328 POSTER

Clinical and molecular studies of the effect of imatinib on advanced aggressive fibromatosis (desmoid tumors)

M. Heinrich[1], G.A. McArthur[2], H. Joensuu[3], G.D. Demetri[4], S. Dimitirjevic[5], C.L. Corless[1], S. Dirnhofer[6], Z. Nikolova[5], J.A. Fletcher[4], Imatinib Target Exploration Consortium[7]. [1]*OHSU Cancer Institute, Division of Hematology/Medical Oncology, Portland, OR, USA;* [2]*Peter MacCallum Cancer Centre, East Melbourne, Australia;* [3]*Helsinki University Central Hospital, Helsinki, Finland;* [4]*Dana Farber Cancer Institute and Harvard Medical School, Boston, MA, USA;* [5]*Novartis Pharma AG, Clinical Research Oncology, Basel, Switzerland;* [6]*Kantonspital, Basel, Switzerland;* [7]*Imatinib Targeted Study Group, ITEC Study B2225, Basel, Switzerland*

Aggressive fibromatosis (AF, also known as desmoid tumor) is a fibroproliferative neoplasm that typically arises in the abdomen but can present as a primary tumor of extrabdominal site, most notably in an extremity location. These tumors have a relatively high local failure rate after primary treatment using surgery and/or radiotherapy but rarely give rise to distant metastases. Most AF have abnormalities in the regulation of WNT pathway signaling, due either to germline/somatic inactivation of APC **or** somatic gain-of-function mutations of beta-catenin. Enforced expression of mutant beta-catenin in murine mesenchymal cells results in formation of AF lesions. The optimal medical therapy of recurrent and/or unresectable AF is uncertain, although some activity of has been reported for small series of patients treated with tamoxifen, NSAIDSs, or chemotherapy. Mace el al. reported the response of two patients with *extra-abdominal* AF to imatinib mesylate (IM) (Cancer 95:2373, 2002). In the current study, we treated 19 patients with with unresectable AF enrolled in a phase II trial investigating the effects of IM on multiple types of cancers, including a variety of sarcomas. The mean patient age was 28 years (range 17–63) with 18 of 19 patients having undergone one or more prior surgeries and14 of 19 patients having undergone prior medical therapy, including tamoxifen, NSAIDS, and/or chemotherapy. Patients were treated with 400 mg bid of IM and clinical activity was assessed using CT/MRI imaging and conventional SWOG (pre-RECIST) response criteria. All patients had progressive disease at the time of study entry. 3/19 patients (16%) had a partial response (all with abdominal primary sites) and an additional 3 pts had SD > 18 months (2 abdominal, 1 extremity primary site), giving an overall clinical benefit rate of 32% (6/19). Tumor specimens were evaluated for genomic or proteomic evidence of activation of IM target kinases and genomic evidence of beta-catenin mutations. There was no association of response/nonreponse with underlying APC or beta-catenin mutations. IHC and immunoblotting revealed no evidence of significant KIT or PDGFRA expression or kinase activation. No intragenic mutations of *KIT, PDGFRA*, or *PDGFRB* were found. Low amounts of PDGFRB activation were seen in immunoblotting experiments, consistent with a possible autocrine/paracrine mechanism of activation. We conclude that IM appears to have significant activity in treating refractory AF arising either in abdominal or extra-abdominal sites, possibly through inhibition of PDGFRB kinase activation. Further studies are needed to 1) better define the clinical activity of IM for the treatment of AF, especially in comparison to other medical therapies; 2) identify the mechanism of action underlying IM response; and 3) identify biomarkers to aid in patient selection for treatment with IM.

