First-Generation Epidermal Growth Factor Receptor Inhibitors in Non-small Cell Lung Cancer: Clinical Impact of the Epidermal Growth Factor Receptor Fluorescence In Situ Hybridization Assay

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Epidermal growth factor receptor (EGFR) inhibitors have been proven effective in some patients with advanced non-small cell lung cancer (NSCLC) previously treated and progressed on chemotherapy.1,2 Most data are published on the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. Objective response rates in phase II studies with gefitinib and erlotinib are 10 to 18% in western populations and up to 27% in Asian populations.2–4 In addition, a substantial fraction of NSCLC patients, who failed previous chemotherapy, achieve long-term stable disease, leading to disease control rate exceeding 50% of patients and often associated with symptomatic improvement and prolonged survival. Thus, developing a biomarker capable of predicting disease control rate is equally if not more important than one predicting only those who have objective response.

Two large placebo controlled randomized studies were performed with EGFR TKIs as second or third line therapy.1,5 The BR-21 study with erlotinib showed for the first time a survival benefit for a targeted therapy in NSCLC, whereas the ISEL study with gefitinib did not demonstrate a significant survival advantage, although subset analysis in the latter study did show survival benefit in certain clinical subgroups (i.e., never smokers and Asians).5,6 In both studies, clinical effect of EGFR TKIs on survival was seen also in patients with “unfavorable” clinical characteristics (i.e., males, smokers, and patients with tumors of squamous histology).5,7 Thus, clinical features seem insufficient for identifying those patients who would and who would not have survival benefit from these new agents.

After encouraging results of EGFR TKIs in relapsed patient categories it was natural to test these drugs in combination with standard first line chemotherapy of advanced NSCLC. In large prospective randomized studies standard chemotherapy doublets were given in combination with EGFR TKIs or placebo, followed by maintenance with active drugs or placebo.8–11 No benefit from adding EGFR TKIs to
standard chemotherapy was observed in any of the four studies, and again clinical characteristics (except for never-smoking status in the TRIBUTE study) could not identify subsets of patients who benefited from combination therapy. Thus, the search for other measures, i.e., biomarkers, for selection of patients for the EGFR TKIs was mandatory.

**EGFR Gene Copy Number**

In breast cancer patients, amplification of the HER2 gene detected by fluorescence in situ hybridization (FISH) is a strong predictive factor for treatment benefit from anti-HER2 monoclonal antibody, trastuzumab, and recommended a strong predictive factor for treatment benefit from anti-HER2 monoclonal antibody, trastuzumab, and recommended.

**Association Between EGFR FISH and Outcome with EGFR TKIs as Second-Line Therapy**

Cappuzzo et al. analyzed 102 gefitinib-treated patients according to EGFR protein expression, phospho-Akt expression, EGFR gene copy number by FISH, and EGFR mutations. Patients who were FISH positive had significantly higher response rate (36% versus 3% in FISH negative patients), median time-to-progression (9.0 versus 2.5 months, respectively) and median overall survival (18.7 versus 7.0 months, respectively). The association of FISH positivity and superior survival was confirmed in multivariate survival analysis. Evaluation of EGFR gene copy number by FISH was also performed in tumor samples from 81 participants of the Southwest Oncology Group 0126 study, which assessed the role of gefitinib in bronchioloalveolar carcinoma and adenocarcinoma with bronchioloalveolar features. In this study, FISH positive patients had about 50% reduction in the risk of death as compared with FISH negative patients. Data evaluating FISH in the ISEL trial are based on a subset of 370 patients, representing the largest evaluation of this biomarker.

**TABLE 1. Epidermal Growth Factor Receptor Gene Copy Number and Sensitivity to Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Non-small Cell Lung Cancer**

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Patients</th>
<th>Drug (dose)</th>
<th>Method of Gene Copy Number Evaluation (endpoint)</th>
<th>Proportion Positive</th>
<th>Response Rates; Positive vs. Negative</th>
<th>Survival Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cappuzzo et al.</td>
<td>102</td>
<td>Gefitinib (250 mg)</td>
<td>FISH (high polysomy and gene amplification)</td>
<td>32.3%</td>
<td>36% vs. 3%</td>
<td>0.44* (0.23–0.82)</td>
</tr>
<tr>
<td>Hirsch et al.</td>
<td>81</td>
<td>Gefitinib (500 mg)</td>
<td>FISH (high polysomy and gene amplification)</td>
<td>32.0%</td>
<td>26% vs. 11%</td>
<td>0.50b (0.25–0.97)</td>
</tr>
<tr>
<td>Tsao et al.</td>
<td>125</td>
<td>Erlotinib (150 mg) vs. placebo</td>
<td>FISH (high polysomy and gene amplification)</td>
<td>45%</td>
<td>20% vs. 2%</td>
<td>0.44* (0.23–0.82)</td>
</tr>
<tr>
<td>Hirsch et al.</td>
<td>370</td>
<td>Gefitinib (250 mg) vs. placebo</td>
<td>FISH (high polysomy and gene amplification)</td>
<td>30.8%</td>
<td>16.4% vs. 3%</td>
<td>0.61* (0.36–1.04)</td>
</tr>
<tr>
<td>Douillard et al.</td>
<td>374</td>
<td>Gefitinib (250 mg) vs. docetaxel</td>
<td>FISH (high polysomy and gene amplification)</td>
<td>47%</td>
<td>13.0% vs. 7.4%</td>
<td>1.09* (0.78–1.51)</td>
</tr>
<tr>
<td>Crino et al.</td>
<td>158</td>
<td>Gefitinib (250 mg) vs. vinorelbine</td>
<td>FISH (high polysomy and gene amplification)</td>
<td>34%</td>
<td>NR</td>
<td>2.88* (1.21–6.83)</td>
</tr>
<tr>
<td>Goss et al.</td>
<td>84</td>
<td>Gefitinib (250 mg) vs. placebo</td>
<td>FISH (high polysomy and gene amplification)</td>
<td>38%</td>
<td>NR</td>
<td>0.44* (0.17–1.12)</td>
</tr>
<tr>
<td>Bell et al.</td>
<td>90, 453</td>
<td>Gefitinib (250 mg and 500 mg)</td>
<td>Quantitative PCR (&gt;4)</td>
<td>8%, 7%</td>
<td>29% vs. 15%, 56% vs. 53%</td>
<td>NR, 2.03* (0.67–6.13)</td>
</tr>
<tr>
<td>Dzidzuszko et al.</td>
<td>82</td>
<td>Gefitinib (250 mg)</td>
<td>Quantitative PCR (&gt;median)</td>
<td>51%</td>
<td>12% vs. 10%</td>
<td>1.04* (0.61–1.76)</td>
</tr>
<tr>
<td>Takano —retrospective</td>
<td>66</td>
<td>Gefitinib (250 mg)</td>
<td>Quantitative PCR (≥3)</td>
<td>44%</td>
<td>72% vs. 38%</td>
<td>0.80* (0.42–1.50)</td>
</tr>
</tbody>
</table>

