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