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The proteomic classifier VeriStrat® identifies advanced non small cell lung cancer (NSCLC) patients gaining clinical benefit from treatment with first line sorafenib and erlotinib

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Introduction: We recently reported the result of a phase II study of first line erlotinib and sorafenib in unselected patients with advanced NSCLC (Lind et al Clin. Cancer Res 16,3078,2010). In an attempt to find biomarkers predictive for overall survival we analyzed pretreatment derived serum samples for proteomic signatures in the previously described serum matrix-assisted laser desorption ionization proteomic classifier (VeriStrat). 50 patients were enrolled in the phase II study of which 49 pretreatment serum samples could be analyzed.
Methods: Overall survival (OS) data was analyzed using conventional survival analysis methods. Univariate analysis was performed using the Mantel-Haenszel method and log-rank p values (with PRISM) and the Cox proportional hazards method, withassociated p values (SAS). Confidence intervals for median survival estimates were calculated using SAS.
Results: VeriStrat classification identified 33 (67%) as predicted to have "good" and 15 (31%) predicted to have "poor" outcomes; 1 sample was of indeterminate classification VeriStrat classification was not predictive of disease control rate nor of EGFR mutation (n = 7) status in this cohort. VeriStrat good patients had statistically significantly improved OS compared with VeriStrat poor patients. The hazard ratio (HR) between groups was 0.30 (95% CI: 0.12–0.74), log-rank p = 0.009. The median OS was 13.7 months (95% CI: 12.0 months-undefined) for VeriStrat good patients and 5.6 months (95% CI: 1.6−7.6 months) for VeriStrat poor patients. In addition, center and histology (adenocarcinoma vs squamous and large cell carcinoma) were statistically significant in univariate analysis. For analysis by histology, the HR was 2.81 (95% CI: 1.05–7.51), with median OS of 12.4 months (95% CI: 6.3 months − undefined) for adenocarcinoma patients and 4.7 months (95% CI: 1.6–10.9 months) for squamous and large cell carcinoma patients. Stratifying by histology, only adenocarcinoma patients have sufficient numbers to attempt further analysis. For VeriStrat good vs poor the HR is 0.45 and log-rank p value = 0.21; however this result has limited value due to only 7 of the 34 adenocarcinoma patients being classified as VeriStrat poor.
Conclusion: The proteomic classifier VeriStrat may identify a subset of patients that derives significant clinical benefit from upfront treatment of advanced NSCLC patients with EGFR/VEGF inhibitors. These findings should be confirmed in prospective studies.

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The potential to personalize targeted therapy shown by in vitro kinase activity profiling in pancreatic xenograft models

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Background: Protein tyrosine kinase inhibitors (PTKIs) have in recent years emerged as beneficial treatment options for various cancers, also holding promise for pancreatic cancer. Often however, only subcategories of patients respond to treatment, notably those harboring specific mutations or depending on low or high expression of specific kinases. Time is a major limiting factor in the treatment options for pancreatic cancer since it is frequently diagnosed at a late stage. Therefore it would be advantageous to develop biomarkers that could predict tumour response to PTKIs. Xenograft tumours retain the most important biological features of the tumour of origin and can be highly predictive of the efficacy of chemotherapeutic drugs with clinical activity in humans. The aim of the current study is to differentiate PTKI responses among pancreatic xenografts by an *in vitro* phosphorylation-based kinase activity profiling assay.
Methods: The porous flow-through PamChip arrays of the PamGene platform comprise of 144 tyrosine-containing peptides derived from known human kinase phosphorylation sites. Kinase activity profiles measured by substrate peptide phosphorylation in real time, were generated for 13 pancreatic xenograft protein lysates. Select lysates were treated *in vitro* with erlotinib, dasatinib, sunitinib and sorafenib and data compared to vehicle-treated controls.
Results: A clear kinase activity profile was determined for all the xenograft lysates, with no differential kinase activity evident in the absence of PTKIs. Dasatinib showed the highest level of phosphorylation inhibition, including Src and ephrin-related peptide substrates. Erlotinib treatment resulted in differential response among the lysates, of which relative insensitivity correlated to high expression of p-AKT. We also show that sunitinib response correlated with VEGF expression level in the xenografts. We determined the *in vitro* response profile for the pancreatic xenografts and currently studies are underway to correlate and validate these signature profiles with *in vivo* efficacy studies in the xenograft mouse model.
Conclusions: Kinase activity profiles of pancreatic xenograft tumours exposed to various kinase inhibitors could identify differences in sensitivity to different PTKIs. The results could potentially be extrapolated to *ex vivo* kinase profiles in patient tumours in response to treatment with kinase inhibitors. The method paves the way to enable prediction of treatment outcome individually, without extensive analysis of mutation status and other biomarker information.

