Kinase activity profiling combined with genotyping as a tool for predictive biomarker discovery for the treatment of gastroesophageal adenocarcinoma (GEC)

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Aim

Profiling cells from malignant ascites fluid in patients with gastrointestinal cancer.

Molecularly targeted therapy holds promise for the treatment of GEC, but interpatient molecular heterogeneity has proven to be a hurdle towards their successful implementation. Here we describe a new approach towards patient selection where tumor genotyping is combined with kinase activity profiling. Kinases are the main targets for the new precision drugs being developed currently. The possibility of *ex vivo* testing of kinase inhibitors is a useful fapplication of the PamChip kinase assay, which may be predictive for treatment. This profiling was used to determine i) if there are discriminatory profiles that identify tumor cells with various known genomic drivers; ii) if specific inhibitors abrogated these activity profiles selectively; iii) if patient samples (including malignant ascites fluid, cultured patient cells human tumor xenografts, and/or frozen samples directly from patients) were amenable to this analysis with reproducible results.

Methods

Nine GEC cell lines/explanted tumors were genotyped (cMET, kRAS, PI3K, HER2, FGFR2, EGFR) and evaluated for kinase activities by analyzing cell lysates using dynamic peptide microarrays (PamChip assay).

Cells or tissue cryosections were lysed in buffer with phosphatase and protease inhibitors. Protein tyrosine kinase (PTK) activity profiles were generated on PamChip® peptide microarrays comprising peptidic substrates derived from known human phosphorylation sites.

The direct enzymatic effect of the kinase inhibitors crizotinib, affatinib and a dual cMET/RON kinase inhibitor was tested by directly spiking these compounds (at 0.2, 2.0 and 20 uM) into the lysate just before analysis and comparing the results to DMSO control. Peptide phosphorylation was monitored using fluorescently labeled antibodies and quantified with Bionavigator software.

PamChip® Tissue-Based Kinase Activity Assay

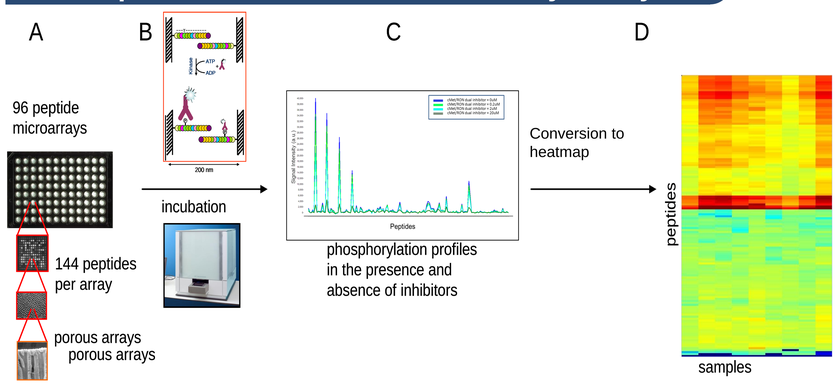
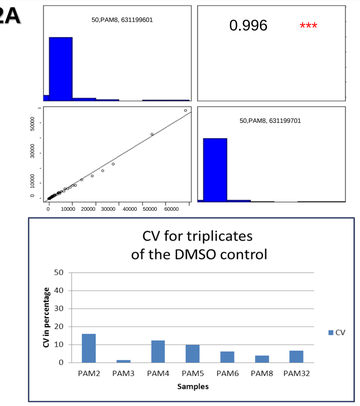


Fig 1. The PamChip® peptide microarray is based on a porous material spotted with 144 peptides derived from known phosphorylation sites in human proteins (A). Peptides inside the pores are phosphorylated by kinases in a cell or tissue lysate (B). Peptide phosphorylation is monitored in real time using an anti-phosphotyrosine antibody. The effect of inhibitors can be detected in situ by spiking inhibitor into the lysates. This results in changes in phosphorylation profile (C). Signals of technical replicates are averaged per array and per peptide. 2log values of these results are calculated. These values are converted in to colors. Blue indicating values of 1 and red values of 16 (D). Each row represents a peptide, each column a sample.