329 POSTER

Antitumor activity in preclinical xenograft models of OSI-930, a novel selective tyrosine kinase inhibitor

S.L. Winski[1], M.A. Bittner[1], P. Briner[2], E.N. Brown[1], A.R. Cooke[1], A. Crew[3], C. Hidden[2], C. Pirrit[1], M.S. Srebernak[1], D.L. Emerson[1]. [1]*OSI Pharmaceuticals Inc., Pharmacology, Boulder, CO, USA;* [2]*OSI Pharmaceuticals Inc., Chemistry, Oxford, UK;* [3]*OSI Pharmaceuticals Inc., Chemistry, New York, USA*

Tyrosine kinases have been implicated in many cellular pathways, including growth, survival and apoptosis, and are consequently considered to be biologically relevant cancer targets. We have identified a series of 2,3-substituted thiophene compounds with potent inhibitory activity against the closely-related Kit, KDR, PDGFRα and PDGFRβ enzymes. These targets are implicated in a broad range of tumor types including GIST, small cell lung carcinoma (SCLC), renal cell carcinoma, colorectal carcinoma and glioblastoma. OSI-930 is the lead potential clinical candidate from this series, which potently inhibits (IC50<1 μM) the *in vitro* tyrosine kinase activity of Kit, KDR, PDGFRα/β and other potentially important kinase targets. OSI-930 has good oral bioavailability in mice and prolonged plasma exposure, which allows for single daily dosing in xenograft efficacy studies. Dose-dependent tumor growth inhibition was observed in a number of models, including SW48 colon carcinoma and NCI-H526 and NCI-H209 SCLC. In these studies, maximal effects on tumor growth were observed when OSI-930 was delivered daily at 200 mg/kg. This dose and schedule were well tolerated and minimal body weight loss was observed when OSI-930 was delivered up to 38 consecutive days. Antitumor activity of OSI-930 as a single agent was explored further in a variety of growth-staged xenograft models. Response was evaluated by tumor growth delay, defined as the delay in time for treated tumors to reach a predetermined size (500% of original size) compared to vehicle matched controls. Growth delay was considered biologically relevant when it was at least equivalent to the number of days that the drug was administered. With this criterion, OSI-930 delivered at 200 mg/kg was effective in delaying tumor growth in SCLC (NCI-H209, WBA), colorectal carcinoma (HT29, HCT-116, LS180, DLD-1, COLO 205, SW48), head and neck carcinoma (KB), gastric carcinoma (NCI-SNU-5), glioblastoma (U251) and renal cell carcinoma (SN12C). In the most sensitive of these models (WBA, U251, NCI-SNU-5 and KB), OSI-930 induced tumor regression and durable cures. These results highlight the utility of an agent, which can directly inhibit key targets and suggest that OSI-930 may have broad clinical utility.

330 POSTER

Magnetic resonance spectroscopy confirms the mechanism of action of the choline kinase inhibitor MN58b in human breast cancer cells

N. Al-saffar[1], L.E. Jackson[1], A. Ramirez de Molina[2], P. Workman[1], J.R. Griffiths[3], I.R. Judson[1], J.C. Lacal[2], M.O. Leach[1]. [1]*Institute of Cancer Research, Royal Marsden NHS Foundation Trust, Surrey, UK;* [2]*Consejo Superior de Investigaciones Cientificas, Madrid, Spain;* [3]*St George's Hospital Medical School, London, UK*