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Imaging heterogeneity of hypoxia: spatial and temporal

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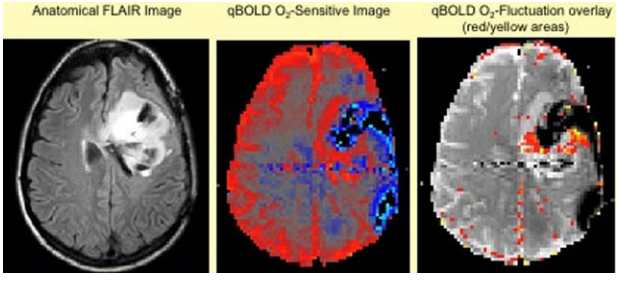
Introduction: Reduction in PO2 reduces cell viability in healthy tissues if it is acute, severe and prolonged. In tumors, hypoxia evolves more slowly as the cancer cells outgrow their blood supply. Thus, cancer cells adapt to low PO2 through genetic changes that promote survival in the hypoxic environment. Because hypoxia is heterogeneous within a tumor mass and between sites in a patient, imaging methods have advantages over sampled biomarkers. Our group developed PET imaging with ^{18}F-misonidazole, (FMISO) and has tested hypoxia imaging as a predictor of outcome in head & neck cancer, sarcoma and brain tumors. In all of these situations, FMISO-PET provided an independent predictor of outcome. Temporal variation in tissue hypoxia when acute hypoxia is superimposed on a background of chronic hypoxia deserves investigation; test-retest FMISO studies are not useful for this purpose.
Materials and Methods: We used an MRI protocol to measure tissue hypoxia variation over relatively short times using the blood oxygen level dependent sequence (BOLD) that was developed to image regional changes in oxygenation during cerebral activation. The signal changes rapidly in response to metabolism so imaging can be repeated every ~5 min to probe the extent to which acute fluctuations in oxygenation add to the stress of chronic hypoxia. The BOLD sequence consisted of TR/TE 100/36 msec, 95 gradient echos with the 5th echo positioned at the peak of the SE over the tumor. Each of the echos was reconstructed as a separate volume. Scan duration was 194 sec/2 averages; repeat 6×. A measure of BOLD fluctuation was calculated from a time series of images to measure biological variation of oxygenation. The std dev was calculated on a pixel basis.
Results: Most of the brain had very low levels of FMISO uptake (normoxic regions) and minimal fluctuations but a small number of voxels around the tumor's edge showed high fluctuations as evident in the figure.



The image shows the ability of BOLD MRI to monitor fluctuations in PO_2 over the course of an hour; the BOLD signal in uninvolved brain remained constant.
Conclusion: This technique allows testing whether temporal heterogeneity in tissue hypoxia is predictive of time to tumor progression and overall survival and whether this finding is independent of the FMISO-PET result. Acute hypoxia followed by reoxygenation may lead to high levels of reactive oxygen species that eventually increase metastasis. Thus temporal variations in regional oxygenation represent an important imaging objective.
Supported by NCI P01 CA42045−21.