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

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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)

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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

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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

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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

shown responses ranging from regression to tumor stasis and growth delay. CHIR 200131 has an absolute oral bioavailability of > 90% in mice and rats, 17% in dogs, and 28% in monkeys. The apparent elimination t* ranged from 1.5 to 5.5 hours in plasma following an IV dose. In general, this compound exhibited high plasma clearance relative to hepatic plasma flow and was also widely distributed as indicated by a large V_{ss} relative to total body water in each species evaluated. Tissue concentrations were higher than those in plasma following a single or multiple oral doses in mice and rats. Maximum plasma and tissue concentrations occurred between 2 and 4.5 hours and following T_{max}, tissue concentrations declined in parallel with those in plasma. After multiple dosing in a human colon tumor xenograft model at 30 mg/kg (ED_{50}), plasma concentrations of approximately 500 ng/mL were obtained 2 hr post-dose and by 24 hr were generally undetectable. However, tumor concentrations of CHIR 200131 were 15 fold higher than plasma at 2 hr and at 24 hr significant levels remained. Multiple dose plasma pharmacokinetics in mice, rats, and monkeys demonstrated time- and dose-independent pharmacokinetics. These data indicate that CHIR 200131, with its combined cytostatic and antiangiogenic activities, has potential as an effective therapy for solid and metastatic tumors.

197

Potent *in vivo* activity of MCR peptides against chemotherapy-resistant human small cell lung cancer (SCLC)

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Previous studies had shown that MCR peptides containing retinoblastoma protein (RB) fragment LFYKKV suppress human non-small cell lung cancer growth (NSCLC) *in vivo*. Since the current prospects for an efficient treatment of human small cell lung cancer (SCLC) are even poorer than those for an effective therapy of NSCLC, we have now also investigated the performance of MCR peptides against human SCLC xenografts in nude mice. As such, we chose the human RB-negative SCLC cell line H82 as a model. A first *in vivo* experiment performed with the MCR peptide coined MCR-4 (sequence: (all-D) LFYKKVRQIKIWFQNRRMKWKK, molecular weight (MW): 3026) showed that this compound is active against s.c. H82 tumors that had been allowed to reach a large size (ca. 400 mm3) before treatment was initiated (this tumor size was attained after 12 days from the initial inoculation of 10 million H82 cells s.c. into each nude mouse). Specifically, MCR-4 achieved 67% tumor growth inhibition vs. controls when injected i.p. at 10 mg/kg every other day over a 2-week-period (i.e. in altogether only 7 doses). In contrast, the chemotherapeutic etoposide (MW: 589), also known as VP-16, when administered at 1 mg/kg i.p. every other day over the same time period was found to have no significant activity in this *in vivo* test. In a different experiment, another MCR peptide termed MCR-14 (sequence: (all-D) KRKRSPVRSLFYKKVYRLAPKT, MW: 2722) at a dose of 5 mg/kg and given via the i.p. route daily over 10 days also performed excellently against s.c. H82 lung tumors by causing about a 70% growth inhibition whereas the chemotherapeutic VP-16 at 1 mg/kg i.p. was again completely inactive. This MCR-14 performance is particularly remarkable given that the 5 mg/kg dose is usually rather a suboptimal dose for an MCR peptide. In contrast, VP-16 failed against H82 tumors which markedly differs from its effectiveness at the same dose against A549 NSCLC tumors in previous tests we had conducted and reported. Taken together, our data suggest that RB-negative SCLC could be a well-suited target tumor for a successful MCR peptide therapy in a clinical setting. Furthermore, our results indicate that MCR peptides should be tested as therapeutics against other RB-negative tumors as well since these tumors frequently display chemoresistance and, moreover, are not treatable with the novel type of agents termed cyclin-dependent kinase (cdk) inhibitors that, by their nature, are active only in RB-positive tumor cells.