Choline kinase (ChoK) is the enzyme responsible for the generation of phosphocholine (PC) from its precursor choline to form phosphatidylcholine, the most abundant component of the plasma membrane. Increased levels of ChoK activity and PC production in human cancers have been reported (1). Recently, ChoK has also been implicated in cell proliferation playing an important role in mitogenic signal transduction pathways. These observations have resulted in the development of an anti-tumoural strategy focused on ChoK inhibition. MN58b is one of the most potent ChoK inhibitors showing anti-proliferation activity both in vitro and in vivo (1). This work set out to test the hypothesis that phosphorus magnetic resonance spectroscopy (31P MRS) could measure changes in PC levels in MN58b treated cells as an indication that the in vivo 31P MRS might be able to monitor the pharmacodynamics of this drug in patients during clinical trials. The human mammary carcinoma cell line MDA-MB-231 was treated with the specific ChoK inhibitor MN58b (6 uM) for 4, 13, 19, 30 and 48h. MDA-MB-231 cells were also treated with the inactive MN58b analogue ACG20B (6 uM) for 48h. Adherent cells were extracted using a dual phase extraction method. 31P MR spectra were acquired at room temperature on a 500 MHz Bruker spectrometer. Metabolite content was determined by integration and normalised relative to internal standards and cell number. The number of MN58b treated MDA-MB-231 cells was reduced to 78% of control cells at 48h, consistent with decreased proliferation. 31P MRS showed that MN58b treatment led to a significant time-dependent drop in PC levels which started as early as 4h (80.9±5.7%, P=0.04) and was down to (39.5±1.8, P=0.00001) at 48h relative to controls (Fig. 1). In contrast, no statistically significant change in PC level was observed in MDA-MB-231 cells following treatment with ACG20B. This indicates that the 31P MRS detected drop in PC is due to the inhibitory effect of MN58b on ChoK. These results provide further support for the necessary proof-of-principle for the hypothesis that the inhibitory effect on proliferation of MN58b is correlated with its ability to inhibit the production of PC in cells. It also provides an evidence for the potential of MRS as a non-invasive tool for the assessment of anti-tumour activity of ChoK inhibitors in early stage of clinical trials. 1. Lacal, J. C. (2001) IDrugs 4:419–426 This work is supported by Cancer Research UK (C1060/A808/G7643)

331 POSTER

Key roles for HSP90 in tumour neoangiogenesis

S. Eccles, S. Brader, W. Court, G. Box, M. Valenti, S. Gowan, L. Patterson, Z. Ahmad, A. de Haven Brandon, P. Workman. *Institute of Cancer Research, Centre for Cancer Therapeutics, Sutton, UK*

Heat shock protein 90 (Hsp90) chaperones several key signalling molecules involved in angiogenesis, including c-*erb*B-2, Akt, c-met, eNOS and HIF-1α. We have used Hsp90 inhibitors including 17-allylamino,17-demethoxygeldanamycin (17AAG) and geldanamycin (GA) to probe the effects of HSP90 inhibition on key endothelial cell functions required for neoangiogenesis *in vitro*. We also explored the role of Hsp90 in the transcriptional upregulation of angiogenic cytokines via c-erbB oncogene activation and hypoxia in tumour cells, and on angiogenesis in xenograft models *in vivo*.

The expression of client proteins in human endothelial cells (EC) was inhibited in response to 17AAG and geldanamycin in a concentration-

and time-dependent manner. EC in log phase growth were found to be particularly sensitive to the growth inhibitory effects of 17AAG, with IC50's in the low nanomolar range, however, quiescent endothelial cells were relatively resistant. We then tested EC migration using a "haptotaxis" (wounded monolayer) assay and a real-time fluorescence based chemotaxis assay. 17AAG inhibited both EC haptotaxis and chemomigration towards FCS, VEGF, HGF, bFGF and EGF at concentrations below those required to inhibit proliferation. Invasion of Matrigel-coated filters was more potently inhibited than migration, suggesting possible additional effects on matrix proteolysis. We found no effects on MMP-2 activity, but 17AAG inhibited uPA production, as shown previously for tumour cells. 17AAG also significantly reduced EC tubule differentiation on Matrigel. In addition, in several human tumour cell lines, 17AAG inhibited the upregulation of VEGF mRNAs and proteins induced by ligand activation of c-*erb*B oncogenes or hypoxia. *In vivo* we found that murine endothelial cell client proteins were downregulated by 17AAG and growth inhibition of human tumour xenografts was associated with reduced microvessel density.
These results identify HSP90 as an important protein chaperone in tumour cell production of, and functional responses to VEGF and other EC activators. HSP90 inhibitors may have a useful role in cancer therapy not only by directly inhibiting tumour cell proliferation but also via interference with several distinct rate-limiting steps in the angiogenic cascade. The fact that rapidly proliferating EC are more sensitive to Hsp90 inhibitors than quiescent EC suggests that normal vasculature may be spared relative to "angiogenic" vasculature.

