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


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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).

Experimental setup

For this study, fresh-frozen tumor tissue was used from NSCLC patients stage IA in the M06NEL study (1). Preoperative treatment comprised one tablet of 100 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 radiological changes and histological assessment of the surgical specimen according to Jankov et al (2), e.g. residual vital tumor, signs of therapy related regression. Patients showing stable disease and no progression of disease were included in the study. All specimens were analyzed for EGFR and KRAS mutation status. For this study, fresh-frozen tissue microarrays were used in Nanorefixation supplement with phosphatase and protease inhibitors. Kinase activity profiles in the lysates were phosphorylated by the kinases in the lysates. In the presence of a kinase inhibitor, kinase activity is reduced. For each peptide ratio of inhibited/non-inhibited signals were calculated and represented in heatmap.

Background

Subcategories of NSCLC patients may benefit from epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs). 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 are unlikely to benefit (2). In the current study, we aimed to evaluate the classification on patient tumor samples of these subcategories, of which the response was disclosed only after classification as responder or non-responder.

Methods

For this study, fresh-frozen tumor tissue was used from NSCLC patients stage IA in the M06NEL study (1). Preoperative treatment comprised one tablet of 100 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 radiological changes and histological assessment of the surgical specimen according to Jankov et al (2), e.g. residual vital tumor, signs of therapy related regression. Patients showing stable disease and no progression of disease were included in the study. All specimens were analyzed for EGFR and KRAS mutation status. For this study, fresh-frozen tissue microarrays were used in Nanorefixation supplement with phosphatase and protease inhibitors. Kinase activity profiles in the lysates were phosphorylated by the kinases in the lysates. In the presence of a kinase inhibitor, kinase activity is reduced. For each peptide ratio of inhibited/non-inhibited signals were calculated and represented in heatmap.

Discussion

Samples used in this study had been reacted 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 hypothesized that the molecule make up of the tumor cells is not essentially different in pre-treatment disease. Interestingly, non-responders show in general more inhibition in the assay than responders, suggesting hyperactivation EGFR signaling that is not completely inhibited in vivo. In addition, for some patients IGRF treatment indicates that a larger number of patients is eligible for anti-treatment than historically current inclusion criteria. This test assesses 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 anti-EGFR kinase activity profiles in the patient’s own tumor tissue.

• These data strongly suggest that for patients with a responder profile, adjunct chemotherapy alone or in combination with chemotherapeutic drugs should be considered, also in cases without EGFR mutations.

• Measuring kinase inhibitor effects at the kinase level in tumor material of individual patients promise to be an important enabler for personalized medicine approaches.

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