Kinase activity based biomarkers: Identification of prognostic and erlotinib response prediction markers in NSCLC patients

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Abstract

Background

Reliable diagnostic tests are needed to identify early stage non-small cell lung carcinoma (NSCLC) patients with poor prognosis. Concomitantly there is a clear need for tests that enable the selection of patients who will benefit from targeted therapy with kinase inhibitors. We evaluated kinase activity profiles in two groups of early stage NSCLC patients, either for prognosis of long- or short-term survival, or for predicting of erlotinib drug response.

Method

Retrospective studies were performed on fresh frozen resection material of two groups of early stage NSCLC patients. The first group consisted of 48 short- and long-term survivors who underwent a complete surgical resection (5+ years follow-up). The second group consisted of 14 NSCLC patients who received 3 weeks of neo-adjuvant treatment with erlotinib prior to complete surgical resection. Response evaluation to neo-adjuvant treatment was based on histopathological examination of the surgical specimens. For both studies, kinase activity profiles of lysed cryosections of tumor tissues were generated in the presence and absence of protein tyrosine kinase inhibitors on PamChip® peptide micro-arrays, comprising 144 tyrosine containing peptides, derived from known phosphorylation sites of human proteins. Partial least square discriminant analysis was used to construct prediction models. ClustalW alignment algorithms were used to investigate the most informative phosphorylation sites.

Results

Kinase activity profiles obtained in the absence of inhibitor did not distinguish between subgroups (long- versus short-term survival, responder or non-responder to TKI), whereas ratios of inhibited versus non-inhibited signals resulted in distinct classifiers predicting survival for the first group, and response for the second group. Multivariate unsupervised analysis with leave-one-out cross-validation resulted in an error rate for survival prediction of 29%. In the drug response prediction 1 (responder) out of 14 patients was misclassified.

Conclusion

This is the first study to show that kinase activity profiles of tumor tissue exposed to a kinase inhibitor can be used to identify NSCLC patients likely to respond to erlotinib treatment. Furthermore, based on kinase activity profiles of early stage NSCLC tumors, a prognostic classifier, for a set of 48 patients, was obtained.

Identification of prognostic biomarkers

A retrospective study was performed on two groups of early stage NSCLC patients (stage IA-IIIA) who underwent a complete surgical resection. The groups consisted of short term survivors and long term survivors (survival duration from diagnosis < 24 months (n=22) and > 48 months (n=26). Follow-up was at least 5 years for all patients.

Table 1. Patients characteristics.

	Short term survivors			Long term survivors		
	Adeno	Squamous	Large Cell	Adeno	Squamous	Large Cell
Patient demographics						
Total number of	7 (6)	9 (8)	6 (6)	9 (3)	11 (1)	6 (0)
Age, median	68	65.6	63.4	55.9	67.0	67.5
Male	6 (5)	9 (8)	4 (4)	6 (3)	10 (1)	5 (0)
Female	1 (1)	0	2 (2)	3 (0)	1 (0)	1 (0)
Disease Free Su	8.1	9.0	6.0	81.3	65.1	77
Overal survival,	13.6	10.7	10.9	81.3	66.4	83
Primary tumor TNM stage						
T1N0M0				2 (1)	1 (0)	2 (0)
T1N1M0		1 (1)		1 (0)	1 (1)	1 (0)
T2N0M0	4 (4)	1 (0)	3 (3)	6 (2)	6 (0)	1 (0)
T2N1M0	2 (2)	3 (3)			2 (0)	2 (0)
T3N0M0		3 (3)	2 (2)			
T3N1M0		1 (1)	1 (1)		1 (0)	
T4N0M0	1 (0)					

Phosphorylation profiles obtained in the absence of gefitinib did not distinguish between patients with short survival and long survival, whereas ratios of inhibited vs. non inhibited signals resulted in a classifier able to predict survival. Interestingly, the inhibitory effects of gefitinib were stronger in patients with short survival times as compared to patients with long survival times (fig.1).

Multivariate unsupervised analysis (fig.2) with leave-one-out cross-validation resulted in the correct prediction of outcome for 33 out of 48 patients (71 %)

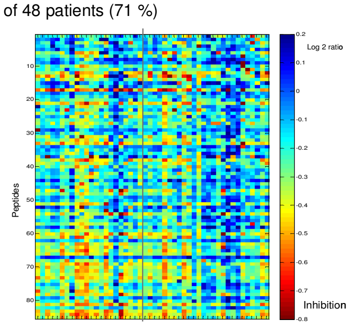

Fig. 1. Classification of samples based on kinase inhibition profiles obtained by spiking gefitinib into the tumor lysates. Each horizontal line represents a peptide, each column a sample. Right: PLS-DA class prediction for 22 short term survivors and 26 long term survivors based on leave one out cross validation. Each point represents a sample and is colored according to survival group. Samples with prediction < 0 are predicted to be short-term survivors, prediction > 0 are predicted to be long-term survivors.

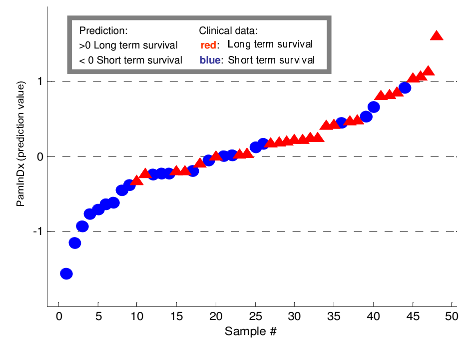

Fig 2. PLS-DA class prediction for 22 short term survivors and 26 long term survivors based on leave one out cross validation. Each point represents a sample and is colored according to survival group. Samples with prediction < 0 are predicted to be short-term survivors, prediction > 0 are predicted to be long-term survivors.