198

Antitumor activity of the epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) ZD1839 ('Iressa'), alone or combined with gemcitabine and vinorelbine platinum-based chemotherapy, in human non-small-cell lung cancer (NSCLC) xenografts

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Objectives: The benefit of chemotherapy in NSCLC remains modest. High expression of EGFR in NSCLC provides an opportunity to improve chemotherapy by combination with anti-EGFR compounds. ZD1839 ('Iressa') is an orally active, selective EGFR-TKI undergoing clinical evaluation in a range of tumor types, including NSCLC. This *in vivo* study aimed to evaluate the benefits of ZD1839, alone or combined with standard platinum-based chemotherapy, using NSCLC human xenografts.
Methods: Five NSCLC biopsies with different levels of EGFR mRNA (1 squamous carcinoma and 4 adenocarcinomas) were obtained from pts and grown as subcutaneous xenografts in nude mice. Two chemotherapy regimens were used: either cisplatin (CDDP, q 3 wks) + gemcitabine (GZ, wkly) at doses of 0.5 and 60 mg/kg ip, respectively, or CDDP (q 3 wks) + vinorelbine (VNR, q 10 d) at 0.5 and 1 mg/kg ip, respectively. ZD1839 was given po at doses of 40 or 120 mg/kg daily for 2 wks, alone, or at 120 mg/kg when combined with chemotherapy. Individual tumor growth rate was measured and % tumor growth inhibition (TGI) was calculated by comparison with control mice.
Results: ZD1839 alone produced significant responses in 4/5 tumors, with mean TGIs of the tumor IC8 of 63 and 40% at high and low dose, respectively. At the high dose, TGIs of the other NSCLCs were 27, 54, 64 and 80%. Response to ZD1839 occurred independently of EGFR expression or histology. Three NSCLCs (IC8, LC131, IC9) responded to CDDP/GZ with TGIs of 40, 41 and 90%, 1 (LC131) was improved by ZD1839. Two NSCLCs did not respond to CDDP/GZ alone, but 1 (IC14) showed marked response to CDDP/GZ + ZD1839 (mean TGI 77%). The CDDP/VNR regimen alone produced a significant response in only 1 NSCLC (IC8), not improved by ZD1839. A marked TGI of LC131 was observed when CDDP/VNR was given with ZD1839 (mean TGI 63% vs 12 or 54% with CDDP/VNR or ZD1839 alone, respectively). No improvement of TGI of IC1 was obtained by combination with ZD1839.
Conclusions: These results suggest that ZD1839 has a significant benefit in NSCLC, independent of histological type and EGFR expression levels of the tumor. The NSCLC xenografts selected had different although modest responses to standard chemotherapy. The CDDP/GZ regimen was the most active, but ZD1839 did not potentiate its efficacy except in tumors insensitive to chemotherapy alone. The CDDP/VNR regimen was inactive, even when combined with ZD1839. 'Iressa' is a trademark of the AstraZeneca group of companies

199

Functional characterization of novel epidermal growth factor receptor(EGFR) and HER2 inhibitors based on pyrrolo[2,3-d]pyrimidinone structure

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Overexpression of epidermal growth factor receptor family members has been implicated in a variety of tumors including breast, lung and ovarian. This overexpression is associated with tumor aggressiveness and poor patients prognosis, partly due to an impaired response of cancer patients to chemotherapy. Therefore blockage of EGFR (HER1) and HER2 signaling by small-molecule compounds is a beneficial therapeutic approach to induce growth inhibition of human carcinoma cells. We have investigated the potency and specificity of two 5,7-dihydro-pyrrolo[2,3-d]pyrimidin-6-one derivatives, namely D-69491 and D-70166, in respect of tyrosine kinase inhibition and carcinoma cell proliferation. In biochemical assays the kinase activities of HER1, HER2 and HER4 were inhibited with IC_{50} values in nanomolar range. Inhibition of HER1 and HER2 phosphorylation was confirmed by western blot analysis of inhibitor-treated A431 epidermoid carcinoma cells and HER2-overexpressing NIH3T3 cells. Both inhibitors impaired ligand-stimulated HER1 and HER2 phosphorylation in a