in vivo, we examined the growth of PC-9 and PC-9/ZD tumor xenografts treated with ZD6474 in athymic mice. Chronic administration of ZD6474 was well tolerated and produced significant growth inhibition of both tumors (12.5-50 mg/kg/day) during the treatment period (21 days). Treatment was well tolerated as evidenced by no instance of body weight loss >5%. There was no macroscopic evidence of remaining tumor in mice transplanted with ZD1839-sensitive PC-9 cells following treatment with ZD6474 at doses of 25 and 50 mg/kg/day. ZD6474 also produced significant growth inhibition in ZD1839-resistant PC-9/ZD tumors in mice, consistent with its anti-angiogenic mode of action, although in this case, all of the mice had some evidence of remaining tumor. These results suggest that ZD6474 is a potent antitumor agent and support further investigation of ZD6474 as a potential therapeutic option in EGFR-TKI resistant disease. 'Iressa' is a trademark of the AstraZeneca group of companies

193

CHS 828 inhibits the activity of the IkBβ kinase *in vitro* and the transcriptional activity of NF-kB in the human monocytic leukaemia THP-1 cells

L.S. Olsen[1], R. Larsson[2], L. Binderup[1], M.W. Madsen[1]. [1]*Leo Pharma, Dept. of Biochemistry, Ballerup, Denmark;* [2]*Uppsala University, Division of Clinical Pharmacology, Uppsala, Sweden*

CHS 828 belongs to a series of pyridyl cyanoguanidines with a significant anti-tumour effect in preclinical tests *in vitro* and *in vivo*. To determine possible genes that are affected by CHS 828, a DNA array was performed to establish expression profiles in human U-937 myeloid leukemia cells made resistant to CHS 828 compared to the CHS 828 sensitive parental cells. A subset of differentially expressed genes could be identified, including genes from the NF-kB signal transduction pathway. NF-kB is a transcription factor that mediates the expression of a variety of cellular genes regulating inflammation, immune responses, and sensitivity to apoptosis. In non-stimulated cells, NF-kB is sequestered in the cytoplasm and is bound to IkB, thereby preventing nuclear transport. Stimulatory signals such as LPS, TNFalpha and certain anticancer agents induce the degradation of IkB, and NF-kB consequently enters the nucleus and activates gene transcription. NF-kB translocation to the nucleus requires IkB phosphorylation, ubiquitination and ultimately proteolytic degradation. The phosphorylation of IkB is regulated by the activation of a 700-900 kDa IKK complex consisting of two catalytic units, IKKalpha and IKKbeta. Inhibitors of this process are likely to become new anti-inflammatory and anti-cancer agents. We tested the effect of CHS 828 on LPS-induced NF-kB activation in human monocytic THP-1 cells. CHS 828 inhibited the LPS-induced activation of NF-kB in a luciferase reporter gene assay with an IC_{50} of 47 nM. This reduced activity could be explained by a low amount of NF-kB in the nucleus. Indeed, the amount of NF-kB binding to kB responsive elements after LPS stimulation was reduced in nuclear extracts from THP-1 cells treated with 1μM CHS 828. NF-kB translocation to the nucleus requires IkB phosphorylation and subsequent degradation. Treatment of THP-1 cells with 1 μM CHS 828 blocked the LPS-induced degradation of IkBs. Also, CHS 828 inhibited the LPS-induced IKKβ activity *in vitro* with an IC_{50} of 8 nM. In conclusion, CHS 828 potently inhibited the LPS-induced activation of NF-kB possibly by inhibiting the activity of the IKKβ.

194

Mechanism of action and biomarker studies of SU11248, a selective inhibitor of split kinase domain receptor tyrosine kinases (including VEGF receptors, PDGF receptors, c-Kit, and Flt3)

O. Potapova, A. Laird, M. Nannini, G. Li, K. Moss, J. Cherrington, D. Mendel. *SUGEN, Inc., Preclinical Research and Translational Medicine, South San Francisco, USA*

