by Iressa; Akt activity is maximally inhibited by induction of PTEN and PI3 kinase is not regulated by EGFR in these cells. Instead the synergy seems to be secondary to inhibition of two parallel pathways that both inhibit the function of the proapoptotic protein Bad. Bad is phosphoryated on serine 112 and serine 136 in these cells. Serine 112 phosphorylation is EGFR-MEK-MAP kinase dependent, whereas phosphorylation of serine 136 is sensitive to inhibitors of PI3 kinase, Akt kinase signaling. Bad function is inhibited and apoptosis is induced maximally only if both serines are dephosphorylated in response to inhibitors of both pathways. Furthermore, conditional expression of PTEN function results in inhibition of Akt phosphorylation in MDA-468 xenografts and a delay in tumor growth. These findings suggest that EGFR activation is not required for cell survival in cells with PTEN deficiency and that selective EGFR inhibitors will be ineffective in such tumors. However, if PI3 kinase, Akt kinase signaling is inhibited, cells become dependent for survival on EGFR driven, Akt independent, inactivation of Bad. Inhibition of EGFR and PI3 kinase signaling by combinations of drugs may be useful for the treatment of such tumors.

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Clinical and molecular features of neuroblastoma tumors that can be targeted through c-Kit

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Background: In neuroblastoma (NB) recent reports show that the selective inhibition of c-Kit signaling by STI571 (Gleevec) is associated with significant tumor growth inhibition in *in vitro* and *in vivo* preclinical models (Vitali R et al, 2003; Beppu K et al 2004). This study was aimed at investigating in a large clinical series of NB primary tumors the clinico-biological characteristics of that subset which utilizes the SCF/c-Kit pathway and may thus be responsive to selective inhibitors.
Material and Methods: Primary tumor site samples were obtained at diagnosis from 168 untreated children with NB. Expression of mRNA and protein for c-Kit and its ligand Stem Cell Factor (SCF) was determined by Northern blot and immunohistochemistry, resp. In selected cases sequencing of c-Kit exon 11 was also carried out in order to identify possible mutations. MYCN amplification and allelic loss for 1p36 (1p36 LOH) were evaluated by Southern blot and demonstrated in 27 and 36 tumors, resp.
Results: Expression of mRNA and protein for c-Kit was detected in 22% and 13% of tumors, resp. Immunostaining was confined to neoplastic neuroblasts. Expression of mRNA and protein for SCF was documented in 31% and 28% of tumors, resp., with 66% of the c-Kit-positive tumors also expressing SCF. Mutations in exon 11 of the c-kit gene were not found in the 9 c-Kit-positive and 9 c-Kit-negative tumors that were analyzed. Expression of c-Kit correlated with advanced stage (3 and 4), MYCN amplification and 1p36 LOH ($p<0.001$). Expression of SCF correlated with adrenal primary ($p<0.05$), MYCN amplification and 1p36 LOH ($p<0.001$). Overall survival (OS) probability was 17% in c-Kit-positive cases vs. 68% in c-Kit-negative, 43% in SCF-positive cases vs. 78% in SCF-negative ($p<0.001$). Using Cox multiple regression analysis however neither c-Kit nor SCF expression were independently associated with a shorter OS.
Conclusions: The SCF/c-Kit pathway is expressed overall in 20% of NBs, but this represents 60% of the most aggressive tumors (i.e., metastatic disease with unfavorable clinical, histological and molecular features). At present these children cannot be cured by high-dose cytotoxic chemotherapy. Preclinical studies suggest that these patients may benefit from the administration of selective inhibitors of c-Kit, and human clinical trials are therefore now warranted.
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C-fos mRNA levels predict response to Iressa therapy

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The response to EGFR inhibitors has been measured using clinical and radiological parameters. In this abstract we present the development of a molecular response assay, which predicts sensitivity to EGFR blockade *in vitro*, and tumor response to therapy *in vivo*.