Identification of predictive biomarkers

Investigations were performed on 14 samples of early stage (stage 1A-IIIA) NSCLC patients included in the M06NEL study (fig.3). This is an open label, multicenter, non-comparative phase II study.

Table 2. Data for 14 (stage IA-IIIA) NSCLC patients included in the M06NEL study. Operable patients, 18 years and over, with resectable NSCLC (cT1-3, N0-1) were eligible for the study.

	Responders	Non-responders
Patients	6	8
Male	2	6
Female	4	2
Tumor Type		
Adenocarcinoma	5	7
Squamous	0	1
Large Cell	1	0
Smoking status		
Never smokers	2	1

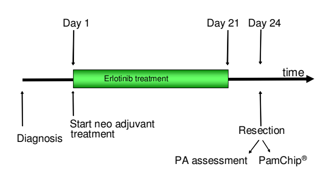

Fig. 3. Clinical study design. Preoperative treatment comprised of 150 mg erlotinib daily for a period of 3 weeks. Surgical resection involved a radical resection of the tumor. Response evaluation to neo-adjuvant treatment was performed by histopathological assessment of the surgical specimens according to Junker et al (3).

Ratios of inhibited vs. non inhibited signals, were used as input for multivariate unsupervised analysis with leave-one-out cross validation. This resulted in misclassification of 1 sample (responder). The results are represented as prediction values in figure 4.

The classification in patients with EGFR or KRAS mutations was coherent with mutation data (among 6 responders 1 showed EGFR ex19 mutation and among 8 non-responders 1 had EGFR ex 20 (9BP in-frame deletion) and 3 KRAS codon 12 mutations).

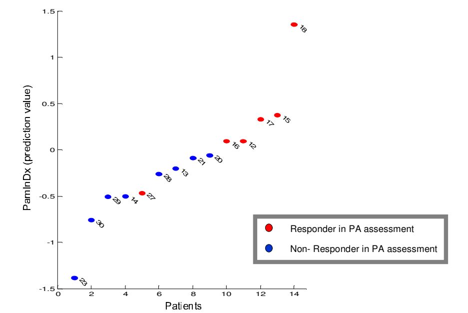

Fig. 4. PLS class prediction for 14 samples based on double Leave One Out Cross Validation.

PamChip® Cell-Based Kinase Assay

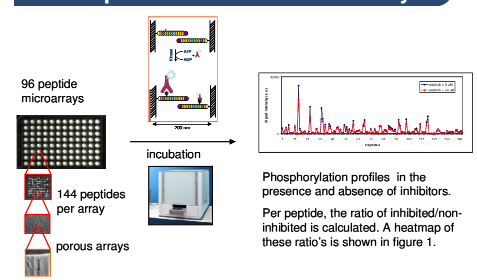

Fig.5. PamChip® peptide microarray technology (see references below).

References

1. Sigurd Folkvord, Kjersti Flatmark, Svein Dueland, Rik de Wijn, Krystyna Kotanska Grøholt, Jahn M. Nesland, Knut H. Hole, Piet J. Boender, Rob Ruijtenbeek, Marianne Johansen, Karl-Erik Giercksky, and Anne Hansen Ree, **Prediction of Response to Preoperative Chemoradiotherapy in Rectal Cancer by Multiplex Kinase Activity Profiling**. Int J Radiat Oncol Biol Phys. 2010 Oct 1;78(2):555-62
2. Maat W, el Filali M, Dirks-Mulder A, Luyten GP, Gruis NA, Desjardins L, Boender P, Jager MJ, van der Velden PA. **Episodic Src activation in uveal melanoma revealed by kinase activity profiling.**, Br J Cancer. 2009 Jul 21;101(2):312-9.
3. Versele M, Perera T, Rockx C, Lavrijssen T, Geerts T, Page M, King P, Göhlmann HW, Janssens B, Talloen W. **Response prediction to a multi-targeted kinase inhibitor in cancer cell lines and xenograft tumors using high-content tyrosine peptide arrays with a kinetic read-out.** Mol Cancer Ther. 2009 Jul;8(7):1846-55
4. Bratland A, Boender PJ, Høifødt HK, Ostensen IH, Ruijtenbeek R, Wang MY, Berg JP, Lilleby W, Fodstad O, Ree AH. **Osteoblast-induced EGFR/ERBB2 signaling in androgen-sensitive prostate carcinoma cells characterized by multiplex kinase activity profiling.** Clin Exp Metastasis, 2009;26(5):485-96
5. Junker, K., Langner, K., Klinke F., Bosse, U. and Thomas, M., Morphology and Prognosis. Chest 120 (2001) 1584-1591.
6. Arja ter Elst, Sander H. Diks, Kim R. Kampen, Peter M. Hoogerbrugge, Rob Ruijtenbeek, Piet J. Boender, Arend H. Sikkema, Frank J. G. Scherpen, Willem A. Kamps, Maikel P. Peppelenbosch, Eveline S. J. M. de Bont, **Identification of new possible targets for leukemia treatment by kinase activity profiling.** Leuk Lymphoma. 2011 Jan;52(1):122-30
7. Arend Sikkema, Sander Diks, Wilfred den Dunnen, Arja Ter Elst, Frank Scherpen, Eelco Hoving, Rob Ruijtenbeek, Piet Boender, Rik de Wijn, W. Kamps, Maikel Peppelenbosch, Eveline de Bont, **Kinome profiling in pediatric brain tumors as a new approach for target discovery.** Cancer Res. 2009 Jul 15;69(14):5987-95