PamGene's Kinase activity profiling of tumor tissues of different origin

Introduction:

Many new anti-cancer drugs target kinase activity. Unfortunately, methods to monitor the drug effects at the enzymatic level in patient derived tumor tissue are limited. Here, a novel molecular profiling method for application in biomarker discovery is presented that is based on measuring kinase activities in tumor tissue extracts. An *ex vivo* activity-based approach which involves assessment of inhibition by a drug of interest, is illustrated for leukemia, locally advanced rectal cancer, lung cancer and melanoma tumors. The discovery of prognostic and predictive biomarkers is illustrated with case studies of LARC and breast and lung cancer. Here we present the applicability of this approach for biomarker discovery in multiple tumors of different origin.

Technology:

All these studies are enabled by dynamic peptide PamChip® microarrays comprising of peptides, which are known substrates for phosphorylation by protein kinases. A Pamstation®12 enables 12 microarray incubations per run and the PamChip96 plate format enables 96 per run. The PamChip® disposable consists of 4 identical arrays, each array containing up to 144 peptides immobilized on a porous ceramic membrane. The 13 amino acid peptide sequences harbor phosphorylation sites derived from literature or computational predictions and are correlated with one or multiple upstream kinases, allowing for multiplex measurements. Fluorescently labelled anti-phospho antibodies are used to detect phosphorylation activity of kinases present in the assay sample. During the assay, the sample solution is pumped up and down the porous membrane allowing for faster kinetics and real-time measurements. When the solution is underneath the array, the CCD camera in the workstation takes an image of each array which are later used by the BioNavigator® software to generate kinetic data curves of each peptide. The data workflow consisting of image quantification, quality control, statistical analysis, visualization and interpretation is performed using the BioNavigator® software.

Methods:

Patient-derived fresh frozen tissues of different tumor types (xenograft tissue for the breast cancer example) are used for extraction of total protein in lysis buffer. Equal amounts of total protein are analyzed for kinase activity on dynamic PamChip peptide microarrays. Protein amounts ranging from 1 - 5 µg are used per microarray analysis. Tumor extracts are analysed in the presence and absence of kinase inhibitor drugs added *ex vivo* in the assay (E.g.: Leukemia, AKN-028; LARC, sunitinib; NSCLC, gefitinib; Melanoma, vemurafenib) or *in vivo* in xenograft models (E.g.: Breast cancer bevacizumab and/ or doxorubicin)

Results:

We show here that from different tumors ATP-dependent kinase activity profiles could be generated. In addition, inhibitor dependent modulation of the peptide phosphorylations can be observed in all case studies described. In the leukemia study, concentration dependent inhibition is shown. Furthermore, differential kinase activity profiles were obtained that could be correlated to different patient subgroups. For example, in the LARC example, sunitinib inhibition profiles could classify tumors into DTC (- or +) phenotypes. In Lung cancer, correlation to clinical data (long vs short term survival) prediction is clearly evident. In the melanoma study, vemurafenib inhibition profiles could distinguish BRAF v600E mutant samples from wild-type. The breast cancer study shows potential of combination therapy predictions in clinical samples.

Conclusion:

A novel molecular profiling approach was successfully applied for classification of different tumors. This approach is based on detection of kinase activities as well as inhibition of kinase activity in tumor tissues. Application of this method in the discovery of biomarkers for diagnosis, prognosis or prediction of drug response is foreseen.

Information:

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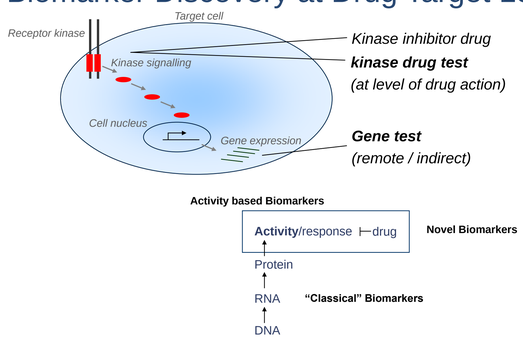
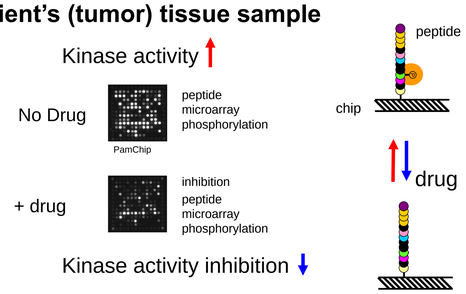
Biomarker Discovery at Drug Target Level

