well as objective clinical response. The surrogate biomarker pERK showed dramatic downregulation in patients that responded to TKI therapy. Using biomarkers to predict and monitor response to TKIs can help stratify best patient populations for TKI treatment. Strategies combining the effectiveness of chaperone-mediated degradation with the selectivity of TKIs hold promise for breast cancer therapy.

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Rapamycin enhances radiation-induced apoptosis in human glioma cells with constitutive activation of the PI3K/PKB signalling pathway

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It has been shown recently that rapamycin and its ester CCI-779 are cytostatic and could potentiate cytotoxic chemotherapy in tumors bearing PTEN deletions. Its efficiency in radiotherapy has not been investigated in detail. The protein kinase B (PKB/Akt)-dependent anti-apoptotic pathway is activated by phosphatidylinositol-3'-kinase (PI3K), and the phospholipid-phosphatase PTEN is the most important inhibitor of PKB/Akt. We and others have demonstrated previously that PKB/Akt is constitutively activated in human glioblastoma multiforme (GBM) not only by deletions of the PTEN gene but also due to overexpression of the EGF receptor. The aim of the present study was to determine the effect of rapamycin treatment on experimetnal radiotherapy of human GBM cells with and without constitutive activation of PKB/Akt due to PTEN deletion and/or EGF receptore overexpression. Using this approach we found different responses between cell lines with and without constitutive activation of PKB/Akt. Cell lines with PKB/Akt-activation were more resistant against radiation after serum starvation. The efficiency of radiation could be increased in all cell lines by pre-treatment with rapamycin and with the specific PI3K-inhibitor Wortmannin but not by the MAP-Kinase inhibitor PD89059. However, rapamycin was more effective in those cell lines showing PKB/Akt activation by PTEN deletions and EGF receptor overexpression.

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Ansamycins inactivate AKT and enhances the anti-tumor effects of paclitaxel

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Ansamycins and radicicol are natural products that bind to a conserved ADP/ATP pocket in Hsp90 and modulate its function. Treatment with these agents causes the degradation of proteins that require Hsp90 function for their proper maturation and stability. HER2 is one of the most sensitive targets and cells that overexpress HER2 are particularly sensitive to the antiproliferative effects of these agents. In breast cancer cells with high levels of HER2 expression, Akt activation is dependent upon the formation of HER2/HER3 heterodimers. In these cells, inhibitors of Hsp90 cause Akt inactivation by two mechanisms. Degradation of HER2 by ansamycins leads dephosphorylation of HER3, an uncoupling of active PI3k and a rapid loss (within 1 hr) of Akt activity. Akt forms a complex with Hsp90 and cdc37 and functional Hsp90 is required for Akt stability. Treatment with inhibitors of Hsp90 thus results in Akt inactivation due to ubiquitination and degradation of the protein. As Akt activity has been demonstrated to sensitize cancer cells to apoptotic stimuli, we evaluated whether inhibition of Akt activity by the ansamycin 17-AAG could enhance the anti-tumor effects of paclitaxel. 17-AAG and paclitaxel could be administered in combination at their maximally tolerated doses (MTD). 17-AAG enhanced the activity of paclitaxel in a dose and schedule dependent manner. 17-AAG at doses of 50-150 mg/kg/wk in combination with paclitaxel (25 mg/kg qwk $\times$ 5 wks) resulted in 25-40% complete responses and mean tumor regression of greater than 90%. By immunoblot, the 50 mg/kg dose of 17-AAG caused a maximal (>95%) reduction in Akt activity. Higher doses of 17-AAG did not increase the magnitude or duration of Akt inactiviation and did not result in additional enhancement of paclitaxel anti-tumor activity. To further define the contribution of Akt activity in mediating this effect, SkBr3 cells were transfected with a constitutively membrane bound form of PI3k. For up to 24 hrs, this construct prevented the effects of 17-AAG on Akt activity. Longer-term exposure to 17-AAG resulted in Akt inactivation due to Akt protein loss. Enhancement of paclitaxel-induced apoptosis was partially abrogated in the p110-transfectants suggesting a role for Akt in mediating this effect. These results suggest that 17-AAG/taxane combinations may represent a new strategy for the treatment of patients with advanced breast cancer.

