prevent oxidant (K3)-induced upregulation of NFκB DNA-binding in ER-positive breast cancer cells (MCF-7) included the IKK inhibitor parthenolide (PA), the antioxidant dithiocarbamate (PDTC), and proteasome inhibitors (MG-132, PS-341/bortezomib). PA given in combination with tamoxifen (Tam) to ER-positive breast cancer cells with reduced Tam sensitivity (BT474, MCF-7/HER2) produced greater than additive reduction in cell survival ($p<0.001$) as compared to treatment with Tam or PA alone. In conclusion, clinical studies indicate that ER-positive breast cancers may be prognostically subdivided according to NFκB activity. Breast cancer cell culture studies indicate that inhibition of NFκB activity by several different drug strategies is feasible, and that inhibition of NFκB activity may synergistically enhance the antitumor activity of ER-targeted endocrine agents like tamoxifen.

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IGF-I receptor kinase inhibitor NVP-AEW541-NX-7 abolishes MCF-7 breast cancer cell responsivity to estradiol

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There is prior evidence for interaction of estrogen receptor and IGF-I receptor signalling in breast cancer. A recent report (Mitsiades, C. et al, *Cancer Cell* 5: 221–230, 2004) showed progressive reduction in survival of estrogen receptor positive MCF-7 human breast cancer cells induced by the IGF-I receptor kinase inhibitor NVP-ADW-742 (Novartis) in concentrations ranging from 0.1 to 10 micromolar, in 10% charcoal-stripped fetal calf serum culture conditions. We observed 40% growth stimulation of MCF-7 cells by 0.1 nM estradiol under 5% charcoal-stripped fetal calf serum culture conditions, and carried out experiments to determine if estradiol influences the activity of NVP-AEW541-NX-7 (a IGF-1 receptor inhibitor related to NVP-ADW-742). We observed that NVP-AEW541-NX-7 at 0.1 micromolar completely blocks estradiol induced cell proliferation. Conversely, estradiol alters the dose-response characteristics of NVP-AEW541-NX-7 as a suppressor of MCF-7 cell proliferation as assessed by MTT assay.
Under our conditions, NVP-AEW541-NX-7 had detectable anti-proliferation effects in the absence of estradiol over a wide concentration range (25% growth inhibition at 0.01 uM to 87% growth inhibition at 1uM). estradiol protected cells, particularly at low NVP-AEW-NX-7 concentrations. Equal growth inhibition was observed at 0.01 micromolar NVP-AEW-NX-7 in the absence of estradiol and 0.15 micromolar in its presence. These results are consistent with recently reported IGF-I receptor – estradiol receptor interactions (Song, R. et al, *PNAS* 101:2078–81,2004), and raise the possibility of therapeutically useful co-blockade of estrogen and IGF-I receptors. Studies of the influence of estradiol on phosphorylation changes induced by NVP-AEW541-NX-7 at the IGF-1R and downstream are in progress.

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Inhibitors of the Ras/Raf/MAPK signaling pathway sensitize pancreatic cancer cells resistant to EGFR inhibitors

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The epidermal growth factor receptor (EGFR) is frequently over-expressed in pancreatic cancers and is currently exploited as a novel therapeutic target in early clinical trials. Ligand binding to the EGFR mediates biological responses through activation of downstream signaling pathways, including the Ras/Raf/MAPK signaling cascade. Over 90% of pancreatic cancers have also activating K-Ras mutations, which can result in constitutive activity of the Ras/Raf/MAPK signaling cascade and represent a possible mechanism of resistance to EGFR inhibition. The aim of this study was to determine the role of constitutively active Ras/Raf/MAPK signaling in pancreatic cancer cells which demonstrate resistance to EGFR inhibitors. Constitutive activity of MAPK was determined by Western blot analyses of pancreatic cancer cell lines, which contain activating K-Ras mutations. MTT survival assays were used to analyzes a panel of pancreatic cancer cells for response to treatment with AG1478, which is an irreversible inhibitor of the EGFR tyrosine kinase domain. These assays demonstrated that pancreatic cancer cells were relatively resistant to EGFR inhibition. By Western blot analyses, treatment with AG1478 failed to inhibit MAPK phosphorylation, expression of SKP2, a mediator of p27Kip1 degradation in the proteasome, was also unaffected. Co-treatment with the MEK1 inhibitor PD98059 or lovastatin, a prenylation inhibitor, sensitized pancreatic cancer cells to treatment with AG1478. Combination therapies were found to block MAPK phosphorylation, SKP2 expression and rendered cells sensitive on a molecular level. In conclusion, these data provide the rationale to explore combined targeted therapies in clinical trials in K-Ras mutated pancreatic cancer.

