 0.1 mg/m², however, drug related adverse events have been unremarkable at the highest dose level tested (0.112 mg/m²). Preliminary pharmacokinetic data indicate an elimination half life of approximately 3-4 minutes with clearance at 8-21 mL/min and Vz of 44-99L. Proteasome inhibition has been assayed in whole blood from patients treated in the 0.0125 mg/m² through 0.075 mg/m² dose cohorts, demonstrating inhibition of CT-L activity up to 63% and indications of a dose/response relation. No clinical responses have been confirmed, however, 4 patients have had stable disease for at least 4 months, including patients with hepatocellular carcinoma (6 months), adenoid cystic carcinoma (each at 4 months) and cervical carcinoma (7 months - investigator reported complete resolution of target lesion(s) [adenopathy] to palpation).

Conclusions: NPI-0052 appears to produce dose-dependent pharmacologic effects at doses below the MTD. Dose escalation continues to define a recommended phase 2 dose. Safety and clinical benefit will continue to be evaluated to further characterize the significance of the initial findings in larger numbers of patients in each of these different populations, inclusive of 10 patient Recommended Phase 2 Dose Cohorts.

B2 A phase 2 study of AZD2171 in recurrent or persistent ovarian, peritoneal or fallopian tube cancer. A study of the PMH, Chicago, and California Phase II Consortia. L. Vitalis, H. Hirtz, G. F. Fleming, A. Sugimoto, R. J. Morgan, J. Biagl, L. Wang, C. Dick, P. Ivy, A. M. Oza. 1. Princess Margaret Hospital, Toronto, Ontario, Canada; 2Juravinsky Cancer Centre, Hamilton, Ontario, Canada; 3University of Chicago Medical Centre, Chicago, IL; 4London Regional Cancer Centre, London, Ontario, Canada; 5City of Hope National Cancer Centre, Duarte, CA; 6Kingston Regional Cancer Centre, Toronto, Ontario, Canada; 7CTEP, NCI, Bethesda, MD.

Introduction: AZD2171 is a novel, orally-administered, highly potent inhibitor of all three vascular endothelial growth factor receptors (VEGFRs) as well as c-kit and PDGFR-α tyrosine kinase activity. VEGFR is over-expressed in human ovarian tumors and this is associated with a poor prognosis. Preclinical and early clinical data from phase I studies indicates that AZD2171 is well tolerated and shows activity against ovarian cancer.

Methods: A two-stage, multicenter phase II clinical trial is underway for women with recurrent ovarian, peritoneal or fallopion tube cancer. Eligibility criteria include patients (pts) with up to 1 prior platinum chemo regimen; ECOG performance status ≤2; normal marrow, liver and kidney function and exclude pts with uncontrolled blood pressure or any other intercurrent disease. Treatment consists of AZD2171 30 mg PO QD continuously for 4 weeks (one cycle). The primary endpoint is objective response rate, with response evaluated every 8 weeks. This study is stratified into two separate arms: platinum sensitive and platinum resistant pts.

Results: 43 pts have been recruited to date. Total number of cycles: 84 (median 3, range 1-9). Median age: 58 (range 31-79), ECOG 0-1:35 pts. 29 pts had ovarian cancer. 19 pts were platinum-sensitive and 24 pts were platinum-resistant. Reasons for off-treatment (sensitive/resistant pts) consisted in PD (2/7), adverse events (AEs) (3/6), investigator decision (1/0) and patient withdrawal (0/1). 22 pts were initially treated at 45 mg with a median (range) of dose intensity of 37. 14 pts required dose modifications and only 8 pts (36%) received the full dose as planned. Dose reduction was necessary in virtually all patients beyond cycle 2. Because of excessive toxicity of the 45 mg dose, the starting dose was reduced to 30 mg PO QD. Grade 3-4 AEs attributable to AZD2171 included hypertension (12), fatigue (6), increased transaminases (3), anorexia (2), headache (2), myocardial infarction (2), proteinuria (1), abdominal pain (1) and intracranial hemorrhage (1). Of 14 platinum sensitive pts evaluable for response, 2 pts had a confirmed PR, 7 had SD (median 6 months) and 1 pt was inadequately assessed. 4 pts on study are too early for evaluation. Of the 17 evaluable platinum resistant patients, 1 pt had an unconfirmed PR, an additional 7 pts had SD (median 4 months) and 3 pts had PD. 6 pts are too early to be assessed.

Conclusions: The results show some single agent activity with tolerable toxicity at the 30 mg PO QD dose. This trial continues to accrue to a second stage in both arms. Updated results will be presented.

B3 Effect of SPI-1620 on tumor perfusion and uptake of paclitaxel in a melanoma model. N.V. Rajeshkumar1, George Matwyshyn2, Anil Gulati1, Guru Reddy2, Luigi Lenaz3, 1Midwestern University, Downers Grove, IL; 2Spectrum Pharmaceuticals, Inc., Irvine, CA.

Background: SPI-1620 (also known as IRL-1620) is a selective ETα receptor agonist that has been reported to selectively enhance tumor perfusion and potentiate the therapeutic potential of anti-cancer agents in breast and prostate tumor bearing rats. The present study was conducted to evaluate the effect of SPI-1620 on tumor perfusion and uptake of paclitaxel in a melanoma model.

Study Design: Male nude mice were subcutaneously inoculated with one million human melanoma cells (UISO-MEL-2). Mice with tumor volume of 200-400 mm³ were selected for the study. Tumor perfusion was measured with Periflux PF2b 4000 Laser Doppler Flowmetry. Mice (n=4/group) were anesthetized using ketamine (150 mg/kg) and xylazine (2mg/kg) as a combined single i.p. injection. SPI-1620 (3 nmol/kg) or saline was injected via tail vein and perfusion was measured for 3 hours. For tumor uptake, the animals were randomly grouped (n=4/group) to receive saline or SPI-1620 (3 nmol/kg) via tail vein. Mice from each group received [3H]paclitaxel 15 minutes after SPI-1620 was administered. The concentration of [3H]paclitaxel was determined in the tumor, heart, kidneys, liver, lungs and spleen.

Results: Administration of saline did not produce any significant change in tumor blood perfusion of melanoma mice. However, administration of SPI-1620 (3 nmol/kg) increased tumor perfusion by 154, 189, 198, 172 and 94% from the baseline at 30, 60, 90, 120 and 150 minutes, respectively. Concentration of [3H]paclitaxel in tumors was significantly increased in SPI-1620 treated mice compared to saline treated mice. There was a 570% increase of [3H]paclitaxel in the tumor of the animals treated with SPI-1620 compared to vehicle treated mice. However, SPI-1620 administration did not produce significant increase in the accumulation of [3H]paclitaxel in the heart, liver, lungs and spleen compared to saline treated animals.
Conclusion: SPI-1620 significantly increased tumor blood perfusion in melanoma compared to saline. SPI-1620 significantly enhanced the uptake and delivery of paclitaxel to the tumor tissues without affecting other organs.

Based on extensive preclinical studies and safety studies in animals, Phase I clinical trials will be initiated soon.

B4 A new microtubulin inhibitor with vascular disruption activity, EPC2407, enhances the antitumor activity of antiangiogenic or chemotherapeutic agents in xenograft tumor models. Ben Tseng1, Chris May1, John Whisnant1, Stephen Anthony2, Daniel Von Hoff2, Paul Gonzales3, Bernardo Chavira3, Stephen Gately3. 1EpiCep Corp., San Diego, CA; 2TGen Clinical Research Services, Scottsdale, AZ; 3TGen Drug Development Services, Scottsdale, AZ.

EPC2407 is a small molecule new chemical entity vascular disruption agent (VDA) which also induces apoptosis. The biochemistry, biology, and mechanism of action studies have been published previously (Mol Cancer Ther. 3: 1365 and 1375, 2004). EPC2407 is currently being evaluated in a phase 1 clinical trial as a single agent to demonstrate the MTD for later phase 1 and 2 studies. EPC2407 disrupts the tumor blood flow and causes central tumor necrosis while leaving the outer rim of the tumor viable, presumably due to oxygen and nutrients from the surrounding interstitial fluids. Also, circulating endothelial cells have been reported to repopulate tumors after VDA treatment (Shaked et al, Science 313:1785, 2007). To identify a combination therapy for preventing regrowth of tumors after EPC2407 treatment, we have examined combinations of EPC2407 with anti-angiogenic agents (bevacizumab or sunitinib) or with the DNA damaging agent (cisplatin) in different cancer tumor xenograft models. Combination and single agent treatments of human lung (Calu 6 or H460) and breast cancer (MX1) xenografts in nude mice were carried out in the doses and schedules described below. Tumor growth inhibition (TGI) and the proportion of animals tumor free (TF) at 80 days post-treatment were assessed for each agent alone and for the combinations. Treatment of Calu 6 tumors with EPC2407 (20 mg/kg, d. 1-5) alone was 78% TGI and 0% TF, with cisplatin (4 mg/kg, d. 1) was 26% TGI and 0% TF whereas combination treatment, with the same doses and schedules, was 98% TGI and increased the proportion of tumor free animals to 40% TF (p<0.01). In the MX1 model, treatment with EPC2407 (40 mg/kg, d. 1-3) resulted in 5% TGI and 60% TF, with cisplatin (2 mg/kg, d. 1) was 40% TGI and 0% TF; whereas the combination treatment was 6%-TGI and 90% TF and the increase in number of tumor free animals was significant (p<0.05). Treatment with anti-angiogenic agents was carried out in H460 tumor model. Treatment of H460 tumors with EPC2407 (40 mg/kg, d. 1-3) resulted in 46% TGI, with bevacizumab (100 ug, d. 1, 14) led to 53% TGI, and the combination resulted in 80% TGI. Treatment with sunitinib (40 mg/kg, d. 1-5, 4 wk) led to 52% TGI and the combination with EPC2407 was 77% TGI. There were no tumor free animals after treatment in this model. The median survival (time to reach 1 g tumor weight or death) of the animals in the H460 model was determined. For the vehicle group, median survival was 6 days, whereas EPC2407 group was 12 days and bevacizumab group was 14 days. With the combination of bevacizumab + EPC2407, the median survival was increased to 21 days (p=0.02). With sunitinib treatment, the median survival was 13 days and the combination with EPC2407 was 16 days (p=0.17). These studies indicate that the combination of EPC2407 with antiangiogenic agents or chemotherapeutic agent cisplatin can lead to additive or synergistic activity against tumor growth.

B5 Protection against mammary tumor metastasis by inhibition of PKCε. JeeWon Kim1, Stephen H. Thorne1, Lihan Sun1, Daria Mochly-Rosen1, 2Stanford, Stanford, CA.

Important events during metastasis is breakdown of extracellular membrane, migration, adhesion and proliferation of cancer cells. Therefore, development of new pharmacological treatments inhibiting these different stage(s) of metastasis and the accompanying molecular events are greatly needed. Protein kinase C (PKC) ε had been shown to be critical in all the stages of metastasis and its over-expression makes breast cancer cells to become more invasive and aggressive. The aim of the study was to determine the effects of PKCeε on tumor metastasis in vivo. Female BalbC mice were semi-orthotopically injected with 4T1 mouse mammary cancer cells conjugated with luciferase at 6 weeks of age. After 1 week, using subcutaneous osmotic pump, the animals were systemically infused with saline, control peptide TAT and εVS-3 (0.5ml/hr, i.e. 35 mg/kg/day). Each week, tumor volumes were measured and ongoing metastasis was monitored by whole-body imaging of luciferase activity. After 4 weeks of peptide treatment, mice were sacrificed. The effects of εVS-3 on lung and whole-body metastasis, lung adhesion, metalloproteinase (MMP) activity, cell surface expression of CXCR4 (a major chemokine receptor on tumor cells that determines their site of metastasis) and beta1 integrin (known to interact with PKCeε in breast cancer cells to direct motility of cancer cells) was studied. Also, inhibition of PKC ε reduced migration and invasion of human breast cancer cells in vitro using isozyme-specific peptide inhibitors of PKC ε and ε. We found εVS-3 treatment reduced tumor metastasis after 4 weeks of treatment (p<0.05, n=9-10, each). Furthermore, εVS-3 decreased lung adhesion of tumor cells (n=9-10, each), indicating that εVS-3 inhibits adhesion step of the metastasis. εVS-3 also decreased MMP2 activity (p<0.05) and CXCR4 expression (p<0.05), indicating its anti-metastatic activity against metastatic extracellular membrane breakdown and migration to distant organs. Furthermore, surface expression of integrin β1 was inversely correlated with the degree of metastasis and had a tendency to decrease with εVS-3 treatment. These findings suggest that εVS-3 provides protection against metastasis in the stages of adhesion, extracellular membrane breakdown and migration and may provide novel targets for therapy. Furthermore, εVS-3 normalized the levels of liver AST/ALT enzyme (markers of liver damage or toxicity) in the serum compared to untreated animals, indicating that the dose used was non-toxic and even protective against liver damage.

B6 Assessing variation in response to anti-VEGFR TKI AV-951 across a population of genetically defined tumor populations to dissect phenotypic and genetic context of sensitivity and resistance. Jie Lin1, Karupiah Kannan1, Joerg Heyer1, Hongmei Yang1, Nan Liu1, Gaoyuan Meng1, Yinghui Zhou1, William M Rideout III1, Angela Bressel1, Tong Zhi1, Sireeshayalavarthi1, Roujie Wang1, Murray O. Robinson1, 2AVEO Pharmaceuticals Inc., Cambridge, MA.

Clinical development of antiangiogenic therapies of cancer have been largely empirical, resulting in high failure rates, long time lines and significant exposure of patients to inappropriate or ineffective therapies. The observed variation in clinical antiangiogenic drug response across tumor types is likely underpinned by extensive and complex genetic variation across tumor types, subtypes and individuals. Traditional preclinical models of human xenografts have been of limited predictive utility in that successful inhibition of a particular xenograft tumor provides little insight into which clinical population is more likely to respond to treatment. In addition, extensive variation in genetic background across different cell lines, the lack of sufficient numbers of well characterized cell lines and the inappropriate cell culture context remain as significant hurdles for the effort to dissect genetic context of response and discovery of biomarkers.

To address these challenges, we have created an alternate approach for preclinical antiangiogenic response prediction and biomarker discovery. Using novel ES cell based chimeric approach, a large population of more than 100 genetically engineered murine breast tumors were generated, in which defined genetic alterations, i.e., HER2 overexpression & INK4A-/-, are allowed to be combined with spontaneous genetic alterations to more accurately model human cancer populations. Characterization of this tumor population revealed a surprising degree of variation in key parameters such as angiogenesis, microvascularization, VEGF expression, microarray gene expression and chromosomal gain/loss by array CGH.

Having developed methodology to expand and archive these tumors, we have now begun testing various antiangiogenic agents including AV-
B7 A phase I study of the safety, tolerability and antitumor activity of escalating doses of combretastatin A4 phosphate (CA4P) given in combination with bevacizumab to subjects with advanced solid tumors. Paul Nathan1, Ian Judson2, Anwar Padhani3, Adrian Harris4, Rajesh Sinha2, Boxall Jane1, Jon Smythe5, Nita Fisher6, Jane Taylor7, David Collins8, Hillori Connors9, Rustin Gordon1. 1Mount Vernon Cancer Centre, Middlesex, United Kingdom; 2Royal Marsden Hospital, Sutton, United Kingdom; 3Paul Strickland Scanner Centre, Middlesex, United Kingdom; 4John Radcliffe Hospital, Oxford, United Kingdom; 5National Blood Transfusion Service, Oxford, United Kingdom; 6Oxigene Inc., Boston, MA.

Background: The vascular disruptive agent (VDA) CA4P induces significant tumor necrosis as a single agent. Vascular shut down is reversible and tumors can re-grow due to vascularisation of the surviving tumor rim. Pre-clinical models have demonstrated that the addition of an anti-VEGF antibody to a VDA significantly increases anti-tumor activity, possibly by inhibiting neo-vascularisation of the surviving rim. The purpose of this first clinical study combining a VDA with an anti-angiogenic drug was to establish the safety of the CA4P/bevacizumab combination and to investigate potential mechanisms of synergy.

Methods: Patients with advanced solid malignancies received CA4P at 45mg/m2 (cohort 1), 54mg/m2 (cohort 2) or 63mg/m2 (cohort 3) every 14 days in combination with bevacizumab at 10mg/kg. Principal inclusion criteria included no significant history of ischaemic heart disease, PS ECOG 0/1 and no prior exposure to agents acting on the VEGF pathway. Pharmacokinetics, toxicity and anti-tumor responses were assessed. Functional imaging with DCE-MRI was performed at baseline, after CA4P alone and after CA4P + bevacizumab in the 1st cycle. Circulating endothelial cells (CECs) and progenitors (CEPs) were enumerated and serum/plasma biomarkers measured.

Results: At the time of abstract submission, cohorts 1 and 2 were complete (n=6). No dose limiting (grade 3/4) toxicities were seen. Most common grade 1/2 toxicities were headache, hypertension, pruritis, and pyrexia. Asymptomatic transient self-limiting ECG changes were seen in 2 patients with no evidence of myocardial damage. DCE-MRI showed statistically significant reductions in tumor perfusion/vascular permeability (36% group reductions in Ktrans) in 5 out of 7 evaluable patients (2/7 patients with 100% reduction) which reversed after CA4P alone but which were sustained following bevacizumab. CECs, CEPs, VEGF and SDF-1 all increased following CA4P administration. 3/6 evaluable patients experienced stable disease (1 >2 months and 2 >4 months ongoing). Tumor markers in three patients improved.

Conclusion: CA4P in combination with bevacizumab appears safe and well tolerated in this dosing schedule with evidence of early clinical activity. CA4P induced profound changes in tumor perfusion/permeability which were maintained by the presence of bevacizumab. CA4P induces an acute rise in CECs and CEPs. This therapeutic combination warrants further investigation.

B9 Physiological changes induced by combretastatin and their significance for the therapeutic application. Michael R. Horsman1, Anja B. Schmidt1, Thomas Nielsen1, Marianne Skals2, Tobias Wang2, Morten Busk1. 1Dept. Experimental Clinical Oncology, Aarhus University Hospital, Aarhus C, Denmark; 2Dept. Zoophysiology, Aarhus University, Aarhus C, Denmark.

Introduction: Combretastatin A-4 disodium phosphate (CA4DP) can induce physiological changes which may not only lead to unwanted side-effects, but may also influence its anti-tumor efficacy, especially when combined with conventional therapies. The aim of this pre-clinical mouse study was to investigate the physiological changes induced by CA4DP and how these influenced radiation therapy.

Material and Methods: Male and female CDF1 mice, either non-tumor bearing or with a 200 cubic mm C3H mammary carcinoma growing in the right rear foot, were used in all experiments. CA4DP was dissolved in saline and injected intraperitoneally (i.p.) at 0.02 ml/kg mouse body weight. Blood samples (100 ul) were collected from the sub-orbital sinus of individual non-tumor bearing mice and analyzed on an ABL300 Acid-Base Laboratory and OSM3 Hexometer. Mean arterial blood pressure (MABP) was measured in similar animals following cannulation of the carotid artery. After cannulation the animals were allowed to recover before being transferred to special restraining jigs that enabled measurements on non-anesthetized animals; MABP being continuously monitored by connecting the cannula to a pressure transducer. Tumor bearing animals were also restrained in jigs and the tumors locally irradiated (240 kV x-rays) with single graded radiation doses. The percentage of mice in each treatment group showing local control at 90 days was recorded and the TCD50 values (radiation dose producing local control in 50% of treated animals) estimated from full radiation dose response curves. Statistical analysis was performed with a Chi-squared test (TCD50) or Student’s t-test (Blood analysis and MABP), with the significance level being p<0.05.

Results: For the blood analysis the most obvious change was in hematocrit. In controls the mean (+/- 1 S.E.) hematocrit was 48.3% (48.1- 48.5). This was significantly increased to 54.5% (53.8-55.2) within 1 hour after injecting 25 mg/kg CA4DP, and remained elevated for several hours before returning to normal by 8 hours. Similar peak levels of 54.7% (54.5-54.9) and 54.8% (54.7-54.9) were seen with doses of 100 and 250 mg/kg, respectively, with full recovery by 8 (100 mg/kg) or 12 (250 mg/kg) hours. CA4DP induced changes in MABP were very similar. Control MABP was 110 mmHg (109-111), and this was significantly increased to 123 mmHg (116-130), 127 mmHg (122-132), and 128 mmHg (121-136) within 1-hour after injecting 25, 100 and 250 mg/kg CA4DP, respectively. Full recovery was seen within 4 hours at the 25 and 100 mg/kg doses, and by 6 hours at the 250 mg/kg dose. The increase with 100 mg/kg could be eliminated by simultaneously injecting hydralazine (HDZ; 0.2 mg/kg; i.p.). The TCD50 value (with 95% confidence interval) for tumors given radiation alone was 53 Gy (51-55). Irradiating tumors and then injecting CA4DP 30 minutes later enhanced the tumor radiation response, with respective TCD50 values of 48 Gy (45-51), 50 Gy (46-54), and 46 Gy (42-50), obtained with 25, 100 and 250 mg/kg; these increases were significant except for the 100 mg/kg dose, and the TCD50 value with this dose was unaltered by HDZ.

Conclusions: CA4DP significantly altered hematocrit and MABP, but these changes were not dose dependent although the recovery may have been. These changes did not appear to influence the tumor response to the combination of CA4DP with radiation.
B10 Determination of a 35 gene signature predictive for the effectiveness of Bevacizumab

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Bevacizumab (BV), an antibody against the human VEGF, was recently approved in combination regimens for metastatic colorectal, adenocarcinoma and large cell lung as well as breast cancer patients. Activity was also demonstrated in renal cancers. The costs of this new targeted therapy are high and also side effects have been observed. The FDA strongly recommends developing companion diagnostic tests for targeted drugs. Developing such predictive tests in the clinic is difficult for BV since it is not only borderline active as single agent (remission rates 30 patient derived solid tumor models growing sc in nude mice. For 220 tumors gene expression profiles of ~38,500 human genes were determined using the Affymetrix HG-U133 plus 2.0 mRNA expression array. In a previous study we have demonstrated a high correct prediction of in vivo xenograft testings for cytotoxic standard agents when the 80 responses in the donor patients were compared to the response in the nude mouse and in a limited number for BV and Cetuximab.

We investigated the hypothesis that correlating drug response against BV with gene expression would identify a gene signature that predict the response of individual tumors. BV was investigated in 72 established cancer xenografts originating from colon (18), NSCLC (34), breast (10) and renal (10) cancer patients. When the tumors reached about 100 mm³ BV was applied as single agent iv 40 mg/kg/d on day 0, and 20 mg/kg/d on day 7 and 14, respectively. Antitumor activity was evaluated at the minimum T/C value or after 28 days. Using a T/C of <34% as a cut-off for activity 29,2% (21/72) of all tumors were sensitive, 12/34 NSCLC, 4/18 colon, 3/10 breast and 2/10 renal cancers. However, most of the tumors grew progressively and doubled the initial tumor volume within 2-3 weeks. A remission was found only in 1 NSCLC. No lethalties or body weight losses were observed. The 72 test results were randomly divided into a training and a testing set (47 and 25 tumors). The obtained signature was validated using the leave-one-out cross-validation method (LOOCV) and on an independent testing set. The bio-informatic analysis yielded an optimal gene signature consisting of 35 genes. In the LOOCV 15 tumors out of 47 were predicted to respond from which in the real testings 10 responded. Therefore the response rate increased from 30% (14/47) to 67%, a 2.2 fold increase. Conversely, from the predicted 32 non-responders 28 (88%) were resistant in real testings.

In the independent testing set the correct prediction was 71% for the predicted responders and 89% for the non-responders. This gene signature gave very promising predictions compared to 8 proteins associated with angiogenesis which we have also investigated in this study. The 35 genes found belong to different classes. Remarkably, 21 are associated at least indirectly with angiogenesis in the literature. In addition Oncotest has now developed predictive gene signatures for 11 cytotoxic drugs (Cancer Genomics & Proteomics 4:187-196 & 197-210 2007) and for Cetuximab. 1600 genes from which ~900 are predictive for these compounds are spotted on a customized gene expression array which is ISO17025 certified.

This Oncotest proprietary customized Chip is now being evaluated in a pilot clinical study to predict and compare the response of heavily pretreated patient tumors.

B11 Interim results from a phase I trial of the vascular disrupting agent OXi4503.

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Background: OXi4500, the active product of OXi4503, is a vascular disrupting agent acting at the colchicine binding site on the β subunit of tubulin. A phase I trial has commenced, which includes molecular imaging to determine the mechanism of action at an early stage.

Methods: OXi4503 was administered by 3 weekly IV infusions to a maximum of 6 cycles. Starting dose was 0.06mg/m². 100% dose escalation in single patient cohorts proceeded to 3.84mg/m², when cohorts were expanded to three patients and 30% escalation due to emergence of grade 2 drug-related toxicity (thrombocytopenia).

Functional MRI or PET imaging with [18F]fluorodeoxyglucose (FDG) and 150-labelledd water (H2O) were performed prior to and after the first infusion of OXi4503.

Results: 19 patients have so far received OXi4503 (median age 53y). Common adverse events include short-lived pyrexia, lethargy and hypertension. Pharmacokinetic profiles show a dose-dependent linear increase in peak plasma concentrations and AUC of both OXi4503 and OXi4500. OXi4500 concentrations at 6.5mg/m² are close to that seen at the MTD in animals. DCE-MRI showed significant reduction in Ktrans in one patient. Changes in standard uptake values were observed in all four patients studied with FDG-PET at 28 days. H2O-PET showed that the distribution volume of water was reduced in 3 out of the 5 metastases in one of the three patients studied. Of the 16 patients fully assessed, 2 have stable disease and 14 disease progression.

Conclusions: OXi4503 has been tolerated at dose up to 8.5mg/m² with no dose limiting toxicity. MTD has not been reached and recruitment is ongoing. Molecular imaging studies suggest activity of the compound.

B12 The effect of vandetanib, a VEGFR-2 and EGFR tyrosine kinase inhibitor, in an orthotopic nude mouse model of human adeno cystic carcinoma.

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Purpose: Adenoid cystic carcinoma (ACC) is one of the most common types of salivary gland cancers, and it is characterized by local recurrence, hematogenous metastasis and poor response to conventional chemotherapy. Despite recent progress in molecular medicine, the disease-specific 10 years specific survival rate for patients with ACC remains 20-40%. A better understanding of the molecular mechanisms involved in ACC carcinogenesis and tumor progression is required for the development of new and effective therapies in ACC. We hypothesized that the epidermal growth factor receptor (EGFR) and vascular endothelial growth factor receptor-2 (VEGFR-2) signaling pathways may be important for the progression and metastasis of human salivary ACC, and inhibition of these pathways may be beneficial as part of an integrated treatment strategy for this tumor.

In this study, we evaluated the therapeutic effect of vandetanib (ZD6474, ZACTIMA™), a potent inhibitor of VEGFR-2 and EGFR tyrosine kinases, alone and in combination with paclitaxel in an orthotopic nude mouse model of human adenoid cystic carcinoma.

Experimental design: The in vitro effects of vandetanib on cell growth, apoptosis and VEGFR-2 and EGFR phosphorylation levels were examined in ACC cells. The in vivo anti-tumor activity of vandetanib was examined in an orthotopic nude mouse model of ACC. Nude mice bearing parotid gland tumors were established by intragland injection of ACC cells. These mice were treated for 4 weeks with vandetanib (50mg/kg, once per day), paclitaxel (200ug, once a week), vandetanib (50mg/kg, once per day) + paclitaxel (200ug, once a week), or placebo.

Results: In vitro, vandetanib treatment of ACC cells resulted in a dose-dependent inhibition of phosphorylation of both VEGFR-2 and EGFR, as well as phosphorylation of MAPK and AKT. Vandetanib also inhibited the proliferation of ACC cells and induced dose-dependent apoptosis of ACC cells. In animals receiving vandetanib alone or vandetanib + paclitaxel in combination for 4 weeks, tumor volumes were decreased significantly compared with those in the time-matched placebo control group (p=0.009, and p=0.006 respectively).

Conclusion: These results suggest that vandetanib inhibits the growth
of ACC cells in vitro and in vivo, making it a promising novel agent for the treatment of ACC. Based on these data, vandetanib warrants further preclinical evaluation for salivary gland cancers.

B13 A Phase Iib study of telatinib (BAY 57-9352), a VEGF-R2 inhibitor, in combination with docetaxel in patients with advanced solid tumors. Heather M. Shaw1, Rhoda Molife1, Vasilios Karavasilis1, Teng J. Ong2, Laura Tate2, Peter C. Fong1, Tim A. Yap1, Rohit Lal1, Catherine Ludwig2, Erich Brendel3, Olaf Christensen4, Johann S. de Bono1. 1Royal Marsden Hospital, Sutton, United Kingdom; 2Bayer Schering Pharma, Newbury, United Kingdom; 3Bayer HealthCare AG, Wuppertal, Germany; 4Bayer Pharmaceuticals Corporation, New Haven, CT.

Background: Telatinib (BAY 57-9352) is an oral, multitargeted tyrosine kinase inhibitor of VEGF-R-2, PDGFR-β and c-Kit receptors, with IC50 (nM) of 6, 4, 15 and 1 respectively. This phase I study assesses the safety, tolerability and pharmacokinetics (PK) of telatinib in combination with docetaxel (D).

Methods: Patients (pts) with advanced solid tumors refractory to standard treatment, with adequate renal, hepatic, and bone marrow function, and ECOG PS ≤1 were eligible. Following informed consent pts were treated with D 75mg/m2 IV on day (d) 1 of q 21d for 6 cycles if tolerated. Telatinib was administered twice daily (bid) continuously from d3 of cycle 1 until tumor progression or intolerable toxicity. Dosing of telatinib was escalated from 600mg bid (cohort 1, n=6) to 900mg bid (cohort 2, n=8). Pharmacokinetic (PK) sampling was performed on d1 and d21 of cycle1, and d1 and cycle 2. The primary objectives were to determine safety, tolerability, and the maximum tolerated dose (MTD) for the combination. The secondary objectives were to evaluate biomarkers, PK, and tumor response.

Results: 14 patients commenced D and telatinib and were evaluated for safety (M/F 9/5, median age 56 years (range 34-75), ECOG PS 0/1/2/1 4/10). Study-treatment-related adverse reactions leading to a dose reduction or interruption in cycle 1 were transaminitis (n=1, 7%), fatigue (n=1, 7%), and neutropenia (n=2, 14%). In cycle 2 or beyond these were neutropenia (n=2, 14%), and hypertension, nausea, fatigue, pneumonitis and dizziness (each n=1, 7%). Overall, treatment-emergent adverse events of CTC grade ≥3 (as defined by NCI-CTC v3.0) occurring in ≥10% of subjects were: neutropenia (n=11, 79%); fatigue (n=3, 21%); and GI disturbances (n=4, 29%). 12 pts completed ≥1 treatment cycles and were evaluated for efficacy. Out of these, 9 (75%) achieved at least stable disease (SD) at 12 weeks; 2 pts (14%) remain on the study, with partial response (PR) (cervical cancer) and SD (renal cancer) lasting >6 months after enrollment. Biomarkers are currently being evaluated. PKs showed that the concomitant administration of telatinib and D resulted in slight to moderate decreases in AUC(0-12) and Cmax of both telatinib and its metabolite, BAY 60-8246, while no consistent changes of AUC and Cmax of D were observed.

Conclusions: The combination of D with telatinib was generally tolerated without reaching the toxic dose at D 75mg/m2 and telatinib 900mg bid. The frequency of neutropenia was not increased compared to historical data for treatment with D alone. PK data did not reveal an increased exposure to D upon co-administration with telatinib. The combination treatment demonstrated promising anti-tumor activity with a 75% disease control rate, with 2 patients ongoing in treatment after 10 months.


Background: NPI-2358 is a novel tumor vascular disrupting agent (VDA) acting on β-tubulin that destabilizes tumor vascular endothelial cells with an additional direct cytotoxic activity. NPI-2358 selectively induces tumor vascular collapse and tumor regression in multiple murine tumor models and also potentiates the effects of chemotherapeutic agents and radiation. Preclinical data suggest NPI-2358 may have advantages in terms of safety profile and activity (tumor blood flow remains markedly reduced after 24 hours).

Methods: A Phase 1 study of NPI-2358 is being conducted in patients with advanced solid tumors and lymphomas. Patients enrolled were treated with a weekly IV infusion of NPI-2358 for 3 weeks in 4-week cycles. Seventeen subjects have been enrolled. The study uses a dynamic accelerated dose titration design in which the dose of NPI-2358 is escalated in cohorts of patients dependent on observed adverse events. Any cohort may consist of 1 patient provided no Grade 2 AE is reported in the prior cohort. If a ≥Grade 2 AE is reported in the prior cohort, the cohort consists of at least 3 patients. A cohort is expanded to 6 patients if a DLT is reported. Escalation was initially conducted in 100% intervals and decreased to 50% intervals once a Grade 2 adverse event was reported. In addition to weekly safety monitoring (including ECGs, Troponin I and blood pressure), echocardiography and pharmacokinetics were performed on Days 1 and 15 (D1 & D15) and a DCE-MRI obtained 4 hours after the first dose was compared to baseline. Additional DCE-MRIs may also be performed on D2 and every 2 cycles.

Results: The dose has been escalated from 2 mg/m² to 20 mg/m² (predicted minimum efficacious dose = 7.5 mg/m²). One DLT of pulmonary embolus occurred at 13.5 mg/m². Nausea, tumor pain and transient elevations in blood pressure have been reported. Significant toxicities likely to be related to drug, including cardiac (assessed by echocardiography, ECG and Troponin I) or neurotoxicity, have not been reported. Intrapatient values for Kranz from DCE-MRIs have been highly reproducible and a consistent 12-23% decrease in Kranz was seen in all patients evaluated at 13.5 mg/m². No responses have been reported, however, three patients had stable disease (SD) for two or more cycles: pancreatic adenocarcinoma (4 cycles), colorectal carcinoma (2 cycles) and squamous cell (2 cycles). Average Cmax and AUCinf have increased from 34 to 223 ng/ml and from 101 to 1159 ng/ml*h respectively. No drug accumulation was observed; for all patients T1/2 was 5.3 ± 2.98 h, CL was 40.7 ± 30L/h and distributive volume was 229 ± 57 L.

Conclusions: NPI-2358 is well tolerated at doses above the minimum predicted efficacious dose. Systemic exposure assessed by Cmax and AUC increased with dose escalation and no drug accumulation was evident with weekly administration. Pharmacodynamic measures (DCE-MRI) suggest effects of the drug on tumor blood flow at current doses.
B15 Phase I dose-escalation study of the anti-VEGFR-2 recombinant human IgG1 MAb IMC-1121B administered every other week (q2w) or every 3 weeks (q3w) in patients (pts) with advanced cancers. 

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Background: VEGFR-2 is a key regulator of angiogenesis and cancer cell growth. IMC-1121B is a recombinant human IgG1, MAb targeted to VEGFR-2. Anti-VEGFR-2 antibodies inhibit the action of VEGF in a variety of preclinical leukemia and solid tumor models.

Methods: Five dose-escalating cohorts of 3-6 patients (ECOG PS ≤ 2) with advanced cancer and no significant cardiovascular, thorobitic or bleeding disorders received IMC-1121B q2w (6, 8, and 10 mg/kg) or q3w (15 and 20 mg/kg). Patients in the q2w cohorts received three doses of IMC-1121B followed by a 2-week observation period in the initial treatment cycle. The q3w cohorts were treated without a 2-week observation period. All patients in all cohorts were performed every 6 weeks. DLT was determined during the initial treatment cycle for Cohorts 1-3, and the first 2 cycles for Cohorts 4 and 5. Patients with an objective response or stable disease (SD) received additional 4-week treatment cycles until PD or withdrawal. Anti-IMC-1121B antibodies and PK analyses were assessed with the initial and final infusions of each treatment cycle.

Results: To date, a total of 14 pts (9 M; 5 F), have entered the study at the first 4 dose levels: Cohort 1 (n=4), Cohort 2 (n=5), Cohort 3 (n=4), and Cohort 4 (n=1). All patients in Cohorts 3 and 4 are presently receiving study drug. Enrollment continues in the q3w cohorts. Adverse events ≥ grade 3 considered to be at least possibly-related to IMC-1121B were reported for two pts: one pt (10 mg/kg q2w) with Grade 3 hypertension during Cycle 2; and one pt (8 mg/kg q2w) with prior history of peptic ulcer disease reported a Grade 4 duodenal ulcer hemorrhage during Cycle 2, however the event resolved after drug discontinuation. No DLT occurred in the first 3 cohorts. Preliminary efficacy data reveal 4 pts have stable disease of ≥ 3 months duration with cancers of the colon (1 pt), ovary (1 pt), liver (1 pt), and kidney (1 pt). No anti-IMC-1121B antibodies have been detected in cohorts 1-3. Preliminary antitumor activity data reveal 4 pts have stable disease of ≥ 3 months duration with cancers of the colon (1 pt), ovary (1 pt), liver (1 pt), and kidney (1 pt). Anti-IMC-1121B antibodies and PK analyses were assessed with the initial and final infusions of each treatment cycle.

Conclusion: We obtained a set of 247 genes that efficiently clustered the 17 patients according to therapeutic benefit status. The intersection of the 17 gene sets corresponded only to 4 genes (NM_172229, NM_003129, NM_006403, and AI612529). Interestingly, the expression of these 4 genes was predictive of therapeutic benefit. The gene expression of the 7 known sorafenib targets was not modified. However, the biologic pathway analysis among the 247 genes set revealed that 15 genes were related directly to sorafenib biologic activity: RAP1 pathway (8 genes); KDR pathway (4 genes), KIT pathway (2 genes), and PDGFR β pathway (1 gene).

Conclusions: In this study, we showed the potential value of expression analysis in a phase I trial combining sorafenib and dacarbazine.

Despite the high heterogeneity of tumors and the limited number of patients, we demonstrated that gene expression profiling of sequentially-obtained biopsies is a powerful approach to discover putative biomarkers in early clinical trials. The biomarkers discovered in this study still require validation. Nevertheless, it appears that they may be useful to predict therapeutic benefit at the end of the first cycle of the sorafenib and dacarbazine combination. This study also confirmed the feasibility of the genomic strategy based on sequential biopsies in terms of sample size, RNA extraction, and early detection of gene differentially expressed in response to chemotherapy.

B17 Synergistic antitumor activity of Bevacizumab in combination with HIF-1 inhibition. 

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Antiangiogenic strategies have limited clinical activity as single agents. Bevacizumab, an anti-VEGF antibody, can have different effects on tumor vasculature either stabilizing tumor vessels, therefore improving delivery of chemotherapy or decreasing tumor vascularization, thus increasing intra-tumor hypoxia. Hypoxia is associated with induction of HIF-1 and selection of therapy resistant, more invasive and metastatic clones. Topotecan inhibits HIF-1α expression and tumor growth in U251-HRE xenografts. To assess whether the addition of a HIF-1 inhibitor increased the therapeutic efficacy of Bevacizumab, we tested the effect of daily low dose topotecan (0.5mg/kg x 10) alone or in combination with bevacizumab (50-100 µg/mouse Q2wk) on tumor growth, HIF-1α expression and angiogenesis in U251-HRE xenografts. Bevacizumab alone increased intra-tumor hypoxia, measured by pimonidazole staining. HIF-1α protein accumulation and HIF-1 dependent gene expression in tumor tissues. Addition of topotecan to Bevacizumab inhibited HIF-1α protein accumulation and HIF-1 dependent gene expression, consistent with the inhibition of the compensatory hypoxic responses induced by Bevacizumab. Furthermore, microvessel density was significantly decreased in mice treated with Bevacizumab alone or in combination with topotecan. Notably, Topotecan or Bevacizumab alone decreased tumor weight by 34% and 40%, respectively; however the combination of Topotecan and Bevacizumab...
decreased tumor weight by 82% relative to vehicle treated controls. In addition, GADD45 mRNA expression (a gene induced in response to DNA damage) was increased in animals treated with the combination TPT-Bevacizumab.

These results suggest that the combination of topotecan with bevacizumab may have synergistic antitumor and anti-angiogenic activities and is a therapeutic strategy that warrants further clinical investigation. Funded by NC1 Contract N01-C0-12400.

B18 Plasma biomarkers in a phase II trial of sorafenib in advanced non-small cell lung cancer. Carol Peña1, Ulrich Gatzeimeier2, Chetan Latia1, Frank Rossi2, Martin Reck2, James Elting1, George R. Blumenschein1. 1Bayer HealthCare Pharmaceuticals, West Haven, CT; 2Thoracic Oncology, Hospital Grosshansdorf, Grosshansdorf, Germany; 3University of Texas, MD Anderson Cancer Center, Houston, TX.

Background: Sorafenib is a potent inhibitor of the serine/threonine kinases c-Raf and B-Raf and the angiogenic receptor tyrosine kinases (VEGFR-1, -2 and -3 and PDGFR-β) with activity against both tumor cell proliferation and tumor angiogenesis. Sorafenib has shown antitumor activity in several tumor types and is currently under clinical evaluation in several solid tumors, including non-small cell lung cancer (NSCLC). Here, we investigated several biomarker candidates in NSCLC patients to determine their relationship with clinical endpoints.

Methods: 52 patients with relapsed or refractory, advanced NSCLC were enrolled in a phase II, single-arm, multicenter study, and received sorafenib 400 mg orally bid, taken orally on a continuous schedule. Plasma samples were collected at baseline (BL), cycle1/day15 (C1D15), and C3D15 and assayed for total VEGF, VEGF-165, soluble VEGFR-2 (sVEGFR-2), PDGF-BB, soluble PDGFR-beta (sPDGFR-β), soluble EGFR (sEGFR), soluble HER-2 (sHER-2), UPA, PAI-1, uPAR, TIMP-1, and circulating Ras p21.

Results: Sorafenib treatment resulted in significant changes in the levels of VEGF-165, sVEGFR-2, PDGFB, PDGFR-β, sEGFR, PAI-1, uPAR, and TIMP-1. The mean level of sVEGFR-2 decreased by 17% at C1D15 and 27% at C3D15, as compared with baseline (n=25, p<0.001; n=24, p<0.001, respectively), consistent with previous results from a phase III trial of Sorafenib in RCC (Bukowski et al., ASCO 2007). sVEGFR-2 levels decreased in 34/35 patients at C1D15, and 24/24 patients at C3D15. At C1D15, significant decreases in mean levels of PDGFB (7.3%, n=32), sPDGFR-β (7.4%, n=35), TIMP-1 (28.9%, n=35), and uPAR (9.8%, n=32), and an increase in sEGFR (11.2%, n=35), were observed. PDGFB (43.1%, n=21) and TIMP-1 (22.4%, n=24) remained decreased at C3D15, and this timepoint also showed a decrease of PAI-1 (27.2%, n=21). High levels of plasma VEGF, VEGF-165, PDGFB, Ras, and TIMP-1 at baseline correlated with poor outcome (short time to death, TTD and/or progression free survival, PFS) compared with low levels. An increase in VEGF, VEGF-165, PDGFB, or TIMP-1 at C1D15/ C3D15 correlated with better outcome (long TTD and/or PFS). Conversely, an increase in Ras at C1D15 correlated with short PFS.

Conclusion: Baseline level and/or pharmacodynamics of VEGF, VEGF-165, PDGFB, Ras, and TIMP-1 correlated significantly and consistently with clinical outcome of NSCLC patients treated with sorafenib. These results are promising and further biomarker investigation in larger, placebo-controlled studies is warranted to confirm the observed correlations and determine their clinical utility.

B19 A phase I study of the recombinant human IGF1 anti-IGF-IR monoclonal antibody (Mab) IMC-A12, administered on a weekly basis to patients with advanced solid tumors: Interim analysis. Celestia Higano1, Patricia LoRusso2, Michael Gordon3, Evan Y. Yu1, Samuel H. Whiting1, Floyd Fox4, Terry Katz5, Eric Rovinsky6, Hagop Youssoufian6. 1University of Washington, Seattle, WA; 2Wayne State, Detroit, MI; 3Arizona Cancer Center, Tucson, AZ; 45mClone Systems Incorporated, New York, NY.

Background: The insulin-like growth factor-I receptor (IGF-IR) plays a critical role in tumor cell proliferation and survival, and is overexpressed in multiple tumor types. IMC-A12 is a recombinant human Mab of the immunoglobulin G, subclass 1, which targets the IGF-IR with high affinity. In preclinical studies, IMC-A12 has been shown to inhibit the growth of multiple tumor types in vivo and in vitro.

Methods: This multicenter, first-in-human trial enrolled patients age = 18 with advanced refractory or untreatable solid tumors, ECOG PS ≤ 2, and measurable disease. All patients received intravenous IMC-A12 once a week for 4 weeks, with a 2-week observation period following the first 4 week cycle only. The starting dose for the first cohort was 3 mg/kg, dose escalation in subsequent cohorts was to be 6 mg/kg, 10 mg/kg, and 15 mg/kg. Radiological evaluation of tumor response was performed following the 2 week observation period after the first treatment cycle, and every 8 weeks thereafter until documentation of progressive disease (PD), withdrawal of consent, or toxicity necessitating study cessation. Blood samples for pharmacokinetic and serum pharmacodynamic marker analyses and evaluation of any antibodies against IMC-A12 were drawn throughout the study.

Results: A total of 24 patients were enrolled (14/nm11f), including seven, nine, six, and two in the 3 mg/kg, 6 mg/kg, 10 mg/kg, and 15 mg/kg dose groups, respectively. Eleven patients (46%) had a best overall response of stable disease (SD), including five of nine (56%) treated at 6 mg/kg. The patients with stable disease included a heavily pretreated male patient with breast cancer (SD of approximately 9 months), a patient with hepatocellular carcinoma (SD approximately 10 months), and a patient with pheochromocytoma (SD ≥ 7 months). A total of 18 patients (75%) experienced at least one adverse event (AE) thought to be possibly, probably, or definitely related to IMC-A12; the most common of these was hyperglycemia, which affected a total of four patients (17%), was grade ≥ 3 in two patients, and resulted in study discontinuation in one patient in the 3 mg/kg cohort and one patient in the 10 mg/kg cohort. Infusions of 6 mg/kg and above produced IMC-A12 serum concentrations at or above those associated with activity in preclinical models (trough concentration of approximately 60 to 158 μg/mL). Pharmacodynamic analysis showed that IGF-I levels increased following infusion and remained elevated in the presence of IMC-A12. No evidence of anti-IMC-A12 antibody development has been detected in clinical study to date.

Conclusions: In this phase I study of IMC-A12 in patients with advanced, treatment-refractory solid tumors, disease stabilization was observed in nearly half of enrolled patients, with several patients experiencing stable disease ≥ 6 months. Treatment with IMC-A12 produced sustained elevation in IGF-I levels, suggesting successful blockade of the IGF-IR. Dose escalation has been completed for this trial. Because target IMC-A12 serum concentrations have been met or exceeded at a weekly dose of 6 mg/kg, and because IMC-A12 was well-tolerated at this dose level, 6 mg/kg has been selected as the recommended dose for phase II study of weekly IMC-A12.

B20 [F-18]-Fluoromisonidazole (FMISO) PET demonstrates reduced hypoxia in recurrent malignant gliomas that respond to bevacizumab and irinotecan. Alexander M. Spence1, Mark Muzi1, Maciej M. Mrugala1, Tom C.H. Adamsen1, Jeanne M. Link1, Kenneth A. Krohn1. 1University of Washington, Seattle, WA.

Background: Hypoxia is associated with resistance to radiotherapy and chemotherapy in malignant tumors including gliomas. Due to the extremely low retention in normal brain, FMISO is an effective quantitative imaging agent for hypoxia in brain tumors. We report our preliminary experience measuring the change in hypoxia in recurrent malignant gliomas with FMISO PET before and after one cycle of the antiangiogenic antibody bevacizumab, plus irinotecan. Bevacizumab is a humanized anti-VEGF-A antibody that has direct antiproliferative effects on endothelial cells. Its actions may also include normalization of delivery of nutrients and therapeutic agents.

Materials and Methods: Four patients were studied at the point of recurrence following conventional surgery, radiotherapy and chemotherapy with temozolomide. Three had anaplastic astrocytoma and one had glioblastoma multiforme. Recurrence was documented based on MRI and clinical criteria. Each patient had FMISO PET imaging both before and after one cycle of bevacizumab and irinotecan. Each scan was 20 min long, 2
hours after iv injection of 7.0 mCi of FMISO. Regions of interest over tumor and normal brain areas were constructed on co-registered MRI T1 + gadolinium images applied to the PET images. Venous blood samples taken during imaging were used to create tissue to blood concentration (T/B) ratios. T/B values above 1.2 were used to determine the hypoxic volume for each patient’s tumor and brain regions. (Rajendran, Eur J Nucl Med Mol Imaging, 2003) Maximum T/B values (T/Bmax) were determined from the pixel with the highest uptake.

Results: All tumors demonstrated a decrease in hypoxic volume ranging from 12% to 85% (12, 70, 85) and a decrease in T/Bmax of 5% to 20% (5, 10, 12, 20). There was corresponding reduction of the MRI T1 + gadolinium and T2 tumor volumes in each tumor.

Conclusions: In this small selected series, FMISO PET provided an important pathophysiological dimension of response not identified by MRI imaging. These results suggest that antiangiogenic therapy may have a significant positive impact on reducing hypoxia and its treatment resistance effects in malignant gliomas. (Supported by NIH grants Nos. PO1 CA42045 and S10 RR17229)

Apoptosis and Autophagy

B22  Apoptosis induced in PaCa-2 cancer cells with novel Bacillus fermented extract. Ruel Michelin¹, Cynthia Johnson², Frederick Oladeinde³, Antony Kinyua⁴, Wolfgang Leitner³, Nathaniel Knox², Kathleen Lobban⁵, Imad Shureiqi⁶, Marshall Jenkins⁵, Joseph Whittaker⁵. ¹Morgan State University, Walden U. and IUHS School of Medicine, Baltimore, MD; ²Morgan State University, Baltimore, MD; ³NCI/NIIH, Bethesda, MD; ⁴University of Technology and UWI, Kingston, Jamaica; ⁵M. D. Anderson Cancer Clinic, University of Texas, Houston, TX.

Pancreatic cancer is the fifth leading cause of cancer mortality in females and fourth in males in the United States. Diagnosed later than other cancers, there is poor prognosis and incidence and mortality has not experienced many changes. Approximately 30,000 new cases are diagnosed each year, and the incidence is highest in African Americans. Less than 5% of patients survive more than 5 years after an initial diagnosis. Natural sources are continually being explored and several novel compounds including Doxorubicin (Adriamycin) produced by a member of the genus Streptomyces, and Paclitaxel (Taxol) originally derived from plant source are presently employed in therapy. However, members of the genus Bacillus, already established producers of antimicrobial compounds, have not received tremendous investigation regarding their production of antitumor compounds. In response to this we investigated the induction of apoptosis in MIA PaCa-2 pancreatic carcinoma cells, treated with varying concentrations of the extract fermented by a novel Bacillus subspecies 14135. In previous experiments we have demonstrated the filtrate’s antineoplastic ability employing MCF7 breast adenocarcinoma, NCI-H526 lung carcinoma and LNCaP-clone FGC prostate carcinoma cells. 25cm² flask cultured MIA PaCa-2 cells were plated with 5.0 x 10⁴ cells/ml in 96-well cell culture plates, incubated for 72hrs, at 37°C, 5% CO₂, and under controlled humidity. At approx 95% confluence selected wells were treated with definite quantities of the extract containing precise concentration of native protein. In earlier studies employing GC-MS fractionation we identified Taxol (Paclitaxel) treated and untreated tumor cells, along with vehicle treated and non-tumor treated reference line cells were employed as controls. Following incubation at 37°C, for 24hr to 48hrs, cells were then checked to confirm cytotoxicity and apoptosis. These were assessed through microscopic observations, employing trypan blue dye exclusion analysis, CellTiter-Blue® cell viability assay, and Annexin V- FITC® apoptosis assay. The latter is an indicator of plasma membrane destruction, which was revealed following fluorescence microscopy. In earlier studies where we employed GC-MS fractionation, several compounds were identified including Pyrrol[1, 2-a] pyrazine-1, 4-dione and Pyrrol[1, 2-a] piperazine-3, 6-dione. We feel that these compounds may be involved in the cytotoxic activity. Results were conclusive regarding the demonstration of cytotoxicity, as well as very strong evidence indicative of associated cellular shrinkage. Untreated, vehicle treated and extract treated non-tumor reference line cells remained unaffected. These results indicate the presence of compounds, which appear to selectively induce apoptosis in PaCa-2 tumor cells, and could greatly impact future cancer research.

B23  6-(1-oxoalkyl)-5, 8-dimethoxy-1,4-naphthoquinone-S17 induced apoptosis via mitochondrial dependent pathway in human leukemic U937 cells. Suk-Hyun Won¹, Min-Jong Park¹, Hee-Young Kwon², Hyo-Jung Lee², Eun-Ok Lee², Sung-Hoon Kim¹. ¹Cancer Preventive Agent Development Research Center, KyungHee Univ., Seoul, Republic of Korea; ²Cancer Preventive Agent Development Research Center, Kyung Hee Univ., Seoul, Republic of Korea.

6-(1-oxoalkyl)-5, 8-dimethoxy-1,4-naphthoquinone-S17 (DMNQ-S17) was synthesized to develop a potential antitumor agent. In the present study, the apoptotic effect of DMNQ-S17 in human leukemic U937 cells was evaluated as an antitumor candidate. DMNQ-S17 was cytotoxic to U937 cells with IC₅₀ of 4 µM. TUNEL assay revealed apoptotic morphological changes in U937 cells by DMNQ-S17. DMNQ-S17 also increased sub-G₁ DNA contents in U937 cells. DMNQ-S17 also activated caspases 8, 9 and 3 and cleaved poly (ADP-ribose) polymerase (PARP) as
well as released cytochrome c at 4 µM, while it did not affect the expression of Bax and Bcl-2 in U937 cells. Furthermore, DMNQ-S17 inhibited reactive oxygen species (ROS) production and mitochondrial membrane potential in U937 cells. These results suggest that DMNQ-S17 can be an antitumor candidate via mitochondrial dependent apoptosis in U937 cells.

**B24 Enhanced susceptibility to TRAIL-mediated apoptosis in oral squamous cell carcinoma cells through down-regulation of cellular FLIP.** Masayasu Iwase¹, Sayaka Takao¹, Makiko Uchida¹, Sayaka Yoshida¹, Tatsuo Shinoda¹, Masashi Hatori¹, Satoru Shintani¹. ¹Showa University, Tokyo, Japan.

In general, oral squamous cell carcinoma (OSCC) cells are relatively resistant to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis during culture in vitro. In this study, we investigated the roles of phosphatidylinositol 3-kinase (PI 3-K), epidermal growth factor receptor (EGFR), proteasome, and histone deacetylase (HDAC) in TRAIL-mediated apoptosis of OSCC cells. The PI 3-K inhibitor wortmannin and LY294002, EGFR inhibitors AG1478 and C225, proteasome-inhibitor MG132, and HDAC inhibitors suberylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) markedly accelerated TRAIL-mediated apoptosis in OSCC cells. The addition of TRAIL to these inhibitor-treated cells resulted in caspase-8 activation and the loss of mitochondrial membrane potential. Furthermore, the inhibitors of caspase-3, -8, and -9 reduced the acceleration effect of these inhibitors on TRAIL-mediated apoptosis. These results suggest that the pro-apoptotic effect of these inhibitors on TRAIL-mediated apoptosis may contribute to both the extrinsic and intrinsic pathways of apoptosis. Although the PI 3-K and EGFR inhibitors did not affect expressions of the TRAIL receptors DR4 and DR5, the proteasome and HDAC inhibitors enhanced the expressions of these receptors. Furthermore, we observed a marked reduction in the expression of cellular FLICE inhibitory protein (c-FLIP) with these inhibitors. The knockdown of c-FLIP in OSCC cells with a small interfering RNA (siRNA) strongly enhanced TRAIL-mediated apoptosis. Although these inhibitors also modulated the expressions of members of the Bcl-2 and inhibitor of apoptosis protein (IAP) family, common mechanisms of modulation were not observed. These results suggest that the down-regulation of c-FLIP may represent a novel strategy for overcoming resistance to TRAIL-mediated apoptosis in OSCC cells.

**B25 Downregulation of XIAP expression induces apoptosis and sensitizes a panel of pediatric tumor cells to cytotoxic agents.** Sarah V. Holt¹, Karen E. Brookes¹, Jon Durkin², Caroline Dives³, Guy W. J. Makin¹. ¹Paterson Institute for Cancer Research, Manchester, United Kingdom; ²Aegera Therapeutics Inc., Quebec, Quebec, Canada.

Cancer is one of the most common causes of death in children. Traditionally pediatric cancers have been treated, with reasonable success, by a range of cytotoxic agents. However, several tumor types fail to respond to conventional therapy and remain resistant to cytotoxic agents. One explanation for this is the inability of the cells to successfully execute the apoptotic pathway in response to cytotoxic drug exposure. The endogenous inhibitor of apoptosis proteins (IAP’s) function by inhibiting effector (−9) and initiator (−3 and −7) caspases. Interestingly, the X-linked inhibitor of apoptosis (XIAP) is observed to be overexpressed in several tumor types. Furthermore, down regulation of XIAP sensitises adult tumor cells to chemotherapeutic agents and radiotherapy. In adult cells, a novel XIAP antisense oligonucleotide (ASO), AEG 35156 (Aegera Therapeutics Inc.), had been shown to induce cell death, sensitise cells to cytotoxics and delay growth of tumor xenografts. AEG 35156 is currently in adult Phase I clinical trials in North America and the UK.

A role for XIAP in pediatric tumors has not been elucidated but high expression levels correlate with poor survival in childhood AML. We have screened a panel of pediatric cancer cell lines and shown that XIAP is widely expressed. In order to study the role of XIAP in pediatric cancer cell lines, we have used the novel XIAP ASO, AEG 35156, and demonstrated that XIAP can be down regulated in osteosarcoma, neuroblastoma, rhabdomyosarcoma and Ewing’s sarcoma cells. We have observed that in response to AEG 35156 there is an increase in cell death. Subsequent analysis demonstrated increased levels of cleaved caspase 3 and PARP suggesting the cell death observed was due to apoptosis. In long term clonogenic assays, AEG 35156 sensitised 791T (osteosarcoma), SH-SY5Y (neuroblastoma) and A673 (Ewing’s sarcoma) to clinically relevant cytotoxic agents. We are currently extending our in vitro panel of cell lines and evaluating the XIAP ASO in pediatric xenograft models as a single agent and in combination with cytotoxics.

**B26 Effect of radiation on colon cancer cell lines KM12C, KM12SM and KM12L4a with different metastatic potential.** Daniella Pfeifer¹, Åsa Wallin¹, Birgitta Holmlund¹, Xiao-Feng Sun¹. ¹Linköping University, Linköping, Sweden.

We previously found p53, p73, survivin and PRL implicated in the outcome of radiotherapy in rectal cancer patients. In the present study, we tried to understand mechanisms of colon cancer cell lines response to radiation based on protein expression related to proliferation and apoptosis. KM12C, KM12SM and KM12L4a, cell lines with different metastatic potential, were radiated with 0, 10 or 15Gy γ-radiation. Radiosensitivity was determined with cell cycle and apoptosis analysis. Protein expression patterns of p73, ΔNp73, mutated p53, survivin and PRL-3 were determined with Western blot. KM12C, the least radiosensitive phenotype, showed upregulation of resistance factors such as PRL-3 and favourable patterns of the p73 isoforms at higher dose. KM12SM had relatively moderate sensitivity and upregulation of anti-apoptotic survivin and downregulation of pro-apoptotic TAp73. The radioresistance factor PRL-3 was downregulated. KM12L4a, the most sensitive cell line, had favourable patterns with upregulation of TAp73 and downregulation of upregulation of resistance factors such as ΔNp73, survivin and PRL-3 after radiation. In conclusion, the KM12C cell line was less aggressive from an invasive point of view, but more radioresistant than the metastatic counterparts. The radiosensitivity of KM12L4a might at least partly depend on the lack of upregulation of proteins negative for the outcome of radiotherapy.

**B27 The RbBP6 expression in uterine cervix squamous cell carcinoma.** Zodwa L Dlamini¹, Zukile Mbita¹. ¹University of the Witwatersrand, Johannesburg, South Africa.

RbBP6 is a gene that encodes RbBP6 protein product and has been reported to be involved in mitotic apoptosis and also interacts with pRb as well as p53. This strongly suggests the involvement of this gene in the cell cycle regulation. It has also been reported that this gene is involved in mRNA processing and has a ubiquitin ligase activity. The aim of this study was to determine the expression of the RbBP6 gene in uterine cervix squamous cell carcinoma.

RbBP6 localisation studies were done on paraffin sections using in situ hybridization and immunocytochemistry. Camptothecin was used for apoptosis induction in cell lines to see if there was any change of the level of expression in RbBP6 using real time PCR and the ApoPercentage assay was used to check if cells were dying due to apoptosis. Both RbBP6 mRNA and protein localization using in situ hybridization and immunocytochemistry showed the accumulation of the RbBP6 gene products in the cytoplasm and very little localization was seen in the nucleus as compared to normal controls. More than 50% of Hela cells underwent apoptosis when grown in media with 20µM Camptothecin and showed increased expression levels of RbBP6. These cells died by apoptosis as measured by an ApoPercentage assay. Real-time PCR showed that RbBP6 mRNA is increased under Camptothecin apoptotic inducing conditions.

Cytoplasmic localization of the RbBP6 may suggest that it is translocated to the cytoplasm in order to impede it from performing its apoptotic or cell cycle regulating functions.
Interestingly, c-FLIPsiRNA-induced apoptosis was not dependent on the receptor involved in regulating apoptosis induced by c-FLIP silencing. Binding of DR5 to its ligand TRAIL. Overexpression of c-FLIP L, but not c-FLIP S, significantly decreased spontaneous and chemotherapy-induced apoptosis in HCT116 cells. Further analyses indicated that DR5 was the principal death mediator of c-FLIP L in regulating apoptosis in HCT116, H630 and neuroblastoma (SH-SY5Y) cells that express varying Mcl-1 levels. c-FLIP targets for apoptosis in colorectal cancer cells and potentiate the anti-tumour effect of tamoxifen in ER-negative cells. In combination with the use of an inhibitor in vivo. α-TEA and 9NC combination treatments administered by liposomal aerosol, resulted in effective inhibition of tumor growth and lung metastases. Combination treatments reduced tumor volume by 31, 57, 66, 80 and 82 % after 7, 9, 19, 27 and 29 days of liposomal aerosol treatment, respectively. Mice treated with α-TEA or 9NC alone reduced tumor volumes of 51% and 72 % respectively by 29 days. Analysis of fluorescent micrometastatic lung lesions showed that mice treated with aerosol combination results in 95% fewer micrometastatic lung lesions per lobe when compared to mice receiving the aerosol control. Individual treatments with α-TEA or 9NC resulted in 87% and 86% reduction respectively. α-TEA and 9NC in combination shows 79% fewer lymph node micrometastatic lesions when compared to the control group, (1.62 ± 0.4 vs. 7.9 ± 1.5, in vivo, is shown to be via decreasing cell proliferation and inducing apoptosis. The animals treated with α-TEA or 9NC alone, as well as in combination, show an increase in the number of TUNEL positive cells and a reduced number of Ki-67 stained cells. Together this data suggests that individual treatments, as well as combination treatments of α-TEA and 9NC, are acting through different apoptotic pathways. The data we have studied using α-TEA and 9NC and 9-Nitro-Camptothecin (9NC) in combination with 9-nitrocamptothecin (9NC) is able to inhibit the growth of mouse mammary tumor cells invitro and in vivo in a mouse mammary tumor model. α-TEA has been shown to be non-toxic and can induce DNA synthesis arrest and apoptosis in cells from BALB/c mouse mammary tumor line 66 clone 4 stably transfected with green fluorescent protein (66cl-4-GFP) in a dose responsive and time dependent manner. Studies presented here show that combinations of α-TEA and 9-Nitro-Camptothecin (9NC) are able to produce increased levels of cell death in 66cl-4-GFP mouse mammary tumor cells in vitro. Treatment with α-TEA and 9NC separately and in combination, increased levels of membrane bound FAS, Caspase 8, 9 and p-JNK, suggesting that α-TEA and combination treatments induce cell death in a caspase and mitochondrial dependent manner. To evaluate the role of JNK, protein levels were knocked down with the use of an inhibitor in tumour producing a significant decrease in apoptosis suggesting a pivotal role in programmed cell death. To investigate the role of anti-apoptotic players such as c-FLIP and Survivin, cells where transfected to overexpress these proteins. Reduced levels of apoptosis were seen in both cases, highlighting the importance of c-FLIP and Survivin in tumor cell survival.

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B28 α-TEA and 9-nitro-camptothecin alone and in combination, induces caspase dependent apoptosis in 66cl-4-GFP mouse mammary tumor cells invitro and reduces mouse mammary tumor burden and metastases in vivo. Paul Latimer1, Rachel M. Snyder1, Marla Simmons-Menchaca1, Bob G Sanders1, Kimberly Kline1. 1University of Texas at Austin, Austin, TX.

Previous studies have shown that a novel, nonhydrolyzable other analog of RRR-α-tocopherol, 2, S, 7, 8-tetramethyl-2R-(4R, BR-12- trimethyltridecyl)(chroman-6-yloxyacetic acid, α-TEA) exhibits anti-tumor activity both in vitro and in vivo in a mouse mammary tumor model. α-TEA has been shown to be non-toxic and can induce DNA synthesis arrest and apoptosis in cells from BALB/c mouse mammary tumor line 66 clone 4 stably transfected with green fluorescent protein (66cl-4-GFP) in a dose responsive and time dependent manner. Studies presented here show that combinations of α-TEA and 9-Nitro-Camptothecin (9NC) are able to produce increased levels of cell death in 66cl-4-GFP mouse mammary tumor cells in vitro. Treatment with α-TEA and 9NC separately and in combination, increased levels of membrane bound FAS, Caspase 8, 9 and p-JNK, suggesting that α-TEA and combination treatments induce cell death in a caspase and mitochondrial dependent manner. To evaluate the role of JNK, protein levels were knocked down with the use of an inhibitor in tumour producing a significant decrease in apoptosis suggesting a pivotal role in programmed cell death. To investigate the role of anti-apoptotic players such as c-FLIP and Survivin, cells where transfected to overexpress these proteins. Reduced levels of apoptosis were seen in both cases, highlighting the importance of c-FLIP and Survivin in tumor cell survival.

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Methods: The potentiation of tamoxifen by HDAC inhibitors was evaluated using MTS, colony forming and apoptotic assays. The induction of autophagy by HDAC inhibitors and/or tamoxifen was evaluated by transmission electron microscopy as well as the expression of autophagic markers, such as LC3 and Beclin 1 by microarray and Western blot analyses.

Results: Treatment of breast cancer cell lines with either tamoxifen or HDAC inhibitors showed morphological features consistent with autophagy and expression of autophagic markers. The morphological characteristics of autophagy were detected in cultured breast cancer cell lines as well as in xenograft tumors from mice treated with HDAC inhibitors. Microarray and Western blot analyses of cells treated with HDAC inhibitors and/or tamoxifen showed modulation in the expression of autophagic markers.

Conclusions: The anti-tumor effects of tamoxifen in breast cancer cell lines are enhanced by the co-administration of HDAC inhibitors irrespective of ER expression status. This synergistic interaction may be explained in part by the induction of autophagy by both modalities. To delineate between apoptotic and autophagic cell death may guide the future clinical development of HDAC inhibitors and the design of accompanying correlative studies.

B32 Activity of Apomab, a fully human agonistic DR5 monoclonal antibody, in models of non-Hodgkin's lymphoma. Dylan L Daniel1, Becky Yang1, Klara Totpal1, Klaus Wagner1, Sharon Yee1, Sarajane Ross1, Avi Ashkenazi1, Genentech, Inc., South San Francisco, CA.

Activation of the pro-apoptotic receptor DR5 triggers apoptosis through the cell-extrinsic pathway. We have generated fully human, optimized IgG1/l3 monoclonal antibody (Apomab) that induces tumor cell apoptosis through DR5, and investigated its anti-tumor activity in xenograft models of non-Hodgkin’s lymphoma (NHL). Initially, a panel of NHL cell lines that included Burkitt’s lymphomas, follicular lymphomas (FL) and diffuse large B cell lymphomas (DLBCL) were tested for DR5 expression. All of the NHL cell lines tested were found to express DR5, and thus, were rational candidates for analysis of Apomab activity in vitro. We tested Apomab on the NHL cell lines in vitro and observed apoptosis in a subset of the lines, including BJAB, SU-DHL-4 and OCI-Ly19. Furthermore, apoptosis in those cell lines was accompanied by activation of the effector caspases, caspase-3 and -7.

In vivo, Apomab showed single-agent activity against several types of NHL xenografts grown in SCID mice. Rituximab is a CD20 antibody used to treat FL and DLBCL in humans. We observed significant cooperation between Apomab and rituximab in vivo against metastatic BJAB, SU-DHL-4 xenografts. Remarkably, Toledo cells were resistant to Apomab in vitro, but sensitive in vivo to the combination of Apomab and rituximab. These findings demonstrate activity of Apomab against NHL xenografts and provide a strong rationale for clinical investigation of Apomab in combination with rituximab as a novel strategy for NHL therapy.

B33 Effects of ShetA2 on thymidine phosphorylase expression and NF-κB activity. Sty leth Chengedza2, Tongzu Lu1, Doris M Benbrook1.

1University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Introduction: Current cancer chemotherapy strategies are toxic and demonstrate limited efficacy, especially in ovarian and renal cancers. Therefore, new molecular targets are needed for drug development. Thymidine phosphorylase (TP) is a potential target because it is overexpressed in ovarian cancer, induces angiogenesis, and suppresses apoptosis induced by hypoxia and chemotherapeutic drugs. TP catalyzes reversible phosphorylation of thymidine, deoxyuridine and their analogues to their respective bases and 2-deoxyribose-1-phosphate. Microarray analysis indicated that TP mRNA expression is inhibited by the Flexible Heteroarotinoid (Flex-Het) drug, ShetA2, which targets the mitochondria leading to increased ROS production. NF-κB is another potential molecular target that is constitutively activated in many cancers, is regulated by the redox state of the cell, and is implicated in regulation of TP expression.

Hypothesis: It was hypothesized that ShetA2 effects on mitochondria alter the cellular redox state leading to inhibition of NF-κB activity, TP expression, and subsequent apoptosis.

Methods: The effects of ShetA2 on TP expression and apoptosis in ovarian (A2780 and SK-OV-3) and renal cancer (Caki) cell lines and normal endometrial cells were evaluated with Western blot, real time rt-PCR and flow cytometric analyses. ShetA2 effects of on NF-κB activity were measured in ovarian cancer cells using NF-κB specific reporter and gel retardation assays.

Results: ShetA2 inhibited TP mRNA and protein expression in all cancer cell lines in dose and time-dependent manners. Inhibition was observed as early as 2 hours and was maximal by 24 hours. The lowest concentration that inhibited TP expression over 24 hours of treatment was 3 μM. Induction of apoptosis by ShetA2 could be observed within 2 hours. ShetA2 inhibited TP expression in the same dose and time frame as it induced apoptosis in both ovarian and renal cancer cell lines. Inhibition of both constitutive and TNF-induced NF-κB activity by ShetA2 was both time and dose dependent. ShetA2 affected NF-κB activity by altering the DNA binding activity of NF-κB transcription factors.

Conclusions: ShetA2 inhibits TP mRNA and protein expression in correlation with induction of apoptosis. Studies are planned to validate the role of TP in apoptosis by determining if inducible expression of exogenous TP cDNA can block ShetA2-induced apoptosis. ShetA2 also regulates NF-κB DNA-binding and transactivation activities. Current studies are underway to determine which of the five members of NF-κB family members are involved in ShetA2 action on ovarian cancer cells. Future studies are planned to evaluate ShetA2 effects on the TP gene promoter and to determine if NF-κB plays a role in the mechanism of TP inhibition.

B34 Inhibition of glycolysis and induction of autophagy in glioblastomas by 2-fluoro-deoxy-D-glucose. Leposava Antonovic1, Izabela Fokt1, Slawomir Szymanski1, Charles A Conrad2, Waldemar Priebe1, Timothy Madden1.

1UT M. D. Anderson Cancer Center, Houston, TX.

The metabolism of fast growing tumor cells requires increasing amounts of energy to support rapid proliferation, which provides an opportunity for targeting metabolism as therapeutic approach. In addition to apoptosis (extensively studied Type I programmed cell death), autophagy (Type II cell death), under normal physiological conditions serves as a natural way of disposing of unwanted cellular material, regeneration of both cellular building blocs and energy resources. However, when the cells undergo metabolic stress, such as nutrient deprivation, the process of autophagy dramatically increases and ultimately leads to cell death. In this study we investigated the potential of inhibiting the glycolytic pathway in glioma U87 cell lines using 2-fluoro-deoxy-D-glucose (2-FG) and determined whether or not 2-FG induced autophagic cell death. U87 cells were treated with increasing concentrations of 2-FG and allowed to grow under normoxic (21% O₂) or hypoxic (0.1% O₂) conditions. The cytotoxicity of 2-FG was assessed in treated cultures at various time points (24, 48 and 72 h). Our results show that 2-FG induces Type II cell death in U87 glioma cells in dose dependent manner and that it is initiated even in the absence of nutritional stress. The cytotoxicity studies show IC50 (2-FG) to be 3.4 mM and that U87 sensitivity towards 2-FG increases 40% in cells grown under hypoxic conditions. Additionally, to test the cytotoxicity of 2-FG in U87 cells during glucose deprivation, this cytotoxicity analysis was repeated with cells grown in low glucose (5.6 mM) media. Under these conditions U87 cells were increasingly sensitive to 2-FG effects; growth was inhibited by 78% under normoxic conditions and by 86% when grown under hypoxic conditions. Confirmation of autophagy in 2-FG treated cells was demonstrated using transmission electron microscopy (TEM). U87 cells treated with 5 mM 2-FG X 72 hrs showed the presence of autophagic vacuoles in the cytoplasm - a hallmark of autophagy. Cell cycle analysis of U87 cells treated with 5 mM 2-FG X 72 hrs showed marked accumulation in G2/M phase with no increase in Sub G0/G1, demonstrating that cell death following treatment is not attributable to apoptosis. These studies show 2-FG to be a potent inhibitor of cell proliferation and an inducer of autophagic cell death in the U87 cell line and that targeting autophagic survival response using inhibitors of glycolysis should be a reasonable therapeutic approach to the treatment of cancers that are heavily dependent on glycolysis for survival.
B35 2-Deoxy-D-glucose induces Type II cell death in pancreatic cancer. Leposava Antonovic1, Mary J. Johansen1, Izabela Fokt1, Slawomir Szymanski1, Charles A. Conrad2, Waldemar Priebe1, Timothy Madden1. 1UT M. D. Anderson Cancer Center, Houston, TX.

Inhibition of glycolytic pathway is becoming an important strategy in cancer therapeutics for targeting a major survival pathway in hypoxic cancers solely dependent on glucose as an energy source. 2-Deoxy-D-glucose (2-DG) has been shown to be a potent inhibitor of glycolysis in glioma cancer cells which depend almost exclusively on glucose for metabolism. Here we investigated the effect of 2-DG as an inhibitor of cell growth in a panel of pancreatic cancer cell lines including MiaPaCa2 and Panc1. To asses the cytotoxicity of 2-DG, MiaPaCa2 and Panc1 cells were treated with increasing concentrations of 2-DG for 24, 48 and 72 hour periods under normoxic (21% O2) and hypoxic (0.1% O2) conditions. The results from MiaPaCa2 treated cells revealed the IC50 (2-DG) to be 2.3 mM and that sensitivity of the MiaPaCa2 cells towards 2-DG increases by 37% under hypoxic conditions. When MiaPaCa2 cells were grown under same conditions in low glucose media (5.6 mM), sensitivity of MiaPaCa2 cells to 2-DG treatment further increased 82%, however the use of low glucose media under hypoxic conditions did not further increase the sensitivity of MiaPaCa2 cells to 2-DG. 2-DG cytotoxicity studies in Panc1 treated cells demonstrated a 3 fold higher resistance to 2-DG treatment (IC50 6.9 mM) than observed in the MiaPaCa2 cell line suggesting sensitivity to the monosaccharide based antimetabolites like 2-DG is cell specific as demonstrated by increased activity in the highly glycolytic MiaPaCa2 cell line. We also assessed the induction of autophagy in MiaPaCa2 cells using acridine orange staining to determine the acidic vesicular organelles (AVO), a hallmark of autophagy cell death. Here we found supporting evidence of 2-DG as an inducer of autophagic cell death, showing a significant increase in AVOs following treatment with 5 mM 2-DG. These findings support our hypothesis that 2-DG acts as a potent inhibitor of cell proliferation in MiaPaCa2 pancreatic cancer cell line, and is not as potent in cell lines less dependent on glycolysis like the Panc1 cell line. These data in total provide strong evidence that inhibitors of the glycolytic pathway could be a significant therapeutic strategy in the treatment of pancreatic and other highly glycolysis-dependent tumor types.

B36 Investigations into the cellular mechanism of action of transplanaranamines of the type trans-[Pt(O2CR)2(L)(L')] by Sheena M. Aris1, Ken Knott1, John Ryan1, David Gewertz2, Nicholas Farrell1. 1Virginia Commonwealth University, Richmond, VA.

The current work investigates the influence of transplanaranamine (TPA) platinum compounds of structure trans-[Pt(O2CR)(L)(L')], (L=pyridine, quinoline, isoquinoline; L= pyridine, quinoline, isoquinoline; L= L' = pyridine; R = H, CH3, CH2OH) on growth and viability of HCT116 human colon carcinoma and A2780 human ovarian carcinoma cells as well as their putative mechanism(s) of cytotoxicity. A series of structural analogs was examined with a focus on the contribution of the carboxyamide leaving group to drug action. The compounds, as a class, have been shown to induce cell death through caspase-dependent apoptosis, with activation of both caspase 3 and caspase 9 and concomitant PARP cleavage. Both A2780 and HCT116 WT cell lines are most sensitive to trans-[Pt(O2CH)(NH3)(tsoq)], which may be related to its enhanced cellular accumulation. The enhanced cytotoxicity of trans-[PtO2CH2(NH3)(tsoq)] compared to its isostructural -O2CH analog may be a consequence of its increased hydrolytic activation, enhanced cellular uptake, interstrand cross-linking and abortive efforts by the cell to repair the cross linked DNA. Previous studies have shown higher levels of cytotoxicity specific tumor types when compared to cisplatin. Here we examine the contributions of molecular pathways to the cytotoxic effect of transplanaranamines as compared to cisplatin and oxaliplatin. This data suggests that the trans-[Pt(O2CH)(NH3)(tsoq)] compound is a possible candidate for clinical development.

B37 Anticancer drug potential of “noncovalent” platinum compounds. The biology of the phosphate clamp. Ralph Kipping1, Peyman Kabolizadeh1, Frederic Frezzard2, John J. Ryan3, Nicholas P. Farrell1. 1Virginia Commonwealth University, Richmond, VA; 2Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

The development of all clinically used platinum anti-cancer drugs has been predicated on the concept that irreversible platinum-DNA adduct formation is the ultimate molecular mechanism of cytotoxicity. The necessity for substitution-labile ligands (Cl, dicarboxylato) for Pt-DNA bond formation also means that many ‘side-reactions” occur with protein residues which may affect toxicity and/or metabolism. Recently, using highly charged “non-covalent” polymeric platinum agents denominated TriplatinNC, a discrete new mode of DNA binding, the phosphate clamp, has been reported [1]. TriplatinNC is a direct analog of the Phase II drug BBR3464, with which it shares many structural similarities. The mode of DNA-binding of TriplatinNC requires only backbone and minor groove interactions between platinum-am(mijnine units and phosphate oxygens reminiscent of the arginine-RNA recognition motif, the arginine fork [1]. In terms of drug development, TriplatinNC is of interest because it does not contain substitution-labile ligands and is a further advance away from the structure-activity paradigm of platinum drugs. This contribution summarizes the biology and potential anti-cancer utility of TriplatinNC.

Remarkably, cytotoxicity in human tumor cells is similar to cisplatin. The effect of chain length (n =4,5) is not significant with respect to cytotoxicity and emphasis has been placed on the n=6 derivative. Cytotoxicity is significantly less dependent on p53 status. TriplatinNC induced p53 expression in HCT116 cells; however, its cytotoxicity was not affected by p53 deletion. TriplatinNC further differs from BBR3464, by inducing G1 rather than G2 cell cycle arrest in HCT116 cells. Unlike c-DDP, oxaliplatin, and BBR3464, TriplatinNC was able to circumvent deactivation by glutathione since its cytotoxicity was not affected by inhibition of glutathione synthesis. The antitumor activity of TriplatinNC was also examined in vivo showing higher activity against the 2008 ovarian carcinoma cells than c-DDP at the doses administered. The study illustrates fundamental differences in the mechanisms of action between noncovalent binding drugs like TriplatinNC and structurally related covalent binding drugs such as c-DDP, oxaliplatin, and BBR3464.

1. S. Komeda et al., J. AM. CHEM. SOC. 2006, 128, 16092-16103

B38 Antitumor activity of a novel small molecule agent PRLX 93936 againstinstitute human tumor xenograft models. Paul Robbins1, Chris Clemens1, Michael Pierce1, John Peltier1, Robert Selliah1, Raj Gopal Venkat1, Matt Rebentisch1, Shaping Lai1, Robert Becklin1, Anna Senina1, Vlad O’Me1, Tracey Erekki1, Sudhir R. Sahasrabudhe1. 1Prolexys Pharmaceuticals, Inc., Salt Lake City, UT.

PRLX 93936 was identified as a potential anti-cancer therapeutic in a synthetic lethalf screen againstinstitute isogenic cell lines engineered to differentially express several oncogenes including activated RasV12. Subsequent testing againstinstitute a series of normal and tumor cell lines derived from tumors with dissimilar causative mutations and limited treatment options, indicated potent and selective activity againstinstitute a wide variety of tumor cell lines (many with activated Ras pathway). Mass spectrometric analysis of proteins selectively pulled-down from tumor lysates by PRLX 93936 immobilized on bead surfaces led to the identification of mitochondrial outer membrane protein VDAC (Voltage Dependent Anion Channel) as a potential target. PRLX 93936 caused tumor regression in human xenograft models representing cancers such as pancreatic (PANC-1) and fibrosarcoma (HT-1080). Tumor regression was demonstrated with oral (PO), intravenous (IV), and intraperitoneal (IP) routes of administration. In vivo, PRLX 93936 produces efficacy ranging from tumor-growth inhibition to complete regression in a dose-dependent fashion. Toxicology studies with mice, rats, dogs, and monkeys indicate drug tolerance at potentially therapeutic levels based on body surface area scaling. Although its mechanism of action is still under investigation, results of pharmacodynamic studies demonstrate its promise as a novel
anti-cancer drug. As a result of successful IND submission, Phase 1 clinical trials are scheduled for September 2007.

B39 A novel molecule targeting VHL-deficient renal cell carcinoma that induces autophagic cell death. Sandra Turcotte1, Patrick D. Sutphin1, Denise A. Chan1, Michael P. Hay2, William A. Denny2, Amato J. Giaccia3. 1Stanford University, Stanford, CA; 2Auckland Cancer Society Research Centre, The University of Auckland, Auckland, New Zealand, New Zealand.

Background: Renal Cell Carcinomas (RCCs) are highly vascular, metastatic tumors that pose a significant clinical challenge because they are refractory to standard therapies. The von Hippel-Lindau (VHL) tumor suppressor gene is mutated or inactivated in 75% of RCCs. In this study, we investigated a therapeutic approach targeting VHL-defective cells. Methods: The drug STF-62247 was identified from a screen of small molecules that target the loss of VHL. Viability was measured by clonogenic assay in cells that lack VHL versus matched controls. The effect of STF-62247 on tumor growth was evaluated in vivo in an xenograft model. The vacuole formation observed during drug treatment was associated with autophagy and characterized using western blotting and immunofluorescence for LC3, a biological marker. Acidification of the vacuoles by lysosomal fusion was stained with acridine orange and measured by flow cytometry. Results: We found that STF-62247 induces selective cytotoxicity in VHL-inactivated cells. A small library of analogs was synthesized and also demonstrated VHL-deficient lethality. The STF-62247-stimulated toxicity occurs in a HIF-independent manner through autophagy, a lysosomal degradation process. Both deficient and wild-type VHL cells treated with STF-62247 accumulate the lipidated form of LC3, a biological marker for autophagosomes. However, VHL-deficient cells had higher acidification of mature autolysosomes, suggesting that VHL can protect cells against autophagic acidification and cell death induced by STF-62247. simultaneous treatment of VHL-deficient cells with STF-62247 and 3-methyladenine (3-MA), a specific autophagy inhibitor, reduced LC3-lipidation and increased cell viability. Moreover, loss of Atg5, an essential component of the autophagy pathway also protected cells since VHL-deficient cells with siRNA against Atg5 are resistant to STF-62247-induced cell death. Conclusion: Taken together, these data suggest that STF-62247 induces autophagic cell death in VHL-deficient cells. Importantly, STF-62247 specifically reduces tumor growth of VHL-deficient RCC in vivo, indicating that it could be clinically useful in selectively targeting VHL-deficient RCC therapeutically.

Biological Therapeutic Agents

B40 A monoclonal antibody targeting melanoma-associated chondroitin sulfate proteoglycan demonstrates antitumor activity in human melanoma, ovarian and breast cancer models. Ashwani Gupta1, Alison Ferry3, Daad Sayegh1, Luis A. G. da Cruz2, Ningping Feng1, Susan Hahn1, Daniel S. Pereira1, Daniel B. Rubinstein1, David S. Young1. 1ARIUS Research, Inc., Toronto, Ontario, Canada.

CD44 is a widely distributed cancer-associated membrane glycoprotein, believed to play a critical role in cellular adhesion, migration, invasion and tumorigenicity. CD44 was recently shown to be expressed in cancer stem cells from several cancer types, including breast, colon, prostate, pancreas, and AML. A monoclonal antibody targeting CD44, CD44H460-16-2, was identified using the ARIUS’ FunctionFIRST™ platform. The chimeric version of the antibody demonstrated potent dose-dependent tumor growth inhibition in an in vivo established subcutaneous model of breast cancer and in an established orthotopic model of metastatic liver cancer at all doses tested (20, 2 and 0.2 mg/kg). IHC staining of human cancers from breast, prostate, colon, and liver demonstrated that the epitope for ARH460-16-2 is present on the majority of human adenocarcinomas. Limited binding to normal human tissues was observed, predominantly in the bone marrow myeloid series with no binding to erythroid cells, megakaryocytes, endothelium, fibroblasts, smooth muscle, eye, heart, liver, ovary and colon epithelium. The binding pattern was not substantially different in cyromolgus monkey tissues, although some differences in intensity were seen. To enable the clinical development of ARH460-16-2, a dox-finding toxicology study was carried out in cyromolgus monkeys using the chimeric version of the antibody. In the first phase, monkeys (2 per group) were given a single 1 hr infusion of 10, 30 or 95 (high dose) mg/kg chARH460-16-2. A second cohort of 3 monkeys was infused with 25 mg/kg chARH460-16-2 once per week for 2 doses. Animals were observed daily, and clinical chemistry, hematology and coagulation parameters were assessed 24 hr after injection, and then twice more after 9 and 28 days for the first cohort. For the second cohort, analysis was similar except that the final blood collection was carried out at Day 15, at which point the repeat-dose animals were sacrificed for necropsy. In one of the high-dose treated monkeys, and all subjects in the repeat-dose group, there was a transient reddening of the skin which resolved after a few days. There were small decreases in the indicators of circulating erythrocyte mass which were attributable to blood sampling for clinical pathology and toxicokinetic assessments. These changes were accompanied by expected and appropriately increased reticulocyte counts. Other fluctuations in hematology, coagulation, body weight and clinical chemistry parameters were sporadic, transient, observed prestudy, and/or of a magnitude of change that is commonly observed in laboratory-housed cyromolgus

B41 Toxicology and pharmacokinetics of chimeric ARH460-16-2, a therapeutic monoclonal antibody targeting CD44. Susan E. Hahn1, Luis A.G. daCruz2, Nadine Chouinard1, Daniel S. Pereira1, Daniel B. Rubinstein1, David S. Young1. 1ARIUS Research, Inc., Toronto, Ontario, Canada.

