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)

detectable changes in glycolysis that could serve as biomarkers of target suppression in human cancer cells.
Methods: We used the MEK inhibitor CI-1040 and three human cancer cell lines, HCT116 (colorectal), SKMEL-28 and WM 266.4 (melanoma). Cells were treated with 20 μM of CI-1040 for 16 h and 24 h (HCT116) or with 1 μM of CI-1040 for 24 h (SKMEL-28 and WM 266.4). Post-treatment, 10^7-10^8 cells were extracted with dual phase extraction and ^{1}H MRS spectra of the water soluble metabolites were acquired on an 11.7 T Bruker Avance spectrometer. In addition, cell culture media from 16 h treatments with HCT116 cells were analysed.
Levels of P-ERK and downstream targets cyclin D1 and pRB, were evaluated with Western blotting to confirm successful MEK signalling inhibition in response to treatment.
Results: Exposure to CI-1040 led to a marked decrease of ERK phosphorylation as well as levels of cyclin D1 and pRB as shown by Western blotting. Post-treatment, ^{1}H MRS data revealed decreased intracellular lactate levels to 44±10% at 16 h ($n = 3$, $P = 0.01$) and 62±14% at 24 h ($n = 2$) in HCT116 cells. Intracellular lactate levels decreased to 45±18% in SKMEL-28 cells and to 45±2% in WM 266.4 cells at 24 h post-treatment ($n = 2$). Extracellular lactate levels were unchanged 16 h post-treatment in HCT116 cells.
Conclusion: These results demonstrate that MEK inhibition leads to modulation of glycolysis in human colorectal cancer and melanoma cells. Our findings need to be evaluated further in vivo and by the use of additional MEK inhibitors, nevertheless they suggest a role for lactate as a potential non-invasive MRS biomarker of response to MEK targeted therapeutics.
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Preclinical pharmacodynamic markers of MGCD265, a potent orally active c-Met/VEGFR multitargeted kinase inhibitor in Phase I clinical trials

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Background: MGCD265 is a novel multitargeted receptor tyrosine kinase inhibitor in Phase I clinical trials. MGCD265 inhibits the activation of key regulators of cancer development and progression: the receptor tyrosine kinases c-Met, all three members of the VEGFR family, Tie-2 and Ron. This unique profile distinguishes MGCD265 from other agents that target c-Met or VEGFRs alone. Preclinical evaluations demonstrate that MGCD265 abrogates c-Met and VEGFR-mediated cell-based responses with nanomolar potencies and displays potent anti-tumor activities in multiple human xenograft models. In support of the clinical development of MGCD265, we have analyzed pharmacodynamic markers in tumor tissues and plasma from human xenograft mouse models.
Methods: Pharmacodynamic markers in xenograft tumor tissues were analyzed by immunohistochemistry and western blotting. Plasma markers were assessed by ELISA.
Results: We demonstrate that in correlation with potent tumor growth inhibition, MGCD265 abolishes target enzyme phosphorylation and the activation of downstream signaling pathways in xenograft tumor tissues. These occur with a concomitant reduction in the mitotic index and an increase in the level apoptotic markers. Moreover, it has been suggested that increased tumor malignancy correlates with an increase in the plasma level of proteolytically cleaved c-Met receptor (shed-c-Met). Consistent with this notion, we demonstrate a diminution in the level of circulating c-Met following MGCD265 treatment in mice bearing xenografts, in parallel with tumor shrinkage. Furthermore, the plasma levels of the angiogenic factors, VEGF and HGF, that are regulated by the targets, and that have been linked to poor patient prognosis, are also reduced in MGCD265 treated animals.
Conclusion: Thus, as revealed in preclinical models, monitoring MGCD265-mediated target enzyme inhibition in tumor tissues, and assessing levels of circulating angiogenic factors that are target-regulated, may be used as exploratory markers in Phase I clinical trials of MGCD265.

