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Head and neck squamous carcinoma cell lines exhibit an intact EGFR signaling pathway and variable response to EGFR agonist and antagonist

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Head and neck cancer is an epithelial malignancy expressing receptor tyrosine kinases of which the epidermal growth factor receptor (EGFR) has demonstrated clinical significance in this disease. Cetuximab (Erbitux) has resulted in survival benefits when added to standard radiotherapy in locally advanced head and neck cancer (Bonner et al. N Engl. J Med. 2006 Feb 9; 354(6): 567−78). We embarked on a study to examine a panel of head and neck cell lines, all of squamous cell histology, to determine the phosphorylation status of several signaling molecules of the EGFR pathway in relation to growth of the cells and the effect from EGFR agonist (transforming growth factor, TGF-α) and commercially available antagonist erlotinib (Tarceva).
Experiment 1: On day one, cells were seeded on 24 well plates with 10,000−40,000 cells per well. On day two, cells were treated in triplicate with either 5umol erlotinib or no treatment control (DMSO alone). On day five, cell count assays were performed to determine growth. This experiment was repeated two additional times to ensure reproducibility of the data. Experiment 2 consisted of 4 treatment arms of the complete cell line panel: erlotinib, TGF-α, erlotinib and TGF-α and no treatment control. Western blot analysis was performed on cell lysates for (phosphorylated) forms of ERK (pERK), AKT (pAKT), and RAF (pRAF), all downstream transducing proteins of the EGFR signaling pathway, as well as EGFR and human EGF receptor 2 (HER2). Degree of signal intensity was determined via phosphorimaging.
Results of the experiments were as follows: erlotinib was found to decrease all downstream phophorylated signal transducers of the EGFR pathway when compared to no treatment control in every cell line tested. In addition, inhibition of growth of cell lines was also consistently seen when compared with no treatment control. This growth inhibition in the presence of antagonist, however, was found to be independent of both EGFR/HER2 levels and the basal levels of the phosphorylated downstream transducers studied. Furthermore, it was also found that lower expression of HER2 in these cell lines correlated with greater inhibitory effect of antagonist upon the phosphorylated form of AKT, while higher levels of HER2 showed a lesser inhibitory effect of antagonist upon the prevalence of the phosphorylated form ($R^2 = 0.71$).
Variability of EGFR, HER2, and basal activated transducer levels as well as growth inhibition data between these cell lines suggest they are heterogeneous in their growth patterns, signaling pathways, and expression of receptors and transducers. This heterogeneity likely reflect biologic diversity of head and neck squamous cell carcinoma and supports the need for further study, data from which should improve our understanding of this disease as well as improve patient selection for this targeted therapeutic approach.

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Pharmacokinetics of Akt inhibitor NSC 728209 in the rat by LC/MS/MS method

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Purpose: NSC 728209 (OSU-03012, **I**) is a celecoxib derivative that was designed through molecular modeling to disrupt Akt signaling pathways by inhibiting PDK-1 with an IC_{50} of 5.0 μM and possesses antitumor activity against prostate and chronic lymphocytic leukemia. It was selected for preclinical development under the NCI RAID program and previously we reported the assay development and pharmacokinetics in mice (AACR04). The objective of the current study was to characterize the pharmacokinetics and oral bioavailability of **I** in the rat in comparison with that in the mouse and its metabolism in rat liver microsomes.
Methods: A sensitive and specific LC-MS/MS method previously developed for **I** in mouse plasma was adapted to rat plasma. The precursor/product ion pairs at m/z 461–404 for NSC 728209 and at m/z 446–429 for an analog NSC 728210 as the internal standard were used for the quantifications in rat plasma and microsomal extracts. Six Fisher 344 rats were given i.v. bolus of **I** and another six given p.o. doses, all at 10.0 mg/kg formulated in PEG400/ethanol/normal saline. Plasma pharmacokinetics were characterized and oral bioavailability determined based on the AUC method. Metabolism in rat liver microsomes and stability in rat plasma of NSC 728209 were also studied.
Results: Plasma concentration-time profile of **I** after an i.v. bolus dose reached a mean concentration of 4.4 μM at 5 min, which declined triexponentially with an initial half-life of 4.1 min, an intermediate half-life of 5.2 h and a terminal half life of 12.7 hr. Its pharmacokinetics are similar to those previously found in the mouse. The drug was detectable in plasma at 72 hr. After oral administration, the plasma level reached a mean C_{max} of 0.23 μM between 4–24 hr and was detectable at 48 hr. The total clearance in the rat was 37 ml/min/kg. The oral bioavailability of **I** was found to be 50.2% and in the mouse it was essentially complete at this dose. No evidence of metabolism was found in rat liver microsomes and the compound was stable in rat plasma at 37ºC, contrary to that in mouse plasma, which degrades with a half-life of 14 hr.
Conclusion: Plasma pharmacokinetics of **I** was similar between the mouse and the rat following i.v. dosing. The oral bioavailability shows species difference and no metabolism was found in rat liver microsomes or in rat plasma.
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Effect of the SRC tyrosine kinase inhibitor dasatinib in combination with erlotinib and in cells with acquired resistance to erlotinib