AR11BD-2E11-2 showed significantly lower weight gain of 40% (p=0.03) corresponding to decreased ascites formation, as well as having a significantly longer mean survival time (p=0.02). Immunohistochemistry analyses revealed that the staining pattern of the epitope recognized by AR11BD-2E11-2 on frozen human breast cancer and melanoma sections was found to be highly specific for malignant cells. With respect to normal tissue expression, epithelial staining with AR11BD-2E11-2 was generally minimal. Taken together, the anti-MCSP antibody AR11BD-2E11-2 has exhibited significant anti-tumor efficacy in human xenograft tumor models of melanoma, ovarian and breast cancer. Since MCSP was recently identified as being expressed on breast cancer stem cells, the epitope of AR11BD-2E11-2 may be a particularly important antibody target for the treatment of breast cancer. The fact that this epitope on MCSP was identified using the ARIUS FunctionFIRST™ platform highlights the importance of this platform in identifying functional epitopes within important cancer targets.
monkeys undergoing similar study procedures. Together with the potent anti-tumor effect in models of human cancer, these results support the clinical development of a therapeutic monoclonal antibody targeting the cancer stem cell antigen CD44.

B42 Sorafenib-mediated Mcl-1 and cFLIP\textsubscript{L} down-regulation synergistically increase TRAIL lethality in human leukemia cells. Roberto R. Rosato\textsuperscript{1}, Jorge A. Almenara\textsuperscript{1}, Stefanie Coo\textsuperscript{2}, Steven Grant\textsuperscript{1}.

In the present study, interactions between the Raf and multi-kinase inhibitor Sorafenib and TRAIL (TNF-related apoptosis-inducing ligand) were investigated in malignant hematopoietic cells. Pretreatment (24h) of U937 leukemia cells with 7.5 \( \mu \)M sorafenib, a concentration that was minimally toxic, dramatically and synergistically potentiated apoptosis induced by sub-lethal concentrations of TRAIL/Apo2L (e.g., 75ng/ml). Similar interactions were observed in other malignant hematopoietic cells including Raji, Jurkat, Karpas, K562, U266 cells and primary AML blasts. Notably, the combination was minimally toxic to normal CD34\textsuperscript{+} bone marrow cells. Exposure of sorafenib-pretreated cells to TRAIL potently and rapidly induced mitochondrial injury and release of cytochrome c, Smac and AIF into the cytosol, and caspase -9, -3, -7, and -8 activation. Sorafenib pretreatment also downregulated Bcl-xl and abrogated Mcl-1 expression, while co-administration of TRAIL strikingly increased Bid activation, conformational change of Bak (ccBak) and Bax (ccBax), and Bax translocation. Ectopic Mcl-1 expression significantly attenuated sorafenib/TRAIL-mediated lethality and dramatically reduced ccBak while minimally affecting levels of ccBax. Similarly, inhibition of the receptor-mediated apoptotic cascade (i.e., in U937 cells ectopically expressing a caspase-8 dominant-negative construct) significantly blocked Sorafenib/TRAIL-induced lethality but not Mcl-1 down-regulation or Bak/Bax conformational change, indicating that TRAIL-mediated receptor pathway activation is required for maximal lethality. No changes were observed in levels of DRAHDR or recruitment of procaspase-8 or FADD to the death-inducing signaling complex (DISC); however, sequential administration of sorafenib/TRAIL synergistically increased activation of DISC- associated procaspase-8. Attention then focused on FLIP\textsubscript{L} (FLICE-inhibitory protein), a potent procaspase-8 inhibitor. Pre-exposure of cells to sorafenib led to marked cFLIP\textsubscript{L} down-regulation, but no changes in expression of the short form of this protein (cFLIP\textsubscript{S}). Moreover, sorafenib-mediated cFLIP\textsubscript{L} downregulation involved a translational mechanism associated with diminished eIF4E phosphorylation. Notably, ectopic expression of cFLIP\textsubscript{L} significantly reduced sorafenib/TRAIL lethality. Together, these results, taken in conjunction with earlier findings, suggest that in human leukemia cells, sorafenib potentiates TRAIL-induced lethality by down-regulating Mcl-1 and cFLIP\textsubscript{L}, most likely at the translational level, and these events cooperate to promote simultaneous engagement of the intrinsic and extrinsic apoptotic cascades, culminating in pronounced mitochondrial injury and apoptosis. They also suggest that a strategy combining sorafenib with TRAIL warrants attention in leukemia and possibly other hematologic malignancies.

B43 Combination effect of CMB, Cordyceps militaris fruiting bodies, with cordycepin on antitumor activity

Cordyceps militaris (C. militaris), which belongs to the class ascomycetes, has long been used as a traditional oriental medicine due to its diverse biological activities, including immunostimulatory, anti-tumor and hypoglycemic activity. In vitro cytotoxicity assays of CMB, Cordyceps militaris fruiting bodies (CMB) and cordycepin exhibited on dose dependent inhibitory effect on the proliferation of mouse melanoma cell line, B16BL6. Combination effects of the CMB with cordycepin on in vivo tumor growth were monitored after implantation of B16BL6 cells into C57BL/6 mice. Purpose of this research was to examine the anti-proliferative and cytotoxic activities of combination CMB with cordycepin in mouse melanoma cell line, B16BL6 in vitro and B16BL6 melanoma in C57BL/6 mice. CMB, in particular, had the most potent effect against mouse melanoma cell line. Simultaneous administration of CMB (50 mg/kg) and cordycepin (2 mg/kg) showed much stronger anti-tumor effect on B16BL6-induced melanoma in C57BL/6 mice than cordycepin only, causing about 52% decrease of tumor size over 7 days. Our results suggest that the C. militaris has strong anti-tumor activity and is a potential source of natural anti-tumor agents.

B44 In vitro assessment of the IGF-1R inhibitor, PQIP, alone and in combination with chemotherapy, against human colorectal cancer cell lines: Antiproliferative, molecular, and metabolic effects.

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Background: Signaling through the insulin-like growth factor 1 receptor (IGF-1R) promotes cell growth, migration and survival in several human tumors. IGF-1R is overexpressed in colorectal cancer (CRC) and is associated with a poor prognosis and resistance to chemotherapy. In this study, we tested PQIP, a small molecule IGF-1R tyrosine kinase inhibitor, alone or in combination with standard chemotherapeutic agents in proliferation and apoptosis assays using human CRC cell lines. Based on our prior data demonstrating distinctive metabolic responses of cancer cells to tyrosine kinase inhibitors, we also investigated changes in glucose and choline metabolism in de novo sensitive and insensitive CRC cells after treatment with PQIP.

Methods: The antiproliferative effects of PQIP, as a single agent and in combination with 5-fluorouracil, oxaliplatin, or SN38 were assessed using a sulforhodamine B assay. Synergy was evaluated between PQIP and chemotherapy using the Chou and Talalay method. Apoptosis was analyzed using bioluminescent caspase 3/7 detection, and downstream effectors were analyzed by immunoblotting lysates from cells stimulated with IGF-II following pretreatment with PQIP. NMR-based metabolomic analysis was performed on one de novo sensitive and one insensitive cell line after PQIP exposure.

Results: PQIP demonstrated antiproliferative effects against CRC cell lines with a range of IC50’s of 0.3 to > 5\( \mu \)M. A sensitive cell line (HT29; IC50 = 300 nM) and an insensitive cell line (HCT116; IC50 > 5\( \mu \)M) were chosen for further analysis. Interestingly, both cell lines demonstrated synergy, as reflected by CI values of <1 for all combinations. PQIP alone or in combination with SN38 did not promote apoptosis. PQIP inhibited IGF-II-induced activation of proteins of the PI3K and MAPK pathways, such as p-AKT and p-ERK, in both cell lines. Differential metabolic responses were noted in response to PQIP, with the sensitive HT29 cells demonstrating decreased glucose uptake and lactate production, as well as a decrease in phosphocholine (marker for cell proliferation) and an increase in glycerophosphocholine (marker for membrane degradation), whereas the insensitive HCT116 displayed no significant changes in cell metabolism. Discussion: PQIP exhibits differential antiproliferative activity against CRC cell lines in vitro and inhibits molecular and metabolic pathways associated with survival. Preliminary studies comparing a sensitive and insensitive cell line indicate that inhibition of IGF-1R in combination with chemotherapy may have synergistic antiproliferative effects in both settings, but differential metabolic effects. Studies are ongoing with other sensitive and insensitive cell lines to further define molecular and metabolic differences in response to PQIP.

B45 Novel antagonist of the HGF-Met pathway displays antitumorigenic activity.

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The c-Met tyrosine kinase receptor and its ligand, the hepatocyte growth factor/scatter factor (HGF/SF), are critical components of the hepatocyte growth factor (HGF) signaling pathway in development and adult physiology. Mutations in the c-Met tyrosine kinase are associated with a variety of human cancers, including ovarian, prostate, breast, and lung cancer. Novel Met inhibitors would therefore be of great clinical significance. The human HGF/Met pathway is a target for the development of novel cancer therapies. In this study, we report the discovery and characterization of the novel small molecule HGF antagonist, PQIP. PQIP binds to the extracellular domain of Met with high affinity (IC50 = 2 nM) in an orthosteric manner, without affecting the Met receptor dimerization and activation. PQIP blocks HGF-induced cell migration and anchorage independent growth in human tumor cell lines. PQIP also inhibits HGF-induced activation of proteins of the PI3K and MAPK pathways, such as p-AKT and p-ERK, in both cell lines. Furthermore, PQIP induces apoptosis in several human tumor cell lines. The combination effect of PQIP and standard chemotherapy agents was synergistic in the sensitizing cell line. Differential metabolic responses were noted in response to PQIP, with the sensitive HT29 cells demonstrating decreased glucose uptake and lactate production, as well as a decrease in phosphocholine (marker for cell proliferation) and an increase in glycerophosphocholine (marker for membrane degradation), whereas the insensitive HCT116 displayed no significant changes in cell metabolism. Discussion: PQIP exhibits differential antiproliferative activity against CRC cell lines in vitro and inhibits molecular and metabolic pathways associated with survival. Preliminary studies comparing a sensitive and insensitive cell line indicate that inhibition of IGF-1R in combination with chemotherapy may have synergistic antiproliferative effects in both settings, but differential metabolic effects. Studies are ongoing with other sensitive and insensitive cell lines to further define molecular and metabolic differences in response to PQIP.
growth factor (HGF), are involved in a wide range of biological activities, including cell proliferation, motility, invasion, and angiogenesis. The HGF-Met pathway is frequently activated in a variety of cancers, and uncontrolled c-Met activation correlates with highly invasive tumors and poor prognosis. Using our alternative splicing modeling platform LEADS, we have identified a novel splice variant of the c-Met receptor, which encodes a truncated soluble form of the receptor. This variant was produced as an Fc-fused protein in a mammalian expression system and was designated Cgen-241A. The biological activity of Cgen241A was assessed in various cell-based assays, reflecting different outcomes of Met activation. Cgen241A significantly inhibited HGF-mediated c-Met phosphorylation as well as cell proliferation and survival, indicating an anti-mitogenic activity. In addition, Cgen241A showed a profound inhibitory effect on cell scattering, invasion and urokinase upregulation, indicating anti-tumorigenic and anti-metastatic activities. These inhibitory effects of Cgen-241A were shown in multiple human and non-human cell types, representing different modes of ligand-dependent and ligand-independent c-Met activation. In agreement, binding studies revealed that Cgen241A binds both HGF and c-Met receptor in its membranal form, pointing to a dual mechanism of action. Preliminary results show that Cgen-241A inhibits xenograft tumor growth in vivo, suggesting a therapeutic potential. Taken together, our results indicate that Cgen241A is a potent antagonist of the HGF-Met pathway. Its dual mechanism of action may confer a therapeutic advantage, due to its ability to inhibit the diverse modes of c-Met activation existing in human malignancies.

B46 Enhanced antitumor activity with anti-epidermal growth factor receptor monoclonal antibody cetuximab in combination with carboplatin in preclinical human ovarian carcinoma models. Xiaoqiang Kang1, Dipa Patel1, Stanley Ng1, Maxine Melchior1. 1ImClone Systems, New York, NY.

Ovarian carcinoma frequently expresses the epidermal growth factor receptor (EGFR) and this expression correlates with disease progression and patient survival. Cetuximab is an IgG1 monoclonal antibody that specifically targets the EGFR and inhibits its activation in tumor cells. In this study, we evaluated the activity of cetuximab in combination with carboplatin in two human ovarian carcinoma cell lines. Flow cytometry showed that OVCAR5 and OVCARB expressed significant EGFR on cell surfaces. Cetuximab treatment of ovarian cells in vitro inhibited EGFR stimulation and induced cell proliferation in a dose-dependent manner. In vivo effects of Cetuximab on tumor growth were evaluated in ovarian xenograft models in athymic mice. Aymic mice with established (200 mm3) OVCAR5 or OVCARB subcutaneous tumor xenografts were treated with cetuximab (40mg/kg; q3d), carboplatin (20mg/kg; q3d), or the combination. Treatment with cetuximab or carboplatin resulted in moderate tumor inhibition of OVCAR5 (31% and 61%, respectively) and OVCARB (58 and 65%, respectively) compared to saline control. Greater tumor inhibitions were observed when mice treated with the combination. The combination of cetuximab and carboplatin resulted in significantly enhanced antitumor activity in the OVCAR5 (81%) and OVCARB (78%) models compared to monotherapy treatment. These results suggest that the combinations of cetuximab and carboplatin may be effective approach for the treatment of ovarian carcinoma.

B47 A phase I and pharmacokinetic study of CR011-vcMMAE, an antibody toxin conjugate drug, in patients with unresectable stage III/IV melanoma. Mario Sznol1, D Sanders2, H Kluger1, L Rink1, N Phousaphone1, K Kim1, A Bedikan1, N Papadopoulos1, Wl Huvz1, T Hawthorne1, T Mansfield1, Y Halvorsen1, P Huvz1, J Melanoma Unit, Yale Cancer Center, New Haven, CT; 2MD Anderson Cancer Center, Houston, TX; 3CuraGen Corporation, Branford, CT.

Background: Glycoprotein NMB (GPNMB) expression on the cell surface of melanoma cells is substantially greater than most normal tissues. CR011-vcMMAE is a fully human monoclonal antibody against GPNMB conjugated to the cytotoxic monomethyl auristatin E (MMAE). The toxin is released upon internalization and proteolytic cleavage within antigen-expressing cells. CR011-vcMMAE shows anti-tumor activity in vitro and in melanoma xenograft mouse models, therefore, we conducted a phase I multi-center study to evaluate its safety, maximum tolerated dose (MTD), and pharmacokinetics (PK) in advanced melanoma patients with 1 prior systemic cytotoxic therapy.

Methods: CR011-vcMMAE was administered every 3 weeks. The dose was doubled in successive cohorts of 3 patients until grade (gr) 2 toxicity was observed, followed by 40% increments until determination of MTD using a standard Phase I design. Response was evaluated by RECIST.

Results: To date, 21 patients in 7 cohorts received 2 to 8 cycles at dose levels from 0.03 and 1.34 mg/kg. All patients tolerated the infusion well. No drug limiting toxicity (drug related CTC gr 4 hematological toxicity or gr 3 non-hematological toxicity during cycle 1) has been observed. No drug-related > gr 2 toxicity was reported up to the 0.48 mg/kg dose. At doses of 0.96 and 1.34 mg/kg, 4 of 6 patients developed gr1 or 2 transient diarrhea. At 1.34 mg/kg, 2 of 3 patients developed gr1 rash and 1 patient developed gr3 and 4 neutropenia in cycles 1 and 2 approximately 20 days after dosing. Of the 18 assessable patients, 3 (16.7%) had stable disease for > 12 weeks. One patient who received 8 treatments of 0.24 mg/kg CR011-vcMMAE exhibited a 20% reduction of the target lesions at 18 weeks. PK analysis confirmed dose dependent exposure. Concentration-time profiles were similar between cycles 1 and 2. Terminal phase 1/2 ranged from 16.7 hrs at 0.03 mg/kg to 37.9 hrs at 0.96 mg/kg. The increase in 1/2 with dose suggested target-mediated disposition. Levels of free MMAE were approximately 1.4% of total CR011 at later collection points in all cohorts ( mole percentage) and remained measurable at 2 weeks after dosing suggesting low spontaneous dissociation of MMAE. Preliminary staining of biopsy samples from 2 patients showed that GPNMB was present in both samples.

Conclusions: CR011-vcMMAE up to 1.34 mg/kg dose was well tolerated and dose escalation continues. Preliminary IHC and PK results confirmed the presence of target antigen and drug exposure. Once a MTD is established, a phase II component of the study will evaluate activity.

B48 Inhibiting FGFR3 for enhancing the cytotoxic effects of cisplatin on bladder cancer cells and possible mechanisms. Dhanvantthi S. Deevi1, Roberto DiRenzo1, Huling Lu1, Maria Malabunga1, Marie C. Prevett1, Rajiv Bassi1, Weiqiang Wang1, Larry Witte1, James R. Tonra1, Haijun Sun1, 1ImClone Systems Incorporated, New York, NY.

Previously we demonstrated that inhibition of human fibroblast growth factor 3 (FGFR3) with a fully human IgG1, IMC-D11, enhanced the anti-tumor effects of cisplatin in subcutaneous bladder cancer xenograft models (AACR Annual Meeting 2007, Abstract# 2080). In the present study, we investigated the underlying mechanism for this combination effect by evaluating the effects of cisplatin and IMC-D11 on cultured RT112 and RT4 bladder cancer cell lines. Treatment effects reported below were consistent across cell lines, unless otherwise noted. Cytotoxic effects of cisplatin +/- IMC-D11 were evaluated by flow cytometry to quantify the percentage of cells in sub-G0, or by measuring the cell survival utilizing a sulphorhodamine B method. Cells were treated with IMC-D11 beginning one hour prior to the start of cisplatin treatment. IMC-D11 had no apoptotic effects on its own, but IMC-D11 (30 µg/m) increased cisplatin (10 or 30 µM) mediated cytotoxic effects by 20-30%. Cisplatin at 1 µM did not cause apoptosis with or without IMC-D11 co-treatment. Since FGFR3 is a tyrosine kinase receptor that can activate the ERK1/2 survival/proliferation pathway, we evaluated the effect of cisplatin and IMC-D11 on ERK1/2 activation. Cisplatin at 10 µM significantly increased the phosphorylation of ERK1/2 at 24 hours of treatment by western blotting. Strikingly, IMC-D11 prevented ERK activation by cisplatin. This finding indicates cisplatin induced mAPK activation may be mediated through FGFR3 signaling. To confirm this, we utilized a receptor tyrosine kinase array analysis of samples treated with either cisplatin (30 µM) or cisplatin +/- IMC-D11 (30 µg/m). Our results show that cisplatin indeed activated FGFR3 in RT4 cells and this activation was blocked by IMC-D11. Interestingly, while FGFR3 activation was not detected after cisplatin treatment in RT-112 cells, EGFR and c-Met were phosphorylated by...
cisplatin. IMC-D11 blocked EGFR and c-Met activation in this cell line, pointing to the potential importance of receptor transactivation mediating a survival mechanism during cisplatin treatment. While we continue to evaluate the molecular biology underlying the beneficial effects of inhibiting FGFR3 in combination with cisplatin, results already suggest a link between cisplatin and FGFR3 signaling at the level of receptor tyrosine kinase activity and downstream ERK activation, allowing IMC-D11 to increase the apoptotic effects of cisplatin against bladder cancer cells.

B49 Apparent lack of immunogenicity of human antibody adecatumumab in breast and prostate cancer patients. Andreas Wolff1, Micromet AG, Munich, Germany.

The human antibody adecatumumab (MT201) targets the tumor-associated antigen EpCAM (CD326). Adecatumumab is currently explored in metastatic breast cancer patients in combination with docetaxel, and has earlier been evaluated in two phase II studies in metastatic breast and early-stage prostate cancer patients. In breast cancer, exploratory subgroup analyses found time-to-progression to increase with both antibody dose and the level of EpCAM target expression, indicating activity of this EpCAM-targeting cytotoxic agent.

Here we have analyzed the immunogenicity of adecatumumab following long-term biweekly treatment of breast (N=112) and prostate cancer patients (N=84) as an antibody monotherapy. Patients had no concurrent immunosuppressive therapies. The longest therapy was for 26 infusions during a treatment period of 48 weeks, and the shortest for one infusion. Mean number of infusions was 7.5 with a median of 5. Immunogenicity was assessed earliest 6 weeks and latest 14 weeks after beginning of treatment.

A sandwich ELISA for detection of a human anti-adecatumumab response with a lower limit of detection of 20 ng/ml was developed and validated. The assay is biased for detection of higher affinity immune responses in sera from patients with no concurrent immunosuppressive therapies. The longest therapy for 26 infusions during a treatment period of 48 weeks, and the shortest for one infusion. Mean number of infusions was 7.5 with a median of 5. Immunogenicity was assessed earliest 6 weeks and latest 14 weeks after beginning of treatment.

In the two clinical phase II studies with a total of 196 patients analyzed for immunogenicity only one patient apparently developed a detectable antibody response to adecatumumab. Further analysis of sera from the immune-reactive patient showed that addition of excess adecatumumab was not capable of reducing the ELISA signal, indicating that the single low-affinity immune response in the presence of high residual serum levels of antibody may thus have escaped detection. Calculation of the assay cut point used the 95th percentile and the mean value of 55 individual sera from untreated patients.

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B50 TRAIL-receptor antibodies synergize with chemotherapy to enhance anti-tumor activity in cholangiocarcinoma. Robin C. Humphreys1, Carol Poortman1, Kathy McCormick1, Lindsey Shepard1, Jeff Carroll1, Lenny Taslim1. 1Human Genome Sciences, Inc., Rockville, MD.

Tumor necrosis factor related apoptosis-inducing ligand (TRAIL) death receptors, TRAIL-R1 and TRAIL-R2, are expressed on the cell surface of many human tumor cells. Activation of these receptors induces programmed cell death. Mapatumumab and lexatumumab are fully human agonistic monoclonal antibodies (mAbs) that target and activate cell death via TRAIL-R1 or TRAIL-R2, respectively. Both mAbs are currently being evaluated in clinical trials.

We assessed the in vitro and in vivo efficacy of mapatumumab or lexatumumab alone and in combination with chemotherapeutic agents in several cholangiocarcinoma (CCA) cell lines in cytotoxicity assays and xenograft tumor models.

Three CCA cell lines representing intra- (HuCCT1) or extra- (EGI, TFK-1) hepatic, biliary duct cancer expressed low to moderate levels of both TRAIL-R1 and TRAIL-R2 on the cell surface and were either resistant (HuCCT1), weakly (EGI) or moderately sensitive (TFK-1) to treatment with mapatumumab or lexatumumab.

Co-treatment with cisplatin, gemcitabine or 5-flourouracil increased the cytotoxicity of both mAbs in most cell lines; in some cases a synergistic enhancement was observed. Co-treatment with the triple of cisplatin and gemcitabine and either mAbs was more effective than either single agent alone in all cell lines with most treatment triplets inducing >90% cytotoxicity. We examined the effect of pre-treating CCA cell lines with a single chemotherapeutic agent 24 hours before treatment with either mapatumumab or lexatumumab. Interestingly, pre-treatment with a single chemotherapeutic agent followed by mapatumumab or lexatumumab increased cytotoxicity more than that observed in co-treatment with the same single agent and either mAbs in most cell lines. In some cell lines, pre-treatment with cisplatin followed by mapatumumab was as effective as co-treatment with cisplatin, gemcitabine and mapatumumab. We conclude that pre-treatment with chemotherapeutic agents can alter the sensitivity of CCA cell lines to TRAIL-R mAbs.

Similarly, in a xenograft model of CCA, mapatumumab and either concurrent cisplatin and gemcitabine were more effective than either chemotherapy or mapatumumab alone. In addition, pre-treatment with chemotherapy was as effective as co-treatment with the triplet in inhibiting tumor growth. This in vivo result confirms the conclusion that chemotherapy can enhance the anti-tumor activity of mapatumumab and shows that pre-treatment with chemotherapy is a potential strategy for enhancing this activity. We continue to investigate the mechanism of increased TRAIL-R mAb sensitivity to pre-treatment with chemotherapy in CCA. These results demonstrate the significant anti-tumor activity of the TRAIL-R mAbs mapatumumab and lexatumumab as therapeutic agonist monoclonal antibodies that can augment the activity of cytotoxic agents used in the treatment of human cancer. The preclinical data supports the evaluation of these chemotherapeutic agents in combination with TRAIL-R mAbs in future clinical studies, particularly in biliary cancer.


The oncoproteins E6 and E7 of human papillomavirus type 16 (HPV-16) efficiently immortalize cervical keratinocytes, induce tumors in transgenic mice and correlate with cervical cancer. For this purpose we have utilized hairpin ribozymes derived from the negative strand of the tobacco ring spot virus (-TRSV). Previously, we reported engineered hairpin ribozymes (R419 and R434) caused down-regulation of HPV-16 E6/E7 mRNA and inhibited growth of both HPV-16 immortalized cells and tumor cells. Due to the exquisite specificity of ribozymes, target sequence variability remains an issue concerning ribozyme performance in vivo. To overcome HPV-16 variability, R419 and R434 were incorporated into two different triplex expression systems to allow for multiple ribozyme expression. One system used a canonical three-ribozyme cassette (G1) and the second system was based on a single hairpin ribozyme (G2) to perform trimming and trans-cleaving functions. Both triplex configurations facilitated individual activity of multiple trans-acting ribozymes resulting in increased degradation of E6 mRNA in vitro. Duplex G2 R434 was more efficient in cleaving E6 mRNA than duplex R434, suggesting that the release of individual ribozymes enhanced G2 trans kinetics. A multiplex G2 cassette was constructed with both R419 and R434 resulting in more efficient and independent trans-
cleavage kinetics than the duplex R343-based G2 ribozyme. Analysis of G2-R343 in SihA and C33-A cells showed correct releasing of R343 in the intracellular environment indicating that the G2 system performed adequately in vivo to produce the specific down-regulation of E6/E7 mRNA. These ribozymes have potential therapeutic value in direct application to the cervix.

B52 A phase I study of IMC-11F8, a recombinant human anti-epidermal growth factor receptor (EGFR) IgG, monoclonal antibody in patients with solid tumors. Bart Kuenen1, Petronella Oda Witteveen2, Rita Ruijter1, Manuel Tijn-A-Tor2, Hopop Youssofian2, Eric Rownisky3, Floyd Fox1, Guojun Wang1, Giuseppe Giaccone1, Voest Emile1. 1 Free University Hospital of Amsterdam, Amsterdam, The Netherlands; 2 University Medical Center Utrecht, Utrecht, The Netherlands; 3 ImClone Systems Incorporated, Branchburg, NJ.

Background: This phase I study was conducted to determine the safety profile and recommended dose of IMC-11F8, a recombinant human IgG, monoclonal antibody that targets the EGFR.

Methods: Patients (pts) with advanced solid malignancies who were refractory to or had no available standard therapy received IMC-11F8 intravenously at 100, 200, 400, 600, 800, or 1000 mg either weekly (Arm A) or every other week (Arm B) for 6 weeks (1 cycle). Prior to the initial cycle, pts received one IMC-11F8 infusion at their assigned cohort followed by a 2-week pharmacokinetic (PK) period. Pts with stable disease or response after cycle 1 were eligible to receive additional cycles of IMC-11F8.

Results: 60 pts were enrolled in the 100-, 200-, 400-, 600-, 800-, and 1000-mg cohorts. 29 pts (M/F 11/18, median age 60 years) received IMC-11F8 weekly and 31 pts (M/F 14/17, median age 59 years) received IMC-11F8 every other week. Dose limiting toxicities (DLTs) of nausea, vomiting, and headache were observed in the peri-infusional period in 2 patients in the 1000-mg cohort with IMC-11F8 administered every other week. Grade 3-4 adverse events considered at least possibly related to IMC-11F8 include fatigue and acne in the weekly dosing schedule and anemia, diarrhea, nausea, vomiting, fatigue, decreased blood magnesium, hypokalemia, headache, and acne in the every other week dosing schedule. Two pts achieved partial responses including 1 pt with melanoma in the 200-mg cohort after 14 weeks of treatment with IMC-11F8 administered once a week (duration of response=15.6 months) and 1 pt with rectal cancer in the 400-mg cohort after 12 weeks of treatment with IMC-11F8 administered every other week (duration of response=5.6 months). An additional 16 pts (Arm A=8 pts, Arm B=8 pts) in the 200- to 1000-mg cohorts had stable disease and received from 10 to 49 weeks of IMC-11F8 treatment; cancer types include colorectal, renal, prostate, melanoma, ovarian, cholangiocarcinoma, and sinus piniformis. A noncompartamental analysis of 39 pts (Arm A=17, Arm B=22) demonstrated that IMC-11F8 exhibits nonlinear PK. As IMC-11F8 escalated from 100 to 1000 mg, T1/2 increased from 70 to 351 hrs and 30 to 138 hrs, Cmax increased from 40 to 804 µg/mL and 33 to 885 µg/mL, AUCinf increased from 2968 to 304457 hr*µg/mL and 1224 to 147879 hr*µg/mL, and CL decreased from 41.6 to 3.8 mL/hr and 90.2 to 7.1 mL/hr in Arm A and Arm B, respectively.

Conclusion: IMC-11F8 is well tolerated and no unanticipated safety signals were observed. The maximum tolerated dose of IMC-11F8 was determined to be 800-mg administered every other week. Antitumor activity included 2 pts with tumor regression and several others with prolonged disease stabilization.

B53 Preclinical evaluation of huC242-DM4 in tumor xenograft models of canag-positive human gastric cancer. Christina N. Carrigan1, Michele F. Mayo1, Hongsheng Xie2, John M. Lambert1, Chari V. J. Chari1, Gillian Payne1, Robert J. Lutz1. 1 ImmunoGen, Inc., Cambridge, MA.

HuC242-DM4 is a novel, targeted antitumor agent in development for the treatment of CanAg-expressing tumors. It is formed by the conjugation of the potent maytansinoid cell-killing agent, DM4, to the CanAg-binding humanized antibody, huC242. HuC242-DM4 currently is in Phase I testing for the treatment of gastric and gastroesophageal junction cancer. The rationale for the clinical trial is provided by in vitro characterization including immunohistochemistry studies, and in vivo preclinical evaluation of the compound. To identify the distribution of the CanAg on human esophageal and gastric cancer, immunoreactivity of the murine C242 antibody was assessed with formalin-fixed, paraffin-embedded human tumor samples. Nine out of eleven (82%) patients with adenocarcinoma and eight out of nine (89%) patients with squamous cell subtype stained positive with murine C242 antibody on esophageal tumor specimens. Thirteen of twenty-one (62%) gastric patient specimens stained positive with the antibody. Our preclinical assessment of its activity included evaluation in xenograft tumor models. In mice bearing NCI-N87 (human gastric carcinoma, CanAg+) tumors, huC242-DM4 caused complete tumor regressions with single intravenous administration as low as 3.5 mg of conjugate protein per kg, well below the estimated maximal tolerated dose of about 50 mg of conjugate protein per kg in mice. The lowest single dose tested, 1.8 mg/kg, resulted in log10 cell kill of 1.27. At the highest dose tested, a single injection of 18.9 mg/kg, huC242-DM4 caused complete tumor regressions in 5 of 6 mice with a mean tumor-free period of 55 ± 36 days. No body weight loss was observed at any dose. Similar results were found in a second model of gastric carcinoma using xenografts of the SNU 16 cell line. For active doses, the pharmacokinetics and the area under the curve (AUC) of tumor drug exposure in mice were compared to the pharmacokinetics and AUC of huC242-DM4 in Phase I human testing. The analysis indicates that the Phase II dose of 168 mg/m2 in humans is likely to achieve the blood levels (plasma exposure) found to be efficacious in preclinical evaluation.

B54 Preclinical evaluation of PEGylated tumor necrosis factor alpha in dogs with spontaneous tumors: Results of phase-I investigation. Douglas H. Thamm1, Mike A. Clark1, Ilene D. Kurzman2, Susan L. Kraft1, Daniel L. Gustafson1, Susan Plaza1, Christie Anderson1, David M. Vail1. 1 Colorado State University, Fort Collins, CO; 2 University of Wisconsin - Madison, Madison, WI.

Tumor necrosis factor (TNF) is a cytokine capable of potent antitumor activity, but its toxicity and short half-life following systemic administration have limited its utility as an anticancer agent. Conjugation of various biotherapeutics to polyethylene glycol (PEG) has been shown to alter beneficially their immunogenicity, bioactivity and half-life. We previously demonstrated that Pegylation of TNF (PEG-TNF) results in significantly increased plasma half-life and diminished toxicity in mice, translating into enhanced antitumor activity. We furthermore have demonstrated significantly diminished toxicity in normal dogs, when compared with unconjugated TNF. The goal of the current study was to evaluate the toxicity and biologic effect of escalating doses of PEG-TNF in dogs with spontaneous neoplasia.

This phase-I clinical trial enrolled dogs with measurable spontaneous malignancies of any histotype, for which standard therapy had failed or had been declined by the owner. Dogs received escalating doses of PEG-TNF, administered as a rapid intravenous bolus once every 3 weeks, according to a standard 3+3 dose escalation scheme. Vital signs, blood pressure, blood oxygenation, and hematologic and biochemical parameters were evaluated serially following treatment, and a subset of patients underwent dynamic contrast-enhanced MRI (DCE-MRI) following treatment to assess changes in tumor blood flow and perfusion. Fifteen dogs were enrolled in the study. PEG-TNF doses of 20, 23.3, 26.7, and 30.0 mcg/kg were administered. In the 30.0 mcg/kg dose cohort, 2 of 3 dogs experienced dose-limiting toxicity, consisting of vascular leak in 1 and hypotension in 1, establishing 26.7 mcg/kg as the maximum tolerated dose for subsequent study. Mean elimination half-life was 14.1 +/- 5.4 h, and peak plasma concentration correlated weakly with administered dose (ρ = 0.059). Evidence of biologic activity, in the form of transient fever and profound but transient leukopenia, was observed at all dose levels. Changes in tumor blood flow were demonstrated in the majority of dogs whose tumors were evaluated by DCE-MRI. Minor or transient antitumor responses were observed in dogs with melanoma, head and neck squamous cell carcinoma and mammary carcinoma, and a partial response, persisting 3 months, was observed in a dog with angiosarcoma.
In conclusion, using a clinically relevant, spontaneous large animal model of neoplasia, we have demonstrated that biologically relevant doses of PEGylated TNF can be administered safely, and that PEG-TNF administration is associated with encouraging antitumor activity. Ongoing canine studies are evaluating the antitumor effect of postoperative PEG-TNF in canine angiosarcoma and the efficacy and tolerability of PEG-TNF in combination with the traditional chemotherapeutic agent doxorubicin.

**B55** FP-1039 (FGFR1:Fc), a soluble FGFR1 receptor antagonist, inhibits tumor growth and angiogenesis. Hongbing Zhang1, Masuoka K. Lorianne1, Kevin P. Baker1, Ali Sadra1, Elizabeth Boschi1, Tom Brennan1, Stephen Dobnerstein1, Gilbert Goodworth1, Kevin Hestir1, Diane Hollenbaugh2, Li Long1, Minmin Qin1, Lewis T Williams1. 1Five Prime Therapeutics, Inc., San Francisco, CA; 2Schering Plough, Palo Alto, CA.

FP-1039 is a soluble fusion protein consisting of the extracellular domain of human fibroblast growth factor receptor 1c (FGFR1) linked to the Fc portion of human IgG1. In these studies, we investigate the anti-tumor effect of FP-1039 in cell line-derived and primary human tumor-derived xenograft models, and quantify the anti-angiogenic effect on both fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) induced angiogenesis. Most of the known 23 human FGF ligands bind to more than one FGF receptor subtype; therefore, a drug that specifically targets the FGFR1 receptor, such as an anti-FGFR1 monoclonal antibody, will not prevent binding of FGF1 ligands to other FGF receptor subtypes. Thus, FP-1039 was engineered to prevent all FGFR1 ligands from binding to any of the four FGF receptors.

The FGF-FGFR signaling pathway is implicated in the pathogenesis of many cancers. The gene encoding FGFR1 is located within a region on chromosome 8 (8p11-12) that is amplified within specific subsets of breast cancer patients for whom the amplification strongly predicts shorter metastasis-free survival. FGFR1 ligands, such as FGF-2, possess not only mitogenic activity, but also have potent angiogenic activities that promote tumor progression. This dual cancer promoting mechanism of tumor growth stimulation and angiogenesis makes FGF-FGFR signaling an attractive target for therapeutic intervention.

FP-1039 inhibits the growth of several human cell-line derived tumors in xenograft models, including Caki-1, A549 and MCF-7. It also inhibits tumor growth in specific human primary tumor derived xenografts which are well characterized with respect to key cell surface markers associated with the FGF-FGFR pathway. Such models may greatly facilitate our ability to identify tumor markers associated with response specificity to FP-1039, and may facilitate patient selection criteria for clinical trials.

To assess the anti-angiogenic activity of FP-1039 in vivo we employed a model in which neovascularization is driven by the presence of human angiogenic factors in a matrigel plug that is implanted subcutaneously into mice. In a dose dependent fashion, FP-1039 completely inhibited the neovascularization induced by either FGF-2 or VEGF alone, and that induced by a combination of FGF-2 plus VEGF. Endothelial staining of tumor sections from a Caki-1 xenograft in which FP-1039 treatment resulted in a dose dependent inhibition of tumor growth also revealed qualitative differences in the tumor vasculature including a marked reduction in vessel density compared to animals treated with control.

The preclinical studies performed thus far demonstrate the anti-tumor effect of FP-1039 in both cell line-derived and primary human tumor-derived xenograft models, and the potent inhibition of both FGF and VEGF-induced angiogenesis. These data indicate the potential for FP-1039 to be a selective and potent anti-cancer drug for the treatment of certain cancers in which the FGF-FGFR pathway plays an important role in supporting the tumor/host interaction, while possibly avoiding some of the dose limiting toxicities identified previously with oral tyrosine kinase inhibitors that block multiple tyrosine kinases.

**B56** Development of a recombinant single-chain variable fragment (scFv) antibody against MRP3 (multi drug resistance protein) for immunotargeting of GBM (glioblastoma multiforme). Nidhi Srivastava1, Chein-Tsun Kuan1, Wayne Marascan1, Darell D Bigner1. 1DUMC, Durham, NC; 2Dana-Farber Cancer Institute, Boston, MA.

MRP3 is a membrane bound, active transport carrier which confers resistance to chemotherapeutic agents. It has been identified as one of the cell surface expressed molecular targets for GBMs, where it is over expressed but not present in normal brain. Use of recombinant antibody fragments, armed or unarmed, has recently emerged as an important tool in therapy for a variety of cancers. The goal of this project is to develop a single chain Fv fragment antibody capable of binding to MRP3 with high affinity. In the present study, we have demonstrated isolation of a novel recombinant scFv, MS8 from a naive human scFv phage display library, againstiste the extracellular NH2 terminus of the human MRP3, Pep I (1-20 aa), with the affinity of Kd, 4.8 X10^-8 M. In ELISA it only reacted specifically with the immobilized Pep I antigen of the human MRP3 but did not cross react with other MRP3 derived peptides and furthermore, the reactivity was found to be dose dependent. Flow cytometry revealed that MS8 scFv binds specifically to fixed MRP3 expressing GBM cells (D54 MG) and after labeling using iodogen, 53% of radioactive scFv was found to be immunoactive. It was further characterized for its surface binding by inhibition assay with D54 MG cells, viable cells were found to inhibit 50-57% binding of MS8 scFv to the Pepl. With it’s smaller size, selective binding and good affinity to the extracellular epitope of MRP3, MS8 scFv may possibly be a good candidate for use in immunotargeting for GBM.

**B57** Antibodies generated by conditioned cell immunization technology bind to beta 5 integrin and synergize with chemotherapy and radiation to inhibit the growth of human tumor cells. Kathleen L. King1, Mark Armanini1, Francine Chen1, Adam Cunha2, Key Kang1, Tony Liang1, Monica Licea1, Irene Ni1, Beverly Potts1, Penelope Roberts1, George Schreiner1. 1Raven Biotechnologies, South San Francisco, CA.

Monoclonal antibodies are generally used clinically in conjunction with standard therapies for the treatment of cancer. As such the repertoire of potentially clinically relevant cell surface targets consists not only of those antigens on the surface of untreated tumor cells, but also those targets on the surface of cells that are responding to a cytotoxic insult from radiation and/or chemotherapy. We recently reported the development of a novel technology, conditioned cell immunization, to take advantage of this critical functional association between cell surface target and approved therapies. To date we have generated 150 monoclonal antibodies by immunizing mice with human tumor progenitor cell lines pretreated with chemotherapy or radiation and derived from prostate, lung, colon and breast resections. These antibodies were selected on the basis of minimal normal tissue binding. A subset of these antibodies has shown dose dependent inhibition of the growth of human tumor cell lines in monolayer culture. Three of these antibodies bind to the integrin subunit beta 5, reported to be involved in tumor neovascularization and growth. The data presented here demonstrate synergism in combination studies with these beta 5 integrin antibodies and both chemotherapy and radiation for the inhibition of human tumor cell growth in monolayer culture. These data constitute proof of concept for the use of the conditioned cell immunization technology for the production of monoclonal antibodies that could serve as adjuvant therapy when combined with standard approved chemotherapeutic drugs or radiation.

**B58** PCNA is a sensitive target of photodynamic damage. Soo In Bae1, Robert M Snapka1. 1The Ohio State Univ., Columbus, OH.

Photodynamic therapy (PDT) employs a combination of photoactivatable drugs and light to generate highly reactive molecules capable of damaging crucial biological targets and killing tumor cells, fungi, bacteria or viruses. Singlet oxygen is thought to be the major cytotoxic species in PDT. Numerous molecular and cellular targets for PDT
have been suggested, including cellular membranes, lysosomes, mitochondria, DNA, RNA and proteins. Photodynamic damage to these targets varies with the intracellular localization of the photodynamic drug. We have found that PCNA is an extremely sensitive target of singlet oxygen generated by photodynamic drugs. Photodynamic damage causes covalent trimerization of PCNA which is easily detected as a 95 kDa PCNA reactive band by Western blotting. The photodynamic covalent trimerization of PCNA by the tested drugs was suppressed by histidine, a singlet oxygen quencher and enhanced by D2O which increases the lifetime of singlet oxygen by ~20X. Using this biomarker for photodynamic damage, we were able to demonstrate singlet oxygen production in cells by drugs such as camptothecin and doxorubicin, which are extremely weak photodynamic generators of singlet oxygen. Cytoplasmic localizing photodynamic drugs such as hypericin and NP6 caused PCNA covalent trimerization comparable to that caused by DNA binding photodynamic drugs such as acridine orange, proflavine, methylene blue and ellipticine, suggesting that the reactive species can easily access the nucleus. The photodynamic action of these drugs also caused crosslinking of additional proteins to PCNA. One of these species, migrating as a 148 kDa band on gels, contained only PCNA as determined by nano-LC/MS/MS and may be either a PCNA tetramer or a double trimer. Other high molecular weight bands appear to be heterologous proteins crosslinked to PCNA. The kinetics and dose-response of crosslinking of the PCNA trimer differed greatly (orders of magnitude) from the crosslinking of the 148 kDa species and the heterologous proteins. The identity of the photodynamic drug also affected the kinetics of crosslinking slightly. The pattern of high molecular weight PCNA bands resulting from photodynamic damage was identical for all the drugs and cell lines studied. The covalent trimerization of PCNA provides an extremely sensitive biomarker for photodynamic singlet oxygen damage which can be used to evaluate new drugs and antioxidants, and may also be useful for evaluation of the efficiency of photodynamic therapy. This work was supported by NIH NC1 CA97107.

B59 A human monoclonal antibody to a novel ovarian cancer target inhibits tumor growth. Steven B. Kanner1, Nandini Ganguly1, Kendall R Morrison1, Zili An1, Karen J Morrison1, Pia M Chalilla-Eid1, Arthur B Raitano1, Daulet Satpaev1, Xiao-Chi Jia1, Jean M Gudas1, Aya Jakobovits1.1 Agensys, Inc., Santa Monica, CA.

We identified a novel human cell surface protein, AGS-8, which is expressed in >70% of ovarian cancers, predominantly in advanced stages of serous adenocarcinoma. Amongst normal tissues, AGS-8 expression is restricted to testis and the red pulp of spleen. In vitro studies with AGS-8 expressing cells indicated an involvement of AGS-8 in tumor cell survival, migration, invasion and cell-cell interaction. Furthermore, a purified AGS-8 extracellular domain (ECD) protein mediated Ca2+-dependent binding to human umbilical vein endothelial cells (HUVEC), supported their survival and induced tube formation in vitro. Recombinant expression of AGS-8 in murine fibroblasts was oncogenic, leading to aggressive tumor growth and metastasis in mice. Additionally, these cells induced significant angiogenesis when implanted in vivo. Recombinant AGS-8 expression in ovarian carcinoma cells (OVCAR-5) led to enhancement of their intrinsic tumor growth in vivo.

XenoMouse® technology was employed to generate a panel of high affinity fully human monoclonal antibodies (mAbs) to AGS-8, targeting diverse epitope groups. One mAB that exhibited potent inhibitory activity in all functional assays was AGS-8M4, an IgGκ MAb with a Kd = 5 x 10^{-9} M for cell surface AGS-8. AGS-8M4 dose-dependently inhibited tube formation in vitro. In addition, AGS-8M4 inhibited the migration, invasion and cell-cell interaction of AGS-8 expressing cells. Significant anti-tumor efficacy of AGS-8M4 in vivo was observed in both recombinant models and in endogenous models, either during tumor formation or in well-established tumors. Efficacy of AGS-8M4 was maintained after MAb de-glycosylation, indicating an intrinsic anti-tumor activity of the MAb without significant contribution by immune effector functions. AGS-8M4 inhibited lung metastases in the 3T3/AGS-8 tumor model, further suggesting a role of AGS-8 in tumor cell migration and invasion. Studies evaluating the anti-tumor efficacy of AGS-8M4 with drug combinations are underway. Together, these data establish AGS-8 as a novel and attractive ovarian cancer target and AGS-8M4 as a potential therapeutic MAB for this cancer indication.

B60 Humanized anti-CD19 auristatin antibody-drug conjugates display potent antitumor activity in preclinical models of B-cell malignancies. Hans-Peter Gerber1, Carol Morris-Tilden1, Ivan Stone1, Mechthild Jonas1, May Kung-Sutherland1, Jamie Miyamoto1, Lindsay Brown1, Lori Westendorf1, Damon Meyer1, Django Sussman1, Paul Carter1, Che-Leung Law1, Iqbal S Grewal1.1 Seattle Genetics, Bothell, WA.

CD19 is a pan-B cell membrane glycoprotein that is expressed from early stages of pre-B cell development through terminal differentiation, regulating B lymphocyte proliferation and function. Expression of CD19 was identified on most cancers of lymphoid origin, on the vast majority of Non-Hodgkin’s Lymphoma (NHL) and on leukemias, including Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL) and Waldenstrom’s Macroglobulinemia (WM). Despite major improvements in the treatment of NHL and CLL patients, the majority will continue to relapse and salvage regimens with non-cross resistant compounds are required to improve patient survival. Here we describe an anti-CD19 antibody with rapid internalization characteristics, that induces potent tumor cell killing when conjugated with derivatives of the anti-tubulin agent auristatin. Potent antitumor activities starting in the sub-nanomolar range were observed when anti-CD19-mcMMAF and -vcMMAE conjugates were tested on a variety of human lymphoma cell lines grown in culture. The anti-CD19 antibody was humanized and binds with sub-nanomolar affinity to both human and cynomolgus monkey CD19. Antibody-drug conjugates (ADCs) consisting of mcMMAF or vcMMAE displayed typical PK characteristics when tested in rodents and both compounds were equally effective when administered to mice xenografted with various NHL tumor types, including Ramos (Burkitt), DoHH2 (Mantle Cell) and a model of ALL (Nalm-6). The antitumor effects were associated with an increase in tumor cell apoptosis and frequently resulted in durable tumor regressions in subcutaneous or disseminated models. Combined, our data suggest that anti-CD19-mcMMAF and -vcMMAE conjugates are promising therapeutics for the treatment of NHL and ALL indications.

B61 The use of pharmacokinetic and pharmacodynamic data to optimize the dose and schedule of an EphA2 antibody drug conjugate in vivo. Krista Kinner1, Laura Camara1, Shenlan Mao1, John Gooya1, Ruth Moser2, Damon Meyer2, Peter Senter2, Peter Kiener2, Steve Coats3, Bahija Jallal1, David Tice1, Dowdy Jackson1.1 MedImmune, Gaithersburg, MD; 2 Seattle Genetics, Bothell, WA.

The EphA2 receptor tyrosine kinase is selectively expressed on the surface of many different human tumors. We have previously demonstrated that the EphA2 on tumor cells can be targeted by monoclonal antibodies and that these antibodies function, in part, by inducing EphA2 internalization and degradation. Monoclonal antibodies that selectively bind and internalize into tumor cells provide a vehicle for targeted delivery of cytotoxins. Thus, antibody-drug conjugates (ADC) increase the therapeutic index of the attached cytotoxins by reducing systemic toxicity and enhancing tumor targeting and efficacy. Here we explore the relationship between the pharmacokinetics of the EphA2 ADC, the pharmacodynamics of EphA2 in the prostate tumor (PC3) and tumor efficacy.

The EphA2 ADC has a serum half life of approximately 50 hours and a tumor half life of approximately 60 hours. The tumor exposures were approximately dose proportional while the serum exposures were not. Following treatment with the EphA2 ADC, the EphA2 receptor is degraded in a time and dose dependent manner. We used this observation as our pharmacodynamic marker. A single dose of the EphA2 ADC at the 1, 5 and 10 mg/kg dose levels resulted in rapid and prolonged degradation of the EphA2 receptor in a PC3 tumor xenograft model, which began one hour post dose and lasted for at least six days at the higher doses. The 10
mg/kg dose of the EphA2 ADC did not result enhanced degradation of EphA2, thus setting our dosing limits between 1 and 5 mg/kg. We show that once/week administration of the EphA2 ADC gave comparable efficacy to a twice/week dosing schedule in the PC3 tumor model. In conclusion we report that the use of pharmacokinetic and pharmacodynamic data can provide guidance in the planning of tumor efficacy studies using the EphA2 ADC.

B62 Tyrosine kinase of insulin-like growth factor receptor as a target for chemoprevention and treatment strategy in NSCLC. Ho-Young Lee 1, Young Lee 1. 1UT M.D. Anderson Cancer Ctr., Houston, TX.

NSCLC is the leading cause of cancer-related death in the United States and worldwide. Recent application of EGFR tyrosine kinase inhibitors in the treatment of NSCLC could only benefit a small fraction of NSCLC patients. To find new therapeutic options for those who are insensitive to EGFR TKI, we investigated roles of IGF-1R signaling in NSCLC. We analyzed antitumor activity of PQIP, a novel small molecule IGF-1R tyrosine kinase inhibitor, in cigarette carcinogen induced lung cancer models in vitro and in vivo, single and in combination with erlotinib, an EGFR tyrosine kinase inhibitor. We found malignant human bronchial epithelial (HBE) cells have increased dependence on IGF-1R signaling for proliferation and survival. IGF-1R is mainly coupled with Akt pathway, but not MAPK pathway in NSCLC cells. PQIP inhibited cancer cell proliferation through G1 cell cycle arrest and apoptosis. IGF and EGFR pathways can transactivate each other and cooperate to stimulate cancer cell proliferation. Finally, we demonstrated PQIP inhibited lung tumor formation and progression in vivo in a transgenic mouse, in which IGF-1 is exclusively expressed in the lung. Taken together, IGF-IR-targeting agents or in combination with EGFR targeting agents, may be effective chemopreventive and therapeutic approaches for NSCLC.


CD40, a TNF receptor family member, is expressed on normal B lymphocytes, non-Hodgkin’s lymphoma (NHL), multiple myeloma, and carcinomas. SGN-40, a humanized anti-CD40 antibody, is a partial agonist that induces apoptosis and also mediates ADCC against CD40+ NHL B cell lines, resulting in antitumor activity in human lymphoma xenograft models. Previous work demonstrated that SGN-40 activates multiple signaling cascades including the MAP Kinases p42/44 (ERK1/2) and stress-responsive JNK. The current study reveals two potential mechanisms by which SGN-40 signaling through CD40 contributes to apoptosis in transformed lymphoma cells. SGN-40 treatment resulted in a significant and sustained downregulation of BCL-6, a protein implicated in lymphomagenesis. Constitutive activation of ERK1/2 MAPK signaling by SGN-40 correlated with the loss of BCL-6 protein, consistent with phosphorylation-induced proteasomal degradation. BCL-6 is a transcriptional repressor which prevents B cell maturation by blocking Blimp-1 and BAX expression, resulting in upregulation of c-Myc. As predicted by this model, SGN-40 mediated BCL-6 downregulation was associated with a decrease in c-Myc protein and increased expression of an active BAX-1 splice variant characteristic of plasma cell differentiation. Through a distinct molecular mechanism, it was found that SGN-40 strongly upregulates the p53 family member TAp63α, in three of five lymphoma lines examined. SGN-40 also induces expression of CD95 (Fas), a TAp63α transcriptional target, suggesting that SGN-40 can promote apoptosis in lymphoma by the direct upregulation of pro-apoptotic proteins in the extrinsic apoptotic signaling pathway. Furthermore, TAp63 has been reported to sensitize cells to cytotoxic drugs. Consistent with this, SGN-40 treatment was found to sensitize lymphoma cells to chemotherapeutic agents, resulting in increased cell death. Collectively, our data suggest that SGN-40 signaling may contribute to cell death in NHL by at least two mechanisms: 1) BCL-6 degradation, triggering cell cycle exit and differentiation and 2) increased expression of TAp63α and pro-apoptotic proteins. This is the first instance reported of a therapeutic antibody able to modulate these two pathways, providing a novel approach to the treatment of B cell lymphoma. Future studies will assess functional perturbation of the described signaling events as well as investigate their prevalence in a larger panel of cell lines and ultimate importance for the observed clinical activity of SGN-40.

B64 CT-322, a specific VEGFR-2-blocking protein, significantly alters the morphology and reduces the density of tumor vasculature in conjunction with its anti-tumor activity in a preclinical tumor model. Irvinth M. Carvajal 1, Janette Knowlton 1, Brent Morse 1, Jennifer Jobin 1, Miguel Moreta 1, Henry Wong 1, Moritz Konrerdz 1, Roni Mamluk 2, John Mendlein 2, Eric Furfin 3, 1Adnexus Therapeutics Inc., Waltham, MA; 2University of Mainz, Mainz, Germany; 3Chisima, Jerusalem, Israel.

VEGFR-2 is a key regulator of endothelial cell functions in both physiological and pathological angiogenesis. Blockade of VEGFR-2 function in tumor angiogenesis has been extensively studied and the pathway has been validated as target for cancer therapy. CT-322 is a pegylated Adnectin that selectively blocks VEGFR-2 thereby inhibiting VEGFR-2-mediated endothelial cellular functions in vitro and in vivo. Adnectins are a new class of protein therapeutics designed from a domain of human fibronectin. CT-322 is the first product in this class to enter clinical trials and is currently in Phase 1 development in cancer patients. CT-322 binds to the extracellular domain of VEGFR-2 and blocks its activation by all of its known ligands: VEGF-A, -C and -D. In vivo, CT-322 has demonstrated antitumor activity in a wide variety of tumor models as a monotherapy in both xenograft and orthotopic models. In addition, the combination of CT-322 with cytotoxic chemotherapies showed additive effects on the inhibition of tumor growth in tumor models. In this study we evaluated the effects of CT-322 on tumor vasculature density and morphology in a xenograft tumor model. The results indicated an association between CT-322-mediated inhibition of tumor growth and relevant structural changes in tumor vasculature. Quantitative 2D immuno-histochemical analyses of an endothelial vascular staining marker, CD31, demonstrated a significant reduction of microvascular density in tumors from CT-322-treated animals as compared to vehicle-treated animals. Parallel 3D vascular casting studies corroborated the 2D findings confirming the decreased tumor vascular density under CT-322 treatment. The 3D vascular casting data enabled quantitative morphometric analysis of both intervessel and interbranching vessel tumor vessel distances; these measurements showed a significant reduction of both parameters in treated compared to control animals. In CT-322-treated animals immature blood vessels were trimmed and the abnormal appearance was reversed, as measured by a reduced chaotic tumor vessel branching. These observations support the hypothesis that CT-322-mediated inhibition of VEGF-2 signaling leads to reduction and remodeling of tumor vasculature in connection with its inhibitory effects on tumor growth.

Chemoprevention

B65 Preventive effects of the EGFR inhibitor Iressa on chemically-induced urinary bladder cancers: Histopathological and biochemical endpoints. Clinton J Grubbs 1, Ann M Bode 1, Vernon E Steele 1, M Margaret Juliana 1, Ronald A Lubet 2. 1The Hormel Institute, Austin, MN; 2National Cancer Institute, Bethesda, MD.

The hydroxybutyl(butyl) nitrosamine (OH-BBN) induced urinary bladder cancer model in rats has been used to examine potential chemopreventive agents. Our previous studies showed that a variety of genes associated with urinary bladder cancer in man (including KI 67, Cyclin D1, Myc and certain genes associated with the EGFR pathway) are similarly over-expressed in this rat model [Neoplasia 9: 207-21, 2007]. Female Fischer 344 rats were administered OH-BBN 2x/week for 8 weeks beginning at 56 days of age. The preventive efficacy of Iressa (Geftinib), 10 mg/kg BW/day,
was evaluated; administered by gavage beginning one week after the last dose of OH-BBN (115 days of age). This treatment reduced the incidence of large cancers from 48% (14/29) in OH-BBN controls to 7% (2/30). Virtually all the rats, however, developed microscopic bladder lesions irrespective of their treatment. In a second experiment, the ability of delayed administration of Iressa (10 mg/kg BW/day) beginning three months after the last dose of OH-BBN (192 days of age) was tested and found to reduce palpable cancers from 52% (control) to 4%. Prevention studies with two lower doses of Iressa (4.5 mg and 1.5 mg/kg BW/day) showed reductions in the incidence of large cancers from 52% in controls to 4 and 30%, respectively. Employing immunohistochemistry, it was found that Iressa (10 mg/kg BW/day) significantly decreased phosphorylated EGFR, MAPK, and AKT, as well as Ki 67, staining in bladder tumors from animals treated with Iressa. These studies show that an EGFR inhibitor was highly active in preventing urinary bladder cancer, and that delaying administration of Iressa until a time when a substantial number of microscopic lesions already existed (192 days of age) did not decrease the efficacy of this class of agents.

B66 A novel synthetic tricyclic compound with an acetylgen group at C-8a and cyano enones in rings A and C is a potent multifunctional agent in vitro and suppresses the formation of aflatoxin-DNA adducts in vivo. Karen Liby1, Tadashi Honda2, Mark M. Yore1, Chitra Sundararajan2, Hidenori Yoshizawa2, Rene Risingson3, Charlotte R. Williams1, Darlene B. Royce1, Alben T. Dinkova-Kostova1, Katherine K. Stephenson3, Patricia A. Egner4, Melinda S Yates4, John Groopman5, Thomas W. Kensler6, B. D. Roebuck7, Gordon W. Gribble1, Michael B. Sporn1, Dartmouth Medical School, Hanover, NH; 2Dartmouth College, Hanover, NH; 3Dartmouth Medical School, Baltimore, MD; 4Johns Hopkins University School of Medicine, Baltimore, MD.

TBE-31 is the lead molecule in a series of tricyclic compounds containing cyano enone functionalities in the A and C rings. Originally screened for its anti-inflammatory activity, TBE-31 inhibits the production of nitric oxide (\(\text{IC}_{50} = 1 \text{ mM}\)) and the inducible nitric oxide synthase protein in RAW264.7 macrophage-like cells stimulated with either interferon-gamma or lipopolysaccharide. Low nanomolar concentrations of TBE-31 also induce the phase 2 cytoprotective proteins heme oxygenase-1 and NAD(P)H quinone oxidoreductase and reduce the formation of reactive oxygen species in cells treated with the tert-butyl hydroperoxide. In U937 human leukemia cells, TBE-31 increases expression of CD11b, a marker of monocytic differentiation; inhibits proliferation; and induces apoptosis, as shown by increased PARP cleavage and annexin V staining. These results are seen with higher concentrations (100-1000 nM) than needed for the anti-inflammatory activity and phase 2 enzyme induction. The multifunctional activities of TBE-31 are similar to those of the synthetic oleanene triterpenoids, including CDDO-methyl ester and CDDO-imidazolide; CDDO-methyl ester is currently in phase II clinical trials for the treatment of melanoma and pancreatic cancer. The alpha, beta-unsaturated carbonyl groups in TBE-31 suggest it can form Michael adducts with protein targets containing reactive cysteine residues. Indeed, TBE-31, like the triterpenoids, directly interacts with dithiothreitol, as determined by a spectral shift when dithiothreitol is added to TBE-31. Identifying the direct molecular targets of TBE-31 is an area of active investigation. To determine whether biologically meaningful tissue levels could be obtained in vivo, CD-1 mice were gavaged with 1 micromole TBE-31. Six hours later, blood and tissues were harvested, extracted in acetonitrile and analyzed by liquid chromatography–mass spectrometry. The levels of TBE-31 in blood were 1 micromolar. Of the organs surveyed, the highest concentrations were found in the liver and pancreas, with levels of 0.7 and 0.5 micromole/kg, respectively. Because of the high drug levels in the rats, livers were gavaged with TBE-31 and then, 48 hours later, challenged with aflatoxin. At both 10 and 100 micromole/kg, TBE-31 significantly reduced the formation of aflatoxin-DNA adducts. In these studies, TBE-31 was more potent than CDDO-imidazolide, one of the most active compounds ever tested in this assay. Studies evaluating the ability of TBE-31 to block hepatic pre-neoplastic lesions induced by aflatoxin are ongoing. The exquisite potency of TBE-31 in vitro and in vivo suggest it should be tested for both chemoprevention and chemotherapy in relevant models of cancer and other chronic, degenerative diseases where inflammation and oxidative stress are known factors in the pathogenesis.

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B67 Regulation of growth of renal clear cell carcinoma by peroxisome proliferator-activated receptor gamma. Emad Zaki1, Mary L. Taub2, McMaster University, Hamilton, Ontario, Canada; 2State University of New York at Buffalo, Buffalo, NY.

Renal Clear Cell Carcinoma (RCC) is the tenth most prevalent cancer, accounting for 80% of all renal cancers. Recent results of a 15.3 year epidemiological study indicate that a diet of fatty fish offers significant protection against RCC, results which were attributed to omega-3 fatty acids. The question arises as to the molecular mechanisms by which such omega-3 fatty acids exert their protective effect. One possible mechanism is through the activation of Peroxisome Proliferator-Activated Receptors (PPARS) by omega-3 fatty acids, which presumably is altered during the process of tumorigenesis. In order to examine this hypothesis, normal renal proximal tubule cells have initially been examined with regards to their growth responses to PPAR agonists (the RCC is of renal proximal tubule origin). Towards these ends the effects of PPAR alpha and PPAR gamma agonists on the growth of a well-characterized primary renal proximal tubule cell culture system have been examined. The primary cultures were initiated from purified rabbit renal proximal tubules in serum free medium supplemented with 5 microgram/ml bovine insulin, 5 microgram/ml human transferrin and 5 x 10-8 M hydrocortisone. Fibrate drugs and thiazolidinediones (TZDs) have been identified which have a high affinity for PPAR alpha and PPAR gamma, respectively. The effects of the TZD drug troglitazone on the growth of primary renal proximal tubule cells were examined over a concentration range between 10-7 and 10-4 M. After a 7 day incubation period, cells were removed from culture dishes by trypsinization, and counted in a Coulter Counter. The results of independent studies indicated that troglitazone caused a 4-fold increase in cell number over a concentration range of 10-6 to 5 x 10-6 M. A physiologic PPAR gamma agonist, Prostaglandin J2 (PGJ2), was also growth stimulatory, albeit to a lesser extent than troglitazone. Both PPAR alpha and PPAR gamma are expressed in the renal proximal tubule. However, fenofibrate, a PPAR alpha agonist, had no significant effect on renal proximal tubule cell growth over a concentration range of 10-7 to 10-4 M. Unlike the results that we have obtained with our normal renal proximal tubule cells, several recent studies have indicated that troglitazone, and other TZDs (PPAR gamma agonists) induce growth arrest and apoptosis in RCC, as well as other urological cancers. Presumably, PPAR gamma signaling is altered in RCC. Indeed PPAR gamma is included amongst the proteins that are up-regulated in RCCs as a consequence of mutations in the von Hippel-Lindau suppressor gene (VHL). Thus, omega-3 fatty acids may act to prevent the progression of the tumor stem cells which have arisen as a result of such mutations in the VHL gene. Alternatively, omega-3 fatty acids may act to prevent metabolic processes necessary for the emergence of RCC cells, with their distinctive lipid droplets.

B68 Transcriptional activation of FoxO by resveratrol in prostate cancer. Sharmila Shankar1, Qinghe Chen1, Suthakar Ganapathy1, Rakesh K. Srivastava1, 1UT Health Science Center, Tyler, TX.

FoxO (forkhead box 0) transcription factors are crucial regulators of cell fate. This function of FoxO proteins relies on their ability to control diverse functions such as proliferation, differentiation, DNA repair and apoptosis. Constitutively active AKT in cancer cells has been shown to phosphorylate FoxO transcription factors (FKHR, FKHL1 and AFX), which bind to protein 14-3-3 and sequester in the cytoplasm. In the absence of survival signals, FoxO proteins dephosphorylate, translocate to the nucleus and upregulate a series of target genes, thereby promoting cell cycle arrest and apoptosis. The stilbene resveratrol exerts antiproliferative and proapoptotic actions on a number of different cancer cell lines through diverse mechanisms. The
Mechanisms by which resveratrol inhibits cell proliferation and induces apoptosis are poorly understood. The present study was designed to examine the regulation of phosphoinositide 3-kinase (PI3-K) signaling, and FoxO transcription factors by resveratrol in prostate cancer cells. Resveratrol inhibited viability and induced apoptosis in androgen-sensitive LNCaP and -insensitive PC-3 cells. Treatment of LNCaP cells with resveratrol resulted in induction of PTEN and inhibition of AKT activity. Downregulation of AKT by inhibitors of PI3K (Wortmannin and LY294002) and AKT, or by dominant negative AKT enhanced resveratrol-induced apoptosis, whereas transfection of constitutively active AKT attenuated this effect. Furthermore, resveratrol inhibited phosphorylation of FKHR, enhanced translocation of FKHR from cytoplasm to the nucleus, and increased FoxO-DNA binding and transcriptional activity. Overexpression of wild type FKHR and phosphorylation deficient mutants of FKHR (Thr-24, Ser-256 and Ser-319 replaced with alanine) enhanced resveratrol-induced apoptosis. These data suggest that resveratrol induces apoptosis through regulation of FoxO transcription factor, and it could be used as a chemopreventive agent for prostate cancer.

**Clinical Trials**

**B69**  A phase I dose-escalation study of the safety, pharmacokinetics, and pharmacodynamics of XL820 administered orally daily (QD) or twice daily (BID) to patients (pts) with solid malignancies. Mark Stein1, Salim Yaziji2, Jordi Rodon3, Dale Miles4, Pat O’Rourke5, Kyrilakas Papadopoulos2, Alain C. Mitra2. 1The Cancer Institute of New Jersey, New Brunswick, NJ; 2Exelixis, South San Francisco, California; 3Institute for Drug Development, San Antonio, TX.

**Background:** XL820 is a small molecule inhibitor of several receptor tyrosine kinases (RTKs) with growth-promoting and angiogenic properties. XL820 inhibits KIT, VEGFR2/KDR, PDGFR-α and -β in preclinical models in vitro and in vivo. In addition, in vitro XL820 inhibits wild-type and mutationally-activated KIT frequently found in tumors resistant to wildtype FKHR and phosphorylation deficient mutants of FKHR (Thr-24, Ser-256 and Ser-319 replaced with alanine) enhanced translocation of FKHR from cytoplasm to the nucleus, and increased FoxO-DNA binding and transcriptional activity. Overexpression of wild type FKHR and phosphorylation deficient mutants of FKHR (Thr-24, Ser-256 and Ser-319 replaced with alanine) enhanced resveratrol-induced apoptosis. These data suggest that resveratrol induces apoptosis through regulation of FoxO transcription factor, and it could be used as a chemopreventive agent for prostate cancer.

**Methods:** The purpose of this phase I dose escalation study is to define the maximum tolerated dose (MTD), pharmacokinetics (PK), and to assess the pharmacodynamic (PD) effects of XL820 in pts with advanced solid malignancies. Pts are enrolled in successive cohorts to receive XL820 orally on a 28 day schedule (QD or BID) dosing schedule. Safety is assessed continuously. Tumor response is assessed every 8 weeks by RECIST criteria. PK and PD blood samples are collected from all pts. Additional tissues, including hair, are also collected for PD analysis.

**Results:** As of 15 August 2007, 22 pts have been enrolled in 5 cohorts at 200 mg QD (n=5), 400 mg QD (n=3), 800 mg QD (n=3), 1600 mg QD (n=4), and 300 mg BID (n=7). Two DLTs have been observed at date 1, 1 pt with Grade 3 nausea in the 1600 mg QD cohort, and 1 pt with Grade 3 fatigue in the 300 mg BID cohort. Fourteen pts are currently evaluable for safety: the most common adverse events (AEs) deemed possibly or probably related to XL820 observed in >10% of pts consisted of nausea in 4 pts (29%), vomiting in 4 pts (29%), fatigue in 2 pts (14%), peripheral edema in 2 pts (14%), pyrexia in 2 pts (14%) and thrombocytopenia in 2 pts (14%). These side effects were generally mild or moderate (grade 1-2). Possibly or probably related SAEs occurred in two pts, one with Grade 2 rash in the 1600 mg QD cohort and one with Grade 3 fatigue in the 300 mg BID cohort. Three pts had stable disease (SD) for ≥16 weeks, 1 pt with bladder cancer in the 400 mg QD cohort, 1 pt with sarcoma and 1 pt with parotid gland cancer in the 1600 mg QD cohort. Preliminary non-compartmental PK analysis of the four QD dosing cohorts indicated rapid absorption of XL820 following oral administration (t max ranged from 1.3 to 6.5 hours on Day 1). Based on the concentration of XL820 in pre-dose samples, XL820 appeared to accumulate approximately 1.0 to 1.5-fold (based on C max) versus Day 1 with repeated QD dosing. Exposure to XL820 (ie, C max and AUC) generally increased less than in proportion to dose. Concentrations of XL820 for the 300 mg BID cohort on Day 8 appeared generally consistent with those seen for the 400 and 800 mg QD dosing cohorts. The PD effects of XL820 are currently being explored in patient tissues, including blood and hair follicles. Preliminary PD analyses of plasma samples demonstrated increased levels of both VEGF and PGF, and decreased levels of sVEGFR2 in some pts after administration of XL820. Correlations between plasma biomarker levels and plasma concentrations of XL820 were sometimes observed in individual pts, suggesting a potential exposure/response relationship.

**Conclusions:** XL820 was generally well tolerated when administered once or twice daily. The maximum administered dose was 1600 mg once daily. Preliminary pharmacodynamic analyses indicate that administration of XL820 potentially results in the inhibition of VEGF pathway signaling in some pts. Further evaluation of BID dosing is currently ongoing.

**B70**  A phase I and pharmacokinetic study of a CanAg-targeted immunoconjugate, HuC242-DM4, in patients with CanAg-expressing solid tumors. Kamalesh K. Sankhala1, Alain C. Mitra1, Alejandro D. Ricart1, Monica M. Mitra1, Chia C. Lin1, Leslie S. Wood1, John Sarantopoulos1, Chris H. Takimoto2, Amita Patnaik2, Albert Qin3, Sybil Zildjian3, Anthony W. Tolcher1. 1Institute for Drug Development, Cancer Therapy and Research Center, San Antonio, TX; 2South Texas Accelerated Research Therapeutics, San Antonio, TX; 3ImmunoGen Inc., Cambridge, MA.

**Background:** HuC242-DM4 is a novel, targeted anti-cancer agent in development for the treatment of CanAg-expressing tumors. The compound is formed by the conjugation of the potent cytotoxic maytansinoid drug, DM4, to the humanized monoclonal antibody, huC242. This agent was highly active across a broad spectrum of CanAg-expressing human tumor xenograft models.

**Methods:** Patients were enrolled with metastatic or inoperable colorectal, pancreatic, and other CanAg-expressing tumors who have failed standard therapy (about 95% of pts. had received ≥4 prior chemotherapy regimens).

**Results:** Thirty-six patients were treated with huC242-DM4, receiving a single intravenous (IV) infusion once every three weeks. Cohorts of 3 patients initially were enrolled on each dose level. Patients received huC242-DM4 at 183 pts (36, 60, 90, 126, 168, 223 (all)) mg/m2 dose level. The maximum tolerated dose (MTD) appears to be 168 mg/m2 every three weeks. Dose limiting toxicity (DLT) including decreased visual acuity, corneal deposits and keratitis was experienced by 2 of 6 patients treated at 223 mg/m2. The ocular adverse events have appeared to improve in patients where follow-up data is available. Additional patients are being enrolled at 168 mg/m2 dose level using preservative-free, lubricating eye drops as an exploratory preventative treatment for ocular toxicity. In addition, there were 12 grade 3 and 4 AEs that were considered likely drug-related. Of sixteen patients treated at the 168 mg/m2 dose level (including two patients who were dose reduced to 168 mg/m2), 11 patients have received at least two cycles of treatment. 4 of these 11 patients went on to receive three cycles of treatment and 3 of these 4 patients went on to receive at least four cycles of treatment. No partial or complete tumor responses have been observed to date. At the 168 mg/m2 dose level preliminary pharmacokinetic data reveal a half-life of huC242-DM4 of about 5 days, Cmax of 105.8 μg/mL, and a clearance of 16.7 mL/h/m2 in patients (9) with low plasma CanAg levels (< 800 units/mL). In patients (4) with high plasma CanAg levels (> 900 units/mL) at the 168 mg/m2 dose level, the half-life is 2.5 days, Cmax is 62.5 μg/mL, and the clearance of huC242-DM4 is 46.1 mL/h/m2. There has been no clinically significant myelosuppression and no formation of antibody to humanized antibody (HAHA) or drug (HADA) has been observed.

**Conclusions:** HuC242-DM4 at the 168 mg/m2 dose level has an acceptable safety profile and this dose level appears to be the MTD. Additional patients with pancreatic cancer and cholangiocarcinoma are planned to be enrolled at the MTD to gain more experience with huC242-DM4 in other CanAg-expressing tumor types. In addition, a phase II study with huC242-DM4 in patients with gastric and gastroesophageal junction CanAg-expressing cancers has been initiated.
B71 Phase I pharmacokinetic and pharmacodynamic study of the inhibitor of activation of Akt (p-Akt), triciribine phosphate monohydrate (TCN-PM), in solid malignancies. Christopher R. Garrett1, Domenico Coppola1, Robert M. Wenham1, Christopher L. Cubitt2, Anthony M. Neuger3, Timothy J. Frost4, Daniel M. Sullivan5, Jin Q. Cheng6, Said M. Sebti7.1H. Lee Moffitt Cancer Center, Tampa, FL.

Background: Akt is an important signal transduction kinase that is frequently dysregulated, and occasionally mutated, in cancer cells. Triciribine phosphate monohydrate (TCN-PM) is a tricyclic nucleoside that inhibits the levels of phosphorylated Akt (p-Akt) and suppresses selectively the growth of tumors with hyperactivated pAkt.

Methods: We conducted a phase I dose escalation study of TCN-PM administered intravenously weekly over one hour days 1, 8 and 15, with treatment cycles every 28 days, in adult subjects with advanced solid tumors refractory to standard therapy, whose tumors had been demonstrated to have high levels of p-Akt by immunohistochemistry (IHC). Tumor biopsies were obtained prior to initiation of therapy and on day +16, and assessed for the presence of p-Akt by IHC and Western blot analysis.

Results: Thirteen subjects have enrolled to date (M:F 5:8, mean age 60 years, range 46-81): eight subjects are evaluable for radiographic response (see table below).

Conclusions: Toxicity has been modest at the doses evaluated to date: accrual is ongoing. Updated pharmacokinetic and pharmacodynamic data will be presented at the meeting.

B72 A Phase 1b study to assess the safety of lexatumumab, a human monoclonal antibody that activates TRAIL-R2, in combination with gemcitabine, pemetrexed, doxorubicin or FOLFIRI. Branimir I. Sikic1, Heather A. Wakelee1, Margaret Von Mehren2, Nancy L. Lewis2, E. Ruth Plummer2, A. Hilary Calvert3, Norma Lynn Fox4, Elizabeth A. M. Neuger1, Suzanne F. Jones5, Howard A. Burns1, Stanford University, Stanford, CA; 2Fox Chase Cancer Center, Philadelphia, PA; 3Newcastle General Hospital, Newcastle, United Kingdom; 4Human Genome Sciences, Rockville, MD; 5The Sarah Cannon Research Institute, Nashville, TN.

Background: Lexatumumab (HGS-ETR2) is a fully-human agonistic monoclonal antibody that targets and activates the Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand Receptor 2 (TRAIL-R2). TRAIL-R2 is a member of the Tumor Necrosis Factor Receptor (TNFR) superfamily that, when activated, induces apoptosis via the extrinsic pathway. Lexatumumab shows promising anti-tumor activity in preclinical models, particularly in combination with chemotherapeutic agents. Single-agent lexatumumab was well-tolerated in Phase 1 trials. This is the first study of the safety of a TRAIL-R2 agonist in combination with chemotherapy.

Methods: Patients for whom gemcitabine, pemetrexed, doxorubicin or FOLFIRI was considered an appropriate treatment received one of the full-dose chemotherapy regimens plus lexatumumab every 2 weeks (for gemcitabine and FOLFIRI) or 3 weeks (for pemetrexed and doxorubicin). Four to 6 patients were treated with 5 mg/kg lexatumumab in each chemotherapy cohort prior to dose escalation to 10 mg/kg.

Results: 41 patients with a wide range of cancer types received 190 courses of lexatumumab over the 2 dose levels. The majority (33/41) received at least 2 courses (range 1 to 24). Lexatumumab was well tolerated. Severe adverse events considered at least possibly related to lexatumumab included neutropenia, fatigue, anemia, increase in blood amylase levels, stomatitis and dehydration. Tumor shrinkage was observed, and 3 patients had confirmed partial responses (2 colorectal cancer patients in the FOLFIRI arm and 1 small cell lung cancer patient in the doxorubicin arm). None of the chemotherapeutic regimens affected lexatumumab disposition, nor did lexatumumab affect the pharmacokinetics of gemcitabine, doxorubicin or irinotecan.

Conclusions: Lexatumumab can be safely administered in combination with a wide range of chemotherapeutic agents. Evaluation of the efficacy of lexatumumab in combination with chemotherapy in Phase 2 trials is warranted.

B73 Selective inhibition of CYP17 with abiraterone acetate is well tolerated and results in a high response rate in castration-resistant prostate cancer (CRPC). Gerhardt Attard1, Alison H.M. Reid1, Rajesh Sinha2, Rhoda Molife2, Florence Raynoud2, Mitch Dowsett2, Mary Barrett2, Emilda Thompson3, Timothy A. Yap1, Sarah Settare4, Vanessa Martins3, Christopher Parker2, David Dearmaley2, Elizabeth Folkard2, Gloria Lee2, Johann S. De Bono1.1The Royal Marsden NHS Foundation Trust and the Institute of Cancer Research, London, United Kingdom; 2The Royal Marsden NHS Foundation Trust, London, United Kingdom; 3Institute of Cancer Research, London, United Kingdom; 4Cougars Biotechnology, Los Angeles, CA.

Introduction: Despite androgen deprivation therapy, recent studies demonstrate high levels of intra-tumoral androgens and continued androgen receptor signalling in CRPC. AA is an oral, selective inhibitor of CYP17, a key enzyme in androgen synthesis.

Methods: AA was administered once daily, continuously: (i) to chemotherapy-naïve CRPC patients (pts) in a dose escalation phase I/II trial with phase II cohort expansion at the optimal dose (ii) at the optimal dose to pts previously treated with docetaxel chemotherapy in a parallel phase II study. All pts were resistant to castration, antiandrogens and frequently diethylstilboestrol and steroids. The primary objective of both studies was to evaluate the anti-tumor activity of AA with rejection of the null hypothesis if ≥7 pts from a maximum of 35 pts had a PSA decline ≥50% (Ho: ≤10%, Ha: >30% [power 86%; α 5%]). Secondary objectives included evaluating: (i) safety; (ii) CYP17 inhibition; (iii) pharmacokinetic (PK) profile; (iv) response rate by RECIST; (v) median time to progression (MTTP); (vi) reversal of resistance by addition of dexamethasone (D) 0.5mg od at progression on AA; (vii) circulating tumor cell (CTC) counts; (viii) ERG and PTEN tumor and CTC gene status.

Results: AA was well tolerated at all doses (250mg - 2000mg). Although not dose-related, the anticipated toxicities secondary to mineralocorticoid (M) excess were successfully managed with an M-receptor antagonist. Anti-tumor activity was observed at all doses but 1000mg was selected for phase II evaluation owing to a plateau in pharmacodynamic effect at 1000mg. The null hypothesis was rejected in both phase II studies. 44 chemotherapy-naïve pts received AA (median baseline PSA: 75, range: 8.8 - 964; 31/44 had bone metastasis on bone scan). 27/44 (61%) had ≥50% PSA decline confirmed ≥1 month, 22/44 (50%) and 11/44 (25%) had ≥75% and ≥90% declines in PSA respectively. MTTP by PSA: 252 days. 21/44 pts had measurable disease on CT scan - by RECIST: 12/21 partial response (PR) (57%), 7/21 stable disease (SD) (33%). 18 pts continue on AA alone; median follow-up (FU): 171 days (range: 581 - 108). Addition of D reversed resistance in 3/13 pts. 28 docetaxel-treated pts received AA (median baseline PSA: 523, range: 33.3 - 10325; 22/28 had bone disease). 14/28 (50%) had ≥50% PSA decline confirmed ≥1 month, 9/28 (32%) and 5/28 (18%) had ≥75% and ≥90% declines in PSA respectively. 18/28 pts had measurable disease on CT scan - by RECIST: 3/18 PR (17%); 11/18 SD (61%). MTTP by PSA: 165 days. 11 pts continue on AA; median FU: 213 days (range: 248 - 103). Symptom relief and falls in CTC counts were observed. At all dose levels, treatment was associated with a rise in ACTH and steroids upstream of CYP17 as well as significant suppression of downstream androgenic steroids, testosterone and estradiol. PK analysis on 21 pts showed a consistent terminal half-life (mean: 10.3 hours) and a non-linear increase in AUC and Cmax with dose (r² 0.186, 0.049 respectively). When administered with high fat food, drug exposure was significantly increased (4.4 fold) in comparison with fasting (p=0.0049).

Conclusion: The significant anti-tumor activity observed with AA suggests that 50-60% of CRPC remain hormone driven. Selective CYP450C17 inhibition with AA could represent a novel therapeutic option for pts with CRPC. Randomized Phase III trials will start in 2008.
B74 Phase I and pharmacokinetic study of ABI-007, a Cremophor®-free nanoparticle formulation of paclitaxel, administered weekly in Japanese patients with solid tumors. Masashi Ando1, Kan Yonemori1, Eriko Nakano1, Mayu Yunokawa1, Tsutomu Kouno1, Chikako Shimizu1, Kenji Tamura1, Noriyuki Katsumata1, Yasuhiro Fujisawa1, Mamoru Oyane2.

1Breast and Medical Oncology Division, National Cancer Center Hospital, Tokyo, Japan; 2Clinical Development Department, Taiho Pharmaceutical Co., Ltd., Tokyo, Japan.

Purpose: ABI-007 is a novel Cremophor®-free nanoparticle albumin-bound paclitaxel. The Cremophor®-free formulation allows for a higher dosage of paclitaxel in shorter infusion period without premedication to prevent hypersensitivity reaction. The primary objective of the study was to determine maximum tolerated dose (MTD) and recommended dose (RD) at weekly administration. Efficacy/safety profiles and pharmacokinetics (PK) were also assessed as secondary objective.

Patients and Methods: The study was designed to perform standard 3+3 dose escalation on solid tumors patients recruited regardless of pre-treatment history. The examined doses included four levels: Level 1 80 mg/m², Level 2 100 mg/m², Level 3 125 mg/m², and Level 4 150 mg/m². Treatment schedule per course was a 30-minutes IV infusion once a week for 3 weeks followed by 1 week rest. Evaluation of toxicities and antitumor responses was done in accordance with on Common Terminology Criteria for Adverse Events (CTCAE v3.0) and Response Evaluation Criteria in Solid Tumors (RECIST) guidelines respectively. The specified toxicities that occurred during the first course of treatment were considered dose limiting toxicity (DLT) such as thrombocytopenia (grade 4), thrombocytopenia with blood transfusion (grade 3), neutropenia lasting over 4 days (grade 4), febrile neutropenia, non-hematologic toxicity (grade 3 or 4) as well as treatment skip during the first course. Treatment skip was considered as DLT only in this Japanese study.

Results: Out of 16 accrued patients (from May 2006 to June 2007), 15 patients were evaluated for MTD/RD and 12 patients subjected to efficacy/safety/PK assessment. MTD was determined at 125 mg/m² (level 3) via DLT of treatment skip presented by 2 out of 6 patients at MTD dose level. RD was thus 100 mg/m² (level 2) equal to the RD of heavily pre-treated cohorts in Western studies. Other common adverse drug-related events were grade 1 or grade 2 peripheral neuropathy and alopecia. Abnormalities based upon laboratory values occurred with respect to hemoglobin, lymphopenia, and neutropenia; the severity grade was ranging from grade 1 to grade 3. Median number of courses administered was 3.5 courses per patient (3 administrations/course), and stable disease was observed with 7 patients by investigator assessment. As for PK analyses exhibited the area under the curve (AUC) of paclitaxel in Japanese patients increased with increasing the dosage. A comparison with PK reported for Western patients confirmed the similarity across the two ethnic groups.

Conclusion: Weeklyschedule of ABI-007 demonstrated the feasible tolerability as well as efficacy and safety in the Japanese patients. Moreover, the similar results between the Japanese and Western studies in terms of MTD/RD and PK profile indicate that the same dose and schedule of ABI-007 were applicable to global studies involving different ethnic groups. (Data cutoff as of July 31, 2007)

B75 Phase I and pharmacokinetic study of ABI-007, a Cremophor®-free nanoparticle formulation of paclitaxel, administered once every 3 weeks in Japanese patients with solid tumors. Toru Mukohara1, Hikaru Nakajima1, Shunji Nagai1, Hirobono Minami1, Noboru Yamamoto2, Kazuhiko Yamada2, Yasuhide Yamada2, Tomohide Tamura2, 1National Cancer Center Hospital East, Kashiwa, Japan; 2National Cancer Center Hospital, Tsukiji, Japan.

