mg/day group, in IDEAL 1 and 2, respectively. In pts who were symptomatic at entry, a symptom improvement (SI) (*/=2 point increase, */=4 weeks) was observed in 40% and 37% of pts receiving 250 and 500 mg/day, respectively, in IDEAL 1, and in 43% and 34% of all pts, respectively, in IDEAL 2. A positive association was observed between SI and both radiologic response and survival in both trials. Overall, quality of life (QoL) was improved in 24% and 22% of pts receiving 250 and 500 mg/day, respectively, in IDEAL 1, and in 34% and 23% of pts, respectively, in IDEAL 2. Most drug-related adverse events (AEs) were mild grade 1/2 diarrhea and skin disorders. Drug-related AEs were more frequent in the higher dose group. Withdrawal due to drug-related AEs was 2% and 9% for pts receiving ZD1839 250 and 500 mg/day, respectively, in IDEAL 1, and 1% and 5%, respectively, in IDEAL 2. In conclusion, in pretreated pts with advanced NSCLC, oral ZD1839 250 mg/day resulted in clinically significant antitumor activity, had an acceptable tolerability profile and provided improvement in disease-related symptoms and QoL. 'Iressa' is a trademark of the AstraZeneca group of companies

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ABX-EGF, a fully human anti-epidermal growth factor receptor (EGFr) monoclonal antibody: phase II clinical trial in renal cell cancer (RCC)

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EGRr is a transmembrane glycoprotein that promotes cell growth in a variety ofnormal and transformed tissues. ABX-EGF is a high-affinity, fully human IgG2monoclonal antibody to EGFr generated in Xenomouse mice. Part 1 of a two-partphase 2 trial consisting of 8 weekly infusions of ABX-EGF was performed inpatients (pts) with RCC who failed or were unable to receive IL-2/IFN-alfa.Stable or responding pts were eligible for extended weekly treatment at theassigned dose for 8 additional months or until disease progression. In Part 1,88 pts received at least one dose of ABX-EGF at the following dose levels: 1.0mg/kg (22 pts), 1.5 mg/kg (22 pts), 2.0 mg/kg (23 pts), and 2.5 mg/kg (21 pts).Overexpression of EGFr was documented in 95% of pts enrolled. Eleven percent of the pts had received no prior biotherapy or chemotherapy, whereas 56% and 33%were more heavily-pretreated, having received 1?2 and at least 3 prior regimens,respectively. All pts have completed one 8-week cycle of ABX-EGF and areevaluable for response. Three pts (1 each at 1, 1.5 & 2.5 mg/kg) had partialresponses. Two patients (1 each at 1.0 & 2.5 mg/kg) had minor responses. Fiftypercent of pts had stable disease as their best response. A transient acneiformskin rash, which is a potential pharmacodynamic surrogate of EGFr blockade, wasobserved in 70%, 91%, 95% and 100% of pts treated with at least 3 doses ofABX-EGF at 1.0, 1.5, 2.0 and 2.5 mg/kg, respectively. Other >/= grade 2 adverseevents in over 2% of pts included asthenia, pain, abdominal pain, back pain,constipation, cough and dyspnea. An analysis of peak and trough serum ABX-EGFconcentrations indicates low intrapatient variability and consistent drugexposure in individual pts throughout the 8-week treatment period in all dosinggroups which is consistent with the lack of human anti-human antibody (HAHA)formation in pts tested to date. The inter-patient variability in ABX-EGFexposure was extremely low and trough concentrations at ABX-EGF doses of atleast 2 mg/kg consistently exceeded IC90 values determined for human tumorsxenograft models. The relationship between the incidence of skin rash and dosewas well described by a sigmoidal model, which predicted a 90% incidence of skinrash at an ABX-EGF dose of 1.5 mg/kg (ED90) in agreement with the results ofphase 1 studies. In conclusion, ABX-EGF is well tolerated and preliminaryevidence of antitumor activity was observed in heavily-pretreated RCC pts.