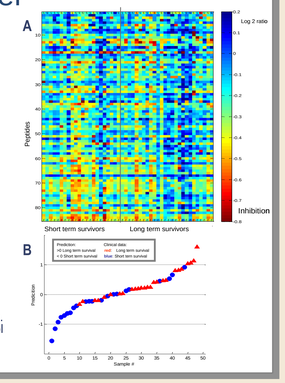


Drug testing on tissue derived kinases



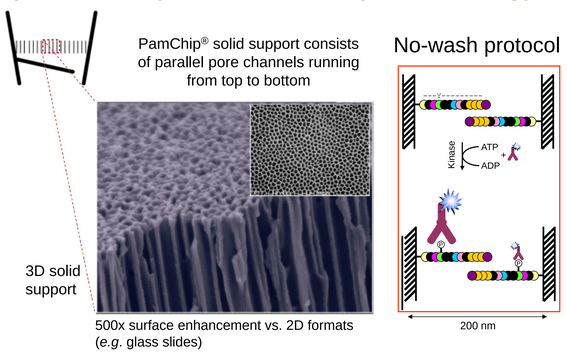
Lung Cancer

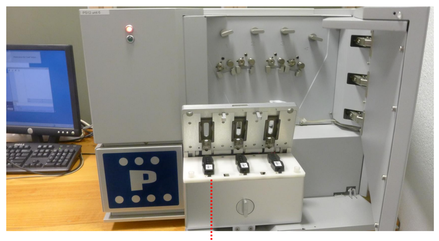
Early stage NSCLC tumour lysates from a group of short term survivors vs. long term survivors (< 24 months post diagnosis (n=22) and > 48 months (n=26)) were tested in the presence and absence of the PTK inhibitor gefitinib on PamChip® arrays. (Inhibition ratios (A) could classify samples and predict survival (B) using a PLS-DA class prediction.



Ruijtenbeek R. et al., J. Clin. Oncol. 28:7s, (2010) (suppl abstr 10642)

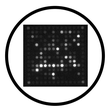
Dynamic Peptide Microarray Technology





PamStation® 12

PamChip® 4



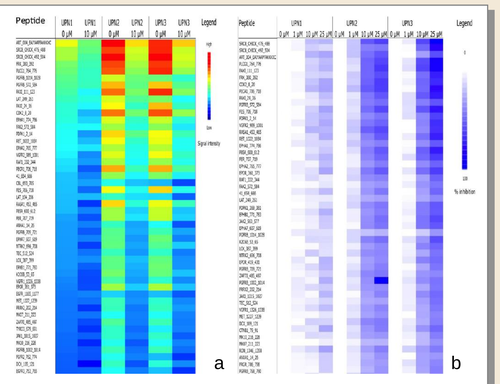
1 array per well

Leukemia

3 AML primary patient samples showing

(a) Tyrosine kinase activity profiles with AKN-028 (10µM) or vehicle (DMSO)

(b) Inhibition ratios with increasing concentrations of AKN-028 (added *ex vivo* in assay)



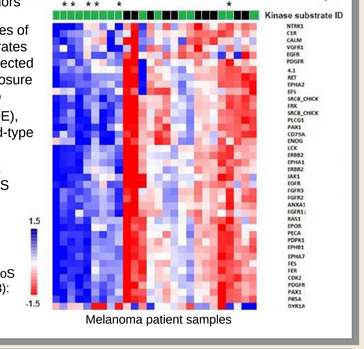
Eriksson A *et al.* Biochem. Pharmacol. (2014) 87: 284-91

Melanoma

Supervised clustering of BRAF(V600E) and BRAF wild-type melanoma tumors

Inhibition profiles of 40 PTK substrates significantly affected by *ex-vivo* exposure to vemurafenib

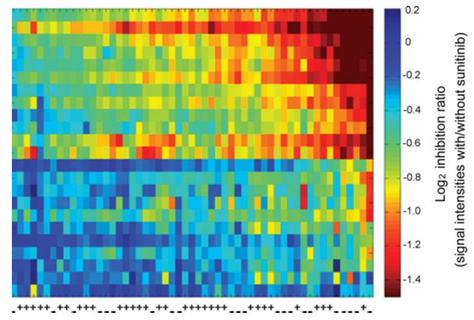
- BRAF(V600E), (black); Wild-type (green)
- (*) samples harbor NRAS mutations.



Tahiri A *et al.* PLoS ONE. (2013) 8(8): e72692

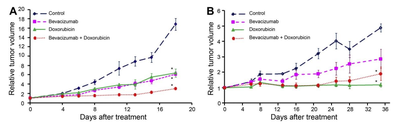
Locally Advanced Rectal Cancer (LARC)

Ex vivo sunitinib inhibition profiles from 23 angiogenesis-related kinase substrates (rows). 55 patient tumor samples (columns) annotated by (-) or (+) status for disseminated tumor cells (DTC) to bone marrow. Inhibition is higher is DTC(-) patient samples.



Saelen MG *et al.* Angiogenesis (2011) 14: 481-9

Breast Cancer

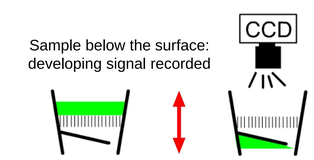


The *in vivo* inhibition by the combination of bevacizumab and doxorubicin is higher than monotherapy in (A) basal-like breast cancer but not in (B) luminal-like breast cancer xenografts. (C) Kinase activity profiles of basal-like xenografts show higher inhibition of the combination.

Lindholm EM *et al.* Mol. oncol. (2012) 6: 418-27

Kinetic Readout

Sample below the surface: developing signal recorded



repeated cycling of the sample through the array enables diffusion-independent binding kinetics