Purpose: ABI-007 is a novel Cremophor®-free nanoparticle albumin-bound paclitaxel. The Cremophor®-free formulation allows for a higher dosage of paclitaxel in shorter infusion period without premedication to prevent hypersensitivity reaction. The primary objective of the study was to determine maximum tolerated dose (MTD) and recommended dose (RD) when administered once every 3 weeks. Efficacy / safety profile and pharmacokinetics (PK) were also assessed as secondary objective.

Patients and Methods: This study was designed to perform standard 3+3 dose escalation on solid tumor patients recruited regardless of pre-treatment history. The examined doses included three levels: Level 1 200 mg/m², Level 2 260 mg/m², and Level 3 300 mg/m²; The study did not intend to probe further beyond Level 3 because it was MTD found in the overseas PI study. Treatment schedule per course is 30-minutes IV infusion once every 3 weeks. The specified conditions occurring during the first course of treatment were considered dose limiting toxicity (DLT) such as thrombocytopenia (grade 4), thrombocytopenia with blood transfusion (grade 3), neutropenia lasting over 4 days (grade 4), febrile neutropenia, and non-hematologic toxicity (grade 3 or 4). In the absence of DLT until Level 3, investigators and sponsor were to co-decide on potential RD and evaluate additional 3 patients to elucidate RD.

Results: A total of 12 patients were accrued (from August 2006 to June 2007) and all were evaluable for safety, PK and efficacy. Median number of courses delivered was 2.5. No DLT was evident through the dose escalation to the highest Level 3. In the absence of MTD, potential RD was to be re-evaluated. Considering the frequency of grade 1 and 2 peripheral neuropathy induced in Level 2 and Level 3 patients and the situation of the Western patients dosed at the same Level 3, Level 2 was selected as potential RD and subsequent 3 patients showed no DLT. On the other hand, anemia, leukopenia, neutropenia, and lymphopenia were commonly experienced and the latter 3 categories achieved grade 3 or greater in most patients. Common non-hematologic toxicities included anemia, peripheral neuropathy, myalgia, alopecia, rash, and arthralgia. Febrile neutropenia or hypersensitivity reaction did not occur. Two out of 3 patients who were enrolled in Level 3 experienced grade 3 peripheral neuropathy in 3rd or later courses. As for PK analyses, the area under the curve of paclitaxel in Japanese patients increased with increasing the dosage. A comparison with PK reported for Western patients confirmed the similarity across the two ethnic groups. In efficacy assessment by investigator, partial response was observed with 3 lung cancer patients.

Conclusion: As a result of no DLT observed at any level, MTD was not determined. RD was deemed accordingly at 260 mg/m² in consideration of efficacy observed in Japanese patients, and frequency of peripheral neuropathy reported inside and outside of Japan. This dose is also the RD found in Western studies. Regarding PK profile, similarity between the Japanese and Western population was endorsed. Consistent RD and similar PK profile indicate that the same dose and schedule of ABI-007 can be used in global studies involving different ethnic groups. (Data cutoff as of July 31, 2007)

B76 Results of a first-in-man phase I study assessing the safety and pharmacokinetics of a one hour intravenous infusion of DTS-201 every 3 weeks in patients with advanced or metastatic solid tumors. Patrick Schöffski1, Etienne Braun1, Herlindo Dumez1, Jacques Robert2, Vincent Dubois2, Véronique Amani2, André Trouet2, Jamal Gasmi3, Jean-Pierre Delord2, 1University Hospitals Leuven, Leuven, Belgium; 2Centre René Huguenin, Saint Cloud, France; 3Institut Bergonie, Bordeaux, France.

Introduction: DTS-201 is a doxorubicin prodrug which has shown encouraging results in experimental models in terms of both efficacy and safety compared to conventional doxorubicin. The drug is licensed in by Diatos from Medarex Inc with exclusive European rights. A Phase I study has been conducted with a 3 weekly-schedule of the anthracyclin analogue to assess the safety and pharmacokinetics and to determine the maximum tolerated dose (MTD) for consecutive trials.

Methods: DTS-201 was administered as a 1 hour iv infusion every 3 weeks in eligible patients. Dose escalation was performed according to a modified Fibonacci schema. PK profiles were obtained in each patient after the first administration.

Results: Three centers enrolled 25 pretreated pts; 12 females and 13 males, median age 58 years (range, 30-72), WHO PS<2; main tumor types: prostate, breast, head and neck, lung, sarcoma and pancreatic cancer. A total of 86 cycles (median 3; range, 1-8) were administered at 4 dose-
levels (DL), ranging from 80 to 400 mg/m² three-weekly dose, which is equivalent to 45–225 mg/m² of conventional doxorubicin. All patients were evaluable for safety. The treatment was well tolerated and no unexpected side effects occurred. No dose limiting toxicity (DLT) occurred up to 160 mg/m². Three DLTs were observed in DL3 and 4 (diarrhea in DL3, vomiting and neutropenia on DL4). Three-weekly DTS-201 400 mg/m² was considered MTD. A total of 12 patients received this dose.

Myelosuppression was the main toxicity. Short-lasting and reversible NCI-CTC grade 3–4 neutropenia was common at MTD. Only one patient developed febrile neutropenia. Non-hematological adverse reactions were mild to moderate including nausea, anorexia, asthenia and alopecia. No drug-related cardiace adverse events have been reported. One patient with cardiac risk factors suffered from cardiac ischemia that was managed medically. PK analysis demonstrated that DTS-201 was metabolised to alanyl-leucyl-doxorubicin, leucyl-doxorubicin and doxorubicin. The AUC and the Cmax of DTS-201 and doxorubicin increased in a dose-linear fashion. At MTD, the plasma AUC of doxorubicin was similar to the AUC in patients exposed to conventional doxorubicin at a dose of 60 mg/m². One patient with soft tissue sarcoma achieved a partial response and 9 pts had stable disease for at least 4 treatment cycles.

**Conclusions:** DTS-201 was well tolerated and safe. High doses of the anthracyclin derivatives could be delivered without obvious drug-related cardiace toxicity. DTS-201 showed some evidence of antitumoral activity. Phase II studies will explore the efficacy of DTS-201 in several indications.

**B77 PNU-166196 induces DNA damage measured by histone H2AX phosphorylation independent of the nucleotide excision repair pathways.** Josée Guirouilh-Barbat1, Kazutaka Takagi2, Daniel D. Von Hoff3, Yves Pommier4, 1Center for Cancer Research, NCI, Bethesda, MD; 2Translational Research Institute, Scottsdale, AZ.

PNU-166196 is a DNA minor groove binder in Phase II clinical development. Data suggest that PNU-166196 is activated by glutathione to form a highly reactive bromine derivative that can alkylate DNA reversibly. Because this mechanism is reminiscent of the minor groove DNA alkylaton by eucinecsidin 743, the aim of the present study was to elucidate the molecular and cellular effects of PNU-166196 in various cell lines known to be differentially sensitive to eucinecsidin 743. Potent cytotoxicity in the submicromolar range was observed in a panel of cell lines. Only minor sensitization was observed by stable transfection of the nucleotide excision repair (NER) gene XPD in XPD-deficient cells indicating that the activity of PNU-166196 is dependent on NER than it is for eucinecsidin 743. Furthermore, the previously identified HCT116-ERS cells selected for resistance to eucinecsidin 743 and defective for XRQ showed no cross-resistance to PNU-166196. Because we previously reported that eucinecsidin 743 induces the phosphorylation of histone H2AX (referred to as gamma-H2AX), we also investigated gamma-H2AX response. We found that PNU-166196 induces the formation of histone gamma-H2X foci at submicromolar pharmacological concentrations in all cell lines examined even in the NER-deficient cells. Our study demonstrates clear differences between PNU-166196 and eucinecsidin 743 with respect to NER-dependency and cross-resistance. Our results also suggest that gamma-H2AX, a major regulator of cellular responses to DNA damage, could be considered a pharmacodynamic biomarker for the rational clinical development of PNU-166196.

**B78 A biological and pharmacologic phase I study of NGR-TNF, a vascular targeting agent, in patients with refractory solid tumors (EORTC 16041).** Carla Van Herpen1, Walter Fiedler2, Sandrine Marreaud3, Hanneke Van Laarhoven4, Marlies Peters5, Anne-Sophie Govaerts6, Salvatore Toma2, Claudio Bordignon5, Arend Heerschap6, Kees Punt1, 1Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; 2University Hospital Hamburg-Eppendorf, Hamburg, Germany; 3EORTC, Brussels, Belgium; 4Molmed S.P.A., Milan, Milan, Italy.

**Background:** NGR-TNF is a novel agent exploiting a tumour homing peptide (cNGRCG) that selectively targets CD11 that is expressed on the neovascularature of solid tumors. Preclinical data show that its antitumour activity is achieved by a change of vascular permeability (at low doses) and damage of tumour-associated blood vessels (at high doses). This two-centre, open-label, phase I study assessed the safety, PK, PD, MTD, and optimal biological dose of NGR-TNF in patients (pts) with advanced solid tumours.

**Experimental design:** 67 patients (pts) with malignant solid tumours refractory to standard chemotherapies were treated with escalating dose of NGR-TNF administered once every 3 weeks by a 20 min IV infusion to cohorts of 3-6 pts. The starting dose was 0.2 µg/m². Dose escalation was performed with a doubling of the dose until grade 2 (G2) toxicity was observed; thereafter a modified Fibonacci schedule was used. PK and PD analyses were performed in blood during the first 4 cycles. DCE-MRI was performed at cycle 1 at baseline and 2 hours after start of the infusion to document modification of the tumour vascularity. Anti-tumour activity was assessed by CT scan every 2 cycles.

**Results:** 67 pts (median age, range: 60 years (34-79); median performance status: 0) with a range of advanced solid tumours received a total of 162 cycles (mean 2.9, range 1-12). 17 dose levels (DL) have been visited (0.2 to 60 µg/m²). At DL4 (1.3 µg/m²) during cycle 1, one DLT out of 7 pts, bronchospasm G3 was observed. As 3 out of the first 18 pts experienced chills G2 during infusion, the study was amended and infusion time increased to 1 hour. At DL10 (8.1 µg/m²) a second DLT abdominal pain G3 occurred (1/6 pts). At DL 17 (60 µg/m²) 2/3 pts experienced DLTs. Those consisted of G3 hypoxia with rapid shift hypotension-hypertension and G3 dyspnea with fever.

Other toxicity was generally mild and the most frequently related adverse events included (in the first 56 pts): chills 77%, fever 41%, pain 23%, nausea 14%, constipation 9%, diarrhoea 11%, anorexia 5% and hypotension 4%. The PK data were linear with dose. Intrapatient variability was low and interpatient variability was moderate. PD analysis showed an increase for the plasma levels of TNF-R1 and TNF-RII. After the 20 minutes infusion of NGR-TNF the raise of TNF-R1 and TNF-RII was relatively higher than after the 60 minutes infusion.

At DL ≥ 1.3 µg/m² most pts showed a decrease in kG and the number of pixels with a low fraction of kep and K trans significantly increased (p<0.05) in the metastases, as seen with other anti-vascular agents. No significant changes in d-MRI kG and K trans were observed in normal tissues. Nine out of 55 evaluable patients had stable disease. No responses were observed.

**Conclusions:** The recommended dose of NGR-hTNF administered alone once every 3 weeks is 45 µg/m² (DL 16 (9 pts) no DLT). NGR-hTNF was generally well tolerated with promising antitumour activity and some biological activity observed by DCE-MRI.

**B79 Phase I/II trial of the novel Hsp90 inhibitor, IPI-504, in patients with relapsed and/or refractory stage IIB or stage IV non-small cell lung cancer stratified by EGFR mutation status.** Lecia V. Sequist1, Pasi A. Jänne2, John Walker3, Jennifer Sweeney3, David Grayzel3, Thomas J. Lynch1. 1Massachusetts General Hospital Cancer Center, Boston, MA; 2Dana Farber Cancer Institute, Boston, MA; 3Infinity Pharmaceuticals, Inc, Cambridge, MA.

**Introduction:** Inhibition of the Heat Shock Protein 90 (Hsp90) chaperone protein results in selective destruction of the mutated epidermal growth factor receptor (EGFR) kinase in human non-small cell lung cancer (NSCLC) cell lines and significantly reduces tumour growth in vivo models, including those resistant to tyrosine kinase inhibitors (TKIs). We designed a phase I/II trial of IPI-504, a water-soluble Hsp90 inhibitor, for patients (pts) with advanced NSCLC who had received prior TKI therapy.

**Methods:** Patients with Stage IIB (with malignant effusion) or Stage IV NSCLC who had received prior therapy with an EGFR TKI (e.g., gefitinib or erlotinib) for ≥12 weeks, and had available tissue for EGFR mutation analysis were eligible for treatment. The goal of the Phase I portion was to evaluate the safety and maximum tolerated dose (MTD) of IPI-504 in pts with advanced NSCLC. Once dose-escalation is completed, the Phase II portion will begin with a goal of determining the potential anti-tumor activity.
activity of IPI-504 in NSCLC pts, stratified by EGFR mutation status. Pts received IPI-504, IV in 250 cc of normal saline over 30 mins, twice weekly on a four-week cycle. Subjects were evaluated for clinical benefit and radiographic improvement or stabilization by RECIST. Pharmacokinetic profiling of IPI-504 and its major active metabolites (17-AAG and 17-AE) was also performed on all pts.

Results: 8 NSCLC pts have been entered at 3 dose levels (150 mg/m^2 IPI-504 [n=3], 225 [3], 300 [2]); 7 females, 1 male; age range 47-83; 7 were positive for EGFR mutation (exon 19del or L858R); avg. # prior therapies 3.7 (range 1-6); All pts completed at least 1 cycle and were evaluated. No objective responses have been observed yet; however, 2 pts had stable disease including 1 pt on therapy for >5 cycles (20 weeks). IPI-504 has been well-tolerated to date; one DLT (grade 3 AST elevation, reversible) was observed at 300mg/m^2. The MTD has not been reached, and dose escalation in the phase I portion of the study is ongoing.

Conclusions: Targeting Hsp90 represents a novel therapeutic strategy in advanced, metastatic NSCLC patients who have failed prior therapy with TKIs. IPI-504 has been well-tolerated overall, and encouraging preliminary biological activity has been observed with the occurrence of extended stable disease.

B80 A phase II study of erlotinib as a first-line therapy for non-small-cell lung cancer patients with favorable clinical predictors and/or molecular predictors. Dae Ho Lee¹, Joo Hee Shin¹, Jeong-Sook Hong¹, Sang-We Kim¹, Cheolwon Suh¹, Yoon-Koo Kang¹, Jung Shin Lee¹. ¹Asan Medical Center, Seoul, Republic of Korea.

Background: Many studies of EGFR TKIs suggest positive and negative predictors for response and survival. We conducted the study to evaluate the efficacy of erlotinib as a first-line therapy for patients with non-small cell lung cancer with favorable clinical predictors, such as never-smoking, adenocarcinoma or female gender, and/or molecular predictors, such as EGFR gene mutation or gene amplification.

Methods: The eligible patients should have at least two of three favorable clinical predictors, including female gender, adenocarcinoma, non-smoking history; or have at least one of favorable molecular predictors, including EGFR gene mutations or gene amplification. Additional inclusion criteria were as follows; stage IIIIB or IV, ECOG PS of 0-2, adequate organ functions, and measurable lesions. Neither prior chemotherapy or targeted therapy nor radiotherapy to measurable disease was allowed. Treatment consisted of erlotinib 150 mg orally given once daily till disease progression, unacceptable toxicity or patient’s refusal. Objective tumor responses were assessed one month after the commencement of erlotinib and then every two months.

Results: Between 10/2006 and 6/2007, all 29 patients enrolled (median age: 58 years; M/F 8/21; ECOG PS 0/1/2 2/24/3; stage IIIIB/IV 26/3, never/former/current smoker 19/6/4) were evaluable for response. There were 14 PRs (48.3%) while there were 5 SDs (17.2%) and 10 PD (34.5%), giving overall response rate of 48.3% and disease control rate of 65.5%. The response rate in female patients was 57.1% (12/21) while that in never-smokers was 57.9% (11/19). Before the start of treatment, 5 patients were known to have EGFR gene mutation. Among them, interestingly, 2 patients whose tumor also had EGFR gene amplification showed a PR while 2 of 3 patients without gene amplification showed PDs. After a median follow-up of 4.5 months, 12 progressive diseases and 4 deaths were observed. The most common toxicity was skin rash, which was manageable. Retrospective biological study for patients enrolled is still being analyzed and will be presented at the meeting.

Conclusions: Erlotinib showed promising response rate as a first-line therapy for selected subsets of NSCLC patients based on favorable predictors.

B81 A Phase I study to evaluate the feasibility, safety and biological effects of intratumoural administration of wild-type Reovirus (REOLYSIN®) in combination with radiation in patients with advanced malignancies. Dean Harris¹, Laura Vidal¹, Alan Melcher², Kate Newbold¹, Alan Anthony³, Vasiliros Karavasilis³, Roshan Agarwal¹, Chris White¹, Katie Twigger², Matt Coffey³, Karl Mettinger³, Brad Thompson³, Hardev Pandha³, Johann De-Bono¹, Kevin Harrington¹. ¹Royal Marsden Hospital, Sutton, Surrey, United Kingdom; ²St James Hospital, Leeds, United Kingdom; ³Institute of Cancer Research, Sutton, Surrey, United Kingdom; ⁴Oncolytics, Calgary, Alberta, Canada; ⁵University of Surrey, Guildford, Surrey, United Kingdom.

Background: Wild-type reovirus (REOLYSIN®) is a dsRNA virus which results in asymptomatic infection in humans and has been demonstrated to replicate in and prove cytotoxic to Ras activated cells, while sparing normal cells.

Methods: An open label, dose escalating, multicentre phase I study of intratumoural injections of REOLYSIN with concurrent fractionated radiotherapy in patients with advanced solid tumours. The primary objectives are to determine the feasibility and safety of this intervention as well as assessing the antitumour activity, viral replication and the development of immune response. Patients were treated in sequential 3-patient cohorts. The treatment plan initially involved local irradiation of 20Gy in 5 consecutive daily fractions in combination with two intratumour injections of REOLYSIN (10⁹ TCID₅₀ on days 2 and 4 in week 1) with successive cohorts receiving escalating doses of virus to a maximum 10¹⁰ TCID₅₀. As no dose-limiting toxicity (DLT) was encountered the radiotherapy dose was increased up to 36Gy in 12 fractions. The number of REOLYSIN injections were escalated in conjunction from two doses of 10⁹ TCID₅₀ (days 2 and 4 in week 1) for the first cohort then four doses of 10¹⁰ TCID₅₀ (days 2 and 4 in week 1 and days 9 and 11 week 2) and finally 6 doses of 10¹¹ TCID₅₀ (days 2 and 4 in week 1 and days 9 and 11 week 2 and days 16 and 18 in week 3).

Tumour evaluation using imaging(RECIST criteria) or clinical examination was performed at days 33 and 61 in the 20Gy cohorts and days 29 and 59 in the 36Gy cohorts. Blood was collected for assessment of T cell subsets, T cell proliferation and cytokine production in association response to tumour associated antigens.

Results: 24 patients have been enrolled to date with 15 having completed the study (7 withdrawals/replacements and 2 patients still on study) with one patient required to complete the study. Treatment has been well tolerated with grade 3 toxicities of fatigue(4), hypokalaemia(3), tumour pain(1), tumour necrosis(1), diarrhea(1), dysphagia(1), cellulitis(1) and anorexia(1) seen. Common grade 1 and 2 toxicities included lymphopenia, fatigue, vomiting, fever, rash and neutropenia. In the low dose cohort two pts (oesophageal and skin squamous carcinoma) had significant partial responses (PR) (70% and 50%) and stable disease(SD) of treated site was seen in 8 of 11 evaluable patients with disease progression noted at non treatment lesion sites in 2 of those with stable disease. Of note the patient with oesophageal cancer had a PR in non-irradiated mediastinal disease. In the high dose cohort 1 partial response(PR) (in colo rectal cancer) in the 7 evaluable patients, and 6 SD were seen with one showing progression at non-treated sites.

Conclusion: The use of intratumoural injections of REOLYSIN combined with radiotherapy is tolerated without significant toxicity. The administration of multiple injections in association with radiotherapy is feasible in selected patients. The data on immune response and viral replication is ongoing at present with the study nearing planned completion.
B82 Phase I trial with a novel orally administered synthetic triterpenoid RTA 402 (CDDO-Me) in patients with solid tumors and lymphoid malignancies. David Hong1, Razelle Kurzrock1, Jeffrey G. Supko2, Donald Lawrence2, Jennifer Wheeler1, Joseph P Eder1, Chana Ng1, James Mier3, Marina Konopleva3, Sergej Konoplev4, Suhendan Ekmekcioglu1, Michael Andreef5, Elizabeth A. Grimm1, Donald Kufe6, Colin J. Meyer7, Geoffrey I. Shapiro8, Bruce J. Dezbere4. 1The University of Texas MD Anderson Cancer Center, Houston, TX; 2Massachusetts General Hospital Cancer Center, Boston, MA; 3Dana-Farber Cancer Institute, Boston, MA; 4Beth Israel Deaconess Medical Center, Boston, MA; 5Reata Pharmaceuticals, Inc., Irving, TX

Background: RTA 402 (CDDO-Me) is a novel synthetic triterpenoid with potent anticancer and anti-inflammatory activity mediated through direct and indirect inhibition of NF-κB and STAT3. The drug suppresses reactive oxygen species-mediated signaling through induction of antioxidant and detoxification systems via Keap1 binding/Nrf2 induction and suppression of pro-inflammatory and anti-apoptotic mediators. Based on broad spectrum preclinical data demonstrating activity against tumors and associated stroma, a Phase I dose-finding and pharmacokinetic study was initiated.

Methods: The primary objectives of the study were 1) to determine the dose-limiting toxicity (DLT), maximum tolerated dose, and recommended phase II dose of RTA 402 in patients with refractory cancer and 2) to characterize the pharmacokinetics of RTA 402 administered orally once daily for 21 days every 28 days. Secondary objectives were to 1) document any preliminary antitumor activity of RTA 402 in this patient population and 2) determine the in vivo molecular and biological effects of RTA 402 by measuring changes in markers of inflammation and apoptosis in PBMCs, blood plasma, and tumor biopsies. Dose escalation proceeded according to an accelerated titration design until a DLT was reached.

Results: RTA 402 was administered to 27 patients at 10 dose levels (5 to 1,300 mg/day). DLT was observed in two patients at 1,300 mg/day who experienced asymptomatic, Grade 3 liver transaminase elevations without concomitant increases in total-bilirubin. Both patients were dose reduced and continued on study. At the higher dose levels (> 900 mg/day), occasional fatigue and anorexia were also reported. The median biological half-life of RTA 402 was 31 h (range, 18-67 h), and all patients receiving doses >20 mg/day were continuously exposed to plasma levels of the drug exceeding 1 ng/mL. Evidence of antitumor and biological activity was observed in multiple patients. Of 12 patients evaluable for efficacy, 9 (75%) had stable disease for 2 months or longer, including patients with melanoma (N = 4; stable for 3, 4, +4, 6, 12, and 11+ months), renal cell (N = 2; stable for 7 and 8 months), and medullary thyroid cancer (N = 1; stable for 10 months). Four of the patients with stable disease had large increases in neutrophils and platelets. Six patients consented to have biopsies, and suppression of NF-κB and pSTAT3, as well as downstream target genes iNOS, cyclin D1, COX-2, and arginase, was observed after treatment. Relative to baseline, apoptosis as measured by TUNEL positivity was increased up to 3-fold, and the presence of macrophages was increased up to 10-fold. Induction of the Keap1/Nrf2/ARE system was demonstrated in PBMCs by increases in NQO1 and gamma-GCS mRNA. Additionally, circulating plasma cytokine profiles suggested a switch from a Th2 to a Th1 phenotype.

Conclusions: Orally administered RTA 402 was well tolerated up to 900 mg/day and with prolonged exposure up to 11 months. Data from initial patients indicate appropriate modulation of targets NF-κB, STAT3, and Keap1/Nrf2 and suggest clinical benefit, with prolonged disease stabilization in several patients with previously progressing disease and high tumor burden. Phase 2 trials in patients with melanoma and pancreatic cancer have begun.


Introduction: MER-104 (acyline) is a gonadotropin-releasing hormone (GnRH) antagonist which effectively suppresses luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion in man. Injectable forms have been administered successfully in man in Phase II studies as part of a potential contraceptive regimen. MER-104 is an enteric coated acyline tablet developed by Merion Pharmaceuticals using GIPET™ I technology to enable the oral administration of this decapeptide. GIPET™ I is based on proprietary penetration enhancers which improve the uptake of such drugs in the small intestine. There is no chemical modification to the active drug. The enhancer system is comprised of food-based material on the US GRAS list. These aspects are important factors in reducing regulatory requirements and the time to market. GIPET™ technology is equally applicable to small molecules, peptides and other macromolecules, and is broadly applicable over a wide range of marketed and emerging products.

Experimental Procedures: Eight healthy men were enrolled in the study at a single site. After determination of baseline gonadotropin levels, MER-104 10mg enteric coated tablets were administered at weekly intervals over a 3 week period. A single dose of 10, 20, and 40mg was given in order over the 3 week period. Blood levels were taken pre-dose, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, and 48 hours after dosing for assay of serum LH, FSH, testosterone, and acyline. Side effects were assessed by exam and routine laboratory tests.

Data Summary: Treatment with oral MER-104 tablets resulted in measurable serum levels of drug at all doses and dose-related suppression of serum LH and serum testosterone. Laboratory assessments, including liver function tests and creatinine, were unaffected by treatment. Conclusions: Therapeutically-effective levels of a decapeptide GnRH antagonist, acyline, can be achieved using an oral delivery platform, GIPET™. These data indicate that MER-104 tablets may have potential in the treatment of prostate and breast cancer, endometriosis, prostate hypertrophy, and as part of a male contraceptive regime.

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B84 A Phase II trial of VEGF-Trap in previously treated patients with metastatic colorectal cancer (PHL 050). A study of the PMH Phase II Consortium. Neesha C. Dhani1, Steven J. Cohen2, Georg A. Bjarnason3, Christian Kollmannsberger4, Kiran Virik3, Jasmine Brown1, Lisa Wang1, Amit Ozai1, Alice Chen6, Malcolm J. Moore5, 1Princess Margaret Hospital, Toronto, Ontario, Canada; 2Fox Chase Cancer Center, Philadelphia, PA; 3Toronto Sunnybrook Regional Cancer Center, Toronto, Ontario, Canada; 4BC Cancer Agency: Vancouver Cancer Centre, Vancouver, British Columbia, Canada; 5QEI Health Sciences Centre, Halifax, Nova Scotia, Canada; 6NIH/NCI, Rockville, MD.

Background: VEGF-Trap is a recombinant humanized fusion protein containing epitopes from VEGF-R1, VEGF-R2 and IgG. Advantages over other VEGF inhibitors include higher affinity binding to VEGF, longer circulating half-life and activity against all other angiogenic factors including PI GF (placental growth factor). Preclinical efficacy has been demonstrated with tumour growth inhibition in xenograft models treated with VEGF-Trap. The efficacy of bevacizumab in colorectal cancer provides rationale for this multi-center Phase II study to evaluate rates of response and 4-month PFS in patients with advanced colorectal cancer treated with VEGF-Trap.

Methods: Patients with good performance status (ECOG 0-2) having previously received at least one line of therapy for metastatic colorectal cancer were enrolled in 2 cohorts (based on prior treatment with bevacizumab) to receive single agent VEGF-Trap (4 mg/kg iv) on a 14 day
cycle. A maximum of 2 dose reductions per patient was allowed for drug-related toxicity. Treatment continued until evidence of disease progression, unacceptable toxicity or patient withdrawal. Correlative studies included assessment of levels of VEGF-Trap (bound and free) and anti-VEGF-Trap antibody.

**Results:** Follow-up is available for 50 of 51 patients accrued from Nov 2006 to April 2007 for 232 cycles of treatment. 53% (27) patients had received prior bevacizumab. Median age was 59 (39-80) with 59% (30) of patients being male. 94% (48) were ECOG 0 or 1. 51% (26) had received prior adjuvant therapy and 37% (19) radiotherapy. This was a heavily pre-treated group with 75% (38) having received ≥ 3 prior lines of systemic therapy. Median number of cycles per patient was 4 (1-13). Of the 24 patients in the bevacizumab-naïve (BevN) group, 17 were evaluable for tumor response by RECIST. There were no PRs but 8 SDs (with 4 prolonged responses lasting greater than 4 months) with a 4-mth PFS rate of 24%. In patients previously treated with bevacizumab (PBev) patients, 25 of the 27 were evaluable. There was 1 PR and 12 SDs (6 exceeding 4 mths) for a similar 4-mth PFS rate of 24%. Of the 13 patients continuing on study, 5 are in the bevacizumab-naïve group. 19 patients in each group have discontinued treatment, 12 (BevN) and 14 (PBev) respectively for progressive disease, 3 for symptomatic progression (BevN) and 2 in each group for adverse events. Each group had 2 patients withdraw consent. There was 1 death on study, secondary to PD and unrelated to study treatment. The most common NCi CTC V3 grade 3 or greater toxicities across both groups were fatigue in 14% (7 pts), hypertension in 10% (5), headache in 8% (4) and biochemical changes (hyperglycemia (3), increased liver enzymes (1), cytopenia (1), elevated INR (1) and proteinuria (2)) A further 61% (31), 41% (21) and 37% (19) experienced grade 2 or lower fatigue, hypertension and proteinuria respectively, and these were the most commonly observed drug related adverse effects. Other common (and < grade 2) toxicities included dysphonia (29%) anorexia (20%) and nausea (14%).

**Conclusions:** The study is currently on hold at the end of stage I and will continue to stage II if ≥ 2/22 patients have a response in either arm or > 10/22 are progression free at 4 months per protocol design.

**B85 Phase I trial of BAY 73-4506, a kinase inhibitor that targets oncogenic and angiogenic kinases, in patients with advanced solid tumors.** A. Frost1, S. Hedbom1, S. Steinbild1, M. Bürchert2, C. Unger1, O. Christensen3, D. Voliotis4, R. Heinig4, K. Mross1. 1Tumor Biology Center, Wuppertal, Germany. 2University Hospital, Freiburg, Germany; 3Bayer Healthcare Pharmaceuticals, West Haven, CT; 4Bayer Schering Pharma, Wuppertal, Germany.

**Background:** BAY 73-4506 is an orally active kinase inhibitor that targets both the tumor and its vasculature. BAY 73-4506 is a potent inhibitor of the oncogenic RTK (KIT, RET, PDGFR), angiogenic RTK (VEGFR-1, -2 and -3 and PDGFR-β) and serine/threonine kinases (p38MAPK and RAF). In preclinical cancer xenograft models, BAY 73-4506 demonstrated a broad spectrum anti-tumor activity.

**Methods:** The phase I study described here is an ongoing dose-escalation trial investigating the safety, pharmacokinetic (PK), and pharmacodynamic profile of BAY 73-4506 given orally in 21 days on/7 days off cycles until discontinuation due to toxicity or tumor progression. PK is assessed on days 1 and 21 of cycle 1. Pharmacodynamic markers, including dynamic contrast-enhanced MRI (DCE-MRI), circulating soluble VEGF-2 (sVEGFR-2) and plasma VEGF level, are assessed at each cycle. Tumor response is evaluated per RECIST criteria.

**Results:** 41 patients with solid tumors and progressive disease were enrolled in this trial and treated with BAY 73-4506 at doses of 10 mg to 220 mg once daily. Most frequent tumor types (≥ 5%) included colorectal carcinoma (n=11, 27%), malignant melanoma (n=5, 12%), renal cell carcinoma (n=4, 10%), and soft tissue sarcoma (n=4, 10%). The median treatment duration was 41 medication days (min. 1, max. 375). Commonly reported drug-related adverse events (AEs) (≥10% of pts) were hoarseness (n=21, 51%), dermatological toxicities (n=17, 41%, CTC grade 3 in 5 pts 12%), mucositis (n=11, 27%), fatigue (n=8, 20%, CTC grade 3 in 1 pt 2%), diarrhea (n=8, 20%), anorexia (n=7, 17%, CTC grade 3 in 1 pt 2%), hypertension (n=6, 15%, CTC grade 3 in 1 pt 2%), and weight loss (n=5, 12%). Treatment-related AEs leading to dose reduction, interruption or discontinuation were dermatological toxicities (n=6, 15%), fever without documented infection (n=2, 5%), leucopenia / thrombopenia (n=1, 2%), diarrhea (n=1, 2%), and fatigue (n=1, 2%). BAY 73-4506 exposure increased with dose up to 120 mg reaching a plateau at higher doses; the AUC target exposure level of 13 mg*hr/l (from preclinical xenograft models) was reached at 30 mg. The major metabolites of BAY 73-4506 (active in vitro) showed dose-dependent increases in exposure up to 220 mg and reached similar AUC(0-24h), as the parent drug at 220 mg. Two patients (RCC and osteosarcoma) out of 30 evaluable patients achieved RECIST partial response (7%). Seventeen patients (57%) had stable disease at least 7 weeks after start of treatment and 7 of these patients (23%) had stable disease for more than 15 weeks. Pharmacodynamic parameters (decrease in sVEGFR-2 level, decrease in IAUC60s of Cd-DTPA by DCE-MRI) correlated with drug exposure.

**Conclusions:** BAY 73-4506 was well tolerated when given at doses up to 160 mg once daily. The toxicity evaluation with the 220 mg dose is ongoing with additional patients being enrolled. In nineteen out of 30 evaluable patients (63%) antitumor activity has been demonstrated. Optimal dose and regimen are under evaluation in preparation for phase II trials.

**B86 UGT1A1*28 genotype determines the maximally tolerated dose (MTD) of OSI-774 and CPT-11 in patients with advanced solid tumors.** Henry Pitot1, Joel Reid2, Matthew Goetz2, Rui Qin3, Matthew Ames4, Stephanie Safgren4, Renee McGovern5, Sarah Buhrow6, Ravi Rao7, Charles Erlichman1. 1Mayo Clinic, Rochester, MN.

**Background:** Previously, we demonstrated marked dose limiting hematologic toxicity associated with the UGT1A1*28 genotype when we combined CPT-11 and OSI-774, which precluded the further development of this regimen (Rao et al, Proc ASCO 23:93053, 2004). Based on our demonstration that UGT1A1*28 genotype determined the MTD of CPT-11 based chemotherapy (Goetz, et al, GI ASCO 2007), we performed a dose-escalation study of CPT-11 (once every 3 weeks) and OSI-774 within UGT1A1 TA promoter genotypes (6/6, 6/7 and 7/7). Secondary aims included pharmacokinetic evaluation for drug interactions.

**Methods:** Eligibility criteria included solid tumors known to overexpress EGFR, EOCG performance scores 0-2, adequate hematologic, renal and hepatic function. OSI-774 was fixed at 100 mg/d with escalating doses of CPT-11 at 60, 90, 120 and 150 mg/m² for 6/6 cohort; 30 and 60 mg/m² for the 6/7 & 7/7 cohorts. CPT-11 was started on day 7 of OSI-774 therapy in cycle 1. Dose-limiting toxicities (DLT) were defined as grade 4 neutropenia, grade 3 thrombocytopenia or ≥ grade 3 non-hematologic toxicities despite maximal supportive care.

**Results:** 38 pts underwent UGT1A1*28 genotyping: 6/6 (20), 6/7 (13), and 7/7 (5); 33 pts, (median age 57) were eligible, treated, and evaluable for cycle 1 toxicity (table 1). Tumor primaries were esophagus (6), breast (6), pancreas (5), and other (16). Previous therapies were chemotherapy(100%), radiation therapy(45.5%) and surgery (51.5%). 84 cycles in total were delivered (median 2). DLT (neutropenia) was observed at a CPT-11 dose of 30 mg/m² (1 of 4 pts in UGT1A1 7/7 cohort) and DLT (neutropenia-1 pt, rash-1 pt) at 60 mg/m² (2 of 6 pts in UGT1A1 6/7 cohort). In contrast, UGT1A1 6/6 pts did not experience hematologic DLT, and non-hematologic DLT (diarrhea, nausea, anorexia and dehydration) was not observed until 150 mg/m² dose level (2 of 3 pts). Other Gr 3 toxicities included elevated liver function tests, anorexia, diarrhea, fatigue, and anemia. Pharmacokinetic analyses show mean (range) SN-38/CPT-11, SN-38CPT-11 and SN38GSN-38 AUC ratios were 0.100 (0.034-0.241), 0.192 (0.068-0.473) and 2.419 (0.448-4.88), respectively. Spearman correlations between genotype and AUC ratios were determined. The UGT1A1*28 variant (6/7 or 7/7 genotype) was significantly correlated with increased SN-38/CPT-11 (p=0.0005) and decreased SN-38GSN-38 (p=0.0001). There were no significant differences in the median SN38 AUC comparing UGT1A1 6/6 patients receiving CPT-11 120 mg/m² (302 ng/ml*hr) with 6/7 patients receiving 30 mg/m² (216 ng/ml*hr) and 7/7.
patients receiving 30 mg/m² (317 mg/m²·hr). One partial response was seen (colon), and 3 pts had stable disease for ≥5 cycles.

**Conclusions:** CPT-11 and OSI-774 can only be safely administered by dosing according to UGT1A1*28 genotype, which determines MTD of this combination. Compared to UGT1A1 7/7 patients, 6/6 patients are tolerating 4-fold greater CPT-11 doses despite comparable SN 38 AUC. Supported by CA9912-10, P30-CA15083, MM01-RR00585 and the Commonwealth Cancer Res. Fdn.

**B87** Clinical and biomarker responses in a Phase I continuous dose study of telatinib (BAY 57-9352), a VEGFR-2 inhibitor, in patients with colorectal carcinoma. K. Mross1, M. E. Scheulen2, A. Frost3, S. Steinfeld1, M. Büchert1, O. Christensen4, P. Rajagopalan5, G. Wensing1, D. Strumberg2,1.1Tumor Biology Center, Freiburg, Germany; 2West German Cancer Center, Essen, Germany; 3University Hospital, Freiburg, Germany; 4Bayer Pharmaceuticals Corporation, West Haven, CT; 5Bayer HealthCare, Wuppertal, Germany; 6Marien hospital Herne, University of Bochum, Herne, Germany.

**Background:** Telatinib (BAY 57-9352) inhibits VEGFR-2, VEGFR-3, PDGFR-β and c-Kit receptor tyrosine kinases. Telatinib showed anti-tumor activity in colon, breast, pancreatic, and NSCLC preclinical models.

**Methods:** This phase I expansion study investigated the safety, pharmacokinetics (PK), and pharmacodynamics of continuous oral dosing of telatinib. PK was assessed on day 1 and 14. DCE-MRI was performed at baseline and on days 2, 14, 35, and 56. Plasma biomarkers (VEGF, soluble sVEGFR-2) were assessed at each 21-day cycle.

**Results:** Twenty-seven patients with actively progressing colorectal carcinoma (CRC) were enrolled. Twenty-four patients were treated with telatinib 900 mg twice daily (bid), 2 patients at 1200 mg bid and 1 patient at 1500 mg bid. Frequent treatment-emergent adverse events (≥5% patients) of CTC a3 (NCI-CTC v2.0) were diarrhea (22%), hypertension (22%), infection without neutropenia (15%), fatigue (11%), tumor pain (7%), hepatic pain (7%), increased bilirubin (7%), and dyspnea (7%). Study treatment-related adverse events leading to a dose reduction or interruption were diarrhea (15%), nausea (4%), and vomiting (4%). Geometric mean Cmax and AUC (n=26) were 0.98 mg/L and 12.58 mg*hr/L at day 14, respectively. Telatinib exposure in CRC patients was comparable to that observed in patients enrolled in the corresponding dose range of the dose escalation portion of this study. The best response according to RECIST in 25 evaluable patients was stable disease (assessment at day 35, 68%) and progressive disease (32%). After 11 weeks of telatinib treatment 42% of the evaluable patients had stable disease. DCE-MRI showed a day 14 decrease in IAUC0–60 Gd-DTPA of ≥35% in 9 of 22 evaluable patients (41%). Biomarker responses (increase of VEGF and decrease of sVEGFR-2) correlated with telatinib AUC.

**Conclusions:** Telatinib treatment at the recommended dose level of 900 mg twice daily was well tolerated in CRC patients. Although the tumor shrinkage potential was limited, the stable disease rate of 42% after 11 weeks of treatment indicates therapeutic potential for telatinib in CRC patients. Phase Ib studies of telatinib with FOLFOX and irinotecan/capecitabine are ongoing.

**B88** Phase I study of CNF1010 (lipid formulation of 17-(allylamino)-17-demethoxygeldanamycin: 17-AAG) in patients with advanced solid tumors. M. Wasif Saif1, Charles Erlichman2, Tomislav Dragovich1, David Mendelson1, Lisle Nabel1, Francis Burrows2, Denise Trone2, James Woodworth6, Rachelle Perea2, Chris Storgard2, Daniel Von Hoff1,1.1Yale University School of Medicine, New Haven, CT; 2Mayo Clinic, Rochester, MN; 3Arizona Cancer Center at UMC North, Tucson, AZ; 4Premiere Oncology of Arizona, Scottsdale, AZ; 5University of Alabama, Birmingham, Birmingham, AL; 6Biogen Idec, Inc, San Diego, CA; 7Gen Clinical Research Services, Scottsdale, AZ.

17-AAG is a benzoxquinone ansamycin that binds to and inhibits the molecular chaperone Hsp90 leading to the proteasomal degradation of client proteins critical in malignant cell proliferation and survival. Previous 17-AAG formulations contained cemphor and DMSO, likely contributing to the hepatotoxicity and adverse event (AE) profile of 17-AAG. CNF1010 is an oil-in-water nanoemulsion of 17-AAG. Here we report final results of a CNF1010 Phase 1 study in patients (pts) with solid tumors (ST). Methods: Pts with advanced ST, ECOG PS 0-2, and adequate hematologic, hepatic, renal and cardiac functions received CNF1010 by infusion, twice-a-week, 3 weeks out of 4, starting at 6 mg/m² per dose. Doses were escalated sequentially in single pts (6 and 12 mg/m²) and 3-6 pts (≥25 mg/m²) cohorts according to a modified Fibonacci's schema. Plasma pharmacokinetic (PK) profiles were obtained on days 1 and 18. Results: As of June 9, 2006, 35 pts (MF 17/18, median age 60 years, range 39-78) with advanced ST were exposed to CNF1010. 19 pts experienced 50 SAEs. 26 pts reported treatment-related (tr) AEs. 10 pts reported gr3 tr AEs: fatigue, increased AST, increased ALT, increased alk phos, increased GGT, cancer pain, tumor ulceration, anemia, thrombocytopenia, hypocalcemia, hyperalbuminemia, hypomagnesemia, hyponatremia, hypophosphatemia, hypoxia, hyperbilirubinemia, acute hepatitis, acute renal failure, atrial fibrillation, and pain in extremity. 6 of these 10 pts received 175mg/m² CNF1010. 3 pts reported gr4 tr AEs: metabolic encephalopathy, shock, and hyperglycemia. 2 of the 3 pts received 225 mg/m². 3 DLTs were originally reported (hypoxia at 175 mg/m², metabolic encephalopathy and atrial fibrillation at 225 mg/m²). A retrospective review of the safety data by Biogen Idec (BIIB) identified an additional 5DLTs. (fatigue x 2 (83 mg/m² and 175 mg/m²), increased transaminases and shock at 175 mg/m² and pain in extremity (225 mg/m²), PK data were available through 225 mg/m²; The PK appears dose linear and median t1/2 values ranged from 2.1 to 6.8 hours. Hsp70 induction in PBMCs and inhibition of serum Her2 extracellular domain indicated biological effects of CNF1010 at doses >83 mg/m². Tumor response data were available for 24 pts. Best response was stable disease (n=6) and progressive disease (n=18). Conclusions: CNF1010, an oil-in-water nanoemulsion of 17-AAG demonstrates biologic activity at 83mg/m² and is toxic at doses of and above 175 mg/m² administered twice weekly. Because of toxicity, development of 17-AAG by BIIB has stopped. Phase 1 studies of BIIB021, an oral, fully synthetic, non-ansamycin derived Hsp90 inhibitor are on-going.

**B89** Phase I dose escalation study of sodium stibogluconate (SSG), a protein tyrosine phosphatase inhibitor, in combination with interferon-α for patients with solid tumors. Luis H. Camacho1, Aung Naing1, Claire F Vescraegen2, Funda Meric-Bernstam2, David S Hong3, Stacy Moulder1, Cataliner Gutierrez4, Saneese Stephen1, Chadnip Chandhasin1, Hui Gao5, Evans N Cohen6, Bang-Ning Lee6, Maritza Martinez5, Lawrence Akinsammi2, Razelle Kurzrock1, James Reuben4,1.1Phase I Program, Division of Cancer Medicine, University of Texas M. D. Anderson Cancer Center, Houston, TX; 2Department of Hematology Oncology, University of New Mexico Cancer Center, Albuquerque, NM; 3Division of Surgical Oncology (Breast), University of Texas M. D. Anderson Cancer Center, Houston, TX; 4Department of Hematopathology, University of Texas M.D. Anderson Cancer Center, Houston, TX; 5Vloquest Pharmaceuticals, Inc., Basking Ridge, NJ.

**Background:** SSG is a potent inhibitor of key IFN-α signaling protein tyrosine phosphatases (PTPases) and has potential anti-cancer activity by enhancing the antiproliferative effects of IFN-α. SSG+IFN inhibits the growth of melanoma, myeloma, colon, prostate, and breast carcinoma cell lines in vitro. We designed a dose-finding trial to assess the toxicities, MTD, pharmacokinetics, PTPhase modulation, and immune effects of this combination.

**Methods:** Phase I trial testing a 6 week regimen consisting of IV SSG for 5 days of the first week of cycle 1. IFN-α 2b is administered SQ 3 times/wk on week 2. Both drugs are given in combination thereafter on a 2 wk-on/1 wk-off schedule. SSG was given at five different dose (cohort) levels (400, 600, 900 & 1350), the next dose → 2025 mg/m² is ongoing (not included). Fourteen patients were treated; 4 melanoma, 3 colon, 3 pancreas, 1 prostate, 1 neuroendocrine, 1 ovarian and 1 mesothelioma. Cellular immunology studies were conducted first week pre and post SSG administration (Days 1 & 8), and after the administration of SSG and IFN-α on day 12 of first course (cycle 1) at all dose (cohort) levels. The
immunology studies evaluate subsets of T cells, natural killer cells (NK), and dendritic cells (DC) at all dose (cohort) levels, whereas intracellular cytokines syntheses by subsets of T cells (CD4, CD8) and DCs (myeloid/DC1 and plasmacytoid/DC2), as well as the expressions of granzyme A (GA) and perforin (PE) by NK and CD8+ T cells were determined for dose (cohort) levels above 400 mg. All measurements were obtained by flow cytometry (FACS). Molecular signaling markers SHP-1, SHP-2, P tyrosine, p-Jak2, p-STAT1, and p-STAT3 are being measured (not included).

**Results:** A total of 23 courses (cycles) have been delivered for all dose (cohort) levels attained (400 - 1350) at a range of 1-3 cycles. Median patient age is 58 yrs (range 33-82). Toxicities include elevation of serum lipase and amylase, lymphopenia, fatigue, low grade fever and abdominal pain. Although very few grade III blood count reductions and a skin rash were observed, there were no dose limiting toxicities identified that could prevent cohort progression (dose escalation). Cmax on Day 8, 30 min post-administration is 72, 428 µg/L. Significant changes in immune parameters were noted only in subjects receiving 900 mg of SSG and above. In these subjects, particularly those having significant decreases in the percentage (%) of CD4+ T cells and % CD4+CD25hi T-regulatory cells, significant increase in % DC2 (dendritic cell type 2) expression of CCR7 (p = 0.029) and CCR (p = 0.034). With respect to function, they demonstrated significant increases in % NKT, % NK, and CD8+ T cells that synthesized GA and/or PE (p < 0.05); moreover, there was a near statistical increase (p = 0.086) in the % PDC/DC2 that secreted IFN-α in response to activation through TLR7/8 by CL097.

**Conclusions:** SSG in combination with IFN has been overall well tolerated. Most common toxicities are elevation of serum amylase and lipase. Dose escalation continues. PK data suggest a linear accumulation of SSG with rapid excretion after 120 minutes. Preliminary immune monitoring suggests that the combination of SSG and IFN-α is capable of augmenting cellular immunity positively. Nevertheless additional studies are necessary to confirm these observations.

**B90** A phase I study: Immunotoxin targeting the epithelial glycoprotein 2 (ESA/Ep-CAM) in cancer patients. Olav Engbeka1, Svein Dueland1, Paal F Brunsvig1, Siri Juel1, Øystein Fodstad1, Steinar Aamdal1,1 The Norwegian Radium Hospital, Oslo, Norway.

Targeted treatment with monoclonal antibodies has successfully been used in cancer therapy. The antibodies may also be used as carriers for radioactive compounds and immunotoxins. Such conjugates may be more potent, but their use have often been associated with increased toxicity. A phase I protocol has been initiated to investigate the treatment related toxicity of immunotoxin treatment with an antibody targeted to the epithelial cell marker EGFP (ESA/Ep-CAM), conjugated to pseudomonas exotoxin A (PE). A total of 27 patients with antigen positive epithelial tumors were treated with up to 8 injections of the immunono conjugate, given every second week. On the current dose level, 6.5 ng/kg, one patient experienced dose limiting toxicity (DLT), a grade 4 elevation in liver enzymes after the first administration of the immunotoxin. No other serious adverse events have been associated with the study treatment. A large number of the treated patients developed anti-immunono conjugate antibodies. To what extent this inhibit the anticancer activity of the immunotoxin is under investigation. Pharmacokinetic analysis demonstrated that the immunono conjugate have been reached after intravenous administration. The inclusion of patients into the study continues.

**B91** A phase I dose escalation study and signs of anti-metastatic activity of ARQ 197, a selective c-Met inhibitor. Lee Rosen1, Neil Senzer2, John Nemunaitis1, Tarek Mekhail2, Ronald Bukowski3, Chiang Li4, Nigel Rulewski5, April Dovholuki5, Feng Chai6, Thomas Chan5, 1Premiere Oncology, Santa Monica, CA; 2Mary Crowley Medical Research Center, Dallas, TX; 3The Cleveland Clinic Hematology & Medical Oncology, Cleveland, OH; 4Boston Biomedical Institute, Norwood, MA; 5ArQule, Inc, Woburn, MA.

ARQ 197 is a selective, non-ATP competitive inhibitor of the c-Met receptor tyrosine kinase, a high-affinity receptor for hepatocyte growth factor (HGF). c-Met is dysregulated in a broad spectrum of human cancers and may contribute to cancer cell proliferation, apoptosis, angiogenesis, invasive growth, dissemination, and metastasis.

A phase 1 dose escalation study of ARQ 197 was initiated in adult patients with metastatic solid tumor in 2006, from which a recommended phase 2 dose (RP2D) of 120 mg bid was established based on pharmacokinetic data. Doses of up to 180 mg bid were administered orally with one of two dosing schedules, bid for 2 weeks following by 1 week of rest (schedule A) or bid continuously (schedule B). Maximum tolerated dose (MTD) was not reached, and dose escalation was terminated based on pharmacokinetic data.

Fifty-five patients (37 schedule A, 18 schedule B) have been treated with no dose-limiting toxicity observed. The safety profiles are similar between the two dosing schedules. The most commonly reported drug-related adverse events were fatigue and nausea.

Tumor types in the patient population included colon/colorectal (16.4%), renal (7.3%), thyroid (7.3%), ovarian (7.3%), pancreatic (7.3%), prostate (5.5%), non-small cell lung cancer (NSCLC, 3.6%), nasopharyngeal (3.6%), neuroendocrine (3.6%), breast (3.6%) and others (34.5%).

Of 47 patients evaluable per the study protocol, 3 (6.4%) had partial responses. Twenty-eight (59.6%) had stable disease (SD) for more than 2 months, 19 (40.4%) had prolonged SD for more than 4 months (range from 4 to 17 months).

Consistent with preclinical data, signs of anti-metastatic activity were observed. Based on Investigators’ evaluations, 33 (94.3%) of 35 patients who had received more than two cycles (6 weeks) of treatment showed no evidence of new metastatic lesions developing during their treatment with ARQ 197. Two patients (5.7%), one with renal cell carcinoma and the other with paraspinal sacral sarcoma, developed new lesions after approximately 10 and 12 months of treatment, respectively. Seven (35.0%) of other 20 patients who had received 2 or fewer cycles of treatment with ARQ 197 developed new lesions. The tumor types of these 7 patients were colon cancer (3) with new lesions (developed in lung, peritoneal and lung respectively), colorectal (1) with new liver lesions, NSCLC (1) with a new liver lesion, breast (1) with a new liver lesion, and spindle cell sarcoma (1) with new lung lesions. New lesions occurred only in organs with documented preexisting metastatic disease, except in one colon cancer patient with new peritoneal metastases who had lung and liver metastases as study enrollment. All patients’ original images are under review by an independent reviewer to assess the anti-metastatic activity of ARQ 197. Updated data will be presented. In summary, ARQ 197 is well tolerated with an acceptable safety profile, and MTD was not reached. RP2D was established based on pharmacokinetics data. Early signs of anti-tumor and anti-metastatic activities of ARQ 197 have been observed.

**B92** Phase I study of gefitinib and ramapycin in adult patients with recurrent glioblastoma multiforme (GBM). Surasak Phuphanich1, Ray Chiu1, John S. Yu1, Keith L. Black1, 1Johnnie Cochran Brain Tumor Center, Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA.

Purpose: To evaluate the safety and toxicity of gefitinib and ramapycin, while secondarily determining time to tumor progression, overall survival and quality of life in recurrent GBM. GBMs overexpress epidermal growth factor receptor (EGFR) and gefitinib inhibits EGFR tyrosine kinase. The related mTOR antagonists. Therefore, the combination of gefitinib and rapamycin should have a synergistic effect in treating GBM because a possible mechanism of EGFR TKI resistance may be targeted by mTOR antagonists.

Patients and Methods: Patients aged 18 years or older with recurrent progressive GBM treated previously with surgery, radiation, chemotherapy with or without immunotherapy and KPS > 40% are eligible. Gefitinib is dosed at 500 mg/day for patients, who are not taking enzyme inducing anti-epileptic drugs (EIAED) and patients with enzyme inducing anti-epileptic drugs (EIAED) the dose is escalated to 1000 mg/day. Ramapycin is...
and that whole blood concentration could be up to fivefold more than whole blood. Revealed that quarfloxin associates reversibly with blood cells, plasma quarfloxin concentration. The pharmacokinetic analysis protocol was amended to include assessment of whole blood and plasma concentration in ten phase I patients at the MTD level of 360 mg/m². At the MTD, Day 5 quarfloxin plasma and whole blood terminal half lives are 75.1 hours and 28.3 hours, respectively.

Results: To date 21 patients have enrolled and 18 are evaluable. There are 13 male and 5 female with a median age of 51.6 years (range 23-72 years), a median KPS of 60% (range 50-80%) and 12 patients were on EIAED. Only 5 patients received vaccine immunotherapy in addition to surgery, radiotherapy and chemotherapy. There were 3 patients with grade 3/4 non-hematological toxicities with rash, diarrhea, renal failure, hypotension, dyspnea, coagulopathy, and elevated LFT. One patient developed wound infection and it was not related to these drugs. There were 2 (11%) minor response and 6 (33%) stable disease (range 1-18 months) with a clinical benefit of 44%.

Conclusion: Oral daily co-administration of gefitinib and ramapycin is safe, tolerable in this heavily treated group of patients. The median progressive free survival time and time to tumor progression is to be determined.

B93 Pharmacokinetic findings from the phase I study of Quarfloxin (CX-3543): A protein-rDNA quadruplex inhibitor, in patients with advanced solid tumors. Kyriakos Papadopoulos1, Alain Mitza2, Alejandro Ricart2, David Hufnagel2, Donald Northfelt3, Daniel Von Hoff3, Levan Darjaniaa, John Lim4, Claire Padgett4, Robert Marschke1. 1South Texas Accelerated Research Therapeutics, San Antonio, TX; 2Cancer Therapy and Research Center, San Antonio, TX; 3Mayo Clinic Arizona, Scottsdale, AZ; 4Cylene Pharmaceuticals, Inc., San Diego, CA.

Background: Ribosomal RNA (rRNA) biosynthesis and subsequent ribosome assembly determine the proliferative state of cells, and these processes are highly amplified in cancer due to mutations in regulatory signaling pathways that control rRNA biogenesis. Quarfloxin, a novel fluoroquinolones derivative, directly inhibits aberrant rRNA biogenesis in cancer cells by disrupting an essential protein-ribosomal DNA interaction that is over-expressed, thereby selectively triggering apoptotic cell death in tumor cells but not in normal cells. A phase I study of quarfloxin has been completed in patients with solid tumors, including an elucidation of this agent's pharmacokinetic attributes.

Material and Methods: This phase I study was designed to identify the maximum tolerated dose (MTD), the dose limiting toxicities and the pharmacokinetic profile of quarfloxin when administered as an IV infusion for 5 consecutive days every 21 days, to patients with advanced solid tumors. Serial blood samples were collected for analysis on Day 1 and Day 5 of dosing during the first cycle of drug administration. A twelve hour urine sample was also collected with Day 1 drug administration to determine urinary clearance of quarfloxin.

Results: A total of 48 patients from 8 escalating dose cohorts of quarfloxin had samples collected for pharmacokinetic analysis. Twenty of these patients were dosed at the maximum tolerated dose level (360 mg/m²). Area under the plasma concentration versus time curve generally increased in proportion with each dose level escalation. Urinary clearance was noted to be consistent throughout all dose levels. Plasma terminal half life was observed to be longer on Day 5 when compared with Day 1, and a trend of increasing plasma terminal half life with each subsequent dose escalation was also evident. The increase in plasma terminal half lives did not result in significant plasma quarfloxin accumulation, and were thought unlikely to be due to saturated elimination. In vitro tests using human whole blood revealed that quarfloxin associates reversibly with blood cells, and that whole blood concentration could be up to five fold more than plasma quarfloxin concentration. The pharmacokinetic analysis protocol was amended to include assessments of whole blood and plasma concentration in ten phase I patients at the MTD level of 360 mg/m². At the MTD, Day 5 quarfloxin plasma and whole blood terminal half lives are 75.1 hours and 28.3 hours, respectively.

Conclusions: The reversible association of quarfloxin with blood cells creates a "reservoir" of drug that is gradually released into plasma, thus extending the plasma terminal half life. This attribute supports drug administration on a weekly basis, and a phase I study of weekly quarfloxin administration is in progress.

B94 Vorinostat (NSC 701852) in patients (pts) with relapsed non-small cell lung cancer (NSCLC): A Wisconsin Oncology Network phase II study. Anne M. Traynor1, Sanita Dubey2, Jens Eckhoff2, JILL M. Kolesar3, Kathleen Schell4, David L. Groteluschen5, Sarah M. Marcotte1, Courtney M. Hallahan3, Hilary R. Weeks3, James Zwiebel5, Joan H. Schiller5. 1University of Wisconsin Paul P. Carbone Comprehensive Cancer Center, Madison, WI; 2UCSF Cancer Center, San Francisco, CA; 3Green Bay Oncology, Green Bay, WI; 4NCI, Bethesda, MD; 5UT Southwestern Cancer Center, Dallas, TX.

Background: Vorinostat (SAHA) is a small molecule inhibitor of histone deacetylase (HDAC). HDAC inhibitors have shown preclinical activity in lung cancer and are postulated to have an antitumor effect by alteration in acetylation status of histone and non-histone proteins.

Methods: Pts with relapsed NSCLC who failed no more than 1 prior cytotoxic therapy were eligible. Treatment: vorinostat, 400 mg po daily in a 21 day cycle. Primary objective: response rate (RR), with goal of at least one responder in first 14 evaluable pts according to the Simon-optimal two-stage design. Secondary objectives: time to progression (TTP), overall survival (OS), safety, and correlative assays.

Results: 16 pts enrolled from 12/05-4/07. Median age 59.5 yrs (range 47-79). 13 females (81%). PS 0:1, 10 pts (63%); 6 pts (37%). Fifteen of 16 pts had only 1 prior cytotoxic regimen; 1 pt had only prior erlotinib. Best response to prior treatment: partial response (1 pt), stable disease (SD; 12 pts), progressive disease (PD; 3 pts). Median time since last prior therapy: 2.7 mo (range 0.2-78.5). Vorinostat treatment compliance: 96.3%. Two pts were not evaluable for response due to not completing Cycle 1 of treatment due to PD. No objective antitumor responses were seen in the first 14 evaluable pts. Eight pts experienced SD (median 3 mo, range 1.7-10.7). Median TTP: 2.4 mo (range 0.9-17.9+); median OS 6.5 mo (range 1.4-17.9+); estimated 6 mo OS rate 54% (SE 13%) and estimated 1 year OS rate 18% (SE 11%). Adverse events possibly or probably related to vorinostat: Grade (Gr) 5 toxicity: CVA (1 pt). Gr 3/4 toxicities: neutropenia (Gr 4-1 pt; Gr 3-1 pt), lymphopenia (Gr 3-3 pts), fatigue (Gr 3-2 pts), elevated alk phos (Gr 3-1 pt), memory impairment (Gr 3-1 pt), PE/DVT (Gr 3-1 pt; Gr 4-2 pts), dehydration (Gr 3-1 pt), vomiting (Gr 3-1 pt). Flow cytometry was performed on 1 patient using buccal mucosal cells pre- and post-treatment: no change was seen in the percentage of cells in S phase after treatment with vorinostat. Data from the following additional correlative studies will be updated: p53 status of archived tumor cells, gene expression in baseline peripheral blood mononuclear cells (PBMCs), H3 acetylation in PBMCs pre- and post-treatment, transcription of p21, Nur77, Hsp70, erbB1 and 2, and Akt in PBMCs pre- and post-treatment, and assay of plasma isoprostanes generated by treatment with vorinostat.

Conclusions: Vorinostat 400 mg daily is tolerable with more than 87% of patients completing Cycle 1. No objective antitumor activity was detected with vorinostat in this setting; however, it yields TTP in relapsed NSCLC similar to that of other targeted agents. Treatment exerted no effect on buccal mucosal cell cycling in the one patient sampled. Further studies in NSCLC should focus on combining vorinostat with other antitumor agents.

Drug Metabolism, Transport, and Biodistribution

B95 Effect of ketoconazole, a potent CYP3A4 inhibitor, on the pharmacokinetics of temsirolimus administered by intravenous infusion. Joseph Boni1, Cathie Leister1, Jaime Burns1, Bruce Hug1. 1Wyeth Research, Collegeville, PA.

Background: Intravenous (IV) temsirolimus, a novel inhibitor of the mammalian target of rapamycin (mTOR), has been approved by the US Food and Drug Administration for the treatment of advanced renal cell carcinoma. Intravenous temsirolimus also is being studied in patients with other solid tumors and hematologic malignancies. Representative
pharmacokinetics (PK) for temsirolimus in patients with cancer and healthy subjects are available. Both temsirolimus and its metabolite sirolimus are substrates for cytochrome P450 (CYP) 3A4. Orally administered sirolimus-structural analogs are CYP33A4 substrates and exhibit substantial drug interaction with ketoconazole (KETO), a potent CYP3A4 inhibitor. The objective of this study was to characterize the effect of ketoconazole (KETO), a potent CYP3A4 inhibitor, on the PK profile of IV temsirolimus.

Methods: This was an open-label, nonrandomized, 2-period, sequential study in healthy adult subjects. A 5-mg dose of temsirolimus was chosen for combination with KETO to mitigate safety concerns of the temsirolimus 25-mg clinical dosage. On day 1, a single 5-mg dose of temsirolimus was administered via 30-minute IV infusion. After a 14-day washout period, oral 400 mg KETO was administered once daily for 7 consecutive days (days 15-21). A 5-mg IV dose of temsirolimus was administered 2 hours after the KETO dose on day 15. Concentrations of temsirolimus and sirolimus in whole blood were measured using a validated, dual, LC/MS/MS bioanalytic assay. PK analyses utilized a noncompartmental model. Least squares geometric mean (LSGM) ratios of test to reference treatments and their 90% confidence intervals (CI) for Cmax, AUC, and AUC were determined.

Results: 17 subjects (mean age, 34 years) were enrolled. PK profiles were available for 16 subjects who received temsirolimus alone and for 14 subjects who received concomitant KETO with IV temsirolimus. Coadministration of KETO with IV 5-mg temsirolimus resulted in no meaningful change in mean temsirolimus Cmax or AUC. However, coadministration of KETO with IV 5-mg temsirolimus resulted in large increases in sirolimus PK parameters, a 2.2-fold increase in mean Cmax and a 3.2-fold increase in mean AUC. The 90% confidence intervals for LSGM ratios of Cmax, AUC, and AUC for temsirolimus were within 80% to 125%. In this regimen, KETO coadministration with temsirolimus was well tolerated and there were no unexpected safety results in these healthy subjects.

Conclusion: Concomitant administration of KETO with temsirolimus resulted in a 3.2-fold increase in mean AUC for sirolimus compared with temsirolimus administration alone.

B96 Lack of pharmacokinetic interaction between intravenous temsirolimus and the cytochrome P450 2D6 substrate desipramine. Joseph Boni, Cathie Leister, Jaime Burns, Bruce Hug. Wyeth Research, Collegeville, PA.

Background: Intravenous (IV) temsirolimus is a novel inhibitor of mammalian target of rapamycin (mTOR) and recently has been approved by the US Food and Drug Administration for the treatment of patients with advanced renal cell carcinoma. Intravenous temsirolimus is also being evaluated for the treatment of patients with mantle cell lymphoma. In studies with pooled human liver microsomes, the cytochrome P450 (CYP) 2D6 was inhibited by temsirolimus with KI >1.5µM and was associated with a moderate Cmax/KI of 0.38 of the anticipated peak temsirolimus concentration following IV dosing. This study investigated whether temsirolimus inhibited the activity of CYP2D6 in humans. The effect of IV temsirolimus on the pharmacokinetic (PK) profile of desipramine (DES), a substrate of CYP2D6, was determined.

Methods: In this open-label, nonrandomized, 2-period, sequential study, healthy adults were genotyped for CYP2D6 polymorphisms. In period 1, subjects received 1 DES 50-mg tablet. In period 2, subjects received 1 oral DES 50-mg tablet coadministered with 1 temsirolimus 25-mg dose given via 30-minute IV infusion. Plasma and urine samples for PK analyses were collected in both periods. DES and its metabolite 2-hydroxy-DES were measured using a validated LC/MS/MS assay. PK analyses utilized a noncompartmental model. Least squares geometric mean (LSGM) ratios of test to reference treatments and their 90% confidence intervals (CI) for Cmax, AUC0–24, and AUC were determined.

Results: 26 subjects (mean age 34 y; n=25 men) received oral DES and 23 of these received DES coadministered with IV temsirolimus. One subject had a poor metabolizer CYP2D6 genotype. Mean DES Cmax and AUC were 13% and 4% lower and mean 2-hydroxy-DES Cmax and AUC were 9% and 5% lower, respectively, with temsirolimus coadministration. Although temsirolimus coadministration resulted in statistically significant pharmacokinetic effects for DES (Cmax, P<0.001; AUC, P=0.043; V/F P/F =0.023), the 90% CIs for LSGM ratios of DES Cmax, AUC, and V/F were within 80% to 125% (whether or not the poor metabolizer was included). This indicates that the observed PK differences did not manifest into clinically relevant changes. Temsirolimus PK values were similar to those observed in other studies. A single 25-mg dose of IV temsirolimus, alone or with DES, was well tolerated in healthy subjects.

Conclusion: Temsirolimus single 25-mg IV dose did not alter disposition of DES, a sensitive CYP2D6 substrate, in a clinically relevant way. Therefore, temsirolimus 25-mg IV may be safely coadministered with agents metabolized through the CYP2D6 pathway.

B97 Stability and proteolysis of immunotoxins in patient urine after treatment. Roberta Traini, Inger Margulies, Robert J. Kreitman. NIH, Bethesda, MD.

BL22 and LMB-2 are Immunotoxins (ITs) composed of the Fv portion of an antibody (scFv) of anti-CD25 and anti-CD22 respectively and a truncated fragment of Pseudomonas exotoxin A (PE38), which inactivates Elongation Factor 2. Patients with leukemia and lymphoma have been treated with these ITs with major responses in CLL and complete remissions in hairy cell leukemia. The half-life of these ITs in blood is about 2-3 hours. However, there is little known about the excretion of ITs into urine by humans. We know that in mice, 6 h after an injected dose of LMB2, one-third of the injected dose is excreted in urine, and over two-thirds after 24 h. Studying the renal excretion of ITs in humans may be critical to understanding hemolytic uremic syndrome, a limiting toxicity of BL22 but not of LMB-2.

To evaluate the pattern of urinary excretion of immunotoxins in humans, we evaluated the rate of IT excretion in patient urine and the catabolism occurring in the urine. We recovered the total protein from urine by TCA precipitation or purified the ITs from urine using Q-Sepharose ion-exchange chromatography. We detected the presence of ITs by Western Blot Assay. We studied the stability of BL22 added to urine at different time points as a function of pH. Finally, we quantified the amount of ITs in patient urine using ELISA assay. The ELISA assay was able to test the presence of ITs in patient’s urine or ITs exogenously added to healthy donor urine. The accuracy of the ELISA assay was >80% in the absence of urine, but accuracy decreases to <20% due to decreased sensitivity in the presence of urine. Thus purification by Q-Sepharose is needed to obtain a good quantification of ITs in urine.

BL22 catabolism/breakdown is variable not only between patients, but also with the same donor at different times, possibly due to variability in proteases present in urine. By reducing SDS-PAGE, BL22, composed of VL disulfide-bonded to a fusion of VH with PE38, undergoes proteolysis in urine with PE38 appearing as at least 5 different fragments, corresponding to 1) VH+PE38 (51 kDa) 2) VH plus amino acids 253-279 of VH (16 kDa), 3) PE35 (amino acids 280-364 and 381-613 of PE, 35 kDa), and slightly truncated forms of 1) and 2) indicating proteolysis within VH. BL22 also undergoes proteolysis in urine ex vivo. Interestingly, LMB-2 could not be recovered in urine after injection of 40 ug/Kg, although additional patients will need to be tested to determine if the two immunotoxins are different with respect to renal excretion.

B98 Enhanced CPT-11 hydrolysis by a modified human carboxylesterase improves antitumor drug response. Monika Wierd, Lyudmila Tsurkan, Janice Hyatt, Carol Edwards, M. Jason Hatfield, Christopher Morton, Peter J. Houghton, Mary K. Danks, Matthew R. Redinbo, Philip M. Potter. 1St. Jude Children’s Research Hospital, Memphis, TN; 2University of North Carolina, Chapel Hill, NC.

We have developed a human carboxylesterase (CE) that is highly proficient at CPT-11 activation for use in enzyme produg therapy approaches with this drug. Whilst CPT-11 is an effective anticancer agent, only ~5% of the drug is converted to the active metabolite SN-38 in patients. Potentially therefore, selective activation of CPT-11 within the
B99 Metabolism and pharmacokinetics of the 2ME2 Analog, ENMD-1198, an orally bioavailable microtubule destabilizing agent, in rats, dogs, and humans. Theresa LaValle1, Glenn M. Swartz2, Gizachew Kiffe3, William E. Fogler4, Anthony M. Treston1, Daniel L. Gustafsson3, Carolyn Sidor1, D. Ross Camridge4, 1EntreMed, Inc., Rockville, MD; 2Colorado State University, Fort Collins, CO; 3University of Colorado Cancer Center, Denver, CO.

Background: Clinical studies of 2-methoxyestradiol (2ME2, Panzem®), a microtubule destabilizing agent, demonstrate an excellent safety profile for 2ME2 with evidence of clinical benefit, including complete and partial responses and prolonged stable disease across a range of patients with different advanced cancers. 2ME2 is metabolized by conjugation at the 3- and 17-positions as well as oxidation at the 17-position. A series of analogs of 2ME2 modified at positions 3 and 17 was generated. ENMD-1198 (2-methoxyestra-1,3,5,(10),16-tetraene-3-carboxamide), was selected as the lead molecule for further clinical development due to improved metabolic stability and more potent antiproliferative properties in preclinical models compared to 2ME2. Preclinical toxicity studies evaluating daily oral dosing of ENMD-1198 for 28 days indicated a 10-fold difference in the maximum tolerated dose (MTD) between rats and dogs. In female rats the MTD is 60 mg/m²/d, which results in a Cmax of 927 ng/mL and an AUC of 3724 ng·h/mL, whereas the MTD in female dogs is 600 mg/m²/d, which results in a Cmax of 4396 ng/mL and an AUC of 29,223 ng·h/mL. The organs affected by ENMD-1198 in both species were those with cell populations of high mitotic potential, consistent with its mechanism of action: bone marrow, lymph nodes, spleen, thymus, GI tract, and testes.

Methods: To assess metabolic differences between rats and dogs, radiolabeled compound was synthesized and metabolite profiles were evaluated. A first-in-man Phase 1 clinical trial of ENMD-1198 in patients with advanced cancers is ongoing with a starting dose based on data from the most sensitive species (rats). Metabolite profiles from the ENMD-1198 dosed rats and dogs were compared to the plasma samples from patients dosed with 30 mg/m² ENMD-1198.

Results: While there were quantitative differences between rats and dogs, overall, metabolism of ENMD-1198 appears to be formation of metabolites via hydroxylation of the B or D ring or des-methylation of the O-methyl group followed by conjugation with glucuronide or sulfate. Consistent with preclinical data from both the rat and dog, once daily oral dosing of ENMD-1198 in humans demonstrates dose proportionality across the dose range 5-30 mg/m². At 5, 10, 20, and 30 mg/m², mean peak plasma exposures are 68.8, 141.2, 418.7, and 677.8 ng/mL, respectively. The plasma concentration time curves have resulted in AUCs of 390.0, 1373.8, 2515.4 and 5880.8 ng·h/mL, respectively and a half-life of approximately 13 h (range 10.6 to 19.5).

Similar to dogs, ENMD-1198 was the major component in the human plasma at all time points. There were no dominant metabolites (>20% of total area of the identified peak) found in human, rat or dog plasma. M7a/b and M10, hydroxylated ENMD-1198, are the most abundant plasma metabolites in all three species.

Conclusions: ENMD-1198 is a 2ME2 analog with improved preclinical pharmacokinetics and antitumor activity compared to 2ME2. There is a significant species difference in sensitivity, but no differences in dominant metabolites. A Phase 1 dose escalation study in humans is ongoing, which to date has demonstrated dose proportional PK exposure from 5 to 30 mg/m², and a metabolite profile that appears similar to both rats and dogs.

B100 Linker-dependent metabolites of antibody-maytansinoid conjugates in livers of CD-1 mice. Xiaxiong Sun1, Hans K. Erickson1, Wayne C. Wildison1, Michele F. Mayo1, Robert J. Lutz1, Ravi V. Chari2, Rajeeva Singh3, ImmunoGen Inc., Cambridge, MA.

Antibody-maytansinoid conjugates (AMCs) are anticancer agents composed of three components—a monoclonal antibody that binds specifically to a target antigen, a maytansinoid cell-killing agent (e.g., DM1 or DM4), and a linker that stably connects the two. Three AMCs currently in phase II clinical trials for the treatment of cancer - huC242-DM4, huN901-DM1, and trastuzumab-DM1 - displayed different dose limiting toxicities in Phase I clinical trials. AMCs can differ in toxicity for reasons related to their antibody component, such as different expression patterns for the target antigen on normal tissue. Tolerability differences can also potentially be due to differences in the link between the maytansinoid (may) and antibody. HuC242-DM4, huN901-DM1, and trastuzumab-DM1 employ three different linkers: huC242-DM4 and huN901-DM1 both utilize disulfide-based links but differ in the hindrance surrounding the link, while trastuzumab-DM1 utilizes a thioether linker. The current evidence from preclinical animal models suggests that AMCs are cleared by hepatobiliary elimination. To investigate the impact of these three linkers on this process, we administered huC242-4H-may conjugates with these three linkers to non-tumor-bearing CD-1 mice and then isolated and characterized the may metabolites from their livers. The conjugates were 4H-labeled at the C-20 methoxy group and administered in a single dose of 15 mg/kg. Mice were sacrificed at 8 h, 2 d, 4 d, and 7 d following treatment. The blood was collected, the systemic circulation was flushed, and the livers were collected and analyzed for may metabolites following homogenization and extraction. Metabolites were identified by LCMS and quantified by HPLC and liquid scintillation counting. The sole metabolite observed in livers of mice treated with the thioether conjugate, huC242-4HDM1, was a lysine-linker-may metabolite, lysine-Nε-SMCC-[3H]DM1. The initial fate of the disulfide-linked conjugates appears to be similar, with catabolism of the antibody component to yield the respective lysine-linker-may metabolites that were also isolated from the livers. However, these metabolites appear to then be cleaved to the respective DM1 and DM4 metabolites which in turn are S-methylated, as these metabolites also were isolated from the livers. These initial steps appear to be similar to the metabolism of the conjugates within target cancer cells, with the exception that the liver S-methyl transferases appear to be more active. In the liver, the S-methyl derivatives are then rapidly oxidized by an NADPH-dependent CYP to the corresponding S-methyl-sulfides and ultimately the S-methyl-sulfones. We synthesized the major metabolites and assessed them for cytotoxicity. The lysine-Nε-SMCC-DM1 metabolite of huC242-4HSMCC-DM1 is
nearly 3 logs less potent than the S-methyl-DM4 in cytotoxicity assays, while the sulfoxide and sulfone metabolites of the disulfide-linked conjugates are nearly 2 logs less potent than S-methyl-DM4. The different metabolites formed during hepatobiliary elimination may account for the better tolerability of the thioether-linked conjugate versus the disulfide-linked conjugates in preclinical evaluation. The thioether-linked huC242-may conjugate was not chosen for development due to its limited in vivo activity.

B101 Phase I clinical pharmacokinetics of RTA 744 (berubicin(B)), a blood-brain barrier penetrating anthracine active against high grade glioma, and evaluation of its 13-hydroxy metabolite, berubicinol (B-ol). Reza Kazerouni1, Charles Conrad1, Mary J. Johansen1, Mick Sakamoto1, Neil C. Thapar2, Colin Meyer2, Waldemar Priebe1, Timothy Madden1. 1U M D Anderson Cancer Ctr., Houston, TX; 2Reata Pharmaceuticals, Irving, TX; 3Reata Pharmaceutical, Irving, TX.

Preclinical studies demonstrated that Berubicin (B), a 4’-O-benzyl-doxorubicin designed to effectively cross the BBB by circumventing P-gp and MRPI-mediated efflux, is retained in brain & brain tumor tissue for >24 hrs, and has demonstrated in vivo activity against glioblastoma multiforme (GBM) in an orthotopic model. Berubicinol (B-ol) is the 13-hydroxy derivative, determined as the primary metabolite. A multicenter, phase I dose-escalation study of B, administered as a short IV infusion, designed as 2 arms: 3 consecutive days, every 3 weeks, or weekly x 4, every 5 weeks. Patients enrolled in the study were adult patients with recurrent or refractory GBM, anaplastic astrocytoma, or other primary brain tumors. Peripheral blood samples for PK analysis were collected prior to and at selected timepoints after drug administration with parent and B-ol quantified by LC/MS/MS. PK parameters describing B disposition were determined by fitting compartmental models to individual patient plasma concentration-time data. An integrative parent-metabolite PK model is being developed to explain the clinical pharmacokinetics of B-ol. Thirty-four patients have been enrolled at daily x 3 doses of B ranging from 1.2 to 9.6 mg/m^2 with the MTD for this dosing schema determined to be 7.5 mg/m^2. Mean (range) population terminal half-life is 31.9 (11.0-89.2) hrs, plasma drug clearance is 49.7 (22.3-107.5) L/hr/m^2, and Vss is 1842 (583-4722) L/m^2 for the daily arm; and terminal half-life is 44.0 (34.9-52.2) hrs, plasma drug clearance is 26.9 (13.8-35.8) L/hr/m^2, and Vss is 173 (661-1800) L/m^2 for six patients enrolled on the weekly regimen, with doses ranging from 7.5 to 10.0 mg/m^2. Regimen related toxicity was benign with the most common adverse event being myelosuppression. Percentage of unchanged parent drug renally eliminated was 3.8% (0.4-14.9%). Several partial responses and one complete response have been noted, even at dose levels below the observed MTD of 7.5 mg/m^2/day for the daily arm. The results of B are similar to what we have observed in our preclinical studies, demonstrating increased lipophilicity and enhanced biodistribution of drug when compared to doxorubicin. Direct confirmation of drug penetration into the CNS is the focus of an ongoing clinical trial in patients with leptomeningeal malignancies. In mice, B-ol has shown poor bioavailability after oral administration, and 30 to 50-fold lower brain tissue penetration than the parent drug, when each were given as 40 mg/kg IV injections. Parent-metabolite studies are ongoing to adequately determine the in vivo conversion rate, thus far, based on clinical comparisons of AUC, exposures of metabolite range from 14-37% of that of the parent. With in vitro evidence suggesting metabolite cytotoxicity to be 40% and 49% that of B in U87 and DS4 glioma cell lines, respectively, further study of the clinical role of B-ol is warranted.

EGFR/Her2/ras/raf/MAPK Pathways

B102 Combinations of trastuzumab with either pertuzumab or bevacizumab show higher efficacy than lapatinib-based combinations against HER2-positive breast cancer xenografts progressing on trastuzumab monotherapy. Thomas Friess1, Werner Scheuer1, Max Hasmann1. 1Roche Diagnostics GmbH, Penzberg, Germany.

The humanized monoclonal antibody (mab) trastuzumab, which specifically binds to the extra-cellular domain of HER2 has become standard of care for patients with HER2-positive breast cancer. Unfortunately, some patients progress while on trastuzumab and chemotherapy combination treatment. One way to avoid progression is to block HER pathways more completely by targeting other HER-family growth factor receptors like HER1/EGFR or HER3. We investigated whether HER2-positive tumours progressing under trastuzumab therapy can be inhibited by the combination of trastuzumab in multiple lines with other HER-family receptor targeting agents (pertuzumab and lapatinib) that are in clinical development, and together with clinically established cytotoxic drugs (paclitaxel and 5-FU). Since up-regulation of VEGF has been suggested to promote tumour progression, we also tested the combination of trastuzumab with the anti-huVEGF targeting mab bevacizumab. We used the HER2-positive human breast cancer xenograft model KPL-4 which is known to shed the extra-cellular domain of HER2 and to co-express HER1 and HER3.

Mice carrying well established KPL-4 xenograft tumours of about 100-120 mm^3 size were treated once weekly (q7d) with 15mg/kg i.p. trastuzumab, following an initial 2-fold loading dose. After 2 weeks of trastuzumab treatment, those mice with progressively growing tumours of about 160-250 mm^3 in size were randomly distributed into various groups in order to test different treatment options. The compounds used for second line treatment included: (1) pertuzumab (15mg/kg, i.p. q7d), a novel HER dimerisation inhibitor; (2) bevacizumab (5mg/kg, q3d, i.p.) which inactivates huVEGF-A; (3) lapatinib (25mg/kg, 2qd, p.o.), which is described as a dual HER1 and HER2 tyrosine kinase inhibitor, and two cytotoxic drugs, (4) paclitaxel (15mg/kg, q7d, i.v.) and (5) 5-FU (50mg/kg, q7d, i.v.).

While trastuzumab treatment was continued after progression, the addition of either pertuzumab or bevacizumab for combined second line treatment demonstrated significant antitumour activity compared to untreated or trastuzumab single agent controls. Adding paclitaxel or 5-FU on top of the dual antibody combinations was clearly superior, resulting in complete tumour remissions and long term survival. In contrast, various combinations of trastuzumab with lapatinib were not efficacious and less well tolerated, especially with the addition of paclitaxel or 5-FU. In summary, our data indicate that there are several effective treatment options for HER2 positive tumours to be used in treatment in multiple lines of trastuzumab with monoclonal antibodies targeting HER-family receptors and/or angiogenesis. The addition to continued trastuzumab therapy of either the HER dimerisation inhibitor pertuzumab or the anti-VEGF mab bevacizumab together with cytotoxic drugs, resulted in superior antitumour activity with complete tumour remission and long term survival, compared to lapatinib-based combinations.

B103 High Amphiregulin and Eripegulin expression in K-ras wild type colorectal primaries predicts response and survival benefit after treatment with cetuximab and irinotecan for metastatic disease. Sabine Yeffar1, Wendy De Roock1, Bart Biesemans1, Jef De Schutter1, Hubert Pissyeaux2, Yves Humbert2, Marc Peeters2, Ilhan Celik3, Eric Van Cutsem1. 1KULeuven, Leuven, Belgium; 2UCL, Bussels, Belgium; 3UGent, Ghent, Belgium; 4Merck Serono, Darmstadt, Germany.

To date no validated markers of outcome to cetuximab (CTX) in metastatic colorectal cancer (CRC) exist. The potential use of molecular markers determined on the primary tumor needs to be explored. We determined the KRAS mutation (Mut) state and measured the EGFR ligands’, Amphiregulin (AR) and Eripegulin (ER), mRNA expression levels in 95 formalin Fixed Paraffin embedded (FFPE) primary CRC specimens of
patients (pts) treated with CTX standard dose and trinitotecan (Iri) in clinical trials for metastatic disease and correlated these variables with response and overall survival (OS).

**Methods:** Tumor areas were manually dissected for RNA and DNA extraction. AR and ER mRNA expression was assessed by real-time quantitative RT-PCR (TaqMan). Expression levels (ΔCt) represent the average value of duplicate reactions, normalized for GAPDH expression. Lower ΔCt values indicate higher expression. The median ΔCt values was used as cut-off between high and low expression. KRAS exon 2 Mut were analyzed by allele specific TaqMan and confirmed by PCR and sequencing. RECIST criteria for tumor response were used.

**Results:** The expression of AR and ER in these primaries showed a normal distribution. Mean Ct was 4.516 (SD 1.493) (95%CI 4.211-4.820) for AR and 4.797 (SD 1.588) (95%CI 4.473-5.120) for ER. AR and ER expression were significantly correlated (r = 0.712, p < 0.0001). KRAS Mut were found in 35 % of pts. KRAS wild type status (WT) and high ligand expression were significantly correlated (χ²; p < 0.0001). Estimated median OS was 16.7 w (95%CI[12.2-21.2]) in KRAS Mut pts vs. 45.9 w (95%CI[38.4-53.3]) (Log-rank: p = 0.0001) in WT pts. Estimated median OS was 43.0 w (95%CI[37.2-48.8]) in high AR expressors vs. 22.9 w (95%CI[17.0-28.7]) (p = 0.008) in low AR pts. Estimated median OS of KRAS WT pts with high AR expression was 49.4 w (95%CI[27.9-70.9]) vs. 30.6 w (95%CI[13.9-47.3]) (p = 0.01) in low expressors. In KRAS Mut Pts OS was similar in high AR expressors 15.86 w (95%CI[13.7-18.1]) vs. 15.86 w (95%CI[11.2-20.5]) (p = 0.405) in low AR expressors. Data for ER were similar. A Cox regression analysis looked at the following predictor variables: age, sex, KRAS, AR and ER expression and maximum grade of skin toxicity on OS. Skintoxicity (HR (per increase ∆Ct) (95%CI[0.618-0.907]; p = 0.003) were retained in the model.

