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Title

DIVERGENT ACTIVITY OF AFATINIB (AFAT) AND CETUXIMAB (CET) IN PATIENT-DERIVED XENOGRAFT (PDX) MODELS OF ACQUIRED ERLOTINIB RESISTANCE.

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Conclusion: We have performed broad proteomic analysis of NSCLC cell lines treated with MEK inhibitor BAY86-9766. Baseline activation of the PI3K/Akt pathway predicts for resistance to MEK inhibition. Sensitive cell lines, but not resistant cell lines, show suppression of mTOR activity with treatment with BAY86-9766. The effects of MEK inhibition of mTOR may be modulated by p90RSK through an LKB1 dependent pathway. This suggests a basis for combining targeted agents to overcome resistance, such as combinations of MEK inhibitors with PI3K inhibitors or mTOR inhibitors.

Keywords: MEK inhibitors, Targeted therapeutics, Preclinical models

PRECLINICAL THERAPEUTIC MODELS II
WEDNESDAY, OCTOBER 30, 2013 - 10:30-12:00

MO20.03 DEVELOPMENT AND CHARACTERIZATION OF A PANEL OF GDC-0980 RESISTANT NSCLC CELL LINES

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Background: The PI3K-Akt- mTOR pathway regulates cell growth and proliferation and is often dysregulated in cancer due to mutation, amplification, deletion, methylation and post-translational modifications. PI3K pathway activation in NSCLC has been shown by us and others to lead to a more aggressive disease correlating to poor prognosis for patients. Multiple novel agents, targeting different regulators within the pathway are currently under development. GDC-0980 is a selective dual inhibitor of PI3K and mTOR, which demonstrated excellent downstream inhibition of the PI3K pathway *in vitro*, with the strongest effects being observed in lung, breast and prostate cancer cell lines. There are 12 clinical trials ongoing for this drug, with Phase I studies in solid tumours and Phase II studies in endometrial carcinoma, renal cell carcinoma, prostate cancer and breast cancer. As with all targeted therapies, acquired resistance to GDC-0980 is anticipated to be a major hurdle in the success of this drug. Multiple mechanisms of resistance to GDC-0980 may develop while a patient is being treated with this drug. The aim of this project is to develop four cell line models of resistance to GDC-0980, each representing a different molecular subtype of NSCLC, in order to predict which mechanisms of resistance may occur in patients. This will allow us to identify biomarkers of response/resistance to the drug that may dictate beneficial treatment strategies.

Methods: H460, A549, H1975 and SKMES-1 cells were treated with a dose response curve of GDC-0980 and BrdU proliferation assays determined IC50 values for each cell line. Each cell line was then cultured in GDC-0980 at IC50 concentrations over a period of several months, along with matched 'parent' cell lines. Each month, BrdU proliferation assay were carried out in order to track the development of resistance to the drug. When a log fold difference between the parent and resistant IC50s was observed, the cells were deemed to be resistant. Matched parent and resistant cells were then screened for a panel of mutations. Cells lines were also screened for gene alterations using a human cancer drug resistance PCR array. Identified genes of interest were validated at the RNA and protein level by PCR and Western blot, respectively.

Results: All four cell lines exhibited a dose-dependent decrease in proliferation when treated with GDC-0980. H1975 cells (adenocarcinoma; PIK3CA mutant) were most sensitive to GDC-0980, however they developed resistance to the drug more rapidly than the other 3 cell lines. Results from mutational analysis and investigation of the gene and protein expression of each of the 4 pairs of parent and resistant cell lines will be presented.

Conclusion: While the panel of four NSCLC cell lines all responded well to GDC-0980 treatment initially, resistance to the drug developed rapidly. As such, understanding the mechanisms involved in the development of resistance to this drug will be crucial so that we may design optimal treatment strategies. Specific conclusions regarding the mechanisms of resistance in this panel of cell lines will be drawn based on identified genes and proteins of interest.

Keywords: GDC-0980, PI3K, resistance, NSCLC

PRECLINICAL THERAPEUTIC MODELS II
WEDNESDAY, OCTOBER 30, 2013 - 10:30-12:00

MO20.04 DIVERGENT ACTIVITY OF AFATINIB (AFAT) AND CETUXIMAB (CET) IN PATIENT-DERIVED XENOGRAFT (PDX) MODELS OF ACQUIRED ERLOTINIB RESISTANCE.

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Background: The combination of AFAT and CET has demonstrated remarkable clinical activity in patients with acquired resistance to erlotinib. Preclinical modeling in genetically engineered mice and cell lines predicted activity in cases where erlotinib resistance was mediated by the EGFR T790M gatekeeper mutation. However, in the clinic, patients lacking T790M-positive tumors showed equivalent benefit from this combination, suggesting alternative mechanisms of synergy. We explored the individual and combined molecular and growth inhibitory activity of these agents in PDX models derived from NSCLC patient tumors with distinct mechanisms of acquired resistance to erlotinib. These models were developed by the UC Davis - Jackson Laboratories Consortium, which has xenotransplanted over 170 NSCLC models using the nod/scid/IL2Rgamma chain-null (NSG) mouse.

Methods: EGFR-mutant PDX models LG0703 (T790M-negative) and LG1049 (T790M-positive) were established from tumor biopsies from patients who progressed following durable responses to erlotinib. Both patients were subsequently treated with AFAT+CET, with the LG0703 donor patient exhibiting a prolonged response and the LG1049 donor patient exhibiting a transient response followed by rapid progression. Excised tumors from passage 1 PDXs were fragmented and implanted into treatment cohorts. When tumors reached 300mm^3, mice were randomized to erlotinib (50 mg/kg qd po), AFAT (20 mg/kg qd po), CET (10 mg/kg twice weekly iv), AFAT-CET, or vehicle control (n per arm = 12) for 3 weeks followed by a 75-day monitoring period. In a parallel cohort, tumor pharmacodynamic changes in signal transduction mediators and RTKs were assessed after 6 and 24h treatment exposures using kinase arrays (R&D systems) and immunoblotting.

Results: In LG0703, AFAT, CET and AFAT-CET resulted in complete tumor response (CR) during the 21-day treatment period. After cessation of treatment, mice treated with CET or AFAT-CET remained in complete remission; whereas AFAT-treated mice progressed within 2 weeks. Clinical activity in this model was associated with