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Phase Ib and pharmacokinetic studies of Everolimus (RAD001), a novel oral mTOR-inhibitor, with paclitaxel in patients with advanced solid tumors

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Background: RAD001 (everolimus) is a novel mTOR inhibitor that displays in-vitro and in-vivo additive or synergistic effects of with paclitaxel when the two drugs were administered simultaneously.
Methods: This study investigates the safety and pharmacokinetic interactions of everolimus given orally at escalated doses simultaneously with a fixed dose of 80mg/m^2 paclitaxel on days 1, 8 and 15 every 4 weeks. In this study, doses of everolimus were selected on the basis of previous phase I single agent safety data with everolimus, pharmacokinetic/pharmacodynamic analysis demonstrating that everolimus inhibits the phosphorylation of $P_{70}S_6$kinase downstream to mTOR at doses ranging 20–30mg, and early evidence of antitumor activity. So far, two dose levels were successively investigated: 15mg (cohort 1) and then 30mg (cohort 2).
Results: Twelve patients have been enrolled onto the study (male/female: 2/10; median age 59y, range 38–69y). Tumor types consisted of breast (5pts), ovarian (2pts), thyroid (1pt) carcinomas; melanoma (1pt); and sarcomas (3pts). All patients were previously treated with chemotherapy, including 9 patients who were previously exposed to paclitaxel. Three pts were entered in cohort 1 and received a range of 3–4 cycles with no dose-limiting toxicity. Therefore 9 pts were included in cohort 2 with so far no dose-limiting toxicity. As expected for paclitaxel-based chemotherapy, the most frequent toxicity was grade 1–2 neutropenia in 50% of patients. Adverse events related to everolimus administration were mild to moderate grade 1–2 skin reactions, mucositis and diarrhea. None of the patients required treatment withdrawal or discontinuation due to toxicity. No clinically relevant pharmacokinetic interaction between the drugs was found in cohort 1. Antitumor activity was observed in a patient with breast cancer previously exposed to a taxane and in patients with paclitaxel-pretreated ovarian cancer (2 stable diseases with decrasing CA125 at 3 and 4 months).
Conclusions: The combination of everolimus to weekly paclitaxel has a safe toxicity profile comparable to that observed with paclitaxel and everolimus when used single agents. So far, no pharmacokinetic interaction between everolimus and paclitaxel has been detectable. Based on early evidence of activity, our results encourage subsequent explorations of this combination in phase 2 studies.

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NFkB expression and disease outcome in prostate cancer

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Background: The activation of nuclear transcription factor NFkB is regulated by the binding to its specific inhibitor IkB. NFkB binds to multiple DNA sequences controlling the downstream expression of cell cycle regulatory proteins, cytokines, angiogenesis factors, cell adhesion molecules, enzymes and pro- and anti-apoptotic proteins. IkB is in turn regulated by degradation through the ubiquitin proteasome pathway.
Methods: Using prostatectomy specimens, immunohistochemical staining (IHC) for NFkB and IkB (Santa Cruz Biotechnology) was performed on formalin-fixed paraffin-embedded sections of 136 cases of PCA. Cytoplasmic immunoreactivity was scored for intensity and distribution and results were correlated with tumor grade, stage, DNA ploidy status (Feulgen spectroscopy) and biochemical disease recurrence.
Results: 49% of PCAs over-expressed NFkB and 63% showed decreased expression of IkB. NFkB over-expression correlated with advanced tumor stage ($p=0.048$), aneuploidy ($p=0.022$), and biochemical disease recurrence ($p=0.001$). Decreased expression of IkB correlated with high tumor grade ($p=0.015$). On multivariate analysis, tumor stage ($p=0.043$) and NFkB expression ($p=0.006$) were independent predictors of disease recurrence.
Conclusions: Over-expression of NFkB in primary PCA predicts advanced tumor stage and independently predicts disease recurrence. Drugs such as proteasome inhibitors that target NFkB should be considered for the treatment of prostate cancer.