Two case studies on kinase activity profiling in non-small cell lung carcinoma tumour tissues:

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Aim

Evaluate the prognostic value of kinase activity profiles and kinase inhibition profiles in early stage NSCLC patients - for prognosis of long- or short-term survival, - for predicting erlotinib response.

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, either for prognosis of long- or short-term survival, or for predicting erlotinib response.

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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>T1N0M0</th>
<th>T1N1M0</th>
<th>T2N0M0</th>
<th>T3N0M0</th>
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<tbody>
<tr>
<td>Short term survivors</td>
<td>12</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Long term survivors</td>
<td>8</td>
<td>2</td>
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Table 2. Data for 14 (stage IA-IIIA) NSCLC patients included in the M0N1EL study. Operable patients, 15 years and over, with resectable NSCLC (cT1-3, N0-1) were eligible for the study.

Identification of biomarkers that predict the response to erlotinib

Investigations were performed on 14 samples of early stage (stage IA-IIIA) NSCLC patients included in the M0N1EL study. Operable patients, 15 years and over, with resectable NSCLC (cT1-3, N0-1) were eligible for the study.

Table 2. Data for 14 (stage IA-IIIA) NSCLC patients included in the M0N1EL study. Operable patients, 15 years and over, with resectable NSCLC (cT1-3, N0-1) were eligible for the study.

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PamChip® Kinase Assay

Fresh frozen tissue cryosections were lysed in M-Per buffer supplemented with phosphatase and protease inhibitors. Kinase activity profiles were determined in the presence and absence of erlotinib or gefitinib on PamChip® arrays comprising 144 tyrosine containing peptide sequences derived from known human phosphorylation sites. Peptide phosphorylation was detected with anti-phosphotyrosine antibodies and monitored in time. Signal intensities per peptide were quantified using proprietary software. Log Ratio’s of inhibited vs. non-inhibited signals were used as input for multivariate analysis to classify samples.

Results

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

Conclusion

Using a novel biomarker discovery platform we show that kinase activity profiles of tumour tissue exposed ex vivo to a kinase inhibitor can support - the identification of (early stage) NSCLC patients who are likely to respond to TKI treatment, - the prognosis for early stage NSCLC patients.

References