332 POSTER

Fragment-based and structure based optimisation of potent PKB/AKT inhibitors

P. Wyatt[1], D. Barford[2], T. Davies[1], S. Woodhead[1], G. Saxty[1], D. Davis[1], M. Garrett[2], F. Raynaud[2], S. Eccles[2], V. Berdini[1]. [1]*Astex Technology Ltd, Medicinal Chemistry, Cambridge, UK;* [2]*Institute of Cancer Research, Cancer Research UK Centre for Cancer Therapeutics, Sutton, UK*

The serine/threonine kinase PKB/AKT is a major downstream target of PI3K. Extensive studies of this protein kinase show that it plays a key role in promoting cancer cell proliferation and survival via inhibition of apoptosis. Growth factor over-expression, mutations in the ras genes, overexpression of the lipid kinase PI3 kinase and loss of the lipid phosphatase tumour suppressor gene PTEN lead to activation of PKB and have been identified in multiple forms of cancer, implicating this kinase pathway in tumour development. The identification of small molecule inhibitors were sought in order to develop molecules useful for the treatment of cancer.
An integrated fragment-based approach utilizing virtual screening, X-ray crystallography and NMR was applied to identify novel leads for PKB. From a library of ~300,000 fragments including our drug fragment set and kinase biased set, 8 key fragments were identified and validated by structural studies. The fragment hits had a spread of potency in-vitro (16uM-1mM), low molecular weights and were considered to have drug-like properties.
Further structure-based design identified 2 lead series with single digit nano-molar potencies, whilst maintaining drug-like properties and low molecular weights (<400). Furthermore, using SBD, we took one of our original hits (80uM) and identified a 30nM lead compound from the synthesis of only 14 analogues.
In summary, we have identified a number of novel, potent and drug like inhibitors of PKB using fragment-based discovery.. We will present our approach in detail and the associated biological data for the lead compounds.

333 POSTER

Akt pathway siRNA screening using automated fluorescence imaging

R.A. Blake, P. Le, K. Ward, L. Friedman. *Exelixis Pharmaceuticals Inc, Signal Transduction Research, South San Francisco, USA*

Background: The Akt-family of kinases constitutes a major node of a signaling pathway that regulates cell-growth and apoptosis and is implicated in the process of tumorigenesis. We have combined RNAi gene knockdown techniques and automated fluorescence imaging of multiple nodes of the Akt pathway to investigate the interplay of various pathway proteins and to facilitate a screen for novel components of the pathway.
Material and Methods: siRNA transfections were carried out using 25nM/well (96-well plate) of siRNA using oligofectamine. Cells were fixed in formaldehyde 72 hours post transfection and prepared for immunofluorescence staining of various phospho-epitopes of proteins downstream of Akt signaling. Samples were analyzed using automated fluorescence imaging on a Cellomics ArrayScan II measuring the distribution of fluorescence stain within different components of the cell.
Results: RNAi of Akt2, mTOR or p70S6Kinase had the expected result reducing phosphorylation levels of their substrates and inhibiting signaling events lower in the pathway. Unexpectedly RNAi knockdown of certain downstream components of Akt signaling pathways such as mTOR, p70S6Kinase and EIF-4E-BP1 also modulated the phosphorylation of proteins higher in the classic Akt pathway.
Conclusions: Our data suggest that the various signaling pathways downstream of Akt are not simple linear pathways but involve feedback loops and cross-talk that complicate the positional interpretation of novel components of these pathways.

334 POSTER

First-in-human study of the safety, tolerability, pharmacokinetics, and pharmacodynamics of oral cp-724, 714, a selective, small molecule inhibitor of her2 in patients with advanced cancer

P.N. Munster[1], A.W. Tolcher[2], C.D. Britten[3], K. Gelmon[4], S. Moulder[1], S. Minton[1], M. Mita[2], D. Noe[5], K.J. Pierce[5], S.P. Letrent[5]. [1]*H. Lee Moffitt Cancer Center, Interdisciplinary Oncology, Tampa, USA;* [2]*Cancer Therapy and Research Center, San Antonio, USA;* [3]*UCLA Medical Center, Los Angeles, USA;* [4]*British Columbia Cancer Agency, Vancouver/Victoria, Canada;* [5]*Pfizer Global Research and Development, New London, USA*

