Blind prediction of response to erlotinib in early stage non-small cell lung cancer (NSCLC) in a neoadjuvant setting based on kinase activity profiles

R. Hilhorst[&], E.E. Schaake[#], R. van Pel[#], P.M. Nederlof[#], L. Houkes[&], M. Mommersteeg[&], R. de Wijn[&], M.M. van den Heuvel[#], P. Baas[#], R. Ruijtenbeek[&], H.M. Klomp[#].

[&] PamGene International BV, Wolvenhoek 10, 5211 HH 's-Hertogenbosch, The Netherlands. Phone +31736158076, rhilhorst@pamgene.com, www.pamgene.com

[#] Netherlands Cancer Institute/Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. h.klomp@nki.nl

Aim

Predict response to pre-operative erlotinib in a blinded test set (n=13) of NSCLC patients with phosphorylation-based peptide-biomarkers obtained in a training set (n=15).

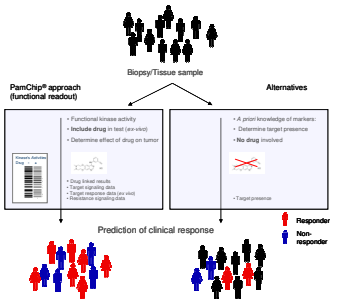

Fig.1. Approaches to personalized medicine.

Experimental setup

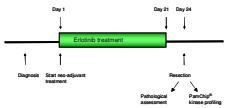

Fig.2. Treatment schedule of patients in neoadjuvant study M06NEL.

Table 1. Summary of patients of training and test set included in the M06NEL study. In brackets: responders.

	Training set	Test set
Patients	15 (6)	13 (6)
Female	7 (5)	8 (6)
Adenocarcinoma	12 (4)	5 (3)
Never smoker	6 (3)	2 (2)
Mutation status		
EGFR (exon 19/20/21)	2 (1)	3 (2)
kRAS (codon 12)	2 (0)	2 (0)

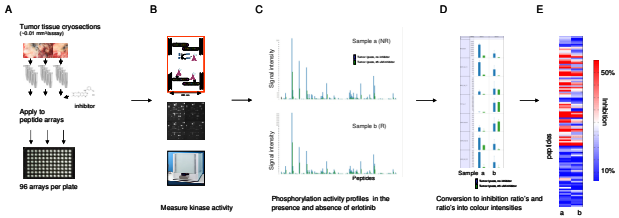

Fig.3. Lysates from fresh frozen tumor tissue are applied onto PamChip® arrays on a 96 array plate (A). Peptides are phosphorylated by the kinases in the lysates (B). In the presence of a kinase inhibitor, kinase activity is reduced (C, D). For each peptide ratio's of inhibited/non-inhibited signal are calculated and represented in heatmaps (E).

Results: response class prediction

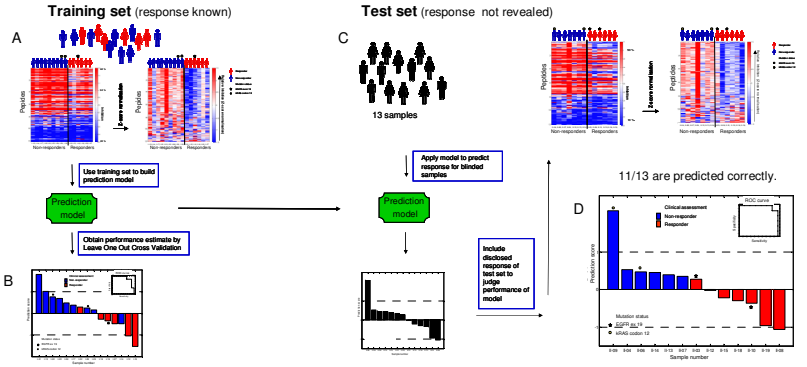

Fig.4. The response of a test set of tumor samples with blinded response (C) is predicted using a prediction model developped for a training set with known response. Ratio's of inhibited/non-inhibited signals (A) (shown in a heatmap in two representations) for a peptide set are used to build the model. Predictions for the training set are used to validate the model (B) that is applied to the test set (D).

Background

Subcategories of NSCLC patients may benefit from epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI's). Patients with cancers that harbor mutated EGFR have a higher chance of response, whereas those with kRAS mutations are unlikely to respond. However patients lacking EGFR mutations may respond as well (1). At present, there are no tests to identify likely responders more reliably than by mutation testing. Previously (2), we have shown that a prediction model based on kinase activity profiles in the presence and absence of a kinase inhibitor predicted erlotinib response of NSCLC patients in a neoadjuvant setting. The aim of the current study was to evaluate this classifier on patient tumor samples (test set), of which the response was disclosed only after classification as responder or non-responder.

