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ZD1839 ('Iressa') improves the antitumour activity of tamoxifen ('Nolvadex') and ICI 182, 780 ('Faslodex') in antihormone responsive breast cancer

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While many oestrogen receptor (ER)-positive breast cancer patients initially respond to antihormonal agents such as tamoxifen ('Nolvadex'), resistance may develop. We have previously demonstrated that the selective epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) ZD1839 ('Iressa') is a potent inhibitor of the elevated EGFR activity, which is essential for proliferation of breast cancer sub-lines that have acquired antihormonal resistance. In contrast, EGFR is barely detectable in the antihormone responsive parental cells (MCF-7) and ZD1839 has little effect on cell growth. In anticipation of the increased dependence on EGFR signalling with development of antihormonal resistance, the present *in vitro* study investigated whether co-treating breast cancer cells with tamoxifen (1x10-7 M) or ICI 182, 780 ('Faslodex') (1x10-7 M) and ZD1839 (1x10-6 M) can promote additional growth inhibitory activity compared with antihormone alone. Tamoxifen decreased cell growth compared with untreated MCF-7 cells, with MIB1 immunostaining and FACS analysis revealing decreased proliferative activity and a reduced S-phase fraction, respectively. Tamoxifen modestly enhanced apoptosis (as measured by annexin V-FITC binding and the ApoAlertTM Mitochondrial Membrane Sensor Kit), with some decreases in the cell survival protein Bcl-2. Co-treatment of MCF-7 cells with tamoxifen plus ZD1839 enhanced anti-proliferative and pro-apoptotic activity compared with tamoxifen alone, while ZD1839 alone had no effect. ICI 182, 780 plus ZD1839 was similarly superior to ICI 182, 780 alone. Studies in ER-positive T47D breast cancer cells confirmed that co-treatment increased inhibitory activity. In parallel with improved growth inhibition, ZD1839 plus tamoxifen blocked antihormone-induced increases in EGFR protein expression, with greater depletion of activated ERK 1/2 MAP kinase (pMAPK) and AKT. These studies demonstrate that ZD1839 in combination with antihormonal agents provides superior antitumour activity in antihormone-responsive breast cancer cells *in vitro*. Indeed, combination treatment may prevent antihormonal resistance, since the obvious outgrowth of EGFR and pMAPK positive cells after 5 weeks' tamoxifen treatment was absent in cultures treated with tamoxifen plus ZD1839. Clinical studies using such combinations of antihormonal agents and EGFR-TKIs are ongoing. 'Iressa', 'Nolvadex' & 'Faslodex' are trademarks of the AstraZeneca group of companies

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In vitro studies evaluating the interaction between ZD1839 ('Iressa') and ionizing radiation

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Introduction: Tumor repopulation during treatment is a major problem in radiation therapy and may be the cause of treatment failure. Over the past decade, treatments combining cytotoxic antitumor drugs and radiation have been developed to take advantage of the synergistic interaction between these modalities. Here we present results from *in vitro* studies to determine whether the cytostatic agent ZD1839 ('Iressa'), an orally active, selective epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), interacts with radiation treatment.
Methods: Three epithelial cancer cell lines with different levels of EGFR expression were used (A431, vulvar carcinoma, EGFR++; A549, lung carcinoma, EGFR±; HeLa, cervix carcinoma, EGFR+). The effect of ZD1839 was investigated as a function of both the concentration (up to 30 μM) and the length of drug exposure (up to 6 days). Irradiation of cells was performed with a 137Cs irradiator. Growth inhibition and cytotoxicity were measured by cell count and colony-forming assays, respectively. DNA double-strand breaks (DSB) were determined by neutral filter elution, and cell cycle progression was measured by flow cytometric analysis with bromodeoxyuridine labeling.
Results: ZD1839 alone reversibly blocked cell proliferation in the three cell lines of interest. The drug consistently elicited S-phase depletion and G1 phase accumulation at concentrations in excess of the IC_{50} determined from cell growth assays (A431, 0.3 μM; A549, 6 μM; HeLa, 8 μM, 48-h contact). No cytotoxicity was observed for drug alone, except in HeLa cells. ZD1839 did not affect the cells' response to ionizing radiation, even after prolonged contact (up to 60 h prior to radiation) with $1xIC_{50}$ of ZD1839. Furthermore, ZD1839 did not affect the incidence and repair of radiation-induced DSB.
Conclusion: The data show that ZD1839 does not impair the rejoining of radiation-induced DNA DSB, and does not alter cell survival following radiation. This suggests that it could be useful to combine ZD1839 with radiotherapy, concomitantly or in close temporal proximity, as it should provide strictly additive interaction without the local and systemic adverse effects inherent in treatments with radiation sensitizers (Balosso J et al, Bull Cancer Radiother 1995; 82: 101-112). 'Iressa' is a trademark of the AstraZeneca group of companies