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Prevalence of G12R or Q61H K-Ras mutations in pancreas cancer and development of Ras-targeted immunotherapy

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Background: Aberrant constitutive signaling by Ras oncoproteins with mutations at codons 12, 13 and 61 drive uncontrolled cell proliferation and tumorigenesis. We have developed targeted immunotherapy to specifically eliminate tumor cells expressing mutated Ras and are genotyping pancreas cancer for the incidence of specific ras point mutations.
GI-4000 tarmogens for immunotherapy with whole, heat-killed yeast expressing mutated Ras proteins were originally a three product series targeting tumors expressing ras codon 12 mutations encoding G12V (GI-4014), G12C (GI-4015) or G12D (GI-4016). Each of the original products also targeted Q61R and Q61L mutations. In preclinical studies, the original GI-4000 tarmogens stimulated mutation-specific ablation of tumor cells when the Ras antigen mutation expressed in yeast matched that found in the tumor. Ras mutation-specific cellular immune responses were shown in 90% of subjects from a Phase 1 study of GI-4000 in colorectal and pancreas cancer.
Experimental procedures: Tissue was obtained by surgical resection of tumors from 124 US and India subjects with Stage I and II pancreas cancer screened for enrollment in a placebo-controlled Phase 2 trial of adjuvant gemcitabine chemotherapy plus immunotherapy with GI-4000 tarmogens. Tumors were genotyped for K-, N- and H-ras DNA sequences by nested PCR amplification including peptide-nucleic acid oligomer clamping, followed by DNA sequencing. The GI-4020 tarmogen was engineered to express G12R- and Q61H-mutated Ras protein. CD8 T cells from mice immunized with GI-4020 or control yeast were mixed with tumors expressing G12R- or Q61H-mutated Ras, then implanted and monitored for control of tumor growth.
Results: Tumor genotyping for the Phase 2 study revealed that 83% of pancreas cancers had K-Ras mutations, predominantly G12V or G12D. However, 19% of these harbored G12R or Q61H mutations, where 5−6% of tumors harbored the Q61H mutation, which was at much greater frequency than previously documented. Preclinical studies demonstrated that administering GI-4020, which targets these mutations, specifically activated T cells against tumors with G12R- or Q61H-mutated Ras.
Conclusions: G12R- or Q61H-mutated Ras is found in pancreas cancer with much higher incidence than previously reported. GI-4020 immunotherapy targets these 2 mutations and is being tested in the ongoing Phase 2 trial. The current four GI-4000 products now cover 92% of mutated Ras-bearing pancreas cancers.

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A robust and quantitative biomarker assay for SB939, a potent, orally-active HDAC inhibitor

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Background: Histone deacetylase inhibitors (HDACi) are therapeutic agents, which induce tumor cell cytostasis, differentiation and apoptosis. SB939 is a pan-HDACi with superior pharmaceutical and pharmacokinetic properties that is currently in Phase I trials. We have developed a sensitive and quantitative Western blot assay for acetylated histone 3 (AcH3) as a pharmacodynamic (PD) readout for the target efficacy of SB939.
Material and Methods: The assay was validated by determining the effects of ex-vivo treatment with SB939 on human tumor cell lines as well as on human peripheral blood mononuclear cells (PBMCs) from healthy donors. In addition, AcH3 was determined in normal as well as tumor tissues after oral treatment of tumor bearing mice with SB939. The doses and schedules used corresponded to those in the ongoing Phase I trials where this assay is currently being employed to assess target efficacy on patient PBMCs.
Results: AcH3 could be detected after ex-vivo treatment of tumor cell lines or PBMCs with SB939 for 24 h. The lowest concentration of SB939 yielding a detectable signal was 60 nM for RAMOS cells and 125 nM (44 ng/ml) for PBMCs. The minimum amount of protein needed to detect AcH3 in cells was 1.56 μg. In the animal studies orally dosed SB939 led to a strong induction of AcH3 only in tumor tissue with no basal level detected in other tissues. In all normal tissues tested, the induction of AcH3 was less than in the tumor tissues indicating selectivity of SB939 for tumor tissue. In the clinical samples assessed, a dose-dependent AcH3 response was detected with the strongest signal observed 3 h post-dose. Comparison of