References
1. Klomp and Schaake, J Clin Oncol 29:2011 (suppl; abstr 7030)
2. Hilhorst et al, J Clin Oncol 28:7s, 2010 (suppl abstr 10566)
3. Junker et al, Grading of Tumor regression in Non-smal Cell Lung Cancer. Morphology and Prognosis. Chest 120 (2001) 1584-1591.
4. Versele et al. Response prediction to a multitargeted kinase inhibitor in cancer cell lines and xenograft tumors using high-content tyrosine peptide arrays with a kinetic readout. Mol Cancer Ther 8 (2009) 1846-1855.
5. Bratland et al. Osteoblast-induced ErbB3/EGFR-ErbB2 signaling in androgen-sensitive prostate carcinoma cells characterized by multiplex kinase activity profiling. Clin Exp Metastasis 26 (2009) 485-496.

Methods

For this study, fresh frozen tumor tissue was used from NSCLC patients (stage IA-IIIA) included in the M06NEL study (1). Preoperative treatment comprised one tablet of 150 mg erlotinib daily for a period of 3 weeks. Surgical resection involved a radical resection of the tumor and regional lymph nodes. Response evaluation to neo-adjuvant treatment was based on metabolic changes and histological assessment of the surgical specimens according to Junker et al (3), e.g. residual vital tumor, signs of therapy related regression. Patients showing stable disease and progressive disease were grouped as non-responders. All specimens were analyzed for EGFR and kRAS mutation status.

Fresh frozen tissue cryosections were lysed in M-Per buffer supplemented with phosphatase and protease inhibitors. Kinase activity profiles in the presence and absence of spiked in erlotinib were determined on PamChip® peptide microarrays essentially as described in (4) and (5). Signal intensities were quantified using proprietary software. ^{2}Log ratio's of inhibited vs. non-inhibited signals were used as input for multivariate analysis.

Results

Previously (2) we have shown that a partial least square-discriminant analysis (PLS-DA) prediction model based on kinase activity profiles in the presence (in the assay) and absence of erlotinib, was able to distinguish responders and non-responders in a training set. The study was repeated with inclusion of 13 blinded samples. The training set data (n=15) were used to build a prediction model that was applied onto the blinded test set (n=13) to predict response.

Leave-one-out cross validation of the prediction model on the training set resulted in misclassification for 2 of 15 samples. Application of the prediction model to the 13 blinded test set samples resulted in correct prediction of response for 11 samples. One of the two incorrectly classified samples had an EGFR exon 19 mutation and was a complete responder, i.e. tumor cells were not present anymore in the resected sample. This sample, that should be considered a control sample, was classified as non-responder The other EGFR mutant was correctly predicted as being responder. All kRAS mutants were predicted correctly as non-responders. EGFR and kRAS wild type tumors could be classified as either responders or non-responders, which is not possible based on mutation analysis

Discussion

Samples used in this study had been resected after exposure to erlotinib. Unfortunately, no pre-treatment material was available. Since we established that a control group of untreated patients could not be distinguished from the training set (data not shown), we hypothesize that the molecular make up of the tumor cells is not essentially different in pre dose from post dose. Interestingly, non-responders show in general more inhibition in the assay than responders, suggesting hyperactive EGFR signalling that is not completely inhibited *in vivo*.

The results of this functional test of a drug in the patients' own tumor tissue indicate that a larger number of patients is eligible for erlotinib treatment than indicated by current inclusion criteria.

This test assesses the effect of a kinase inhibitor directly at its target. Response prediction based on the effect of a drug on a molecular profile is a welcome approach to complement and extend mutation detection tests. Kinase activity profiling may be a promising method in bringing personalized medicine into the clinic.

Conclusions

- This study, involving blinded samples, validates the use of a classifier to assess/predict molecularly the response to treatment, based on inhibition by erlotinib of kinase activity profiles in the patients' own tumor tissue.
- These data strongly suggest that for patients with a responder profile, adjuvant erlotinib alone or in combination with chemotherapy should be considered, also in cases without EGFR mutations.
- Measuring kinase inhibitor effects at the kinase level in tumor material of individual patients promises to be an important enabler for personalized medicine approaches.

Citation: J Clin Oncol 29:2011 (suppl; abstract 10521)