**Discussion:** We show that AR and ER mRNA expression can be quantified on FFPE primary tumor samples. Only in KRAS WT pts they significantly influence tumor response and OS, when subsequent metastatic disease is treated with CTX and IRI. The identification of these response predictors early on in the disease process and their persistence in metastases (1), suggests AR, ER and KRAS mutation status are essential players in tumor biology and stresses the importance of the EGF pathway in this setting. The combined use of these markers on primary tumors may provide an improved prediction of outcome so far in mCRC treated with CTX and IRI.


B104 An ErbB receptor-derived peptide, Inherbin3, inhibits EGF-induced receptor activation, cell proliferation and cell migration.

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ErbB receptor tyrosine kinases play crucial roles in tumourigenesis, and hence constitute attractive targets for structural drug design (Normanno et al., 2003). The ErbB receptors are activated by ligand-induced homo- and heterodimerization. Structural studies have revealed that ErbB receptor dimers are stabilized by receptor-receptor interactions, primarily mediated by a region in the second extracellular domain, termed the “dimerization arm” (Schlessinger, 2002). The present study demonstrates that a peptide constituting the dimerization arm of ErbB3, termed Inherbin3, binds to the extracellular domains of all four ErbB receptors, with highest peptide binding affinity exhibited by the extracellular domains of ErbB1 and ErbB3. At the cellular level, Inherbin3 functions as an antagonist of epidermal growth factor (EGF)-ErbB1 signalling. Specifically, we show that Inherbin3 inhibits EGF-induced ErbB1 phosphorylation and serum-induced growth of two human ErbB1 overexpressing tumour cell lines, the head and neck cancer cell line HN-5 and the non-small cell lung cancer cell line A549. Furthermore, the present study shows that Inherbin3 inhibits serum-induced migration of A549 cells in a Boyden Chamber invasion assay. Finally, the effects of Inherbin3 are compared to the effects of known ErbB1 kinase inhibitors and the anti-ErbB1 monoclonal antibody Cetuximab, which are shown to have a stronger inhibitory effect on HN5 cell proliferation than Inherbin3, whereas their effects on A549 cells is considerably weaker than the effects of Inherbin3. In summary, the Inherbin3 peptide and similar ErbB-derived peptides constitute a potential therapeutic strategy for preventing ligand-induced dimerization of ErbB receptors, thereby interfering with their functions in tumourigenesis. Specifically, the present study indicates that Inherbin3 is a potential anti-cancer drug for treatment of ErbB1 overexpressing cancers.

B106 Three-dimensional (3D) overlay culture models of human breast epithelial malignant progression reveal a critical dependence on MAP kinase activation for Ras-driven proliferation. Quanwen Li1, Albert B. Chow1, Raymond R. Mattingly1,1Wayne State University, Detroit, MI.

Although Ras mutations are common in many human cancers, they are rare in breast cancer. Nevertheless, functional Ras activation is prevalent in human breast cancers, presumably due to over-expressed growth factors and their receptors, and there is evidence that therapeutics directed to inhibit the Ras pathway may be useful in breast cancer treatment. We have screened drugs that inhibit several pathways that may be downstream of Ras activation, including MAP kinase, PI 3-Kinase, Rac1 and Rho/myosin light chain kinase (MLCK) for their effects in both traditional, two-dimensional (2D) cell culture and overlay 3D cultures in reconstituted basement membrane (rBM). We tested the agents against: control MCF10A cells, which are a spontaneously immortalized human breast epithelial cell line that retains a normal phenotype by most criteria; a progression series from MCF10A that is termed NeoT, AT1, and DCIS cells (which are aspontaneous immortalized human breastepithelial cell that retainsanormal phenotype bymost criteria;aprogression series fromMCF10A thatis termed NeoT, AT1, and DCIS cells (which model hyperplasia, atypical hyperplasia, and ductal carcinoma in situ xenograft studies, respectively); and transformed MCF10A that express high levels of activated H-Ras or N-Ras. We used spheroid size to measure cell growth in 3D overlay culture, and MTI (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to determine proliferation in 2D culture. The MAP kinase kinase inhibitor U0126 potentially blocked proliferation of the MCF10.H-Ras and MCF10.N-Ras cells in 3D overlay culture, with EC50 values of 2.1 and 3.7 µM, respectively. A second MAP kinase kinase inhibitor, PD184352, was significantly more potent. Interestingly, U0126 had almost no effect on control MCF10A cells, or the NeoT or AT1 variants, in 3D overlay culture, while having a moderate effect on the MCF10.DCIS cells at the highest concentration tested (10 µM). Western analysis showed that DCIS cells express higher levels of H-Ras protein than the MCF10A, NeoT, and AT1 cells, but less than that in MCF10.H-Ras cells. Remarkably, the selective effect of the MAP kinase kinase inhibitors to inhibit the proliferation of the more transformed cells that was observed in the 3D overlay cultures was not seen in 2D cultures, where 10 µM U0126 produced a similar degree of inhibition of proliferation (30-70%) in all the cell lines tested.

In addition to activation of MAP kinase, Ras signaling may lead to other pathways, including activation of PI3K, Rac1 or Rho/MLCK, that could further contribute to malignant progression. In this study, we found no significant growth inhibition in the 3D overlay cultures in response to wortmannin (200 nM, a PI3 kinase inhibitor), NSC23766 (75 µM, a Rac1 inhibitor) or ML-7 (10 µM, a MLCK inhibitor). MTT assays showed only slight effects of these inhibitors in 2D-Culture.

The results from this study suggest that 3D overlay culture may be a good model for the screening of Ras pathway inhibitors. Using this model, our results indicate that MAP kinase kinase inhibition may be an effective means to selectively suppress Ras-driven proliferation and malignant transformation of breast epithelial cells. Unlike 2D culture, where there was little selectivity for action against the more transformed variants, 3D overlay culture apparently reveals that dependence on MAP kinase activation increases during progression.
B107  Expression of Her-2/neu in gastric adenocarcinoma. Sung Bae Jee, Yong Seok Kim, Hoon Huh, Hae Myung Jeon. Catholic University of Korea, College of Medicine, Seoul, Korea, Republic of Korea. Purpose: HER-2 oncogene plays a role in cellular oncogenic transformation and is related to aggressive tumour behaviour. Several studies addressed the prognostic and predictive roles of HER-2 status in gastric cancer; however, HER-2 gene has not been used as a therapeutic target because opinions about its prognostic role are controversial. Methods: Between January 1994 and December 1997 (1995 group), 192 patients who underwent curative resection for gastric adenocarcinoma in St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, were retrospectively reviewed from their paraffin embedded tissues and medical records. One hundred twenty-seven patients, with the same characteristics and operated on between January and December 2002 (2000 group) were compared. The expression of HER-2 was analyzed by means of immunohistochemistry, with regard to the clinicopathological features and survival rate. Results: The average age of patients in the 1995 and 2000 groups were 56.3 and 59.1 years, respectively. In the 1995 group, 40.1, 26.5, 24.0 and 9.4%, had stage I, II, III and IV diseases. Whereas, in 2000 group, these figures were 57.5, 18.1, 19.7, 4.7%, respectively. The expression of HER-2 was positive in 12/192 patients (6.3%) in the 1995 group, and in 7/127 (5.5%) in the 2000 group. There was no relation between the overexpression of HER-2 and pathologic findings. However, the 5-year survival rates of patients with negative and positive HER-2 expression were 91.7% and 75.0%, in stage I and II gastric cancers, respectively. There was a significant difference in the survival rates between the two groups (p = 0.010). Conclusion: HER-2 overexpression is an early event in gastric cancers, appearing at all stages of the disease, and is related to the poor prognosis of stage I and II diseases. This fact is thought to be the basis of monoclonal antibody treatment and chemotherapy following a surgical operation for early gastric cancer.

B108  Coordinated over-expression of genes in the EGFR pathway predicts sensitivity to EGFR inhibition in pancreatic cancer. Antonio Jimeno, 1 Aik Choon Tan, 1 Jordy Coffa, 2 N V Rajeshkumar, 1 Peter Kulesza, 1 operation for early gastric cancer. monoclonal antibody treatment and chemotherapy following a surgical

Results:

Between January 1994 and December 1997 (1995 group), 192 patients who underwent curative resection for gastric adenocarcinoma in St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, were retrospectively reviewed from their paraffin embedded tissues and medical records. One hundred twenty-seven patients, with the same characteristics and operated on between January and December 2002 (2000 group) were compared. The expression of HER-2 was analyzed by means of immunohistochemistry, with regard to the clinicopathological features and survival rate.

Results: The average age of patients in the 1995 and 2000 groups were 56.3 and 59.1 years, respectively. In the 1995 group, 40.1, 26.5, 24.0 and 9.4%, had stage I, II, III and IV diseases. Whereas, in 2000 group, these figures were 57.5, 18.1, 19.7, 4.7%, respectively. The expression of HER-2 was positive in 12/192 patients (6.3%) in the 1995 group, and in 7/127 (5.5%) in the 2000 group. There was no relation between the overexpression of HER-2 and pathologic findings. However, the 5-year survival rates of patients with negative and positive HER-2 expression were 91.7% and 75.0%, in stage I and II gastric cancers, respectively. There was a significant difference in the survival rates between the two groups (p = 0.010).

Conclusion: HER-2 overexpression is an early event in gastric cancers, appearing at all stages of the disease, and is related to the poor prognosis of stage I and II diseases. This fact is thought to be the basis of monoclonal antibody treatment and chemotherapy following a surgical operation for early gastric cancer.

B109  Pharmacokinetic (PK) and pharmacodynamic (PD) results of a phase I study of PD-0325901, a second generation oral MEK inhibitor, in patients with advanced cancer. Weiwei Tan, 1 Samuel DePrimo, 1 Smitha S. Krishnamurthi, 1 John J. Rinehart, 1 Lisle M. Nabel, 1 Dana Nickens, 1 Steven Bentivegna, 1 Keith D. Wilner, 1 Alejandro D. Ricart, 1 Patricia M. LoRusso, 1 Pfizer Inc., San Diego, CA; 2 Case Western Reserve University, Ireland Cancer Center, Cleveland, OH; 3 University of Alabama at Birmingham, Birmingham, AL; 4 Wayne State University, Karmanos Cancer Institute, Detroit, MI.

Background: The RAS, RAF, mitogen-activated protein kinase kinase (MEK) and extracellular signal regulated kinase (ERK) pathway represents a promising therapeutic target. It is activated in several human tumors, often through gain-of-function mutations of RAS and RAF family members that might involve similar downstream dependencies. Since ERK is the only known MEK substrate, MEK inhibition is a key target of this pathway. PD-0325901 is a potent and selective MEK1/2 inhibitor.

Methods: PD-0325901 was administered orally at 1 mg QD, and at 1 to 30 mg BID in the first-in-human phase study. Three dosing schedules were evaluated in 28-day (D) cycles (C): intermittent (3 weeks on/1 week off), continuous (every D), and continuous with breaks (5 D on/2 D off). Serial blood samples were obtained on C1D1 and C1D21 (or C1D19) in 58 patients, and urine samples were collected in intervals on C1D21 in 35 patients. Plasma and urine concentrations of PD-0325901, its carboxylic acid metabolite (PD-0315209), and its S-enantiomer (PD-0326116) were determined. The effect of food on PD-0325901 PK was evaluated in 16 patients. Tumor tissue was sampled at baseline and C1D15 (or C1D19) from 27 patients and assessed by immunohistochemistry (IHC) for pERK modulation. Other biomarker evaluations included: Ki-67, pAKT, and PTEN methylation. Mutational analysis of select regions of BRAF, NRAS, and KRAS genes were also performed. Plasma drug concentrations were also determined at tumor tissue collection.

Results: In general, both C max and AUC 0-24 of PD-0325901 increased proportionally with the doses tested. The time to peak plasma concentration occurred within 1-2 hours after administration on an empty stomach, and was delayed for 2-8 hours with a meal. The mean elimination plasma half-life (t 1/2) was 8.8 hours. The exposure of PD-0315209 was comparable to that of the parent; the mean ratios of PD-0315209 to PD-0325901 plasma AUC 0-24 were approximately 124% following repeated dosing. The terminal t 1/2 of PD-0315209 appeared to be longer than that of the parent drug. About 2.5% of the administered PD-0325901 dose was excreted from urine as unchanged. PD-0325901 suppressed tumor pERK in a dose-dependent trend. Doses ≥ 2mg BID, at
which the plasma concentration exceeded the minimum target level based on xenograft mouse models, consistently caused >60% suppression of pERK in tumors. Ki-67 was also reduced in some cases. BRAF and KRAS or NRAS mutations were detected in 20 of 41 cases screened and were mutually exclusive. Fourteen of 30 patients with melanoma had mutations in the BRAF gene, all of which were at the V600E site.

**Conclusion:** The PK of PD-0325901 was characterized by rapid absorption, with generally dose-proportional increases in Cmax and AUC. PD-0325901 suppresses pERK in tumor tissue. Correlations between mutational status and clinical benefit will be presented.

**B110 A pilot phase II study of PD-0325901, an oral MEK inhibitor, in previously treated patients with advanced non-small cell lung cancer.** Eric B. Haura1, Timothy G. Larson2, Philip J. Stella3, Lyudmila Bazhenova4, Vincent A. Miller5, Roger B. Cohen6, Peter D. Eisenberg7, Paulina Selaru8, Keith D. Wilner8, Alejandro D. Ricart9, Sirish M. Gadge8. 1H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL; 2Hubert H. Humphrey Cancer Center-North Memorial Health Care, Robbinsdale, MN; 3St. Joseph Mercy Hospital, Ann Arbor, MI; 4University of California at San Diego, San Diego, CA; 5Memorial Sloan-Kettering Cancer Center, New York, NY; 6Fox Chase Cancer Center, Philadelphia, PA; 7California Cancer Care Inc., Greenbrae, CA; 8Pfizer Global Research and Development, San Diego, CA; 9Karmanos Cancer Institute, Detroit, MI.

**Rationale:** The RAS-RAF-MEK-ERK MAP kinase signaling pathway is activated in a variety of human tumors. Mutational activation of KRAS or BRAF has been detected in ~25% of NSCLC (BRAF mutation in about 3%; Brose et al. 2002; Davies et al. 2002; Naoki et al. 2002). ERK1/2 activation has also been associated with tumor aggressiveness (Vicent et al. 2004).

**Methods:** This study was undertaken to determine the activity of PD-0325901 in advanced NSCLC (*"wet"* stage IIIb or IV) as measured by objective response according to RECIST. PD-0325901 was initially administered at 15 mg BID intermittently (3 weeks on/1 week off). This schedule was not well tolerated. Consequently, additional breaks consisting of weekends off drug were added (ie, 5 days [D] on/2 D off for 3 weeks, followed by 1 week off). Each cycle consisted of 28 D. Blood samples for PK were collected on D 0 and 15 (or 19) of cycles 1, 2, 3, 5 and 7 at predose and 2 to 8 hours postdose.

**Results:** All patients had received prior systemic therapy with a median of 2 regimens (range 1-4), 26% had received EGFR-TKIs. Thirteen patients (8 M/5 F, median age 64 [range 47 - 69]) were treated on the initial schedule, but 4 of them came off study early for adverse events (visual disturbances, pulmonary infiltrates, fatigue and hallucinations). Only one patient had stable disease. Twenty-one patients (10 M/11 F, median age 64 [range 36-82]) received the revised intermittent schedule. Patients received a median number of 2 cycles (range 1-10). Major toxicities included: reversible visual disturbances (6 patients, 29%), grade (G) 2-4 diarrhea (6 patients, 29%), G2 rash (6 patients, 29%), and G2-3 fatigue (5 patients, 24%). Two patients (10%) had pulmonary embolism and 1 patient (5%) had congestive heart failure. Fourteen patients were evaluable for response on the intermittent schedule with breaks. There were no objective responses. Five patients had stable disease, the longest lasting 10 months with a 25% reduction in tumor size. Nine patients had early progressive disease (<2 months) or clinical deterioration. The mean trough concentration of PD-0325901 was 108 ng/mL at steady state after multiple doses, and exceeded the minimum target level based on xenograft mouse models (11.5 ng/mL).

**Conclusion:** Limited clinical activity was observed in this heavily pretreated population. It will be important to refine the dosing of PD-0325901 further to minimize toxicity. Further evaluation using a lower dose on the intermittent schedule may be warranted in selected patients with KRAS or BRAF mutations.

**B111 Activity of mevalonate pathway inhibitors against institute ovarian and breast cancers in the ATP-based tumour chemosensitivity assay.** Louise A. Knight1, Sharon Glaysher2, Christian M. Kurbacher2, Ralf Reichelt2, Augusta Fernand01, Marta Polak1, Ian A. Cree1. 1University of Portsmouth, Portsmouth, United Kingdom; 2L.a.n.c.e. Inc., Bonn, Germany.

Prenylation of Ras and other oncoproteins is an important facet of their activation and is susceptible to inhibition of the mevalonate pathway that provides the necessary metabolites required. Previous data suggest that lipophilic statins such as fluvastatin and N-bisphosphonates such as zoledronic acid have anti-cancer effects in vitro and in patients. We have examined the effect of fluvastatin alone and in combination with zoledronic acid in the ATP-based tumour chemosensitivity assay (ATP-TCA) for effects on breast and ovarian cancer tumour-derived cells. In addition, the effect of these drugs on inhibition of Rap1a prenylation in MCF-7 cells was assessed by Western blotting following exposure to the individual agents. Both zoledronic acid and fluvastatin showed activity in the ATP-TCA against breast and ovarian cancer, though fluvastatin was less active, particularly against breast cancer. The combination of zoledronic acid and fluvastatin was more active than either single agent in the ATP-TCA with some synergy against breast and ovarian cancer tumour-derived cells. Western blot studies using MCF-7 cells demonstrated an increase in unprenylated Rap1a with both drugs compared to untreated cells. Sequential drug experiments showed that pre-treatment of ovarian tumour cells with fluvastatin resulted in decreased sensitivity to zoledronic acid. These data suggest that the combination of zoledronic acid and fluvastatin may have activity against breast and ovarian cancer. A clinical trial to test this is in preparation.
such as SPARC, MGC11242, NDRG1 and ACTN1 showed to be modulated after EGFR (epidermal growth factor) treatment in the HB4, C5.2 and SKBR3 cells. Our study provides a comprehensive view of the expression changes induced in two human mammary carcinoma expressing different levels of ERBB2 in response to docetaxel that might contribute to elucidate the mechanisms involved in ERBB2-mediated chemoresistance in breast cancer. Supported by FAESP.

B113 Clinical aspects of a phase I study of PD-0325901, a selective oral MEK Inhibitor, in patients with advanced cancer. Patricia A. LoRusso1, Smitha S. Krishnamurthi2, John J. Rinehart3, Lisle M. Nabel1, Gary A. Croghan4, Paul B. Chapman5, Paulina Selaru6, Sinil Kim6, Alejandro D. Ricart7, Keith D. Wilke4, 1Wayne State University, Detroit, MI; 2Case Western Reserve University, Ireland Cancer Center, Cleveland, OH; 3University of Alabama at Birmingham, Birmingham, AL; 4Mayo Clinic and Foundation, Rochester, MN; 5Memorial Sloan-Kettering Cancer Center, New York, NY; 6Pfizer Global Research and Development, San Diego, CA.

Background: PD-0325901 is a highly potent and selective oral inhibitor of the dual-specificity kinases, MEK1/2 (MAPK/ERK/Kinase), thereby preventing phosphorylation and subsequent activation of mitogen-activated protein kinase (pMAPK/pERK). MEK inhibition seems to be a rational therapeutic strategy for BRAF mutant tumors.

Methods: This evaluation was designed to determine the maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) and dose limiting toxicities (DLT) of PD-0325901. Pharmacokinetic and pharmacodynamic results are presented in a separate abstract. PD-0325901 was administered at 1 mg QD, and at 0.5 to 10 mg BID. Each cycle consisted of 28 days and 3 schedules of administration were evaluated: intermittent (3 weeks on/1 week off), continuous (every day), and continuous with breaks (5 days on/2 days off).

Results: Sixty six patients were enrolled; 33 M/33 F; median age 57 (range 29 - 86), breast (7), colon (4), non-small cell lung cancer (NSCLC) (5) and melanoma (50). All except 4 patients had received prior systemic therapy with a median of 4 (range 0-14). Patients received a median number of 3 (range 1-9) cycles of PD-0325901. The most common toxicities included rash, diarrhea, fatigue, nausea, visual disturbances and vomiting. Acute neurotoxicity was frequent in patients receiving ≥15 mg BID (all schedules). Escalation in the intermittent schedule proceeded until DLT was observed in 2 patients at 30 mg BID: grade (G) 3 rash and G3 syncope. There were also 1 case of optic neuropathy at 20 mg BID and 1 case of optic nerve ischemia at 15 mg BID. Based on this early experience, 20 mg BID (intermittent) was originally chosen as the RP2D. However, emerging phase 2 data indicated that this dose was not well tolerated (G3-4 confusion, neck/foot drop, slurred speech) and continuous with breaks schedule was evaluated with an initial dose of 10 mg BID. Of the 13 patients on this dose, there was one case of retinal vein occlusion (RVO). Continuous dosing was evaluated concurrently, and two patients had RVO (at 15 and 10 mg BID). Contrast to the neurotoxic effects, all episodes of RVO presented quite late, after 13, 15 and 36 weeks of therapy. A subsequent analysis of relevant visual episodes found predisposing factors for retinopathy in all patients with RVO, but no correlation with cumulative dose. There were 3 PRs (melanoma) and 24 SD ≥3 months (22 melanoma, 2 NSCLC).

Conclusion: PD-0325901 demonstrated preliminary activity in heavily pretreated patients with melanoma. Nevertheless, two different continuous regimens of PD-0325901 have been associated with RVO. Predisposing factors for retinopathy may have increased risk. Further evaluation at doses ≤10 mg BID may be conducted in selected patients with an intermittent schedule.

B114 KRAS mutational status is associated with clinical response in patients with metastatic colorectal cancer receiving panitumumab monotherapy. Dan Freeman1, Todd Juan1, Neal J. Meropol1, J. Randolph Hecht2, Jordan Berlin3, Eric Van Cutsem4, Maureen Reiner5, Robert Radinsky1, Rafael G Amaud1, Marc Peeters5, 1Amgen Inc., Thousand Oaks, CA; 2Fox Chase Cancer Center, Philadelphia, PA; 3UCLA School of Medicine, Los Angeles, CA; 4Netherland University Medical Center, Nashville, TN; 5University Hospital Gastuisberg, Leuven, Belgium; 6Ghent University Hospital, Ghent, Belgium.

Background: Panitumumab is a fully human monoclonal antibody targeting the epidermal growth factor receptor (EGFr) and is indicated in patients (pts) with refractory metastatic colorectal cancer (mCRC) (Van Cutsem 2007). Since anti-EGFr therapy does not benefit all pts with mCRC, identifying biomarkers of responsiveness has become a forefront clinical effort as it would allow physicians to target therapy to those pts most likely to benefit. We evaluated the association of KRAS mutational status with clinical efficacy to panitumumab in ptc samples from 4 large mCRC studies of safety and efficacy with panitumumab monotherapy.

Methods: Tumor sections from 66/709 pts from three phase 2 studies and one phase 3 extension study were consented for genetic sequencing, had response data, and were available for analysis of KRAS gene mutations. Genomic DNA was isolated from FFPE tumor sections (pretreatment), PCR was performed on KRAS (exons 2 and 3) to determine the prevalence of activating mutations. For exons 2 and 3, more than 30 independent colonies from each cloning procedure were sequenced and resolved on a Genetic Analyzer. Best objective response was assessed using RECIST (2 studies) or WHO criteria (2 studies) at prespecified timepoints; the three phase 2 studies were assessed by blinded central review and the extension study was assessed by local review.

Results: Of the 66 pts, 41 (62%) pts had wild-type (WT) KRAS and 25 (38%) pts harbored a KRAS mutation (codon 12 & 13). In the mutant KRAS group, 6 pts had SD (24%) and 19 pts had PD (76%) as their best OR; there were no responses. In the WT KRAS population, the PR rate was 12% (95% CI: 2 to 22), the SD rate was 54% (95% CI: 38 to 69), and the PD rate was 34% (95% CI: 20 to 49). The association between KRAS mutation status and lack of response to panitumumab was statistically significant (Fisher’s exact test, p = 0.003). From a Cox PH model, the hazard ratio for WT:mutant KRAS was 0.6 (95% CI: 0.34 to 0.95) for PFS and 0.5 (95% CI: 0.29 to 0.91) for OS.

Conclusion: Our findings suggest that KRAS mutational status may be a predictive marker of clinical benefit with panitumumab in mCRC pts. Pts with activating KRAS mutations in exon 2 may be less likely to respond to treatment with panitumumab monotherapy. These findings warrant further investigation in a controlled clinical trial to determine the predictive value of KRAS mutational status for response to panitumumab treatment.

B115 Aquaporin 5 (AQPS) is a novel signaling molecule triggering Ras/ERK/retinoblastoma (Rb) signaling pathway in colon cancer cell lines. Sung Koo Kang1, Janghee Woo1, Young Kwang Chae1, Myoung Sook Kim1, Jin Hyen Baek1, Jong Chul Park1, Juna Lee1, Minjoo Park1, Barry Trink1, Edward Ratovitski1, Taekyu Lee1, Beomsoo Park1, Jean Charles Soria2, Joseph Califano3, Se Jin Jang2, David Sidransky3, Chulsoo Moon4, 1Johns Hopkins University, Baltimore, MD; 2Gustave Roussy Institute, Villejuif, France; 3Asan Medical Center, Seoul, Republic of Korea.

Overexpression of several aquaporins (AQPS) has been reported in different types of human cancer; however, the role of AQPS in human carcinogenesis has not yet been clearly defined. Here, we demonstrate that overexpression of human AQPS (hAQPS) induced many phenotypic changes characteristic of transformation both in vitro and in vivo.

Overexpression of wild-type (WT) hAQPS increased cell proliferation and phosphorylation of ERK1/2 in colon cancer cell line HCT116. However, this phenomenon in AQPS mutants (N185D and S156A) was diminished compared with WT AQPS, indicating the requirement of both membrane association and serine/threonine phosphorylation of AQPS. Overexpression of AQPS and AQPS showed no difference of ERK1/2 phosphorylation, suggesting that AQPS, unlike AQPS, might be a water channel involved in
signaling transduction. Moreover, in the nucleus, hAQP5-overexpressing cells showed an increase of phosphorylation of retinoblastoma (Rb) protein through complex of cyclin D1 and CDK4. Small interfering RNA (siRNA) analysis showed that AQP5 activates the Ras signaling pathway in DLD1 and HCT116 cell lines. Furthermore, hAQP5-transfected NIH3T3 cells revealed tumorigenicity in athymic mice in vivo as well as in vitro colony formation. Therefore, our findings for the first time suggest that hAQP5 plays an important role in human carcinogenesis through Ras/ERK/Rb signaling pathway.

B116 A phase I study of Lonafarnib, a farnesyl transferase inhibitor in combination with herceptin plus paclitaxel in Her2 / neu overexpressing breast cancer. Jan H. Schellens1, Véronique Dieras2, Marja Roelvink1, Ahmad Awada3, Anne-Sophie Govaerts4, Jan Bogaerts4, Sandrine Marréaud2, Denis Lacombe1. 1The Netherlands Cancer Institute, Amsterdam, The Netherlands; 2Institut Curie, Paris, France; 3Institut Jules Bordet, Brussels, Belgium; 4EORTC, Brussels, Belgium.

Background: Lonafarnib (SCHOOL 66336) is a farnesyl transferase inhibitor. This phase I study assessed the safety and tolerability of Lonafarnib (L) administered in combination with herceptin (H) plus paclitaxel (P) every 21 days in Her2/ neu metastatic breast cancer (MBC).

Patients and Methods: Patients (pts), first or second line MBC, were treated with escalating doses of L administered orally twice daily in combination with H (4 mg/kg loading dose, 2 mg/kg weekly thereafter) and P (175 mg/m² except 135 mg/m² at dose level 1 (DL)) with cycles repeated every 3 weeks. Lonafarnib was administered continuously at DLs of 75, 100, 125 mg/bid and from d1-d7 and d15-21 at DL of 150 mg/bid. At least three pts were treated at each dose level, and the maximum tolerated dose (MTD) was defined as the highest DL that was not associated with an unacceptable severe toxicity (dose associated with a probability of acute Dose Limiting Toxicity (DLT) closest to 20%).

Results: Twenty three pts with a median age of 49 years (range 34-68) received 185 cycles of treatment with a median duration of 6 cycles (range 1-26) and 6 pts are still on treatment. The median PS was 0 and 14 pts had prior exposure to anthracycline. Five DLs were investigated and the trial was interrupted before reaching the MTD upon the sponsor’s request. To date, nineteen pts went off protocol, 10 due to toxicity, 2 upon patient’s request, 3 upon investigator decision and 4 for PD. The adverse events for which patients went off protocol were: neuropenia (6), neuropathy (2), allergic reaction (1) and ischemia and increase QTc (1). At the highest DL (L 300 mg/d) one out of eight pts experienced DLT of grade 3 allergic reaction (1) and ischemia and increase QTc (1). At the highest DL (L 300 mg/d), one out of eight pts experienced DLT of grade 3 allergic reaction (1) and ischemia and increase QTc (1). At the highest DL (L 300 mg/d), one out of eight pts experienced DLT of grade 3 allergic reaction (1) and ischemia and increase QTc (1). At the highest DL (L 300 mg/d), one out of eight pts experienced DLT of grade 3 allergic reaction (1) and ischemia and increase QTc (1).

Conclusions: The toxicity profile observed was consistent with that expected for this regimen. Preliminary efficacy results are very promising. Thus, this combined treatment merits further investigation.

B117 The role of ADAM-17 in chemotherapy-induced EGFR activation. Joan N. Kylia1, Sandra Van Schaeybroeck1, Caitriona Holohan1, Daniel B. Longley1, Patrick G. Johnston1. 1Queen’s University, Belfast, United Kingdom.

Human cancer cells may respond to chemotherapy by activating the epidermal growth factor receptor (EGFR) and survival pathways. Recently, we have shown that colorectal cancer (CRC) and non-small cell lung carcinoma (NSCLC) cells respond to chemotherapy by activating EGFR and are thereby sensitized to EGFR inhibitors. In light of these results, we have investigated the mechanism by which EGFR is activated following chemotherapy treatment in CRC and NSCLC cells. We found that the MMP (matrix-metalloproteinase) and ADAM (a desintegrin and metalloproteinase) inhibitor GM6001 abrogated chemotherapy-activated EGFR phosphorylation in CRC and NSCLC cells, indicating that EGFR activation was mediated by metalloproteinases. Further studies indicated that ADAM-17 was the principal ADAM involved in chemotheraphy-induced EGFR activation. Furthermore, we found that ADAM-17 regulated TGF-alpha shedding following chemotherapy treatment and that TGF-alpha was the main ligand involved in chemotherapy-induced activation. In addition, silencing of ADAM-17 or TGF-alpha sensitized CRC and NSCLC cells to chemotherapy-mediated apoptosis. The importance of ligand shedding for chemotherapy-induced EGFR activation was further demonstrated by the use of the EGFR-targeted antibody cetuximab (C25), which blocks ligand binding. Co-treatment with C225 attenuated chemotherapy-induced activation of EGFR and sensitized CRC and NSCLC cells to chemotherapy. Our findings indicate that increased EGFR activity following chemotherapy is mediated by ADAM-17-mediated shedding of TGF-alpha, suggesting that specific metalloproteinases in combination with chemotherapy may have therapeutic potential for the treatment of CRC and NSCLC tumours.

B118 Pediatric preclinical testing program (PPTP) evaluation of the EGFR and ErbB2 inhibitor lapatinib. Peter J. Houghton1, John M. Maris2, Joshua Courtright2, Henry S. Friedman3, Stephen T. Keir3, Richard B. Lock4, Hernan Carol5, Richard Gorlick5, E. Anders Kolb6, Nino Keshelava7, C. Patrick Reynolds7, Christopher L. Morton1, Malcolm A. Smith8, 1St. Jude Children’s Research Hospital, Memphis, TN; 2Children’s Hospital of Philadelphia, Philadelphia, PA; 3Duke University, Durham, NC; 4Children’s Cancer Inst, Randwick, Australia; 5Albert Einstein College of Med, New York, NY; 6duPont Hospital for Children, Wilmington, DE; 7Children’s Hospital of Los Angeles, Los Angeles, CA; 8NCl-CTEP, Bethesda, MD.

Background: Lapatinib is a small molecule reversible tyrosine kinase inhibitor of EGFR and ErbB2 that shows in vitro and in vivo activity against a range of EGFR- and ErbB2-dependent adult cancer cell lines and that has clinical efficacy against ErbB2-overexpressing breast cancer. Lapatinib was studied by the PPTP to develop data concerning the relevance of EGFR family members as therapeutic targets for childhood cancers.

Methods: The PPTP includes a molecularly characterized in vitro panel of cell lines (n=27) and in vivo panel of xenografts (n=61) representing most of the common types of childhood solid tumors and childhood ALL. Lapatinib in vitro testing used media containing 10% FCS and evaluated concentrations from 1.0 nM to 10 µM, with viable cell numbers for treated and control replicates evaluated at 96 hours using the DIMSCAN fluorescence-based method. Lapatinib was tested against the PPTP in vivo panels using a twice-daily oral administration schedule for six weeks (5-days on, 2-days off) at a dose of 160 mg/kg (320 mg/kg/day). Three measures of antitumor activity were used: 1) response criteria modeled after the clinical setting; 2) treated to control (T/C) tumor volume at day 21; and 3) a time to event (4-fold increase in tumor volume) measure based on the median EFS of treated and control lines (intermediate activity required EFS T/C > 2, and high activity additionally required a net reduction in median tumor volume at the end of the experiment).

Results: EGFR and/or ErbB2 were expressed at detectable levels in most of the PPTP’s cell lines and xenografts at the RNA level based upon data from Affymetrix U133 Plus 2.0 arrays. The median IC50 value for lapatinib against the entire PPTP cell line panel was 6.84 µM. IC50 values ranged from a low of 2.08 µM (Ramos, NHL) to a maximum exceeding 10.0 µM in seven cell lines. Lapatinib was well tolerated in vivo, with toxicity in only 1.5% of the treated animals. Lapatinib induced significant differences in EFS distribution compared to controls in 1 of 41 evaluable xenografts tested. No xenografts met the criteria for intermediate activity for the PPTP EFS activity measure (EFS T/C value > 2.0 and a significant difference in EFS distribution). No objective responses were observed in any of the solid tumor panels or in the ALL panel. The best response observed was a single example of PD2 (progressive disease with growth delay).

Conclusions: The response of the PPTP cell lines to lapatinib corresponds to the pattern of response described previously for adult cancer cell lines that do not overexpress EGFR or ErbB2 and that have IC50 values exceeding 1 µM. Thus, the lapatinib in vitro activity against the PPTP cell lines likely represents “off-target” kinase
inhibition effects. The lack of in vivo activity for lapatinib is consistent with previous reports that EGFR mutation and ErbB2 amplification are uncommon for childhood cancers. These results do not preclude a role for lapatinib in a biological subtype of a pediatric cancer that is not represented within the PPTP panel. However, when combined with preclinical and clinical experience to date for EGFR small molecule inhibitors, the results do suggest a limited role for EGFR family members as therapeutic targets for childhood cancers. (Supported by NCI N01CM2216)

B119 Phase I pharmacokinetic study of ECO-4601, a novel bificação targeting agent. Pierre Faradadou,1 Sharyn D. Baker,2 Petr Kavan3, Benoît Samson3, Robert Bélanger3, Maxime Ranger1, Michael D. Harvey1, Gerald Battist1.1Thallion Pharmaceuticals, Montreal, Quebec, Canada; 2St. Jude Children’s Research Hospital, Memphis, TN; 3Segal Cancer Center, Jewish General Hospital, Mc Gill University, Montreal, Quebec, Canada; 4Charles LeMoine Hospital, Greenfield Park, Quebec, Canada; 5M2 Research, Saint-Bruno, Quebec, Canada.

Background: ECO-4601 (U.S. Patent 7,101,872) is a structurally novel farnesylated dibenzodiazepinone with broad µM in vitro cytotoxic activity and in vivo antitumor activity in rat glioma and human hormone-independent prostate and breast tumor xenografts. Preclinical data suggest ECO-4601 is a targeted anticancer drug with dual activity: selective binding to the peripheral benzodiazepine receptor (PBR), resulting in apoptosis, and inhibition of the Ras-MAPK pathway, which is involved in cellular proliferation and migration. Antitumor activity is dependent on continuous exposure, and a target plasma ECO-4601 efficacy concentration of 2 µM was determined. The pharmacokinetic profile ECO-4601 has been tested in a Phase I clinical trial in patients with advanced solid tumors.

Methods: ECO-4601 was administered as a 2-week continuous i.v. infusion (CIV) followed by 1 week off in repeated 21 day cycles. The trial included dose-escalation and dose-extension portions, with comprehensive pharmacokinetics (PK) during the first and second cycles. ECO-4601 doses of 30, 60, 120, 180, 270, 360, and 480 mg/m2/day were evaluated in 14 patients in the escalation portion and in 12 additional patients treated at 480 mg/m2/day. ECO-4601 plasmatic concentrations were determined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) using EDTA-K2 as anti-coagulant agent. The lower limit of quantitation (LLOQ) was established at 1 ng/mL.

Results: The first six (6) patients dosed at 30 mg/m2/day had a mean ECO-4601 plasmatic concentration of 0.50 µM, which was in good agreement with a PK simulation calculated from PK studies in animal species (mouse, rat and monkey). Apparent volume (Vapp) of distribution was 111 ± 26 L and clearance (CL) was 9.4 ± 2.4 L/hr. Inter-patient PK variability was moderate (26% CV for CL). After the infusion ceased, ECO-4601 was rapidly eliminated from the bloodstream within 24 hr at all dose levels. The mean terminal half-life was ~9.4 hours and the post-infusion half-life (t1/2 alpha) was less than 30 min.

ECO-4601 concentrations increased linearly as a function of dose and clearance, half-life and volume of distribution appeared to be independent of dose level. Doses of 270, 360 and 480 mg/m2/day in the dose escalation portion resulted in ECO-4601 steady-state plasma concentrations of 2.4, 3.1 and 4.7 µM, respectively. These plasma concentrations are above the estimated therapeutic threshold determined in animal efficacy studies. An additional twelve (12) patients were dosed in the dose extension portion at the selected dose of 480 mg/m2/day. Plasma concentrations varied between 4.5 and 7 µM. Blood samples were collected during cycles 1 and 2, and steady-state concentrations in human plasma were comparable between both cycles, indicating that no drug accumulation had occurred. Post-infusion half-life (t1/2 alpha) of ECO-4601 was similar in both cycles.

Conclusions: ECO-4601 is a bification targeting agent, against a novel combination of targets, for which estimated therapeutic plasma concentrations are reached at doses of 270 mg/m2/day or greater. ECO-4601 is well tolerated and demonstrates preliminary evidence of biological activity in an early phase clinical trial. These PK data, together with Phase I safety data, and non-clinical data, support further development of ECO-4601 in a Phase II trial.

B120 RDEA119: A potent and highly selective MEK inhibitor for the treatment of cancer. Paul Weinigarten1, Robert Hamatake1, Sonny Gunawan1, Li-Tain Yeh1, Hong Kim1, Kathy Tieu1, Gary Larson1, Chon Lai1, Cory Iversen1, Todd Vo1, Shunji Yan1, Jean-Michel Vernier1,2.1Ardea Biosciences, Costa Mesa, CA.

Introduction: The RAS-RAF-MEK-ERK pathway has emerged as a significant focus for molecular targeted cancer therapy, and MEK inhibitors have the potential for broad utility in the treatment of human cancers driven by activation of this pathway. We have identified a novel series of potent and highly selective MEK inhibitors with favorable pharmacological properties. RDEA119 was selected from this series for clinical development.

Methods/Results: A radiometric enzyme assay that measured RDEA119 inhibition of MEK phosphorylation of ERK resulted in IC50 values of 17 nM for MEK1 and 43 nM for MEK2. Kinetic analysis indicated MEK inhibition was non-competitive with respect to ATP. X-ray crystallography of the complex of MEK1-ATP-Mg-RDEA119 showed binding of RDEA119 to an allosteric pocket in MEK. Of 205 kinases tested, only MEK1 & 2 were significantly inhibited. ELISA analysis of cellular phospho-ERK levels was used to determine MEK inhibition by RDEA119 in A375 human melanoma cells (EC50 = 7 nM, 1% FBS). In human cell lines A375 (melanoma), A431 (epidermoid carcinoma), Colo205 (colon cancer), and HT29 (colon cancer), RDEA119 inhibited anchorage independent proliferation with mean Gl50 values of 71, 86, 89, 70 nM, respectively. RDEA119 also inhibited anchorage independent growth in cell lines A375 and Colo205 at mean Gl50 values of 84 nM and 40 nM, respectively. Flow cytometry revealed a G1/S phase arrest in RDEA119 treated A375 cells, and at doses up to 10 µM, RDEA119 showed negligible cytotoxicity to primary human cells. When RDEA119 was dosed orally qd for 14 days, significant tumor growth inhibition and tumor growth delay was seen in nude mice bearing various subcutaneously implanted human tumor xenografts (e.g., A375, Colo205, A431) with tumor regressions evident in some treated animals.

Conclusions: RDEA119 is a potent and selective MEK inhibitor with favorable in vitro and in vivo anti-tumor properties in preclinical model systems. RDEA119 is now in development for the treatment of cancer.

B121 Epitope mapping and elucidation of the mechanism of action of panitumumab, a fully human monoclonal antibody targeting the epidermal growth factor receptor (EGFR). Dan Freeman1, Jilin Sun1, Randall Bass1, Ken Chong1, Selam Ogbagabriel1, Gary Elliott1, Robert Radinsky1. 1Amgen Inc., Thousand Oaks, CA.

Background: Understanding an antibody’s binding epitope may shed light on its mechanism of action (MOA). Panitumumab, a fully human monoclonal antibody directed against the epidermal growth factor receptor (EGFR), has demonstrated anti-tumor efficacy as a monotherapy in both preclinical models and in clinical trials. The objective of this study was to identify the residues on EGFR that are critical for panitumumab binding and to gain further information on the mechanism of action of panitumumab.

Methods: The extracellular region of EGFR (amino acids 1-618) was expressed as an avidin fusion protein in 293T cells and purified using biotin coated polystyrene beads. Fusion proteins were used to determine the binding affinity of panitumumab using BIACore technology and were used as a control for further epitope mapping studies using a flow cytometry based assay. EGFR truncation mutations, including individual domains I, II, III and IV, were also expressed to determine the domain on EGFR that was necessary for panitumumab binding. Replacement scanning was performed on solvent-accessible amino acids in domain III (ligand binding domain) to further define the critical residues involved in panitumumab binding. Using mutagenesis from the panitumumab Fab’2, the crystal structure of the EGFR and the residues determined to be critical for panitumumab binding, a model for panitumumab binding that minimize steric clashes was built. To evaluate the potential MOA, A549 NSCLC cells were treated with 20ug/ml of panitumumab 1 hour prior to a 15 minute stimulation with known EGFR ligands (TGF-α, amphiregulin, epiregulin, HB-EGF, betacellulin and EGF). Inhibition of ligand-induced phosphorylation of EGFR was determined using a specific anti-pEGFR (pY1068) antibody.

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Results: The binding affinity of panitumumab for the extracellular domain of EGFR was 50 pM by BIAcore. Further truncation analysis by flow cytometry narrowed the binding epitope of panitumumab to domain III of the EGFR. Point mutations revealed that amino acids 349, 355, 412 and 438 were critical for panitumumab binding. A 2.9 Å Angstrom crystal structure of the panitumumab Fab’2 was solved. A model of the panitumumab-EGFR complex was generated and predicts that the CDRs for both light and heavy chains of panitumumab would be proximal to domain III of EGFR. In vitro studies determined that panitumumab can inhibit receptor activation of all known EGFR ligands in A549 NSCLC tumor cells.

Conclusion: From our model using the point mutation and crystal structure data, we determined that panitumumab binds to surface exposed amino acids in the ligand binding domain of EGFR (domain III). Panitumumab inhibits all known EGFR ligands, resulting in inhibition of receptor activation.

B122 Integration of functional proteomics with discrete non-parametric token-based modeling to identify combinational therapies targeting EGFR homeostatic loops in breast cancer cells. Melissa M. Muller, Derek Ruths, Jen-Te Tseng, Luay Nakhleh, Gordon B. Mills, Prahald T. Ram. 1MD Anderson Cancer Center, Houston, TX; 2Rice University, Houston, TX.

The epidermal growth factor receptor (EGFR) signaling system is a highly interconnected, dynamic network containing multiple positive and negative feedback loops. EGFR is overexpressed in many breast, lung, colon, and pancreatic cancers and plays a key role in driving the proliferation of cells.

In this work we take a combined experimental and computational modeling based approach to understanding signal propagation through EGFR network. Functional proteomic studies using reverse-phase protein microarray (RPPA) technology highlighted several key molecules in the EGFR signaling network which were upregulated in response to treatment with three different MEK inhibitors. We coupled this to the development of a discrete non-parametric token-based simulation to model the EGFR network. The model provided evidence that targeted agents disrupt regulatory loops leading to consequential increases in other components of the network. Therefore, in order to disrupt the regulatory loops, we determined which combination of targets may be more efficient. The combinational targets identified by the model were then tested experimentally in a panel of breast cancer cell lines using quantitative immunoblotting and RPPA for experimental measurements of signaling dynamics.

Biological signal transduction networks are complex, dynamic systems, whose analyses are greatly facilitated by quantitative modeling in conjunction with traditional experimental techniques. The results of this study and further implementation of our approach in the EGFR signaling network have the promise to inform the development of new, targeted pharmaceuticals for cancer treatment.


Background: Trastuzumab is a monoclonal antibody therapeutic in use for treatment of breast cancers overexpressing HER2/neu. The high molecular weight and binding affinity of trastuzumab, in combination with tumor specific microenvironmental factors, may limit its distribution and impact its efficacy. Unfortunately analytical methods make investigations of extravascular drug distribution challenging.

Methods: Mice bearing HER2/neu overexpressing MDA-435-LCC6 xenografts were systemically administered 4 or 20 mg/kg trastuzumab, with tumor harvest at various time points thereafter. Bound trastuzumab was imaged directly in frozen tumor cross-sections using fluorescently tagged anti-human secondary antibodies. Combinations of other tumor features, including HER2/neu, CD31, perfusion marker carboxyline, desmin and collagen IV were also identified on the same tumor sections. An automated x-y stage and robotic microscope enabled image tiling to generate images of whole tumor sections. Up to five images from different markers per section were overlayed to create composite images for quantitative analysis of trastuzumab distribution relative to marker expression and nearest blood vessel location.

Results: Imaging trastuzumab directly permits the characteristics of penetration over time and distribution within tumors in the context of other microenvironmental features. A single dose of 4 mg/kg is detectable in tumors at 3 hours, peaks by 24 hrs and is in decline by 48 hrs. The highest intensity of bound trastuzumab is near vessels, but is above background intensity at distances up to 150 µm from nearest vasculature; drug presence decreases with time homogeneously through the tumor cord. At early timepoints extravasation of trastuzumab is seen in most tumor vessels, although considerable inter-vessel heterogeneity is seen. Analysis of sections obtained at 1 mm intervals from the gross tumor edge (perpendicular to the caudal-rostral axis) showed higher bound trastuzumab closer to the tumor ends, 3 or 26 hrs after dosing. HER2/neu expression is seen to be homogeneous in these sections and therefore does not account for heterogeneity of bound trastuzumab. The vascularity of these sections was also analyzed, with no changes seen in microvessel density (MVD) or fraction of perfused vessels. The endothelial-cell specific collagen type IV association with vasculature was also consistent throughout the tumor. A marker of vascular maturity, desmin, showed a higher fraction of vessels positively associated in sections proximal to the tumor edge, in correspondence with those tumor sections displaying higher trastuzumab saturation.

Conclusions: Trastuzumab penetration and distribution into tumor tissue has been analyzed in addition to microenvironmental features using direct immunohistochemical techniques. Here we show trastuzumab penetration detectable at 3 hrs and peaking at 24hrs; the distribution through tumor microenvironment demonstrates heterogeneity with tumor margins saturating more thoroughly in the doses and time-points analyzed. This heterogeneity is independent of HER2/neu expression and microvessel characteristics with exception to the fraction of vessels found to be associated with pericytes.


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Background: XL647 is an orally bioavailable small molecule inhibitor of receptor tyrosine kinases involved in tumor growth and angiogenesis, including EGFR/ErbB1, HER2/ErbB2, and VEGFR2/KDR. Oral administration of XL647 results in dose-dependent and sustained inhibition of these targets in preclinical pharmacodynamic studies. In addition, XL647 has preclinical activity in the H1975 NSCLC tumor model, which harbors the T790M mutant form of EGFR that is resistant to erlotinib and gefitinib.

Methods: Patients (pts) with previously untreated advanced NSCLC (Stage IIIIB with malignant pleural effusion or Stage IV) with adenocarcinoma histology are eligible. Pts must have an activating mutation of the EGFR gene in tumor tissue or meet at least one of the following three demographic eligibility criteria: Asian, female (F), minimal (<15 pack-years) or no smoking history (within the last 25 years). XL647 is administered orally as a single dose of 350 mg on days 1-5 of each 14 day cycle. Tumor response assessed every 8 wks by RECIST is the primary end point. The mutational status of EGFR and other relevant genes in tumor tissue is being analyzed. Plasma markers reflecting effects of antiangiogenic therapy such as VEGF-A, sVEGFR2 and Ang2 are being analyzed.

Results: Thirty-three pts (23F/1 Asian/10M) with a median age of 66yrs (range 44-86) have been enrolled. Sixteen pts were never-smokers, 11 had ≤15 pack years (pk-yr) smoking history, and 6 had >15 pk-yr. The best responses to date include 7 partial responses (PR, 5 confirmed [cPR]), 11 stable disease (SD), and 12 progressive disease (PD). One pt is too early to
evaluate. In total, 16 pts have been discontinued with PD (including 4 with stable disease [SD] or better), 1 pt discontinued due to Grade 3 GI hemorrhage, and 1 was withdrawn due to an ineligible screening QTC value. A preliminary pharmacokinetic analysis indicated that mean pre-dose and 4-hr concentrations (i.e., approximate trough and peak) of XL647 on the 5th day of cycle 1 were (mean ± std-dev) 346 ± 108 and 577 ± 208 ng/ml, respectively, and appeared generally unchanged from cycle to cycle. Deletions in exon 19 of the EGFR gene have been detected in tumor tissue from 4 pts. Two of these pts experienced cPRs and two unconfirmed PRs. Two pts with EGFR L858R mutations have SD (9% and 21% reduction, respectively). EGFR mutations were not detected in tumor DNA from 3 additional pts who experienced a cPR. No EGFR or KRAS gene mutations were detected in 5 pts with PD. Further mutational analysis is ongoing. The most common adverse events reported to date are Grade 1/2 diarrhea, fatigue, rash, and nausea.

Conclusions: XL647 has anti-tumor activity with >50% of pts experiencing either PR or SD, and is generally well-tolerated in a NSCLC patient population clinically enriched for likelihood of having an EGFR mutation. All 6 pts with EGFR activating mutations have experienced tumor shrinkage, including 4 with PRs. 3 patients without an EGFR mutation experienced a cPR. Updated results including mutational analysis and further characterization of pharmacokinetic and pharmacodynamic parameters will be presented.

B125 JAB1 confers resistance to trastuzumab by inhibition of p27.

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The development of the humanized monoclonal antibody, trastuzumab targeting the HER2 (ErB2) receptor has provided promising treatment to patients with aggressive HER2 positive breast cancer. However, resistance is common with only 26% of HER2 overexpressing tumors responding to treatment and many tumors that do initially respond will regress within one year. Recent findings indicate that HER2 overexpressing breast carcinoma cells proliferate at a higher rate because of activated AKT phosphorylation of p27 resulting in cytoplasmic localization. Trastuzumab increases nuclear p27 protein levels by inhibiting AKT. Once in the nucleus, p27 inhibits Cdk2 activity and the cells remain in the G0/G1 phase of the cell cycle. Trastuzumab-resistant cells retain HER2 amplification and overexpression as well as inhibition of AKT when treated with trastuzumab. However, reduced nuclear p27 protein levels were observed in the resistant cells suggesting this may be a mechanism of resistance to Herceptin. The Jun activation domain-binding protein 1 (JAB1) was previously found to negatively regulate p27 through nuclear to cytoplasmic relocation and subsequent degradation. JAB1 overexpression is seen in breast cancer and correlates with low p27 expression as well as poor prognosis. We examined whether JAB1 facilitated degradation of p27 may be one mechanism of resistance against Herceptin treatment. Overexpression of JAB1 decreased p27 levels in a protesome dependent manner and increased the percentage of cells in S phase in the HER2-overexpressing breast cancer cell lines, BT-474 and SKBR-3. When SKBR-3 cells were treated with trastuzumab, p27 levels increased and G1 arrest was observed, but this was not seen when JAB1 was overexpressed. Increased JAB1 levels were observed in two BT-474 trastuzumab resistant clones, C5 and C6. Targeted silencing of JAB1 increases p27 protein levels, reinstates a G1 checkpoint in tumor cells, and reduces cellular proliferation. We have observed that JAB1 overexpression is involved in breast tumorigenesis and overexpression protects against trastuzumab treatment by facilitating p27 degradation. Further, inhibition of JAB1 sensitizes resistant cells to treatment, thus inhibition of JAB1 is a novel strategy to sensitize tumors to trastuzumab-induced tumor growth arrest.

B126 Multi-targeted siRNA galinstinate c-Src, and HSP90 combined with Cetuximab circumvent oncogene addiction, transactivation, and resistance due to mutations, and deletions in the kinase domain of EGFR in mCRC.

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Introduction: CRC cells can develop resistance to Cetuximab through insertion mutations at exon 20, and c-Src which activates EGFR in the absence of EGFR ligand despite treatment with the blocking anti-EGFR chimeric human mouse mAb. We aim to circumvent these two types of resistance.

Materials and Methods: CRC cells were obtained from metastatic patients resistant to Cetuximab due to insertion mutations at exon 20, and overexpressed c-Src. Orthotopic mouse CRC models generated from our patients’ tumor cells were injected with multi-targeted siRNA galinstinate HSP90, and c-Src.

Results: Multi-targeted siRNA inhibited expression of HSP90 resulting in degradation of EGFR with kinase domain deletion type mutations in exon 19, substitutions in exon 21, and resistant insertion mutations at exon 20. Simultaneous inhibition of c-Src circumvented transactivation, and inhibited EGFR mediated signaling inhibiting tumor proliferation, and metastases to the liver, and peritoneum. Addition to the EGFR oncogene was circumvented. Dual-inhibition of EGFR blocked the activation of downstream mediators including STAT3, AKT, Erk/MAPK, and PI3K, while IRF-1 was upregulated. There was enhanced cell to cell adhesion, and membrane localization of b-catenin, while MMP-9 active invasivity was blocked. Furthermore, the HIF-1a/Met pathway was blocked downregulating CAIX. VEGFR-2 and VEGFR-3 were blocked inhibiting vascularization and lymphangiogenesis, respectively. Finally, we observed type I, II and III PCD in tumor cells.

Conclusion: These results indicate that systemic treatment of multi-targeted siRNA galinstinate c-Src, and HSP90 circumvented resistance to Cetuximab suppressing tumor growth, and metastasis in orthotopic mouse mCRC model.

B127 Differential effects of Mek1 and Mek2 inhibition on cellular proliferation of malignant and non-malignant human breast cells.

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Stimulation or inhibition of the Raf-Mek-Erk kinase cascade signaling pathway via cell surface receptors results in downstream effects on genes controlling cell cycle progression, motility and apoptosis. Because misregulation of each of these processes is a critical step in cancer progression, there has been much interest in using Mek inhibitors as anti-cancer therapeutics. These drugs target both the Mek1 and Mek2 kinases, which are assumed to act similarly on the signaling pathway. However, there is reason to believe that Mek1 and Mek2 have differential effects on proliferation, therefore complicating drug target strategies. Ussar and Voss (JBC, 279: 43861-9, 2004) showed that Mek1 causes proliferation, whereas Mek2 induces growth arrest at G1. Additionally, Scholl et al. (Cancer Research, 64: 6035-40, 2004) over expressed Mek1 or Mek2 and found that only Mek1 increased proliferation. These data are consistent with our preliminary data in cultured human mammary epithelial cells (HMECs) which indicate that cells constitutively expressing short hairpin RNAs (shRNAs) galinstinate Mek2 proliferate more rapidly than controls. We are determining the individual contributions of Mek1 and Mek2 to proliferation and Mek inhibitor drug sensitivity in malignant and non-malignant human breast epithelial cell lines. To date, four cell lines (MCF7, MCF10A, 184A1 and 184v-TER) have been successfully transformed with lentiviruses expressing shRNAs galinstinate Mek1, Mek2, or both Mek1 and 2. The resulting knockdowns were robust and specific, as determined by immunoblotting. Surprisingly, these lines grew well despite decreased Mek expression, even in the case of the double knockdown. In addition, the shMek2 lines grew significantly faster than controls, regardless of cell lineage. Interestingly, immunoblotting measuring total Erk and phosphorylated Erk (P-Erk) levels, indicated that P-Erk levels remain
inhibition of PI3-kinase signaling. In contrast, multi-level inhibition of the PI3-kinase pathway prevents apoptosis upon additional treatment with shRNAs targeting Mek1 and/or Mek2.

B128 Synergistic effects of multi-level targeting of the MAPK and PI3-kinase pathways in breast cancer cells. Olga Mirzoeva1, Rina Gendelman1, Zachary Knight1, Richard Neve2, Heidi Feiler2, Ju Han2, Wen-Lin Kuo2, Norah Bayani2, Doris Siwak1, Gordon Mills1, Paul Spellman3, Kevan Shokat1, Frank McCormick1, Joe W Gray2, W Michael Korn1. 1UCSF Comprehensive Cancer Center, San Francisco, CA; 2Lawrence Berkeley National Laboratory, Berkeley, CA; 3MD Anderson Cancer Center, Houston, TX.

The MAPK and PI3-kinase pathways play a central role in promoting cell proliferation and cell growth in cancer cells while inhibiting apoptosis. In breast cancer, these pathways are frequently constitutively activated through various mechanisms. The discovery and extensive evaluation of novel, highly active and specific compounds targeting the PI3-kinase makes it possible to assess the interplay of both pathways and to test the therapeutic utility of combined inhibition of multiple key-molecules within these pathways. We performed extensive time-course analyses of the response of breast cancer cells to inhibition of MEK using the specific small-molecule inhibitors U0126 and CI-1040. We observed that these compounds induced G1 arrest in asynchronous cell cultures of breast cancer cells. This was accompanied by activation of PI3-kinase signaling as determined by reverse phase protein lysate array analysis. We therefore investigated the effect of combining MEK inhibitors with PIK90, a specific inhibitor of the p110-alpha subunit of PI3-kinase and mTOR. Interestingly, this combined treatment did result in even more pronounced G1 cell cycle arrest, compared to inhibition of MEK alone. No induction of apoptosis was detectable. In contrast, treatment of breast cancer cells with PI103 or with a combination of PIK90 and rapamycin (a specific inhibitor of mTOR) resulted in apoptosis induction. We conclude that inhibition of MEK induces a negative feedback loop that activates the PI3-kinase pathway and prevents apoptosis upon additional inhibition of PI3-kinase signaling. In contrast, multi-level inhibition of the PI3-kinase pathway promotes induction of apoptosis in breast cancer cells.

Epigenetic Targets

B129 Expression of the chromatin remodeling factor Rsf-1 in breast carcinoma effusions predicts poor survival. Ben Davidson1, Tian-Li Wang2, Le-Ming Shih2, Aasmund Berner1. 1Riks-hospitalet-Radiumhospitalet Medical Center, Oslo, Norway; 2Johns Hopkins University Medical Institutions, Baltimore, MD.

Objective: We recently identified Rsf-1, a chromatin-remodeling gene, as a potential oncogene that is frequently amplified and overexpressed in ovarian serous carcinoma, and demonstrated that its expression in carcinoma cells in effusions is associated with poor prognosis. In the present study, we assessed the clinical significance of Rsf-1 overexpression in breast carcinoma effusions.

Methods: Formalin-fixed paraffin-embedded sections from 52 effusions were analyzed for Rsf-1 expression using immunohistochemistry. Matched primary tumors (n=30) and solid metastases (n=26) from 30 patients were additionally studied. Rsf-1 expression in tumor cells in effusions was analyzed for possible association with clinicopathologic parameters and survival.

Results: Rsf-1 protein expression was found in carcinoma cells in 38/52 (73%) effusions, 24/30 (80%) primary carcinomas and 24/26 (92%) metastases. Rsf-1 immunoreactivity in effusions showed no association with HER-2 or hormone receptor status. Rsf-1 expression level was significantly lower in effusions compared to primary tumors (p=0.013 and p=0.011 for extent and intensity, respectively) and lymph node metastases (p=0.037 and p=0.02 for extent and intensity, respectively). However, higher staining extent in cancer cells in effusions was associated with poor overall (p=0.037) and disease-free (p=0.031) survival in univariate survival analysis. In Cox analysis, Rsf-1 expression independently predicted shorter disease-free survival (p=0.02).

Conclusions: Rsf-1 expression is downregulated in breast carcinoma cells in effusions. However, conserved expression of this chromatin-remodeling protein in tumor cells at this site is associated with aggressive clinical course. Rsf-1 may be a novel prognostic marker and therapeutic target in advanced breast cancer.

B131 Sodium valproate to enhance doxorubicin sensitivity in osteosarcoma. Luke A Wittenburg1, Barb Rose1, Liam Bisson1, Douglas H Thamm1. 1Colorado State University, Fort Collins, CO.

Osteosarcoma (OS) remains an incurable and ultimately fatal disease in many patients, and novel forms of therapy are needed. Although current therapies including surgery and adjuvant chemotherapy improve outcome, there have been no major treatment breakthroughs for OS in the last 20 years. The histone deacetylase (HDAC) enzymes are powerful new targets for epigenetic cancer therapy. The acetylation of histones, controlled by multiple histone acetyltransferases and HDACs, is important in governing chromatin structure and thereby can modulate expression of genes associated with cellular proliferation, differentiation and survival. Pharmacologic inhibition of HDAC has multiple anti-tumor effects that stem from the ability to affect cellular processes that are dysregulated in neoplastic cells. Furthermore, the effect of HDAC inhibition on chromatin structure has been shown to increase access of DNA-binding molecules such as transcription factors as well as DNA damaging agents. One available and inexpensive drug with HDAC inhibitory activity is the anticonvulsant sodium valproate (VPA). VPA has been shown to be a broad-spectrum inhibitor of HDAC, blocking the catalytic activity of class I enzymes and inducing proendothelial degradation of some class II enzymes. We hypothesized that VPA can be used to inhibit HDAC and potentiate the anti-tumor effects of doxorubicin (DOX) in OS cells in vitro and in vivo. Canine and human OS cell lines were incubated with and without VPA alone and in combination with DOX, and antiproliferative effects were evaluated using bioreductive and clonogenic assays. Effects on apoptosis were evaluated using TUNEL assay. Immunofluorescence cytometry and western analysis were used to evaluate changes in histone H3 acetylation. In vivo efficacy was evaluated in a canine OS xenograft model in athymic
mice utilizing single-agent DOX or VPA as well as combination therapy. Treatment with VPA resulted in significant increases in acetylated histone H3. Incubation with VPA alone had modest antiproliferative effects, while pre-incubation with VPA followed by a short-term exposure to DOX resulted in significant chemosensitization. Exposure to VPA also resulted in increased nuclear DOX accumulation and potentiated DOX-induced apoptosis. Mice receiving combination therapy had statistically significant reductions in tumor growth and improvements in overall survival compared to mice receiving either agent alone or placebo. Based on the encouraging in vitro and xenograft data generated, we are currently performing a phase I clinical trial in canine patients with spontaneously occurring tumors, in which dogs scheduled to receive a standard dose of DOX are pre-treated for 48 hours with escalating doses of oral VPA. In conclusion, pretreatment of canine and human OS cells with clinically relevant doses of VPA results in histone hyperacetylation, enhanced nuclear DOX accumulation, and sensitization of these cells to the antiproliferative and proapoptotic effects of DOX in vitro and in vivo. These results justify evaluation of HDAC inhibitor/anthracycline combination therapy in canine and human patients with OS.

B132 Characterization of the apoptotic and therapeutic activities of the histone deacetylase inhibitors LAQ824 and LBHS89 using a mouse model of B cell lymphoma. Leigh Ellis¹, Ralph Lindemann¹, Andrea Newbold¹, Kate Whitecross¹, Leonie Cluse², Marc Pelligreni², Andrew Wei², Clare Scott², Peter Atadja³, Scott Lowe³, Ricky W Johnstone³.

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Histone deacetylase inhibitors (HDACi) can elicit a range of biological responses that affect tumor growth and survival including induction of tumor cell cycle progression, induction of tumor cell-selective apoptosis, suppression of angiogenesis and modulation of immune responses and show promising activity against hematological malignancies in clinical trials. Using the Eµ-myc model of B-cell lymphoma and the HDACi LBHS89 and LAQ824 we demonstrated a direct correlation between induction of tumor cell death in vivo and therapeutic efficacy. Neither HDACi required p53 activity or a functional death receptor pathway, but mediated lymphoma cell death via the intrinsic apoptotic pathway as demonstrated by decreased apoptosis and therapeutic activity of LBHS89 and LAQ824 against Eµ-myc/Bcl-2 and Eµ-myc/Bcl-XL cells. Interestingly, both LBHS89 and LAQ824 effectively killed Eµ-myc/caspase-9/- and Eµ-myc/Apaf-1/- lymphomas, which lack a functional apoptosis and thus have a defective apoptotic program downstream of the mitochondria. These cells did not display classic morphological or biological features of apoptosis following treatment with LBHS89 and LAQ824 however their clonogenic capacity was significantly reduced and interestingly electron microscopy analysis indicated that these HDACi-treated cells underwent autophagy. Importantly, both Eµ-myc/caspase-9/- and Eµ-myc/Apaf-1/- responded to LBHS89 and LAQ824 in vivo indicating that in the absence of an effective apoptotic program downstream of mitochondrial membrane perturbation, tumor cells can undergo autophagy and this is sufficient to mediate therapeutic efficacy. Our studies provide important information regarding the mechanisms of action of LBHS89 and LAQ824 that may have broader implications regarding future stratification of patients receiving therapy with these agents and the use of these compounds in combination with other anti-cancer agents.

B133 Selective inhibition of HDAC isotypes decreases radiation survival of human carcinoma cells: Potential role of isotype specificity. In Ah Kim¹, So Yeon Kim¹, Jeong Ah Hong¹, Sung Ho Mun¹, Jae Sung Kim¹, Il Han Kim¹. ¹Seoul National Univ, Seongnam-si, Republic of Korea.

Background & Purpose: Histone Deacetylases (HDACs) play critical roles in an immense number of biological pathways, and the characterization of specific functions of individual isotype of HDACs are clearly required in terms of optimal therapeutic efficacy. In this study, we tried to identify the specific isotype(s) of HDACs which play important roles in the response of tumor cells following ionizing radiation.

Materials & Methods: Since the most of the HDAC inhibitors have generally lacked specificity affecting both class I and II HDACs, we applied the RNA interference for specific inhibition of individual isotype. This allowed us to investigate the relative contributions of each isotype of HDACs towards the response to ionizing radiation. The specific siRNAs targeted each HDAC isotype and nonspecific control siRNA were transfected into SQ20B cells and radiation survival was evaluated via clonogenic assay.

Conclusions: These results add to growing evidence for differential roles of individual isotypes of HDACs and support the development of new isotype-specific inhibitors which confer the ideal pharmacological profiles with least unintended effects as a proof of concept.

B134 A phase I study of oral belinostat (PXD101) in patients with advanced solid tumors. Rhoda Molfie¹, James Lee², Daniel Petrylak³, George Blumschein, Jr.⁴, Ulrik Lassen⁵, Jayme Clark³, Timothy Yap⁶, Elin Rowen⁶, Jocern Ang⁷, Elizabeth Crowley⁸, Anne Clarke⁶, Thomas Hawthorne⁶, Peter Buhl-Jensen⁷, Johann de Bono⁸, Wm Kevin Kelly⁹. ¹Royal Marsden Hospital, Sutton, Surrey, United Kingdom; ²Yale Cancer Center, New Haven, CT; ³Columbia University Medical Center, New York, NY; ⁴MD Anderson Cancer Center, Houston, TX; ⁵University Hospital Rigshospitalet, Copenhagen, Denmark; ⁶CuraGen Corporation, Branford, CT; ⁷TopoTarget, Copenhagen, Denmark.

Background: Belinostat (PXD101) is a pan hydroxamate histone deacetylase (HDAC) inhibitor which demonstrates broad anti-neoplastic activity in vitro and in vivo. In Phase I studies in patients (pts) with advanced cancer, IV belinostat is well-tolerated up to 1000 mg/m² days (d) 1-5, q21d.

Methods: Pts with advanced solid tumors refractory to standard therapy, or for which no standard therapy exists, adequate renal and hepatic function and Karnofsky PS 70-100 were eligible. Primary objectives were to evaluate safety and tolerability, and secondary objectives, to determine pharmacokinetics (PK) and explore anti-tumor efficacy of oral belinostat. Pts were dosed in sequential cohorts of 3-6 pts on 1 of 4 schedules: once (QD) or twice (BID) daily in 28d cycles (continuous schedule), or QD or BID d1-14 q21d (discontinuous schedule). The dose was escalated in 250mg increments. Fasting and non-fasting PK studies were performed on all pts along with serial ECGs to evaluate effects on QTc interval.

Results: Sixty pts, median age 60 (range 32-80) have been treated: 46 pts on continuous schedules at 250mg QD (20 pts), 500mg QD (6 pts) and 250mg BID (20 pts), and 14 pts on discontinuous schedules at 500mg QD (3 pts), 750mg QD (7 pts) and 500mg AM/250mg PM (4 pts). Median number of cycles is 2 (range 1-11); 15 pts continue on study. Two of 6 pts on continuous schedule of 500mg QD developed dose limiting toxicity (DLT) of grade (gr) 3 dehydration and gr 3 fatigue, and thus the MTD was set at 250mg QD. Dose escalation from 250mg to 500mg BID was deferred.
and the MTD set at 250mg QD. One of 6 pts each at 750mg QD and 500mg AM/250mg PM discontinuous schedule developed DLT of gr 3 fatigue. In available safety data from 41 pts, the most frequent adverse events (AE) occurring in >25% of pts included fatigue, anorexia, nausea, constipation and weight loss. Fatigue is the only drug-related grade 3/4 AE in >1 pt, occurring in 9 pts. Symptoms usually resolved after drug was withheld. In >2400 EGs collected, no QTcF >500ms or QTcF increase >60ms above baseline was seen. Most common tumor types treated were colorectal (n=15), prostate (n=10), bladder (n=6) and lung (n=5). To date, 7 pts have SD lasting >4 months (one pt each with adenoid carcinoma, chondrosarcoma, renal, lung, prostate, thyroid). PK studies detected belinostat in serum, with exposure generally correlating with either dose, or frequency of dose (i.e., QD vs. BID). The apparent t1/2 of QD oral belinostat ranged from 1.16 - 1.59 hrs and did not appear to be influenced by food. T_max ranged from 1.26- 3.68 hrs.

Conclusions: Oral belinostat at 250mg QD and 250mg BD continuous, and 750mg d1-14 in a 21d cycle is tolerated. Dose escalation on discontinuous schedules continues.

B135 Hypermethylation of p16INK4a is associated with cervical neoplasia. Woong Ju1, Eun Ji Kang1, Sa Ra Lee1, Shi Nae Lee1, Seung Cheol Kim1, Jong Sub Choi2. 1Ewha Womans University, College of Medicine, Seoul, Republic of Korea; 2Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Republic of Korea.

Objective: Epigenetic mechanisms such as DNA methylation play an important role in the development of cervical preinvasive lesion and invasive cancer. The aim of this study is to examine the methylation status of p16INK4a in carcinoma in situ (CIS) and invasive squamous cell carcinoma of uterine cervix and the correlation between methylation status and disease progression.

Methods: The present study included 59 patients in all, 7 non-cancer controls, 21 patients with CIS, and 31 patients with invasive squamous cell carcinoma (Ia6,Ib15,IIa6,IIb3, and IVb1 patient). Cervical tissues were selectively microdissected. DNA was isolated using DNA isolation kit. Methylation status of p16INK4a gene was analyzed by methylation-specific PCR (MSP) using primer, p16 M1. Comparison of each variable were performed using the χ² test or Fisher’s extract test.

Results: Of the 52 cervical neoplasia investigated, 29 (55.8%) tumors showed methylation. The methylation rate for CIS and invasive SCC was 57.1% (12/21) and 54.8% (17/31), respectively compared to 14.2% (1/7) in controls. Promoter hypermethylation ranged from 1.16-1.59hrs and did not appear to be influenced by food. T_max ranged from 1.26-3.68 hrs.

Conclusions: Oral belinostat at 250mg QD and 250mg BD continuous, and 750mg d1-14 in a 21d cycle is tolerated. Dose escalation on discontinuous schedules continues.

B136 Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. Sonia Lain1, Jonathan Hollick2, Johanna Campbell1, Oliver Staples1, Maureen Higgins1, Aputhala Mustapha1, Anna McCarthy2, Virginia Appleyard1, Karen Murray1, Thompson Alastair1, Joanne Mathers1, Stephen Holland1, Mike J.R. Stark1, Julie Woods1, David Lane1, Nicholas J. Westwood1, 1University of Dundee, Dundee, United Kingdom; 2University of St Andrews, St Andrews, United Kingdom.

A 30,000 compound library was screened using a p53-activation cell-based assay. To date, 2 hit compounds from this screen have proven active in animal models. Here we focus on one of these hits, tenovin-1, along with its more water-soluble analogue, tenovin-6. Via a yeast genetic screen, biochemical assays and validation studies in mammalian cells we demonstrate that tenovins act through inhibition of the protein-deacetylase activities of SirT1 and SirT2 sirtuin family members. Tenovins are significantly more potent in mammalian cells than sirtuin inhibitors discovered using other approaches, decrease tumor growth in vivo as single agents and have led to the finding that wild-type and mutant p53 differ in their acetylation status. This study shows that using p53 as a sensor for compound activity in cells and exploiting the vast amount of available information on p53 regulation, rapidly leads to the discovery of small-molecule tools with potential as therapeutics.

B137 SFRP1 promoter methylation and its contribution to endometrial tumorigenesis. Bharati V Bapat1, Genna Luciani1, Vaijayanti Pethe1, Michele Zysman1, Aaron Pollett1. 1Mount Sinai Hospital, Toronto, Ontario, Canada.

Purpose: The secreted frizzled-related protein 1 (SFRP1) encodes a wnt/beta-catenin signalling antagonist and is frequently inactivated by promoter hypermethylation in tumors. We hypothesized that epigenetic inactivation of SFRP1 may be causally involved in the development of endometrial cancer. We undertook a study to examine the contribution of SFRP1 promoter methylation and its association with tumor microsatellite instability (MSI) status among patients with endometrial carcinomas of the endometrioid type.

Methods: Formalin-fixed, paraffin-embedded tissue sections of endometrial adenocarcinomas and matched normal tissue specimens were obtained from patients (n=56) and DNA was extracted from microdissected specimens enriched for tumor and normal cell populations. MSI status was determined using five National Cancer Institute (NCI) consensus panel markers and the methylation status of SFRP1 promoter was analyzed by methylation-specific PCR (MSPCR) assay of sodium bisulfite treated DNA obtained from these specimens. Beta-catenin expression was assessed in a subset of endometrial tumors by immunohistochemistry.

Results: We found that a majority of tumors (87%) show some degree of SFRP1 promoter methylation at the region examined, however 16% of tumors showed exclusive hypermethylation in tumors. There were 30 MSS and 26 MSI endometrial cancer cases. No significant correlation was observed with either tumor MSI status or with strong nuclear beta-catenin expression.

Conclusion: Epigenetic inactivation of SFRP1 is a common event in endometrial cancer. It is possible that for a small subset of cases, SFRP1 promoter hypermethylation may play a role in tumorigenesis either through Wnt or an alternative pathway.

B138 Selective depletion of HDAC2 is sufficient to reduce heterochromatin protein expression and induce chromatin decondensation. Douglas Marchion1, Elona Bicaku1, Morgen Schmitt1, Pamela N Munster1. 1H. Lee Moffitt Cancer Center & Res. Inst., Tampa, FL.

Background: Treatment of breast cancer cells with histone deacetylase (HDAC) inhibitors results in chromatin decondensation and the sensitization of cancer cells to DNA damaging agents such as topoisomerase II inhibitors. We previously reported that the HDAC inhibitors induced chromatin decondensation through the down-regulation of heterochromatin maintenance proteins such as heterochromatin protein 1 (HP1), structural maintenance of chromosome proteins (SMC) 1-5 and DNA methyltransferase 1 (DNMT1). Here we report the role of HDAC2 in the expression of heterochromatin maintenance proteins, chromatin decondensation and DNA damage induced by topoisomerase inhibition.

Methods: HDAC2 was selectively depleted using siRNA transfection. HDAC2 depleted cells were evaluated by microarray and Western blot analysis for changes in HP1, DNMT1 and SMC mRNAs and protein expression. Chromatin decondensation was evaluated by electron microscopy. DNA damage and cell death induced by the topoisomerase inhibitor, epirubicin, was evaluated by the expression of H2AX and nuclear fragmentation assays. For comparative analysis select experiments were performed with cells depleted of HDAC1 and HDAC6. Negative controls included transfection of non-silencing siRNA. Non-transfected cells treated with VPA served as a positive control.
Results: Transfection of breast cancer cells with HDAC2-specific siRNA resulted in the selective depletion of HDAC2 enzyme without affecting the expression of other HDACs. Depletion of HDAC2, while only modestly inducing histone acetylation, was sufficient to reduce both mRNA and protein expression of several heterochromatin maintenance proteins. Reduced expression of these proteins was associated with decondensation of the chromatin and sensitization of cells to DNA damage and apoptosis induced by epirubicin. In contrast, transfection of cells with non-silencing, HDAC1 or HDAC6 siRNA did not result in chromatin decondensation or sensitize cells to epirubicin.

Conclusions: Our data suggest that HDAC2 may regulate the expression of genes and proteins involved in the maintenance of chromatin structure and dynamics. Inhibition of HDAC2 may play an important role in HDAC inhibitor-induced sensitization of breast cancer cells to agents that target the DNA.

B139 Phase III: The oral isotype-selective HDAC inhibitor MGCD0103 in combination with gemcitabine in patients with refractory solid tumors. Herbert I. Hurwitz1, Emily Chan2, Peter O'Dwyer1, Nashat Gabrail3, Zuomei Li3, Ann Kalita2, M.C. Trachy Bourget2, Manal Tawashi2, R E Martell1, R Ward6. 1Duke University Medical Ctr., Durham, NC; 2Vanderbilt University Medical Center, Nashville, TN; 3University of Pennsylvania, Philadelphia, PA; 4Gabrail Cancer Center, Canton, OH; 5MethylGene Inc., Montreal, Quebec, Canada; 6Pharmion Corporation, San Francisco, California.

Purpose: The primary objective of the phase I portion of this trial was to determine the MTD of MGCD0103 in combination with gemcitabine (gem) and for the phase II portion, to assess the combination in pancreatic cancer patients (pts).

Methods: Multicenter, open-label, phase III, dose-escalation trial in adults (≥18 yrs) with refractory solid tumors. Pts (3-6 per cohort) received oral MGCD0103 3x per wk in 28-day cycles at sequential ascending doses using a 3+3 design targeting a DLT rate of <33%. Gem was administered at a standard dose (1,000 mg/m²) and schedule (1x per week for 3 weeks, followed by 1 week of rest). Before the dose of MGCD0103, on days 2 & 17, peripheral blood mononuclear cells were collected and analyzed for whole cell HDAC activity levels.

Results: At submission, 20 pts were enrolled (mean age, 56.9 ± 14.7 yrs; M:F 7:13). A dose level of 90mg MGCD0103 in combination with gem was undergoing evaluation. Previous dose levels of MGCD0103 evaluated were 50 mg, 75 mg in combination with gem. The MTD was not exceeded and dose escalation was ongoing. The most common non-hematologic toxicities observed were fatigue (n=9), vomiting (n=6), anorexia (n=5), diarrhea (n=5) and nausea (n=4). The most common heme toxicity was decreased hemoglobin (n=4). All toxicities were ≤ grade 3, except one each of grade-4 hyperkalemia and thrombocytopenia. Inhibition of HDAC activity in PBMCs was observed in the majority of pts. Among 6 evaluable for clinical response, 1 PR occurred in a pt with pancreatic ca.

Conclusions: In combination with gem, MGCD0103 was well-tolerated by pts with refractory solid tumors. The 90 mg dose is consistent with the single-agent dose for MGCD0103 currently undergoing investigation in phase II trials in hematologic malignancies; suggesting good compatibility between agents. Preliminary response analysis indicates that the combination may have clinical activity in patients with solid tumors.

B140 The decitabine-derived demethylating dinucleotide, S110 shows improved activity due to increased drug delivery and stability. Steven L. Warner1, Adrienne Clifford2, Krzysztof Swerczek3, Hariprasad Vankayalapati4, Paul Severson1, Jared J. Bears1, Jeremy Lamb1, Donald Lavalle2, Kestis Vaitkus2, Joseph DiSimone1, Chunlin Tang2, Sanjeev Redkar3, David J Bears1. 1SuperGen, Inc, Salt Lake City, UT; 2Department of Medicine, University of Illinois, Chicago, IL; 3SuperGen, Inc, Dublin, CA.

DNA demethylating agents have proven to be clinically effective in prolonging survival of patients with hematological disorders such as myelodysplastic syndrome (MDS). Decitabine (5-aza-2'-deoxy-cytidine) is a FDA approved, effective treatment for MDS, although it is subject to degradation by hydrolytic cleavage and to deamination by cytidine deaminase. To address this, we set out to improve the stability and thereby, increase the in vivo efficacy of decitabine by incorporating 5-aza-2'-deoxy-cytidine into a guanine dinucleotide to generate a second generation decitabine agent called S110. In drug stability studies, the deamination of S110 was significantly decreased compared to decitabine; however, hydrolytic cleavage was not improved (Cancer Res 2007;67:6400-8). In cell-based systems, S110 showed equivalent activity to decitabine in real-time PCR re-expression studies looking at genes commonly methylated in cancer (p16, p15, and MLH1). Interestingly, MLH1 expression went from undetectable levels in untreated cells to substantially higher levels in S110-treated cells. In additional studies, the effect of S110 on fetal hemoglobin (Hbf) levels was tested in vivo in non-human primates as a biomarker for DNA methylation. Hbf levels significantly increased in baboons treated with S110 compared to pretreatment levels. We conclude that due to the improvements in drug delivery, cellular uptake, and protection from deamination, S110 is a more potent and effective DNA demethylating agent with the potential to be used in a wider variety of hematological cancers and solid tumor types.

Functional and Molecular Imaging

B141 NVP-BEZ235, a pan class I PI3K inhibitor, impairs microvascular permeability and tumor growth as detected by magnetic resonance imaging and interstitial fluid pressure. Christian R. Schnell1, Allegirini R. Peter2, McSheehy Paul1, Stumm Michael1, Stauffer Frederic1, Brueggen Josef1, Cozens Robert1, Maira Michel Sauveur1, Garcia-Echeverria Carlos1. 1Novartis Institutes for Biomedical Research, Basel, Switzerland.

Tumor blood vessels are distinctly abnormal in structure and function. When compared to normal vessels, they are enlarged and tortuous and leaky, poorly covered by pericytes and with a defective basement membrane. VEGF is a prominent cytokine responsible for the hyperpermeable state of tumor microvasculature to plasma macromolecules. This increase in tumor vessel permeability is thought to contribute to the deposition of plasma proteins, which may provide a provisional matrix for the inward migration of fibroblasts and endothelial cells into tumors and amplify the signals important for tumor growth. The phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway functions downstream of VEGF and can activate the production of the free radical gas nitric oxide (NO) through the enzyme endothelial NO synthase (eNOS). The stimulated release of NO is central to the in vivo biological activity of VEGF since VEGF-driven vascular leakage and angiogenesis are markedly reduced in eNOS-deficient mice. PI3K inhibitors might therefore impair tumor induced angiogenesis.

In the present study, we investigated the effect of NVP-BEZ235, a novel, synthetic low molecular mass compound, which potently inhibits class I PI3K and mTOR activity, on vascular leakage in tumors and its impact on tumor progression in an orthotopic breast cancer model (BN472) in syngeneic rats. Effects on the vasculature were investigated in normal and tumor tissues with a variety of technologies: dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), tumor interstitial fluid pressure (IFP) measurements in conscious animals via radio-telemetry and a modified Miles assay, representing non invasive, minimally invasive and invasive methods, respectively. Tumor histology and IHC were used to confirm pathway inhibition.

NVP-BEZ2235 induced dose-dependent and significant decreases in VEGF-induced vascular leakage in normal and tumor tissues; IFP and tumor fractional Ktrans (vascular permeability transfer constant measured by DCE-MRI) in tumor tissue. The reduced tumor Ktrans measured after just 2 days of treatment preceded a significant inhibition of tumor growth (T/C=0.17 at 20 mg/kg p.o.) at day 6, both parameters showing a strong positive correlation (Pearson R=0.84; P=0.00009; n=15). Moreover, histological analysis showed that there was a significant increase in tumor necrosis of 2-3 fold.
In conclusion, our data demonstrate that in BN472 tumors, NVP-BEZ235 decreases tumor microvascular leakage as detected in situ using DCE-MRI, IFP and histology. Changes in $K_{\text{TRM}}$ preceded and correlated with tumor response, suggesting that $K_{\text{TRM}}$ may be a clinical biomarker, potentially useful to predict responsiveness towards NVP-BEZ235. Further studies are required to test this hypothesis.

**B142 18$\text{F}_{-}$Z$_{\text{HER2}}$-Affibody: A new tracer for molecular imaging of HER2-expressing tumors by PET.** Gabriela Kramer-Marek$^1$, Dale O Kiesewetter$^2$, Lucia Martinova$^1$, Yessenia Rodrigues$^1$, Jacek Capala$^1$.

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Expression of HER2 receptors in cancers is correlated with poor prognosis. Since it might be different in distant metastases as compared to the primary tumor, it is crucial, for adequate diagnosis and selection of proper therapy, to assess the global expression of these receptors in vivo. Currently, there are no means for repeated monitoring of their expression following targeted therapy. This work describes the synthesis as well as in vitro and in vivo characterization of a novel HER2-specific 18$\text{F}_{-}$Z$_{\text{HER2}}$-Affibody molecule that may allow application of PET imaging to assess in vivo HER2 expression and, thereby, provide means to monitor possible changes of receptor expression in response to therapeutic interventions. We have applied maleimide chemistry to attach the positron emitting 18$\text{F}$ radionuclide to Z$_{\text{HER2}}$-Cy5 Affibody molecule - a very stable protein of relatively low molecular weight (8.3 kDa) that binds to HER2 with high affinity. The in vitro binding of the resulting radioligand was characterized by receptor saturation and competitive binding assays using several human breast and ovarian cancer cell lines expressing different levels of HER2. The possible interference of Affibody molecules with binding of trastuzumab was tested by cell culture competition assays. Pre-treatment with non-labeled Affibody and cells with negligible expression of HER2 were used to test specificity of the binding both in vitro and in vivo. A thymic nude mouse bearing subcutaneous HER2-positive or HER2-negative tumors, were used for biodistribution and PET imaging studies. The in vitro studies revealed that pre-incubation with non-labeled Affibody molecules inhibited the binding of the tracer to the cells in a dose-dependent manner. Saturation analysis of 118$\text{F}_{-}$Z$_{\text{HER2}}$-Affibody molecules indicated a single class of high-affinity binding sites. The data also showed that trastuzumab does not interfere with the tracer supporting the idea that these reagents bind to different epitopes within the HER2 extracellular domain. In vivo, high accumulation of the radioactivity in HER2-positive tumor was observed as early as 20 min after injection as showed by PET imaging and reached the plateau after 40 min. That was confirmed by the results of the biodistribution studies indicating that, already 1 hr post-injection, the concentration of radioactivity (%ID/g) in tumor was 7.5 times higher than that in the blood. Four hours later, that ratio increased to 69. Two hours after injection the tumor uptake was higher than uptake in any other organs and remained steady over studied time. Our results suggest that the described 18$\text{F}_{-}$Z$_{\text{HER2}}$-Affibody radioligand conjugate can be used to assess HER2 expression in vivo by PET imaging and to monitor possible changes of receptor expression in response to therapeutic interventions.