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Epidermal growth factor receptor (EGFR) expression on NSCLC is not useful to predict response to ZD1839 therapy: Preliminary results of the Istituto Clinico Humanitas, Rozzano, Milano

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Background: ZD 1839 (Iressa) is an orally active, selective epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor which blocks signal transduction pathways implicated in cancer growth. Overall response rate in NSCLC is around 18% in recent phase II trials being principal adverse events mild diarrhoea and skin rash.
Aim: To evaluate a correlation between response and EGFR status in a series of patients with NSCLC treated with ZD 1839. EGFR was detected by immunocytochemistry using the MoAb Ab-10 (Clone 111.6- Neomarkers) in paraffin embedded material. ZD 1839 was provided on a named-patient basis.
Results: From February 2001 88 pts were treated in with oral ZD 1839 250 mg/daily. 73 pts were evaluable for response and toxicity (M/F 57/16, median age 63, range 32-76).One pt had complete response, 5 pts partial response (PR), one pt minor response (MR) and 24 pts stable disease (SD) for an overall disease control of 54%. Duration of both PR and SD was 4 months (range 2-13 mos). Median survival was 5.2 mos (2-13). At this moment, EGFR analysis has been performed in 26 pts: 4 PR, 10 SD, 12 progressive disease (PD). Responses and EGFR expression were as follows: PRs: 1 pt 40%, 3 pts 0%; SD: 4 pts 0%, 2 pts 10%; 4 pts >20%. Mean expression rate of EGFR for PR, SD and PD was 10%, 13.9% and 17.3% respectively.
Conclusion: This preliminary results suggest that expression of EGFR in paraffin embedded material fails to predict response to ZD 1839 and may not be used to select NSCLC pts for treatment with this novel agent. Definitive results will be presented

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Biomarkers of anticancer activity of R115777 in combination with paclitaxel in a human breast cancer model *in vitro*

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Overexpression of HER2 (c-erb-2, neu) gene observed in 30% of human breast cancers is associated with a poor prognosis for the overall and disease-free survival. Since most patients who overexpress HER2/neu fail to respond to single agent herceptin or paclitaxel therapy, novel combination therapies are being evaluated. R115777 is a potent, selective, non-peptidomimetic inhibitor of farnesyl transferase that inhibits the growth of H-, K-, and N-ras transformed and some wild-type ras xenograft tumors. R115777 has already shown clinical activity in breast cancer. This study evaluated the efficacy of R115777 and paclitaxel as single agents and in combination against HER2/neu overexpressing human breast cancer cell line BT-474 *in vitro* on cytotoxicity, prenylation status (DJ-2, Rho B), expression of biomarkers of tumor growth (Raf/MEK/Erk) and survival (PI3K/Akt), and secretion of pro-angiogenic factors (VEGF, FGF-2, MMP-2, MMP-9). The IC_{50} concentrations of R115777 (5 μM) and paclitaxel (10 nM) were not affected by the drug combination. BT-474 cells continuously exposed to R115777 + paclitaxel at respective IC_{50} doses for 1-4 days had no significant change in Rho B prenylation but showed >90% inhibition of DJ-2 farnesylation. Secretion of VEGF, FGF-2, MMP-2, and MMP-9 by the drug-treated cells was not different from the untreated control over 4 days. Notably, over two-fold transient decrease in the ratio of phosphorylated Erk to total Erk and phospho-Akt to Akt expression occurred at day 2 in the drug combination in comparison with R115777 alone. The enhanced inhibitory effect of the drug combination was gradually lost by day 4. At that time, R115777 + paclitaxel reduced total Raf-1, MEK and Erk without a decrease in the ratios of the phosphorylated to total proteins. The results are consistent with the ability of R115777 to interfere with tumor signaling pathways relevant for growth and survival and suggest that the dual drug therapy might offer clinical advantage over R115777 and paclitaxel used as single agents. Supported by Janssen and Shelby Rae Tengg Foundation.