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Phase II study of OSI-774 in patients with metastatic colorectal cancer

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Epidermal Growth Factor Receptor overexpression is seen in upto 75% of colorectal cancers, and has been implicated in the development and propagation of the malignancy. OSI-774 is a potent epidermal growth factor receptor tyrosine kinase inhibitor (EGFR TKI) which is being evaluated in a phase II study to assess the activity in patients with metastatic colorectal cancer. Primary endpoints are response or disease stabilization, with a multinomial stopping rule and secondary endpoints are the assessment of molecular changes with therapy. The study is currently ongoing. Sixteen evaluable patients with metastatic colorectal cancer have been treated with OSI-774 at a dose of 150 mg PO daily continuously on a four weekly cycle. Paired biopsies of the tumors and skin have been obtained in 15 patients. Radiologic evaluation was done every 8 weeks and skin and tumor biopsies were performed prior to treatment and on day 8. Eleven (69%) were male and the median age was 59 years with a range from 43-76 years. Eight (50%) of patients had an ECOG status of 1 and 8 (50%) had an ECOG status of 0. Apart from adjuvant chemotherapy, patients had only received chemotherapy for metastatic disease with one line irinotecan/5FU in combination or sequentially. The most common sites of disease were liver in 13 (81%), lymph nodes in 10 (63%) and lung in 7 (44%). Thirteen patients were evaluable for efficacy and toxicity. There are 3 (23%) patients with stable disease (2 confirmed and one pending confirmation) who remain on study (5, 4 and 3 cycles to date). Ten patients have progressed. The two most common toxicities observed were diarrhea [grade 1: 4pts (31%), grade 2: 1pt (8%) grade 3: 1pt (8%)] and rash [grade 1: 4pts (31%), grade 2: 5pts (38%) grade 3: 1pt (8%)]. There were no grade 4 toxicities related to this drug. There were 4 grade 3 toxicities assessed to be possibly or probably related to this drug including diarrhea, rash, elevation in INR and elevation in ALP. Treating patients with flamazine cream and minocycline antibiotic improved rash from OSI-774 in 70% of patients with a grade 2 or greater rash. Conclusion: Patients are being evaluated and correlative studies are ongoing.

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The use of predicting factors and surrogate markers in breast cancer biopsies treated with targeted erbB tyrosine kinase inhibitor

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Over-expression of erbB receptors is associated with aggressive breast cancers. Therapeutic strategies targeting these oncoproteins are in clinical trials. One approach is the use of a monoclonal antibody to erbB2, Herceptin. Studies performed *in vitro* have attributed the therapeutic potential of Herceptin to enhance intracellular degradation resulting in a functional inhibition of erbB2. Another effective approach is the use of tyrosine kinase inhibitors (TKIs) that block the nucleotide-binding site of the erbB kinases, specifically erbB1 and erbB2. An alternative way to enhance degradation and inhibit activity of erbB proteins involves targeting the heat shock protein 90 (Hsp90) using benzoquinone ansamycins such as geldanamycin (GA). Hsp90 forms complexes with erbB2 proteins and stabilizes them. GA blocks ATP binding to Hsp90 resulting in poly-ubiquitination and destruction of the erbB2. However, GA's broad effect is of concern. In contrast, the TKI group of drugs is highly selective to erbB receptors blocking only the nucleotide-binding site of tyrosine kinase proteins. Consequent to blocking kinase activity, most downstream signaling pathways are inhibited leading to growth arrest. In this work, we used cancer tissue biopsies from patients before and after TKI treatment to understand the mechanism and the factors associated with response or non-response to TKI treatment. Breast cancer biopsies from patients, before and after TKI treatment, were immunostained for erbB1 and erbB2. Their phosphorylated forms and phosphorylated ERK (pERK) (a downstream signal) were used as a surrogate marker of response (antibodies were purchased from Cell Signaling and Ventana). Levels of staining were quantitated by microscope based image analysis. Patients with high levels of EGFR, HER-2 and pERK responded to TKI. Their response was confirmed by using surrogate biomarkers as

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.