**B143 Design and evaluation of novel glutamate-urea heterodimers that target PSMA as molecular imaging agents for prostate cancer.**

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The expression of distinct proteins on the surface of tumor cells offers the opportunity to diagnose and characterize disease by probing the phenotypic identity and biochemical composition of a tumor. Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein that is primarily expressed in normal human prostate epithelium and is highly upregulated in prostate cancer, including metastatic disease. The development of small molecule ligands that interact specifically with PSMA and carry an appropriate radionuclide may provide a promising and novel molecular targeting option for the detection, staging, and potential treatment of prostate cancer. Therefore, a series of halogen-containing glutamate-urea heterodimers (glu-urea-X) originally described by Kozikowski et al J Med Chem. 2001 Feb 1;44(3):298-301, where X = F-N-(o-l, m-l, p-l, p-Br, or p-F)-benzyl-lys or e-(p-I)-phenyl ureido-lys, were designed, synthesized and shown to bind to the N-acetyl-linked-α-acidic-dipeptide (NAALADase) region of PSMA. The affinity of the analogs was determined by screening in a competitive binding assay against N-[N-[S]-1,3-dicarboxypropyl]-carbamoyl]-[S]-3-[131 I]-iodo-L-tyrosine for binding to PSMA on LnCaP prostate cancer cells. The most potent compounds, 2-[(1-carboxy-5-(4-iodobenzylamino)-pentyl)-ureido]-pentanedioic acid (MIP-1072) and (S)-2-((R)-1-carboxy-5-(3-(4-iodophenyl)ureido)pentyl)ureido)pentanedioic acid (MIP-1095), exhibited an IC$_{50}$ of 25 ± 8 and 10 ± 3 nM, respectively (IC$_{50}$ values of the series ranged from 8 to >1,000 nM). MIP-1072 and MIP-1095 inhibited the NAALADase enzymatic activity of PSMA with $K_v$ values of 6 ± 2 and 0.3 ± 0.1 nM, respectively. In a saturation binding experiment both compounds bound to PSMA with high affinity (Kd of [125 I]-MIP-1072 = 0.25 ± 0.11 nM, Kd of [125 I]-MIP-1095 = 1.08 ± 0.03 nM). An acidity-insensitive time and temperature dependent increase in cell association of both compounds indicated cellular internalization. Both compounds were stable in plasma and liver microsomes for 1 hour. In nude mice bearing PSMA (+) xenografts, the peak uptake of MIP-1095 in the tumor was 34% injected dose per gram (ID/g) at 4 hours and the compound persisted in the tumor for 24 hours (29% ID/g). MIP-1072 achieved peak uptake at 1 hour (17 %ID/g) and washed out more rapidly (5% ID/g at 24 hr). Both compounds exhibited excellent target to background ratios with >30:1 for tumor:blood and >60:1 for tumor:muscle by 2 hr. Co-injection of the animals with a structurally unrelated NAALADase inhibitor reduced in vivo tumor uptake of both compounds $>$30-fold. SPECT imaging demonstrated localization of [125 I]-MIP-1072 and [125 I]-MIP-1095 in PSMA(+) tumors and kidney tissue but not PSMA(-) tumors or other tissues. In conclusion, 2 novel iodine containing Glu-urea-lys analogs were synthesized that bind with high affinity to PSMA; upon binding, the compounds are internalized in prostate cancer cells that express PSMA and accumulate in human prostate cancer xenografts in vivo. As MIP-1095 and MIP-1072 exhibit distinct pharmacokinetic profiles, both compounds will be further investigated in patients for localization in prostate cancer.

**B144 Whole body MRI at 7 tesla using a 1H/19F elliptic body coil with whole-body, fat-signal insensitive, three dimensional magnetic field shim algorithm.**

Leonard Liebes$^1$, Ray Lee$^1$, Songtao Liu$^1$, Michael T. Buckley$^1$, Howard Hochster$^1$, Oded Gonen$^1$. $^1$NYU Medical Center, New York, NY.

**Introduction:** We are employing fluorine-19 magnetic resonance spectroscopy (19F-MRS) for the study of gemicateline metabolism in patients with adenocarcinoma of the pancreas. To take advantage of high signal to noise ratio (SNR) at 7T, a 19F/1H double tuned 7T elliptic body coil has been designed and constructed. The novelty of this coil is that the ellipticity is used as the double tuning mechanism to achieve homogeneous excitation at both $^1$H and $^{19}$F resonance frequencies. This strategy deviates from the traditional circular coil design where the goal was just to optimize its efficiency. $^{19}$F MRS, however, requires corrections for several acquisition parameters. Key among these are instrumental, e.g., static and radiofrequency ($B_0$ and $B_1$) field inhomogeneities and molecular environment factors, as reflected in the longitudinal ($T_1$) and transverse ($T_2$) relaxation times. While $B_0$ and $B_1$ are handled by field mapping, sophisticated line-fitting postprocessing and internal water reference, $T_1$ and $T_2$ are sample-specific.

**Methods:** In order to correct the $B_0$ field inhomogeneities caused by the magnet and the patient's body susceptibility differences between air, water, and different-tissue, we applied automatic shimming. The approach performs a three dimensional $^{19}$F-MRS measurement at low spatial and temporal resolution. We then obtain the distribution of water lines'
frequency at each voxel, and automatically correct, using all 8 shim currents (3 linear and 5 non-linear) for all to resonate at one frequency. The procedure (measurement and calculation) takes less than 1 minute per iteration and 3-5 are sufficient.

**Results:** Four cycles of our signal processing algorithm with $^{19}$F has been applied with a normal male subject with good Lorentzian lineshape (from the whole body slice) of 96 Hz full-width at half-maximum. Since there are 16 voxels across the field of view (FOV), the individual voxel linewidth is approximately 8-10 Hz, which is sufficiently homogeneous for $^{19}$F MRS. Field shimming also improves the sensitivity of the MRI as shown in Fig. 2, where the pancreas (as well as the liver and kidneys) is clearly seen at the center of the abdomen.

**Conclusions:** Human body MRI and $^{19}$F MR are feasible at 7 T given the appropriate dual-tuned coil and shimming paradigm necessary to overcome the severe susceptibility distortions induced by the high field and different tissue types in the coil. Sub-ten Hz proton linewidths in (3 cm)$^3$ voxels are sufficient for effective proton and/or fluorine spectroscopy at this ultra-high field.

**B145 MicroPET imaging of heat-activated transgene expression in oral squamous cell carcinoma.** Jesse J. Parry 1, Vijay Sharma 1, Eduardo G. Moros 2, David Piwnica-Worms 3, Buck E. Rogers 1. 1Washington University School of Medicine, Saint Louis, MO; 2University of Arkansas for Medical Sciences, Little Rock, AR.

A replication-deficient adenovirus was constructed to conditionally express a fusion protein of a mutated Herpes Simplex Virus Type 1 thymidine kinase (HSV-mTK) to enhanced green fluorescent protein (EGFP), both under control of the full length human heat shock (HS) 70b promoter. The virus (AdHSV-mTK-EGFP) was evaluated both in vitro and in vivo in oral squamous cell carcinoma SCC-9 cells for expression of both mTK and EGFP. The heating time, heating temperature, and post-heating times were determined for optimal expression of the transgenes. The cells were infected at 0, 50, 100, or 500 plaque forming units (pfu)/cell and then heated for 0.5, 1, 2, and 4 h at 41°C at 1, 2, and 3 days after infection. A $^{3}$H-penciclovir uptake assay was performed 1 day after heating. Maximal expression of mTK activity was 12788 ± 1979 disintegrations per minute/mg at 1 h heat shock, 1 day after infection. Transgene expression was then evaluated 1, 2, and 3 days after heating for 1 h at 41°C, 1 day after infection, using the $^{3}$H-penciclovir uptake assay. Maximal expression was observed at 1 day post-heat. Expression of EGFP was determined using fluorescence-activated cell sorting (FACS), in which the SCC-9 cells were infected as before, but were only heated for 1 h at 41°C. Supporting the data obtained via tracer techniques, maximal EGFP expression was seen in cells 1 day post-heating after 1 day of heating at a mean fluorescent intensity of 1671 ± 172. These results provided the basis by which to heat tumors in an animal model as well as showing the efficacy of viral infection plus heat treatments on activity of both components of the mTK-EGFP chimera. To evaluate the expression of mTK-EGFP in vivo, female severe combined immunodeficient (SCID) mice were implanted with SCC-9 cells in the flanks and allowed to form tumors for 18-21 days. Either AdHSV-mTK-EGFP, at either 1 x 10$^5$ or 1 x 10$^6$ pfu/tumor, or AdCMVM-mTK-EGFP, at 1 x 10$^5$ pfu/tumor, were then injected intratumorally followed by no heat or ultrasound heating of the tumors for 1 h at 41°C, 1 day after injection. A set of uninfected tumors injected with diluent served as a negative control. On the following day, $^{3}$H-penciclovir (2 µCi) was injected intravenously into each animal, and the animals were sacrificed 4 h post-injection, tissues harvested, and counted for quantification. In control tumors expressing mTK, the percent injected dose per gram (% ID/g) was 1.7 ± 0.3, as compared to 1.0 ± 0.1 and 1.6 ± 0.36% ID/g for 1 x 10$^5$ and 1 x 10$^6$ of AdHSV-mTK-EGFP, respectively. These values were significantly greater (p < 0.05) than the negative controls (≤ 0.5% ID/g). Expression of EGFP in virally-injected, heated tumors was also observed using confocal microscopy. Based on these results, microPET imaging of SCID mice was conducted using $^{18}$F-labeled 9-[4-fluoro-3-(hydroxymethyl)butyl]guanine (FBHG) in infected SCC-9 tumors with and without heat. Supporting the biodistribution data, PET imaging demonstrated significant (p < 0.05) uptake of the radiotracer at 4 h post-injection as determined by the standard uptake values (SUV) of 0.041 ± 0.006 versus 0.013 ± 0.001 for heated and non-heated tumors, respectively. These in vivo results, which are the first to determine HS-inducible transgene expression using PET imaging, lend insight to the potential applicability of this system to be used as a non-invasive means of monitoring heat-induced gene therapy in local tumors, such as oral squamous cell carcinomas.

**B146 In living color: Optical imaging of multiple molecular targets in vivo and ex vivo.** James Mansfield 1, Richard M. Levenson 2. 1CRI, Woburn, MA.

Insights gained in characterizing intracellular pathways and other cellular phenotypes have led to increased demands on all kinds of imaging systems, which now are being asked to report on the status of multiple targets simultaneously. One factor that has interfered with the ability to image fluorescently labeled markers in vivo has been unwanted autofluorescent signals. Multispectral imaging (MSI) methodologies can spectrally characterize and computationally eliminate autofluorescence, revealing otherwise invisible molecular targets. Application of MSI can increase sensitivity by orders of magnitude, allowing much less abundant (or dimly labeled) targets to be detected and measured. MSI also provides a perfect complement to multiplexed analyses, with as many as five exogenous probes being imaged in vivo simultaneously. In addition, we will present a simple but powerful in vivo optical imaging approach that can create co-registered small animal anatomical maps using standard optical imaging instrumentation. The technique of Dynamic Contrast Enhancement (DyCE) takes advantage of the differing biodistribution dynamics of a small bolus of a tracer dye (such as indocyanine green). A time series of multi-view images acquired following injection can be analyzed to generate a high-resolution delineation of the major anatomical organs of nude mice, providing enhanced anatomic context for locating specifically labeled targets. Microscopy-based multiplex immunohistochemistry, in brightfield or fluorescence, has many potential applications in the field of drug-target evaluation. However, accurate imaging of two or more co-localized antigens, especially chromogenically labeled ones, has been hindered by difficulty in discriminating and quantifying overlaying signals. MSI can resolve overlapping labels and generate quantitative images of individual analytes. As in the in-vivo case, MSI is well suited to detecting and removing autofluorescent bands seen in fluorescence microscopy, allowing more sensitive and quantitative studies. Assessment of simultaneous (per-cell) expression of ER, PR and Her-2 expression in breast cancer using chromogenic labels and the imaging highly multiplexed quantum-dot-labeled immunofluorescent signals in tissue will be shown. The advantages of linking in-vivo and ex-vivo studies will be developed.

**B147 Circulating tumor cell analysis in patients with metastatic breast cancer.** Glenn Deng 1, David Burgess 1, Edward Mann 2, David Krag 1, Julian Burke 1, Michael Herrler 1. 1Applied Imaging Corp.-A Genetix Company, San Jose, CA; 2University of Vermont/College of Medicine, Burlington, VT; 3Genetix, New Milton, United Kingdom.

Circulating tumor cells (CTCs) may offer useful clinical information for screening, prognostication, monitoring of response to therapy, and for predicting disease recurrence. Neither imaging (CAT, PET, bone scan etc.) nor blood tests (CA15.3, CEA, PSA, etc.) meet the needs of the oncologist treating the cancer patient in respect of sensitivity, specificity and time to results. It has been shown that the quantity of CTCs is an independent predictor of survival and treatment response in breast cancer patients. To detect 1 CTC in a blood sample with >10$^6$ leukocytes, a highly sensitive and reproducible assay is needed.

Various strategies are applied to isolate and enrich the number of CTCs from blood samples. A high sensitive assay is required to test if CTCs have the potential to enable "real-time biopsies". Relatively successful approaches for CTC enrichment and detection are based on binding to anti-EpCAM antibodies. It is clear that some tumor cells are lacking or have low expression of EpCAM that will cause a variation in sensitivity.
and reproducibility with this method of CTC enrichment.

Currently, the CellSearch system is the only regulatory approved test for CTC monitoring in metastatic breast cancer patients. We recently developed a new method for CTC enrichment and analysis using the Arior SL-50 system, an automated image analysis platform. We have successfully applied our method to metastatic breast cancer patient’s blood samples and showed higher sensitivity than the CellSearch assay. We used anti-cytokeratin alone or in combination with anti-EpCAM antibodies linked to microbeads for CTC enrichment. An automated image analysis platform was used for image capture and characterization of CTCs on standard microscope glass slides. Fluorescence labeled anti-cytokeratin, anti-CD45 and DAPI were used for CTC identification. In addition, the morphology of CTCs was visualized by brightfield staining.

In a blinded comparison study, we tested approximately 50 metastatic breast cancer patients. Our method showed a higher overall CTC detection rate (46% vs. 27%) and a wider dynamic range (1-571 vs. 1-270 CTCs) than the CellSearch system. The correlation coefficient between our method and the CellSearch system was 0.95. No CTCs were detected in blood samples from normal individuals.

Our data indicate that enrichment with anti-EpCAM and anti-cytokeratin antibodies enhances assay sensitivity. The Arior SL-50 system has demonstrated a higher quality of CTC images by combining three fluorescent channels and brightfield images. It should be noted that the brightfield image provides additional morphological information to distinguish between intact cells and debris or fragments. Furthermore, we will discuss how changes of CTC counts in individual patients correlate over time with changes of CA27.29 levels.

**Gene and RNA Therapies**

**B148 Silencing EGFRvIII as a therapeutic strategy against glioma**
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Epidermal growth factor receptor (EGFR) undergoes a well-characterized deletion resulting in the creation of a constitutively active mutant receptor, named EGFR variant-III (EGFRvIII). EGFRvIII is expressed in several malignancies, including those of the brain, prostate, lung and breast. We designed a siRNA molecule against the glioma cell line U87 to specifically silence EGFRvIII and evaluate its influence on brain tumor cells as a model. Anti-EGFRvIII siRNA was introduced into EGFRvIII expressing glioma cells (U87) by electroporation, resulting in complete inhibition of expression of EGFRvIII as early as 96 hours post-treatment. During the window in which EGFRvIII was silenced, the proliferation rate of glioma cells decreased to ~20% of control levels. SiRNA treatment had no perceptible effect on the proliferation of glioma cells that do not express EGFRvIII (U87 cells). The invasiveness of siRNA-treated U87Δ cells was reduced (p<0.05), while the apoptosis rate was increased. Furthermore, glioma cell colony formation was decreased by exposing them to a lentivirus expressing anti-EGFRvIII siRNA (p<0.05). We also evaluated the signal transduction machinery downstream of EGFRvIII, including activation of the small monomeric G-protein Ras and its downstream pathway ERK (extracellular-signal regulated kinase). The levels of Ras-GTP and phosphorylated-ERK were reduced upon EGFRvIII silencing. Based on our findings, we conclude that gene silencing of EGFRvIII shows major potentials for being pursued as a translational strategy against glioma cancer.

**B149 Phase I study of the ribosome inactivating protein produg TST10088 (TST88) in patients with advanced solid tumors**
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**Background:** Ricin is a ribosome inactivating protein that inhibits protein synthesis through deproteinization of a single adenosine in ribosomal RNA. TST88 is a novel recombinant ricin-based produg activated by matrix metalloproteinases (MMPs). TST88 is activated through an MMP-specific recognition sequence, selectively targeting tumor cells where high levels of MMPs are expressed. This study aimed to determine the maximum tolerated dose (MTD) and the recommended phase II dose (RPTD) of TST88 when given intravenously on days 1, 8 and 15 every 4 weeks. Secondary endpoints include pharmacokinetics (PK), immunogenicity, safety and antitumor activity.

**Methods:** Patients with advanced solid tumors received increasing doses of TST88. Dose escalation was conducted in two phases. In the initial phase, single patients received increasing (2x) doses of TST88 from 0.01 mg/m² to 0.64 mg/m². Thereafter, a modified Fibonacci scheme was used with sequential cohorts of 3-6 patients to escalate doses from 1.1 mg/m² to 5 mg/m². Dose limiting toxicity (DLT) was defined as any grade ≥4 hematological or any grade ≥3 non-hematological toxicity. PK and anti-TST88 antibodies in plasma were determined by ELISAs.

**Results:** Twenty-four patients have been enrolled to date; complete data are available for 21: median age=59 (range: 21-78); F:M=11:10; ECOG 0:1:2=4:16:1. Primary tumor sites were colorectal (5), cholangiocarcinoma (3), ovary (2), breast (2), lung (1), pancreas (1) and others (7). Forty-five cycles of TST88 have been administered (median 2; range: 1-8). Adverse events (AEs) at least possibly related to TST88 in over 15% of the patients included fatigue, hypophosphatemia, anorexia, fever, nausea, vomiting and flu-like symptoms. AEs were generally CTCAE grades 1-2 and reversible. Two patients at 2.1 mg/m², experienced grade 2 fever, rigor and chills during day 1 cycle 2 infusion that responded well to conventional treatment. No DLT has been observed to date. Cmax and AUC levels correlated well with TST88 dosage. PK curves were biphasic with a terminal drug half-life of approximately 4 hours. Anti-TST88 antibody titers were detected in most patients during the second cycle of therapy; median value was 19.3 µg/ml (range: 1.6-104.6 µg/ml) and titers generally plateaued at below 20 µg/ml. Serum TST88 levels remained measurable in PK assays at doses up to 2.8 mg/m² despite the presence of antibodies, and antibody titers did not appear to correlate with drug dose. Response data is available for 22 patients. Four patients achieved stable disease as the best response, with one patient (melanoma) having near complete response, with one patient (melanoma) having near complete response. Four patients achieved stably disease as the best response, with one patient (melanoma) having near complete response.

**Conclusions:** At the doses evaluated thus far, TST88 is tolerable and exhibits favorable PK. Further dose escalations continue and updated clinical and PK data will be presented.

**B150 In vivo inhibition of tumor angiogenesis by systemic delivery of siRNA against VEGF pathway using TargeTran technology**
Frank Y. Xie, Yijia Liu, Ying Liu, Puthuppampillai Scarina, Martin C. Woodle, Qing Zhou, Xiaodong Yang. 1Intradigm Corp, Palo Alto, CA.

RNA interference (RNAi) is a post-transcriptional specific gene silencing mediated by double stranded small interference RNA (siRNA). RNAi therapeutics carry tremendous promise for the treatment of a wide range of diseases such as cancer, however systemic delivery of siRNA remains a challenging issue to be solved. To overcome the hurdle, we developed TargeTran technology which is a ligand-directed and sterically stabilized polymer nanoparticle (NP) for systemic delivery of siRNA. TargeTran NP is constructed with polyethyleneimine (PEI) which is PEGLyated and conjugated with Arg-Gly-Asp (RGD) peptide. PEI functions as a siRNA carrier and condenser while PEI serves a protective coating and RGD ligand mediates tumor vasculature-specific targeting and acts as integrin
receptor-mediated endocytosis. We examined the anti-angiogenic efficacy of TargeTran NP carrying siRNA against several VEGF pathway genes, including VEGF, VEGFR1, VEGFR2 or cocktails of different VEGF and VEGFR siRNA combinations in two tumor matrigel angiogenesis models in nude mice. NP carrying VEGF pathway siRNA (2mg/kg) were administered i.v. twice (2 and 5 days post tumor inoculation) into nude mice bearing human colorectal cancer cell LS-174T or renal cell carcinoma cell 786-O matrigel plugs. Matrigels were collected 8 days post tumor inoculation and tumor-induced angiogenesis was determined by immunohistochemistry staining of mouse CD31. All VEGF pathway siRNA NP treatments resulted in a significant inhibition of tumor angiogenesis comparing to the control siRNA treatment in both LS174T and 786-O tumor models. The inhibitory effect is more profound in LS174T than 786-O model. For single siRNA treatment, mouse VEGE; mouse VEGFR1 and VEGFR2 siRNA appeared to be more potent than human VEGF siRNA in both models. Treatment with cocktail siRNAs completely blocked the tumor-induced angiogenesis and showed an efficacy comparable to that of bevacizumab in LS174T model. Furthermore, RT-PCR analysis demonstrated the knockdown of target gene expression. In conclusion, TargeTran technology is capable of delivering siRNA to tumor vasculature and tumor cells, leading to silencing of target genes and suppression of tumor angiogenesis. RNAi targeting VEGF pathway represents a novel approach for antiangiogenesis-based cancer therapy.

**B151 Suppression of renal cell carcinoma growth and metastasis with sustained antiangiogenic gene therapy.** Matthew J. Mellon1, Kyung-Hee Bae2, Chinghai Kao3, Thomas A. Gardner1. 1Indiana University, Indianapolis, IN.

**Introduction and Objective:** Renal Cell Carcinoma (RCC) is the third most common urologic neoplasm. This aggressive malignancy has proven refractory to conventional treatment options. Recently, antiangiogenic agents have shown early success in treating metastatic disease. The highly vascular nature of RCC appears particularly susceptible to this approach. This study investigates the potential of sustained expression of an endostatin-angiostatin fusion protein in an early-stage model of RCC to inhibit tumor growth and metastasis.

**Methods:** Subcutaneous RCC-29 tumors were induced in 7-week old athymic nude male mice by injecting 2x10⁶ cells bilaterally into each flank region. Mice were divided into two groups. Once tumors reached volumes of 10 mm³ and 25 mm³ respectively, subjects received intratumoral injections in the right flank only of a non-replicating adenoviral vector every 20 days until the conclusion of the trial. The mice were randomly assigned to three treatment groups: saline control, viral Ad-GFP control and Ad-EndoAngio, encoding a chimeric endostatin-angiostatin fusion protein. Tumor volumes were measured twice weekly for 80 days. During days 40-50 of the trial, subjects were administered fluorescent rhodamine-BSA dye and underwent dual-photon optical imaging of the tumor vasculature to ascertain antiangiogenic effects of treatment. All animals underwent post-mortem histopathological analysis to assess for metastatic disease in the kidney, lung, liver, brain, lymph nodes and spleen.

**Results:** The 10 mm³ and 25 mm³ tumor arms when treated with Ad-EndoAngio displayed 97% and 92% growth reduction respectively (p<0.001) as compared to saline and viral controls. Further, in vivo tumor vascular imaging illustrated a reduction in blood vessel number and lumen diameter size in the Ad-EndoAngio treatment groups. Kaplan-Meier analysis illustrated statistically significant survival advantage of Ad-EndoAngio treatment. Importantly, histopathological examination demonstrated marked lung and liver metastases suppression in the treatment arms as compared to controls.

**Conclusions:** These results suggest that sustained EndoAngio gene therapy has effective antiangiogenic action against human RCC tumors and possesses potential as a novel treatment for metastatic renal cell carcinoma.

**B152 Pancreatic cancer targeted C-VISA-Bik-DD: DNA-liposome complex results in minimal toxicity in vivo.** M. M. Javle1, X. Xie1, W. Xial2, Z. Li3, H. Kuo1, Y. Liu4, Q. Ding5, S. Zhang1, B. Saphn1, Y. Yang1, Y. Wei1, J. T. Lang1, J. L. Abbuzzesi1, M. C. Hung1, M. D. Anderson Cancer Institute, Houston, TX.

**Introduction:** Gene therapy for pancreatic cancer is complicated by the need for a pancreatic cancer-specific promoter and the desmoplastic reaction within the tumor, thus making local delivery unreliable. We developed a versatile expression vector "VISA" (VP16-GALA-WPRE integrated systemic amplifier) and a CCARK (cholesterol kinase type A receptor) gene-based, pancreatic-cancer-specific promoter VISA (CCARK-VISA): liposome composite to target transgene expression systemically in pancreatic adenocarcinoma [Cancer Cell. 2007;12(1):52-65]. The acute toxic effects induced by systemic administration of this DNA:liposomal complex in C57BL/6 mice were studied.

**Methods:** C57BL/6 mice randomly received 0.9 % normal saline (control:ctrl) i.p. or cerulein i.p. 50 µg/kg injections (to induce acute pancreatitis: positive ctrl) or 100 µl (2.5 mg/kg or 5 mg/kg) of the DNA:lipo complex. Pancreas was removed, frozen in liquid nitrogen, and stored at -80°C. Tissue sections were also fixed in formaldehyde for histological and TUNEL analysis. Levels of serum AST, ALT, BUN and serum amylase activity [Phadebas Amylase Test Kit (Mable Life Sciences, Lund, Sweden)] were measured. To examine active trypsin, 25-µl aliquots of the pancreatic homogenate was incubated at 37°C for 5 minutes in assay buffer and 50 ul of 400 uM trypsin substrate (Sigma, St. Louis, MO). The plate was read using the Cytofluor Multi-Well Plate Reader (PE Biosystems, Foster City, CA) with an excitation at 380 nm and an emission at 440 nm. Trypsin activity was calculated according to a standard curve from purified trypsin (Sigma).

**Results:** No differences were observed of the levels of serum amylase (p = 0.6) and pancreatic active trypsin activity (p = 0.1) between the C-VISA-Bik-DD-treated mice and the ctrl mice. In the positive ctrl group, cerulein resulted in elevated levels of serum amylase and pancreatic active trypsin activity. Histology of the pancreas from mice treated with C-VISA-Bik-DD showed no evidence of parenchymal edema, pancreatic epithelial vacuolization, necrosis, apoptosis or inflammatory cell infiltration. However, there was clear evidence of acute pancreatitis induced by cerulein. No TUNEL-positive cells appeared in the acinar and ductal cells of the pancreas of mice treated with C-VISA-Bik-DD in contrast to apoptotic positive cells of the pancreas of mice treated with cerulein (4.5% TUNEL-positive cells). Treatment with C-VISA-Bik-DD produces virtually no systemically acute toxicity or acute pancreatitis.

**Conclusion:** Systemic administration of C-VISA-Bik-DD:DNA-liposome complex appears to be safe. Clinical study in pancreatic cancer is planned.
explants of the human non-small cell lung cancer (NSCLC) cell line A549. Pemetrexed is used clinically to treat NSCLC and other tumours. Immunodeficient (nude) mice (CRL:NU-Foxn1nng) were injected with 7.5 x 10^6 A549 cells subcutaneously (left and right flank), and tumours were allowed to grow to 200 mm^3 prior to initiating treatment. Mice were then treated with: (a) the antisense ODN SARI 083 or a scrambled control ODN (SARI 032) at 25 mg/kg intraperitoneally (IP) on Monday, Wednesday, and Friday of each week; (b) pemetrexed at 50 or 100 mg/kg, IP, once per week; (c) combinations of ODN (083 or 032) with 50 or 100 mg/kg pemetrexed; or (d) saline as a control. Based on Kaplan-Meier plots of the length of time tumours took to increase their size by 100%, ODN SARI 083 significantly enhanced the antitumour effect of 50 mg/kg pemetrexed, and that of the combined pemetrexed treatment groups (P < 0.05). The median length of time for tumors to double in size was 11 to 26 days for controls, single treatments and combinations that did not include SARI 083. Median tumor size doubling time was increased to greater than 50 days when SARI 083 was added to pemetrexed. In the groups with ODN alone, 50 mg/kg pemetrexed alone, or control ODN 032 plus 50 mg/kg pemetrexed, 40-50% of tumours increased in volume by 300% or more over the 50-day period. By contrast, none of the tumors treated with SARI 083 plus pemetrexed did. To our knowledge, this is the first demonstration in an in vivo setting of an antisense ODN directed towards a drug target that enhanced the antitumor activity of a drug against that target. (Funded by the Canadian Institutes for Health Research and the London Regional Cancer Program.)

Genomics, Proteomics, and Target Discovery

B154 UNBS1450-mediated c-Myc down-regulation and nucleolar targeting in carcinoma cells through over-expressed sodium pump alpha-1 subunit. Tatjana Mijatovic1, Nancy De Nève1, Véronique Mathieu2, Eric Van Quaquebeke1, Francis Darro1, Robert Kiss1.

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Epidermal growth factor receptor (EGFR) is one of the tyrosine kinase receptors that is frequently over-expressed in cancer cells and that represents a major therapeutic target. However, the role of the sodium pump alpha-1 subunit (Na+K+ATPase) in cancer is not yet fully understood. This proteasome inhibitor UNBS1450, developed at Bio-Rad Laboratories, is an effective anti-cancer drug which induces apoptosis in a variety of cell lines. It has been shown that UNBS1450 down-regulates the expression of c-Myc and inhibits the growth of LNCaP prostate cancer cells. In order to identify the mechanisms underlying the anti-tumour activity of UNBS1450, we investigated the effects of this compound on the nucleolus, a dynamic sub-cellular organelle that is important for control of gene expression, cell proliferation, and cell cycle regulation. The nucleolus is a common target of anti-cancer drugs and it is often involved in the process of cell death. It has been demonstrated that the nucleolus is a potential target for the treatment of cancer. In this study, we investigated the effects of UNBS1450 on the nucleolus in human carcinoma cells, focusing on the expression of nucleolar proteins and their localization.

In conclusion, UNBS1450-mediated c-Myc down-regulation paralleled by severe nucleolar targeting, results in non-apoptotic cancer cell death and in marked in vivo anti-tumor activity.

B155 Profiling of human angiogenesis biomarkers in sera of cancer patients using Bio-Plex™ suspension array system. Vinita Gupta1, Li Ma1, Joyce Eldering1, Woel Tan1. 1Bio-Rad Laboratories, Hercules, CA.

Introduction. Cancer development is a malignancy that involves non-reversible genomic change. The disease has been a significant cause of mortality in the Western Hemisphere. One of the criteria for the evaluation of tumor progression is the study of circulating biochemical markers elevated during angiogenesis. The aim of this preliminary study was to evaluate the link between the on-set of tumorigeneses and the elevation of a series of angiogenic markers in sera collected from a selected group of cancer patients. Initial study includes profiling of Angiopoietin-2, Follistatin, G-CSF, IL-8, HGF, Leptin, PDGF-BB, VEGF and PECAM-1.

Methods. The profile of the nine biomarkers in sera collected from four different types of cancer patients (N=10) for colorectal cancer; N=15 for each of Breast, Lung and Prostate cancer was evaluated using a magnetic bead based flow cytometry assay. The level of each marker was compared to a population of control sera collected from healthy individuals (N=25).

Results. The results of a two-tailed t-test showed that the levels of IL-8, VEGF, and PDGF-BB in cancer samples were statistically higher than the levels detected in the normal population. The disease was associated with colon and prostate cancer types only. The levels of leptin were lower in lung and prostate cancer. In addition the levels of IL-13, IL-17 and FGF basic were found to be elevated in lung and breast cancer where these samples were evaluated by a separate cytokine assay. IL-10 was elevated in lung and prostate cancer. The overall presentation of these markers showed that IL-8, VEGF and PDGF-BB were elevated and Leptin was reduced in the cancer types evaluated. G-CSF was exclusively reduced in breast cancer.

Conclusion. The simultaneous analysis of nine circulating angiogenic markers was demonstrated using the Bio-Plex™ Suspension Array System. The multiplex immunoassay platform is capable of measuring the levels of multiple targets in a single test sample with as little as 12.5 µL of serum volume. This significantly reduces the labor and time spent on initial screening of serum samples for biomarker profiling. Preliminary evaluation showed altered levels of at-least six biomarkers in the disease state samples relative to the levels in the normal population. The potential prognostic value of these markers will be further evaluated by testing the clinically diagnosed serum samples on selected predicate devices.

B156 Gene expression profiling differences between synchronous and metachronous liver metastases of colorectal cancer. Maria A. Pantaleo1, Annalisa Astolfi2, Margherita Nannini1, Giorgio Ercolani1, Giovanni Brandi4, Silvia Fanello4, Serena Formica4, Carla De Giovanni2, Pier-Luigi Lollini4, Andrea Pession2, Antonio D Pinna2, Guido Biasco2.

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The liver is the most common site of metastases from colorectal cancer. Liver metastases may be synchronous, at diagnosis of cancer, or metachronous, at recurrence after surgery of primary tumors. Actually the medical treatment of these two clinical setting is not differentiated. We studied the gene expression profiling of synchronous and metachronous lesions using Affymetrix platform in order to identify molecular patterns as possible basis for choice of systemic therapies and for response prediction. We collected fresh tissues specimens from liver metastases of 18 patients undergone liver surgery. They were classified as 10 synchronous and 8 metachronous lesions. RNA was extracted from frozen tumor specimens, labelled and hybridized to HG-U133Plus 2.0 Affymetrix arrays. Raw data were background-subtracted, normalized and summarized with the RMA algorithm. Routine quality controls were performed to check for the presence of artifacts and for the consistency of normalization across arrays. Probes poorly expressed or not differential in all the samples were excluded from further analysis. A moderated t-statistic test was used to identify genes differentially expressed between synchronous and metachronous metastases, setting the significance threshold at p < 0.01. All the analyses were performed with R and Bioconductor packages. PCA analysis and hierarchical clustering was performed with TIGR MeV and functional classification of differential genes with EASE tool.

At a global gene expression level the synchronous or metachronous characteristic of metastases was not the major determinant of gene expression differences among samples. Supervised analysis identified 245 probes differentially expressed between synchronous and metachronous lesions: genes upregulated in synchronous metastases belong to functional categories strongly implicated in the metastatic process, as cell adhesion, response to wounding, cell motility, immune response, angiogenesis and matrix remodelling, thus may suggest a more aggressive phenotype of this kind of metastases. In addition the two groups seem to originate from colorectal tumors with different biological features and with different potential therapeutic targets since synchronous metastases overexpress COX2, FLT1 and Cyr61, while metachronous overexpress EGFR and genes downstream its signaling pathway.

In conclusion, we found some interesting differences of the gene expression profiling of synchronous and metachronous metastases. The present study may suggest that medical treatments of patients affected by metastatic colorectal cancer should be differentiated considering the different biological background, for example therapies based on EGFr pathway inhibition (such as cetuximab or panitumumab) for metachronous metastases and based on angiogenesis inhibition (such as bevacizumab or VEGF tyrosine-kinase inhibitors) for synchronous metastases. These preliminary results need to be confirmed first of all in larger series and secondly by the on going quantitative measurements of the overexpressed proteins, EGFr, COX-2, FLT1 with Western Blotting and ELISA techniques, and in the future these different evaluated approaches may be also investigated in clinical trials.


In prostate cancer, the skeleton is the principal organ for metastasis formation, and bone metastases are predominantly osteoblastic. Prostate cancer progression is androgen-driven, however, most patients respond only temporarily to androgen ablation therapy. Further insight into regulatory mechanisms underlying the establishment of prostate carcinoma cells within an osteoblastic microenvironment may eventually lead to more effective therapies to prevent or treat metastatic disease. To study regulatory mechanisms involved in the interaction of prostate carcinoma cells with osteoblasts, a model system to identify activated intracellular signaling pathways was established. Human androgen-sensitive prostate carcinoma cells (LNCaP) were cocultured with human osteoblast-derived cells (osteosarcoma OHS cells), to simulate the direct cellular interaction, and the LNCaP cells were subsequently isolated from coculture by immunomagnetic target cell selection. In addition, monocultured LNCaP cells were treated with medium conditioned by OHS cells or a synthetic androgen analog, to specifically examine the biological context of paracrine and androgenic influence, respectively. Multiplex profiling of LNCaP kinase activity was performed using flow-through microarrays with peptide substrates (Tyrosine Kinase Pamchip® arrays; www.pamgene.com), a technological platform that allows rapid, real-time measurement of phosphopeptide signatures generated by the biological samples. Network interaction analysis of substrates with significantly increased phosphorylation levels showed that signaling pathways mediated by EGFR and ERBB2 were activated in LNCaP cells under the influence of both osteoblastic cells and androgen treatment. Similarly, signaling by EGFR and ERBB2 was found to be induced in LNCaP cells cocultured with osteoblastic cells that had been differentiated from human mesenchymal stem cells, but not in androgen-independent LNCaP-19 cells cocultured with OHS cells. Given that EGFR/ERBB2 signaling was activated by osteoblastic cells in the androgen-sensitive LNCaP cells but not in the androgen-independent derivative cell line, we believe that a functional androgen signaling axis is permissive for activity of these pathways in prostate cancer. These results may explain why recently conducted trials using therapies inhibiting EGFR or ERBB2 in hormone-refractory prostate cancer have not shown benefit. Of importance, our experimental data suggests that targeted inhibition of the signaling pathways directed by EGFR or ERBB2 may simultaneously ablate androgen-driven proliferation and the survival responses of prostate carcinoma cells within an osteoblastic microenvironment, and provides a strong biological rationale for the use of EGFR or ERBB2 inhibition in systemic prevention or treatment of metastatic prostate cancer in the androgen-sensitive stage of the disease.

Subsets of these data were presented at the 2007 Annual Meeting of the American Association for Cancer Research (abstract #4936).

B158 A combined proteomics and cell biology approach to elucidate the function of BCL11B in T cell lymphoma cell lines. Karanam Narasimha Kumar, Piotr Grabarczyk, Maren Depke, Franziska Schöbel, Christian Scharf, Christian A. Schmidt, Uwe Völker. 1Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany; 2Clinic for Inner Medicine C, Greifswald, Germany.

The B-cell chronic leukemia/lymphoma 11bgene (BCL11B) is known to be involved in T cell development and undergoes chromosomal rearrangements in human T cell leukemias or hematopoietic malignancies. The exact function of this gene is still debated in scientific community. It was shown recently that BCL11B is crucial for the survival of human Tcell leukemia and lymphoma cell lines (1), and its loss of function results in DNA replication stress and damages (2) and finally cell death by apoptosis(1). But the precise reason and detailed molecular mechanism of cell death is yet to be elucidated. Here we used combined functional genomics and cell biology methods to study the role of BCL11B in Jurkat and HuT, T cell lymphoma cell lines. We knocked down the BCL11B gene using sequence specific si RNA and analyzed BCL11B-dependent differential expression using proteomics and transcriptomics approaches. In our study we demonstrated that the cell death/apoptosis caused upon BCL11B knockdown occurs by both mitochondrial and non mitochondrial pathways.Apart from already established caspase substrates such as Myosin, Spectrin,Vimentin and Kinectin, we demonstrated new putative protein cleavage sites of MCM4 and MCM6 with detailed analysis by mass spectrometry. Myosin was shown to be hyperphosphorylated after BCL11B knockout, using phosphospecific Diamond ProQ staining thus providing a potential link to the ACE signaling cascade and cytoskeletal rearrangement. Applying the DIGE technology for differential expression studies we revealed that deoxyuridine nucleotidohydrolase (dUTPase) and...
Urdeine-ctidine kinase 2 which are key enzymes in the uracil misincorporation pathway, were up regulated. From transcriptomic studies we learnt that thymidylate synthase and dihydrofolate reductase like 1 genes, which are key regulators of salvage pathway, were down regulated. From these results we postulated that BCL11B loss results in uracil misincorporation and confirmed this hypothesis using a Uracil DNA N Glycosylase (UNG) assay, which specifically recognises and degrades uracil containing DNA. We showed using this UNG assay that there is more DNA degradation upon UNG treatment in BCL11B knockout cells compared to control cells, revealing a potential role of BCL11B in uracil misincorporation pathway, thereby leading to increased DNA damage which might be one of the important cause of cell death/apoptosis in BCL11B knockout cells. Taken together, our results not only strongly support our previous report that BCL11B can be used as an efficient therapeutic target but also help in extend the knowledge about the role of BCL11B in cell death, DNA damage and T cell malignancies.

References:

B159 Breast tumor xenografts of luminal subtype switch to the ERBB2+ molecular subtype in response to endocrine therapy: Gene expression profiling may not predict pathways of resistance in pretreatment tumors. Chad J Creighton1, Suleiman Massarweh2, Shixia Huang3, Anna Tsimelzon1, Susan G. Hilsenbeck1, C. K. Osborne1, Rachel Schiff1. 1Baylor College of Medicine, Houston, TX; 2University of Kentucky, Lexington, KY.

Background: Of the breast cancer patients treated with therapies targeting estrogen receptor (ER) or HER2, many do not respond, and of those who do, many acquire resistance over time. Materials and Methods: Previously, we have used a breast cancer xenograft model system to investigate the mechanisms of acquired resistance to targeted therapies in vivo. In this study, we profiled the expression of over 50,000 gene transcripts in xenograft tumors that were either ER+/HER2+ (thought to represent the luminal B subtype) or ER+/HER2- (luminal A subtype) prior to treatment and then after they developed resistance to the various treatment regimens. An important aspect of our study was to compare the gene signatures derived from our model systems with gene signatures derived from breast cancers from patients that define specific molecular subtypes (luminal A, luminal B, basal, and ERBB2+), to assess how our models might represent breast cancer in the clinical setting.

Results: We observed in each of our model systems that acquired resistance to various ER targeted therapies transformed the associated clinically-defined molecular profile subtype of breast cancer from an ER+ tumor (luminal subtype) that relied upon the estrogen pathway for growth to an ER-/HER2+ tumor (ERBB2+ subtype) that relied upon the HER2 growth factor receptor signaling pathway. On the basis of gene expression, it was evident that the ER+/HER2- tumors initially relied upon the estrogen pathway and so would be initially sensitive to estrogen deprivation, but we could not have predicted HER2 as one mechanism of acquired resistance solely on the basis of the profile data from the pre-treatment tumors.

Discussion: One popular idea in molecular oncology is to identify molecular markers predicting tumor response to targeted therapies. Breast cancer treatment is a prime example of this idea being put into clinical practice, with patients whose tumors express ER receiving endocrine therapy and patients whose tumors express HER2 receiving anti-HER2 therapy. At the same time, our study suggests that using gene expression of pre-treatment tumors to predict response might be limited to predicting initial response, and would not necessarily be predictive of possible mechanisms of acquired resistance. Our study lends support to a cancer treatment paradigm that targets not only the tumor in its pre-treatment state, but also the tumor as it might manifest itself during tumor progression and acquired resistance to treatment.

B160 Proteomic analysis of Hsp90 modulation. Jing Wei1, Mark Daniels1, Ben Colson1, Diem Tran1, John Sensintaffar1, Noe Ittmann1, Naveen Dakapagaran1, Adeela Kamal1, Karen Lundgren1, Ingrid Joseph1, Shabnam Tang1, Chris Storgard1, Alessandra Cesano1, Steve French1, Steve French1, Francis Burrows1, Alex Buko1. 1Biogen Idec, San Diego, CA.

Background: Hsp90 is a pleiotropic cytosolic chaperone protein that is involved in stabilization and maturation of over 100 ‘client’ proteins, many of which are upregulated or mutated in human cancers. Preclinical data indicates that not all client proteins are affected by Hsp90 inhibition equally and some proteins such as HER-2 have been identified as more sensitive client proteins than others. The wealth of potential cellular functions in which Hsp90 plays a role potentially offers a plethora of biomarkers for drug sensitivity, and activity. Proteomic analysis represents a comprehensive and efficient method to improve our understanding of the cellular consequences of Hsp90 modulation. Hsp90 and other cellular chaperones function primarily by regulating protein stability, sub-cellular protein location, composition of the secreted proteome, and affects protein phosphorylation, all of which can be monitored globally and simultaneously by proteomic analysis.

Methods: To explore the astounding complexity of proteome, we have developed a robust and fully automated non-labeled tandem multidimensional separation system on-line coupled with ESI-MS (LC/ESI-MS/MS/MS). This platform provides reliable and sensitive analysis and gives over 10,000 high-confidence protein identifications from a typical sample load (100 ug). Multiplex iTRAQ labeling has been applied to provide accurate quantification. This mass spectrometry based platform was utilized to investigate the consequences of small-molecule inhibition of Hsp90. Both in vitro and ex vivo experiments were designed to focus on the changes occurring over 1 to 48 hours by both marginally and strongly active concentrations of the Hsp90 inhibitor BIBO21 in either HER-2+ breast cancer cells or normal human PBMCs. Additionally, tumor interstitial fluid (TIF) was explored as a source of secreted biomarker candidates in an in vivo setting using a xenograft model of tumor regression.

Results: Consistent changes in protein expression level were observed in various metabolic and signaling pathways after BIBO21 exposure, including ribosomal proteins and purine metabolism. In agreement with results from antibody based techniques, BIBO21 induced HER-2 degradation in both a dose and time dependant manner. Both the intracellular and extracellular domains of HER-2 peptides were detected. Additionally, we found other proteins that also decreased in a similar dose and/or time dependant manner. Conversely, antibody based techniques have revealed the upregulation of heat shock protein Hsp70 as a consequence of Hsp90 inhibition. Our results confirmed Hsp70 upregulation but it does not show a dose dependant response. In addition, more proteins were identified that demonstrated a dose dependant upregulation in response to Hsp90 inhibition.

Conclusions: Proteomics analysis of Hsp90 modulation has confirmed and extended our knowledge of the dose dependent changes in abundance for existing biomarkers such as HER-2 and HSP70.

B161 BIBO21 is a small molecule inhibitor of the heat shock protein, Hsp90, that shows potent anti-tumor activity in preclinical models. Karen Lundgren1, Hong Zhang3, Adeela Kamal1, Rachel Lough1, Noelito Timp1, John Sensintaffar1, David Busch1, Cindy Yang1, Laura Neely1, Samina Khan1, Kevin Hong1, Srinivas Kasibhatla1, Marcus Boehm1, Francis Burrows1. 1Biogen Idec, San Diego, CA.

Hsp90 is a widely-expressed molecular chaperone that functions in the maturation and stabilization of cellular proteins. Complexed together with other co-chaperone proteins, Hsp90 catalyzes the conformational changes of client proteins via its ATPase activity. The activity of Hsp90 maintains a variety of client proteins in their active conformation and plays an important role in the regulation of several key oncogenic signaling
proteins. Mutant and over-expressed oncoproteins that drive malignant progression are particularly dependent on Hsp90 chaperone activity. In tumor cells inhibition of Hsp90 results in degradation of these proteins followed by cell death making Hsp90 a target of substantial interest for cancer therapy. The prototypic natural product drug, 17-AAG, has shown promise in early trials but is limited by poor pharmacapeutic properties and hepatotoxicity. Here we describe BIIB021, a novel synthetic inhibitor of Hsp90. BIIB021 binds competitively with geldanamycin in the ATP binding pocket of Hsp90. In a flow cytometric assay designed to detect HER-2 protein levels treatment of MCF-7 cells with BIIB021 induced HER-2 degradation with an EC50 of 32nM. Western blot analysis after treatment of MCF7 cells with BIIB021 demonstrated the degradation of key client proteins including HER-2, Akt, and Raf-1 in a dose-dependent and time-dependent manner. As shown with other Hsp90 inhibitors the upregulation of expression of heat shock proteins Hsp70 and Hsp27 was also observed. In tumor cell growth assays treatment with BIIB021 led to growth inhibition and cell death at nanomolar concentrations. BIIB021 was tested two models that express high levels of the HER-2 oncprotein, the BT474 breast and N87 gastric human xenograft models. Oral administration of BIIB021 led to the degradation of the Hsp90 client protein HER2 and the potent inhibition of tumor growth in these models. BIIB021 also showed anti-tumor activity in the CWR22 prostate tumor model that expresses another Hsp90 client protein, the androgen receptor (AR). BIIB021 is a promising new Hsp90 inhibitor designed to be given orally and has potent preclinical anti-tumor activity. BIIB021 is currently undergoing Phase I clinical trials.

B162 Diverse regulation of Bcl-2 in microvascular endothelial cells leads to altered pattern of gene expression. Charles J. Rossier1, Yoshishia Sakai1, Steve Goodison2, Wengang Cao2, Kazumori Namiki1, Stacy Povasnik1. 1University of Florida, Gainesville, FL; 2University of Florida, Jacksonville, FL.
The tumor microenvironment plays a major role in establishing and maintaining tumoral angiogenesis. Previously we demonstrated that Bcl-2 expressing prostatic tumors, are not only more tumorigenic but are more angiogenic. Increased rates of angiogenesis could be due to these cancer cells overexpressing and secreting more angiogenic factors, specifically Vascular Endothelial Growth Factor (VEGF). We now report that VEGF stimulation induces Bcl-2 expression, stabilizes mRNA Bcl-2, and protects these endothelial cells from apoptosis. Indeed the importance of Bcl-2 expression is evident by the fact that four distinct pathways (i.e., mTOR, PI3K, PKC, and ERK) contribute to Bcl-2 expression in endothelial cells. Microarray analysis of VEGF stimulated Bcl-2 expressing endothelial cells triggered a transcriptional program of genes involved in cellular metabolism, cell recognition and communication, transport, and signal transduction. Collectively, these data suggest that Bcl-2 expression in endothelial cells plays a major role in differentiation, proliferation and resistance to apoptosis. Furthermore, these data provide a possible explanation for the observed association between Bcl-2 expression and enhanced angiogenesis.

B163 A role for Bcl-2 onogene in modulating angiogenesis in prostate cancer. Charles J. Rossier1, Yoshishia Sakai1, Steve Goodison2, Stacy Povasnik1, Wengang Cao1, Satoshi Anal1, Kazumori Namiki1. 1University of Florida, Gainesville, FL; 2University of Florida, Jacksonville, FL.
We previously demonstrated that Bcl-2 overexpression enhances the resistance of PC-3 human prostate cancer cells to radiation by inhibiting apoptosis, increasing proliferation and inhibiting angiogenesis. To further elucidate the relationship between Bcl-2 expression and the angiogenic potential of the PC-3-Bcl-2 cell line, we evaluated whether Bcl-2 could be involved in not only tumorigenicity but angiogenesis as well. Bcl-2 overexpressing clone and a control transfected clone were used for in vitro and in vivo experiments. Bcl-2 overexpression enhanced the tumorigenic ability of prostate cancer xenografts, enhanced the expression and secretion of key angiogenic factors which stimulated synthesis of newosculature (as evident by CD31 immunohistochemical staining). Specifically, the increased angiogenic potential was correlated with increased serum levels of BFGF, IL-8, and MMP-9. In vitro analysis demonstrated Bcl-2 expressing tumors to be resistant to rapamycin induced apoptosis and to secrete bFGF into culture supernatant. Microarray analysis of Bcl-2 expressing PC-3 prostate cancer cells demonstrated increased transcription of genes involved in metabolism, regulators of biologic processes, and transport factors which included interleukins, growth factors, TNF family, and peptidases. These results demonstrate that Bcl-2 overexpression can regulate tumoral angiogenesis. Targeted therapy directed at Bcl-2 expression and angiogenesis may act synergistically to modulate tumor growth. Hence this work validates Bcl-2 role in tumorigenicity and angiogenesis and support our notion that Bcl-2 is an important target in cancer therapy.

B164 In silico screening of differentially expressed microRNAs in lung cancer. Shuta Tomida1, Koyo Tsuchiakawa1, Takashi Takahashi1. 1Nagoya University Graduate School of Medicine, Nagoya, Japan.
Introduction: MicroRNAs (miRNAs) are an abundant class of small non-protein-coding RNAs of ~22 nt that negatively regulates hundreds of genes by base-pairing to the messages of protein-coding genes. Recent evidence including our own suggests that certain miRNAs may function as tumor suppressors or oncogenes. In this study, we describe a novel bioinformatic approach to utilize an already existing enormous number of microarray datasets of miRNAs for studies on miRNAs.
Methods: We constructed a “robust” set of the target genes for each miRNA using two major web-based prediction programs to search for miRNAs associated with given phenotypic distinctions of interest by performing gene set enrichment metric based on the Kolmogorov-Smirnov statistic.
Results: The present approach confirmed lower expression levels of let-7 targets in adenocarcinoma cases with up-regulated expression of let-7. Furthermore, we could correctly predict members of the miR-17-92 cluster as miRNAs with preferential high expression in small cell lung cancer samples using our own expression profiling data of miRNAs, as was predicted from our previous experimental data.
Conclusions: These result suggest that our approach may be useful to utilize pre-existing, thousands of expression profiling datasets of miRNAs in order to accelerate identification of miRNAs with specific association with given phenotypes.

B165 Comparative genomic hybridization-array and real-time PCR reveals new candidate genes for breast cancers. Kweon Haeng Lee1, Jin Soo Choi1, Seung Ho Baik1, Young Lim1. 1The Catholic U of Korea, Seoul, Republic of Korea.
Genomic alterations are important pathogenic mechanisms to induce several cancers including breast cancer. In breast cancer, DNA copy number level was observed in several studies but limited to the cancer tissues not in bloods. Thus, we compared differences in genomic aberration of blood and breast cancer tissue. We used array-comparative genomic hybridization (array-CGH) compared differences for 30 breast cancer bloods and cancer tissues. Samples were staged correctly according to the TNM (tumor, nodes, and metastases) classification of malignant tumors. Array CGH was performed using MACArray™-Karyo 4K BAC-chip (Macrogen, Seoul, Korea) which contains 4,030 bacterial artificial chromosome (BAC) clones on the whole human genome with a resolution of about 1 Mbp. The data analyses were using a MAC Viewer v1.6.3 Software and the relative degree of chromosomal changes was analyzed using log, ratios. For validation, we used real time PCR, the relative genomic copy number was calculated using the comparative Cq method. A large number of regions in the entire genomes were altered. Genomic alterations were detected at 1q, 3q, 4p, 5q, 8q, 10q, 11p, 12p, 15q, 16p, 17p, 17q, 18q, 19p, 20q, and 22q. Generally, the genomic alterations were more frequent in cancer tissues than bloods. The most common gains were found in 5p (60% of bloods, and 80% of tumor tissues) and the most common losses were found in 22q (40% of bloods, and 30% of tumor tissues). The four genes including CDKN1C (11p15.4), HRAS1 (11p15.5), NTRK1 (1q23.1), and RPS5...
189 genes displaying somatic mutations in breast and colorectal cancers. Based on their mutation prevalence, a subset of these genes was identified in melanoma and pancreatic cancers and indicated that CAN genes and their nucleotide variant of OBSCN that was previously reported as a somatic gene in glioblastoma, melanoma, and pancreatic carcinomas are also suggesting PIK3CA targeting in the treatment of MSI colorectal cancers. Among other changes, we found novel somatic mutations in EPHA3, MLL3, TECTA, FBXW7, and OBSCN, affecting amino acids not previously found to be mutated in human cancers. Interestingly, we also found a germline nucleotide variant of OBSCN that was previously reported as a somatic mutation. Our results identify specific genetic lesions in glioblastoma, melanoma, and pancreatic cancers and indicate that CAN genes and their mutational profiles are tumor specific. Some of the mutated genes, such as the tyrosine kinase EPHA3, are clearly amenable to pharmacologic intervention and could represent novel therapeutic targets for these incurable cancers. We also speculate that similar to other oncogenes and tumor suppressor genes, mutations affecting OBSCN could be involved in cancer predisposition.

B166 Clinicopathological analysis of colorectal cancers with PIK3CA mutations in Middle Eastern population. Jehad Abubaker1, Prashant Bavi1, Sayer Al-Harbi1, Muna Ibrahim1, Nasser Al-Sanea1, Alaa Abdul Jabbar1, Luai Ashari1, Samar Alhomoud1, Fouad Al-Dayel1, Shahab Uddin1, Khawla Al-Kuraya1, 1King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia.

Purpose: Activation of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway results in an increase in cell proliferation, survival. Somatic mutations within the PI3K catalytic subunit, PIK3CA are a common cause of increasing PI3K activity and are believed to be oncogenic in many cancer types. In the present study, we have evaluated PIK3CA mutational status in a series of 410 Middle Eastern colorectal carcinomas (CRC) to study the prevalence of PIK3CA mutations in mismatch repair deficient cases, PTEN expression in CRC and possibility of therapeutic targeting of this set of patients.

Experimental Design: In this study, 13 colon cell lines, 410 CRC were analyzed for PIK3CA mutations by direct PCR sequencing. Microsatellite instability (MSI) status was determined using 5 microsatellite markers. Tumors were also analyzed for p53 mutations and PTEN alteration by immunohistochemistry.

Results: PIK3CA mutations were found in 4 of the cell lines tested and 51 colorectal carcinomas (12%). Three of these 4 mutated cell lines were MSI. PTEN was inactivated in 66.1% of the CRC. Furthermore, we observed a strong association between PIK3CA mutations and MSI status (P = 0.0046) while PTEN loss was more frequent in microsatellite stable (MSS) CRC (P = 0.043).

Conclusions: A high prevalence of genetic alterations in PI3K/AKT pathway in Saudi cohort of CRC, predominance of PIK3CA mutations in the MSI subgroup and their possible involvement in development/progression of this subset of CRC are some of the significant findings of our study. We are also suggesting PIK3CA targeting in the treatment of MSI colorectal carcinoma patients.

B167 Novel somatic and germline mutations in cancer candidate genes in glioblastoma, melanoma, and pancreatic carcinoma. Bleeker E Fonnet1, Asha Balakrishnan1, Simona Lambda1, Monica Rodolfo1, Maria Daniotti2, Aldo Scarpal, Angela A Van Tilborg1, Sieger Leenstra1, Carlo Zanon1, Alberto Bardelli1, 1Institute for Cancer Research and Treatment, Turin, Italy; 2Instituto Nazionale Tumori, Milan, Italy; 3University of Verona, Verona, Italy; 4Academic Medical Center, Amsterdam, The Netherlands; 5St. Elisabeth Ziekenhuis, Tilburg, The Netherlands.

A recent systematic sequence analysis of well-annotated human protein coding genes or consensus coding sequences led to the identification of 189 genes displaying somatic mutations in breast and colorectal cancers. Based on their mutation prevalence, a subset of these genes was identified as cancer candidate (CAN) genes as they could be potentially involved in cancer. We evaluated the mutational profiles of 19 CAN genes in the highly aggressive tumors: glioblastoma, melanoma, and pancreatic carcinoma. Among other changes, we found novel somatic mutations in EPHA3, MLL3, TECTA, FBXW7, and OBSCN, affecting amino acids not previously found to be mutated in human cancers. Interestingly, we also found a germline nucleotide variant of OBSCN that was previously reported as a somatic mutation. Our results identify specific genetic lesions in glioblastoma, melanoma, and pancreatic cancers and indicate that CAN genes and their mutational profiles are tumor specific. Some of the mutated genes, such as the tyrosine kinase EPHA3, are clearly amenable to pharmacologic intervention and could represent novel therapeutic targets for these incurable cancers. We also speculate that similar to other oncogenes and tumor suppressor genes, mutations affecting OBSCN could be involved in cancer predisposition.

B168 Proteomic identification of component proteins of orphan nuclear receptor COUP-TFI complexes in mammalian cells. Ling-juan Zhang1, Mark Leid1, 1Oregon State University, Corvallis, OR.

Nuclear receptors, the transcription factors regulated by ligands, have become major targets for drug discovery, including new drug development for chemotherapy. Orphan nuclear receptor chicken ovalbumin upstream promoter transcription factor I (COUP-TFI) possesses the ability to either activate or repress the transcription of a diversity of target genes through undefined mechanisms. A proteomics-based, tandem affinity purification (TAP) procedure was used in this study to identify the component proteins of COUP-TFI complexes in mammalian cells. Several known proteins of transcriptional repressive complexes, including NCoR, HDAC1 and TIF1 (β/KAP-1), were found to co-purify with COUP-TFI, as were other transcriptional regulatory proteins, including the SWI/SNF family member Brhma, and its associated factors BAF155 and BAF170. Proteins not previously implicated in transcriptional regulation were also found to co-purify with COUP-TFI including the DNA repair protein DDB1, a pro-apoptotic protein that is deleted in breast cancer (DBC1), HSP70, HSP90 and the ubiquitin ligase HYD1. Finally, several components of the spliceosome assembly were identified (SF1R, SF3A1 and SF3B1) in COUP-TFI complexes. Collectively, our TAP strategy revealed that COUP-TFI may associate with a number of transcriptional regulatory proteins in HeLa S3 cells as well as other classes of proteins that have not been previously implicated in the regulation of gene expression. Many of these proteins and COUP-TFI were demonstrated to co-occupy the promoter of retinoic acid-induced 1, a newly identified, COUP-TFI target gene in HeLa S3 cells. These results may underlie the complexity of COUP-TFI action in mammalian cells.

B169 The promoter polymorphisms of RET proto-oncogene associated with lung cancer risk in Korean patients. Hyun Kyung Kim1, Jae Sook Sung1, Uk Hyun Jo1, Slegiyo Han1, In Kun Choi2, Kyong Hwa Park2, Sang Chul Oh2, Sang Won Sin2, Jun Suk Kim2, Yeol Hong Kim1, 1Genomic Research Center for Lung and Breast/Ovarian Cancer, Seoul, Republic of Korea; 2Korea University Hospital, Seoul, Republic of Korea.

The RET proto-oncogene, which is located on the long arm of chromosome 10 (10q11.2), comprises 21 exons, encodes a tyrosine kinase receptor and is activated by its physiological ligands, members of the GDNF- family. The activation of this gene contributes to the development of human cancers. Several single-nucleotide polymorphisms (SNPs) of the RET proto-oncogene have been described in the general population as well as in patients with endocrine tumors, papillary thyroid carcinoma, familial and sporadic medullary thyroid carcinoma (MTC), and Hirschsprung disease (HSCR). However, there is no report about the SNPs of the RET in lung cancer. In view of these, we investigated the SNPs of the promoter region of the RET proto-oncogene in Korean lung cancer patients and normal population. And we examined whether the SNPs of the RET proto-oncogene were associated with the risk of lung cancer in a Korean population. The 11 SNPs of RET promoter region were examined by direct sequencing method in the preliminary study. Two SNPs (-527 C/A; rs3097561, -1260 C/T; Novel) out of the 11 candidate polymorphisms showed more than 5% of minor allele frequency (MAF). To analyze the genotype of the RET gene for the 409 Korean lung cancer patients and 409 normal subjects, a polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) was used. The relationships with lung cancer and the genotypic and allelic frequency of the 2 SNPs were analyzed using SPSS 10.0 program. In the analysis, the SNP of the RET promoter region (-527 C/A) did not show statistically significant differences between lung cancer patients and normal subjects. However, the allelic and genotypic
frequency of the novel SNP of the RET promoter region (-1260 C/T) showed statistically significant differences between lung cancer patients and normal subjects. In particular, risk of lung cancer was increased in patients with dominant (CT+TT, OR=1.427; 95%CI,1.083-1.881) of this SNP region. This dominant genotype was associated with increased risk for lung cancer in the cases of male (CT+TT, OR=1.621; 95%CI,1.174-2.388), smoking (CT+TT, OR=1.695; 95%CI,1.522-2.537), alcohol drinking (CT+TT, OR=1.687; 95%CI,1.091-2.609) and older than 60 years old (CT+TT, OR=1.462; 95%CI,1.024-2.086) lung cancer patients in the subgroup analysis. In conclusion, we described novel RET polymorphism in lung cancer and confirmed a relation between the RET promoter polymorphisms and lung cancer risk. Our results suggest that the polymorphisms of RET are associated with the risk of lung cancer in a Korea population. (This study was supported by a grant of the Korea Health 21 R&D project, the Ministry of Health & Welfare, Republic of Korea (A010250))

B170 Genetic polymorphisms in the HER-2 gene and the risk of lung cancer. U.K. HyunJo1, Young Mi Whang1, Jaee Sook Sung1, Slegilo Han1, In Kun Choi2, Kyong Hwa Park2, Sang Chul Oh2, Sang Won Sin2, Hyo Jung Lee1, Jun Suk Kim2, Yeul Hong Kim1. 1Genomic Research Center for Lung and Breast/Ovarian Cancers, Seoul, Republic of Korea; 2Korea University Hospital, Seoul, Republic of Korea; 3College of Political Science and Economics, Korea University, Seoul, Republic of Korea.

The HER-2 (also known as erbB-2 or neu) proto-oncogene has been associated with carcinogenesis and poor prognosis of human cancers, acting as a binding partner of other epidermal growth factor receptor (EGFR) family in the activation of EGFR signaling. Alterations for HER-2 gene are found in human cancers. In this study, we investigated whether the six polymorphisms (-3444C>T, -3396C>T, -3374A>G, -1985G>T, I655A C>G) of HER-2 gene are associated with the risk of lung cancer in a Korean population. The frequencies of 6 SNPs of the HER-2 gene were examined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or the SNP-IT assay for 407 lung cancer patients and 407 normal controls. We found that -3444C>T, -3396C>T and 3374A>G showed complete linkage disequilibrium (LD) and the three SNPs exhibited strong LD with -1985G>T. The frequencies of the 6 polymorphisms were not complete linkagedisequilibrium (LD) and the three SNPs exhibited strong LD with -1985G>T.

In conclusion, this study shows that the polymorphisms of -1260 C/T were associated with increased clinical outcome (in terms of increased disease-free survival, relative to less-proliferative tumours), based on an expression-based gene proliferation signature [1]. The reverse relationship (i.e., high proliferation associated with poor outcome) was observed in multiple microarray data sets from a number of non-gastrointestinal cancers, including breast, glioma and lymphoma, using the same gene-based assessment of proliferation. Earlier work in our laboratory also identified a gene signature for colorectal cancer prognosis prediction in which higher levels of expression of immune response related genes were associated with increased disease free survival [2].

Here we use newly developed bioinformatic methodology to investigate the biology underlying this somewhat counterintuitive relationship between proliferation and prognosis in colorectal and gastric cancers, in the context of an expression-based assessment of both proliferation level and immune response for each tumor. This approach is based on identifying sets of correlated genes which undergo statistically significant alterations in expression between prognosis, proliferation, and/or immune response...
classes in colorectal and gastric cancer, and contrasting these relationships with those observed in breast cancer, for which there is both a large amount of high quality publicly available microarray data, as well as a widely accepted association between high proliferation and poor prognosis.

This approach has revealed that while highly proliferative tumors express immune response related genes in a similar pattern across colorectal, gastric and breast cancers, certain genes appear to be down-regulated in highly proliferative breast cancer tissues (relative to low proliferation tumors) that remain unchanged in colorectal and gastric tumors. Our conclusion is that subtle differences in the host immune response underly the differences in the relationship between proliferation and prognosis in colorectal, gastric and breast cancers.


B173 Proteomic identification of extracellular proteins differentially expressed in prostate cancer reveals novel opportunities for targeting tumor cells. Mehdi Mesri1, Erin Brand1, Yeoun Jin Kim1, Kathy McKinnon1, Charles E. Birse1, Tao He1, Candy Lee1, Karen Van Orden1, William FitzHugh1, Steve Ruben1, Paul A. Moore1.

Prostate cancer is the second leading cause of cancer death in American men. The growing number of elderly people across the major markets will expand the overall prostate cancer incidence population in the coming years. Identifying novel targets by proteomics may lead to opportunities for the development of targeted therapies or biomarkers. Additionally a significant clinical unmet need for prostate cancer is an effective drug therapy that is able to prevent the disease from progressing to androgen-refractory status or that can prolong the survival of patients with androgen-refractory disease.

By employing mass spectrometry (MS) as a tool to identify proteins that are over-expressed in prostate tumor relative to normal prostate cells, we aimed to discover new targets that could be utilized in prostate cancer therapy. We developed proteomic methods that allowed us to focus our studies on the discovery of cell surface/secreted proteins, as they represent key antibody therapeutic and biomarker opportunities. Cell-surface and secreted proteins from normal and prostate tumor cell lines were preferentially captured, digested with trypsin and subjected to MS analysis. Peptides were first quantified, and then sequence composition of differentially expressed peptides was resolved by MS analysis. To date, we have identified in excess of 200 proteins over-expressed in prostate cancer cell lines, including known markers such as PSA, PSMA and a panel of proteins not previously associated with prostate cancer. Our analysis also showed 23 of identified proteins were uniquely found in prostate with no overlap with other oncology indications.

Further analyses identified 37 proteins as over-expressed in androgen-refractory cell lines compared to androgen sensitive cell lines. Independently, a mouse xenograft study of androgen-dependent 22Rv1 prostate cell line was performed to investigate modulation of prostate tumor proteome expression upon androgen withdrawal. Xenografts from pre-castrated or re-grown post-castrated androgen-independent phase of the study were compared. Initial studies identified 21 proteins as over-expressed in 22Rv1 xenografts derived from post-castrated androgen-independent mice compared to pre-castrated mice.

To validate proteins identified by MS, we performed additional confirmatory studies including immunohistochemistry (IHC), mRNA profiling, Flow Cytometry and functional analyses using RNAi-mediated transfection to evaluate the effect on prostate cell proliferation and/or apoptosis. A subset of targets was selected for validation based on criteria such as druggability of the proteins, novelty, and intensity of tumor over-expression. From this subset, 15 targets confirmed over-expression by IHC in prostate tissues while 16 targets demonstrated RNAi function in prostate cell lines. Examples of such validated targets will be presented.

These studies highlight that our large scale proteomic mapping capabilities can provide a platform for identification of novel therapeutics and biomarkers. Together with additional functional and expression characterization, this approach represents a unique opportunity for the discovery of targets that may be exploited in the diagnosis and treatment of prostate cancer.

B174 Analysis of the 70-gene breast cancer prognosis signature in a general hospital population. Ben S. Wittrner1, Dennis C. Sgroi1, Paula D. Ryan1, Tako L. Bruinsma2, Annuska M. Glas2, Anitha Male1, Sonika Dahiya1, Karleen Habin1, René Bernards2, Daniel A. Haber1, Laura J. van ‘t Veer3, Sridhar Ramaswamy1,2 Massachusetts General Hospital Cancer Center, Boston, MA; 2Agendia BV, Amsterdam, The Netherlands; 3The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Introduction: A 70-gene microarray gene expression signature was previously discovered at the Netherlands Cancer Institute (NKI) to identify younger breast cancer patients (age <55 years) with lymph-node negative disease who are at low risk of developing distant metastasis and might therefore be spared further adjuvant chemotherapy (1, 2). This diagnostic test known as MammaPrint was recently validated in an independent cohort including patients up to the age of 61. Many breast cancer patients, however, are older and post-menopausal and this subgroup of patients has a lower overall risk of distant metastasis. Therefore, a molecular diagnostic test with high negative predictive value for distant metastasis could spare many older women treatment with chemotherapy.

Methods: We determined the negative and positive predictive value of the 70-gene signature in unselected breast cancer patients who were consecutively diagnosed and treated at the Massachusetts General Hospital (MGH) between 1985 and 1997. Primary tumors from 101 patients with node negative, invasive breast cancer (median age 62 years) were subjected to microarray expression analysis using the 70-gene MammaPrint assay and were classified as being at either low or high risk for distant metastasis.

Results: MGH node-negative patients differed significantly from previously studied, node-negative NKI patients with respect to age, tumor size, histologic grade, and adjuvant hormonal and/or chemotherapy (p ≤ 0.01). Within the MGH cohort, patients who were classified as low risk for distant metastasis differed significantly from those classified as high risk with respect to histologic grade and estrogen receptor status in univariate analysis (p ≤ 0.01). In considering clinical outcome, the 70-gene signature displayed excellent negative predictive value (NPV), correctly identifying 100% of women at low risk to develop metastases within five years. As expected (see discussion below), the 70-gene signature had lower positive predictive value (PPV) (11% at 5 years) in this older and more heavily treated cohort.

Conclusion: The 70-gene signature was originally designed to identify younger breast cancer patients at low risk for distant metastasis, who might consequently be spared systemic treatment. We show here that the same signature can also identify older breast cancer patients at low risk for metastatic disease. That fewer of the older age high risk patients developed metastatic disease compared to previously reported studies in younger patients may have two causes. First, these results might reflect host differences in younger versus older breast cancer patients that are associated with propensity to metastasize (i.e. the same “seed” may have different metastatic behavior in a different (post menopausal) “soil”). Second, the patients in the MGH cohort received considerably more adjuvant therapy than the patients from the NKI study (mostly untreated patients), which is likely to have reduced recurrence rates in the MGH patients.
B175 A functional genomics approach using high-throughput RNAi to identify genes as gemicitabine sensitizing targets in pancreatic cancer. David O. Azorsa1, Gargi D. Basu1, Irma G. Monzon1, Jeffrey M. Trent2, Daniel D. Von Hoff3, Spyro Mousses1. 1TGen, Scottsdale, AZ; 2TGen, Phoenix, AZ.

Pancreatic cancer is a deadly disease that kills more than 30,000 people in the United States each year. Pancreatic cancer often presents as an advanced, therapy resistant cancer that is refractory to many forms of chemotherapy. Current therapeutics to treat pancreatic cancer includes gemicitabine, yet the one-year survival of pancreatic cancer patients treated with gemicitabine is about 18%, representing a significant but modest advancement in the quality of life. In order to improve gemicitabine response in pancreatic cancer cells, we utilized high-throughput RNAi (HT-RNAi) to identify genes that when silenced would sensitize pancreatic cancer cells to gemicitabine. A HT-RNAi assay was developed to monitor the growth inhibition of Mia PaCa-2 pancreatic cancer cells transfected with siRNA and treated with gemicitabine. For screening, Mia PaCa-2 cells were transfected with siRNA from several small siRNA libraries targeting cancer-associated genes (139 genes), kinases (574 kinases) and toxicologically relevant genes (75 genes). Twenty-four hours after siRNA transfection, an EC50 dose of gemicitabine was added and growth was assessed after 72 hours of drug exposure. Results from the screen using all three siRNA libraries showed that silencing the genes checkpoint kinase 1 (Chk1) and Ataxia-Telangiectasia and Rad3-related kinase (ATR) sensitized pancreatic cancer cells to gemicitabine. Validation of the screening results was done by examining the dose response of Mia PaCa-2 cells to gemicitabine treatment in the presence of six different Chk1 siRNA. These results showed a three to five-fold decrease in the EC50 for Chk1 siRNA-treated cells versus control siRNA-treated cells. Moreover, Chk1 siRNA-treated cells showed a decrease in proliferation with G0/G1 arrest. Similar results were seen using ATR siRNA-treated cells. Chk1 was further targeted with the small molecule inhibitor PD-407824 in combination with gemicitabine. Results showed that pretreatment of Mia PaCa-2 cells with the Chk1 inhibitor also sensitized cells to gemicitabine. These results indicate that Chk1 and possibly ATR could serve as putative therapeutic targets for sensitizing pancreatic cancer cells to gemicitabine. Furthermore, these findings demonstrate the effectiveness of using HT-RNAi as a tool for identifying sensitizing targets to chemotherapeutic agents. This study is now being expanded to genome-wide screening using larger siRNA libraries.

B176 Flow-cytometric isolation of neoplastic clones in clinical carcinomas coupled with aCGH enables profiling of novel and previously undetectable genomic aberrations. Michael T. Barrett1, Alex Robeson1, Jeffrey Kiefer1, Quick Que1, Holly Yin1, David O Azorsa1, Antonio Jimenez2, Manuel Hidalgo2, Elizabeth Stephan1, Kathleen Delgiforno2, Michael J. Demeure2, Haiyong Han2, Jeffrey Trent2, Daniel D. Von Hoff3, Spyro Mousses1. 1TGen, Scottsdale, AZ; 2TGen, Phoenix, AZ.

Normal cell infiltration and clonal heterogeneity of cancer cells in clinical tumors can greatly interfere with efforts to detect and resolve DNA copy number alterations. In addition to masking critical genomic aberrations, the cellular complexity of clonal mixtures of neoplastic cell populations makes it prohibitively difficult to discern which genomic aberrations occur concurrently. To address these issues, we developed a multistep method for preparing clinical samples in a way that isolates clonal populations prior to analysis of DNA by high definition aCGH. Specifically we flow sorted neoplastic cells that represent distinct clonal populations from primary pancreatic adenocarcinoma (PA) and adrenal cortical carcinoma (ACC) tissues. We then used 60mer oligonucleotide 244k CGH arrays (Agilent Technologies) to scan the genomes of these clonal samples as well as a series of PA cell lines and tumor xenografts. The copy number aberrations present in each of these samples were objectively detected and mapped using an aberration calling method based on computing significance scores for all genomic intervals. We used an efficient algorithm (ADMM) to identify all high scoring intervals in the carcinoma genomes. These experiments demonstrated robust and high definition CGH resolution and identified distinct copy number aberrations in each clonal sample including homozygous deletions, focal amplifications, and interstitial deletions. For example homozygous deletions localizing to genes such as CDKN2A on 9p21.3 and PTEN on 10q23.31, as well as focal amplification of DNA repair genes and various kinases were detected and mapped in flow sorted PA and ACC clonal populations. Furthermore examples of several known and novel intragenic transitions in copy numbers, suggesting gene-targeting rearrangements and translocations, were detected in several of the samples. Genes that mapped to regions commonly affected by focal amplifications were being evaluated by high throughput RNAi phenotype profiling experiments to determine which of the genes are essential for PA cell survival, and which might regulate response to Gemicitabine. This functional analysis has allowed us to rapidly prioritize candidate drug targets to selectively treat pancreatic cancers that harbor specific genetic changes. The results of this study provide compelling evidence that coupling methods for isolating clonal populations of neoplastic cells, with aCGH analysis provides a breakthrough for resolving novel genomic aberrations and clinically relevant gene targets that would otherwise be masked by cellular and clonal heterogeneity.

B178 Reverse-phase protein array (RPPA) profiling of non-small cell lung cancer (NSCLC) identifies tumor signatures for sensitivity and resistance to chemotherapy and targeted agents. Lauren Averett Byers1, Meera Nanjundan1, Luc Girard2, Kevin R. Coomes1, Yang Xie2, Michael Peyton2, Yao Ma3, Sunny Zachariah4, Petros Nikolinas5, Ricardo Cigarroa4, Gordon B. Mills1, Jack A. Roth1, John D. Minna1, John V. Heymach1. 1M. D. Anderson Cancer Center, Houston, TX; 2TGen, Phoenix, AZ.

Introduction: Non-small cell lung cancer (NSCLC) is a molecularly heterogeneous disease, with a variety of cell signaling pathways driving progression and therapeutic resistance. Although agents are available that target many of these critical pathways, there is currently no biologically-based method for selecting the best drug for a patient. Using reverse phase protein array (RPPA), a high-throughput technology that systematically measures key signaling proteins, we profiled forty-four NSCLC cell lines for markers of sensitivity and resistance to fifteen cytotoxic and targeted agents. RPPA is a quantitative, antibody-based assay that allows broad and simultaneous profiling of numerous therapeutically relevant targets from small amounts of protein, as is obtained from routine biopsy. Unlike gene expression arrays, RPPAs directly measure protein levels and can discern specific post-translational modifications such as phosphorylation or cleavage. The ability to compare levels of phosphorylated versus non-phosphorylated proteins is particularly critical to determining the activity of signaling proteins such as tyrosine kinases. Methods: The drug concentration required for 50% growth inhibition (IC50) was determined for 44 cell lines using MTS assays. For the RPPA, serial dilutions of pre-treatment cell lysates were printed on nitrocellulose-coated glass slides, and each slide incubated with one of 59 validated, monospecific antibodies. A single, representative logarithmic value for the signal intensity-serial dilution curve was then determined for each sample, representing the quantitative measure of a specific protein. Results: Subsets of sensitive and resistant cell lines were identified for each drug, despite most cell lines having never been exposed to any treatment either in the patient from which they were derived or in vitro. When subjected to unsupervised clustering, mechanistically-related drugs grouped together (such as taxanes, platinum agents, and epidermal growth factor receptor inhibitors), with similar patterns of protein expression characterized sensitivity or resistance. For example, resistance to cisplatin and carboplatin was associated with increased expression of cell adhesion molecules beta-catenin and E-cadherin and with Rab25, a GTPase previously correlated with worse outcome in breast and ovarian cancer. Cell lines treated with pemetrexed showed the greatest variation in IC50’s, with a 1000-fold difference between sensitivity and resistance in 42 of the cell lines. Pemetrexed resistance was strongly associated with increased phospho-Rb (pRb) relative to Rb expression and with increased PKCalpha.
Elevated pRb and Rb levels were also strongly associated with resistance to etoposide, as was high levels of phospho-LKB1. Conclusions: RPPA proteomic profiling identified intracellular signaling pathways and proteins associated with sensitivity and resistance to chemotherapies and targeted agents in NSCLC cell lines. These results suggest biologic mechanisms of therapeutic resistance. Our findings will be further investigated by correlating RPPA of tumor samples with clinical outcomes, with the goal of developing predictive markers that can guide treatment selection and identify new targets in NSCLC.

B179 Identification of driver mutations in gastrointestinal tract cancer. Timothy K Starr1, Raha Allaei1, Rodney A Staggs1, Kevin A Silverstein1, Adam J Dupuy2, Nancy A Jenkins3, Neal C Copeland2, Robert T Cormier4, David A Largaespada1. 1University of Minnesota, Minneapolis, MN; 2University of Iowa, Iowa City, IA; 3Institute of Molecular and Cellular Biology, Singapore; 4University of Minnesota, Duluth, MN.

Recent large-scale sequencing studies have identified an average of 81 somatic mutations in a typical human colorectal tumor. The next step must be to distinguish passenger from driver mutations in carcinogenesis. In this study we employ a synthetic DNA transposon, called Sleeping Beauty (SB), to create random somatic mutations in the gastrointestinal (GI) tract of mice, leading to carcinoma development. Tumor tissue is collected and transposon insertions are mapped. Due to the small number of insertions generated relative to genome size, a common insertion site (CIS) in three or more independent tumors likely represents a bona fide oncogenic mutation. This approach utilizes somatic transposition of an SB transposon vector, called T2/Onc, which can induce gain-of-function or loss-of-function mutations upon insertion within or near genes. Thus, T2/Onc is capable of activating oncogenes or disrupting tumor suppressors. In this study, T2/Onc mutations were directed to the GI tract using a Cre controlled SB transposase knock-in allele, in combination with a Cre recombinase transgene driven by the Villin promoter, plus the T2/Onc transposon transgene array. A cohort of 45 mice containing all three transgenes along with 81 control mice containing two of the three transgenes were aged for 18 months and monitored for tumor development. Greater than 55% of experimental animals became moribund before 18 months compared to 16% of control mice. Triple transgenic mice had an average of 14 GI tract polyps per mouse while control mice had no detectable GI tract tumors. In addition to adenomas, multiple foci of gastrointestinal intraepithelial neoplasia (GIN) were identified by H&E staining. Nine experimental animals also had liver tumors in addition to GI tract neoplasia. Linker-mediated PCR was performed on tumor DNA from 63 individual GI tract tumors from 16 mice to determine SB insertion sites. Preliminary analysis of roughly 6,000 mappable insertions identified approximately 350 common insertion sites, each site representing a candidate cancer locus. The most commonly disrupted site was the APC locus, harboring 42 non-redundant insertions. Other known colorectal tumor suppressor genes were identified as a CIS, including Smad4, p53, and PTEN, indicating that this approach can effectively uncover driver mutations in an epithelial cancer. Most of the candidate genes identified have not previously been associated with GI tract tumor formation. We will be testing a subset of identified genes using human cell lines and transgenic mouse models to validate their oncogenic potential. In addition, we will analyze the list of identified genes to find cooperating mutations/pathways present within the same tumor.

Metastasis, Invasion, and Tumor Microenvironment Targets

B180 The nucleotide analogue cidofovir suppresses lung metastasis of FGF2-overexpressing endothelial cells. Sandra Liekens1, Armando De Palma1, Annelies Bronckaers1, Erik Verbeken2, Roberto Ronca2, Sigrid Hatse1. 1Rega Institute for Medical Research, Leuven, Belgium; 2K.U.Leuven, Leuven, Belgium; 3University of Brescia, Brescia, Italy.

Cidofovir ([S]-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine, (S)-HPMPC) is an antiviral drug that has been approved for the treatment of cytomegalovirus-induced retinitis in AIDS patients (1). Cidofovir was also found to inhibit the growth of human papillomavirus-induced tumors in animal models and patients (1). In addition, we have recently shown that cidofovir induces apoptosis in tumors that are not associated with an oncogenic virus (2, 3). These highly vascularized tumors were induced in mice by inoculation of basic fibroblast growth factor (FGF2)-overexpressing endothelial cells (FGF2-T-MAE). In vitro, cidofovir treatment of FGF2-T-MAE cells resulted in the up-regulation of the tumor suppressor protein p53 and the inhibition of FGF2 expression and signaling through Erk42/44 (3).

Inoculation of FGF2-T-MAE cells, expressing the green fluorescent protein (FGF2-T-MAE-GFP), in the tail vein of SCID mice resulted in the development of lung metastases, which were macroscopically evident 4 weeks after cell inoculation. Microscopic evaluation of the lungs demonstrated the presence of multiple spindle-shaped hemangiona-like tumors, which expressed FGF2. Systemic administration of cidofovir (150 mg/kg ip, once weekly) markedly reduced the number and size of experimental lung metastases, even when treatment was delayed until micrometastases were already present in the lungs (as determined by FACS analysis of lung homogenates). Our findings thus suggest that the clinical use of cidofovir might be expanded to tumors that are not induced by a virus, and that cidofovir might be useful to prevent the progression and metastasis of these tumors.

B182 Significance of proline rich tyrosine kinase 2 (Pyk2) in epithelial-mesenchymal transition reveals the effectiveness of TSU-68 treatment in extended survivility of tumor stroma by the production of soluble factors. Such a process, known as angiogenesis, is a critical event in malignant tumor progression. It has been proposed that growth factors promoting angiogenesis are likely responsible for the overall reduced responsiveness to cytotoxic drug. Vascular endothelial growth factor (VEGF) is a key player for its role in modulating tumor microenvironment and supporting angiogenesis and endothelial cell survival. VEGF expression in tumor tissues and the presence of its soluble isoforms in biological fluids have been associated with poor prognosis in several types of cancer, including ovarian carcinoma.

We have generated a variant of the human ovarian carcinoma 1A9 by stable transfection with the VEGF121 isoform (1A9-VS1) [Manenti et al., Mol Cancer Ther. 4: 715-725, 2005]. 1A9-VS1 cells express VEGF mRNA and the protein is secreted in the culture supernatant. 1A9-VS1 xenografts in nude mice show dilated blood vessels and release human VEGF in the plasma and peritoneal ascites.