Human carcinoma cell lines (A431, HuCCT1, HN11, 29, CAL29 and Hep2) were grown under standard tissue culture conditions. The cell lines were stimulated for 1 hour with recombinant EGF, in presence or absence of inhibitor (Iressa). 2 groups of 5 nude athymic mice were injected (2 tumors per mouse) with $5x10^6$ cells, and treated during 14 days (vehicle or Iressa 150 mg/kg, intraperitoneally). Fine needle aspiration biopsy of tumors was performed according to standard cytopathologic practice at 1, 7, and 14 days of therapy. mRNA was obtained for quantitative PCR analysis. The levels of c-fos expression were normalized to 3 house-keeping genes (HPRT, SDHA and Ubiquitin).
3 cell lines showed markedly elevated levels of c-fos mRNA in response to EGF stimulation (18 to126-fold), which was completely inhibited by the addition of Iressa. Two cell lines (HUCCT1 and Hep2) showed no increase in c-fos levels. None of the cell lines showed a decrease of c-fos levels with inhibitor alone at 1 hour. When grown over 72 hours, A431 showed a 6-fold decrease in c-fos levels in presence of Iressa (HuCCT1 showed no change).
Iressa induced tumor growth arrest in A431 xenografts, and a decrease in c-fos levels was documented in the treated vs. the control arm, showing a significant correlation with response to therapy. The c-fos levels were: baseline at d1: 7.3 (std.error 4.9), d7: 3.6 (1.2), d14: 3 (1.6). The c-fos levels in the control group were 4.5-fold higher than in the treated group: baseline: 3.9 (1.2), d7: 14.3 (3.3), d14: 14.1 (2.8). There were no significant differences between treated and control groups at baseline. HuCCT-1 xenografts showed no difference between treated and control groups in terms of tumor growth and c-fos levels.
C-fos quantitation can be used in an in-vitro EGF stimulation assay for assessment of EGFR blockade by Iressa. Importantly, the assay is useful in predicting lack of response in cases of activating mutations downstream of EGFR proximal phosphorylation events.
C-fos mRNA levels predict tumor response to Iressa therapy in a xenograft model in both sensitive and resistant cell lines. We are in the process of validating the assay in prospective trials in patients receiving Iressa.

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Constitutive activation of Akt/protein kinase B in gastric cancer: its correlation with lower invasiveness and better prognosis

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Background: Gastric cancer is one of the most common malignancies worldwide and a major cause of cancer death in Asia. Thus, it is important to identify genetic alterations that allow malignancy and prognosis of gastric carcinoma to be estimated. Phosphatidylinositol-3 kinase/Akt (protein kinase B) pathway has been reported to promote cell proliferation, survival and tumor progression. However, the role of Akt in the biology of gastric cancer has not been well studied. We sought to investigate the expression of Akt phosphorylation in human gastric carcinomas and its biological significance.
Material and Methods: The expressions of Akt and pAkt were evaluated immunohistochemically in 329 consecutive gastric carcinomas using the tissue-array method, and the associations between pAkt and clinico-pathologic features were assessed. Survival curves were estimated using the Kaplan-Meier product-limit method, and the significance of differences between the survival curves was determined using the log-rank test. We also performed Western blot analysis and cell growth assay using human gastric cancer cell lines which were transduced with retroviral vectors containing constitutively active Akt (CA-Akt) or kinase-dead Akt (KD-Akt).
Results: Akt expression was detected in 74% of the tumors and pAkt expression in 78%. Akt phosphorylation was highly expressed in the early stage gastric carcinomas ($p=0.01$). We also found an inverse association between Akt activation and lymphatic invasion ($p=0.01$) or lymph node metastasis ($p=0.008$). Patients with pAkt-positive carcinomas showed significantly better survival than those with pAkt-negative carcinomas ($p=0.0002$). An evaluation of combined expressions revealed that the group with pAkt-positive plus LN-negative had a better prognosis than the other cases ($p<0.0001$). *In vitro* study showed that constitutive activation of Akt promoted cell growth in gastric cancer cells.
Conclusions: pAkt, which is highly expressed in the early stage of gastric carcinoma, is significantly correlated with cell growth, nodal status and better outcome. These findings suggest that Akt activation may be used as an independent prognostic marker in gastric cancer.