and TGFa induced Erk activation. This evaluation of downstream signaling revealed that E2-induced Erk activation is mediated by a HRG/ HER-2/PKC-d/Ras pathway that could be crucial for E2-dependent growth-promoting effects in early stages of tumor progression.

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17-DMAG (NSC 707545), a water-soluble geldanamycin analog, has superior *in vitro* and *in vivo* antitumor activity compared to the hsp90 inhibitor 17-AAG

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The heat shock protein 90 (hsp90) is involved in the correct folding of several signal transduction kinases such as erbB2, PI3K and raf-1. 17-allylamino-geldanamycin (17-AAG) is an inhibitor of hsp90 and currently in clinical trials. However, difficulties in formulation have lead to the development of the water-soluble 17-(dimethylaminoethylamino-17- demethoxygeldanamycin (17-DMAG). In the present study, the two compounds were compared to contrast their behavior. We had found previously that human melanoma cells were responsive to 17-AAG. Therefore this tumor type was investigated. Growth inhibition (IC_{50}, IC100) was assessed in the melanoma cell lines MEXF 276L, MEXF 462NL and MEXF 514L using a 96 hr SRB assay. 17-DMAG was more potent than 17-AAG with an IC_{50} in the sensitive MEXF 276L of 37 nM for 17-DMAG and 187 nM for 17-AAG. MEXF 514L was resistant to both compounds (IC_{50}>8 μM). Additionally, clonogenic assays were performed on a panel of 13 human tumor xenografts. The mean IC_{50} for inhibition of colony formation was lower for 17-DMAG than for 17-AAG (20 nM vs. 39 nM). These results translated into *in vivo* activity. In 2/3 s.c. growing melanoma xenografts both compounds were active at their MTD, but e.g. the growth delay in mice bearing MEXF 276 tumors was 16 d for 17-DMAG (15 mg/kg given 2x QdX5 i.v. in PBS) and only 11 d for 17-AAG (60 mg/kg, 2x Qdx5 i.p. as a DMSO/PBS suspension). MEXF 514 xenografts however, did not respond. The most marked difference between the sensitive and resistant melanomas is the expression of erbB2. The latter is prominently expressed in the MEXF 276L model but undetectable in MEXF 514L cells. In order to compare the behavior of the agents on a molecular basis, the modulation of hsp90 and its client proteins were assessed via immunoblotting after exposure to IC100. Here, 17-DMAG and 17-AAG were identical: hsp90 protein levels decreased in MEXF 276L whilst in MEXF 514L cells the expression did not change; c-raf-1 protein was reduced in MEXF 276L cells, but not in MEXF 514L. No change in protein expression was observed for PI3K. MEXF 276L showed a decrease in erbB2 protein levels concomitantly with loss of hsp90. Our data demonstrates that while both compounds inhibit signal transduction through hsp90 modulation, the efficacy and pharmaceutical properties for 17-DMAG are superior to those of 17-AAG. 17-DMAG should be regarded as therefore having potential advantages for clinical development in comparison to 17-AAG.

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The role of G1 and G2 checkpoint control proteins involved in cell cycle arrest following treatment with the HSP90 inhibitor 17AAG

