Biomarkers

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Prediction and in vivo validation of AZD0530 sensitivity by gene expression profiling in human pancreatic tumor xenografts

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Background: Pancreatic adenocarcinoma, one of the most lethal human cancers, has been reported to exhibit Src overexpression (70%) and/or increased Src activity. AZD0530 is a Src inhibitor in clinical development for the treatment of several cancers. We evaluated the efficacy and pharmacodynamic effect of AZD0530 in a panel of patient-derived pancreatic tumors, and prospectively validated AZD0530 sensitivity in another set of patient tumors as predicted by the KTSP (kTop Scoring Pair) Classifier.
Methods: Surgical, non-diagnostic pancreatic tumors operatively removed from patients (Johns Hopkins Hospital, Baltimore, MD) were subcutaneously implanted in pathogen-free female nude mice and kept as a live PancXenoBank according to an IRB-approved protocol. Twenty-one patient tumors (16 training set + 5 validation set) in the PancXenoBank were further expanded and cohort tumors in 5−6 mice (10 evaluable tumors) were randomized to vehicle or AZD0530 (50 mg/kg od po) for 28 days. Tumor size was measured twice per week and relative tumor growth index was calculated versus control mice. Src- and pathway-related genes were analyzed by RT-PCR, Western blot, immunohistochemistry, and Affymetrix U133 Plus 2.0 gene arrays. We used KTSP, a novel and innovative rank-based comparison method, to identify a small number of gene pairs from the training set to predict AZD0530 sensitivity in the separate validation set.
Results: RT-PCR, Western blot and immunohistochemistry confirmed that Src was highly expressed in the pancreatic tumors at baseline. Three patient tumors (Panc 291, 410, 420) out of sixteen were found to be sensitive to AZD0530, defined as tumor growth <50% than that of control tumors (100%). The KTSP Classifier identified one gene pair (LRRC19 and IGFBP2) from the training cases with an estimated leave-one-out cross-validation accuracy of 97.8%. The KTSP decision rule is: if LRRC19 has higher expression than IGFBP2, then the tumor is predicted as sensitive to AZD0530, if not it is predicted as resistant. The classifier achieved 100% accuracy in predicting two sensitive (Panc194 and JH131) and three resistant (Panc294, JH010 and JH069) tumors in the independent validation set.
Conclusion: AZD0530 inhibits tumor growth in a subset of human pancreatic xenograft tumors. The KTSP Classifier has high predictive power for AZD0530 sensitivity and potentially can be used as biomarkers for predicting pancreatic tumor sensitivity to AZD0530 in the clinic.

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Tyrosine kinase inhibitors, such as TAK-285, GW572016 or SU11248, protect or damage the heart based on their ability to activate AMPK

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Background: Tyrosine kinase inhibitors (TKI) can elicit secondary effects including heart toxicity. GW572016, an EGFR/ErbB2 inhibitor, has shown low cardiotoxicity in *in vitro* studies and clinical trials, likely due to its ability to activate the protective AMPK pathway. Conversely, kinome analysis has predicted that SU11248 may inhibit the AMPK pathway. This prediction correlates with SU11248-treated patients exhibiting an elevated risk of cardiotoxic symptoms. This report compares TAK-285 to GW572016 and SU11248 in their influence on the AMPK pathway. We have found that TAK-285 activates the AMPK pathway in cardiac cells providing cardiac protection in the presence of ErbB-targeted therapy.
Materials and Methods: Human cardio myocytes (HCM) were treated with 25 μM TAK-285 (Takeda), GW2974 (Sigma) (a GW572016 derivative) or SU11248 (LC Laboratories) for 1 hour for protein analysis or with 5.0 μM TAK-285 or SU11248 for 72 hours for lipid staining. Sprague Dawley rats were treated with 100 mg/kg of TAK-285, GW572016 (LC Laboratories), SU11248 or vehicle for 8 hours. Rat hearts were harvested and used for lipid staining and protein analysis.
Results: TAK-285 and GW2974 treatment of HCMs resulted in AMPK activation shown by increased p-ACC as well as activation of the survival factor NF-kB. Interestingly, SU11248 treatment of HCMs showed no activation of AMPK. Furthermore, both TAK-285 and GW2974 treatment of HCMs demonstrated a reduction in lipids where SU11248 treatment showed massive lipid accumulation. While treatment of rats with TAK-285 or GW572016 showed no cardiac lipid accumulation, SU11248 treated rat hearts demonstrated significant lipid accumulation. Western analysis of rat hearts also showed that TAK-285 and GW2974 activated AMPK while SU11248 treatment failed to do so.
Conclusions: TAK-285 dual EGFR/ErbB2 inhibitor demonstrated in vitro and in vivo activation of cardiac protection mechanisms whereas SU11248 treatment showed lipid accumulation and a lack of AMPK activation. As it has been suggested that SU11248 inhibits AMPK, kinome analysis of TAK-285 showed no predicted interaction with the AMPK pathway, leading us to believe that TAK-285 treatment of EGFR- or ErbB2-driven cancers will not exhibit elevated cardiac risks. From these studies, we strongly believe that new and existing TKIs should be tested for their effects on the AMPK pathway in cardiac cells to determining possible cardiac risks involved with treatment.