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Aspirin induces b-catenin phosphorylation and reduces expression of Akt/PKB

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Constitutive activation of signal transduction pathways like the Akt/PKB or the b-catenin-pathway play a central role in carcinogenesis. We have previously shown that the proapoptotic non-steroidal anti-inflammatory drugs (NSAIDs) aspirin and indomethacin downregulate b-catenin/TCF-signaling. Upon phosphorylation by glycogen synthase kinase 3-b (GSK3-b), b-catenin is suggested to be targeted for ubiquitin-depentent degradation. To elucidate whether the reduced signaling activity of b-catenin in response to NSAIDs was a result of its enhanced phosphorylation we analyzed colorectal cancer cell lysates by phosphorylation-specific antibodies. In SW948 and SW480 colorectal cancer cells, expressing high levels of b-catenin, the phosphorylation of S33, S37, T41 and S45 time dependently increased in response to aspirin and indomethacin. In contrast, in 293 cells, containing low amounts of b-catenin, NSAID treatment did not result in b-catenin phosphorylation. In unstimulated, resting cells, GSK3 is a constitutively active enzyme that is negatively regulated i.e. by the Wnt pathway and by Akt-induced phosphorylation. Phosphorylation of GSK3-b by Akt results in its inactivation and subsequent accumulation of unphosphorylated b-catenin in several cell types. We therefore analyzed whether the aspirin induced b-catenin phosphorylation might be linked to the Akt/PKB survival signaling pathway. We demonstrate that NSAIDs differentially modulated the phosphorylation pattern of GSK3-b and Akt/PKB. Furthermore, expression of Akt/PKB was reduced upon aspirin treatment. The data undeline the importance of the Akt/PKB pathway as a central player in regulation of cell survival and apoptosis and define it as an important target for anti-cancer therapeutics.

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Mechanism of 17-B-estradiol-induced ERK1/2 activation in breast cancer cells: a role for HER2 and PKC-delta

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Activation of mitogen-activated protein kinase (Erk/MAPK) is a critical signal transduction event for estrogen (E2)-mediated cell proliferation. Recent studies from our group and others have shown that persistent activation of Erk plays a major role in cell migration and tumor progression. The signaling mechanism(s) responsible for persistent Erk activation are not fully characterized, however. In this study, we have shown that E2 induces a slow but persistent activation of Erk in MCF-7 breast carcinoma cells. The E2-induced Erk activation is dependent on new protein synthesis, suggesting that E2-induced growth factors play a major role in Erk activation. When MCF-7 cells were treated with E2 in the presence of an anti-HER-2 monoclonal antibody (Herceptin), 60 -70% of E2-induced Erk activation is blocked. In addition, when untreated MCF-7 cells were exposed to conditioned medium (CM) from E2-treated cells, Erk activity was significantly enhanced. Furthermore Erk activity was blocked by an antibody against HER-2 or by heregulin (HRG) depletion from the conditioned medium through immunoprecipitation. In contrast, epidermal growth factor receptor (EGFR) (Ab 528) antibody only blocked 10-20% of E2-induced Erk activation, suggesting that E2-induced Erk activation is predominantly mediated through the secretion of HRG and activation of HER-2 by an autoctine/paracrine mechanism. Inhibition of PKC-d mediated signaling by a dominant negative mutant or the relatively specific PKC-d inhibitor rottlerin blocked most of the E2-induced Erk activation, but had no effect on TGFa-induced Erk activation. By contrast, inhibition of Ras, by inhibition of farnesyl transferase (Ftase-1) or dominant negative (N17)-Ras significantly inhibited both E2