Here we tested the response to chemotherapy in mice bearing 1A9-VS1 or 1A9-VAS3 (undetectable VEGF level) xenografts. Therapeutic response was observed subsequent to paclitaxel treatment (20-40 mg/kg, Q4x3), however the effect was significantly less manifest for 1A9-VS1 (T/C= 40% in mice bearing a sc tumor; ILS= 17% in mice bearing tumor in the peritoneal cavity) than 1A9-VAS3 (T/C= 9%; ILS= 72%). The blockade of VEGF, by the administration of Avastin® (5mg/kg twice a week), greatly improved the antitumor activity by paclitaxel treatment of 1A9-VS1 (T/C=11%). The increase in survival and reduction in tumor volume and ascites were associated with diminished level of plasma VEGF in 1A9-VS1 bearing mice. In vitro 1A9-VS1 and 1A9-VAS3 cells were equally sensitive to paclitaxel, and combination treatments with Avastin® did not affect their responsiveness.

These studies suggest that VEGF expression/production might be associated with a reduced sensitivity to chemotherapy and its blockade might be of benefit in improving therapeutic efficacy.

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B183 Influence of VEGF production on paclitaxel response in a model of ovarian carcinoma xenograft. MariaRosa Banis, Daniela Caronia1, Marta Cesca1, Elizza Naumova1, Raffaella Giavazzi1, Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

Cancer cells promote the formation of new blood vessels within the tumor stroma by the production of soluble factors. We examined the efficacy of one potential molecularly-targeted therapeutic agent, ARQ 197, a small molecule inhibitor of c-met, in a humanized mouse model of breast cancer (BrCa) cases that spread beyond the primary tumor. Studies aimed at understanding the complex mechanisms of interaction between BrCa and the supporting bone stroma hold the promise of identifying potential target molecules for the development of anti-osteotropic therapies. Using a novel humanized mouse model of BrCa metastasis to bone that recapitulates each step involved in the metastatic cascade, we examined the efficacy of one potential molecularly targeted chemotherapeutic agent. ARQ 197 is a small molecule inhibitor of c-Met,
the receptor for hepatocyte growth factor (HGF). The dysregulation of HGF/c-Met signaling correlates with poor prognosis, drug resistance, and the appearance of metastases in a number of human cancers, including breast. To test the efficacy of ARQ 197 at preventing bone metastasis, mice bearing human bone grafts and injected with luciferase-expressing SUM 1315 BrCa cells in their mammary fat pads, were administered ARQ 197 at 30 mg/kg via oral gavage 5x per week for 14 weeks. Use of bioluminescent imaging technology allowed for the detection of luciferase-expressing BrCa cells that had colonized the human bone implants. Approximately 20% of mice treated with ARQ 197 showed metastasis to the human bone grafts, a marked reduction compared to the nearly 50% of mice in the vehicle-treated (control) and paclataxel-treated groups (10 mg/kg, IV, 2x weekly). In our humanized model of BrCa osteotropism, ARQ 197 shows promise as an anti-osteotropic anti-metastasis drug.

B185 Slug protects from apoptosis and reduces invasion in in vitro and in vivo neuroblastoma models. Roberta Vitali1, Barbara Tanno1, Vincenzo Ces1, Camillo Mancini1, Mariateresa Mancuso1, Bruno Calabretta2, Carlo Dominici2, Giuseppe Raschella1. 1ENEA, Rome, Italy; 2Thomas Jefferson University, Philadelphia, PA; 3La Sapienza University, Rome, Italy.

Neuroblastoma (NB) derives from embryonic neural crest precursors that undergo malignant transformation at some stage of differentiation into neural crest derivatives. Despite intensive multimodal treatment, metastatic NB is still characterized by poor prognosis. Slug is a transcriptional regulator that belongs to a small family of development-regulatory genes. During embryonic development, Slug is involved in the delamination of neural crest cells and in their migration to distant sites. In this study, we assessed the relevance of Slug expression for apoptosis resistance and for invasion potential of NB cells in vitro and in vivo. Slug expression was detectable in 8 out of 10 human NB cell lines analyzed. Two Slug-expressing cell lines (LAN-5 and HTLA-230) were infected with the lentiviral vector pLKO-Slug that encodes a miRNA directed against Slug. After selection, both pLKO-Slug infected cell lines expressed strongly reduced levels of Slug. The expression of some pro-apoptotic and anti-apoptotic markers (p53, Bax, Bcl-2), previously described as Slug targets, was analyzed by immunoblotting. Bcl-2 expression was decreased in pLKO-Slug infected cells. Slug down-regulation also caused an increased sensitivity to apoptosis induced by etoposide (1 M) treatment. Invasion capability of pLKO-Slug infected cells was strongly reduced in Matrigel-coated invasion chambers. Interestingly, the selective tyrosine kinase inhibitor Imatinib Mesylate (IM) also reduced invasion of NB cells in similar assays. In vivo, we tested the ability of Slug-down-regulated NB cells to invade using a pseudo-metastatic model. pLKO-Slug HTLA-230 and control (pLKO-GFP infected HTLA-230) cells were infected in the tail vein of SCID mice. Injected animals were divided in 4 groups: pLKO-Slug untreated; pLKO-Slug treated with IM (200 mg/Kg/day); control (pLKO-GFP) untreated; control (pLKO-GFP) treated with IM (200 mg/Kg/day). After 35 days mice were sacrificed and tumor burden was evaluated. Animals injected with pLKO-Slug had fewer tumors than both untreated and IM-treated pLKO-GFP controls. Tumor inhibition was significantly more evident in pLKO-Slug injected animals treated with IM. Our data demonstrate that Slug protects NB cells from apoptosis induced by cytotoxic drugs and increases their invasion capability in vitro and in vivo. Inhibition of Slug expression, possibly combined with exposure to IM, could represent a suitable strategy to decrease NB metastatic potential.

B186 Transforming growth factor-β1 modulates tumor-stromal cell interactions of prostate cancer through insulin-like growth factor-I. Manabu Kawada1, Masayuki Arakawa1, Hiroyuki Inoue1, Isao Momose1, Daishiro Ikeda1. 1Microbial Chemistry Research Center, Shizuoka, Japan.

Insulin-like growth factor-I (IGF-I) secreted from prostate stroma mediates tumor-stromal cell interactions to develop prostate cancer. Transforming growth factor-β1 (TGF-β1) also plays a critical role in tumor-stromal cell interactions, but it has pleiotropic effects and modulates growth of prostate cancer either positively or negatively. To understand such complexity of its actions, we have studied the effect of TGF-β1 on IGF axis in human prostate stromal cells (PrSC). TGF-β1 induced myofibroblasts differentiation of PrSC and upregulated miRNA expression of both IGF-I and IGF-β3. TGF-β1 significantly increased IGBP-3 secretion from PrSC, but it conversely reduced amounts of biologically active IGF-I unbound to IGBP-3. PSA and MMPI-7 acted as IGBP-3 proteins, degraded IGBP-3, and restored the IGF-I action. TGF-β1 actually suppressed the growth of human prostate cancer DU-145 cells capable to respond to IGF-I in the coculture with PrSC, but PSA and MMP-7 restored the suppressed growth of DU-145 cells. Furthermore, immunohistochemical analyses of 29 human prostate cancer tissues showed that IGF-I expression in stroma significantly correlated positively with TGF-β1 expression in stroma (r=0.797, P<0.0001). Taken together, these results indicate that TGF-β1 modulates the tumor growth either positively or negatively through the balance between the amounts of IGF-I and IGBP-3. Furthermore, degradation of IGBP-3 potentially results in the tumor promotion and will be a therapeutic target.

B187 Targeting metastatic tumor cell functions by inhibition of Src signaling using the small molecule Src inhibitor, AZD0530. Meiyu Dong1, Lori Rice1, Dietmar W. Siemann2. 1Departments of Pharmacology and Therapeutics, and Radiation Oncology, Shands Cancer Center, University of Florida, Gainesville, FL.

Introduction: Src is a non-receptor tyrosine kinase that is frequently overexpressed in malignancies, and increased Src activity (regulated by Src phosphorylation) may be associated with poor patient prognosis. Src is involved in many signaling pathways, including mitogen-activated protein kinase (MAPK), focal adhesion kinase (FAK), and Met that regulate cell proliferation, survival, and migration. Since these cellular functions are key components of the metastatic cascade, Src may be a novel target for cancer therapy. In the present study, Src inhibition by AZD0530, a potent, selective and orally available Src inhibitor with Bcr-Abl activity, was evaluated in the highly metastatic rodent KHT sarcoma cell line.

Methods: The effect of AZD0530 treatment on expression of total and phosphorylated Src, FAK, extracellular signal-regulated kinase (ERK), and Met protein was determined by Western blot. Src and FAK cellular distribution were evaluated by immunofluorescence using confocal microscopy. Tumor cell functions, including viability, cell cycle, and migration, were examined by trypan blue exclusion, flow cytometry, and migration assays (wound healing and transwell), respectively.

Results: At the molecular level, AZD0530 doses greater than 2.5 μM significantly reduced Src phosphorylation at tyrosine 418 in KHT cells. Reductions in Src phosphorylation occurred within 2 hours of treatment (~80% at 10 μM). Met and phosphorylated ERK were also decreased at this dose. AZD0530 treatment also altered the localization of phosphorylated Src and phosphorylated FAK within the tumor cells from focal adhesion to cytoplasm. Functionally, AZD0530 significantly reduced the ability of KHT sarcoma cells to migrate into a denuded area or through a transwell membrane (0.5-2.5 μM), and increased the proportion of cells in the G1 phase of the cell cycle.

Conclusions: These results indicate that AZD0530 treatment inhibits functional tumor cell characteristics associated with a metastatic phenotype in the KHT sarcoma cell line. These data serve as a basis for future studies aiming to evaluate the in vivo antimetastatic efficacy of this Src inhibitor.

B188 Intratumoral heterogeneity for expression of tyrosine kinase growth factor receptors in human colon cancer surgical specimens and orthotopic tumors. Toshiro Kuwai1, Toru Nakamura2, Sun-Jin Kim2, Takamitsusu Sasakii1, Yasuhiko Kitadai1, Robert R Langley2, Dominic Fan3, Stanley R. Hamilton3, Isaiah J. Fidler1. 1Kure Medical Center and Chugoku Cancer Center, Kure, Hiroshima, Japan; 2M. D. Anderson Cancer Center, Houston, TX.

The design of targeted therapy, particularly patient-specific targeted therapy, requires knowledge of the presence and intratumoral distribution of tyrosine kinase receptors. To determine whether the expression of such
receptors is constant or varies between and within individual colon cancer neoplasms, we examined the pattern of expression of the ligands epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor-B (PDGF-B) and their respective receptors in human colon cancer surgical specimens and orthotopic human colon cancers growing in the ecal wall of nude mice. The expression of EGF and VEGF on tumor cells and stromal cells, including tumor-associated endothelial cells, was heterogeneous in surgical specimens and orthotopic tumors. In some tumors, the receptor was expressed on both tumor cells and stromal cells, and in other tumors, the receptor was expressed only on tumor cells or only on stromal cells. In contrast, PDGF was expressed only on stromal cells, in both surgical specimens and orthotopic tumors. Examination of receptor expression in both individual surgical specimens and orthotopic tumors revealed that PDGF was expressed only on stromal cells and that patterns of EGF and VEGFR2 expression differed between tumor cells. This heterogeneity in receptor expression between different tumor cells suggests that only therapeutic regimens inhibiting multiple tyrosine kinase receptor pathways are likely to be effective against Institute human colon carcinomas.

B189 Brain metastasis and blood-brain barrier invasion: Involvement of membrane-bound melanotransferrin. Yanneve Rolland1, Michel Demeule2, Laurence Fenart3, Richard Beliveau1.

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The membrane-bound human melanoma antigen p97, also known as melanotransferrin (MTf), regulates cellular motility, migration and invasion by stimulating the activation of plasminogen. In the present study, we investigated the impact of melanotransferrin expression on brain invasion. Transendothelial migration of Chinese hamster ovarian (CHO) cells transfected with the full-length cDNA of human melanotransferrin and with a control vector were measured in a blood brain barrier (BBB) in vitro model. Our results show that MTf-transfected CHO cells, as well as MTF-overexpressing melanoma SK-Mel-28 cells possess a great capacity to cross the endothelial monolayer. The transmigration of MTF-expressing cells through the BBB in vitro is potently reduced by a monoclonal antibody directed against Institute human melanotransferrin (L235) and by the loss of MTF expression following post transcriptional gene silencing. To further demonstrate that membrane-bound melanotransferrin is involved in brain invasion, an in vivo brain metastasis assay was performed. We demonstrate that MTF-expressing cells have a greater capacity to invade the CNS in vivo. Our results also suggest that the MTF-dependent transmigration process requires the activation of the plasminogen system and the participation of the VEGF signaling pathway. Overall, our results indicate that membrane-bound melanotransferrin facilitates the transmigration of cancer cell across the BBB. Furthermore, these are the first results to identify membrane-bound melanotransferrin as a novel therapeutic target for CNS metastases.

B190 The effect of HIF-1 inhibition on the p53 pathway. Lina Franzén1, Kaye Williams1, Ian Stratford1. 1School of Pharmacy and Pharmaceutical Sciences, Manchester, United Kingdom.

Introduction: Hypoxia is a common feature in solid tumors and is a result of rapid proliferation of tumor cells and a disorganised vascular network. It occurs when the oxygen supply to the cell is below required level. Hypoxia inducible factor 1 (HIF-1) is a key transcription factor activated by hypoxia and target genes are involved in adaptive processes, such as metastatic and angiogenic activity. The result of HIF-1 activation is an aggressive and therapy-resistant tumor phenotype and therefore HIF-1 is a popular target in cancer therapy. The mechanisms underlying HIF-1 inhibition using small molecules are poorly understood, mapping pathways involved could have therapeutic benefits since these may be involved in tumor progression. One pathway of interest is the p53 pathway since this protein is an important tumor suppressor protein. The literature is heterogeneous regarding possible interaction between HIF-1 and p53 under hypoxic condition. It has been suggested that HIF-1 indirectly stabilises p53 via binding to MDM2, that the two proteins interact directly and that they compete for the co-activator p300 (reviewed in Schmid T et al. J Cell Mol Med 2004;8:423-31). The purpose of this study is to evaluate the effect of HIF-1 inhibition on p53 activity.

Methods and results: By transiently transfecting a range of cell-lines with a p53-luciferase or HIF-luciferase reporter gene construct and measuring the transcriptional activity using dual luciferase reporter assay, we were able to map the transcriptional activity of the two transcription factors under anoxic conditions (~0.01% oxygen). A 1.3 fold induction of p53 transcriptional activity and a 2 fold induction of HIF-1 transcriptional activity were observed in the colon carcinoma HCT116 cell-line after 24 hours of anoxic exposure. Western blot analysis of p53 protein levels demonstrated an anoxic induction of the p53 protein following 24 hours exposure. The HCT116 HIF-1 dominant negative cells with ablated HIF-1 transcriptional activity demonstrated stronger induction of the p53 transcriptional activity compared to wild type cells under anoxic conditions (1.6 ± 0.8 (SEM) fold induction compared to 1.3 ± 0.2 (SEM) fold induction). These results demonstrate that inhibition of HIF-1 during anoxia results in a higher transcriptional activity of p53. By using computational analysis we found 7 potential HIF-1 binding sites in the promoter region of the p53 gene, suggesting that HIF-1 could have a negative regulatory effect on transcription of the p53 gene. Furthermore, immuno-precipitation analysis using an anti p300 antibody showed that both HIF-1 and p53 bind the p300 protein after 24 hours of anoxic exposure, suggesting that the p53 protein can access a redundant amount of p300 for transcriptional activity during anoxia.

Conclusion: The results from this study show that HIF-1 has a negative regulatory effect on p53 activity under anoxic conditions, since impaired transcriptional activity of HIF-1 results in a higher transcriptional activity of p53. We suggest that the two transcription factors compete for the co-activator p300 as both transcription factors bind p300 after 24 hours of anoxic exposure. The binding site analysis suggests that HIF-1 could have a negative regulatory effect on the transcription of the p53 gene. The effect of small molecule inhibitor molecules of HIF-1 on the p53 activity and downstream targets is subject for ongoing work.

B191 A single amino acid substitution turns Scatter Factor into a potent inhibitor of tumor dissemination. Paolo Michieli1, Massimiliano Mazzone1, Paolo M. Comoglio1. 1Institute for Cancer Research and Treatment, Candiodo, Turin, Italy.

Scatter Factor (SF), also known as Hepatocyte Growth Factor, is ubiquitously present in the extracellular matrix of tissues under the form of inactive precursor (pro-SF). In order to acquire biological activity, pro-SF must be cleaved by specific proteases present on the cell surface, the most characterized of which is urokinase-type Plasminogen Activator (uPA). The mature form of SF controls invasive cues in both physiological and pathological processes through activation of its receptor, the Met tyrosine kinase. By substituting a single amino acid in the proteolytic site, we engineered an unprocessable form of pro-SF (Uncleavable SF). Using an improved lentiviral vector technology, we achieved local or systemic delivery of Uncleavable SF in mice. We provide evidence that: (a) Uncleavable SF forms stable complexes with Met and uPA, thus preventing both Met activation and pro-SF conversion; (b) local expression of Uncleavable SF in tumors suppresses tumor growth, impairs tumor angiogenesis, and prevents metastatic dissemination; (c) systemic expression of Uncleavable SF dramatically inhibits the growth of transplanted tumors and virtually abolishes the formation of spontaneous metastases, without affecting normal physiological functions. These data show that proteolytic activation of pro-SF is a limiting step in tumor progression, thus suggesting a new strategy for treating or preventing malignant conversion of neoplastic lesions.
Activity of signaling pathways in hypoxic pancreatic and cervical tumor xenografts. Nhu-An Pham, Joao Magalhaes, Trevor Do, Joerg Schwock, Richard Hill, David Hedley. Ontario Cancer Institute, Toronto, Ontario, Canada; University of Toronto, Toronto, Ontario, Canada.

Tumor hypoxia is widespread in different cancer types and has been associated with poor prognosis. Current monolayer cell culture models suggest that signaling pathways including Src, STAT3 and AKT influence the transcription activity of hypoxia inducible factor-1α (HIF-1α) which affects biological processes including tumor angiogenesis, metastasis and survival. We determined the activity of these signaling proteins in hypoxic tumor regions compared to non-hypoxic regions as well as global tumor response to continuous in vivo hypoxia. Tumor hypoxia was measured based on the histological distribution of the fluorescently labeled 2-nitroimidazole agent EF5 in four murine xenograft models. In air exposed mice, the percent of hypoxic EF5-stained tumor regions ranged from a mean of 14% (BxPC-3) to 48% (PANC-1) for subcutaneously grown pancreatic xenografts, and 20% (ME180) to 27% (SiHa) for orthotopically grown cervical xenografts. A doubling of EF5-stained regions was achieved based on the histological distribution of the fluorescently labeled 2-nitroimidazole agent EF5 in four murine xenograft models. In air exposed mice, the percent of hypoxic EF5-stained tumor regions ranged from a mean of 14% (BxPC-3) to 48% (PANC-1) for subcutaneously grown pancreatic xenografts, and 20% (ME180) to 27% (SiHa) for orthotopically grown cervical xenografts. A doubling of EF5-stained regions was achieved in BxPC-3 tumors with continuous exposure of mice to 7% O2 for 3 hours compared to air control. Individual EF5-stained serial tissue sections were co-labeled with single antibodies against tissue signaling proteins. Immunofluorescence image analysis (labeled percent area x intensity) showed a significant increase in the levels of total Src protein in EF5 tumor regions compared to non-EF5 regions in all four tumor models (p≤0.05, Wilcoxon test). Additionally, the levels of total and activated Src (Y419) doubled in EF5 tumor regions compared to non-EF5 regions of mice exposed to low O2 levels, suggesting that levels of Src protein and activity are hypoxia inducible. There was a strong co-localization of activated Src and focal adhesion kinase (Y861-FAK) in EF5-stained regions of BxPC-3 tumors (r=0.74, p=0.006) in mice exposed either to air or 7% O2. This suggests that the cellular adhesion substrate FAK is important in Src-mediated hypoxia signaling in vivo. In contrast, BxPC-3 tumor xenografts of mice exposed to 7% O2 showed a decrease in levels of STAT3 (S727) activity (p=0.004, Mann-Whitney test) despite no changes in total STAT3 levels in the entire tumor area. The continuous exposure to 7% O2 did not affect levels of AKT (S473) activity. These results suggest an important role of Src in hypoxia-responsive signaling in vivo. The increased expression and enhanced activity of Src family tyrosine kinases have been associated with carcinogenesis in different tumor types. Also, Src is a common substrate of oncogenic signaling downstream of several growth factor receptors including EGFR, PDGFR and Met, and impacts on tumor cell adhesion, migration and invasion. A therapeutic strategy involving the use of Src inhibitors might be successful at targeting tumor growth as well as hypoxia-induced processes which lead to increased tumor aggressiveness.


Introduction: The purpose of the study was to compare the absorption of two strengths of an investigational oral dosage form of MER-101, a tablet form of zoledronic acid, to the parenteral reference product, commercially-available zoledronic acid intravenous infusion. MER-101 was developed by Merrion Pharmaceuticals using GIPET™ I technology to improve the oral bioavailability of zoledronic acid and thereby enable the development of an oral dosage form. GIPET™ I is based on proprietary penetration enhancers which improve the absorption of such drugs in the small intestine. There is no chemical modification to the active drug. The enhancer system is comprised of food based material which is on the US GRAS list. These are important factors in reducing regulatory requirements and the time to market. GIPET™ technology is equally applicable to small molecules, macromolecules and biologics, and is broadly applicable over a wide range of marketed and emerging products.

Zoledronic acid is a bisphosphonate used in the treatment of bone metastases. Bisphosphonates are synthetic analogs of pyrophosphate that bind to the hydroxyapatite found in bone. The current marketed dosage form of zoledronic acid is given as an infusion to overcome the limitations of oral dosing of bisphosphonates, including low bioavailability, gastric irritation, and gastric reflux.

Experimental Procedures: The study was a single-dose, three-way crossover bioequivalence study in 13 postmenopausal female subjects with osteoporosis. There was a washout period of at least 7 days between dosing days. Cumulative urinary excretion of zoledronic acid over a 48-hour period was used as the basis for the pharmacokinetic analysis. Eleven subjects successfully completed all treatment periods of the study and were included in the final analysis.

The treatments administered during the clinical trial were: MER-101 10mg and 20mg enteric coated tablets and zoledronic acid injection 1mg, administered as a 15-minute infusion in 100mL of normal saline. The treatments were well tolerated in all cases.

Data Summary: The MER-101 20mg tablet had a mean 48-hour urinary excretion of zoledronic acid approximately 44% greater than the MER-101 10mg tablet. The MER-101 20mg tablet had a mean zoledronic acid excretion that was similar to the zoledronic acid 1mg Injection (0.514mg and 0.541mg respectively).

Conclusions: A tablet dosage form of zoledronic acid has been successfully developed which will allow once weekly treatment of patients. The dose administered via a 20mg tablet equals that of a 1mg intravenous infusion. MER-101 was well tolerated and there were no serious adverse events associated with its administration.
B195 Distinct patterns of matrix metalloproteinase expression in human stromal cells. M. Waheed Roomi1, Julio Monterrey1, Vadim Ivanov1, Aleksandra Niedziwicki2, Matthias Rath1. 1Dr. Rath Research Institute, Santa Clara, CA.

The interaction between cancer cells and the surrounding stromal cells plays an important role in the growth of tumors, as well as in subsequent metastasis. Invasion of the surrounding tissue by malignant cells is a complex process mediated by matrix degrading enzymes. Matrix metalloproteinases (MMPs) are a class of metal-dependent endopeptidases, which play a key role in degradation of extracellular and basement membranes. Increased secretion of these proteinases has been associated with invasion, metastasis and angiogenesis. Furthermore, in many solid tumors the expression of MMPs is higher in stromal cells than in the tumor cells per se, suggesting stromal cells as the major source of these enzymes. MMP-2 and MMP-9 are two members of this class whose secretion is elevated in several types of human cancers. The expression of MMPs is regulated by cytokines and signal transduction pathways, including those activated by PMA. The aim of the this study was to examine (1) the pattern of MMP-2 and MMP-9 expression in human stromal cells and the effect of PMA on their secretion, and (2) to determine if either is preferentially expressed in cells of tissues of different origin. The overall goal was to examine the possibility of MMPs as targets for cancer therapy. Fifteen human stromal cells (obtained from ATCC) of epithelial and connective tissue origin were selected, since carcinomas and sarcomas are derived from these two tissue types respectively. They were cultured in the recommended media and supplemented with 10% FBS and antibiotics in 24-well tissue culture plates. At near confluence, the cells were washed and fresh medium added. A parallel set of cultures was cultured in the recommended media and supplemented with 10% FBS and antibiotics in 24-well tissue culture plates. At near confluence, the cells were washed and fresh medium added. A parallel set of cultures was treated with PMA. After 24h incubation, the media were collected and analyzed for MMP-2 and MMP-7 by gelatinase zymography. The results indicate that stromal cells fall into two groups: (1) those secreting only MMP-2 and not stimulated by PMA, and (2) those secreting both MMP-2 and MMP-9 with PMA stimulation of MMP-9 secretion alone. In conclusion, stromal cells, regardless of tissue of origin, secrete MMP-2. PMA has no effect on secretion of MMP-9 of epithelial origin, but induces MMP-9 secretion of connective tissue cell lines. These results suggest the possibility that these enzymes are differentially regulated and that an understanding of this may open up avenues to use these enzymes as targets for therapy.

B196 Fasudil inhibits lysophosphatidic acid-induced invasion of ovarian cancer cells. Seiji Ogata1, Ken-ichirou Morishige1, Kenjiro Sawada1, Kae Hashimoto2, Seiji Mabuchi1, Chiaki Kawase1, Masahiro Sakata1, Tadashi Kimura1, 1Osaka University, Osaka, Japan; 2University of Toronto, Toronto, Ontario, Canada.

The poor prognosis of ovarian cancer comes from the aggressive invasiveness of ovarian cancer cells. Therefore, it is important to clarify the underlying pathological mechanism in the progression of ovarian cancer and to improve the new therapeutic strategies based on it. Rho-associated kinase (ROCK) is one of the main downstream effectors of a small GTP-ase, Rho. The activation of ROCK by GTP-bound form of Rho induces the formation of stress fibers and focal adhesions, and ultimately leads to increased intracellular contractile force which is needed for cell motility. Lysophosphatidic acid (LPA) is one of the physiologically active lipids which exists in the ascites of ovarian cancer patients at a high concentration. Our group have reported that LPA induces the migration of ovarian cancer cells mainly through accelerated formation of stress fibers and focal adhesions mediated by Rho/ROCK pathway and that inhibition of LPA/Rho/ROCK pathway attenuates the migration of ovarian cancer cells both in vitro and in vivo.

Fasudil [1-(5-isouquinolinesulfonyl)-homopiperazine: HA-1077] is a drug that has been in clinical use in Japan for the prevention of vasospasm after subarachnoid hemorrhage. Fasudil is known to be one of the ROCK-specific inhibitors. Therefore we expected that fasudil could attenuate the invasiveness of human ovarian cancer cells by inhibiting LPA/Rho/ROCK pathway.

We first studied the effect of fasudil on the morphology of human ovarian cancer cells. Whereas untreated Caov-3 cells were round and well-spread, treatment with 30μM fasudil induced cell retraction from the substratum and loss of contacts between neighboring cells, resulting in a spindle-shaped morphology. Secondly, the effect of fasudil on the chemotactic directional invasion of human ovarian cancer cells was evaluated using in vitro invasion assay. Fasudil significantly suppressed the LPA-induced cell invasion in a dose-dependent manner. The effect of fasudil on migration ability of human ovarian cancer cells was also assessed by wound healing assay. Fasudil significantly suppressed the LPA-induced migration in a dose-dependent manner.

Intracellular formation of stress fibers and focal adhesions is indispensable for cell motility. Therefore, we investigated the effect of fasudil on the formation of stress fibers and focal adhesions by immunocytochemistry. Whereas untreated Caov-3 cells were round, had network of thin actin filaments and showed diffused localization of paxillin, a focal adhesion protein, LPA-stimulated Caov-3 cells became polygonal, had thick bundles of actin stress fiber and showed peripherally accumulated localization of paxillin. Pretreatment with fasudil significantly attenuates LPA-induced formation of stress fibers and focal adhesions.

Tyrosine phosphorylation of focal adhesion proteins is known to be an essential process in LPA-induced cell migration. Therefore, we analyzed the effect of fasudil on the tyrosine phosphorylation of paxillin, a focal adhesion protein, by Western blotting. Pretreatment with fasudil inhibited LPA-induced tyrosine phosphorylation of paxillin in a dose-dependent manner. In conclusion, we showed that fasudil attenuates LPA-induced invasiveness of human ovarian cancer cells via inhibiting ROCK activity. Our findings suggest that fasudil may have the potential to control the progression of ovarian cancer in clinical settings.

B197 Inhibition of tumor growth and invasion by YC-1 in nasopharyngeal carcinoma (NPC). Bo Hong1, Vivian W.Y. Lu1, Elaine Y.L. Wong1, Edwin Hui1, Anthony TC Chan1. 1Chinese University of Hong Kong, Hong Kong SAR, Hong Kong.

Objective: Nasopharyngeal carcinoma (NPC) is highly prevalent in Southeast Asia. The majority of NPC deaths are due to metastasis. Hypoxia is known to contribute to cancer progression and metastasis in various human malignancies, including NPC. Hypoxia-inducible factor 1-alpha (HIF-1α) plays a major role in promoting cancer growth, metastasis and angiogenesis. We have previously shown that HIF-1α and several related hypoxia markers (CA IX and VEGF) were over-expressed in about 60% of NPC cases and over-expression of HIF-1, CA IX and VEGF are associated with poor survival in NPC patients (Hui et al., Clin. Cancer Res, 2002). In this study, we investigated the effects of YC-1, a compound known to inhibit HIF-1α, on NPC cell growth and invasion. We hypothesize that HIF-1α targeting would be elicit antitumor activity in NPC models.

Methods: Three human NPC cell lines, HONE-1, CNE-2 (derived from poorly differentiated NPC) and HK1 (from well-differentiated NPC) were used. Hypoxia treatment: cell incubation in a hypoxia incubator (0.1% O₂, 5% CO₂, 94.9% N₂). The effects of YC-1 on NPC cell growth was assessed by MITT assay, while its effects on NPC invasion was determined by wound healing assay as well as the Matrigel Invasion assay. Western blotting was employed for the study of the effects of YC-1 on NPC signaling.

Results: YC-1 effectively induced a dose-dependent growth inhibition of a NPC cell line, HONE-1 (IC₅₀ = 535.6 ± 64.6 nM, 48 hrs). YC-1 treatment at 10 μM resulted in 71.6 ± 0.4% and 47.0 ± 1.8% growth inhibition in HONE-1 and CNE-2, respectively. However, no detectable growth inhibition was observed in HK1 cell when treated with equal concentration of YC-1. Using a representative NPC cell line, HONE-1, we also demonstrated that YC-1 was able to effectively inhibit the hypoxia-induced HIF-1α up-regulation in a dose-dependent manner. However, YC-1 did not preferentially enhance NPC growth inhibition under hypoxia in all three NPC cell lines. We also found that YC-1 inhibited NPC motility and invasive migration in vitro. YC-1, at both 350 nM and 700 nM, inhibited the spontaneous motility of HONE-1 cells in the absence of serum. Serum-induced invasive migration of HONE-1 through the Matrigel Invasion
chamber was also significantly inhibited by YC-1 at 250 nM (~60% inhibition when compared to the vehicle control p=0.0007). It is known that epidermal growth factor receptor (EGFR) signaling plays an important role in NPC growth and invasion. However, the relationship between HIF-1α and EGFR in NPC is unclear. In an NPC cell line, HONE-1, hypoxia not only induced HIF-1α up-regulation, but also EGFR up-regulation (at 24 hrs and 48 hrs). Stable clones expressing EGFR shRNA greatly attenuated the hypoxia-induced HIF-1α up-regulation (at 6 hrs of hypoxia treatment). Our results indicated that HIF-1α and EGFR expression are coupled in NPC by unknown mechanism. Combined targeting of EGFR (by an EGFR-targeting monoclonal antibody, C225, 100 nM) and YC-1 (500 nM) resulted in additive growth inhibition of HONE-1 cells in both normoxic and hypoxic conditions (at 48 hrs).

Conclusions: In summary, our data demonstrated that YC-1 targeting is effective in inhibiting NPC growth and invasion. Our data also suggest that HIF-1α and EGFR signaling seem to be coupled in NPC cells. Combination of YC-1 and EGFR monoclonal antibody resulted in enhanced antitumor effects on NPC cells. Supported by RGC of HKSAR (project no: CUHK 4442/06M)

B198 Novel insight into the mechanism of action and selectivity of the Grb2 Src homology 2 domain binding antagonist C90. Alessio Giubbellino1, Lisa Jenkins1, James Vasselli2, Ettore Appella3, Terrence R. Burke, Jr3, Donald P Bottaro1. 1National Cancer Institute, Bethesda, MD.

Metastasis is the primary cause of death in human cancer and its presence defines malignant tumors. Among the critical features that a primary tumor must acquire to metastasize are motility and invasiveness. Cancer cells break away from the primary tumor, migrate to the vasculature, survive in an anchorage independent way in the bloodstream, extravasate and proliferate at the new site. A drug that can effectively interfere with any of these steps could potentially inhibit tumor metastasis. Although many drugs have been developed to control cancer growth in humans, no drugs presently available have been specifically designed to inhibit metastasis using a molecular targeting approach.

Grb2 is a widely expressed adapter protein that participates in multiple cell functions. Its critical roles in motility, invasion and angiogenesis make it a logical molecular target for the development of therapeutics against the spread of solid tumors. We have previously shown that a potent synthetic antagonist of Grb2 SH2 domain binding, C90, blocked growth factor stimulated motility, invasion, and angiogenesis in cultured cell models and in the chick chorioallantoic membrane. More recently we reported that this drug inhibited metastasis in two aggressive animal models without affecting primary tumor growth rate.

To characterize the selectivity of C90 for the SH2 domain of Grb2 over other proteins containing similar motifs, a biotinylated derivative of the compound was developed (S4S). We first confirmed that S4S retained the biological potency of C90, and that it could efficiently capture Grb2 from non-ionic detergent cell extracts. S4S was then used to capture C90 protein targets from cell lysates for subsequent SDS-PAGE, immunoblotting and mass spectrometry analysis. A C90 analog with defective Grb2 binding was biotinylated in parallel and used as a negative control throughout the course of these studies. Our results indicate that Grb2 is the only SH2-domain containing protein the binds with high affinity to C90/S4S in target cells.

To characterize the molecular mechanisms by which C90 inhibits cell motility and invasion, Grb2 interactions with mediators of cytoskeletal rearrangement and focal adhesion formation were analyzed in human tumor cells treated with Hepatocyte Growth Factor (HGF). As visualized using Alexa 488-phalloidin, C90 treatment was associated with a shift in the distribution of actin cables from the leading edges of HGF-treated cells to the cytoplasm. C90 also inhibited HGF-driven activation and nuclear translocation of PAK1, another important effector of cell motility. Coincident with these effects, C90 treatment disrupted the HGF-stimulated physical association between Grb2 with Focal Adhesion Kinase (FAK), and inhibited focal adhesion formation in a dose-dependent manner. These data support the unique selectivity of C90 for Grb2, and provide greater insight into the molecular mechanisms by which antagonists of Grb2 SH2 domain binding block cell motility in vitro and tumor metastasis in vivo.

B199 Estrogen promotes pulmonary metastases of tuberin-deficient cells, associated with extracellular matrix reorganization. Jane J. Yu1, Chunrong Wang1, Lisa Hernandez-Cuevas1, Aristotelis Astrinidis1, Magdalena Karbowiczek1, Eric Ariazi1, Craig Jordan1, Elizabeth Petri Henske1. 1Fox Chase Cancer Center, Philadelphia, PA.

Lymphangioliomyomatosis (LAM) is a rare disease that affects exclusively women. LAM is characterized by the proliferation of abnormal smooth muscle cells and cystic degeneration of lung parenchyma. Genetic studies suggest that LAM pathogenesis involves the metastasis of benign cells. The fact that LAM affects young women suggests that estrogen may promote metastasis. To determine whether estrogen promotes the metastasis of TSC2-deficient cells, rat uterine leiomyoma (ELT3) cells were injected subcutaneously into ovariectomized female or male CB17-scid mice implanted with estrogen or placebo pellets. The number of pulmonary metastases was scored in four 5-micron sections from both lungs. Estrogen significantly enhanced the frequency and the number of pulmonary metastases in both female and male mice. Estrogen-treated mice developed primary tumors that progressed more rapidly and attained a greater size than tumors in placebo-treated mice. The primary tumors and the metastases were strongly immunoreactive with anti-smooth muscle actin and anti-phospho-S6 antibody, as expected. Compared with placebo treated tumors, cells from the estrogen-treated tumors showed increased nuclear localization of phospho-p42/44 MAPK, suggesting a reactivation of signaling pathways that are impaired in tuberin-deficient ELT3 cells. Estrogen-treated tumors also expressed elevated levels of matrix metalloproteinase 2 (MMP2) compared with placebo-treated tumors.

In both male and female mice, the estrogen-treated tumors exhibited an abnormal nuclear morphology and an altered ECM when compared with the placebo-treated tumors. The accumulation of type IV collagen, a major structural component of ECM, was greatly reduced in estrogen-treated tumors compared with placebo-treated tumors. These findings support a model in which estrogen reactivates MAPK in TSC2-deficient cells, leading to degradation of ECM and promoting pulmonary metastasis. To elucidate the signaling pathways responsible for estrogen-induced ELT3 cell metastasis, xenograft mice were treated with the estrogen receptor inhibitor Fulvestrant or the mTORC1 inhibitor RAD001. RAD001 completely blocked both the primary tumor development and the estrogen-induced metastasis. Fulvestrant did not inhibit the primary tumors, but completely blocked estrogen-promoted lung metastases.

In vitro studies using cultured ELT3 cells supported the in vivo findings: estrogen rapidly stimulated phosphorylation and nuclear translocation of phospho-p42/44 MAPK, induced cellular MMP2 expression, and increased MMP2 activity in the conditioned media.

In conclusion, we found that estrogen promotes the pulmonary metastases of tuberin-deficient ELT3 cells. This is associated with estrogen-stimulated MAPK activation and ECM alteration coupled with MMP2 expression and type IV collagen accumulation. RAD001 inhibited both primary tumor development and lung metastases. Fulvestrant inhibited the estrogen-induced lung metastases. We speculate that this animal model may have relevance to both LAM pathogenesis and to the development of estrogen-focused targeted therapeutic strategies for LAM.
B200 Impaired membrane-type 1 matrix metalloproteinase phosphorylation results in blocking 3D-collagen invasion by tumor cells through cell cycle arrest in G0/G1 phase. Carine Nyalendo1, Edith Beaulieu2, Marisol Michaud2, Denis Gingras2, Richard Béliveau2. 1CHU Sainte-Justine, CHUM Notre-Dame, Université du Québec à Montréal, Montreal, Quebec, Canada; 2CHU Sainte-Justine, Montreal, Quebec, Canada; 3CHU Sainte-Justine, Université du Québec à Montréal, Montreal, Quebec, Canada.

Membrane-type 1 matrix metalloproteinase (MT1-MMP) is a transmembrane membrane metalloproteinase (MMP) that degrades several component of the extracellular matrix, and this activity has been shown to be important for both tumor growth and invasion. We have recently shown that MT1-MMP is phosphorylated on its unique cytoplasmic tyrosine 573, and that this phosphorylation is necessary for in vitro tumor cell migration. In this study, we investigate the mechanisms involved in phosphoMT1-MMP- mediated tumor cell proliferation and invasion.

Fibrosarcoma cells (HT-1080) were stably transfected with the wild type or the non-phosphorylable (Y573F mutant) forms of MT1-MMP. Cell proliferation and invasion studies were performed on 2D- or within a 3D-type 1 collagen matrix. MT1-MMP expression was determined by reverse transcription PCR, western blot or zymography. Cell cycle was analysed by flow cytometry.

Flow cytometry analysis of the stable transfecants showed that MT1-MMP was expressed at the cell surface of both the Y573F mutant- or the wild type- expressing HT-1080 cells, the two forms of the enzyme being catalytically active, as monitored by zymography. Although both the wild type- and the Y573F-expressing HT-1080 cells had the same proliferation rate on 2D-type 1 collagen matrix, we observed that HT-1080 cells stably expressing the Y573F mutant form of MT1-MMP grew less within a 3D-type 1 collagen matrix than the cells expressing the wild type form of MT1-MMP. Similar results were observed when cells were allowed to invade a 3D-type 1 collagen matrix. Such an inhibition of both cell growth and invasion in 3D-type 1 collagen was correlated with alteration of the cell cycle. Indeed, flow cytometry analysis show that most of the Y573F mutant expressing HT-1080 cells were arrested in G0/G1 phase cycle, while cells expressing the wild type form of MT1-MMP were predominantly in the G2/M phase. These results thus suggest that phosphorylation of MT1-MMP may play an important role in tumour cell proliferation and invasion through cell cycle regulation.

Given that pharmacological inhibition of MMP catalytic activities has been shown to induce several undesirable side effects, these findings suggest that the inhibition of MT1-MMP tyrosine phosphorylation may represent an unexpected alternative strategy for the development of drugs aimed at the inhibition of tumor invasion and metastasis.

New Molecular Targets

B202 Na+/K+-ATPase as a potential target to combat melanoma. Véronique Mathieu1, Christine Pirker1, Mathieu Vernier2, Tatjana Mijatovic2, Walter Berger2, Robert Kiss1. 1Laboratory of Toxicology, Institute of Pharmacy, Free University of Brussels (ULB), Brussels, Belgium; 2Department of Medicine I, Institute of Cancer Research, Medical University Vienna, Vienna, Austria; 3Unibioscreen SA, Brussels, Belgium.

The sodium pump, Na+/K+-ATPase, could be an important target for the development of anti-cancer drugs as it serves as a versatile signal transducer, it is a key player in cell adhesion and its aberrant expression and activity are implicated in the development and progression of different cancers (Mijatovic et al., BBA Rev Cancer 2007). The sodium pump is composed of two subunits in equimolar ratios: the catalytic α (four known isoforms) and the regulatory β (three known isoforms) which have tissue-specific expression patterns. Cardiotoxic steroids, known ligands of the sodium pump have been widely used for the treatment of heart failure. Their development to date as anti-cancer agents has however been impaired by a narrow therapeutic margin resulting from their potential to induce cardiovascular side-effects. Chemical modification of 2'-oxovorusscharin (a novel cardenolide extracted from Calotropis procera) based on an understanding of the structure activity relationship within the series, has led to the identification of UNBS1450, a molecule characterized by more potent anti-proliferative activity and lower toxicity than classic cardenolides (Van Quaquebeke et al., J Med Chem 2005).

Quantitative RT-PCR was used to determine Na+/K+-ATPase α1, α2 and α3 mRNA levels in primary melanomas and melanoma cell lines. Using immunofluorescence, computer-assisted video-microscopy for cellular imaging, the MTT assay for global growth assessment and western blotting analyses, cardenolide-mediated targeting of over-expressed α1 subunits in melanomas has been assessed.

Investigation of 10 human primary melanomas and two melanoma cell lines revealed the marked over-expression of α1 subunit mRNA in all tested samples. In contrast, only two of these melanoma cell lines revealed α2 subunit mRNA albeit at moderate levels, while no cell line expressed α3. Video-microscopy based cellular imaging revealed that deleting Na+/K+-ATPase α1 subunit expression in melanoma cells by means of an anti-α1 siRNA, markedly impaired both cell proliferation and cell invasion experiments breast cancer cells were treated with trastuzumab (10 µg/mL) mono or trastuzumab (10µg/ml) plus atrasentan (0.1-1.10 µM) for 48 hours. Cell proliferation was measured using the BrDU-incorporation assay. Control cells were treated with the appropriate vehicle for each experiment.

Results: In BT-474 breast cancer cells basal ET-1 mRNA expression was slightly decreased by trastuzumab (10 µg/mL) and 8-fold increased by EGF (100 ng/mL) (p ≤ 0.001). Moreover, EGF increased ET-1 secretion of BT-474 cells with a maximum value of 130 % (p ≤ 0.05) after 8 hours of incubation. Basal and EGF-induced ET-1 secretion was markedly reduced by trastuzumab (p ≤ 0.001). Combined treatment with trastuzumab (10 µg/mL) and atrasentan (10 µM) lead to a further decrease in proliferation in HER2-overexpressing breast cancer cell lines BT-474 and SK-BR-3 (p ≤ 0.05) compared to trastuzumab (10 µg/mL mono) proliferation. MCF-7 was not affected by any treatment.

Conclusions: Our data are the first to suggest an interaction between HER2 and ET pathways. Moreover we have shown an additive inhibition of breast cancer cell proliferation by trastuzumab and atrasentan. Since both HER2 and ETαR are molecular targets known to promote tumorigenesis and tumor progression, combined inhibition of HER2 and ETαR as multi-targeted therapy may represent a highly effective and well tolerated approach in the treatment of HER2-overexpressing breast carcinomas.
migration. Unlike dioxin, UNBS1450 markedly impaired both cell proliferation and cell migration, resulting in similar morphological features as obtained by siRNA transfection. Additionally, UNBS1450 also induced marked disorganization of the actin cytoskeleton leading to non-apoptotic cell death which again was not evident with dioxin. Together, these data suggest that targeting the Na⁺/K⁺-ATPase α1 subunit by using specific cardenolides could represent a novel manner to combat melanomas. We are currently involved in the immunohistochemical determination of sodium pump α1 subunit expression in a large clinical population of human melanoma samples. It should thus be possible to determine the proportion of melanoma patients who could benefit from cardenolide-related treatments. Indeed, melanoma patients whose tumors over-express the α1 subunit far above normal levels could be candidates for such a treatment.

B203 Inhibitory GABA signaling as a novel target for the prevention and therapy of pulmonary and pancreatic adenocarcinoma. Hildegard M. Schuller1, Hussein A. N. Al-Wadei1, Mourad Majidi1. 1University of Tennessee, Knoxville, TN.

Pulmonary adenocarcinoma (PAC) and pancreatic ductal adenocarcinoma (PDAC) are among the most common human malignancies and are highly resistant to conventional cancer therapy. We have shown that both cancers respond to beta-adrenergic stimulation by cAMP-dependent signaling via PKA/CREB and PKA-dependent transactivation of the EGFR, resulting in stimulation of cell proliferation and migration and that the tobacco carcinogen NNK activates these signaling cascades. Our current data show that the neurotransmitter gamma-aminobutyric acid (GABA) completely abrogates this cAMP-dependent proliferation and migration of PAC and PDAC cells and their normal cells of origin (small airway epithelial cells and pancreatic duct epithelial cells). These effects of GABA were mediated by the G protein-coupled GABA-B receptor as evidenced by GABA-B knockdown in Western blots and luciferase reporter assays for the identification of activated CREB and ERK1/2 and also reduced base level proliferation of the unstimulated cancer cells significantly. GABA was underexpressed in tissue microarrays from human PACs and PDACs while beta-adrenergic signaling was overexpressed. Our findings suggest that an imbalance in stimulatory beta-adrenergic and inhibitory GABA-B signaling contributes to the aggressive behaviour of a significant subset of PACs and PDACs. Restoration of this disturbed balance by treatment with GABA or GABA-B agonists appears to offer a promising new target for the prevention and adjuvant therapy of PAC and PDAC.

B204 Thioredoxin-targeted experimental antitumor Quinols exert cytotoxicity following activation of apoptosis signal regulating kinase 1. Charles S. Matthews1, Tracey D. Bradshaw1, Thilo Hagen1, Eng-Hui Chew1, Malcolm FG Stevens1. 1University of Nottingham, Nottingham, United Kingdom.

PMX 464 and PMX 290 are novel 4-hydroxycyclohexadienone “quinols”, that exert potent antitumor activity againstcolin, renal, melanoma and breast cancer models in vitro and in vivo [1,2]. PMX 464 binds reduced cysteine (thiol) residues within the thioredoxin (Trx1) active site - C-S-G-C-X2 and inhibits Trx reductase/Trx1 signaling in a dose-dependent manner (IC50 < 3 μM; [3]). Trx1 is a 12 kDa redox protein upregulated in certain tumors, especially within hypoxic regions. It is a cytoprotective antioxidant whose functions also include regulation of transcription factor activity (e.g. HIF-1α, AP-1) and inhibition of apoptosis by prohibiting complexing apoptosis signal regulating kinase-1 (ASK-1). Downstream consequences of Trx1 inhibition are being investigated. Inhibition of HIF-1 DNA binding, HIF-1-dependent gene transcription, and CA9 and VEGF protein expression have been reported [4]. Herein, we describe consequences of Trx1 inhibition on signaling specifically leading to tumor cell death.

Immunoprecipitation and Western Blots analyses revealed dose-dependent dissociation of ASK-1 from Trx in quinol or H2O2 treated HCT 116 (colon carcinoma) or MCF7 (breast carcinoma) cells. Phospho specific antibodies revealed dose-dependent and sustained activation of mitogen activated kinases (MAPK) downstream of ASK-1, c-Jun N-terminal kinase (JNK) and p38 MAPK. FACS analyses following Annexin V binding demonstrated emergence of apoptotic populations ≥ 12 h exposure PMX 464 (1 μM) and PMX 290 (500 nM). Apoptosis was corroborated by detection of caspase 3- and PARP cleavage. Thus, inhibition of reduced Trx1 may initiate apoptosis signaling via release of ASK-1 leading to phosphorylation of JNK and p38. In addition, in HCT 116 cells we were able to detect transient (3 - 7 h) generation of reactive oxygen species (ROS) following quinol treatment. The inhibition of free Trx1 by quinols may lead to accumulation of unchallenged intracellular ROS, which are able to oxidise Trx already bound to ASK-1, resulting in its release and activation.


B205 C-reactive protein protects tumors cells from apoptosis: Implicating a potential target for cancer therapy. Jing Yang1, Michele Wezeman1, Xiang Zhang1, Pei Lin1, Larry W Kwak1, Qing Yi1. 1UT M. D. Anderson Cancer Ctr., Houston, TX.

Human C-reactive protein (CRP) is an acute-phase protein, and elevated levels of CRP are present in patients with infections, inflammatory diseases, necrosis such as myocardial infarction, or malignancies including multiple myeloma (MM), lymphoma, and carcinoma. CRP is able to bind a variety of ligands and receptors, activating the classical complement pathway. Accumulating evidence strongly suggests that in cardiovascular disease CRP is not only a marker of inflammation, but also contributes to pathogenesis of the disease. These findings led to our hypothesis that CRP may have a functional effect on tumor cells. The present study was undertaken to determine whether CRP might affect tumor cell growth and survival. When added to the culture of primary myeloma cells isolated from patients, CRP promoted cell proliferation and reduced cell apoptosis in a dose-dependent manner. To confirm this result, we examined the effects of CRP on myeloma cell lines under stressed conditions and showed that CRP protected myeloma cells from apoptosis induced by serum starvation or IL-6 deprivation. More importantly, CRP also protected myeloma cells from apoptosis induced by dexamethasone or melphalan, two common chemotherapy drugs for MM. The protection was significant since CRP reduced cell death by 50 to 60%, and may be clinically relevant because the results were reproduced in established myeloma-SCID or SCID-hu mouse models. Injection of CRP prior to treatment of myeloma (including cell lines and primary tumor cells, isolated from MM patients) bearing mice with dexamethasone or melphalan significantly undermined the therapeutic effects of these chemotherapy drugs. Mice receiving CRP with dexamethasone or melphalan had significantly larger tumor burdens compared with mice treated with dexamethasone or melphalan alone, whereas treatment with CRP alone had no effects on the tumor cells. CRP protected tumor cells from apoptosis by downregulating Bax expression, inhibiting phosphorylation of Bcl-2, and upregulating phosphorylation of Bad, which led to inhibited caspase-9, caspase-3 and PARP activation induced by dexamethasone. We next examined cell surface receptors for CRP and found that CRP bound FcRIIIA CD32A and/or CD32C, but not CD32B. Specific siRNAs that inhibited CD32A or CD32C but not CD32B expression, led to inhibited caspase-9, caspase-3 and PARP activation induced by dexamethasone. Antibodies against CD32A and CD32C that blocked receptor-ligand interaction abrogated CRP-mediated protection of cell apoptosis. These results indicate that CRP mediated its effects via activating immunoreceptor tyrosine-based activation motif (ITAM)-containing FcγR.II.

By binding to these receptors, CRP increased the level of phosphorylated Akt, ERK1/2, and IκBα; relocalized NFκB p65 to the nucleus; and inhibited p38 kinase activity. Inhibitors against these signaling molecules blocked the activity of these pathways and abrogated CRP-mediated protection of myeloma cell apoptosis induced by dexamethasone.
Therefore, our results provide strong evidence for a novel effect of CRP on myeloma cells. This study also implicates CRP as a potential therapeutic target for MM or other CRP-related tumors.

**B206 ΔNp63 expression associates with poor survival in ovarian cancer.** Sergio Marchini¹, Mirko Marabese¹, Eleonora Marrazzò¹, Pietro Marianí¹, Dario Cattaneo¹, Roldano Fossati¹, Anna Compagnoni¹, Robert fruscio¹, Andrea Albert Lirosso², Massimo Broggiini¹. ¹Mario Negri Institute for Pharmacological Research, Milan, Italy; ²Ospedale San Gerardo, Universita’ di Milano Bicocca Monza, Italy, Milan, Italy.

P63 belongs to the "p53 family" whose role in cancer progression has been recently revisited in light of the plethora of splicing variants that are generated. The hypothesis is that the TA isoforms share "p53 like" properties while the ΔN isoforms act in a dominant negative fashion. The aim of this study was to measure the levels of TaP63 and its truncated variant Np63 in a cohort of 169 ovarian cancer patients with different grade and stage to unravel the hypothesis that different levels of TaP63 and Np63 can be associated with survival. We examined by real time RT-PCR the expression pattern of both TaP63 and Np63 in 83 stage I ovarian cancer patients, characterised by a good survival and in 86 stage III ovarian cancer patients characterised by poor survival. Data suggest that the TaP63 levels between stage I and stage III were comparable, while in stage III we observed an increase in the levels of Np63 of 77 folds. This is independent of the mutational status of the p53 gene but is strongly associated with poor overall survival. In conclusion data reported in this study, the largest to author’s knowledge in ovarian cancer, strongly suggest to go further in investigating the role of P63 and its variants in tumor progression of ovarian cancer as a potential biomarker to predict patients outcome and in perspective therapy response. This would have particularly clinical relevance in ovarian cancer where the high rate of mortality reflects our lack of knowledge of molecular mechanisms underlying cell progression towards malignancy.

**B207 Expression levels of different p53 and p73 isoforms in ovarian cancer.** Mirko Marabese¹, Sergio Marchini¹, Eleonora Marrazzò¹, Pietro Marianí¹, Dario Cattaneo¹, Roldano Fossati¹, Anna Compagnoni¹, Mauro Signorelli¹, Ute M Moll², Massimo Broggiini¹. ¹Mario Negri Institute for Pharmacological Research, Milan, Italy; ²State University of New York, New York, NY.

Epithelial ovarian cancer (EOC) is one of the worst leading cause of death among gynaecological cancer in western countries. Data collected in the last years failed to identify biomarkers which could be used in early disease detection and disease progression after therapy. Tp53 gene has long been investigated for its role in cancer progression in different solid and haematological cancer as well as in EOC but data collected till now have been contradictory. The emerging evidence that p53 belongs to a family of transcription factors with p63 and p73, characterised by a plethora of isoforms transcribed from a single locus gene, re-opened the interest on this family gene.

In the present paper we investigated by real time RT-PCR the expression levels of TaP53, TaP73 and their relative different splicing variants in a cohort of 169 ovarian cancer patients with different grade and stage. Data obtained revealed that TaP73 levels are almost overlapping between stage I and stage III, with a slight decreased of expression in stage III patients harbouring mutated p53. We observed a loss of expression for DNP73 in stage III. No substantial differences were observed for the p53 and its two main isoforms D40 and D133. Kaplan Meyer analysis did not suggest a correlation between overall survival and changes in DNP73/TaP73 ratio. In conclusion data presented herein suggest that at least in our patient subset p53 and p73 both in the wild type or in any of their splicing variant are not correlated to malignant progression.

**B208 Characterization of a new tumor-specific variant of the heat shock protein GRP78 as target for antibody therapy.** H Peter Vollmers¹, Nicole Rauschert¹, Leo Rasche¹, Stephanie Brändlein¹. ¹University of Würzburg, Würzburg, Germany.

Heat-shock proteins (HSPs) are critical components of a cell’s defense mechanism against injury associated with adverse stresses. Although HSPs are very beneficial to the normal cell, cancer cells often over-express HSPs on the membrane and use them in response to stresses associated with various therapies (hyperthermia, chemotherapy, radiation), diminishing the treatment effects. The fully human monoclonal antibody SAM-6 is a germ-line coded IgM, isolated from a gastric cancer patient by TRIOMA technology. SAM-6 induces an excess of intracellular lipids by overfeeding malignant cells with oxidized LDL. The treated cells over-accumulate depositions of cholesterol-esters and triglycerides and undergo apoptosis. Here we show that the SAM-6 antibody binds to a tumor-specific O-linked carbohydrate moiety expressed on a membrane-bound variant of GRP78, which is a member of the HSP70 family. These data show that cancer-specific modifications of cell surface protection molecules are ideal targets for immuno-therapeutical approaches.

**B209 Intramembrane proteolysis of the p75 neurotrophin receptor is required for glioma invasion.** Limei Wang¹, University of Calgary and Tom Baker Cancer Center, Calgary, Alberta, Canada.

The p75 neurotrophin receptor is a multifunctional signaling protein that interacts with numerous ligands and coreceptors to regulate cellular survival, neurite outgrowth, myelin formation and apoptosis in others. It has recently been shown that p75NTR is a central regulator of glioma invasion which provide a very important evidence for p75NTR as a major contributor to the highly invasive nature of malignant gliomas. The mechanism by which the receptor elicits the invasive effect is poorly understood. It has been shown that the full length p75NTR protein undergoes ectodomain shedding followed by regulated intramembrane proteolysis (RIP). An α-secretase-like activity cleaves p75NTR in the juxtanuclear stalk region to generate a membrane bound 24 kDa C-terminal fragment (CTF). The CTF is then processed by a presenilin-dependent γ-secretase activity to give rise to a soluble 19 kDa product (ICD). The ICD is then targeted for degradation by the proteasome. We assessed if proteolytic processing of p75 is required for glioma invasion. We found that in glioma cells, p75NTR is cleaved to generate both the 24 kDa fragment CTF and a 19 kDa ICD. Treatment of U87p75 cells with compound X, a specific inhibitor of γ-secretase, resulted in the accumulation of the 24 kDa CTF fragment without subsequent cleavage to ICD and inhibition of migration and invasion in vitro. To directly test the role of p75NTR processing in glioma invasion, we constructed chimeric proteins by replacing either the transmembrane or the extracellular stalk domain of p75NTR with equivalent domains from the Fas receptor. Both p75NTR and Fas are members of the TNF receptor superfamily, and although they each contain similar domains, unlike p75NTR, Fas does not undergo RIP. We found that expression of cleavage resistant forms of p75NTR which prevent receptor proteolysis, blocked glioma invasion in vitro and in vivo. These results indicate the p75NTR-mediated glioma invasion requires γ-secretase dependent release of its ICD. The exact mechanism by which the ICD exerts its role is still to be determined. This work highlights the possibility that γ-secretase inhibitors may have clinical application in gloma treatment.

**B210 BIIB022, a fully human nonglycosylated γ4P antibody targeting IGF-1R for cancer therapy.** Kandasamy Haritharan¹, Jianying Dong¹, Stephen Demarest¹, Ingrid Joseph¹, Peter Chu¹, Christlyn Graff², Scott Glaser¹, Kimberly Kramer-Stickland¹, Robert Peach¹, Mitchell Reff². ¹Biogen Idec, San Diego, CA.

Antibodies targeting human type I insulin-like growth factor receptor (IGF-1R) as inhibitors of tumor survival pathway have shown evidence of objective responses in early clinical trials. BIIB022 is a fully human antibody specific to human IGF-1R being developed for the treatment of patients with solid tumors. The Fab of BIIB022 identified from a human
antibody phage display library has a binding affinity of 1.3 nM to human IGF-1R and does not bind to the closely related insulin receptor. BIIB022 was constructed as a nonglycosylated version of IgG4.P antibody both devoid of Fc-effector function to eliminate potential Fc mediated toxicity to the normal vital organs that express IGF-1R and with a gamma 1 like hinge region. BIIB022 binds to the N-terminal region of the fibronectin domain (Frnll-1) of IGF-1R and allosterically inhibits IGF-1R interaction with its physiological ligands, IGF-1 and IGF-2. In functional assays, BIIB022 efficiently inhibited both IGF-1 and IGF-2 ligand-mediated signaling through IGF-1R in tumor cells, resulting in inhibition of phosphorylation of IGF-1R, AKT and ERK at nanomolar concentrations. The ability of BIIB022 to inhibit AKT survival pathway was further confirmed in a panel of cell lines of different tumor origins where inhibition was observed in greater than 60% of the cell lines. In a subset of cell lines, BIIB022 also inhibited in vitro tumor cell growth in the presence of serum where the growth sensitivity to BIIB022 correlated with its ability to disrupt the interaction of insulin receptor substrate (IRS-1) with p85 regulatory subunit of phosphoinoside 3-kinase. In addition, BIIB022 efficiently down-regulated IGF-1R expression in tumor cells. BIIB022 demonstrated single-agent anti-tumor activity in human pancreatic, lung and breast xenograft models at doses ranging from 3.75 to 60 mg/kg on a once weekly schedule. In a pre-clinical 4-week toxicity study, BIIB022 was well tolerated in cynomologus macaque at doses up to 50 mg/kg on a weekly schedule. No treatment related microscopic findings or effects on serum glucose levels were observed. The half-life of BIIB022 in cynomologus macaque was estimated to be 7-10 days, therefore potentially allowing for once every 2 to 3 weeks dosing in human. Overall, BIIB022 exhibits favorable physical and functional properties, demonstrates anti-tumor activity in pre-clinical tumor models and thus warrants evaluation in IGF-1R pathway sensitive tumors in the clinic.

B211 Targeting Mig-7 inhibits carcinoma cell invasion, primary tumor growth, and stimulates killing of breast carcinoma cells. Aaron P. Petty1, Stephen E. Wright2, Kathleen A. Reeves-Felkin2, Michelle A. Yenderrozos1, J. Suzanne Lindsey1, 1Washington State University, Pullman, WA; 2Texas Tech University Health Sciences Center, Amarillo, TX.

Novel Migration induction gene-7 (Mig-7) is expressed by cancer cells of primary and metastatic tumor sites, those circulating in blood, as well as by occult tumor cells that mimic endothelial cells. Multiple tumor microenvironment growth factors induce Mig-7 expression. Gain or loss of Mig-7 functional studies show that its expression is required for aggressive tumor cell behaviors. Because normal adult cells apparently lack Mig-7 expression, we hypothesized that Mig-7 is a target to specifically inhibit the spread of cancer while preventing damage to normal cells. We first tested this hypothesis using an in vitro modified Boyden chamber chemo-invasion assay of tumor cells treated with Mig-7 or control antibodies. Our results show that Mig-7 antibody caused a >60% reduction in tumor cell invasion. We further tested our hypothesis using an in vitro analysis of peptide-stimulated human peripheral blood monocyte cells (MC) and their killing of MCF-7 breast carcinoma cells. Mig-7 peptide treatment increased MC killing of MCF-7 cells >2-fold over control peptide treatments. Furthermore, our in vivo studies show that tumor cells stably expressing Mig-7-specific short interfering RNA (siRNA) resulted in a ~70% reduction in primary tumor size in a xenograft nude mouse model. Based on these collective data, we conclude that Mig-7 is a potential candidate for targeted cancer therapy.

B212 Phase I, pharmacokinetic (PK), and pharmacodynamic (PD) study of 17-dimethylamoethylenamino-17-demethoxygeldanamycin, (17DMAG, NSC 707545), an inhibitor of heat shock protein 90 (HSP90), in patients with advanced solid tumors: final results. Ramesh K. Ramanathan1, Merrill J. Ebinger2, Charles Erlichman3, Scot C. Remick4, Suresh Ramalingam5, 2Percy Lvy6,1, Cynthia Ten Eyck7, 1, Julienne L Holleran2, Cindy L. Nare1, Chandra P. Belani1, 1Gen, Scottsdale, AZ; 2University of Pittsburgh Cancer Institute, Pittsburgh, PA; 3Mayo Clinic, Rochester, MN; 4Ireland Cancer Center, Case Western Reserve University, Cleveland, OH; 5Cancer Therapy and Evaluation Program, NCI, Bethesda, MD.

Background: 17DMAG is a water-soluble and potent geldanamycin analog, which inhibits HSP90. The objectives of this first-in-human study were to: establish the dose-limiting toxicity (DLT); recommend a phase 2 dose; & characterize the PK & PD of 17DMAG.

Methods: Patients (pts) were accrued to a modified accelerated scheme. 17DMAG was given IV over 1 h daily x 5 (schedule A) or daily x 3 (schedule B) every 3 weeks. Plasma 17DMAG concentrations during cycle 1 were quantitated by LC/MS assay. HSP27, HSP70, and 4 client proteins CDK4, RAF-1, AKT, and ILK were assessed by western blot at baseline & 24 h on d 1. Pre- & post-treatment (at 24 h) biopsies were done in selected patients at the phase 2 dose (n=7).

Results: 56 pts were entered. Sequential cohorts of patients on schedule A received 1.5, 3, 6, 9, 12, or 22 mg/m² (n = 26). On schedule B, the starting dose was 2.5 mg/m². Based on safety information from schedule A, subsequent schedule B dose levels were 14, 19, 25, 34 & 46 mg/m² (n = 30). Dose-limiting toxicities on both schedules were pneumonitis, transaminitis, thrombocytopoenia and fatigue. Selected grade ≥ 3 toxicities are liver function test elevation (14%), pneumonitis (9%), diarrhea (4%), nausea (4%), fatigue (4%) & platelets (4%). No objective responses were noted, stable disease was seen in 4 pts. Day 1, 17DMAG PK were linear over 1.5-46 mg/m² and Cmax increased linearly with dose (0.071-1.7 mg/ml). The AUC increased linearly with dose (0.7-14.7 mg/ml•h). Both clearance and T1/2 did not vary systematically with dose (29 ± 40 ml/min/m² 2 and 24 ± 15 h). The 24-h urinary excretion accounted for 20 ± 9% of dose. The mean HSP70 and HSP27 were 114% (range 71-229) of baseline respectively in PBMCs at 24 h at 12, 16, & 25 mg/m². HSP70, HSP27, AKT and CDK4 levels were decreased in the majority of post treatment biopsy samples, but did not correlate to changes in corresponding PBMC samples.

Conclusions: The recommended phase 2 of 17DMAG is 16 mg/m² x 5 days or 25 mg/m² x 3 days every 3 weeks. Therapy was well tolerated at the phase 2 doses. Reversible pneumonitis was a new DLT and not predicted by animal toxicity. Pneumonitis and transaminitis appear to be schedule-dependent. Cardiac toxicity was not seen. 17DMAG plasma PK are linear over the doses delivered to date, and there is a suggestion of a target effect as manifested by HSP and client protein changes in PBMCs and paired tumor biopsies.

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B213 IAP antagonists kill tumor cells by targeting cIAP1, resulting in NF-κB dependent induction of TNFRc and TNF receptor mediated apoptosis. Wendy W Wong1, James Vince1, Nufail Khan1, Afsar U Ahmed1, Diep Chau1, Rebecca Feltham1, Christopher Benetatos2, Srinivas Chunduru3, Stephen Condon4, Mark McKinlay5, Martin Leverkus2, Vinay Tergaonkar6, Pascal Schneider7, Bernard A Callus1, 1Frank Koentgen1, 1David L Vaux1, 1John Silke1, 1LaTrobe University, Melbourne, Australia; 2TetraLogic Pharmaceuticals, Malvern, PA; 3Otto-von-Guericke University, Magdeburg, Germany; 4IMCB, Biopolis Drive, Singapore; 5University of Lausanne, Epalines, Switzerland; 6Ozgene, Bentley DC, Australia.

IAP antagonist ‘Smac-mimetic’ compounds (IAC) were designed to inhibit the caspase inhibitor XIAP to kill tumor cells. Because XIAP inhibits postmitochondrial caspases, caspase 8 inhibitors should not block killing by IACs. We show however that apoptosis caused by IAP antagonists is blocked by the caspase 8 inhibitor crmA, and that they activate NF-κB signaling from TNF-R1 via removal of cIAP1. In sensitive tumor lines, IAP antagonists also stimulated production of TNFRc that killed them in an autocrine fashion. Inhibition of NF-κB reduced TNFRc production, and blocking NF-κB activation or TNFRc allowed tumor cells to survive IACs. Cells treated with an IAC, or those in which cIAP1 was deleted, became sensitive to apoptosis induced by exogenous TNF .
Expression of G-protein-coupled receptor kinase 4 is associated with breast cancer tumorigenesis. 
Jun Matsubayashi1, Masakatsu Takanashi1, Kosuke Okawa1, Mingli Xu1, Kiyoushi Mukai1, Masahiko Kuroda1. 1Tokyo Medical University, Tokyo, Japan.

G-protein-coupled receptor kinases (GRKs) comprise a family of seven mammalian serine/threonine protein kinases that phosphorylate and regulate agonist-bound, activated, G-protein-coupled receptors (GPCRs). GRKs and arrestins are key participants in the canonical pathways leading to phosphorylation-dependent GPCR desensitization, endocytosis, intracellular trafficking and re-sensitization. Here we showed that GRK4 protein is highly expressed in human breast cancer tissues but not in normal epithelium. GRK4 expressions were associated with lymph node metastasis and NIH3T3 cells overexpressing GRK4 showed transforming activity. In addition, GRK4 is associated with β-arrestin and mediates mitogen-activated protein (MAP) kinase signaling pathways in breast cancer cells. To our knowledge, this is the first report of GRK4 implicated in tumorigenesis. Furthermore, repression of GRK4 expression by RNA interference (RNAi) repressed cell growth in various breast cancer cell lines, suggesting that GRK4 may be a novel target for breast cancers.

Anticancer activity of a human monoclonal antibody inhibitor of MMP-14. 
Laetitia Devy1, Laurent Naa1, Reinoud Van Goor1, Douglas Rank2, Csaba Pazmany2, Simone Nicholson2, Chris Tenhoor2, Shafat Rabbani2, Paula Hendriksen2, Daniel Dransfield2,1Dyax s.a., Liege, Belgium; 2Dyax corp, Cambridge, MA; McGill University Health Centre, Montreal, Quebec, Canada; 3Dyax, s.a., Liege, Belgium.

Over the last 30 decades, there has been a marked improvement in certain cancer remission and survival rates, yet a significant unmet medical need exists by way of treating and prolonging the lives of patients with advanced disease and difficult to treat cancers. Therapies that operate on alternative mechanisms offer a new means to not only slow or halt disease progression but also extend survival. Selective inhibition of the activity of matrix metalloproteinases (MMPs) could provide an attractive nontoxic approach towards improving the therapy of cancers. Antibodies provide the potential for a new generation of protease inhibitors with high levels of potency and selectivity. Combining our human antibody phage display library with automated selection and screening strategies, we have isolated DX-2400, a potent and selective inhibitor of MMP-14. DX-2400 binds to MMP-14 expressing cells and potently inhibits human, mouse and rat MMP-14 activity (Ki<1nM). DX-2400 inhibits invasion and blocks proMMP-2 activation of MMP-14/MMP-2 expressing cancer cells. DX-2400 also potently inhibits HUVEC tube formation in vitro. Immunohistochemical analysis on primary human breast tumor tissues reveals an overall MMP-14 detection rate with DX-2400 of 54%. DX-2400 retards tumor progression and metastasis in multiple subcutaneous and orthotopic tumor models in nude mice. Chronic administration of DX-2400 significantly decreases total MMP activity extracted from tumor tissue confirming the proximal role of MMP-14 in activating the cascade of metalloproteinase activity leading to tumor progression. Immunohistochemical analysis of experimental tumor tissue sections reveals a significant decrease in Ki67-positive tumor cells and CD31-positive tumor microvessel hot spots in the tumors from DX-2400-treated animals, demonstrating both anti-tumoral and anti-angiogenic activity of this novel inhibitor. This fully human antibody represents an innovative approach for the inhibition of MMP-14 activity and is a candidate for therapeutic development.

B216 Validating Aurora B as a potential therapeutic target for human hepatocellular carcinoma. 
Zhong-Zhe Lin1, Yung-Ming Jeng1, Hey-Chi Hsu1, Ann-Lii Cheng1, 1National Taiwan University Hospital, Taipei, Taiwan.

Background: We previously demonstrated that Aurora A was overexpressed in 61% of hepatocellular carcinoma (HCC), and that the overexpression correlated well with higher histology grade and more advanced stage of the tumors (Clin Cancer Res 2004;10:2065). We also demonstrated that a novel pan-Aurora kinase inhibitor inhibited both in vitro and in vivo tumor growth of HCC (Proc Am Assoc Cancer Res 2006;LB-279). However, several studies have indicated that Aurora A and Aurora B may be distinct therapeutic targets. In this study, we sought to analyze the significance of Aurora B overexpression in HCC, as well as the therapeutic potential of AZD1152, a novel and selective Aurora B kinase inhibitor, for the treatment of HCC.

Method: Aurora B mRNA levels were measured in 190 HCC and paired non-tumorous liver tissues by reverse transcription-PCR. The Aurora B mRNA levels were determined by the ratio of signal intensity of Aurora B to that of S26 measured by 1D Image Analysis software (Kodak Digital Science, Rochester, NY) and categorized as overexpression if the ratio > 1.0. Aurora B protein levels were measured by Western blot in 8 HCC cell lines. Antitumor effect of AZD1152, a highly selective inhibitor of Aurora B kinase, was tested in four liver cancer cell lines (Huh-7, HepG2, Hep3B PLC/PRF/5). Cell viability was measured by trypan blue exclusion assay. Aurora A and Aurora B signaling were assessed by immunoblotting with antibodies against Aurora A, Aurora B, phospho-histone H3 (Ser10), and phospho-Aurora A (T288). Mitotic perturbation was visualized by immunofluorescence staining and confocal microscopy. Cell cycle profiles and apoptosis were assessed by flow cytometry.

Results: Overexpression of Aurora B was found in 112 of 190 (59%) primary HCC and all 8 HCC cell lines. Overexpression of Aurora B was associated with higher grade (grade II-IV, P < 0.00001), and more advanced stage (stage III-IV, P = 0.0035) of the tumors, as well as high α-fetoprotein levels (> 200 ng/mL, P = 0.006) and early tumor recurrence (< 1 year, P = 0.0005). AZD1152 induced a dose-dependent growth inhibition in Huh-7, HepG2, Hep3B, and PLC/PRF/S cells with IC50 ranging from 5 to 25 nM. Phosphorylation of histone H3 Ser10 was blocked by AZD1152 in a dose- and time-dependent manner. Huh-7 and HepG2 cells exposed to AZD1152 failed to align their chromosomes at the metaphase plate, a finding consistent with loss of Aurora B kinase activity. Further, AZD1152 treatment resulted in accumulation of cells with 4N/8N DNA content and subsequent apoptosis.

Conclusions: Overexpression of Aurora B is frequently found in HCC, and the inhibitor of Aurora B may be a new class of molecular-targeted agents for the therapeutics of HCC. (Zhong-Zhe Lin and Yung-Ming Jeng contributed equally to this work.)

Expression and functional analysis of GPR87, an orphan G protein-coupled receptor overexpressed in non small cell lung cancer. 
Jeff Winston1, Jeff Reagan2, Sheila Scully1, J.C. Xu3, Dan Freeman1, Terri Burgess1, 1Amgen, Thousand Oaks, CA; 2Amgen, San Francisco, CA; 3Amgen, Seattle, CA.

G protein-coupled receptors (GPCRs) function as transducers systems, linking extracellular signals from a variety of ligands to intracellular second messenger pathways. While a role for this receptor super-family in the regulation of cell metabolism has been well established, it has more recently been recognized that GPCRs act as important mediators of several pathways relevant to tumorigenesis, including promotion of cell survival, stimulation of proliferation, and induction of angiogenesis. Previously, it has been reported that a purinergic-like receptor, GPR87 (also known as GPR95), is regulated by the tumor suppressor p53 in an in vitro model of testicular germ cell cancer. However, the function and expression profile of this orphan receptor in vivo are, at present, poorly understood.

We have observed that GPR87 mRNA is overexpressed with high frequency and magnitude in primary human lung tumors. Receptor expression in lung cancer is strictly associated with tumor epithelium, and is not observed in connective tissue, vascular elements, or infiltrating lymphocytes. Overexpression is almost exclusively restricted to adenocarcinomas of the non-small cell subtype of lung cancers. In contrast, receptor expression is low to undetectable in normal lung and most other tissues examined. GPR87 transfected into HEK293 cells is constitutively active, and co-transfection experiments with chimeric G protein strongly suggest that the receptor couples to the Gi signaling pathway. These findings may facilitate characterization of the functional role for GPR87 during lung cancer progression.
**B218** ZM 447439, a novel promising aurora kinase inhibitor, provoking antiproliferative and pro-apoptotic effects alone and in combination with bio- and chemotherapeutic agents in gastroenteropancreatic neuroendocrine tumor disease. Inna Georgieva1, Daniel Kychev1, Yawen Wang1, Judith Holstein1, Martin Zeitz2, Patricia Grabowsk11, Chrité, Berlin, Germany.

Nowadays therapeutic possibilities of gastroenteropancreatic neuroendocrine tumors (GEP-NETs) are still not satisfactory. A new light in treatment options of GEP-NET cancer diseases could be the novel aurora kinase inhibitor ZM447439 (ZM) which was previously reported to interfere with the mitotic spindle integrity checkpoint and chromosome segregation, but does not interfere with other kinases when used up to 5µM.

We evaluated the antineoplastic effects of ZM on growth and apoptosis of the GEP-NET cell lines BON, GPG-1 and MIP-101, representing the different malignancy types of tumors. We demonstrate that ZM inhibited dose-dependently proliferation of all three cell lines with IC50 values in the nM to µM range. Moreover, aurora kinase inhibition by ZM potently induced apoptosis, which was accompanied by DNA-fragmentation and Caspase 3/7 activation in all three cell lines. Furthermore, we observed cell cycle arrest at G0/G1 as well as a block in G2/M transition at ZM concentrations of 0.5-5 µM. In addition, combined treatment with the synthetic somatostatin analogue octreotide for BON cells, as well as phosphodiesteraseI inhibitors seems to be a promising new therapeutic approach in GEP-NETs, which dose-dependently proliferation of all three cell lines with IC50 values in the nM should be evaluated in further clinical trials.

Andrew J Stephen 2, Christophe Marchand 1, Vincent C Njar 3, Yves Georgieva 1, Daniel Kychev 1, Yawen Wang 1, Judith Holstein 1, Martin Zeitz 2, Patricia Grabowsk 11, 1 Charité, Berlin, Germany.

**B219** Discovery and biochemical characterization of tyrosyl-DNA phosphodiesterase I inhibitors. Dexheimer S Thomas1, Smitha Antony2, Andrew J Stephen3, Christophe Marchand2, Vincent C Njar4, Yves Pommier1, 1CCR/NCI/NIH, Bethesda, MD; 2NCI-Fredrick, Fredrick, MD; 3University of Maryland School of Medicine, Baltimore, MD.

Tyrosyl-DNA phosphodiesterase 1 (Tdp1) is a recently discovered enzyme that catalyzes the hydrolysis of 3'-phosphotyrosyl bonds. Such linkages form in vivo following the DNA processing activity of DNA topoisomerase I (Top1). For this reason, Tdp1 has been implicated in the repair of irreversible Top1-DNA covalent complexes and has been regarded as a potential therapeutic co-target of Top1 in that it seemingly counteracts the effects of Top1 inhibitors, such as camptothecin and its clinically used derivatives. By reducing the repair of Top1-DNA lesions, Tdp1 inhibitors have the potential to augment the anticancer activity of Top1 inhibitors. Using novel high-throughput screening assays, we have identified several specific Tdp1 inhibitors from the "Diversity Set" of 1981 compounds from the Developmental Therapeutics Program of the National Cancer Institute. One of the positive hits, NSC 88915, a steroid derivative containing a tosylate substituent, has been confirmed and further characterized by traditional gel-based assays using several artificial Tdp1 substrates. NSC 88915 has been counter-screened against the enzyme APE1 and shown to be selective for Tdp1. In addition, fragmentation of NSC 88915 into discrete functional groups (i.e. the steroid moiety and the tosylate moiety) reveals that both components are required for inhibition of Tdp1 activity. Moreover, the synthesis of NSC 88915 analogues has provided insights into the structural prerequisites for inhibition of Tdp1. Based on the chemical structure of NSC 88915, we propose that the compound is a competitive inhibitor, which mimics the Tdp1 substrate. More specifically, the phosphate participating in the 3'-phosphotyrosyl of the Tdp1 substrate is replaced by the sulfite moiety of NSC 88915, while the phenyl bromide and steroid moieties of the compound substitute for the DNA and peptide groups of the Tdp1 substrate, respectively. In general, biochemical inhibitors of Tdp1 should enhance the cytotoxicity of camptothecins and delay the removal of Top1 cleavage complexes in cell culture, thereby providing a cellular step for compound selection. The overall therapeutic goal is the development of new drugs that would selectively enhance the activity of Tdp1 inhibitors in tumors with preexisting DNA repair and cell cycle checkpoint deficiencies.

B220 Preclinical development of novel small molecule anticancer agents targeted focal adhesion kinase pathway. Elena V. Kurenova1, Darrell Hunt1, Dihua He1, Vita Golubovskaya1, David Ostrov2, William Canc3, 1University of Florida, Gainesville, FL.

Focal Adhesion Kinase (FAK) is an important survival molecule that is upregulated in a broad range of solid tumors and is expressed at very low levels in normal tissues, creating a therapeutic window and making this protein a highly attractive target for the treatment of cancer, as suggested by our lab and recently by other leading authors in the field. We have identified the key binding partners of FAK and peptides from the binding sites that cause apoptosis in cancer but not normal cells. Based on these findings as well as correlative structural and functional data, we suggest that blocking FAK-protein interactions will lead to apoptosis and tumor cell death. We have well-documented data that targeting FAK-protein interactions is important for cell survival and we have used atomic resolution structural data of specific binding sites to identify small molecule lead compounds. We have screened small molecule libraries and identified several lead compounds that disrupt binding of FAK to key signaling molecules and induce apoptosis in breast, colon, pancreatic, lung, as well as melanoma cancer cell lines. Some of these compounds caused apoptosis at low nanomolar concentrations. We also have shown that lead compounds increase the sensitivity of cancer cells to standard chemotherapy drugs.

Our data suggest that peptides and small molecule inhibitors of FAK-protein interaction can be identified as lead compounds to provide the basis for targeted novel cancer therapeutic agents. Such compounds will effectively reduce activation of both molecules involved in survival signaling and will lead to sensitivity to chemotherapy and cancer cell death. We anticipate that our approach (targeting FAK protein-protein interactions) is amenable to more successful drug discovery and development than the typical method of targeting the kinase activity by targeting ATP binding site of tyrosine kinases. Experience shows that it is especially difficult in the case of FAK due to cross-reactivity with other essential tyrosine kinases.

B221 nNAPc2, a novel inhibitor of tissue factor/factor VIIa complex, inhibits tumor growth and metastasis in mouse models of colorectal cancer. Jingsong Zhao1, Gerard Aguilar1, Michael Imperiale1, Walter Funk1, Arie Abo1, 1Nuvleo, Inc., San Carlos, CA.

Recombinant nematode anticoagulant protein c2 (rNAPc2) is a specific inhibitor of tissue factor (TF)/factor VIIa complex with novel anti-metastatic, anti-angiogenic, and anti-thrombotic activities. TF is highly expressed in human colorectal tumors and the level of TF expression positively correlates with disease stage and inversely correlates with survival. To explore the therapeutic potential of nNAPc2 during tumor growth and metastasis, we tested nNAPc2 efficacy in experimental colorectal cancers in mice. Administration of nNAPc2 inhibited pulmonary metastasis in mice systemically disseminated with CT26 murine colon carcinoma cells in a dose-dependent fashion, as measured by either number of lung surface metastases or lung mass. While nNAPc2 treatment alone moderately reduced primary tumor growth, combining nNAPc2 with the cytotoxic agent 5-fluorouracil (5-FU) resulted in synergistic growth inhibition of HCT116 human colorectal tumor xenografts in nude mice. Likewise, nNAPc2 further reduced tumor growth in HCT116 human colorectal tumor xenograft-bearing mice receiving bevacizumab (humanized anti-vascular endothelial growth factor monoclonal antibody). Using CD31 and Ki67 immunohistochemistry, we found that nNAPc2 synergized with either 5-FU or bevacizumab in inhibiting microvessel density and tumor cell proliferation in HCT116 human colorectal tumor xenografts. Furthermore, nNAPc2 synergized with CPT-11 in inhibiting hepatic metastasis in nude mice with portal vein injection of HCT116 human colorectal tumor cells. Long-term administration of nNAPc2 also significantly suppressed formation of intestinal adenomas and adenocarcinomas in ApcMin+ mice. The dosing regimens of nNAPc2 used in these studies were well tolerated up to a three-month period by recipient mice without major hemorrhage or other adverse effects. In conclusion, the
synergistic tumor inhibitory activity of rNAPc2 in pre-clinical colorectal cancer models suggests that rNAPc2 may be an effective anti-tumor agent in human colorectal cancer patients to potentiate chemo- or antiangiogenic therapies.

**B223** Granulin-epithelin precursor (GEP) as molecular target for treatment of hepatocellular carcinoma. Siu Tim Cheung1, Jenny C.Y. Ho1, Sheung Tat Fan1. 1University of Hong Kong, Pokfulam, Hong Kong.

Hepatocellular carcinoma (HCC) is the major histological type of primary liver cancer, and is the third leading cancer killer worldwide. The disease is frequently diagnosed at an advanced stage and thus precludes curative surgical treatment. There is no effective therapeutic option for the majority of the HCC patients. A new therapeutic strategy is essential. We (Cancer Res 2002; Mol Biol Cell 2002) and others have reported the genome-wide expression proﬁles of HCC and their clinical implications. In the earlier study, we demonstrated that granulin-epithelin precursor (GEP, also called progranulin, acrogranin, or PC-derived growth factor), an autocrine growth factor, was overexpressed in more than 70% of HCC on the mRNA and protein levels. We also showed that GEP controlled HCC metastasis, invasion and tumorigenicity (Clin Cancer Res 2004). Here we show that GEP is a potential therapeutic target for HCC. We developed the anti-GEP monoclonal antibody (mAb), and demonstrated that it inhibited the growth of hepatoma cells, but revealed no signiﬁcant effect on normal liver cells. In the nude mice model transplanted with human HCC, anti-GEP mAb decreased the serum GEP level and inhibited the growth of established tumors in a dose-dependent manner. The anti-GEP mAb reduced tumor cell proliferation via the p44/42 MAPK pathway, and reduced tumor angiogenesis to deprive of the nutrient supply with reduced microvessel density and tumor VEGF level. Overexpression of GEP has also been shown in breast, ovarian and prostate cancers, therefore anti-GEP therapy may also be applicable to a broad spectrum of human cancer types. From the expression proﬁling studies, we are able to identify a number of differentially expressed genes have diagnostic and prognostic significance, and reveal the potential to serve as molecular targets for cancer therapy. Genome-wide expression analysis deﬁnitely serves an important role in identiﬁcation of new targets for cancer treatment.

