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.

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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.

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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

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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

concentration-dependent manner without receptor level alteration. Proliferation assays using HER1-, HER2- and non-overexpressing human breast carcinoma cells revealed that treatment with either D-69491 or D-70166 inhibited cell growth to similar extent. Independent of HER1 and HER2 expression levels, D-70166 inhibited proliferation of the human breast carcinoma cells MDA-MB-468, MDA-MB-453, SK-BR-3, MDA-MB-231 and MCF-7 with IC_{50} concentrations ranging between 0,86 and 2,85 μM and average maximal growth inhibition of 79% to control cells. This suggests that in addition to HER2- and HER1-driven cell growth inhibition, D-70166 exhibited unspecific cytotoxicity. Whereas the growth inhibitory effects of D-69491 on SK-BR-3, MDA-MB-453 and MDA-MB-231 cells were comparable to the effects observed after D-70166 treatment, D-69491 achieved lower inhibitory responses on MCF-7 cells and higher responses on HER1-overexpressing MDA-MB-468. Our *in vitro* data demonstrated that both D-70166 and D-69491 are potential clinical candidates, which target EGFR as well as HER2 tyrosine kinase activities.

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The potential role of STI 571 in the treatment of head and neck cancer

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The complete response of head and neck cancer to systemic therapies is often disappointing. The novel agent STI 571 (2-phenyl aminopyrimidine derivative) is designed to be effective against CML via inhibition of Bcr-Abl kinase. However the drug is known to inhibit other tyrosine kinases, including PDGFR and c-kit. There is evidence that c-kit is expressed in certain head and neck tumours including 90% of adenoid cystic carcinomas (ACC). The aim of this study is to provide pre-clinical data on the response of a panel of head and neck squamous cell carcinoma (SCC) cell lines along with primary explanted tumour cell cultures (adenoid cystic and SCC) to STI 571. We have also explored the interaction of STI 571 when given in combination with commonly used chemotherapuetic agents.. STI 571 alone shows significant growth inhibition against in both SCC cell lines and primary cultures. In SCC cell lines STI 571 was also found to be synergistic with several agents and antagonistic with gemcitabine. These Gemcytabine results were mirrored in ACC primary cultures, and a degree of synergy with other drugs was also observed. The growth inhibitory effect of STI 571 in ACC can be explained by inhibition of the c-kit receptor kinase expressed in these tumours. However this cannot explain the toxicity seen in c-kit –ve SCC cell cultures. It is proposed that this effect is mediated via an as yet unidentified kinase pathway. Likewise the reported synergy and antagonism may well be due to inhibition of other kinases. Studies are ongoing to further establish the role of these kinases in the toxicity of STI571. Furthermore work is ongoing to explore the possible role of STI571 in the treatment of c-kit +ve ACC's, both as a single agent and in combination. Clinical studies are planned to follow this.

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Cellular responses to DNA topoisomerase I poisons and the TOR kinase inhibitor, rapamycin

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Genetic analyses of cellular responses to DNA topoisomerase I (Top1) poisons in the budding yeast Saccharomyces cerevisiae suggest common pathways regulate the cytotoxicity of rapamycin, a Tor kinase inhibitor. Eukaryotic topoisomerase I plays an important role in DNA replication and recombination. Camptothecin (CPT) targets the enzyme by reversibly stabilizing a covalent Top1-DNA intermediate. During S-phase, these complexes are converted into irreversible DNA lesions as a consequence of collisions with advancing replication forks. To define cellular factors that recognize or repair Top1-induced lesions, conditional tah mutants were isolated with enhanced sensitivity to top1T722A at 35oC. The self-poisoning top1T722A mutant is a CPT mimetic that avoids issues of drug transport. Nine TAH genes (including CDC45, DPB11, DOA4, TAH11 and SLA1) were identified that protect cells from top1T722A-induced DNA damage. These mutants were hypersensitive to hydroxyurea and exhibited terminal phenotypes consistent with S-phase induced DNA lesions. Remarkably, the majority of tah mutants were also hypersensitive to rapamycin. This macrocyclic antibiotic targets the PI3-related TOR kinase (mTOR in mammalian cells, Tor1 and Tor2 in yeast) and induces cell cycle arrest in G1 phase. Yeast and mammalian Tor kinases regulate protein translation and cell cycle progression in response to growth signals and nutrient deprivation. Rapamycin has demonstrated surprising antitumor activity in clinical trials, consistent with recent reports of rapamycin-induced apoptosis observed in p53 null cells. The enhanced sensitivity of yeast tah mutants to CPT and rapamycin suggest specific alterations in S-phase potentiate the cytotoxicity of both agents. The ability of extragenic repressors of doa4-10 to suppress top1T722A- or rapamycin-induced lethality provides further support for similar mechanisms of drug action, which may be investigated in yeast. Supported by NIH grants CA23099, CA58755, CA77776 and ALSAC.