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Background: SRC tyrosine kinase proteins can regulate oncogenic processes such as cell growth, survival, invasion, and angiogenesis. We previously identified that lung cancer cells dependent on EGFR for survival demonstrate increased sensitivity to dasatinib, a SRC tyrosine kinase inhibitor (TKI). Here we evaluated the efficacy of dasatinib in combination with the EGFR TKI erlotinib in lung cancer cells with defined EGFR status and also examined the effect of dasatinib on lung cancer cells with acquired resistance to erlotinib.
Materials and Methods: Lung cancer cells with defined EGFR status and sensitivity to erlotinib were evaluated for the combination effect of erlotinib and dasatinib using cell viability assays. Combination effects were evaluated by median dose effect method. Cells with EGFR mutation with acquired resistance to erlotinib were used to evaluate the effect of dasatinib on cell viability, cell cycle, and apoptosis. pSRC expression was examined in these cells by western analysis.
Results: Using concentrations of gefitinib and dasatinib that result in concentration-dependent increases in apoptosis, our preliminary experiments suggest that dual EGFR/SRC inhibition additively or synergistically enhances apoptosis in PC9 lung cancer cells with EGFR mutation. We also examined the effect of dual EGFR/SRC TKI on lung cancer cells that do not have EGFR mutation but nonetheless show some degree of sensitivity to EGFR TKI. We identified synergy with erlotinib and dasatinib in both H292 and H358 cells at lower concentrations of both TKI while no effect was seen with either TKI in H441 cells in the dose range used. Both H292 and H358 cells show pSRC protein expression while H441 cells have low levels of detectable pSRC. Finally, lung cancer cells with EGFR mutation that are resistant to EGFR TKI were examined for the effect of dasatinib. These cells do not demonstrate significant amounts of apoptosis with dasatinib but they do undergo a dose-dependent G1 cell cycle arrest despite no observable effect on cell cycle with erlotinib.
Conclusions: The combination of erlotinib and dasatinib results in synergistic inhibition of viability and/or proliferation in lung cancer cells with dependence on EGFR for survival and/or growth. Resistance to erlotinib generally confers resistance to dasatinib although higher concentrations of dasatinib can induce cell cycle arrest, some degree of apoptosis, and reduced cell viability.

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Effect of the histone deacetylase inhibitor LBH589 against epidermal growth factor receptor dependent human lung cancer cells

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Background: The epidermal growth factor (EGFR) activates signal transduction pathways important in lung cancer cell growth and survival. Activating mutations in EGFR selectively activate STAT and Akt survival signaling pathways. Histone deacetylase (HDAC) inhibitors have been suggested to regulate signaling protein interactions via modulation of protein chaperone function. For these reasons, we evaluate the effect of the HDAC inhibitor LBH589 in lung cancer cells with defined EGFR status.
Materials and Methods: Human lung cancer cell lines with defined EGFR status and sensitivity to EGFR TKI were subjected to treatment with HDAC/Hsp90 inhibitor LBH589. Cytotoxicity assays (MTT) as well as assays specific for apoptosis and cell cycle changes were performed. Changes in cell survival were correlated with changes in signaling pathways known