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Development of a gene signature predicting response to Cetuximab in human tumor xenograft models

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Background: Cetuximab, an AB against the EGFR, was recently approved in combination with chemotherapy in metastatic colon cancer and with radiotherapy in head and neck cancer. We investigated the hypothesis that correlating drug response of patient-derived human xenograft models with their gene expression profiles would identify specific gene signatures which predict the drug response of individual tumors.
Methods: For 220 patient-derived solid tumor models growing sc in nude mice, expression profiles of ~38k genes were determined using the Affymetrix HG-U133 plus 2.0 chip. In a previous study, we have demonstrated for approximately 80 tumors that in >90% of cases the drug response of a patient tumor explant passaged in mice matched the response of the corresponding patient tumor to the same drug. Here, nude mice bearing xenografts of 90 different tumors received Cetuximab at 30 mg/kg ip once weekly for 3 consecutive weeks.
Results: For Cetuximab, using a minimum T/C of 35% as cut-off for efficacy, 17% (15/90) of all tumors were rated as sensitive, including 7/32 NSCLC, 4/20 colon, 3/5 head and neck, 1/3 pancreas, and 1/6 gastric cancers. 13 tumors were highly responsive with T/C values <25%, and most of them went into a remission. At minimum T/C values between 25 and 35%, the 2 remaining responders displayed a reduced growth rate compared with tumors in vehicle control mice. The search for a gene signature predictive for Cetuximab response, using bioinformatic tools, was restricted to tumor types where Cetuximab has an established role in the clinic (colon, head & neck, NSCLC, total of 54 tumors). An optimal signature consisting of 21 genes was identified. The gene signature was validated using the leave-one-out cross-validation (LOOCV). Using LOOCV, 18/54 tumors were predicted to respond. In the real testings 11 out of the 18 predicted responders did indeed respond. This corresponds to a response rate of 61% as compared to 24% for random testing. From the predicted 36 non-responders 34 (94%) were resistant in real testings.
Conclusions: A specific gene signature comprising 21 genes and predicting response to Cetuximab in patient-derived tumor models in nude mice was identified. This signature is now being tested, using fresh tumor samples from patients with colon, head & neck, pancreas cancer and NSCLC and as negative control also in other tumor types like breast cancers.

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Inhibition of MEK1/2 signalling results in decreased levels of intracellular lactate in human melanoma and colorectal cancer cells as observed with magnetic resonance spectroscopy

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Background: To meet the energy demands of the tumour micro-environment, cancer cells exhibit an increased rate of aerobic glycolysis which results in intracellular lactate accumulation, also known as the Warburg effect.
MEK1/2 (MEK), a member of the mitogen activated protein kinase (MAPK) signalling pathway, is often deregulated in cancer and represents a significant target for molecularly targeted drugs with inhibitors currently in clinical development. Our aim was to investigate whether MEK signalling inhibition could lead to magnetic resonance spectroscopic (MRS)