**B224** Analyzing the role of the novel kinase Ror2 in renal cell carcinoma. Tricia M. Wright1, W. Kimryn Rathmell1. 1University of North Carolina at Chapel Hill, Chapel Hill, NC.

Sporadic renal cell carcinoma (RCC), a notoriously hard to treat solid tumor malignancy, has minimal sensitivity to traditional chemotherapy and immune system modulation. The most current and effective therapies for renal cell carcinoma involves the use of kinase-specific inhibitors targeted against receptor tyrosine kinases (RTK) and ligands specific to angiogenesis signaling. However, though these agents have shown promise, they remain to be fully optimized. The net effect is to almost double the time to progression of RCC, with tumors eventually developing resistance to these agents. RCC is deﬁned by inappropriate chemokine expression where, for example, platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are highly expressed growth factors in RCC because of inactivating mutations of the von Hippel-Lindau (VHL) tumor suppressor gene. However, VEGF receptor and PDGF receptor, which are present on tumor-associated endothelial cells and pericytes, respectively, are not generally present on RCC tumor cells. Currently, there are no known reported cancer cell speciﬁc kinases expressed on RCC. Our goal was to identify a cancer cell speciﬁc kinase expressed on RCC as a rational target for pharmaceutical development. Using a phospho speciﬁc RTK screen in renal carcinoma cells, we identiﬁed Ror2, a phosphorylated orphan receptor tyrosine kinase previously unknown in renal carcinoma cells. Ror2 is normally expressed in the heart, brain and lungs of developing mice and has also been implicated in the Wnt/β-catenin signaling pathway. By immunoblot and qRT-PCR, we have shown that not only is Ror2 expressed in renal carcinoma cells; it is expressed in a VHL-dependent manner. In addition, we were able to detect transcripts of Ror2 in more than 55% of a set of 19 archival human RCC tumor specimens. Further analysis is currently underway to delineate the importance of Ror2 for RCC tumorigenesis and regulation as it represents a potentially important molecular target for RCC.

**B225** IPI-504, a selective inhibitor of Hsp90, exhibits potent anti-tumor activity against Institute Her2 positive, Herceptin-sensitive and -refractory cell lines. Ching Ching Leow1, Karen Coffman1, Jon Chesebrough1, Shenlian Mao1, Christine Fazenbakker1, David Weng1, John Gooya1, James Porter2, Steve Coats1, Bahija Jallal1, Dowdy Jackson1, Yong S. Chang1. 1MedImmune, Inc, Gaithersburg, MD; 2Infinity Pharmaceuticals, Cambridge, MA.

IPI-504 is a novel, highly soluble small molecule inhibitor of heat shock protein 90 (Hsp90). Hsp90 is a protein chaperone that plays a central role in regulating protein homeostasis. Many of the proteins stabilized by Hsp90 are oncoproteins and cell signaling proteins important in cell proliferation and survival. Her2 (ErbB2) is expressed in a subset of metastatic breast cancers and is a client protein of the Hsp90 complex. Akt is also a client protein of the Hsp90 complex and has been shown to be activated in many Her2 positive breast cancers that are resistant to Herceptin. In this study, we investigated the anti-tumor activity and the mechanism of action of IPI-504 in Her2+, Herceptin-sensitive (SKBr3, BT-474 and N-87) and -refractory (JIMT-1) cell lines in vitro and in vivo. IPI-504 exhibited potent anti-proliferative activities (range of IC50 = 10-40 nM) against all cell lines examined. Mechanistically, IPI-504 dramatically induced the degradation of Her2 (EC50 = 45 to 96nM) and the lung. A mild inhibitory effect on tumor growth was also observed with CTCE-9908 treatment.

The expression of CKXCR4 in human HCC tumor and non-tumorous tissues was investigated by immunohistochemistry. CKXCR4 was highly expressed in tumour tissues with different staining patterns. Diffused nuclear and cytoplasmic staining was identified in the non-metastatic tumor tissues, while strong nuclear staining only was detected in the tumor tissues with secondary lung metastasis.

**Conclusion:** SDF-1/CXCR4 axis plays an important role in the migration and metastasis of HCC. Blockade of SDF-1/CXCR4 interaction is a potential therapeutic strategy for the treatment of HCC metastasis.
expression of Hsp70 (EC50 = 32 to 62nM). In regards to Akt, dramatic degradation was observed in BT-474 cell line (EC50 = 68nM). Finally, IPI-504, administered i.v. and p.o. in multiple schedules in athymic mice demonstrated potent tumor growth inhibition. Studies are underway to delineate the in vivo mechanism of action and to determine the PK/PD relationship of this potential therapeutic in vivo. Taken together, these findings support the development of IPI-504 as a novel therapeutic in the treatment of Her2 positive metastatic breast cancer.

B226 Disrupting the interaction between Hox and PBX causes apoptotic cell death and reduces in vivo proliferation of a non-small cell lung cancer model. Lynn Plowright1, Liesh Shears1, Hardave Pandha1, Richard Morgan1. 1University of Surrey, Guildford, United Kingdom.

The Hox genes encode a family of homeodomain-containing transcription factors that function during embryonic development. Dysregulation of Hox gene expression has been observed in a number of cancers. We have previously described the use of a novel, cell-permeable antagonist (HXR9) of the interaction between Hox proteins and the co-factor PBX to trigger apoptosis and block the proliferation of murine melanoma in vitro and in vivo. In this study, we investigated the activity of HXR9 in the non-small cell lung cancer cell line A549, and its efficacy in A549 xenograft nude mouse models. By fluorescence-activated cell sorting, HXR9 was confirmed to have significant (P < 0.005) pro-apoptotic activity on A549 cells. Transcriptional profiling by microarray of HXR9-treated cells revealed the up-regulation of a number of genes with suspected roles in apoptosis, including the oncogene c-fos. A549 xenograft studies demonstrated that intra-tumoral treatment with HXR9 for 18 days resulted in considerably smaller tumors compared with those in control groups; importantly, no overt toxicity was observed in treated mice. These results suggest that disruption of Hox/PBX dimer formation may trigger apoptosis and reduced cell proliferation in solid tumors of multiple organ systems. Antagonism of this interaction may thus be an important therapeutic target.

B227 Egr-1 and serum response factor are involved in gene expression of E2-EPF UCP that targets VHL tumor suppressor for degradation. Jung Hwa Lim1, Cho-Rok JUNG1, Su-Hyun Nam1, Yoon Jung Choi1, Jae Hee Oh1, Dong-Soo Im1. 1korea institute of Bioscience and Biotechnology (KIRIBB), Daejeon, Republic of Korea.

E2-EPF ubiquitin carrier protein (UCP) was found to be overexpressed in common human cancers (Wagner KW et al., Oncogene 23, 6621-6629, 2004). We recently reported that UCP induced ubiquitin-mediated proteolysis of von Hippel-Lindau (VHL) in cells and thereby stabilized hypoxia inducible factor-1α (HIF-1α) (Jung CR et al., Nat Med 12, 809-816, 2006). Furthermore, UCP was found to be highly expressed in most cancer cell lines. However, it has been known how expression of UCP gene is regulated. The aim of the present study was to explore molecular mechanisms underlying regulation of expression of UCP gene. Here we report that epidermal and hepatocyte growth factors (EGF, HGF), serum, and phorbol 12-myristate 13-acetate (PMA) induce UCP expression at transcriptional level in HeLa cells by the induction of early growth response gene-1 (Egr-1) and serum response factor (SRF). Promoter deletion assays identified binding sites for the transcription factors, Egr-1 and SRF, in the promoter region of UCP gene, which potentially modulated expression of UCP gene. Northern blot and RT-PCR analyses revealed that EGF or serum treatment increased transcripts of Egr-1 or SRF gene and sequentially transcript of UCP gene, but did not greatly affect transcript levels of VHL and HIF-1α in HeLa cells. Western blot analysis demonstrated that EGF, HGF, PMA, or serum increased Egr-1 or SRF and sequentially UCP, which resulted in a decrease of VHL and an increase of HIF-1α. Eletrophoretic mobility shift assays demonstrated that the DNA-binding activities of Egr-1 or SRF were increased when HeLa cells were treated with growth factors, or serum-starved HeLa cells were stimulated with serum. Furthermore, when HeLa cells were treated with EGF or serum-starved HeLa cells were stimulated with serum, the sequence-specific DNA-binding activities of Egr-1 or SRF in the promoter region of UCP were detected by chromatin immunoprecipitation assays. The effect of EGF or serum on the induction of UCP gene expression was decreased in HeLa cells by depletion of Egr-1 or SRF using siRNA against each gene. To examine whether EGF induced expression of UCP gene in mouse liver tissue, EGF was injected into mice by tail vein and liver organs were excised at various times after injection. RT-PCR analysis revealed that EGF treatment induced transcription of Egr-1 gene and moderately increased transcript of UCP gene at moderate level in liver tissues, but did not greatly affect transcript levels of VHL and HIF-1α. Western blot analyses revealed that EGF treatment increased Egr-1 and sequentially UCP in liver tissues, and decreased VHL, which resulted in an increase of HIF-1α. Collectively, the results suggest that growth factors induce Egr-1 and/or SRF that partially are responsible for the increase of UCP level in vitro and in vivo. In this context, UCP may play a positive role in cellular proliferation triggered by growth factors. Besides, our findings may in part explain why UCP is highly expressed in common human cancers. Furthermore, the Egr-1/SRF-UCP-VHL pathway may be responsible for increased HIF-1α expression by growth factors or mitogens.

Small Molecule Therapeutic Agents: Kinase Inhibitors

B228 In vitro and in vivo potentiation of cytotoxic therapy by XL844, an orally bioavailable inhibitor of Chk1 and Chk2. David J. Matthews1, Exelixis Inc., South San Francisco, CA.

Chk1 and Chk2 kinases activate cell cycle checkpoints and promote DNA repair in response to genotoxic stress. Using quantitative RT-PCR (QPQR) and immunohistochemical analysis (IHC), we show here that both Chk1 and Chk2 are substantially overexpressed in many tumor tissues. Although checkpoint activation may have a protective effect on pre-neoplastic cells, chronic expression of Chk1 and Chk2 may confer a survival advantage on established tumors. In particular, upregulation of Chk1 and Chk2 may enable tumor cells to replicate productively despite the profound replication stress induced by genotoxic agents. Therefore inhibiting these checkpoints in combination with chemotherapy may be a particularly effective method of overcoming cellular resistance to treatment.

XL844 is a potent, orally available inhibitor of Chk1 and Chk2. In combination with the S-phase active agent gemcitabine, XL844 treatment leads to premature mitotic entry, increased DNA damage and enhanced anti-tumor efficacy.1-2 We show here that XL844 also potentiates cell killing by several genotoxic agents that activate other cell cycle checkpoints. In vitro, SN38 (the active metabolite of irinotecan) and cisplatin induce Cdc2 phosphorylation and G2 cell cycle arrest. Co-treatment of these agents with XL844 leads to decreased levels of phospho-Cdc2, decreased G2 arrest and an increase in the sub-G1 apoptotic fraction in tumor cells. In clonogenic assays that measure long-term cell viability, XL844 has modest effects as a single agent in most tumor cells but potentiates the activity of cisplatin and other genotoxic agents. In contrast, combining SN38 with XL844 in clonogenic assays has little impact on cell survival in vitro. Cell cycle checkpoints are also activated in human xenograft tumors in vivo following administration of irinotecan and cisplatin. Oral administration of XL844 leads to abrogation of these checkpoints, with extended duration of action as measured by inhibition of Cdc2 phosphorylation. In a xenograft efficacy study in combination with irinotecan, XL844 produced a significant, although transient, increase in tumor growth inhibition compared to irinotecan alone. The in vivo efficacy of XL844 was also assessed using docetaxel, a microtubule-stabilizing agent that causes activation of the mitotic spindle checkpoint. Co-treatment with XL844 substantially enhanced the efficacy of docetaxel without a concomitant increase in toxicity. Collectively, these data indicate that cell cycle checkpoint inhibitors such as XL844 may have substantial utility in combination with a wide variety of cytotoxic agents. A Phase I clinical study of XL844 in combination with gemcitabine is currently in progress in patients with advanced solid malignancies.

B229 First-in-human study of PF-00299804, a small molecule irreversible panHER inhibitor in patients with advanced cancer: Update on safety and pharmacokinetics and initial report on pharmacodynamic responses and clinical benefit. D. R. Camidge\(^1\), C. D. Britten\(^1\), J. H. M. Schellens\(^2\), F. Guo\(^1\), R. Millham\(^1\), S. G. Eckhardt\(^1\), S. G. Wong\(^2\), D. S. Boss\(^3\), J. Lucza\(^4\), P. A. Janne\(^5\), \(^1\)University of Colorado, Denver, CO; \(^2\)UCLA Medical Center, Los Angeles, CA; \(^3\)Netherlands Cancer Institute, Amsterdam, The Netherlands; \(^4\)Pfizer Global Research and Development, New London, CT; \(^5\)Dana-Farber Cancer Institute, Boston, MA.

Background: PF-00299804 is an orally bioavailable, potent, irreversible small molecule inhibitor of the HER tyrosine kinases Her 1, 2, and 4. A first-in-man phase I dose escalation study is ongoing. Preliminary results were reported at ASCO 2007. An expanded data set exploring clinical benefit and pharmacodynamics in cohorts enriched for specific genetic characteristics is included in the current report.

Methods: Assessments were made of the safety, tolerability, PK, PD, and efficacy of PF-00299804 administered orally once daily in 3-week cycles at doses ranging from 0.5 to 60 mg. In an expanded cohort within the phase I dose escalation study additional patients were recruited at the predicted recommended phase 2 dose (RP2D) for continuous dosing. Cohorts of patients dosed at the predicted RP2D were enriched for molecular variations of HER family receptors in multiple tumor types, as well as for wild type K-ras in refractory NSCLC patients. PD measures included assessment of HER-related signaling pathways via IHC analyses of serial skin and, in some patients, tumor biopsies. Serial 18F-FDG-PET/CT was performed on a subset of patients with scans being classified according to modified RECIST criteria by a central reader. Spearman correlations were assessed between AEs and dose, as well as AEs and PK parameters (Cmax, AUC, and Ctrough).

Results: The most common AEs were diarrhea, fatigue, nausea, and rash. As previously reported, 3/6 patients at 60 mg experienced a DLT [hand-foot syndrome (1), dehydration related to diarrhea (1), mucositis (1)]. Other DLTs observed include diarrhea, rash, and pleuritic pain. The MTD was reached at 45 mg QD which was selected as the recommended phase 2 dose (RP2D). The terminal half life was ~85 hr, supporting once-daily dosing. Correlation was observed between severity of rash and dose as well as PK parameters. Objective tumor responses as assessed using RECIST criteria were observed in two patients with advanced refractory NSCLC. Both patients had failed at least 6 regimens of prior therapy; one patient who had progressed on erlotinib treatment had an exon 20 insertion mutation in EGFR. Data on these and on efficacy assessments within additional patients with other tumor types will be reported.

Conclusions: Daily administration of PF-00299804 appears safe and tolerable across multiple dose levels up to 45mg/d. Rash-, Diarrhea-, fatigue, and nausea, are the most frequent AEs, with rash severity correlating with dose and PK parameters. 45 mg/d has been declared the MTD and is being explored in expanded cohorts enriched for genetic variation in HER family receptors as well as in NSCLC patients wild type for K-ras. Clinical and biological activity of PF-00299804 was observed including objective responses in two patients with advanced refractory NSCLC including one erlotinib failure with EGFR mutation. Explorations of relationships between response and tumor molecular profiles will be reported.

B230 Novel small molecule inhibitors of the Axl receptor tyrosine kinase block tumor growth. Sacha J. Holland\(^1\), Yuenming Hu\(^1\), Betty Chang\(^1\), Alison Pan\(^1\), Christian Franci\(^1\), Weigun Li\(^1\), Matt Duan\(^1\), Arthur Bagos\(^1\), Allan Torneros\(^1\), John McLaughlin\(^1\), Jing Zhang\(^1\), Jiaxian Yu\(^1\), Pingyu Ding\(^1\), Thilo J. Heckrodt\(^1\), Joanne Litvak\(^1\), Emily Stauffer\(^1\), George R. Clemens\(^1\), Sarkiz Daniel-Issakani\(^1\), Polly Pine\(^1\), Dane Goff\(^1\), Rajinder Singh\(^1\), Donald G. Payan\(^1\), Yasumichi Hitoshi\(^1\), \(^1\)Rigel, Inc., South San Francisco, CA.

The receptor tyrosine kinase (RTK), Axl, is a novel therapeutic target for solid tumors. Axl is expressed in both neoplastic and stromal compartments and is associated with poor outcomes in several human tumor types. Signaling via Axl drives proliferation, migration and invasion and promotes cells from apoptosis. Genetic inhibition of Axl signaling both blocks tumor growth and suppresses angiogenesis in vivo. These data suggest that Axl inhibitors may suppress tumor growth via multiple mechanisms.

By high throughput screening of a diverse chemical library followed by an extensive structure activity relationship (SAR) study, we have identified small molecule inhibitors of Axl kinase which exhibit potent on-target activity in both cell-based and biochemical assays. The compounds fall into two classes: 1) those that potently block Axl and VEGFR2 signaling but are selective over other RTKs including EGFR and InsulinR, and 2) Axl-selective inhibitors. Both classes of compound inhibit the proliferation of sensitive tumor cell lines in vitro but lack potent generalised antiproliferative activity. Rigel’s Axl inhibitors demonstrate favorable pharmacokinetic properties and are generally well tolerated.

Our lead Axl / VEGFR2 inhibitor potently blocked bFGF-induced angiogenesis in a corneal micropocket assay (p< 0.001). This compound also strongly inhibited the growth of three different tumor cell lines in vivo in subcutaneous murine xenograft models and was able to extend survival of tumored animals. Regressions were noted in one study. Importantly, our lead Axl-selective inhibitor also significantly reduced corneal neovascularization (p< 0.001). Furthermore, partial suppression of tumor growth in vivo was also observed with this compound.

Rigel has generated potent and selective inhibitors of Axl kinase. We propose that small molecule inhibition of Axl represents a novel therapy for a subgroup of sensitive solid tumors.

B232 Acute dasatinib exposure commits Bcr-Abl dependent cells to apoptosis. Jennifer L. Paulson\(^1\), Thomas O’Hare\(^1\), Christopher A. Eide\(^1\), Michael W. Deininger\(^1\), Brian J Druker\(^2\), \(^1\)Oregon Health & Science University, Portland, OR; \(^2\)Oregon Health & Science University / Howard Hughes Medical Institute, Portland, OR.

Pioneering work with the Bcr-Abl inhibitor, imatinib, demonstrated a requirement for constant Bcr-Abl inhibition to achieve maximal therapeutic benefit in treating chronic myeloid leukemia (CML), establishing a paradigm that has guided further drug development for this disease. Surprisingly, the second-generation Bcr-Abl inhibitor, dasatinib, was reported to be clinically effective with only once daily dosing, despite having a short (3-5 h) plasma half-life. Consistent with this observation, we provide direct in vitro evidence that acute treatments with clinically-achievable dasatinib concentrations irreversibly commit Bcr-Abl positive CML cell lines to apoptotic cell death. This is a specific property of dasatinib, as it was not recapitulated by acute treatment with equipotent concentrations of an alternative Bcr-Abl inhibitor, nilotinib. Regardless, these data suggest that continuous kinase inhibition may not be necessary with some kinase inhibitors to achieve therapeutic benefits.
B233 In vitro and in vivo antitumor activities of SGX523, a novel MET inhibitor. Katayoun A. Jessen1, Karen J. Froning1, Jeremy D. Felce1, Andres Gutierrez2, Marshall C. Peterman2, Stephanie Leonard3, Crystal Tang1, Nanni Huser1, Sam Sperry1, Natalie Tran1, Yousif Sahly1, Steven Gessert1, Stephen K. Bulley1, Siegfried Reich1, Sean G. Buchanan1. 1SGX Pharmaceuticals, San Diego, CA. 

MET (c-Met, HGF-R) the receptor tyrosine kinase for hepatocyte growth factor (HGF), has been implicated in oncogenesis and tumor progression, and thus is an attractive target for molecular therapeutic intervention. We have identified SGX523, a novel and remarkably selective, ATP-competitive, small molecule inhibitor of MET. Previously, data was presented which showed that in purified enzyme assays and various cell-based assays, SGX523 inhibited the activity of MET at low nM concentrations. We have now surveyed the activity of SGX523 across a wide number of cancer cell lines. We show that SGX523 can inhibit various HGF/MET-dependent cellular phenotypes. Genomic amplification or constitutive activation of MET is predictive of in vitro response to SGX523. In vivo evaluation of this compound demonstrated that it is orally bioavailable with good pharmacokinetic properties. SGX523 demonstrated potent anti-tumor activity when dosed orally in human xenograft models with no overt toxicity. The pharmacodynamic biomarker, phospho-MET was used to determine the pharmacokinetic/pharmacodynamic relationship. Continuous and discontinuous dosing regimens were used to determine the effectiveness of SGX523 in these models.

Our results demonstrate that SGX523 potently inhibits HGF/MET-dependent cellular phenotypes and is able to inhibit the growth, and in some cases cause regression, of tumor xenografts in mice and that this can be achieved at doses that are well-tolerated. Moreover, the efficacy in the xenograft models appears to correlate well with the pharmacokinetics and the pharmacodynamic marker.

B234 Rates of proliferation of human melanoma may predict sensitivity to small molecule MEK inhibition. Cathrine L Denton1, Daniel L. Gustafson2. 1University of Colorado School of Pharmacy, Denver, CO; 2Colorado State University Animal Cancer Center, Fort Collins, CO.

Melanoma is the most common fatal skin cancer worldwide and, in the United States alone, an estimated 89,400 new cases and an estimated 8,110 deaths are expected in 2007. Regarding malignant melanoma cell lines and the crucial Ras-Raf-MEK-ERK signaling cascade, approximately 60% harbor activating mutations in the BRAF gene and, of these, 91% occur in codon 600 within the kinase domain of the protein. Inhibitors of MEK have been shown to abrogate ERK1/2 activation (via phosphorylation), consequently resulting in a decrease in melanoma cell proliferation and metastatic potential as well as an increase in apoptotic response.

AR389 (Array Biopharma, Boulder, CO) and AZD6244 (AstraZeneca, Macclesfield, United Kingdom) are potent, selective, noncompetitive MEK1/2 inhibitors. Binding of this class of molecules to the MEK protein-ATP complex prevents downstream phosphorylation of ERK1/2 and thus impedes MAPK signaling. To better understand as well as employ these agents in the clinic, cellular and molecular characterization of small molecule MEK inhibitors, such as AR389 and AZD6244, is absolutely imperative.

Using a panel of six human melanoma cell lines with varying BRAF and NRAS mutations, we demonstrate that AR389 and AZD6244 inhibit phosphorylation of ERK1/2 and induce a profound and sustained cell cycle arrest in G1, with a substantial decrease in % cells in S-phase, and a corresponding decrease in proliferation of all cell lines tested. However, AR389 did not increase apoptosis nor decrease cellular clonogenic potential, indicating that the main therapeutic effect of these drugs is to inhibit cell cycle and proliferation. In fact, we found that sensitivity to small molecule MEK inhibitors can be closely correlated with potential doubling time (r²=0.8806) in human melanoma cell lines. Additionally, fraction of cells in S-phase decreases linearly with dose (r²=0.8510) in MEK inhibitor treatment groups. Accordingly, we hypothesize that sensitivity to MEK inhibition may be predicted based on cellular growth rates (Tprod) and fraction of cells in S-phase.

B235 Selective inhibitors of JAK2 kinase as potential therapeutic agents. Vadim V. Markovtsov1, Diane Y. Shuling Fang1, Marina Gelman1, Wayne Lang1, Vanessa C. Taylor2, Stacey Huynh1, Roy Frances1, John McLaughlin1, Sohasekhar Bhamidipati1, Jeffrey Clough1, Rajinder Singh1, Gary Park1, David Sweeney1, Elizabeth Ionkin1, Rena Bahjat2, Betty Chang1, Polly Pine2, Ruby Daniel2, Donald G. Payran1, Sacha Holland1, Yasumichi Hitoshi1,5. Rigel Pharmaceuticals, Inc., South San Francisco, CA.

Limited options provided by the current standard of care for the patients suffering from myeloproliferative diseases (MPDs) prompted an extensive search for the underlying molecular mechanisms of these disorders. Recent discovery of a single activating mutation (V617F) in JAK2 kinase gene associated with the development of the polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF) opened up a possibility to develop highly targeted therapies against these debilitating ailments. To that end, we engineered cytokine-independent Ba/F3 cell line expressing the V617F mutant of JAK2 to screen a focused small molecule library for potential inhibitors of JAK2 V617F -dependent proliferation. We confirmed the ability of hit compounds to inhibit proliferation of JAK2-dependent tumor cell lines using UKE-1 and SET-2 cells carrying the V617F JAK2 mutation. A FACS-based phosphoSTAT5 assay was then used to demonstrate that the hits directly targeted mutant JAK2. JAK3 activity of each compound was evaluated in IL-2-dependent CTL-L2 cell line using phosphoSTAT5 FACS and proliferation assays. To avoid hits with nonspecific antiproliferative activity, the hits were tested in JAK2-independent MOLT4, AS49 and H1299 cell lines. Compound hits with the desirable properties were further evaluated for their ability to inhibit JAK2, JAK3 and other kinases in the context of T cell, B cell, or mast cell activation using a variety of cell-based assays as well as in the in vitro biochemical assays. We identified a number of compounds that potently inhibit growth of the two V617F mutant cell lines with EC50s varying from 20 to 500 nM, but do not affect proliferation of control cell lines MOLT4, AS49 and H1299 to the same degree. These compounds induce strong and highly specific suppression of STAT5 phosphorylation with IC50s of 10 to 200 nM in SET-2 and V617F. JAK2 expressing Ba/F3 cells. One of the hits with the desirable biological and pharmacokinetic profiles was further evaluated in V617F JAK2 Ba/F3 engraftment mouse model where it demonstrated significant extension of survival at 150 and 200 mg/kg bid. Such potent JAK2 inhibitors could become the basis for the next generation of compounds targeting JAK2-dependent myeloproliferative diseases.

B236 Characterization of in vitro and in vivo antitumor activity for novel IGF-1R kinase inhibitors in colon cancer cells. Yi P. Hu1, Ivan Dominguez2, Jennie Hauser3, Elizabeth Sharratt2, Michelle Vinc2, Gillian Howell1, Jing Wang1, Qun-sheng Ji4, Neil W. Gibson4, Ashwani Rajput2, Michael G. Brattain1. 1University of Nebraska Medical Center, Omaha, NE; 2Roswell Park Cancer Institute, Buffalo, NY; 3Roswell Park Cancer Institute, Buffalo, NY; 4Osi Pharmaceuticals, Inc. Farmingdale New York, Farmingdale, NY.

Insulin-like growth factors (IGFs) and their receptor, IGF-1 R, are frequently expressed in human colon cancers and play important roles in promoting malignancy. We demonstrate that colon cancer cells show dependence upon an IGF2/IGF-1 R autocrine loop and have characterized the effects of two novel low molecular weight kinase antagonist inhibitors (designated PQIP and AQIP) of the IGF-1 R in vitro and in vivo. Both compounds abrogated IGF-1 R mediated activation of IRS-1/Akt to inhibit mitogenesis and anchorage-independent growth in soft agarose at concentrations consistent with inhibition of IGF-1 R phosphorylation. Thus, these novel small molecule kinase inhibitors of IGF-1 R showed potent in vitro antitumor activity in colon cancer cells. The effects of PQIP on the growth of orthotopically implanted GEO colon cancer cells were determined following daily treatment with 75mg/Kg of drug by gavage. Decreased tumor burden in nude mice without significant weight loss and...
toxicity was observed. Fluorescence intensity of the GFP labeled tumors was 3-fold higher in control mice than in treated mice. MRI analysis showed a 5-fold decrease tumor volume in treated mice. TUNEL analysis of treated and sham treated tumors indicated an 8-fold higher rate in PQIP treated tumors. Western blot analysis of the treated tissue samples showed inhibition of IGF1R activation and Akt signaling relative to sham treated animals.


Background: Ligand-independent activation of KIT is a critical factor in gastrointestinal stromal tumor (GIST) pathogenesis and patient survival, and also is associated with a subset of melanomas. Activating mutations in KIT are found in 85-90% of GIST tumors, with juxtamembrane domain alterations that result in kinase activation accounting for the majority of observed mutations. Treatment of GIST patients with imatinib or sunitinib results in marked responses, often followed by relapses associated with secondary KIT kinase domain mutations near the ATP binding site or within the activation loop. XL820 is a novel, potent, orally available small molecule inhibitor of both wild-type and mutationally-activated KIT, VEGFR2, and PDGFR. Therefore, we compared the activity of XL820, imatinib, and sunitinib, as a datum of these KIT mutations.

Materials and Methods: XL820, imatinib, and sunitinib, were tested in cellular assays measuring their ability to inhibit autophosphorylation of a broad panel of clinically relevant mutant KIT proteins.

Results: XL820 inhibits 26 of 27 mutated forms of KIT examined with phosphorylation IC50 values generally < 300nM. Only the D816V activation loop variant, which comprises <10% of KIT mutations associated with GIST drug resistance, was relatively resistant to XL820 (IC50 > 1000nM).

Conclusions: XL820 inhibits both the ATP binding region and activation loop classes of resistance mutations, and based on the results of cellular assays is an effective inhibitor against KIT mutations associated with imatinib or sunitinib resistance. These data will support Phase II exploration of XL820 in metastatic melanoma and recurrent GIST (where activation loop mutations comprise approximately 50% of the GIST resistance-associated mutations) following completion of an ongoing Phase I study of orally administered XL820 in patients with advanced solid malignancies.

B238 A new class of thienopyrimidine Src inhibitors demonstrates potent growth suppression of imatinib resistant Ba/F3 cells harboring a wide range of BCR-ABL mutations, including the CML relevant T315I. Thomas O’Har1, Spyro Mousses2, Kandavel Shanmugam2, Vincent T. Bicocca3, Christopher A. Eide1, Jeffrey Jacob1, Tim Williamson1, Patrick Shannon3, Robert B. MacArthur1, Steve Weitman1, Robert B. MacArthur1, Steve Weitman1, Jeffery Trent2, Patrick Shannon3, Robert B. MacArthur1, Steve Weitman1, Jeffery Trent2, Daniel D. Von Hoff4, Brian J. Drucker4, Howard Hughes Medical Institute, Portland, OR; 1T-Gen, Scottsdale, AZ; 2Systems Medicine LLC, Scottsdale, AZ. 

Purpose: Next generation Abi and Src kinase inhibitors (including dasatinib, AP23464, and AMN107) have demonstrated potent activity against some clinically relevant BCR-ABL mutants that lead to imatinib resistance. However, these compounds generally fail to suppress cells harboring some variants, including the T315I mutant, prompting us to investigate a new class of thienopyrimidine Src inhibitors as candidate agents to treat patients with imatinib resistant forms of cancer.

Experimental Procedures: Drug Dose Response (DDR) experiments were performed to evaluate the effectiveness and selectivity of two thienopyrimidine Src inhibitors for inhibiting the growth and survival of a panel of cell lines harboring BCR-ABL mutants associated with imatinib resistance. Specifically, we performed DDR analyses on a panel of Ba/F3 cell lines, including the parental cell line (engineered to express and depend on the native BCR-ABL protein), and 9 variant cell lines expressing specific BCR-ABL mutants (M244V, Y253F, Y253H, E255K, E255V, T315A, T315I, M351T, and F359V). As a control, we tested imatinib, which as expected showed significant inhibition of K562 cells (IC50 = 236.5 nM), native BCR-ABL expressing Ba/F3 cell line (IC50 = 391.6 nM), however, all other Ba/F3 variant lines with BCR-ABL mutants showed resistance (IC50> 2000 nM).

Summary of findings: Two of the Src inhibitors tested, SMi813 and SMi829, showed potent inhibition of survival (IC50 < 100nM) in all Ba/F3 cell lines including the parental, the native BCR-ABL variant, and all of the mutant variants listed above. In fact, the potent cytotoxicity was uniform and non-selective relative to its effects in the various mutant backgrounds. Interestingly, even the cell line harboring the clinically important T315I mutant was sensitive to both SMi813 (IC50 = 49.3 nM) and SMi829 (IC50 = 50.1 nM).

Conclusions: SMi813 and SMi829 represent a new class of Src inhibitors, which demonstrate potent in vitro activity against the clinical CML variants with BCR-ABL mutations warranting exploration of this class of novel agents with the goal of advancing them as a potential new treatment(s) for patients with imatinib resistant forms of cancer. Based on preliminary in vivo data from earlier generation thienopyrimidine analogues, we expect this class of Src inhibitors to be relatively selective for Src, safe and well tolerated in vivo. Therefore, we plan to continue the development of these compounds, SMi813 and SMi829, to more extensively evaluate their selectivity and toxicity profile. The results presented here warrant further investigation to gain insights into the molecular mechanism by which these new Src inhibitors retains potent activity against all tested imatinib resistant BCR-ABL mutants, including the BCR-ABL T315I mutant, which seem to be refractory to the effects of other Src and Aurora Kinase inhibitors.

B239 A phase I dose finding study of a 3-day administration of BIBW 2992, an irreversible dual EGFR/HER2 inhibitor, in combination with 3-weekly docetaxel in patients with advanced solid tumors. Patrick Schöffski1, Herlinde Dumee2, Pascal Wolter1, Alain Hendriks2, Tatiana Besse-Hammer3, Martine Piccart4, Johan Sellelsch4, Mehdi Shahidi5, Martine Tatou6, Ahmad Awadad7, University Hospital Leuven/Leuven Cancer Institute, Leuven, Belgium; 2Jules Bordet Institute, Brussels, Belgium; 3Boehringer Ingelheim, Bracknell, United Kingdom; 4C.S. Boehringer Ingelheim Comm. V., Brussels, Belgium. 

Background: BIBW 2992 is a potent oral, irreversible inhibitor of EGFR and HER2 with IC50 values of 0.5 and 14 nM, respectively. A phase I dose defining study of short course once daily BIBW 2992 in combination with docetaxel is reported.

Materials: Eligible patients had incurable advanced solid malignancies, good organ function and good performance status. Docetaxel 75mg/m² was administered i.v. on day 1, oral BIBW 2992 was administered once daily on days 2 to 4, every 3 weeks. The BIBW 2992 dose was doubled in successive cohorts of 3-6 patients until toxicity > grade 2 CTC (dose limiting toxicity) occurred. Further escalation was with a dose increment of ≤ 50%. After reaching dose-limiting toxicity (DLT), the maximum tolerated dose (MTD) cohort was expanded to 12 patients.

Results: 38 evaluable patients were treated (17 male); median age was 53 years (range 28-79). BIBW 2992 dose was escalated from 10 to 160mg in consecutive cohorts. Administered doses were 10 (6), 20 (3), 40 (6), 60 (4), 90 (12), 120 (5) and 160 (2). [no. of patients in brackets]. The most common adverse events were fatigue (45.0% of patients), diarrhea (32.5%), anorexia (30.0%), stomatitis (27.5%), alopecia (27.5%), vomiting (20.0%), rash (20.0%) and mucosal inflammation (20.0%). Two DLTs were observed; both were grade 4 CTC neutropenia and thrombocytopenia at 120mg of BIBW 2992 and fully recovered upon treatment interruption and dose reduction (docetaxel 60mg/m² / BIBW 2992 90mg ). The MTD was defined as 90 mg of 90 mg of BIBW 2992 administered for 3 days after administration of docetaxel 75 mg/m² is well tolerated and is the recommended dose for further clinical trials. BIBW 2992 in combination with docetaxel.

Three patients (breast, thymus and oesophageal carcinoma) had confirmed PRs (after 4, 2 and 4 courses, respectively, RECIST criteria). One breast cancer patient had a confirmed CR (PR after 4 and CR after 6...
courses). One out of 4 patients had prior treatment with taxanes. Further 11 patients had stable disease and remained on the combination treatment for > 6 courses with 7 remaining on treatment for > 9 courses.

**Conclusion:** The dose of 90 mg of BIBW 2992 administered for 3 days after administration of docetaxel 75 mg/m2 is well tolerated and is the recommended dose for further clinical trials. Objective responses or durable stable disease (> 6 months) was seen in 15 (39.4%) of the patients.

**B240** Lovastatin inhibits VEGFR function and induces synergistic cytotoxicity in mesothelioma cells in combination with VEGFR inhibitors: Potential novel therapeutic approach. Diane Trinh1, Christina L. Addison1, Jim Dimitroulakos1. 1Ottawa Health Research Institute, Ottawa, Ontario, Canada.

In a recent study, we demonstrated the ability of Lovastatin, a potent inhibitor of mevalonate synthesis, to inhibit the function of the epidermal growth factor receptor (EGFR). Lovastatin attenuated ligand-induced receptor activation and downstream signaling through the PI3K/AKT pathway. Combining Lovastatin with gefitinib, a potent EGFR inhibitor, induced synergistic cytotoxicity in a variety of tumor derived cell lines. The vascular endothelial growth factor receptor (VEGFR) and EGFR are both members of the receptor tyrosine kinase (RTK) family that share similar activation, internalization and downstream signaling characteristics. The VEGFRs, particularly VEGFR2 (KDR, Flt-1), play important roles in regulating angiogenesis by promoting endothelial cell proliferation, survival and migration. Angiogenesis plays a critical role in tumorigenesis and certain tumors, such as mesotheliomas, express both the VEGF ligand and VEGFRs that act as an autocrine loop that directly stimulates tumor cell growth and survival. In this study, we have shown Lovastatin inhibits ligand-induced VEGFR2 activation through inhibition of receptor internalization and also inhibits AKT activation in human vascular endothelial cells (HUVEC). Lovastatin treatment re-organized the actin cytoskeleton, inhibited proliferation and induced apoptosis of HUVECs despite addition of exogenous VEGF. In addition, combining SuM Lovastatin treatments with two VEGFR2 inhibitors in the H28 and H2052 mesothelioma derived cell lines demonstrated synergistic cytotoxicity through the induction of a potent apoptotic response. These results highlight a novel mechanism regulating VEGFR2 function and a potential novel therapeutic approach for mesotheliomas.

**B241** Enzastaurin, a protein kinase C\(\beta\) inhibitor, suppresses signaling through the Rsk and Bad pathways and induces apoptosis in human gastric cancer cells. Keun-Wook Lee1, Sang Gyun Kim2, Hwang-Phill Kim2, Euna Kwon3, Jiran You1, Hyung-Jun Choi3, Jung-Hyun Park2. Byeong-Chool Kang1, Seock-Ah Im1, Tae-You Kim1, Yong-Jue Bang1. 1Seoul National University Bundang Hospital, Seongnam, Republic of Korea; 2Seoul National University College of Medicine, Seoul, Republic of Korea; 3Seoul National University Hospital, Seoul, Republic of Korea.

Activation of protein kinase C (PKC) has been implicated in carcinogenesis of gastric cancer. Enzastaurin is an oral ATP-competitive inhibitor of the PKC\(\beta\) isoform. Although enzastaurin was initially advanced to the clinic based on its antiangiogenic activity, it is also known to have a direct effect on a variety of human cancer cells, inducing apoptosis by inhibiting the Akt signal pathway. However, data on enzastaurin for gastric cancer are limited. Therefore this study was performed to assess the antitumor activity of enzastaurin on gastric cancer cells and to investigate the underlying antitumor mechanisms. Enzastaurin suppressed the proliferation of cultured gastric cancer cells and the growth of gastric carcinoma xenografts. Enzastaurin did not have an effect on gastric cancer cell cycle progression; however, it had a direct apoptosis-inducing effect through the caspase-mediated mitochondrial pathway. GSK3\(\beta\) phosphorylation, a reliable pharmacodynamic marker of enzastaurin activity, and Akt phosphorylation were both decreased after treatment with enzastaurin. Although the p90 ribosomal S6 kinase (Rsk) was also dephosphorylated, Erk phosphorylation was not affected in the enzastaurin-treated gastric cancer cells. Enzastaurin activated Bad, one of the Bcl-2 proapoptotic proteins, through dephosphorylation at Ser112, and depletion of Bad activity resulted in resistance to enzastaurin-induced apoptosis in gastric cancer cells. These data suggest that enzastaurin induces apoptosis through Rsk- and Bad-mediated pathway, besides inhibiting the Akt signal cascade. Furthermore, enzastaurin had synergistic or additive effects when combined with 5-fluorouracil, cisplatin, paclitaxel, or irinotecan. These results warrant the further clinical investigation of enzastaurin for gastric cancer treatment.

**B242** A phase I dose-escalation and pharmacokinetic (PK) study of XL647, a novel spectrum selective kinase inhibitor, administered orally daily to patients with advanced solid malignancies (ASM). Julian Molina1, Heather A. Wakelee2, Joanne M. Fehling2, Janet L. Lensing1, John Calcagni2, Roel F. Funke2, Dale Miles3, Branimir I. Skic2. 1Mayo Clinic, Rochester, MN; 2Stanford Univ, Stanford, CA; 3Exelixis, Inc., South San Francisco, CA.

**Background:** XL647 is an orally bioavailable small molecule inhibitor of multiple receptor tyrosine kinases involved in tumorigenesis and angiogenesis, including EGFR/ErbB1, ErbB2/HER2 and VEGFR2/KDR. In a previous Phase I study, the maximum tolerated dose (MTD) of XL647 was 350 mg when given for 5 consecutive days every 14 days. The purpose of the present study is to determine the MTD of XL647 when administered daily to patients (pts) with ASM.

**Methods:** Cohorts of ASM pts received escalating dose levels of XL647 daily for repeated 28 day cycles. Safety is monitored continually and PK sampling is conducted to determine the Cmax and T1/2 at each dose level. Plasma markers reflecting effects of XL647 on angiogenesis are being analyzed. Eyebrow hair bulbs from a subset of pts are being evaluated as a potential surrogate for the effects of XL647 on signal transduction pathways. Tumor response is being assessed every 8 weeks by RECIST. Results: Twenty-four pts have been enrolled to date. Dose levels of 75 mg (n=3), 150 mg (n=3), 200 mg (n=3), 300 mg (n=11) and 350 mg (n=4) have been studied. A single DLT of Grade (G) 3 pneumonitis occurred in 1/6 pts at the 300 mg level. At 350 mg 2/4 pts developed a DLT of clinically asymptomatic G3 QTc prolongation (by machine and G2 by manual re-read) and the 300 mg dose level was declared the MTD. To date, 5 pts have been enrolled in an expanded 15 pt MTD cohort at 300 mg. The majority of pts have discontinued due to progressive disease (PD); 1 pt withdrew consent and 2 pts discontinued due to AEs (G3 pneumonitis and G3/4 drug-induced transaminitis). All ongoing pts are currently receiving 300 mg. Eleven pts achieved stable disease (SD) lasting ≥ 3mo. The most common AEs reported include G1/2 rash, diarrhea, fatigue, dysgeusia, and G1 asymptomatic QTc prolongation. PK analysis indicates rapid absorption but stable state concentrations reached by day 15. Eyebrow samples from 3 pts were analyzed. XL647 caused a significant reduction in the phosphorylation of the EGFR downstream signaling kinases AKT and ERK in 2/3 and 3/3 pts, respectively. Levels of pharmacodynamic plasma markers were not significantly changed after administration of XL647 and no PK/PD relationships have been identified to date in this phase I population. DCE-MRI analysis is ongoing for some patients in the expanded MTD cohort.

**Conclusion:** XL647 is generally well-tolerated when administered daily at doses up to 300 mg. Eyebrow samples represent a potential surrogate tissue for assessment of inhibition of EGFR downstream targets by XL647. Updated safety and PK results will be presented.

**B243** PKC inhibitor, N-Benzoyl staurosporine, paradoxically induces megakaryocytic differentiation and possesses therapeutic potential in human chronic myeloid leukemia. David K Chao1, Yu-Chuen Huang1, Yu-Jen Chen1. 1Department of Medical Research, Mackay Memorial Hospital, Taipei, Taiwan.

**Background:** Previous research has shown activation of PKC leads to differentiation of progenitor cells into megakaryocytes. N-Benzoyl staurosporine, derived from naturally occurring staurosporine, inhibits multiple isoforms of the serine/threonine protein kinase C (PKC). In preclinical studies, N-Benzoyl staurosporine showed broad antiproliferative activity against various tumor cell lines, including acute myeloid
leukemia. We explored its therapeutic effect on chronic myeloid leukemia (CML) and found that in addition to inducing apoptosis and inhibiting the growth of CML cells, N-Benzoyl staurosporine paradoxically promoted leukemia cell differentiation into megakaryocytes.

**Materials and methods:** Human CML K562 cell line was used as an in vitro model. MTT assay was used for assessment of cell viability. Liu’s dyes and immunofluorescent mAbs against alpha-tubulin and DAPI were used for morphological observation. DNA histogram and surface markers were examined by using flow cytometry. C-mpl mRNA expression was evaluated by RT-PCR.

**Results:** N-Benzoyl staurosporine inhibited the growth of K562 cells in a dose- and time-dependent manner with an IC50 around 0.5 μM. Morphological changes such as enlarged contour with multiple nuclei characteristic of megakaryocytes were noted in more than half of N-Benzoyl staurosporine (0.5 - 1.0 μM)-treated cells. DNA histogram shows that cells were arrested with marked 2n, 4n and 8n DNA content. Surface expression of a megakaryocytic marker CD61 was enhanced and by contrast, that of erythrocytic marker glycoporin A was inhibited by treatment of N-Benzoyl staurosporine. The mRNA expression of c-mpl, a gene encoding thrombopoietin receptor, was increased. These results indicate that N-Benzoyl staurosporine may induce K562 cells to differentiate toward megakaryocytes. Both PKC activator 12-O-tetradecanoyl phorbol-13-acetate and PKC inhibitor staurosporine expressing differentiating effects similar to N-Benzoyl staurosporine, indicating that N-Benzoyl staurosporine may induce megakaryocytic differentiation via a PKC-independent pathway. Furthermore, N-Benzoyl staurosporine at higher dose (1.0 μM) induced apoptosis in a smaller population of K562 cells demonstrated by increase in hypodiploid fraction in DNA histogram and by morphological observation.

**Conclusion:** In contrast to previous research, where activation of PKC causes megakaryocytic differentiation, here we observed a paradoxical phenomenon of a known PKC inhibitor (N-Benzoyl staurosporine) promoting differentiation of leukemia cells into megakaryocytes. N-Benzoyl staurosporine also inhibited the growth and to a lesser extent, caused apoptosis in human CML K562 cells. It may have potential as a novel therapeutic agent for CML.

**B244 In vivo evaluation of OSI-906, a novel small molecule kinase inhibitor of the insulin-like growth factor-1 receptor (IGF-1R).**

Maryland Rosenfeld-Franklin1, Andy Cooke1, Caroline Pirritt1, Darla Landfair1, Stacia Silva1, Roy Turton1, Lixin Feng1, Mark J Mulhivili2, Elizabeth Buck2, Jonathan A. Pachter2, Qun-sheng JF2, Robert Wild1. 1OSI Pharmaceuticals, Inc., Boulder, CO; 2OSI Pharmaceuticals, Inc., Farmingdale, NY.

The insulin-like growth factor-1 receptor (IGF-1R) has emerged as a potentially viable target for the development of novel cancer therapeutics. IGF-1R is primarily activated by its cognate ligands, insulin-like growth factor-1 (IGF-1) and -2 (IGF-2). Ligand binding results in receptor autophosphorylation and stimulation of IRS, leading to the activation of PI-3K/Akt/MTOR and Grb2/Sos/Ras/MAPK signaling pathways that are important for tumor cell survival and growth. IGF ligands are upregulated in several tumor types in which IGF-1R is expressed, providing rationale to investigate this target for the development of a novel anti-cancer therapeutic. Several biologic approaches including antibodies targeting the receptor have been pursued. However, very few small molecule inhibitors interfering with the kinase activity of IGF-1R have advanced to the clinical setting. Here we report the preclinical in vivo pharmacology profile of OSI-906, a potent small molecule kinase inhibitor of IGF-1R that has recently entered Phase I clinical trials. OSI-906 has favorable pharmacokinetic properties including linear exposure in rodents and good oral bioavailability in mice and higher species. In a pharmacodynamic model utilizing the GEO colon carcinoma xenograft, a single oral dose of OSI-906 at 60 mg/kg provided significant target inhibition (80%) for up to 24 hours as measured by loss of phospho-IGF-1R protein in xenograft tumors. The PK/PD profile of OSI-906 correlated to substantial tumor growth inhibition in several xenograft models (GEO, Colo-205, cal-62). As the kinase domains of IGF-1R and insulin receptor are highly homologous we also observed hyperglycemia in association with OSI-906 treatment. A once daily schedule of OSI-906 at 60 mg/kg resulted in transient increases in blood glucose levels. A pharmacodynamic study revealed that while OSI-906 inhibited IGF-1R for 24 hours it only transiently inhibited insulin receptor. This was consistent with modest selectivity of the compound for IGF-1R vs. insulin receptor observed at the cellular level. Moreover, we found that a low BID dose schedule of OSI-906 was able to reduce the hyperglycemia while retaining similar anti-tumor efficacy. These results suggest that alternate dosing schedules may be employed to minimize the potential hyperglycemia effects. In summary, our data suggest that OSI-906 is a novel potent inhibitor of IGF-1R with a promising preclinical pharmacology profile. OSI-906 may have utility in tumor types that are dependent on IGF-1R tyrosine kinase activity. Hence, phase I clinical trials are currently ongoing to investigate its clinical potential.

**B245 Design and synthesis of inhibitors of cyclin-dependent kinases as potential antitumor agents.** Francesco Marchetti1, Nicola J. Curtin1, Bernard T. Golding3, Roger J. Griffin1, Ian R Hardcastle1, David R. Newell1, Lan Zhen Wang1. 1Northern Institute for Cancer Research, Newcastle upon Tyne, United Kingdom.

Cyclin-dependent kinases (CDKs) are a class of serine/threonine protein kinases that regulate progression of the eukaryotic cell-cycle, and act especially at cell-cycle checkpoints.[1] Aberrant cell-cycle progression is associated with loss of checkpoint function, which correlates with increased CDK activity in human tumours.[2] On this premise CDK inhibitors may have therapeutic benefits for the treatment of cancer and other proliferative diseases.[3]

A structure-based approach to inhibitor design resulted in the identification of 2-arylamino-9H-alkylxypyrindines as potential inhibitors of CDK2. These were exemplified by NUN102 (4-(6-(cyclohexylmethoxy)-9H-purin-2-ylamino)benzenesulfonamide; IC50 = 5 nM). Structure-activity studies with this inhibitor class revealed that the purine core is not a prerequisite for potent inhibition of CDKs. Thus, compounds containing the 2,6-diamino-4-alkylxypyrindine pharmacophore have been recognized as an alternative class of CDK inhibitors. Comparable activity to the purines has been found in the corresponding pyrimidines, where an intramolecular hydrogen bond between a 5-substituent, e.g. nitroso, and a 6-amino group imposes a ‘purine-mimetic’ structure (4-(4-amino-6-(cyclohexylmethoxy)-5-nitrosopyrimidin-2-ylamino)benzenesulfonamide; IC50 = 1 nM versus CDK2).[4]

Results for the corresponding Oα-alkylxypyrindines guided systematic structural modifications at the pyrimidine 2-, 4- and 5-positions.[5] Replacement of the 5-nitroso substituent of the parent inhibitor by a group that is more acceptable from a toxicological standpoint has been one of the most prominent goals of this work. The synthesis and structure-activity relationships for the new series of CDK inhibitors, exemplified by 4-(4-amino-6-(cyclohexylmethoxy)-5-formylypyrimidin-2-ylamino)benzenesulfonamide (IC50 = 49 nM versus CDK2), will be discussed in this presentation.

B246 The kinase inhibitor sorafenib induces cell death through a process involving induction of ER stress. Mohamed Rahman1, Eric Maynard Davis1, Timothy Ryan Crabtree1, Joseph Reza Habibi1, Tri K. Nguyen1, Paul Dent1, Steven Grant1. Virginia Commonwealth University, Richmond, VA.

Sorafenib, also known as BAY 43-9006, is a multi-kinase inhibitor that induces apoptosis in human leukemia and other malignant cells. Recently, we demonstrated that sorafenib diminishes Mcl-1 protein expression by inhibiting translation through a MEK1/ERK1/2 signaling-independent mechanism, and that this phenomenon plays a key functional role in sorafenib lethality (Rahman et al., Journal of Biological Chemistry 280:35217-27, 2005). Here, we report that inductive expression of constitutively active MEK1 fails to protect U937 human leukemia cells from sorafenib-mediated lethality, indicating that sorafenib-induced cell death is unrelated to MEK1/ERK1/2 pathway inactivation. Notably, treatment with sorafenib induced ER stress manifested by immediate cytosolic calcium mobilization, GADD153 and GADD34 protein induction, PERK and eIF2α phosphorylation, XBP1 splicing, and a general reduction in protein synthesis as assessed by [35S]methionine incorporation. Notably, genetic disruption of PERK (e.g., transfection of cells with DN-PERK), but not PKR, HRI, or CGN2, attenuated eIF2α phosphorylation, identifying PERK as the primary eIF2α kinase in this setting. These events were accompanied by the pronounced generation of reactive oxygen species (ROS) through a mechanism dependent upon cytosolic calcium mobilization and a significant decline in GRP78/Bip protein levels. Interestingly, enforced expression of IRE1α or XBP1 splice variant (XBPs) significantly reduced sorafenib-mediated apoptosis, whereas knockdown of IRE1α or XBP1, disruption of PERK activity, or inhibition of eIF2α phosphorylation enhanced sorafenib-mediated lethality. Such observations suggest that these events comprise a cytoprotective component of the sorafenib-induced ER stress response. Finally, downregulation of caspase-2 or caspase-4 by siRNA or shRNA respectively significantly diminished apoptosis induced by sorafenib in these cells, providing further support for the notion that the lethal actions of sorafenib involve induction ER stress. Together, these findings demonstrate that ER stress represents a central component of a MEK1/ERK1/2-independent cell death program triggered by sorafenib. They also raise the possibility that induction of ER stress by sorafenib in human leukemia cells may play a role in determining interactions between this kinase inhibitor and other targeted agents.

B247 A pan Her inhibitor, BMS-599626, augments radiosensitivity of HNS cells by increasing radiation-induced apoptosis and inhibiting DNA repair. Mylin A. Torres1, Uma Raju1, Luka Milas1, Kie-Kian Ang1. The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Purpose: BMS-599626 is a small molecule kinase inhibitor that primarily inhibits Her1 and Her2. Thus, it has potential to be an effective antitumor agent and improve the efficacy of cytotoxic treatments including radiotherapy, possibly more so than agents that affect a single Her receptor. The present investigation tested whether BMS-599626 enhances in vitro radiosensitivity of human head and neck squamous cell carcinoma cell lines, and if so, what are the underlying mechanisms.

Methods: HN-5 and UMSCC-10 cell lines were chosen for the study, as HN-5 cells express high levels of Her1 and Her2 proteins and UMSCC-10 cells express Her2 only and no detectable amount of Her1 protein. The effect of BMS-599626 on cell proliferation was determined by MTT assay. The cells were irradiated with graded single doses of γ-irradiation with or without the presence of BMS-599626 (300nM or 600nM). The drug was added to cell cultures 6 h before and kept in the medium for 6 h after irradiation. The cells were allowed to form colonies in drug free medium for 10 more days to assess clonogenic cell survival. At specific time points after BMS-599626, irradiation, or both, cells were assessed for apoptosis, cell cycle distribution, and for protein expression related to the cell cycle, apoptosis and DNA repair.

Results: BMS-599626 (50 nM - 1μM, 72 h) inhibited the growth of HN-5 cells but not UMSCC-10 cells. When combined with radiation, BMS-599626 (300nM or 600nM) significantly enhanced the radiosensitivity of HN-5 but not UMSCC-10 cells assessed by clonogenic cell survival. The enhancement factor at survival fraction of 0.5 was 1.30 for 300 nM and 1.43 for 600 nM of BMS-599626. In HN-5, but not in UMSCC-10 cells, BMS-599626 increased radiation-induced apoptosis and induced accumulation in the G1 cell cycle phase. At the molecular level, in HN-5 cells the agent inhibited the expression of EGFR, HER2, cyclin E, pRb, pAKT, pCDK1/2, and Ku70 proteins. In addition, BMS-599626 prolonged the presence of γ-H2AX foci up to 24 h after radiation assessed by immunocytochemistry.

Conclusion: BMS-599626 significantly enhanced the radiosensitivity of HN-5 cells, expressing both Her1 and Her2, but had no significant effect on radiosensitivity of UMSCC-10 cells expressing Her2 only. The mechanisms involved in the observed enhancement were multiple including induction of apoptosis, cell cycle rearrangement and induction of DNA repair. These in vitro data suggest that BMS-599626 has potential to increase tumor response to radiotherapy and warrants further investigation using in vivo tumor models.

B248 A Phase I dose-escalation study of the safety, pharmacokinetics (PK) and pharmacodynamics of XL880, a VEGFR and MET kinase inhibitor, administered daily to patients (pts) with advanced malignancies. Geoffrey I. Shapiro1, Elizabeth Heath2, Lisa Malburg3, Bruce DeZube4, Dale Miles5, Harold N. Keer6, Andrew X. Xu7, Theresa Laeder8, Patricia LoRusso9, 1Dana Farber Cancer Institute, Boston, MA; 2Karmanos Cancer Institute, Detroit, MI; 3Beth Israel Hospital, Boston, MA; 4Exelixis, Inc., South San Francisco, CA; 5Massachusetts General Hospital, Boston, MA.

Background: XL880 is a potent orally available small molecule inhibitor of MET and VEGFR2/KDR, that also inhibits PDGFR, KIT, FLT3, and Tie-2. Significant tumor growth inhibition was observed preclinically following XL880 treatment in multiple tumor models. In a previous phase 1 study (XL880 dosed on days 1-5 every 14 days) anti-tumor activity was observed. This study evaluates daily oral administration of XL880, with results reported for a range of mechanism-related biomarkers.

Methods: This is a Phase 1 cohort dose escalation study. Pts with advanced malignancies were administered XL880 daily. Safety, tolerability, pharmacodynamics and pharmacokinetics are evaluated. Dose limiting toxicities (DLTs) occurring in the first 28 days of treatment are used to determine the maximum tolerated dose (MTD). Response is assessed every 8 weeks by RECIST criteria. Plasma samples were analyzed for XL880 and for pharmacodynamic biomarkers that have been shown in clinical and/or pre-clinical studies to be modulated in response to treatment with anti-angiogenic agents.

Results: A total of 24 pts have been treated across 4 dose levels (60-120 mg/day) in the following order: 60 (6 pts), 80 (3 pts), 120 (3 pts), 100 (3 pts) and 80 (9 pts) mg/day. Reported DLTs were hypertension and dehydration at 120mg/day, diarrhea at 100 mg/day, and fatigue at 80 mg/day. The MTD was considered to be 80 mg/day though some pts have had stable disease, 9 for ≥ 8 months. The safety profile is consistent with that seen in the prior phase 1: the AEs (≥ 10%) considered related to XL880 were hypertension, fatigue, gastrointestinal events (diarrhea, nausea, and vomiting), anorexia, and laboratory abnormalities (ALT, LDH increased, and blood in urine). All these events were of CTCAE Grade 1-2. A preliminary PK analysis indicates a mean Cmax of 26.7 ng/ml and mean AUC of 362 hr*ng/ml for the 80 mg/day cohort on Day 1. AUC generally increased with increasing dose, although high interpatient variability was observed. XL880 accumulated approximately 3- to 5-fold with repeated daily dosing across all cohorts (60 - 120 mg/day). Preliminary pharmacodynamic analysis of plasma samples demonstrated a statistically significant increase in levels of PIGF, as well as increased levels of VEGF-A after administration of XL880. A statistically significant reduction in sVEGFR2, and reduced levels of Ang2 were also observed. In addition, a trend toward increased levels of the potential mechanism-of-action-related biomarker sMET was detected.

Conclusions: XL880 was generally well tolerated at 80 mg/day dosed
for 28 days (protocol-defined MTD). The safety profile observed is similar to that seen with intermittent dosing. Exposure to XL880 generally increased with increasing dose, and XL880 accumulated in plasma with repeated daily dosing. Changes in anti-angiogenic pharmacodynamic markers, in addition to the observed hypertension, indicate anti-VEGFR activity of XL880 in patients. Increased plasma levels of SMET suggest that XL880 is also inhibiting MET in patients. The observed tumor shrinkage or prolonged SD (> 3 months) in patients with various cancers indicate that XL880 may have antitumor activity.

B249 A Phase 2 study of the dual MET/VEGFR2 inhibitor XL880 in patients (pts) with papillary renal carcinoma (PRC). Robert W Ross1, Ramaprasad Srinivasan2, Ulka Vaishampayan3, Ronald Bukowski4, Jonathan Rosenberg5, Peter Eisenberg6, Theodore Logan7, Sandhya Srinivas8, Mark Stein9, Thomas Mueller10, Harold N Keer10. 1Dana Farber Cancer Institute, Boston, MA; 2Urologic Oncology Branch, National Cancer Institute, Bethesda, MD; 3Karmanos Cancer Center, Detroit, MI; 4Cleveland Clinic, Cleveland, OH; 5University of California, San Francisco, San Francisco, CA; 6California Cancer Care, Greenbrae, CA; 7Indiana University Cancer Center, Indianapolis, IN; 8Stanford University Cancer Center, Stanford, CA; 9Cancer Institute of New Jersey, New Brunswick, NJ; 10Exelixis, Inc., South San Francisco, CA.

Background: XL880 is a potent, orally available small molecule inhibitor of MET and VEGFR2/KDR. Activating mutations or amplifications in MET have been described in pts with PRC. In a phase 1 study, XL880 dosed intermitently was generally well tolerated and resulted in partial responses (PR) in 3 of 4 PRC pts enrolled, of which two are durable after 25 and 12+ months respectively.

Methods: This is a Phase 2 study enrolling pts diagnosed with PRC with MET status (presence or absence of activating mutation) characterized in the germ line (all) and tumor (where possible). Pts with PRC, adequate organ function and ECOG 0-2, receive XL880 at 240 mg/day on Days 1-5 of 14 day treatment cycles. Response is assessed every 8 weeks by RECIST criteria. Optimal tumor biopsies are analyzed by immunohistochemistry (IHC) for XL880 effects on drug targets and downstream readouts. Plasma markers reflecting effects of anti-angiogenic therapy such as VEGF-A, sVEGFR2 and Ang2 are analyzed.

Results: Preliminary data are reviewed for 19 pts (17 germ line MET wildtype, 2 with a germ line MET mutation). Of 18 evaluable pts, 14 had decreases in tumor size at the first 8-week evaluation (range 1-25%). Fifteen pts (12 still active) have had stable disease (11 for ≥ 3 months, 3 for ≥ 6 months and 1 still active at 12 months). As of 8-August-07, 1 pt showed uPR (unconfirmed PR). Safety data (cut-off 1-June-07) show the following AEs considered related to XL880, and reported in at least 2 pts: hypertension (6 pts, 38%), diarrhea, dizziness, fatigue, and headache (each in 2 pts, 13%). There were no related AEs of CTCAE Grade 4 and 5. A preliminary pharmacokinetic analysis indicated that mean pre-dose (ie, approximate trough) concentrations of XL880 relative to the 5th day of the first 14 day cycle (ie, 105 ng/mL) were generally consistent with values seen in the MTD in Phase 1 (ie, 132 ng/mL), and Day 5 pre-dose concentrations appeared generally unchanged from cycle to cycle. IHC analysis comparing baseline and post-dose biopsies demonstrated a marked increase in apoptosis and a decrease in proliferation in lymph node metastases from a pt with sporadic PRC with wildtype MET. In the majority of pts analyzed, administration of XL880 resulted in statistically significant changes in plasma levels of PIGF-VEGF-A, sVEGFR2, and EPO: pharmacodynamic biomarkers which have been shown to be modulated in response to treatment with anti-angiogenic agents. A trend toward reduced Ang2 levels after XL880 treatment was also observed. These findings indicate potential pharmacodynamic inhibition of VEGF signaling by XL880, and are consistent with the observation of hypertension in some patients. In addition, the potential mechanism-of-action-related biomarker sMET was increased in a statistically significant manner.

Conclusions: In pts with PRC, XL880 demonstrated antitumor activity. The majority of the pts experienced prolonged stable disease (including tumor shrinkage). One pt had SD for 5 months and showed uPR at Week-23 CT evaluation. XL880 was generally well tolerated in this pt population. PIGF, VEGF-A, sVEGFR2, EPO, Ang2, and sMET may be promising pharmacodynamic markers to monitor the biological activity of XL880.

B250 XL765 targets tumor growth, survival, and angiogenesis in preclinical models by dual inhibition of PI3K and mTOR. A. Douglas Laird1, 1Exelixis, Inc., South San Francisco, CA.

The PI3K pathway is frequently dysregulated in cancer cells and is implicated in multiple aspects of tumor pathobiology, including tumor growth, survival, angiogenesis, and dissemination. In particular, activating mutations in PIK3CA, the gene encoding the catalytic subunit of PI3K (p110α), and/or loss of function/deletion mutations in the gene encoding its antagonist, the PTEN tumor suppressor, have been found in high frequencies over a wide range of tumor types. In addition, resistance to many anticancer agents (including receptor tyrosine kinase inhibitors and genotoxic agents) has been attributed to failure to downregulate PI3K pathway signaling. Current inhibitors of this signaling pathway include rapamycin and related molecules, which specifically inhibit the mTOR/Raptor complex. However, inhibition of mTOR/Raptor can lead to upregulation of PI3K activity, reflecting alleviation of an mTOR/Raptor-dependent negative feedback loop, with consequent activation of AKT-dependent survival pathways.

XL765 is a potent, orally bioavailable inhibitor of both PI3K and mTOR. XL765 inhibits all four Class I PI3K isoforms and mTOR with IC50 values in the nanomolar range in biochemical assays, yet is highly selective against a panel of over 130 other human kinases. XL765 inhibits PI3K-dependent production of the second messenger PIP3, and nutrient-stimulated mTOR-dependent signaling in cellular assays. Moreover, XL765 inhibits PI3K- and/or mTOR-dependent phosphorylation of key PI3K pathway components including AKT, the AKT substrates PRAS40 and GSK3β, the p70S6K substrate S6, and 4E-BP1 in diverse cancer cells. Oral administration of XL765 to mice bearing xenografts of MCF-7 breast adenocarcinoma cells (positive for an activating mutation in PIK3CA) or PC-3 prostate adenocarcinoma cells (PTEN-deficient) resulted in significant inhibition of PI3K and mTOR signaling. XL765 significantly slowed tumor growth or caused tumor shrinkage in multiple xenograft tumor models, including breast, lung, ovarian, prostate, and brain cancers. These effects were correlated with inhibition of tumor cell proliferation and tumor angiogenesis, and with induction of apoptosis. In contrast, rapamycin inhibited proliferation but caused little or no induction of apoptosis. Consistent with these differences, substantial regression of tumors treated with XL765 but not with rapamycin was observed after cessation of dosing in a NSCLC xenograft model. These data suggest that a dual inhibitor strategy, targeting both PI3K and mTOR, may offer significant advantages over specifically targeting the mTOR/Raptor complex. XL765 is currently being administered to patients with solid tumors in a Phase I clinical trial.

B251 AT13148, an orally bioavailable AKT kinase inhibitor with potent anti-tumor activity in both in vitro and in vivo models exhibiting AKT pathway deregulation. John F. Lyons1, Kyla M. Grimshaw1, Steven J. Woodhead1, Ruth E Feltell1, Matthias Reule1, Tomko Smyth1, Lisa C Seavers1, Isobelle Harada1, Jacqueline Higgins1, Donna M. Smith1, Lynsey Fazal1, Paul Workman1, Ian Collins1, Michelle D Garrett2, Neil T. Thompson1. 1Astex Therapeutics Ltd, Cambridge, United Kingdom; 2The Institute of Cancer Research, Sutton, United Kingdom.

The AKT pathway is an important mediator of tumor cell growth and survival and activation of this pathway is associated with several resistant forms of cancer. This activation can occur by a number of different mechanisms targeting AKT itself or components of the pathways leading to its activation.

AT13148 is a novel, small molecule inhibitor of the AGC kinases AKT, ROCKII and PKA developed from a series identified using fragment-based medicinal chemistry linked to high throughput X-Ray crystallography. AT13148 potently inhibits the AKT enzyme with an IC50 of 11nM. Additional kinase cross-reactivities of the compound are limited to a small
number of AGC kinases. A broader selectivity screen confirmed that the compounds activity was restricted to this subset of kinases. Example IC$_{50}$ values versus a range of AGC kinases were as follows: AKT (11nM), ROCKII (3nM), PKA (3nM), PKM2 (12nM), Rsk-1 (350nM), SGK (3200nM).

In vitro studies demonstrate that AT13148 inhibits AKT pathway activation in a range of AKT-dependent tumor cell lines and that this inhibition is closely correlated with an anti-proliferative, pro-apoptotic action. The compound shows oral bioavailability and has moderate to low plasma clearance across several species. In vivo studies using mouse xenograft models demonstrate that AT13148 has antitumor effects at 40-50mg/kg. The compound is especially effective in an endometrial tumor xenograft line (MES-SA) deficient in PTEN, a negative regulator of the AKT pathway. As a consequence of this defect the AKT signalling pathway is hyperactive in this model assay system. Doses of AT13148 that inhibit tumor growth in the MES-SA model also modulate the activity of components of the AKT pathway in the tumors. Translational biology studies demonstrated the dose and time dependent inhibition of activity of several pharmacodynamic pathway markers in tumors taken from treated mice. The induction of apoptosis as a downstream consequence of these pathway effects was also shown.

The in vitro and in vivo efficacy of AT13148 against the AKT pathway supports the progression of this molecule into pre-clinical evaluation as a clinical candidate.

**B254** Phase II trial of BAY 43-9006 (sorafenib-BAY) in metastatic melanoma (MM) including detection of BRAF mutations with mutant specific-PCR (MS-PCR) and altered proliferation pathways. Christina Min1, Leonard Liebes1, Peter Brooks1, Joanne Yoon1, Herman Yee1, Anne Hamilton2, Michael Buckley3, John Wright4, Iman Osman5, Franco Muggia6, Anna C Pavlick7.1NYU Cancer Institute, New York, NY; 2Sydney Cancer Center, Sydney, Australia; 3CTEP/NCI, Bethesda, MD.

**Background:** BRAF mutations (mu) have been commonly identified in MM. A phase II trial of BAY, a multikinase inhibitor that inhibits the BRAF pathway was conducted in MM to: 1) determine if treatment (tx) with BAY alters proliferation as measured by cyclin D1, Ki-67 and ERK 2) assess for differential anti-tumor responses and 3) assess a newly developed assay to detect muBRAF (MS-PCR).

**Methods:** Eligibility: Biopsy (bx)-accessible, untreated MM with measurable disease (RECISt). Stratification: Tumor BRAF status by routine PCR sequencing for codon 600 mu prior to tx and fluorescent MS-PCR for confirmation. (MS-PCR specifically amplifies the muBRAF allele without amplifying the wild-type (wt) allele). Tx: BAY 400 mg po BID D1-28 q4w. Serial studies: Repeat bx on day 28 assessed for Ki-67, cyclin-D1 and ERK; serum collagen cryptic epitopes; re-imaging every 2 cycles and pts treated until progression (PD). Two stage trial design closed at second stage.

**Results:** 37 pts (11-M1a, 10-M1b, 16-M1c) enrolled. 32 wt and 5 muBRAF by routine PCR. Due to the low yield of mu on routine PCR, MS-PCR was done with 6 mu detected by MS-PCR versus 2 on routine sequencing among first 16 pts enrolled. Median age: 68 (range 22-91); LDH $\geq$ 1.5 x n1 in 12.

**Toxicity:** Gr I diarrhea (7), alopecia (4), rash (6), mucositis (4), nausea (4), pain (4), hand-foot (2); Gr II HTN (4), fatigue (2), mucositis (1), rash (3), pain (3), hand-foot (2); Gr III hand-foot (1), rash (1), fatigue (1), intestinal perforation (1). Responses: muBRAF -1 PR (lymph nodes and subcutaneous arm masses) and 4 PD; wtBRAF -2 PR, 6 SD and 12 PD after 2 cycles. Collagen cryptic epitopes correlated with tumor responses; matched paired biopsies demonstrated down regulation of tumor Ki-67, erk and cyclin-D1 in PRs and SD. Assessment was incomplete in 12 (8 early PD, 1 toxic drop-out and 1 withdrew prior to tx and 1 no eval tumor).

**Conclusions:** Few short-term responses were seen in both mu and wt strata in this single agent BAY trial. Down-regulation of proliferation markers was demonstrable, and MS-PCR improved the detection of muBRAF. Supported by NCI N01-CM17103 and TRI 25XS091.
B256 Synergistic combinations of a novel PI3 Kinase inhibitor with Erlotinib and Rapamycin and the associated effects on downstream signalling. Lara Kevorkian\textsuperscript{1}, Andrew Payne\textsuperscript{1}, Colin Stubberfield\textsuperscript{1}. \textsuperscript{1}UCB, Slough, United Kingdom.

It is now common practice to treat cancer patients with a combination of agents to increase upon the therapeutic benefit achievable by a single agent. It is therefore essential to investigate the activity of new agents in combination with existing therapies prior to embarking upon clinical trials. The phosphatidylinositol 3 kinase (PI3K) signalling pathway impacts upon many cellular processes, including cell proliferation, growth and survival. Deregulation of PI3K signalling is common in many types of cancers and hence this pathway has become an attractive target for the development of small molecule inhibitors. Here we investigate in vitro whether combining a PI3K inhibitor with existing therapies might provide additional anti-proliferative effects in tumour cell lines.

In PC3 and SKOV-3 cells we show that PI3K inhibition leads to a synergistic inhibitory effect on proliferation when combined with either the Epidermal Growth Factor Receptor (EGFR) inhibitor Erlotinib, or the mammalian Target Of Rapamycin (mTOR) inhibitor, Rapamycin.

By Western blotting it can be seen that the combination of PI3K and EGFR inhibition results in the blockade of both the PI3K and the MEK pathways and this may be contributing to the synergistic inhibition of cell proliferation observed with these two compounds. In addition, while it is apparent that Rapamycin alone increases the level of phosphorylated AKT (ser473) in PC3 cells, presumably through a feedback mechanism, this effect is blocked by combining with the PI3K inhibitor. Again this may suggest a mode of action by which these two compounds synergistically act to reduce the level of cell proliferation.

In conclusion, the findings indicate that significant benefit can be achieved by combining targeted agents with complementary modes of action.

B257 A Phase 1 study of ARRY-543, a potent, selective, reversible inhibitor of ErbB receptors. Mace L Rothenberg\textsuperscript{1}, K. Kane\textsuperscript{2}, C. Kollmannsberger\textsuperscript{2}, J.D. Berlin\textsuperscript{1}, W. Cooper\textsuperscript{1}, L. Maloney\textsuperscript{3}, G.S. Gordon\textsuperscript{2}, K. S. Litwiler\textsuperscript{2}, S. D’Aloisio\textsuperscript{3}, K. A. Gelmon\textsuperscript{3}. \textsuperscript{1}Vanderbilt Ingram Cancer Center, Nashville, TN; \textsuperscript{2}Array BioPharma, Boulder, CO; \textsuperscript{3}British Columbia Cancer Center, Vancouver, British Columbia, Canada.

\textbf{Background:} ARRY-543 is an ATP-competitive, selective, reversible inhibitor of EGFR and ErbB2, and, in human tumor xenografts, ARRY-543 is active against both EGFR- and ErbB2-driven tumors. This Phase 1 open-label study was designed to determine the MTD and PK of QD and BID dosing schedules.

\textbf{Methods:} Escalating doses of ARRY-543 were administered in successive cohorts (QD and BID regimens) of pts with solid tumors. All pts received a single oral dose followed by one week of safety and PK evaluations. Daily dosing (QD or BID) started on Day 8, with steady state PK assessments on Day 22 and tumor imaging every 2nd Cycle. Results: As of July 2007, 46 subjects have been enrolled: 22M/24F, median age 59 yrs (34-82), ECOG PS of 0 (n=9), 1 (n=32) or 2 (n=5). ARRY-543 was administered at doses of 50, 100, 200 300, and 400 mg QD and 100, 200, 300 and 400 mg BID. The half-life of the drug is approximately 6.6 hours, independent of dose. ARRY-543 showed increasing dose-related drug exposure, with a mean steady-state plasma AUC\textsubscript{24hr} of 71 µg-hr/mL at the 300 mg BID dose and, preliminarily, an AUC\textsubscript{24hr} of 125 µg-hr/mL at the 400 mg BID dose. At both these BID doses, the C\textsubscript{trough} drug concentrations exceeded 2 µg/mL, providing drug levels capable, based on pre-clinical data, of continuously inhibiting the target. In both QD and BID dosing regimes, the most common grade 1/2 adverse events have been rash, fatigue, nausea, diarrhea, and vomiting; both rash and fatigue showing dose-related trends. Two of 4 pts in the 400 mg QD dose cohort experienced DLTs (grade 3 fatigue (1) and grade 3 fatigue and grade 3 diarrhea (1)). Interestingly, no DLTs were observed in the 300 mg BID dose cohort, and only 1 of 6 patients treated at the 400 mg BID dose level has experienced a DLT (rapidly reversible Grade 3 fatigue). This occurred despite substantially higher drug AUCs in both BID cohorts compared to the 400 QD cohort. No objective responses have been observed but, in the 100-300 mg BID cohorts, eight of fifteen patients had stable disease, remaining on therapy for 12 - 32+ wks.

\textbf{Conclusions:} Given once a day, the MTD of ARRY-543 is 300 mg, with fatigue being the DLT at 400 mg. This toxicity was not as prominent on a twice daily dosing schedule and the MTD for ARRY-543 may be ≥ 400 mg BID. Systemic concentrations of ARRY-543 increased with escalating doses at all dose levels tested. Preliminary evidence of clinical activity was long-lasting stable disease. An expansion phase of this study is underway and will provide a preliminary assessment of biological activity in ErbB2+ breast cancer patients and other ErbB-driven cancers.

B258 Preclinical studies and characterization of BMS-698769, a small molecule inhibitor of Met receptor tyrosine kinase. Joseph Fargnoli\textsuperscript{1}, Veraswammy Manne\textsuperscript{1}, Ashok Gupta\textsuperscript{1}, Tai W. Wong\textsuperscript{1}, John T. Hunt\textsuperscript{1}, Johnni Gullo-Brown\textsuperscript{1}, Kristen Kellar\textsuperscript{1}, Robert Jeyaseelan, Sr.\textsuperscript{1}, Barri Wautlet\textsuperscript{1}, Benjamin Henley\textsuperscript{1}, Simone Oppenheimer\textsuperscript{1}, Anne Lewin\textsuperscript{1}, Becky A. Penhallow\textsuperscript{1}, Carmen Raventos-Suarez\textsuperscript{1}, Robert Schmidt\textsuperscript{1}, Daniel Chen\textsuperscript{1}, Robert Borzilleri\textsuperscript{1}.

Bristol Myers Squibb-FRI, Princeton, NJ.

The Met receptor tyrosine kinase is the exclusive high-affinity receptor for the hepatocyte growth factor (HGF) ligand. Met activation can occur by ligand binding or, as identified in multiple types of cancer, by either Met overexpression or a variety of activating mutations. Receptor activation subsequently results in a variety of pleiotropic responses that include cell motility, migration, proliferation, invasion and survival. These processes not only underlie tumor growth and metastasis, but are also critical in normal physiological processes such as wound healing and tissue regeneration.

We have identified a small molecule inhibitor of Met kinase activity, BMS-698769, that inhibits both ligand stimulated and constitutive Met phosphorylation. As a result, HGF induced scattering and migration were observed to be inhibited when cells were treated with this compound. BMS-698769 also suppressed proliferation of tumor cells in which Met was...
constitutively active. Tumor growth inhibition in vivo was observed with the GTL-16 tumor model in which Met is amplified and activated. In addition, Met receptor phosphorylation in tumor tissue from mice treated with varying doses of BMS-698769 was inhibited in both a dose- and time-dependent manner. Because Met conditional knock-out mice exhibit significantly impaired recovery from acute liver injury, we also evaluated the potential of this compound to impair this process in adult mice using a carbon tetrachloride (CCl 4) induced model of liver injury. Our results demonstrate that pharmacologically (maximum antitumor activity) relevant doses of BMS-698769 do not affect recovery from CCl 4 induced liver injury. As a result, these studies suggest a low potential for tissue regeneration liabilities with BMS-698769 administration as well as the potential for targeted therapeutic intervention in cancer.

B259 An exploration of the ability of DCE-CT scans to evaluate blood flow in an open, pharmacokinetic (PK) and mass balance study of [14C]-cediranib. Alison H.M. Reid 1, Adrian Tang 1, James Spicer 1, Elisa Gallarani 1, Dorothy Mears 1, Heather M. Shaw 1, Vasilios Karavasilis 1, Sarah Settattre 1, Timothy A. Yap 1, Cheryl Marriot 1, Thomas A. Puchalski 2, Mike Harrison 1, Jo H. Naish 3, Helen Mann 3, Johann S. De-Bono 1, Royal Marsden School, Sutton, Surrey, United Kingdom; AstraZeneca, Wilmington, DE; AstraZeneca, Alderley Park, Macclesfield, United Kingdom.

Background: Cediranib (AZD2171) is an oral, highly potent and selective vascular endothelial growth factor (VEGF) signaling inhibitor of all three VEGF receptors (VEGFR-1, -2 and -3) that is suitable for once-daily dosing. DCE-CT offers an attractive alternative to DCE-MRI for assessing tumor vascularity as it is widely available, can provide quantitative data and may be more robust in a multi-center setting.

Methods: The primary objective of this open, non-randomized, radiolabeled, single-center study in patients with metastatic solid tumors, was to determine the rates and routes of excretion of cediranib using [14C]-cediranib. Each patient received a single, oral dose of [14C]-cediranib 45 mg; blood samples and all excreta were subsequently collected for PK and metabolic profiling. Once ≥ 90% of the radiolabel had been recovered or there was ≤ 3 x background in urine and feces, patients started once-daily oral dosing with cediranib 30 mg. This study was designed to explore the ability of DCE-CT scans to evaluate blood flow in patients treated with cediranib and to assess the reproducibility of DCE-CT vascular measurements. Antitumor activity (RECIST) was also measured and correlated with DCE-CT outcomes.

Results: Six patients received treatment and remained on study for 64-171 days. The percentage of radiolabeled material recovered in the urine and feces samples within 168 hours post-dosing in 5/6 patients ranged from 84.8-93.0%. The analogous amount recovered in the remaining patient within 168 hours was 34.1%. This may be due to prolonged bowel transit time because of co-administrated morphine. In all patients, mean patient within 168 hours was 34.1%. This may be due to prolonged bowel transit time. The percentage of radiolabeled material recovered in the urine for 171 days. The percentage of radiolabeled material recovered in the urine in whole blood, radioactively material appeared to be confined to total radiolabeled material recovered was 58.8% in feces and 20.8% in urine. In whole blood, radioactive material appeared to be confined to plasma. As previously reported, cediranib was well tolerated. For four of the five DCE-CT parameters measured, a decrease from baseline outside the baseline reference range was recorded for 3 patients at 1 month after the start of daily dosing of cediranib. These reductions were seen in perfusion, permeability surface product (PSP), positive enhancement integral and blood volume, and occurred in the 3 patients (2 renal cell carcinoma; 1 mesothelioma) who had a best response of stable disease (RECIST); including one with a confirmed reduction in maximum tumor diameter of 10% to <30%. These 3 patients also remained on drug for the longest duration (93, 134 and 171 days). For all DCE-CT parameters, intra-patient variability was generally low and was consistently lower than inter-patient variability. The overall component of variability was largest for PSP (69%), whereas the lowest variability was observed for mean transit time (15%).

Conclusions: The primary route of cediranib elimination appears to be hepatic and the majority of radiolabeled material was recovered within 168 hours post-dosing. Cediranib was well tolerated when given as either a single radiolabeled dose (45 mg) or a continuous once-daily unlabeled dose (30 mg). While these are small numbers of patients, cediranib-induced changes in tumor vascular parameters were detected by DCE-CT and reductions in DCE-CT parameters at 1 month were correlated with antitumor activity at 3 months.

B260 Preclinical characterization of BAY 73-4506: A kinase inhibitor with broad spectrum antitumor activity targeting oncogenic and angiogenic kinases. Scott Wilhelm 1, Lila Adnan 1, Claudia Hirth-Dietrich 2, Paul Ehrlich 1, Mark Lynch 1, Bayer HealthCare Pharmaceuticals, West Haven, CT; Bayer HealthCare AG, Elberfeld, Germany.

Background: Tumor growth and progression are dependent of oncogenic signaling networks formed by cellular kinases. Receptor tyrosine kinases (RTK) such as, KIT, RET, PDGFR, and VEGFR are activated in several solid tumors and contribute to tumor progression. Several agents targeting oncogenic RTK have shown anti-tumor activity in the clinic.

Results: BAY 73-4506 is a novel potent inhibitor of the angiogenic RTK (VEGFR-1, -2 and -3 and PDGFR-β), the oncogenic RTK (KIT, RET), and serine/threonine kinases (p38MAPK and RAF). In cellular assays, BAY 73-4506 showed potent activity against VEGFR-2 (IC50 = 4 nM), VEGFR-3 (IC50 = 150 nM), and PDGFR-β (IC50 = 90 nM). Vascular cell proliferation was strongly inhibited in human umbilical vein endothelial cells (IC50 = 4 nM) and human aortic smooth muscle cells (IC50 = 120 nM). BAY 73-4506 inhibited tumor cell proliferation in a subset of human tumor cell lines of different histological types. Furthermore, BAY 73-4506 inhibited activated mutant KIT (KITK642E, IC50 = 20 nM) and RET (RET643W, IC50 = 25 nM) and imatinib-resistant variants of KIT Δ557-558 with a secondary mutation (IC50 range, 12 to 129 nM). BAY 73-4506 inhibited p38MAPK signaling (IC50 = 120 nM) in anisomycin-stimulated DLD-1 cells (phosphorylation of ATF-1 and MSK1/2). BAY 73-4506 demonstrated broad spectrum anti-tumor activity in murine xenograft models, with complete tumor stasis observed in most models when dosed orally at 10 mg/kg QD. Partial tumor responses were observed in Colo-205 (colon), 786-O (kidney), and MDAMB-231 (breast) models at 30 or 100 mg/kg. Immunohistochemistry analysis of BAY 73-4506-treated tumors with an anti-CD31 (endothelial cell marker) antibody showed significant reduction in microvessel area or neoangiogenesis. Consistent with the effects reported for VEGFR signaling inhibitors, using a recombinant VEGF in a rat model, BAY 73-4506 dose-dependently reduced VEGF-mediated decrease in blood pressure (~80% reduction of hypertensive response by 1 mg/kg i.v. BAY 73-4506). Similar results were observed with M2 metabolite of BAY 73-4506.

Conclusions: BAY 73-4506 is a novel kinase inhibitor with potent activity against KIT/RTK, RET, and imatinib-resistant oncogenic and angiogenic receptor tyrosine and ser/thr kinases. BAY 73-4506 possesses a broad spectrum anti-tumor activity in preclinical cancer models and is currently undergoing phase I clinical trials.