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A phase I study of ZD 1839 (Iressa) in combination with oxaliplatin, 5-fluorouracil (5-FU) and leucovorin (LV) in advanced solid malignancies

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Iressa is an oral small molecule tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR-TKI). Preclinical studies demonstrated promising anti-tumor effects using Iressa alone or in combination with chemotherapy agents in a variety of epithelial tumors. We report the results of a phase I dose-escalation study that investigates the tolerability and clinical biology of Iressa in combination with oxaliplatin, and 5-FU/LV. A sequential dose escalation of Iressa and oxaliplatin was performed. From July 2001 to April 2002, 16 patients (10 men: 6 women, median age 50.5 years, range 31-61 years) were treated. The median number of prior chemotherapy regimens was 1.5 (range 0-3). Twelve patients had stage IV adenocarcinoma of the colon, 1 patient an adenocarcinoma of unknown primary, 1 patient a squamous cell carcinoma of unknown primary, and 1 patient a basosquamous cell carcinoma of unknown primary. Three dose levels were tested. A total of 102 cycles were administered (range 1-8). A dose-limiting toxicity was seen at the second dose level (catheter-related bacteremia). One patient at the third dose level experienced a DLT with grade 3 nausea, diarrhea, and hypokalemia requiring hospitalization for intravenous hydration. This dose level (Iressa 500 mg daily, with a standard dose and a every two week schedule of oxaliplatin, 5-FU/LV) established the phase II recommended dose (MTDs) for Iressa and oxaliplatin combined with 5-FU/LV. Additional grade 3/4 toxicities were neutropenia without fever (4). Grade 2 toxicities consisted of acneiform rash (6), vomiting (5), abdominal pain (4), diarrhea (4), nausea (3), fatigue (2), mucositis (2), thrombocytopenia (2), anemia (1), anorexia (1), and personality/behavioral change (1). Grade 1 toxicities included nausea (11), sensory neuropathy (8), fatigue (7), vomiting (7), anorexia (6), diarrhea (6), abdominal pain (1), ALT elevation (1), AST elevation (1), and fever (1). Three patients with colorectal cancer had a partial response. Nine patients had stable disease after a minimum of 4 cycles of treatment. Pharmacokinetic and biological endpoint studies are ongoing. Currently, a phase II study of this regimen in colorectal cancer is in progress and these results will be presented.

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Antitumor activity of the EGFR/TK inhibitor Tarceva™ (erlotinib, OSI-774) in tumor models

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Tarceva™ (OSI-774) is a potent, orally bioavailable, small molecule inhibitor of EGFR (HER1, erbB1) tyrosine kinase. Tarceva™ inhibits phosphorylation of the EGFR tyrosine kinase domain, thereby blocking key signal transduction molecules downstream from the receptor. Currently, Tarceva™ is in advanced clinical trials for several solid tumors, including NSCLC and pancreatic cancer. Treatment of tumor-bearing animals with Tarceva™ results in significant tumor growth inhibition (TGI) and regression in a variety of *in vivo* models of cancer. In the A431 human epidermoid xenograft model (high EGFR expression), Tarceva(TM) treatment causes tumor regression. Treatment of mice bearing H460a and A549 human NSCLC tumor xenografts (moderate EGFR expression) results in approximately 70% TGI. Tarceva™-induced tumor growth inhibition in animal models is dose-dependent, correlates with circulating levels of drug and with inhibition of phosphorylation of EGFR *in vivo*. In addition, using immunohistochemistry, we have evaluated the ability of Tarceva™ to inhibit cell proliferation and induce apoptosis in tumor cells, as well as studied its effect on tumor angiogenesis. Finally, we have studied the characteristic skin lesions observed in Tarceva™-treated