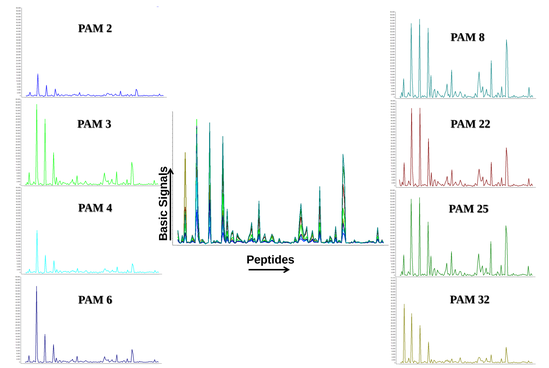
Reproducibility of the assay

2A

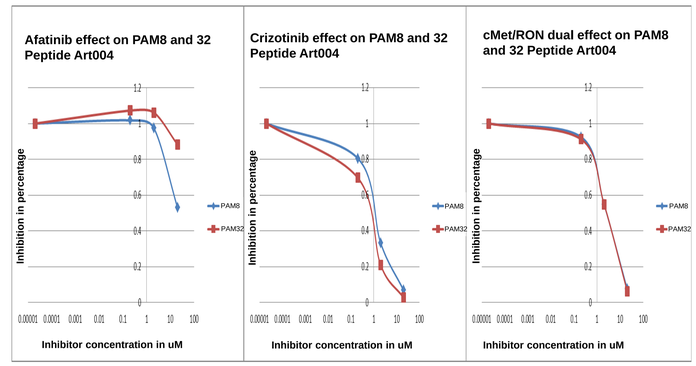


Inhibition curves and IC50 values for 3 inhibitors for Peptide Art004 (Abl)

2B



2C



Inhibition patterns for 3 inhibitors

2D

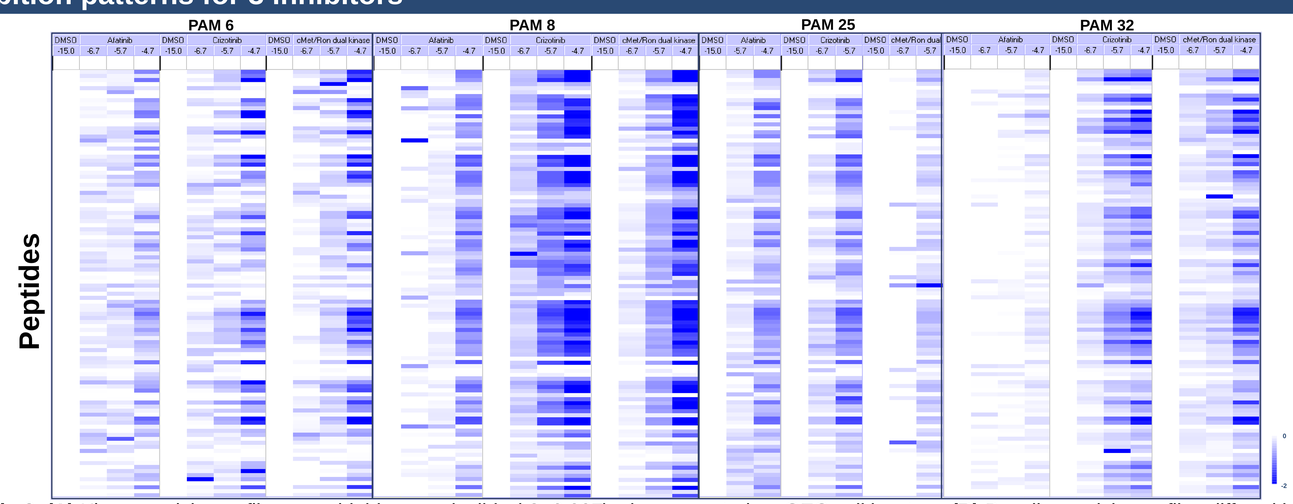


Fig 2. (A) Kinase activity profiles were highly reproducible (r2=0.997) when repeated on GEC cell lysates. **(B)** Baseline activity profiles differed between molecular categories within GEC, including HER2 amplification (amp+), MET amp+, PI3K mutated, FGFR2 amp+ and KRAS amp+/mutated subsets. **(C)** Lysates from tumors with known MET amp+ (PAM 8) or MET activating splice variant (PAM 32) were selectively sensitive to crizotinib and the MET/RON inhibitor, and much less to afatinib demonstrated with peptide 004 (**C)** and heatmap **(D).** Specific inhibitors towards the cMET or EGFR driver events decreased substrate phosphorylation (blue) concentration dependently.

Sample code	Amplification	Mutation status
PAM2	c-MET	CDH1, CDKN2A/B, TP53
PAM3	HER2, MET	TP53
PAM4	HER2	TP53, SMAD4
PAM6	KRAS	CDH1, CDKN2A/B, TP53 mt
PAM8	c-MET	CDKN2A/B, SMAD4
PAM25		KRAS, PIK3CA, CTNNB1, CDH1
PAM32		c-MET (del14)

Conclusions

Kinase activity profiling with the multiplex PamChip kinase assay can categorize cell lines and tumors into different molecular subsets. Treatment of lysates with specific kinase inhibitors and combining this functional proteomics information with genomics data, is a new strategy we propose to predict responses to molecularly targeted therapies.