B261 A phase I dose-escalation and pharmacokinetic study of sunitinib in combination with docetaxel in patients with advanced solid tumors. F. Robert 1, A. Sandler 1, J. H. Schiller 1, J. Ilagan 2, K. Harper 3, W. Vermeulen 2, G. Liu 4, L. Tyer 4, R. Chao 5, L. Verk 5, A. Traynor 6, 1University of Alabama at Birmingham, Birmingham, AL; 2Vanderbilt University Medical Center, Nashville, TN; 3UT Southwestern, Dallas, TX; 4Pfizer Global Research and Development, La Jolla, CA; 5Pfizer Research and Development, La Jolla, CA; 6University of Wisconsin/Paul P Carbone Comprehensive Cancer Center, Madison, WI.

Background: Sunitinib malate (SU) is an oral, multitargeted tyrosine kinase inhibitor approved nationally for the treatment of advanced renal cell carcinoma (RCC) and imatinib-resistant or -intolerant gastrointestinal stromal tumor. In a mouse xenograft model of breast cancer, SU enhanced the antitumor activity of docetaxel (D). The objective of this multicenter, open-label, phase I study was to determine the maximum tolerated dose (MTD), overall safety, pharmacokinetic (PK) profile, and preliminary efficacy of SU in combination with D in patients (pts) with advanced solid tumors.

Poster Session B Small Molecule Therapeutic Agents: Kinase Inhibitors
Materials and methods: Successful cohorts of pts were to receive oral SU on a 6-week (wk) cycle (4 wks on followed by 2 wks off treatment; 4/2 schedule) or 3-wk cycle (2 wks on followed by 1 wk off treatment; 2/1 schedule) in combination with intravenous D given every 21 days (q21d). Doses of SU were 25, 37.5, or 50 mg daily and D doses were 60 or 75 mg/m². The MTD was the highest dose at which 0 of 3 or 1 of 6 pts encountered dose-limiting toxicities (DLTs) during cycle 1. Safety/tolerability was evaluated using adverse event (AE) reports and clinical laboratory analyses. In patients with measurable disease, objective response was determined by Response Evaluation Criteria in Solid Tumors (RECIST). On Schedule 2/1, serial PK collection was performed at the MTD for Cmax and AUC determination.

Results: 10 pts received SU on the 4/2 schedule and the MTD for the combination was determined to be SU 25 mg + D 60 mg/m². This schedule was not further studied, and only data for Schedule 2/1 are presented. As of May 2007, 38 pts, including 10 with non-small cell lung cancer (NSCLC), were enrolled on the 2/1 schedule and treated in 5 dose escalation cohorts. The most frequently observed grade [G] 3/4 AEs (%) included: fatigue/asthenia (21/0%), leukopenia (13/1%), neutropenia (11/58%), stomatitis/oral discomfort and related oral syndromes (8/0%), hyperglycemia (8/0%), and hypokalemia (8/0%). 5 pts (13%) experienced febrile neutropenia. Manageable/ reversible neutropenia (with or without fever; maximum G4) was the most commonly observed DLT (n=5) and occurred at the following dose levels: SU 25 mg + D 60 mg/m² (n=2/9), SU 50 mg + D 75 mg/m² (n=2/2) and SU 37.5 mg + D 75 mg/m² (n=1/2). Other DLTs included G3 gastrointestinal hemorrhage (n=1); SU 37.5 mg + D 75 mg/m². The MTD was established as SU 37.5 mg + D 75 mg/m². PK data from 8 pts revealed no clinically significant drug-drug interaction with co-administration. To date 2 pts (NSCLC; breast cancer) treated with SU 37.5 mg + D 75 mg/m² had documented partial responses and 10 pts (27%) had stable disease.

Conclusions: The combination of oral SU 37.5 mg/day + D 75 mg/m² IV Q21d on the 2/1 schedule has a manageable safety profile and shows preliminary evidence of efficacy. The regimen is undergoing further study in selected pt populations.

B262 MAPKAP kinase-2 acts as a downstream target for modulating bortezomib response in multiple myeloma. Bastien Cauntain1, Estela Cañon1, Paloma Navarro1, Luis A. Tebar1, Songqing Na2, Raymond Gilmour2, Ana Rodríguez-Lopez1. 1Spanish National Cancer Research Centre, Madrid, Spain; 2Eli Lilly, Indianapolis, IN.

Multiple myeloma (MM) is a lymphoproliferative disease that involves plasma cells and accounts for 7% of hematological malignancies. Proteasome inhibitors are a novel class of anticancer agents that have clinical efficacy against hematological malignancies. The 26S proteasome inhibitor, bortezomib (Velcade, PS-341), selectively induces apoptosis in MM cells; however, the nature of its selectivity remains unknown. Bortezomib has displayed efficacy in phase II/III trials in patients with MM, but use as a single agent has been disappointing due to the emergence of resistance mediated via the activation of anti-apoptotic survival pathways.

The mitogen-activated protein (MAP) kinase p38 is a critical component of the cellular pathway in response to a variety of extracellular stimuli including growth factors, cytokines and cellular stresses such as chemotherapeutic agents. MAPKAPK2 (MK2) is a Ser/Thr kinase that is activated specifically by its upstream kinase, p38. We propose that p38/MK2 activation mediates the resistance associated with proteasome inhibition. Inhibition of p38 sensitizes MM cells to bortezomib-induced apoptosis. HSP27, a direct substrate of MK2, is a stress protein with antiapoptotic properties. Here we show that the p38/MK2 pathway is activated in multiple myeloma cells in response to two proteasome inhibitors with different mechanisms of action (bortezomib and MG132). The induction of apoptosis mediated by proteasome inhibition was dramatically enhanced by a small molecule MK2 inhibitor. The inhibitor alone had no effect on cell viability. Furthermore, the enhanced cell death in combination with chemotherapeutic drugs can be recapitulated using a specific inhibitor of the upstream kinase, p38 or following knockdown of MK2 expression in the same cellular models. Taken together, our data indicate that upregulation of MK2 activity mediates a protective response in tumor cells; therefore, MK2 has the potential to be a good target for enhancing the activity of chemotherapeutic agents such as bortezomib.

B263 Phase II study of Cediranib (RECENTIN™, AZD2171) in recurrent epithelial ovarian cancer. Ursula Matulonis1, Suzanne Berlin2, Carolyn Krasner3, Karin Tyburski4, Julie Lee1, Maria Roche1, Percy Ivy5, Richard Penenson6. 1Dana-Farber Cancer Institute, Boston, MA; 2Massachusetts General Hospital, Boston, MA; 3National Cancer Institute, Bethesda, MD.

Background: Cediranib is a highly selective and potent oral tyrosine kinase inhibitor (TKI) of VEGFR1, VEGFR2, c-Kit, and VEGFR3. Angiogenesis is important for ovarian cancer growth, and blocking angiogenesis can lead to ovarian cancer regression.

Methods: This is a CTEP-sponsored phase II study (NCI#7102) of single agent cediranib for recurrent ovarian, peritoneal, or fallopian tube cancer. Eligibility include: previous platinum-based chemotherapy, up to 2 prior lines of chemotherapy in the recurrent setting, ECOG PS of 0 or 1, controlled BP, and normal organ function. 23 patients have been enrolled thus far. Primary endpoint of the study is response rate measured either by RECIST or modified GCIG CA125 criteria, and secondary endpoints include progression-free survival, toxicity, and PD endpoints.

Results: Of the first 23 patients (pts) enrolled, 18 are evaluable for toxicity and tumor response; the remaining 5 pts have not been on study drug long enough for toxicity and response evaluation. 15 of the 18 evaluable pts have recurrent ovarian cancer; 3 had recurrent peritoneal cancer. 8 pts had platinum-sensitive and 10 had platinum-resistant cancer. The starting Phase II dose was 45 mg PO, but because of toxicities observed in the first 11 patients, the dose was lowered to 30 mg for subsequent pts. In the first 18 pts, grade 3 toxicities include: HTN (n=8; 5 pts on 45 mg and 3 pts on 30 mg), fatigue (n=5), abdominal pain (n=1), diarrhea (n=4), vomiting (n=2), oral cavity pain (n=2), nausea (n=1), constipation (n=1), hyponatremia (n=1), and hypothyroidism (n=1). One pt had grade 4 CNS hemorrhage (45 mg dose). No cardiac toxicities nor bowel perforations or fistulas have been reported thus far. Grade III thyroid toxicities were reported. Of tumor responses, 3 pts have had confirmed PR’s (1 pt with platinum-sens and 2 platinum-resistant cancer and all on 45 mg) lasting 11, 12, and 25 weeks (overall RR of 17%), and 2 SD lasting 30 weeks and 16+ weeks. The remaining 13 pts have been removed for either toxicities or PD.

Conclusions: Cediranib is an active drug in the treatment of recurrent ovarian cancer with an acceptable toxicity profile, similar to that observed with other TKI’s and warrants further study.

B264 CEP-11981: A potent TIE-2/Pan-VEGFR inhibitor with broad kinase inhibitory activity exhibits significant antitumor and antiangiogenic efficacy in preclinical tumor models. Bruce A. Ruggieri1, Ted Underner1, Diane Gingrich1, Robert Hudkins1, Thelma Angeles1, Mark Alborn2, Hong Chang3, Candy Robinson1, Kathryn Hunter1, Pawel Dobrzanowski3, Susan Jones-Bolin1, Lisa Aimeone1, Mark Ato1, Jean-Marc Herbert2, Françoise Bon2, Paul Schaefer1, Pierre Casellas1, Bernard Bourie1, John Mallamo1, Jeffry Vaught1. 1Cephalon Inc, West Chester, PA; 2Sanofi-Aventis, Toulouse, France; 3Sanofi-Aventis, Montpellier, France.

The identification of novel anti-angiogenic agents with favorable drug-like properties and the ability to induce effective tumor responses (regressions) across multiple tumor types is a major goal of oncological drug discovery efforts. The therapeutic efficacy of anti-angiogenic agents may be improved by inhibition of a combination of multiple angiogenic and tumor-specific targets. CEP-11981, a C3-(2-aminopyrimidine) dihydroindolocarbazole is a potent and highly-cell permeable multiplex inhibitor of human TIE-2, VEGF-R2, and VEGF-R1 receptor tyrosine kinases (IC50 = 22 ± 6, 4 ± 1, and 3±1 nM, respectively), in enzyme-based assays, with a kinase selectivity index, S(90) against 217 kinases of 0.25 at
B265  Biomarker development for XL765, a potent and selective oral dual inhibitor of PI3K and mTOR currently being administered to patients in a Phase I clinical trial. Amita Patnaki1, Patricia M. LoRusso2, Josep Taberner3, A. Douglas Laird4, Sanjay K Aggarwal5, Kyriakos P. Papadopoulos1. 1South Texas Accelerated Research Therapeutics, San Antonio, TX; 2Wayne State University, Detroit, MI; 3Yale d’Hebreun University Hospital, Barcelona, Spain; 4Exelixis, Inc., South San Francisco, CA.

Activation of PI3K results in increased formation of the lipid PIP3 from PIP2. This results in recruitment of AKT to the plasma membrane and its subsequent activation. In turn, activation of AKT ultimately leads to activation of mTOR, which phosphorylates p70 S6K and 4E-BP, culminating in increased protein translation. In addition, activated AKT elicits prosurvival and other effects via additional signaling pathways. The PI3K pathway is frequently dysregulated in cancer cells and is implicated in multiple aspects of tumor pathobiology, including tumor growth, survival, angiogenesis, and dissemination. In particular, activating mutations in multiple pathway components, including AKT, the AKT substrate PRAS40, the p70 S6K substrate S6, and 4E-BP1. Robust PI3K pathway signaling was evident in the blood of healthy volunteers as assessed by a reduction in phosphorylation of multiple pathway components, including AKT, the AKT substrate PRAS40, p70S6K, and 4E-BP1. Robust PI3K pathway signaling was evident in the blood of healthy volunteers as assessed by a reduction in phosphorylation of multiple pathway components, including AKT, the AKT substrate PRAS40, p70S6K, and 4E-BP1.

XL765 reduced AKT phosphorylation in buccal mucosal smears from healthy volunteers. A Phase I dose-escalation study of the safety and pharmacokinetics of XL765 administered twice daily orally to subjects with solid tumors has been initiated. The primary objectives of this study are to evaluate the safety and tolerability of XL765 and to determine the maximum tolerated dose of XL765 administered twice daily orally for 28 days. Other objectives include evaluation of the pharmacokinetics and pharmacodynamic effects of XL765. Preliminary results from this study will be presented, including available pharmacokinetic and safety data as well as pharmacodynamic analyses of peripheral blood cells and other tissues using the approaches outlined above.

Other Small Molecule Therapeutic Agents

B266  Novel highly potent and selective alpha5beta1 integrin antagonists as anticancer treatments. Claudia Christner-Albrecht1, Doerte Vossmyer1, Roland Strajges2, Gunther Zischinsky3, Grit Zahn1, Ilhan Celik2, Carsten Dietz2, Mohammed Souilaiman2. 1Jenri AG, Berlin, Germany; 2Universitätsklinikum Giessen und Marburg, Marburg, Germany.

The fibronectin receptor alpha5beta1 integrin has been suggested to be a promising oncology target. However, proof-of-concept studies have so far been limited to function-blocking alpha5beta1 antibodies and non-selective peptide antagonists. Jenri AG was first to develop highly potent, orally available small molecule compounds that specifically inhibit alpha5beta1 integrin at sub-nanomolar IC50 values in an ELISA assay. These compounds were shown to affect a variety of oncogenic and metastatic processes in vitro. In particular, they inhibit adhesion of alpha5beta1-positive tumor cells (e.g. K562) to fibronectin at low nanomolar concentrations. Furthermore, the alpha5beta1 integrin antagonists diminish endothelial cell proliferation, migration, sprouting and alpha5beta1 integrin downstream signaling events like Erk phosphorylation.

Different alpha5beta1 antagonists were used as a tool to demonstrate in vivo proof-of-concept in a variety of rodent tumor models. As a result, the compounds have significant in vivo efficacy in multiple mouse tumor models. For example, they inhibit growth of HT29, A549, MDA-MB-231 and BxPC3 tumors in a dose-dependent manner. Besides, the compounds prolong animal survival in the clinically relevant, orthotopic rat prostate tumor model 13762NF MTLn3 by significantly reducing lung metastasis. The alpha5beta1 integrin antagonists exert their effects by targeting of multiple oncogenic processes in vivo including tumor angiogenesis, tumor cell proliferation and metastasis. Treatment with Jenri’s development candidate is well tolerated. No effect on body weight gain and no adverse events were observed at tested doses of up to 200 mg/kg/day.

Taken together, we are presenting sound evidence for the potential of selective alpha5beta1 antagonists as efficacious and safe treatment for multiple tumor indications.

B267  Zinc-binding compounds induce cancer cell death via distinct modes of action. Wei-Qun Ding1, Stuart E. Lind2. 1University of Oklahoma Health Science Center, Oklahoma City, OK; 2University of Colorado Health Science Ctr., Aurora, CO.

Metal binding compounds have been shown to have antitumor activity and are being evaluated clinically as anticancer agents. It is not known if different compounds binding a given metal induce cytotoxicity by similar mechanisms. We have recently found that one such compound, 5-chloro-7-iodo-8-hydroxyquinoline (cliquinol), not only binds metals such as zinc, but is able to transport zinc into cells. We therefore compared the action of cliquinol with two other cytotoxic zinc-binding compounds, N,N,N',N'.
tetraakis(2-pyridylmethyl)ethylenediamine (TPEN) and pyrolidine dithiocarbamate (PDTC). While addition of zinc completely reversed TPEN's toxicity towards human cancer cells, its addition dramatically enhanced cell killing by clioquinol and PDTC. Clioquinol and PDTC down-regulated Akt and NF-κB signaling, effects that were more pronounced in the presence of zinc. In contrast, TPEN had no effects on Akt and NF-κB signaling, whether in the presence or absence of zinc. Although PDTC, like clioquinol, is a zinc ionophore, it did not exhibit synergistic cytotoxicity with the polyunsaturated fatty acid, docosahexaenoic acid (DHA). In the presence of DHA, clioquinol reduced expression of Akt, p65, p44/42, p38, and SAPK/JNK, whereas PDTC had no such effect. These results demonstrate that the three tested zinc binding compounds act through different signaling pathways to induce death of cancer cells, even if they share certain characteristics (such as acting as ionophores). Understanding the signaling mechanisms of each individual metal binding compound will be required in order to optimize the process of developing them into novel anticancer agents.

B268 Novel sugar-cholestanols generate a new therapeutic agent against peritoneal dissemination. Takayuki Asao 1, Shinji Hashimoto 1, Ahmad Faried 1, Toyo Nishimura 2, Takaskhi Nakagawa 2, Takashi Yamachi 3, Noriuki Koyama 4, Hiroyuki Kawano 1, Shin Yazawa 1. 1Dept. General Surgical Science Gunma University, Graduate School of Medicine, Maebashi, Japan; 2Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan; 3Medical Chemistry Research Institute, Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan.

Background: Chemically synthesized sugars attached to hydrophobic aglycones could be used by cancer cells through the cell-mediated priming of such sugars resulting in the suppression of tumor-associated antigen expression on their cell surface and the increase in the susceptibility of cancer cells against anticancer treatments. It was also observed that they showed some cytotoxic activity against cancer cells at their high concentrations (Glycobiology, 12:545, 2002; Glycoconjug., J., 22, 311, 2005).

Methods: A series of chemically synthesized sugar-cholestanols consisting of mono- and oligo-saccharides attached to cholestanol were assayed for their cytotoxic activities against various cancer cells. Along with measuring sugar-cholestanols incorporated into the cells, Western blot analyses of molecules related to the apoptotic cell death were also performed in the cells treated with sugar-cholestanols. Further, using the mouse model for the peritoneal dissemination, sugar-cholestanols were evaluated as an anticancer agent against the peritoneal dissemination.

Results: Sugar-cholestanols mainly consisting of GlcNAc or GlcNAc-Gal attached to cholestanol showed strong activities of cell proliferation inhibition against various cancer cells but cholestanol without sugars moieties did not show any activity. When sugar-cholestanols were added into the culture medium of cancer cells, they were incorporated into the cells through the lipid rafts/microdomains on their cell surface in a short period of time and then into the mitochondria followed by release of cytochrome c from the mitochondria, which resulted in the induction of apoptosis by the activation of mitocondria-dependent intrinsic pathways and the caspase-cascade. Further, in vivo experiments in the mouse model for the peritoneal dissemination using colon26 cancer cells showed that tumor growth was significantly reduced and the survival rates of mice were markedly enhanced by administration of sugar-cholestanols.

Conclusion: Sugar-cholestanols possessed strong cytotoxic activities against various cancer cells in time- and dose-dependent manners and GlcNAc-containing sugar-cholestanols could be applicable to a promising anticancer agent against the peritoneal dissemination.
microarray analysis revealed upregulation of 33 genes and downregulation of 54 genes in K562 cells treated with 100 µM TMPyP4 for 48 hours. We found upregulation of 33 genes, including genes involved in cell cycle regulation (p57); oncogenes (growth-regulated oncogene [GRO] 1 and GRO3); stress-responsive genes (growth arrest and DNA damage-inducible gene [GADD] 45B and GADD34); genes of adhesion molecules (intracellular adhesion molecule-1 [ICAM1]; genes of calcium-binding proteins (calbindin 1, S100 calcium-binding protein A10, and S100 calcium-binding protein A4); and genes for transcriptional regulation (zinc finger protein 165, zinc finger protein 76, CCAT enhancer binding protein [C/EBP] γ, and C/EBP β). On the other hand, we found downregulation of 54 genes, including genes for cell-cycle regulation (cyclin D2, cyclin-dependent kinase [CDK] 5, CDK8, and p18); oncogenes (pim-1, pim-2, and c-syn); genes for DNA damage response (topoisomerase I, topoisomerase II β, chromodomain helicase DNA binding protein 1, and chromodomain helicase DNA-binding protein 4); for kinases (adenylate kinase 2, adenylation kinase 3, Akt, PDZ-binding kinase, and protein kinase C β); for adhesion molecules (ICAM3 and CD58); for receptors (fibroblast growth factor receptor 3 and insulin-like growth factor 1 receptor); for ubiquitination (ubiquitin-conjugating enzyme E2D1, ubiquitin-conjugating enzyme E2M, ubiquitin carrier protein, and ubiquitin specific protease 7); and for transcriptional factors (zinc finger protein 74, general transcription factor IIIC, transcription factor 12, forkhead transcription factor FOXL2, c-myc, and v-myc). Moreover, TMPyP4 decreased c-Myc protein expression, increased the expression of p21<sup>WAF1</sup> and p57<sup>kip2</sup> proteins, and activated p38 mitogen-activated protein kinase, c-Jun N-terminal kinase, and extracellular signal-regulated kinase. These findings may provide a rationale for the development of G-quadruplex-interacting agents as novel antiangiogenic therapies.

**B271** A novel orally active MDM2 inhibitor (MI-219) activates the p53 pathway and is selectively toxic to tumor cells. Sanjeev Shangarp1, Dongyong Qin1, Donny McEachern1, Rebecca S Millier1, Zaneta Nikolovska-Coleska1, Meilan Liu1, Su Qiu1, Ke Ding1, Guoping Wang1, Jianyong Chen1, Yinip Lu1, Deniz Bernard1, Qingguo Yu1, Yi Sun1, Rajal B. Shah1, Kenneth J Pienta1, Xiaolan Lin2, Sanmoo Kang3, Shuguang Zhu2, Min Guo3, Dajun Yang2, Shaomeng Wang1. 1The University of Michigan, Ann Arbor, MI; 2Ascenta Therapeutics Inc., San Diego, CA; 3The University of Michigan, Ann Arbor, MI.

**Introduction:** The tumor suppressor p53 is a key regulator of cell cycle, apoptosis, senescence and DNA repair. Due to its important role in oncogenesis, it is not surprising that p53 function is compromised in all human cancers. In half the cancers, p53 gene is directly mutated/deleted, rendering p53 protein inactive, while in the remaining cancers with wild-type p53, p53 function is effectively inhibited by its cellular endogenous inhibitor the minute double minute 2 (MDM2) protein through direct interaction. Thus, targeting the MDM2-p53 interaction by small-molecule inhibitors is a promising approach for the reactivation of p53 function, and is being intensely pursued as a cancer therapeutic strategy. Since MDM2 is an essential regulator of p53 activity, there is a serious concern that unleashing p53 function by small-molecule inhibitors of MDM2 may result in toxicity to normal healthy tissues, casting doubts on the suitability of MDM2 inhibitors as potential cancer therapeutic strategy.

**Methods:** We employed multiple human cancer cell line and xenograft tumor models, including SISA-1 osteosarcoma and LnCaP prostate cancer, and performed following assays/procedures: 1) Binding affinity was determined in fluorescence polarization-based binding assays, 2) cell growth by WST assay, 3) cell cycle by BrdU incorporation, 4) apoptosis by Annexin V staining, 5) MDM2-p53 interaction by co-immunoprecipitation, 6) p53 was downregulated by siRNA oligonucleotide, 7) oral availability by pharmacokinetic (PK) studies, 8) p53 activation by immunohistochemistry and immunoblotting, 9) cell proliferation in tissues by BrdU incorporation, 10) apoptosis in tissues by TUNEL staining, 11) anti-tumor activity in xenograft models, 12) toxicity by animal body weight and necropsy, and 13) normal tissue damage by histopathology.

**Results:** Employing a computational structure-based approach, we have designed a new, highly potent (K<sub>i</sub> = 5 nM) and selective (>10,000-fold over MDM2 homologue MDMX), orally-available small-molecule MDM2 inhibitor (MI-219) with a distinctively different chemical structure from other known MDM2 inhibitors. MI-219 disrupts intracellular MDM2-p53 interaction in cancer cell lines, leading to accumulation and activation of p53. p53 pathway is activated in both normal and cancer cells with wild-type p53, and leads to cell cycle arrest in both normal and cancer cells with wild-type p53, but selective apoptosis in cancer cells. Induction of cell cycle arrest and apoptosis is strictly p53-dependent. MI-219 has excellent oral bioavailability in mice and rats. A single oral dose of MI-219 induces rapid, but transient, activation of p53 in xenograft tumors and normal mouse tissues, strongly correlating with the plasma levels of MI-219. Multiple oral dosing of MI-219 potently inhibits tumor growth without causing animal weight loss or other signs of toxicity. Importantly, therapeutically effective dose schedules of MI-219 do not result in any damage to normal radio-sensitive and radio-resistant tissues.

**Conclusion:** We conclude that activation of p53 by potent and specific small-molecule MDM2 inhibitors is selectively toxic to tumors without causing damage to normal tissues. Our study thus provides a strong rationale to advance MI-219 or its analogues for clinical development as a new and promising cancer therapeutic strategy.

**B272** The novel HDAC-inhibitor Panobinostat inhibits growth of human hepatocellular carcinoma xenografts in nude mice. Pietro Di Fazio1, Regine Schneider-Stock2, Kinya Okamoto1, Till Wissniowski1, Bernhard Kaufmann1, Axel Schlösser2, Ulrike Haus2, Gabriele Sass1, Eckhart G. Hahn1, Christoph Herold3, Matthias Ocker1. 1University of Erlangen, Erlangen, Germany; 2University of Magdeburg, Magdeburg, Germany; 3Novartis Pharma GmbH, Nuremberg, Germany.

**Background:** Liver cancer (hepatocellular carcinoma, HCC) represents an unmet medical need. Despite its rising incidence, the overall prognosis of HCC is poor as systemic chemotherapy is of low efficacy. Histone deacetylase inhibitors (HDACi) have demonstrated antitumor activity in various cancers. We therefore investigated the effect of the novel HDACi Panobinostat (LBH589) on human hepatocellular carcinoma cell lines in vitro and in a subcutaneous xenograft model in vivo.

**Methods:** HepG2 (p53<sup>+/+</sup>) and Hep3B (p53<sup>−/−</sup>) cells were cultured under standard conditions and incubated with various concentrations of Panobinostat for 6 to 120 h. Cell viability was determined by trypan blue staining, apoptosis was quantified by flow cytometry after propidium iodide staining. Mitochondrial transmembrane potentials (ΔΨ<sub>M</sub>) were determined by JC-1 and DiOC<sub>5</sub> staining. Quantitative RT-PCR and western blotting was used to investigate signaling pathways involved in Panobinostat-mediated apoptosis. CHIP was performed to investigate p53-dependent transcriptional regulations of Panobinostat target genes. In vivo, HepG2 cells were xenografted to male NMRI mice (n/group = 8) and treated with daily intraperitoneal injections of 10 mg/kg Panobinostat. Tumor size and animal weight were determined daily. Tumor samples were obtained for further analysis (immunohistochemistry, RT-PCR, westernblotting). Liver transaminases were determined from blood samples as a surrogate marker for toxicity.

**Results:** In vitro studies showed a pronounced growth inhibitory and pro-apoptotic effect of LBH589 on both HCC cell lines at low micromolar concentrations (IC<sub>50</sub> approx. 0.1 µM). Interestingly, the pro-apoptotic effect of Panobinostat was not paralleled by a breakdown of ΔΨ<sub>M</sub> in p53<sup>−/−</sup> Hep3B cells were more sensitive than the p53<sup>−/−</sup> Hep3B cells. Quantitative PCR and western blotting showed an involvement of the cell cycle regulators p21<sup>WAF1/CIP1</sup> and Chek1 but not the bax/bcl-2 system. Panobinostat regulated the expression of p21<sup>WAF1/CIP1</sup> via a transcriptional upregulation as evidenced by CHIP.

In vivo, the daily application of Panobinostat significantly reduced the growth of HepG2 xenografts (mean tumor diameter: 12 mm vs. 16 mm in untreated controls) and prolonged the overall survival of animals (100% vs. 43% in controls). Macroscopically, a marked reduction of tumor angiogenesis was observed and the PCR results confirmed the in vitro findings, too. No signs of toxicity or elevation of liver transaminases was observed (ALT: 238 UI/I vs. 320 UI/I in controls).
Conclusion: Panobinostat is a potent novel HDACi for the treatment of human HCC.

B273 Targeting imatinib-resistant gastrointestinal stromal tumors by down-regulation of KIT gene expression with quadruplex-stabilizing small molecules. Mekala Gunarathnam1, Francisco Cuenca1, Monica Beltran1, Stephen Swank2, Jonathan Fletcher2, Stephen Neidle1.

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Gain-of-function mutations in the human proto-oncogene receptor tyrosine kinase KIT are found in up to 80% of gastrointestinal stromal tumors (GIST). GISTs are the most common form of gastrointestinal mesenchymal tumor, with ca. 1200 cases per annum in the UK and 5000 in the USA. Conventional cytotoxic treatments do not result in significant clinical responses in GIST patients. However imatinib (Gleevec), a potent inhibitor of the ABL kinase in CML, is also a potent inhibitor of the KIT kinase, and produces dramatic tumor shrinkage in many GIST patients, especially those with KIT exon 11 mutations. These responses are temporary since acquired resistance to imatinib invariably occurs, with consequent tumor regrowth and limitations to longer-term patient survival. In addition a small proportion of GIST patients have primary resistance to imatinib as a consequence of particular mutations in KIT exon 9 or expression of activated wildtype KIT, which is inhibited inefficiently by imatinib.

We present here an alternative strategy for KIT inhibition that is targeted against the enzyme itself rather than the receptor, which would overcome the problems of primary and secondary resistance in the KIT kinase. Two G-rich motifs have been characterized as being capable of forming G-quadruplex (G4) secondary structures within the core promoter nucleosse hypersensitive region of the KIT proto-oncogene, 87bp and 137bp upstream from the transcription start site (Rankin et al. J. Amer Chem Soc, 2005, 127, 10584; Fernando et al., Biochemistry, 2006, 45, 7854). We propose that appropriate small molecules can stabilize these G4s and so inhibit KIT gene expression.

We report here on studies with a novel series of G4-stabilising small molecules that bind with exceptionally high affinity in vitro to the KIT quadruplexes. Short-term cytotoxicity studies show that these ligands have significant activity against all members of a panel of patient-derived GIST cell lines with gain-of-function mutations (GIST882 and GIST62 lines) and also shown activity against the GIST48 cell line, which has acquired secondary resistance mutations following initial imatinib treatment. We have also shown that 24hr treatment of GIST882 cells with compound FC4ND04 at a sub-cytotoxic concentration results in complete loss of KIT mRNA and no detectable expressed KIT protein; imatinib treatment in this cell line does not have any such effects on KIT levels. We conclude that inhibition of KIT oncoprotein expression can be accomplished using G4-stabilizing small molecules, and these novel approaches provide a therapeutic strategy for GISTs with kinase-inhibitor resistance mutations.

B274 STA-4783 induces apoptosis and enhances the anticancer activity of paclitaxel through induction of oxidative stress. Jessica R. Kirshner1, Zhenjian Du1, Jane Kepros1, Vish Balasubramanyam1, Suqin He1, Mei Zhang1, Chiu-Yu Yang1, Kevin P Foley1, James Barsoum1, John Berlin1.

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Background: STA-4783 is a novel small molecule that induces oxidative stress in cancer cells. It is being evaluated in metastatic melanoma with a Phase 3 trial beginning in 2007. In a Phase 2b double-blind, randomized, controlled trial in patients with Stage IV metastatic melanoma, treatment with STA-4783 plus paclitaxel achieved the primary endpoint of increasing progression-free survival relative to treatment with paclitaxel alone (median PFS: 112 days vs. 56 days, respectively; p= 0.035, log-rank). This presentation will describe the mechanism of action of STA-4783.

Methods: Transcriptional profiling was performed on human melanoma cell lines treated with STA-4783. The induction of Hsp70 RNA was monitored by QPCR. Reactive oxygen species (ROS) were measured using the Carboxy-H$_2$DCFDA probe. Apoptosis was assayed by flow cytometry using an Annexin V specific probe. The antioxidants N-Acetyl Cysteine (NAC) and Tiron were used to assess the role of ROS.

Results: STA-4783 treatment induced the generation of ROS and the expression of ROS-regulated genes. ROS induction was correlated with the oxidation of cardiolipin in mitochondria, a decrease in mitochondrial membrane potential, and the release of cytochrome c from the mitochondria leading to caspase activation and apoptosis. The antioxidants NAC and Tiron blocked the induction of ROS by STA-4783 and apoptotic cell death.

Conclusions: STA-4783 is a potent inducer of oxidative stress in cancer cells. Cancer cells produce more ROS and exist under an elevated level of oxidative stress relative to normal cells. This elevated level of oxidative stress makes cancer cells highly susceptible to insults that further increase ROS. In this way, STA-4783 takes advantage of a fundamental and unique property of transformed cells in order to selectively kill them with little effect on normal cells. STA-4783 enhances the efficacy of taxanes, and other chemotherapies, by facilitating the ability of these agents to induce apoptosis without introducing acceptable additional toxicity.

B275 CUDC-101, a synthetic and potent HDAC, EGFR and Her2 inhibitor, effectively inhibits proliferation of cancer cell lines. Cheng Lai1, 1Curis, Inc, Cambridge, MA.

HDAC and receptor tyrosine kinases have emerged as validated cancer targets. We first demonstrated that the epigenetic effects through HDAC inhibition are synergistic with receptor tyrosine kinase inhibitors (RTKis). By integrating the active moieties that inhibit HDAC, EGFR and Her2, we rationally designed and synthesized novel, individual small molecules that simultaneously inhibit these three pathways in the targeted cells. One of the best compounds, CUDC-101, displays anti-proliferation and apoptosis-inducing activities in vitro against a broad range of cancer cell types including lung, breast, prostate, colon, liver and pancreas. CUDC-101 also exhibits an equal or greater potency than a combination of SAHA (a HDAC inhibitor) and erlotinib (an EGFR kinase inhibitor) or lapatinib (an EGFR/Her2 kinase inhibitor) in these in vitro assays. Using in vivo xenograft model, we demonstrated CUDC-101 inhibits all three targeted pathways and induces anti-proliferation and apoptosis effects against diverse cancer types (Bao, R. et al, unpublished data). A favorable safety profiling of CUDC-101 was also reported when assessed by in vitro assays of major receptors, channels and enzymes. These results suggest that CUDC-101 is a potential novel agent suitable for clinical development for various cancer indications.

B276 Indibulin (ZIO-301): An orally active tubulin polymerization inhibitor with a unique molecular mechanism of action. Philip Kormarnitsky1, Lynne Lapierre2, Barbara Wallner1, James Goldenring2.

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Indibulin (N-(pyridin-4-yl)-[1-(4-chlorbenzyl)-indol-3-yl]-glyoxyl-amid; ZIO-301 or D-24851) is a novel synthetic, orally active anti-mitotic agent that binds tubulin, destabilizes microtubulin polymerization, and arrests tumor cell growth at the G2/M phase. Its tubulin binding site is distinct from that of other microtubulin inhibitors such as taxanes, colchicines, and vinca alkaloids. Indibulin does not bind acetylated (neuronal) tubulins and, in contrast to all other microtubulin inhibitors, has not caused neurotoxicity in animal models or in patients enrolled in ongoing Phase I clinical trials. Indibulin is active in a panel of human and rodent tumor cell lines in vitro and in human tumor xenografts including multidrug-resistant tumor cell lines.

We have demonstrated in molecular mechanistic studies that indibulin affects microtubule-dependent processes such as the plasma recycling system in a manner different from other microtubule inhibitors. While taxol caused a shift of the apical recycling system from a central tubulovesicular location in the apical region to the perijunctional corner of polarized cells, indibulin dispersed the apical recycling system throughout the cell, similar to nocodazole. However, unlike nocodazole, indibulin did not disrupt the Golgi apparatus. While nocodazole disrupted all microtubules, indibulin did
not affect acetylated microtubules in intact cells. These findings correlate with the lack of disruption of the Golgi apparatus, which is associated with the more stable acetylated microtubules. These results suggest that indibulin targets specific subsets in cellular microtubules.

Indibulin has potent antitumor activity as a single agent in preclinical animal models. As we continue to translate its biology into the clinic, its cytotoxic activity in combination with approved chemotherapeutics in a series of cell lines is of interest. Strongest synergy was observed in a non-small cell lung cancer cell line, A549, with erlotinib, which is an epidermal growth factor receptor inhibitor. Indibulin also enhanced the effect of carboplatin in the same cell line. In the MCF-7 breast cancer cell line, indibulin had synergistic effects with both vinblastine and paclitaxel; this is of particular interest since indibulin has been previously shown to have antitumor activity in vinca alkaloid- and taxane-resistant tumor cell lines. Indibulin was also synergistic with 5-FU and tamoxifen in MCF-7 cells, and had additive effects with doxorubicin. In the SKOV-3 ovarian cancer cell line, indibulin showed additive effects with cisplatin, carboplatin, and gemcitabine, and in the PC3 prostate cancer cell line with estramustine and prednisol.

These data suggest that indibulin has a unique molecular mechanism, targets specific subsets in cellular microtubules, and has potent oral single-agent antitumor activity that is highly synergistic with a number of widely used anticancer agents. When viewed with the lack of neurotoxicity in the Phase I trials to date, our results would strongly support successful clinical translation in upcoming Phase II studies both as a single agent and in combination.

B278 Design, synthesis and evaluation of microtubule inhibitors phenoxypyridine (PP) and phenyl sulfanyl pyridine (PSP) analogs for cancer therapy. Ravi K. Anchoori1, Madeleene S.O. Kortenhoffs2, Antonio Jimeno1, Taradas Sarkar3, Gurulingappa Hallur1, P.J. Van Diest2, Manuel Hidalgo1, Ernest Hamel3, Saeed R Khan1. 1Johns Hopkins University, Baltimore, MD; 2Utrecht Departments Medical Oncology, Universiteitsweg 100, Netherlands Antilles; 3National Cancer Institute, Frederick, MD.

Introduction: Lack of sensitivity and acquired resistance of tumors to chemotherapy are major obstacles in the treatment of cancer. So, there is a need for the development of new anticancer agents.

Microtubules are key components of eukaryotic cell division. Treatment of cancer cells with agents that interfere with microtubule assembly causes mitotic arrest and eventually cell death. Current microtubule inhibitory agents used in the clinic have severe side effects, and development of resistance is frequent. We have designed and synthesized a 30-compound library of Phenoxypyridine (PP) and Phenyl Sulfanyl Pyridine (PSP) derivatives and studied their effects in pancreatic cancer, breast cancer and Burkitt lymphoma cells.

Results: In MTT assays (J Immunol Methods, 1983; 65:55-63) with pancreatic cancer cell lines Panc1 and HS766T, ARK-62 and ARK-78 showed highest potency of the 30 compounds. The IC50 values after 24h treatment with ARK-62 were 0.8 and 0.15 µM for Panc1 and HS766T, respectively, and with ARK-78, 0.7 and 0.5 µM for Panc1 and HS766T, respectively.

Next, we examined the tubulin inhibitory activity of ARK62 and ARK78 with in vitro tubulin assembly assays (Cell Biochem Biophys, 2003; 38:1-22), using bovine brain tubulin. ARK78 caused a 30% inhibition of tubulin assembly at concentrations of 4 µM and above. ARK62 caused a delay of tubulin assembly at all concentrations tested. Colchicine binding experiments (Mol. Pharmacol. 1998; 53:62-76) with ARK-62 and ARK-78 revealed no inhibitory effect on the binding of radio labeled colchicine to tubulin, indicating that their binding site may be different from the colchicine binding site.

To determine whether the microtubule inhibitory effects of ARK-62 or ARK-78 resulted in mitotic arrest and DNA fragmentation, we used flow cytometry. MCF7 breast cancer cells were treated for 15-27h with either ARK-62 or ARK-78 and stained with propidium iodide (DNA), and MPM2 (mitotic cells). Both ARK-62 and ARK-78 caused a mitotic arrest of 30% in MCF7 cells followed by DNA fragmentation. The response of MCF7 cells to ARK-62 and ARK-78 was comparable to microtubule inhibitory compounds taxol (250 nM) and colcemid (0.1 µg/ml). Treatment of Burkitt lymphoma cells CA46 resulted in a similar response.

Conclusion: The development of microtubule inhibitors can provide novel therapy options. In addition, the development of novel microtubule inhibitors is very useful for the investigation of the biological function of microtubules and microtubule-associated proteins. ARK-62 and ARK-78 are anti cancer agents in the low micro molar range that affect microtubule assembly and cause a strong mitotic arrest followed by DNA fragmentation and cell death in a broad range of cancer cells.

B278 Concomitant and sequential administration of YM155 and docetaxel enhances apoptosis and tumor regression in human tumor xenograft models. Kentaro Yamanaka1, Takahito Nakahara2, Aya Kita1, Masahiro Takeuchi1, Isao Kinoyama1, Akira Matsuhisa1, Hiroshi Koutoku1, Masao Sasamata3, 1Astellas Pharma. Inc., Thukuba-shi, Japan; 2Astellas Pharma. Inc., Thukuba-shi, Japan, Japan.

YM155 is a novel small molecule survivin suppressant currently undergoing phase II clinical trials in patients with various types of cancer. YM155 shows potent antiproliferative activities against various human cell lines regardless of p53 status, and continuous infusion of YM155 induce massive tumor regression in experimental human hormone refractory prostate cancer (HRPC) xenograft model (Cancer Res 2007;67(17):1-8). In this study, we evaluated the antitumor activity of YM155 in both concomitant and sequential combination with docetaxel in experimental human Calu 6 non small cell lung cancer xenograft models. Concomitant administration of YM155 at 2 mg/kg/day (1/5 MTD) and docetaxel at 20 mg/kg/day (MTD) showed massive tumor regression for longer periods than each single compound administration, and tumor volume was significantly reduced in mice treated with YM155 in combination with docetaxel as compared to each single compound treatment. Furthermore, complete tumor regression was observed in all animals by the concomitant administration of YM155 and docetaxel. No decrease in body weight was observed in the combination group as compared to docetaxel group. The same trends were observed in the case of sequential combination of YM155 with docetaxel. Immunohistochemical analysis revealed that YM155 alone suppressed survivin in tumors; however, docetaxel alone did not suppress survivin. In addition, survivin accumulation in microtubules by G2/M cell cycle arrest was observed in tumors obtained from docetaxel treated animals. The tumor regression induced by concomitant administration of YM155 and docetaxel was accompanied by intratumoral survivin suppression and more intense apoptosis induction compared with each single treatment. The results demonstrate that YM155 potentiates the antitumor activity of docetaxel without an increase in toxicity, which provides us a rational approach for combination chemotherapy of YM155 with docetaxel in various types of cancer patients. Further clinical investigation of YM155 as a new antitumor agent, either alone or in combination with taxanes for the treatment of various cancers would be worthwhile. P-II open labeled study of YM155 in combination with docetaxel in patients with HRPC is currently underway.
**B279 NVP-AUY922: A novel small molecule HSP90 inhibitor with potent in vivo antitumor efficacy.**

Michael Rugaard Jensen¹, Joseph Schoepfer¹, Thomas Radimersky¹, Andrew Massey¹, Josef Brueggen¹, Alain Schweitzer², Ulrike Pfarr², Stephan Rueß², Chantal Guy³, Alan Buckler³, Christian Schnell³, Robert Cozens³, Paul Brough⁴, Martin Drysdale⁴, Cornelia Quadt⁵, Carlos García-Echeverría⁵, Patrick Chéne⁶, ¹Novartis Institutes for BioMedical Research, Basel, Switzerland; ²Vernalis Ltd, Granta Park, Great Abington, Cambridge, United Kingdom; ³Novartis Pharma AG, Basel, Switzerland; ⁴Novartis Institutes for BioMedical Research, Cambridge, MA.

Heat Shock Protein 90 (HSP90) is a ubiquitously expressed molecular chaperone with ATPase activity which plays an important role in the conformational maturation and activation of a large number of client proteins that have been implicated in oncogenesis. HSP90 has attracted considerable interest as a therapeutic target for anticancer drugs since HSP90 ATPase inhibition induces simultaneous degradation of multiple oncogenic proteins. The most advanced HSP90 inhibitors are of the benzoquinone ansamycin class which have shown promising activity in human tumor xenograft models. NVP-AUY922, is a novel synthetic resorcylic isoaxazole HSP90 inhibitor which recently entered Phase I clinical trials. In a competitive fluorescent polarization assay, NVP-AUY922 inhibited Hsp90 β with an IC50 of 21 nM and a Ki of 9 nM. Inhibition of HSP90 translates into anti-proliferative effect of human breast cancer tumor cells with GI50 values in the range of 3-10 nM. Importantly, treatment of cell lines with NVP-AUY922 consistently induced dissociation of p23/Hsp90, HSP72 upregulation and client protein depletion - hallmarks of HSP90 inhibition. Intravenous administration of NVP-AUY922 to athymic mice (25mg/kg) resulted in drug levels exceeding 1000-fold cellular GI50 for about 2 days in BT-474 breast cancer xenografts. Tumor stasis was observed when the compound was administered once per week and was accompanied by apoptosis. Therapeutic effects were also concordant with changes in pharmacodynamic markers including p23/Hsp90 dissociation, decreases in ERBB2 and P-AKT as well as increased HSP72. Overall, we demonstrate that NVP-AUY922 is a potent, novel HSP90 inhibitor with long lasting tumor retention and rapid organ clearance. This translates into potent anti-tumor effect with concurrent effects on client proteins when administered once weekly to nude mice bearing human breast cancer xenograft tumors.

**B280 Preclinical evaluation of MLN4924, the first small molecule inhibitor of NEDD8-activating enzyme (NAE) for the treatment of cancer.**

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Neddy-Activating Enzyme (NAE) initiates the conjugation of the ubiquitin-like protein nedd8 to its cellular targets, members of the cullin protein family. Nedd8 conjugation to cullins is known to be essential for the ubiquitination activity of cullin-dependent ubiquitin ligases (CDLs). These CDLs control the timely ubiquitination and subsequent degradation of many proteins with important roles in cell cycle progression and signal transduction. These cellular processes are relevant to tumor growth and survival providing a rationale for inhibiting NAE as an anti-cancer strategy. Targeting NAE for the therapeutic intervention of human cancers is unprecedented. Here we describe MLN4924, the first small molecule inhibitor of NAE. MLN4924 potently inhibits the growth of a variety of human tumor-derived cell lines in vitro and growth inhibition correlates with NAE inhibition. We demonstrate that MLN4924 inhibits the nedd8 conjugation pathway in cells, resulting in stabilization of numerous CDL substrates and disruption of protein homeostasis. In most of the cell lines studied, the mechanism of cell growth inhibition appears to be a consequence of uncontrolled DNA synthesis in the S-phase of the cell cycle followed by induction of a DNA damage response and apoptosis. This phenotype is consistent with DNA re-replication.

**B281 NVP-AUY922 / VER-52296: A novel 3,4 diarylisoaxazole Hsp90 chaperone inhibitor discovered via structure-based drug design.**

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Hsp90 is a molecular chaperone currently the focus of significant research interest as a target for small molecule anti-cancer therapeutic agents. First-class inhibitors based on the natural product geldanamycin (including 17-AAG, 17-DMAG and IPI-504) have entered Phase III clinical trials. These agents confer their inhibitory effect by competitive binding to an ATP binding site on the N-terminal domain of the protein. We have previously described the elaboration of the diarylpyrazole screening hit CCT018159 via structure-based design which led to the identification of the potent pyrazole inhibitor VER-49009 (IC50 = 15 nM; GI50 in HCT116 human colon cancer cells = 260 nM). Further structure-driven medicinal chemistry optimization of VER-49009 identified a novel 3,4 diarylisoaxazole class of inhibitors which had significantly improved potency in growth inhibition assays (in various human cancer cell lines) compared to VER-49009. The isoaxazole series of compounds exhibited tighter binding (as exemplified by a 10-fold slower off rate) compared to the equivalent pyrazole and most likely accounts for their increased cellular potency. The binding mode of these new ligands was established and shown to be consistent with the pyrazole series, making similar key direct and water-mediated interactions with the protein. From a series of highly potent isoaxazole analogues, NVP-AUY922 (VER-52296) was selected for further development due to its high potency for Hsp90, in vitro cellular activity and pharmacokinetic parameters as determined by cassette dosing. In particular NVP-AUY922 demonstrated high tumor uptake and retention compared to plasma when dosed i.p or i.v. and exhibited high potency against the Hsp90 ATPase site with a Ki of 9 nM in an FP assay and significant in vitro growth inhibition (in the range 2-25 nM) against a panel of human cancer cell lines. Its cellular mode of action, as determined by the depletion of Hsp90 client proteins and induction of Hsp72 was clearly consistent with Hsp90 inhibition. In addition, NVP-AUY922 showed excellent efficacy in a range of subcutaneous and orthotopic xenograft models covering major cancer types (including HCT116 colon, U87MG glioblastoma, PC3 prostate, BT474 breast and WM266 melanoma) with diverse oncogenic profiles. Analysis of in vivo pharmacodynamic markers was consistent with an Hsp90 mode of action. NVP-AUY922 has recently entered phase I clinical trials.

**B282 The novel poly(ADP-ribose) polymerase (PARP) -1 Inhibitor, BSI-401, has antitumor activity and potentiates oxaliplatin cytotoxic activity in human pancreatic cancer.**

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Pancreatic cancer (PD) is the fourth-leading cause of cancer mortality among adults in the United States. One of the most promising drugs in PC therapy is oxaliplatin, an organoplatinum molecule, that forms inter- and intrastand DNA adducts/cross-links and induces a high proportion of DNA incorporation.
single strand breaks. However, the gemcitabine and oxaliplatin combination failed to demonstrate a statistically significant advantage compared with single-agent gemcitabine. Development of novel agents and drug combinations are urgently needed. PARP-1 functions as a DNA damage sensor for both single- and double-stranded DNA breaks and plays a key role in many cellular processes including the regulation of DNA repair. PARP-1 also acts as a promoter-specific transcriptional coactivator of NF-κB, a transcription factor constitutively activated in most PC tissues and human PC cell lines. In this study, we describe the antitumor activity of the novel PARP-1 inhibitor BSI-401 alone and in combination with oxaliplatin in PC cell lines and its therapeutic efficacy in PC orthotopic nude mouse models.

The expression of PARP-1 protein was analyzed in thirteen PC cell lines by western blotting. The effect of BSI-401 alone and in combination with oxaliplatin on the proliferation of eight PC cells was determined using a BrdU-ELISA assay. Assessment of synergy was performed according to the method described by Chou and Talalay. To determine whether the PARP-1 activity inhibition by BSI-401 has any inhibitory effects on NF-κB signaling, NF-κB DNA binding activity was evaluated using an electrophoretic mobility shift assay. The therapeutic efficacy of BSI-401 on tumor growth and survival was evaluated in different luciferase-expressing PC orthotopic nude mouse models using an IVIS 100 Imaging System, allowing a quantitative real time monitoring of tumor growth. Mice were monitored daily for signs of toxicity including weight loss, diarrhea, inactivity, ruffled fur, and general appearance.

PARP-1 overexpression was detected in 8 of 13 human pancreatic cancer cell lines but not in immortalized normal pancreatic ductal epithelial cells. In vitro, BSI-401 alone significantly inhibited the growth of eight PC cell lines, with an IC50 ranging from 5 to 10 μM. A synergistic effect (Combination Index <1) was observed with BSI-401 in combination with oxaliplatin on the proliferation of Colo357FG, MiaPaCa2, and ASPC1 human PC cancer cells. Results from the NF-κB DNA binding activity assay suggest that NF-κB activation was affected by BSI-401. In nude mice orthotopically injected with luciferase-expressing Colo357FG or L3.6P1 PC cells, BSI-401 at dose of 100 mg/kg/day significantly reduced the tumor burden and prolonged survival without signs of toxicity.

In conclusion, the PARP inhibitor BSI-401 showed a potent in vitro and in vivo antitumor activity as a single agent and potentiated oxaliplatin cytotoxicity in different PC cell models. BSI-401 is a promising candidate for further preclinical and clinical evaluations.

B283 Translation of indibulin (ZIO-301) preclinical antiangiogenic and antmitotic activity to the clinic. Brian Schwartz1, Gerald Bacher2, Philip Komarnitsky1, Lee Rosen3, Sant Chawla2. 1ZIOPHARM Oncology, Boston, MA; 2mondoBIOTECH AG, Basel, Switzerland; 3Premiere Oncology, Santa Monica, CA; 4Sarcoma Oncology Center, Santa Monica, CA. Indibulin (N-(pyridin-4-yl)-1-(4-chlorbenzyl)-indol-3-yl)-glyoxylamid; ZIO-301 or D-24851) is a novel synthetic, orally active antimitotic agent that binds tubulin, destabilizes tubulin polymerization, and arrests tumor cell growth at the G2/M phase. Tubulin inhibitors such as taxanes and vinca alkaloids are currently widely used to treat cancers although they are associated with serious side effects, most notably neuropathy. Taxanes stabilize tubulin polymers, while indibulin, vinca alkaloids, and colchicines destabilize polymerization. Each class of these agents has distinct tubulin binding sites. However, indibulin is unique in that it does not affect polymerization of mature neuronal tubulins; this property translates into lack of neurotoxicity, as observed in animal models and in ongoing Phase I clinical trials. Indibulin is active in a panel of human, murine, and rat tumor cell lines in vitro and in human tumor xenografts. Indibulin is also active in multidrug-resistant tumor cell lines including taxane- and vinblastin-resistant cell lines.

Angiogenesis is a critical event in tumor growth and metastasis, and tumor cell migration is a prerequisite for metastasis. The effect of indibulin on either of these processes was investigated in assays of cell motility and angiogenesis both in vitro and in vivo. Indibulin inhibited cell migration of MO4 cells, a fibrosarcoma cell line, in a dose-dependent manner with IC150 ~40 nM. In vivo antimitastatic potential of indibulin was evaluated in the murine RENCA tumor model. Indibulin treatment reduced the number of RENCA lung metastases by up to 2.5-fold compared to untreated mice. Antiangiogenic activity of indibulin was indicated by its cytotoxicity for human endothelial cells in vitro. This observation was supported by the demonstration of complete inhibition of endothelial tube formation in vitro at low indibulin concentrations (100 nM). Indibulin’s antiangiogenic properties in vivo are being investigated in the mouse Matrigel plug model. Indibulin concentrations that exhibit activity in these models are well within the plasma concentrations of indibulin observed in patients in ongoing Phase I clinical studies.

Preclinical data suggest that indibulin has a unique molecular mechanism, targeting specific subsets of cellular microtubules and affecting cancer cell growth through several well-defined pathways. In the Phase I studies, several biomarkers are being evaluated to correlate preclinical observations with clinical activity. The effect of indibulin on angiogenesis is being evaluated by measuring plasma VEGF and G-CSF levels. Additionally, functional imaging including PET scans are being performed. Circulating tumor cells and paired tumor biopsies are being analyzed to assess clinical efficacy and correlate with the proposed mechanism of action for indibulin. Preliminary data from these clinical studies will be reported.

B284 NVP-AUY922, a novel diarylisoaxazole resorcinol HSP90 inhibitor, potently inhibits growth and metastasis of human tumor xenografts. Suzanne A. Eccles1, Swee Y. Sharp1, Florence I. Raynaud1, Melanie Valenti2, Lisa Patterson1, Sharon Gowan1, Kathy Boxall1, Wynne Ahern1, Martin Rowlands1, Angela Hayes2, Vanessa Martins1, Frederique Urban1, Christosotros Prodromou2, Laurence Pear3, Karen James1, Thomas P. Matthews1, Kwan-Ming Cheung1, Andrew Kalusa1, Keith Jones1, Edward McDonald1, Paul A Brough4, Andrew Massey4, Brian Dymock4, Martin Drysdale5, Paul Workman6. 1The Institute of Cancer Research, Sutton, United Kingdom; 2The Institute of Cancer Research, London, United Kingdom; 3Vernalis Ltd, Granta Park, Cambridge, United Kingdom.

NVP-AUY922 is a new diarylisoaxazole resorcinol heat shock protein 90 (HSP90) inhibitor that was developed from a structure-based design programme based on an original high-throughput screening hit (CCCT18159). NVP-AUY922 was selected based on its high potency for the HSP90 target (Kd=1.7 ± 0.5 nM) and its excellent uptake and retention in human tumor cells and xenografts. The purpose of the present studies was to explore the antitumor efficacy of this compound in vitro and in vivo. NVP-AUY922 potently inhibited the proliferation of a wide range of human tumor cell lines with G10 values of ~2-2.5nM, inducing G1/G2 arrest and apoptosis. This activity was independent of NQO1/DT diaphorase and P-glycoprotein, and was maintained in drug resistant variants and under hypoxic conditions. The molecular signature of HSP90 inhibition, comprising upregulation of HSP72 and downregulation of CREB, CDK4 and ERBB2 was demonstrated by western blotting in cells treated with NVP-AUY922 in vitro. NVP-AUY922 also inhibited chemomigration (Transwell assay), haptotaxis (scratch wound assay) and matrix invasion of a variety of tumor cells. Daily dosing (50mg/kg i.p. or i.v.) to athymic mice bearing established human tumor xenografts gave statistically significant growth inhibition and a proportion of regressions in many different tumor types with distinct molecular abnormalities, eg BT474 breast carcinoma tumor/control (T/C) 21%, A2780 ovarian carcinoma (T/C 10.5%), U87MG glioblastoma (T/C 37%), PC3L3 prostate carcinoma (T/C 37%) and WM266.4 BRAF mutant melanoma (T/C 31%). Therapeutic effects were concordant with changes in pharmacodynamic markers including depletion of ERBB2, CREB, CDK4, P-AKT, HIF-1α and increased HSP72 as determined by western blot, MitoTEMPO measurement and immunohistochemical analysis of xenografts. NVP-AUY922 significantly inhibited established lung metastases from WM266.4 melanoma (reducing the number and size by 72% and 81% respectively) and lymphatic metastasis from orthotopically implanted PC3L3 prostate carcinoma (mean tumor burden of regional nodes reduced by 52% and distant metastases undetectable). Finally we showed that NVP-AUY922 inhibited
human endothelial cell proliferation (GI50 of 3.9 and 2.5 mM in HUVEC and HDMEC respectively), chemomigration and tubular differentiation (>50% inhibition at 10 mM). This potential antiangiogenic activity was reflected in reduced microvessel density in human tumor xenografts. Collectively the data show that NVP-AUY922 is a potent, novel inhibitor of HSP90, acting via several complementary mechanisms (e.g. cell proliferation, apoptosis, invasion and angiogenesis) to inhibit tumor growth and metastasis. NVP-AUY922 has recently entered Phase I clinical trials.

B285 SN595 potentiates the in vivo antitumor activity of carboplatin, cisplatin, and gemcitabine in solid tumor xenografts. Jeffrey L. Kumer1, Jennifer P. Arbitalo1, Jeffrey J. Jones1, Nirupama Henjarappa1, Ute Hoch1, Jeffrey A. Silverman1, Anthony R. Howlett1, Caroline D. Scatena1. Sunesis Pharmaceuticals, Inc., South San Francisco, CA.

SN595 is currently under clinical investigation in acute leukemia and ovarian cancer. Clinical responses have been observed in these indications, as well as in non-small cell (NSCLC) and small cell lung cancers. SN595 is a replication-dependent DNA damaging agent that causes irreversible G2 arrest, and rapid apoptosis. A secondary mechanism for SN595 is a unique inhibition of topoisomerase II that causes highly selective DNA damage with low dependence on topoisomerase II for its potent anti-tumor activity. The identification of new anti-cancer agents to be used in combination with established cancer therapies is a key step in the development of new treatment modalities. Studies in culture have shown that SN595 combines synergistically with established chemotherapeutic agents (Wright et al., Proc Amer Assoc Cancer Res 47: 2132, 2006). Extending these findings to in vivo studies, we have shown that SN595 acts synergistically with cytarabine, the standard of care in acute myeloid leukemia (AML), to ablate bone marrow (Arbitaio et al., Blood (ASH Annual Meeting Abstract) 108: 2321, 2006). These studies have led to investigation of SN595 in combination with cytarabine clinically in AML patients. In the current study, we examine the potential of SN595 to combine effectively with carboplatin, cisplatin, and gemcitabine in vivo in several solid tumor xenograft models. SN595, cisplatin, carboplatin and gemcitabine were administered alone or in combination to athymic nude mice bearing subcutaneous H460 NSCLC tumors or A2780 ovarian carcinoma tumors. Treatments were initiated when mean tumor volume was greater than or equal to 100 mm3. The animals were monitored for tumor growth and assessed for tolerability of the different treatment regimens. In the H460 NSCLC model, administration of SN595 (10 mg/kg IV qw x5) in combination with carboplatin at 75% of its maximum tolerated dose (MTD, 75 mg/kg IP qw x3) induced significant tumor growth inhibition (TGI) of 77% compared to 32% for carboplatin alone. Similarly, in the A2780 model of ovarian carcinoma, SN595 (10 mg/kg IV qw x3) combined with cisplatin at its MTD (5 mg/kg IP qw x3) resulted in a TGI of 64% compared to 49% for single agent cisplatin. The combination of SN595 (10 mg/kg IV qw x3) with gemcitabine at its MTD (75 mg/kg IP qw x2) results in an 82% TGI and 51 day tumor growth delay compared to 37% TGI and 5 day tumor growth delay for single agent gemcitabine. Body weight losses of 8%, 14% and 8% were observed for the SN595/carboplatin, cisplatin and gemcitabine combinations, respectively, indicating that the dosing regimens were well tolerated. The results from these studies clearly demonstrate that SN595 combines effectively with platinum compounds or anti-metabolites such as gemcitabine, and support clinical exploration of SN595 in combination with these established chemotherapeutic agents.

B286 PCI-27483, a small molecule inhibitor of factor Vila, inhibits tumor growth in vivo. David J. Lourey1, Patti Thienn mann1, Julia E. Prescott1, Joseph Buggy1. 1Pharmacy, Sunnyvale, CA.

PCI-27483 is a selective small molecule inhibitor of activated coagulation factor VII (FVIIa), which is in preclinical development as a novel anti-cancer agent. Tissue factor (TF), the major initiator of the coagulation cascade, has been shown to be over expressed in numerous primary human tumors, including pancreatic, colon, breast and prostate, and this expression has been linked to disease progression. TF binds to and activates FVII initiating both a proteolytic cascade that leads to coagulation as well as intracellular signaling through the G protein coupled receptor PAR2. Such signaling induces phosphorylation of Akt and MAPK and secretion of vascular endothelial growth factor (VEGF) and the autocrine growth factor IL8, known to stimulate chemotaxis and invasion. Others have shown that the nematode anticoagulant protein (rNAPc2), a TF/FVIIa inhibitor, suppresses the growth of B16F10 melanoma and Lewis lung carcinoma (LLC) cells in murine tumor models (Hembrough et al, Cancer Research 2003). Previous work done in vitro has shown that PCI-27483 inhibits the TF/FVIIa complex induced phosphorylation of Akt and MAPK in BaPc3 cells, a human pancreatic cancer line that highly expresses TF (Holsinger et al, AACR Annual Meeting 2006). In order to more directly examine the role of TF/FVIIa signaling in tumor growth, we tested the efficacy of PCI-27483 in mice implanted with TF expressing tumor cells. In a syngeneic model, subcutaneous administration of PCI-27483 at 22.5 and 45 mg/kg bid to C57Bl/6 mice implanted with LLC inhibited tumor growth by 45% and 48%, respectively, after 9 days of dosing. In a xenograft model where CD1 nu/nu mice were implanted with HCT-116 (human colorectal carcinoma) cells, subcutaneous administration of PCI-27483 at 90 mg/kg bid, inhibited tumor growth by 65% during the first 11 days of treatment. These data indicate that PCI-27483 has tumor suppression activity in vivo, more so in the LLC syngeneic model than the HCT-116 xenograft model, and suggests that the inhibition of FVIIa has significant therapeutic potential in the treatment of patients with tumors dependent on FVIIa activity for progression.

B287 Antitumor activity of CP-4055 is enhanced in combination with bevacizumab, cetuximab, and trastuzumab in human NSCLC xenografts. Skjalg Bruheim1, Gunhild M. Melandsmo1, Marit Liland Sandvold2, Finn Myhren2, Øystein Fostad3, 1Rikshospitalet–Radiumhospitalet Medical Center, Oslo, Norway; 2Clavis Pharma ASA, Oslo, Norway.

Background: CP-4055 (cytarabine S’-elaidic acid ester) is a cytotoxic nucleoside analog with broad preclinical antitumor activity in solid tumors and hematologic cancers. CP-4055 uptake is independent of expression of the equilibrative nucleoside transporters. Currently several clinical studies are being conducted with CP-4055 single agent in patients with solid tumors and refractory leukemias. The monoclonal antibodies (mAbs) bevacizumab, cetuximab and trastuzumab have all exhibited clinical activity when administered in combination with cytotoxic chemotherapy. This study was undertaken to evaluate the combinability of CP-4055 with the monoclonal antibodies in animal tumor models.

Methods: The antitumor activity of CP-4055 was tested at MTD (50 mg/kg) and ½ MTD, alone or in combination with bevacizumab (5 mg/kg), cetuximab (20 mg/kg) and trastuzumab (4 mg/kg) in two human NSCLC xenografts, MAKSAX and EKVX in Balb/c nu/nu mice. The EKVX and MAKSAX xenografts were characterized for receptor target expression EGFR, HER-2 and VEGF by immunohistochemistry prior to testing of antitumor activity. A high proportion of the cells in the xenografts MAKSAX and EKVX stained positive for EGFR and HER-2 (+++), and both xenografts stained positive for VEGF (++). Results: The antitumor activity of CP-4055 single agent was of similar magnitude both at MTD and ½ MTD and it was enhanced by adding bevacizumab or trastuzumab and to some extent by cetuximab in the MAKSAX xenograft. In the EKVX model, CP-4055 was highly active as monotherapy and a possible additive effect of combination treatment with each of the mAbs could therefore not be distinguished conclusively. The combination treatment regimens were well tolerated, with no increased toxicity in terms of weight loss compared to monotherapy.

Conclusion: Potential activity warrants clinical studies of regimens including CP-4055, in patients eligible for therapy with mAbs.
B288 Design of cyclic Smac mimic peptide and in vitro characterization of its complex with X-linked inhibitor of apoptosis protein (XIAP). Zaneta Nikolovska-Coleska1, Jennifer L. Meagher2, Chao-Yie Yang3, Sheng Jiang4, Su Qiu5, Jeanne Stuckey6, Peter P. Roller4, Shaomeng Wang3. 1Department of Internal Medicine, Pharmacology, Medicinal Chemistry and Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI; 2Life Sciences Institute, University of Michigan, Ann Arbor, MI; 3Laboratory of Medicinal Chemistry, National Cancer Institute-Frederick, Frederick, MD.

XIAP is a central apoptosis regulator and inhibits apoptosis by binding to and inhibition of effectors caspase-3/-7 and an initiator caspase-9 through its Bir2 and Bir3 domains, respectively. Smac protein in its dimeric form effectively antagonizes XIAP by concurrently targeting both its Bir2 and Bir3 domains. Based on the proposed model for the mechanism of Smac protein we had designed and synthesized cyclic peptide containing two-site interaction N-terminal Smac mimetic tetra peptide binding motif, named as PSmac21.

Using a series of experiments, fluorescence polarization-based binding assay, caspase activity assay, gel filtration chromatography and X-ray study we have characterized PSmac21 on biochemical, structural and functional levels. Our results showed that PSmac21 interacts with XIAP constructs with very high affinity and forms complexes with different stoichiometric ratio. PSmac21 binds to XIAP containing both Bir2 and Bir3 domains with an IC50 of 4 nM, being > 130 and > 2,500 times more potent than its linear counterpart tetrapeptide and the natural Smac AVPI peptide, respectively. Gel filtration experiments with Bir3 domain showed that PSmac21 forms a 1:2 stoichiometric complex, while in the presence of XIAP protein containing both Bir2 and Bir3 domains forms a 1:1 stoichiometric complex. These gel filtration results indicate that the dimerization of the Bir3 is propagated via the bivalent nature of the cyclic peptide, PSmac21, while with the XIAP protein containing both Bir2 and Bir3 domains, PSmac21 can simultaneously interact with the Bir2 and Bir3 domains from a single protein. Interestingly, in the presence of XIAP protein containing both Bir2 and Bir3 domains, but binding to the Bir2 domain was knocked out by mutation, Bir2(E219R)-Bir3, PSmac21 causes dimerization of this protein, similar as in the presence of Bir3 only, indicating that Bir2 domain is involved in the interaction with PSmac21. This interaction not only possesses high binding affinity because of avidity, but also important PSmac21 can efficiently antagonize inhibition of effector and initiator caspases by XIAP containing both Bir2 and Bir3 domains in both, cell-free functional and cell-based assays. Importantly, PSmac21 inhibits cell growth in MDA-MB-231 breast cancer cells, indicating that this cyclic peptide is cell-permeable.

We have determined the crystal structure of PSmac21 in a complex with XIAP Bir3 to 2.1 Å resolution. This high resolution crystal structure clearly shows that cyclic peptide PSmac21 induces homodimerization of XIAP Bir3 and provides a structural basis for the cooperative binding of one PSmac21 to two XIAP Bir3 molecules. Based on our crystal structure we have constructed a structural model between Bir2-Bir3 and PSmac21. This model provides structural information for designing of novel bivalent Smac mimetics inhibitors targeting both domains in XIAP. Our model further suggests small molecule bivalent Smac mimetics may be used as potent inhibitors mimicking the function of the Smac protein agonist in the full length XIAP protein.

B289 Cassette dosing in tumor bearing animals for the discovery of NVP-AUY922, a novel HSP90 inhibitor. Florence J. Raynaud1, Angela Hayes1, Vanessa Martins1, Nicola F. Smith2, Swee Y. Sharp3, Melanie Valentl1, Alan Henley1, Thomas P. Matthews1, Kway-Ming Cheung1, Andrew Kalusa1, Keith Jones1, Karen James3, Edward McDonald1, Paul A Brough4, Andrew Massey2, Brian Dymock2, Martin Drysdale2, Suzanne A Eccles1, Paul Workman1. 1The Institute of Cancer Research, Sutton, United Kingdom; 2Vemalis Ltd, Granta Park, Cambridge, United Kingdom.

Cassette or cocktail dosing increases pharmacokinetic throughput in drug discovery by administering a combination of compounds to animals and measuring each discrete compound by LCMS/MS. We first demonstrated that cassette dosing did not affect the mouse plasma pharmacokinetics (PK) of individual compounds in a series of diarylpyrazole resorincols. Having validated the approach, we used this methodology to evaluate plasma and tumor pharmacokinetics in a diarylisoxazole resorincol series. Combinations of five novel agents were administered together with a pharmacokinetic standard (VER-50589) to NCr athymic mice bearing HT116 human colon carcinoma xenografts using an i.v. dose of 4 mg/kg of a cocktail of each compound in 10% DMSO, 1% tween 20 in saline. Evaluation of plasma and tumor pharmacokinetics showed no more than 2-fold variation in plasma and tissue levels and pharmacokinetic parameters of compound VER-50589 dosed as a standard in 5 different cassettes. The tested compounds showed much greater differences in distribution in tumors with tumor to plasma ratios ranging from 0.2 to 19.6 resulting in significant differences in tumor levels between compounds. Those compounds with the greatest tumor concentrations giving values well above their in vitro cellular inhibitory activity (GI50) were selected for further in vivo evaluation. The compound NVP-AUY922 gave tumor concentrations 35 times above its GI50 and was subsequently shown to have significant antitumor efficacy associated with target modulation in xenograft models. This work exemplifies the power of cassette dosing in tumor bearing animals. NVP-AUY922 has recently entered Phase I clinical trials.

B290 MKC-1 significantly increases survival of mice bearing renal cell carcinoma Caki-1 xenograft tumors through inhibition of the Akt/mTOR pathway. Patricia A. Burke1, Xiaoguo Zhan2, Gizachew Kifle1, Trisha Denny2, William E. Fogler1, Mark R. Bray3, Theresa M. LaVallie1. 1EntreMed, Inc., Rockville, MD; 2EntreMed, Inc., Toronto, Ontario, Canada; 3Laboratory of Medicinal Chemistry, National Cancer Institute-Frederick, Frederick, MD.

MKC-1 (previously Ro 31-7453) is a novel, orally active cell cycle inhibitor with significant in vitro and in vivo activity against a broad range of tumor cell lines. MKC-1 has antiproliferative activities at an IC50 value of 200 nM towards most tumor cell lines. In contrast, renal cell carcinoma cell lines are sensitive to MKC-1, with IC50 values ranging from 20 to 80 nM towards Caki-1, Caki-2, RCC2 and RCC2/VHL cells. Several proteins have been identified as binding targets of MKC-1, including microtubules (colchicine binding site) and members of the importin family (involved in nuclear transport and spindle formation). The importin-β family of proteins are comprised of a series of HEAT repeats, which are tandemly-arrayed structural elements thought to act as "scaffolding" platforms for protein interactions. mTOR is similarly composed largely of HEAT repeats, and is one of the proteins most closely related to the importins phylogenetically. Therefore, we explored whether MKC-1 inhibited the Akt-mTOR pathway. MKC-1 treatment resulted in inhibition of p-mTOR and its downstream target, p70S6 kinase in Caki-1 cells. MKC-1 treatment resulted in antiproliferative activities at an IC50 value of 200 nM towards most tumor cell lines. In contrast, renal cell carcinoma cell lines are sensitive to MKC-1, with IC50 values ranging from 20 to 80 nM towards Caki-1, Caki-2, RCC2 and RCC2/VHL cells. Several proteins have been identified as binding targets of MKC-1, including microtubules (colchicine binding site) and members of the importin family (involved in nuclear transport and spindle formation). The importin-β family of proteins are comprised of a series of HEAT repeats, which are tandemly-arrayed structural elements thought to act as "scaffolding" platforms for protein interactions. mTOR is similarly composed largely of HEAT repeats, and is one of the proteins most closely related to the importins phylogenetically. Therefore, we explored whether MKC-1 inhibited the Akt-mTOR pathway. MKC-1 treatment resulted in inhibition of p-mTOR and its downstream target, p70S6 kinase in Caki-1 cells. Akt activates mTOR and Akt is activated by PDK1 on Thr308 and Akt itself on Thr473. MKC-1 treatment of Caki-1 cells resulted in a dose and time dependent reduction in pSer473Akt, but not p(ser241)-Akt. MKC-1 (previously Ro 31-7453) is a novel, orally active cell cycle inhibitor with significant in vitro and in vivo activity against a broad range of tumor cell lines. MKC-1 has antiproliferative activities at an IC50 value of 200 nM towards most tumor cell lines. In contrast, renal cell carcinoma cell lines are sensitive to MKC-1, with IC50 values ranging from 20 to 80 nM towards Caki-1, Caki-2, RCC2 and RCC2/VHL cells. Several proteins have been identified as binding targets of MKC-1, including microtubules (colchicine binding site) and members of the importin family (involved in nuclear transport and spindle formation). The importin-β family of proteins are comprised of a series of HEAT repeats, which are tandemly-arrayed structural elements thought to act as "scaffolding" platforms for protein interactions. mTOR is similarly composed largely of HEAT repeats, and is one of the proteins most closely related to the importins phylogenetically. Therefore, we explored whether MKC-1 inhibited the Akt-mTOR pathway. MKC-1 treatment resulted in inhibition of p-mTOR and its downstream target, p70S6 kinase in Caki-1 cells. Akt activates mTOR and Akt is activated by PDK1 on Thr308 and mTOR/rictor on Ser473. MKC-1 treatment of Caki-1 cells resulted in a dose and time dependent reduction in pSer473Akt, but not p(ser241)-Akt. MKC-1 was tested for activity against a panel of 100 recombinant kinases, and was shown to have significant activity only towards GSK3 β, with an IC50 value of 7 nM. No inhibition of PI3K, PDK1, or Akt itself was observed. Akt activation results in phosphorylation of a number of substrates in addition to mTOR, including MDM2, FOXO, and I KK. Phosphorylation of MDM2 was also reduced by exposure of cells to MKC-1. The mechanism by which MKC-1 impacts the Akt/mTOR pathway is currently being investigated. However, the data are consistent with inhibiting the mTOR/rictor pathway. In vivo, daily oral treatment with MKC-1 (200 mg/kg) significantly increased the median survival time (MST) of mice bearing Caki-1 renal cell xenograft tumors. The MST for the control treated animals was 49.5 days with no animals surviving on day 88. In contrast, MKC-1 treated animals resulted in a MST of 88 days. In conclusion, our results demonstrate that MKC-1 interferes with the Akt/mTOR pathway important to cancer progression, and has significant activity in preclinical RCC tumor models. MKC-1 may provide clinical benefit in the treatment of renal cell cancer.
B291 Synergy of homoharringtonine with hydroxyurea on leukemia cell lines with and without BCR-ABL. Shawmya Michaels1, Vasu Kanekal1, Stephanie Liu1, Emily Ruby1, Dennis Brown1. 1ChemGenex Pharmaceuticals, Inc., Menlo Park, CA.

Homoharringtonine (USAN/INN designation - omacetaxine mepesuccinate (OMA)) is the semisynthetic version of a natural product alkaloid from the Cephalotaxus evergreen family. Ongoing trials of OMA in CML resistant to tyrosine kinase inhibitor therapies are reporting encouraging results. Identified initially for its inhibitory effects on protein synthesis (elongation step), recent laboratory studies have also demonstrated that OMA upregulates apoptotic pathways and inhibits angiogenic processes. OMA is active in leukemia regardless of BCR/ABL status. Hydroxyurea (HU) is a ribonucleotide reductase inhibitor that is often used to control leukemia in the chronic phase, either alone, or in combination with other leukemia treatments. The use of HU in combination with OMA has not been previously reported and may be important for the future clinical use of OMA. Using a 5 day drug exposure assay, OMA and HU were applied individually and in combination onto K562 (erythromyeloblastic and BCR/ABL positive) and HL-60 (promyelocytic and BCR/ABL negative) human leukemia cell lines. Assessment of cell survival and the percent growth inhibition for the individual agents and the combinations was determined by Coulter Counter analysis. Data was analyzed using CalcuSyn (Biosoft, UK) to determine the IC50 of each agent and whether the treatment combinations were synergistic, antagonistic or additive. The IC50 of OMA was 1.2 nM for K562 and 0.3 nM for HL-60 while the IC50 for HU was 31.0 μM for K562 and 6.7 μM for HL-60. For both K562 and HL-60 cell lines, combinations containing either 1 nM or 10 nM of OMA were synergistic with all concentrations of HU tested. These results support the potential clinical use of HU with OMA.

B292 SB939: A potent and orally active HDAC inhibitor for the treatment of colorectal cancer. Kanda Sangthongpitag1, Gedas Greicirius2, Walter Stünkel1, Zahid Bonday3, Khee Chuan Goh1, Wang Xukun1, Xiaofang Wu1, Chongyang Hu2, Haishan Wang1, Eric Sun1, Michael Entzeroth5, Sven Pettersson2, Jeanette Wood1. 1SBIO Pte Ltd, Singapore; 2Karolinska Institute, Stockholm, Sweden.

Histone deacetylases (HDAC) have emerged as new molecular targets for cancer therapy. Small molecule HDAC inhibitors have been developed and shown to induce tumor cell cytostasis, differentiation and apoptosis in experimental models and efficacy in clinical trials in various hematological malignancies following intravenous and/or oral administration. However, they have not shown promising activity in solid tumors, which may be due to their poor pharmacokinetic properties and dose-limiting toxicities that don't allow adequate tumor exposure to the drug. SB939 is a novel HDAC inhibitor with improved metabolic, pharmacokinetic and pharmacological properties compared to other HDAC inhibitors currently in clinical trials. The objective of this study was to characterize the efficacy of SB939 in models of colon cancer. RNA interference mediated knockdown of class I and class II HDACs in the human colorectal cancer cell line, HCT116, revealed important cancer relevant functions for both classes emphasizing the usefulness of pan-HDAC inhibitors for the treatment of colorectal cancer. SB939 selectively inhibits HDAC class I and II enzymes, with K_i values ranging from 16 to 247 nM and inhibits proliferation of colon cancer cell lines with IC_{50} in the range of 500 nM. In these cell lines, SB939 induced cell cycle arrest leading to apoptotic cell death and also increased acetylation of histone H3 and tubulin, increased the expression of p21, and reduced phosphorylation of retinoblastoma protein (pRb). SB939 also induced apoptosis in primary cells isolated from colorectal cancer patient tumor specimens. SB939 has excellent pharmacokinetic properties and tolerability after oral administration in mice. In a subcutaneous human colorectal cancer (HCT116) xenograft model, SB939 significantly and dose-dependently inhibited tumor growth (%TGI values after once daily oral dosing at 25, 50, 75 and 100 mg/kg for 21 days were 37%, 61%, 76% and 97% respectively). SB939 increased the level of acetylation of H3 in the HCT116 tumor tissue for up to 24 h and reduced the number of perfused blood vessels in the tumors. In APC^-/- mice, a transgenic mouse model of early stage colorectal cancer, SB939 significantly reduced tumor load in both small intestine and colon (by 46% and 55% respectively after a once daily oral dose of 50 mg/kg for 21 days). In contrast, 5FU (40 mg/kg i.p. once daily for 5 days every 2 weeks for 21 days) significantly reduced tumor load in the small intestine only (39%). In conclusion, our data show that SB939 is a potent, orally active anti-tumor drug with potential for the treatment of early and late stage colorectal cancer.

B293 A novel class of mitotic kinesin Eg5 inhibitors: Design, synthesis and characterization of L-cysteine derivatives. Naohisa Ogo1, Yutaka Uehara1, Kenji Matsuno1, Jun-ichi Sawada1, Akira Asai2. 1Center for Drug Discovery, Graduate School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan.

Antimototic agents such as taxol, epothilone, and vinca alkaloids have found clinical utility as cancer chemotherapeutic agents. While effective against most cancer types, these agents can be compromised by undesirable side effects including neurotoxicity and acquired resistance. The microtubule associated kinesin Eg5 also known as kinesin spindle protein (KSP) plays an important role in the early stages of mitosis. It is responsible for the formation and maintenance of the bipolar spindle. Because Eg5 is not expressed in postmitotic neurons and is likely to act only in dividing cells, its inhibitors might provide better specificity than microtubule inhibitors in the treatment of human malignancies. Until now, a number of Eg5 inhibitors have been identified, including S-butyryl-L-cysteine (STLC). We recently reported that STLC derivatives such as S-[4- (methylxoy)phenyl][diphenyl][methyl]-L-cysteine (1) and S-[4- (trifluoromethyl)phenyl][diphenyl][methyl]-L-cysteine (2) were 7-10 fold more potent than STLC itself both in Eg5 ATPase assay and HeLa cells cytotoxicity assay (Bioorg. Med. Chem. Lett. 2007, 13, 987-92). In order to explore the importance of bulkiness or hydrophobicity on the phenyl group, we have synthesized several L-cysteine derivatives focused on modification of the phenyl group in STLC. The methoxy derivative (1) showed selective inhibition against STLC and induced mitotic arrest with characteristic monoaxial spindles in HeLa cells. Because of the potent and selective activities of compound (1), we are interested in the molecular interactions of compound (1) with the cavity of Eg5. Consequently, we conducted as design and prepare the affinity beads immobilized with compound (1) to determine the mode of binding of STLC to Eg5. Further structural optimization, biological evaluation and the mode of action of L-cysteine derivatives will be reported in detail.


SR13654, developed from a dietary anticancer agent, exhibits potent anticancer activity in a variety of human tumor xenograft models, including prostate, breast and lung cancers. Preliminary mechanistic studies show that SR13654 is able to downregulate survivin and EGFR expression in PC-3 prostate cancer cells. Survivin, a member of the Inhibitor of Apoptosis gene family, preferentially expresses in malignant cells over normal cells and has been correlated with aggressive tumor behavior, drug resistance, and shortened survival times. Induction of survivin expression by paclitaxel has been reported to be an early event that initiates and confers paclitaxel resistance in cancer cells. We showed that SR13654 inhibits nuclear survivin expression and increase the efficacy of paclitaxel in human prostate tumor xenografts. Further in-depth mechanistic studies show that SR13654 inhibits Src signaling pathway.
Src tyrosine kinase is overexpressed in a broad range of tumors and is believed to play a critical role in tumor growth, invasion, metastasis and angiogenesis. Src is known to be both an upstream activator and downstream mediator of epidermal growth factor receptor (EGFR), and inhibition of Src activation has been reported to enhance EGFR degradation. Western blot analysis shows that SR13654 selectively inhibits pSrc(Y416) but not pSrc(Y527), and downregulates EGFR expression in a dose-dependent manner. Since Src-family kinases are activated in non-small cell lung cancer (NSCLC) and known to promote the survival of EGFR-dependent NSCLC cells. Moreover, EGFR-mutant NSCLC cell lines were also shown to have different selectivity to Src suppression. We then assessed SR13654’s effect in NSCLS cell harboring distinct EGFR mutations, including A549 (WT EGFR), and H1650 (DelE746A750) and H1975 (L858R + T790M). SR13654 was able to inhibit cell growth in all three NSCLC cell lines, but was more potent in A549 and H1975 cell lines. SR13654 also showed potent oral antitumor activity in A549 lung tumor xenograft. Since mutations in EGFR kinase domain are frequently observed in NSCLC and associate with clinical responses to EGFR inhibitors. Thus, SR13654 could be effective in treatment of NSCLC harboring specific types of mutated EGFR.

In summary, SR13654 is an orally active anticancer agent and has been shown to have potent anticancer activity in various human tumor xenograft models. SR13654 could be used alone or in combination with cytotoxic therapy to delay or prevent survivin-induced drug resistance in tumors, and ensure continued benefit from current therapies. Analysis of samples from a rat and mouse pharmacokinetics (PK) study is in progress.

**B295 Identification of novel inhibitors of USP2 and USP7.** Seth J. Goldenberg1, Craig A. Leach1, Matthew P. Kodrasov1, Tauseef R. Butt1, Michael R. Mattern1, Benjamin Nicholson1.1Progenra Inc, Malvern, PA.

The ubiquitin-proteasome system is critical to normal cellular function, for example, polyubiquitination of proteins typically results in protein degradation by the proteasome and monoubiquitination has been shown to alter protein localization. Ubiquitin is removed from proteins by deubiquitinating enzymes (DUBs) and many of these enzymes have been implicated in a wide range of pathologies including cancer, neurodegeneration, and viral infection. Specifically, USP2a is oncogenic, and its inhibition has been shown to sensitize prostate carcinoma cells to apoptosis. In addition, USP7 (HAUSP) activity has been shown to indirectly reduce the levels of the tumor suppressor p53 in a wide variety of tumors. The approval of the proteasome inhibitor Bortezomib as a treatment for multiple myeloma has demonstrated that the ubiquitin-proteasome system is a viable target for the treatment of cancer. Unfortunately, the efficacy of proteasome inhibitors is limited by toxicity. Selectively modulating DUB activity rather than the proteasome is predicted to result in a more targeted therapy with reduced toxicity.

Progenra has developed a novel assay to measure the proteolytic cleavage of ubiquitin or UBL (ubiquitin like protein) conjugates such as SUMO, NEDD8 or ISG15 by isopeptidases. More than 100 isopeptidases have been reported in the literature and we have tested many of these in the Progenra platform. The technology is driven by a reporter system that remains dormant when conjugated to ubiquitin or UBL. Upon cleavage of the ubiquitin/UBL by its cognate isopeptidase, the previously inactive reporter enzyme is able to turnover its substrate, generating a signal that is a quantitative measure of isopeptidase activity. The Progenra platform is remarkably sensitive and selective for isopeptidases and superior to current assay systems.

We have used this assay to screen several libraries of small molecules to identify inhibitors of USP2 and USP7. Hits from these screens have been profiled in a number of secondary in vitro and cell based assays designed to identify and profile compounds for preclinical development. The most promising compounds are being further studied as clinical candidates for novel therapeutics targeting USP2a and USP7.