Background: HER2 is expressed in a variety of tumor types and plays an important role in oncogenic signaling. HER2 inhibitors have demonstrated benefits in patients with advanced HER2-overexpressing cancers. CP-724,714 is a reversible, highly selective, small-molecule HER2 tyrosine kinase inhibitor currently in clinical development.
Methods: The safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of oral CP-724,714 (250 mg qd, 250 mg bid, 250 mg tid, and 400 mg bid) administered in 3-week cycles were assessed in patients with HER2 positive advanced solid tumors using a dose-escalation design. Safety assessments included adverse events (AEs), clinical laboratories, ECG, and MUGA scans. Blood sampling for PK was performed for up to 48 and 12 hours after the first dose in cycles 1 and 2, respectively. Serum CP-724,714 concentrations were measured by LC/MS/MS following solid phase extraction with PK parameter values estimated using noncompartmental techniques. PD measures included serial assessment of HER2-related signaling pathways via immunohistochemistry analyses of tumor biopsies and ELISA of serum HER2 extracellular domain (ECD) concentrations.
Results: To date, 23 pts have been enrolled, with data available on 17 pts [median (range) age 50.5 (37–71); PS (%) (0 (41.2); 1 (58.8))]. HER2 FISH status evaluations of pretreatment archival tissue: amplified (n=7), non-amplified (n=5), and not reported (n=5). The median number of cycles started was 2 (range 1–5). The most common treatment-related AEs were mild nausea (58.5%), fatigue (35.3%) and hyperbilirubinemia (29.4%). Dose-limiting reversible, grade 3 conjugated hyperbilirubinemia and grade 3 elevated ALT/AST/GGT were noted in 1 patient each in Cycle 1 in the 400 mg bid dose group. No treatment-related cardiomyopathy has been reported. The mean (SD) PK parameter values are AUC 8460 (5230) and 11600 (5900) ng?h/mL, Cmax 3170 (2060) and 3980 (2150) ng/mL and median Tmax 1.5 and 1.6 h, respectively, for a single dose of 250 mg and 400 mg. Systemic exposure steady state in both the 250 and 400 mg dose cohorts exceed the predicted efficacious exposures based on preclinical efficacy experiments. PK/PD analyses using tumor biopsy and serum HER2 ECD data are ongoing. To date, no objective responses have been reported in this population of 16/17 trastuzumab-pretreated patients.
Conclusions: Daily administration of CP-724,714 (250 mg qd and 250 mg bid) appears safe and well tolerated. DLTs, observed at 400 mg bid, are reversible hyperbilirubinemia (1/5) and elevated ALT/AST/GGT (1/5). Systemic exposure exceeds the threshold for efficacy as predicted from preclinical studies. Enrollment is continuing at 250 mg tid.

335 POSTER

SHP-1 protein tyrosine phosphatase as a target molecule in anti-tumor immune therapies: SHP-1 inhibitor SSG interacts withIL-2 to increase anti-murine renal tumor immunity

K. Fan[1], M. Pathak[1], M. Zhou[2], D. Lindner[3], E. Borden[3], T. Yi[1]. [1]*The Cleveland Clinic Foundation, Department Cancer Biology, Lerner Research Institute, Cleveland, OH, USA;* [2]*The Cleveland Clinic Foundation, Department of Pathology, Cleveland, OH, USA;* [3]*The Cleveland Clinic Foundation, Taussig Cancer Center, Cleveland, OH, USA*

SHP-1 is a key negative regulator of cytokine signaling and immune cell activation. This functional role of the protein tyrosine phosphatase suggests that it may have potential as a molecular target for augmenting anti-tumor immunity induced by cytokine- and immune cell-therapies. IL-2 therapy induces responses in advanced renal cell carcinoma (RCC) in connection with its ability to expand and activate immune cells. Based on our recent finding of sodium stibogluconate (SSG) as a SHP-1 inhibitor, the potential of SSG to interact with IL-2 and augment anti-RCC immunity was investigated