Several members of the split kinase domain (Class III) superfamily of receptor tyrosine kinases (RTKs) are implicated in cancer. These include the VEGF receptors VEGFR2/KDR and VEGFR1/Flt-1, the platelet-derived growth factor receptors PDGFRa and PDGFRb, c-Kit, and Flt3. SU11248 is an orally available selective small molecule inhibitor of these RTKs. In biochemical and/or cellular assays, SU11248 inhibited VEGFR2, VEGFR1, the PDGFRs, c-Kit and Flt3 with low nM potency. In human tumor xenografts grown in mice, SU11248 selectively inhibited the phosphorylation of VEGFR2, PDGFRb, c-Kit and an activated mutant form of Flt3 (Flt3-ITD), but did not inhibit EGFR phosphorylation. SU11248 also inhibited biological readouts dependent on the kinase activity of VEGFR2 (vascular permeability) and c-Kit (hair pigmentation) in mice. SU11248 exhibited broad and potent anti-tumor activity in mice, regressing several tumors (including A431 human epidermoid, Colo205 human colon and HT-29 human colon xenografts) and suppressing or delaying the growth of diverse other tumors. Studies were initiated to explore early and late responses to SU11248 treatment in mice bearing tumor xenografts to identify candidate biomarkers of response. Preclinical data will be presented on several candidate tumor biomarkers identified using histological and biochemical approaches and evaluated further preclinically and in the clinic. These include Ki-67 and active Caspase 3, which reflect levels of proliferation and apoptosis, respectively, and phosphoepitopes on several downstream effectors of RTK function. We also report the results of studies using selective inhibitors or VEGF or PDGF receptors, either alone or in combination, to explore the relative contributions of inhibition of these receptor families to the anti-tumor activity of SU11248. SU11248 is currently in Phase I clinical trials in patients with advanced cancer.

195

Comprehensive analysis of epidemiology and clinical significance of egfr amplification and overexpression using a multi-step tissue microarray (TMA) approach

R. Simon[1], K. Wilber[2], A. Nocito[3], P. Haas[3], H. Novotny[3], M. Mirlacher[3], C. Bucher[3], H. Bissig[3], M. Mihatsch[3], G. Sauter[3]. [1]*Institute of Pathology, University of Basel, Basel, Switzerland;* [2]*VYSIS Inc., Downers Grove, Illinois 60515-5400, USA;* [3]*Institute of Pathology, University of Basel, Basel, Switzerland*

Purpose: Several anti Egfr drugs are in phase I and phase II clinical trials. However, a comprehensive overview about tumor types that might benefit from such a treatment is lacking.
Materials and methods: We used a two step tissue microarray (TMA) approach to comprehensively analyze epidemiology and clinical significance of EGFR amplification and immunohistochemically detectable expression. In a first step multitumor and normal tissue TMAs comprising 4987 tissue samples from 128 different tumor types and 76 different normal tissues were utilized to study the epidemiology of EGFR amplification/overexpression. In a second step tumor specific TMAs containing a total of 5491 samples with clinical follow up data were used to analyze the prognostic significance of Egfr alterations breast-, colon-, and bladder cancer.
Results: A strong Egfr expression was found in 71 different tumor types including squamous cell carcinomas of various origins and brain tumors. Gene amplification was found in glioblastoma multiforme, astrocytoma, oligodedroglioma, malignant fibrous histiocytoma, primitive neuroectodermal tumor (PNET), adenocarcinoma of the stomach as well as in squamous cell carcinomas of head and neck, vulva, esophagus, and lung. The Egfr protein expression level was significantly associated with the gene copy number, suggesting a gene dosage dependent increase of expression. Strong Egfr expression was linked to reduced survival in breast and colon cancer.
Conclusion: Large-scale TMA studies provide rapid and comprehensive molecular epidemiology information for potential therapeutic targets.

196

In vitro and *in vivo* characterization of a potent tyrosine kinase inhibitor that modulates angiogenesis and cancer cell proliferation

C. Heise[1], M. Wiesmann[2], J. Vora[3], S. Lee[1], P. Renhowe[4], S. Harrison[2], E. Samara[3], B. Hibner[1], A. Harris[5]. [1]*Cancer Pharmacology,* [2]*In vitro Biology,* [3]*Pharmacokinetics,* [4]*Medicinal Chemistry,* [5]*Drug Discovery Research, Chiron Corporation, Emeryville, USA*

We have identified an orally active amino-benzimidazole-quinolinone, CHIR 200131, that exhibits potent inhibitory activity (10 nM) against Flt-1, KDR, and PDGF receptor tyrosine kinases (RTKs) with significant antiangiogenic properties *in vitro* and *in vivo*. VEGF- or bFGF-induced endothelial cell migration and tube formation were inhibited in a dose-dependent manner. Rat aortic rings showed significant reduction in the number and length of sprouts compared to control. Treatment of endothelial cells with the compounds inhibited MAPK phosphorylation mediated by VEGF or bFGF. Oral administration of CHIR 200131 in the murine FGF matrigel model demonstrated dose dependent inhibition of neovascularization that could be completely blocked over a period of 8 days. In addition to the affects on RTKs of the VEGFR family, these compounds also inhibited bFGFR, Her2/neu and c-Kit, and have been shown to directly inhibit tumor cell proliferation. Activity has been demonstrated in several *in vivo* models of tumor growth and metastases. Established subcutaneous tumors (100-500 mm3) have