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The HSP90 inhibitor 17-allylamino, 17-demethoxygeldanamycin (17AAG) exerts its antitumour effect by inhibiting the intrinsic ATPase activity of the molecular chaperone HSP90. This causes the depletion of key oncogenic proteins via the ubiquitin proteosome pathway, resulting in both cytostasis and apoptosis. However, the effects of 17AAG on cell cycle checkpoint kinase expression have not been explored in any great detail and may be crucial determinants in cell cycle control. In this study, cell cycle kinetics were examined in the A2780, HT29 and Lovo tumour cell lines by continuously labelling cells with bromodeoxyuridine and performing bivariate Hoechst/PI flow cytometric analysis. The expression of a number of key cell cycle checkpoint proteins and upstream signalling proteins were examined using Western blotting and RNase Protection assays (RPA). In the A2780 cell line (p53+/+) cells from all phases of the cell cycle accumulated predominantly in the G1 phase of the cell cycle. In the Lovo (p53+/+) and HT29 (p53-/-) cell lines, G1 and G2 cells were blocked in the cell cycle phases in which they originated, with S phase cells accumulating in G2/M. However, the G2/M arrest was leaky in the HT29 cells and by 16h some cells progress through to the G1 phase of the cell cycle, suggesting differential regulation of the G2 checkpoint by 17AAG in these cell lines. p53 and p21 induction was observed at 24hr in cells expressing wild type p53 whereas mutant p53 was depleted in HT29 cells with no evidence of p21 induction. A decrease in RB phosphorylation in A2780 and HT29 cells was observed consistent with the observed G1/S arrest. However, in Lovo cells there was no obvious phospho RB signal suggesting RB function may be compromised. Following 17AAG treatment, protein expression levels of a number of kinases involved in cell cycle checkpoint control were depleted including CDK4, WEE1 and CHK1. RPA data indicated no drug induced changes in mRNA expression of these kinases, suggesting they are not transcriptionally regulated by 17AAG. To conclude, the G1 or G2 arrests observed with 17AAG in these cell lines are not simply related to the p53 or RB status of the cell line, although these pathways may play a crucial role in the maintenance of the cell cycle response. Depletion of key cell cycle checkpoint kinases may be an important factor in determining cell cycle response following 17AAG treatment.

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Additive interaction of platinum compounds and 17-AAG in colon cancer cell lines depends on intact JNK signaling

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We showed earlier that in the HT29 colon adenocarcinoma cell line 17-AAG antagonizes the action of cisplatin, while exerting additive effects in HCT116 cells. Since the importance of p53 status and integrity of stress signaling pathways in cellular responses to platinum compounds is well established, we investigated if the possible interference by 17-AAG with cisplatin-induced signaling and apoptosis is one of the reasons for their antagonism. To evaluate the role of signaling pathways in the interaction, we studied four colon cancer cell lines with different p53 status: HCT116, with intact p53, and HT29, DLD1 and SW480 cell lines, bearing p53 mutations. Clonogenic assays demonstrated higher sensitivity to 17-AAG and platinum agents in HCT116 and DLD1 cells, compared to those of HT29 and SW480. To assess the effect of combined treatment on signaling through MAPK cascades, cells were treated for 24 hours with 3xIC90 concentrations of each drug alone and in combination. In HCT116 and DLD1 cell lines c-Jun induction by cisplatin was somewhat inhibited by 17-AAG, whereas in HT29 and SW480 cells it was completely abrogated. Further, in HT29 cells the MAPK and JNK signaling pathways were strongly inhibited when cells were exposed to cisplatin in the presence 17-AAG. Treated in the same manner, SW480 cells demonstrated the loss of JNK activation and inhibition of ATF2 and c-Jun phosphorylation, while p38 activation was unaffected. In HCT116 and DLD1 cell lines all major signaling pathways were intact, demonstrating only partial overall inhibition. In addition to disruption of cisplatin-induced JNK pathway activation, 17-AAG treatment led to inhibition of both basal and cisplatin-induced caspase 8 activity in HT29 cells while in the HCT116 cell line it was unaffected. These data suggest that an additive response to combined platinum drug with 17-AAG depends on intact apoptotic signaling, especially through JNK. The data emphasize the care required in combining a stress signal inducer (cisplatin) with a signaling inhibitor (17-AAG).

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Activity of ZD6474, a vascular endothelial growth factor receptor tyrosine kinase inhibitor (VEGFR [KDR]-TKI), in a model of ZD1839 ('Iressa') resistance

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ZD6474 is a novel inhibitor of vascular endothelial growth factor receptor (VEGFR [KDR]) signaling that inhibits angiogenesis and tumor growth in a range of tumor models. In addition, ZD6474 has some activity against EGFR tyrosine kinase. ZD1839 ('Iressa') is an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) that blocks signal transduction pathways implicated in the proliferation and survival of cancer cells. We have established a human lung cancer cell line that is resistant to ZD1839 (PC-9/ZD) and have now investigated the direct tumor inhibitory activity of ZD6474. In an MTT proliferation assay, ZD6474 showed partial cross-resistance to PC-9/ZD cells suggesting that EGFR-inhibitory activity partially contributes to the growth-inhibitory effect of this compound on tumor cells in culture. To elucidate the effects of ZD6474

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

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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.

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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.

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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.

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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