a Comparison between patients with high vs. low EGFR gene copy number.
b Hazard ratio was recalculated from original publication for consistency in the table. NR, not reported.
c Comparison between EGFR tyrosine kinase inhibitor vs. placebo in patients with high EGFR gene copy number.
d Response rates to gefitinib vs. placebo in patients with high EGFR gene copy number.
e Comparison between EGFR tyrosine kinase inhibitor vs. chemotherapy in patients with high EGFR gene copy number.
f Comparison between patients receiving chemotherapy and gefitinib vs. chemotherapy and placebo.
in the study of EGFR TKIs in advanced NSCLC, and favor EGFR gene copy number assessment by FISH as a clinically useful predictor of treatment benefit from gefitinib versus placebo. The response rate in FISH positive patients was 16% as compared with 3% in FISH negative patients, and the median survival was almost doubled (8.3 in gefitinib treated FISH positive patients versus 4.5 months in FISH positive patients treated with placebo, corresponding to a hazard ratio [HR] of 0.61). Patients with high EGFR copy number treated with placebo had slightly inferior survival when compared with patients with low EGFR gene copy number (4.5 versus 6.2 months, respectively), indicating that increased EGFR gene copy number by FISH is purely predictive for the benefit from EGFR TKI and not a prognostic indicator. The lack of prognostic value of EGFR FISH is also supported by the results of EGFR gene copy number assessment from surgically treated NSCLC patients, and in NSCLC patients treated with chemotherapy alone. Molecular analysis of tumor samples from the BR.21 trial was performed using FISH according to the same criteria, although at a different institution. Although FISH result could be obtained only in 125 of 221 samples (57%), the subset of FISH positive patients achieved significant treatment benefit from erlotinib (20% response rate and a HR of 0.44), whereas this benefit was modest in FISH negative patients (2% response rate and a HR of 0.85). However, the survival benefit in FISH-positive patients in this study was not confirmed in the multivariate analysis that included other biomarkers and clinical features. The authors of this article made no conclusions regarding the benefit in FISH-positive subset of patients.

Although the ISEL study and the BR-21 studies both compared EGFR TKI with placebo, randomized studies comparing EGFR TKIs with standard chemotherapy in the second line setting were recently presented. The largest of these studies was the INTEREST study, which compared gefitinib with docetaxel as second line treatment. The trial included more than 1400 patients and the results met the primary end point of noninferiority in the overall survival between the two arms. EGFR FISH analysis was included as a clinical end point, however, EGFR FISH results were available only for 26% of overall study population. No difference in outcome was seen between the FISH-positive and negative patients between the treatment arms. Based on the previous published retrospective data it would be expected that the EGFR FISH-positive patients would perform better with gefitinib as compared with docetaxel. Possible reason for the lack of expected result in the FISH-positive patients could be that this particular subset of patients has a poor prognosis without any systemic therapy, which has been reported by our group, and that this poor overall survival would be improved by chemotherapy per se. Although, the same classification for EGFR FISH assessment was used in the INTEREST study as in previous studies technical differences from one laboratory to another cannot be ruled out on this stage.

Association Between EGFR FISH and Outcome with EGFR TKIs as First-Line Therapy

EGFR TKIs have been studied in combination with standard chemotherapy as first line treatment in patients with advanced NSCLC (outlined in the introduction). Neither of the four studies could demonstrate any survival advantage of adding a EGFR TKI to standard chemotherapy. EGFR FISH analysis has only been performed in the TRIBUTE study and preliminary data have been presented. Although no difference in the overall population could be demonstrated, the FISH positive patients had a statistically significant longer progression-free survival (PFS) compared with the FISH negative patients. Interestingly, the difference in PFS emerged after 6 months, which is the time when the patients stopped chemotherapy and continued with erlotinib alone. Furthermore, a lower response rate was seen in the EGFR FISH positive group receiving chemotherapy and erlotinib as compared with those receiving chemotherapy and placebo (11.6% versus 29.8%, \( p = 0.0495 \)). The immediate interpretation of these results and the raised hypothesis are that during the treatment with the combination of chemotherapy and EGFR TKI the agents are acting antagonistic. This hypothesis remains in agreement with observation previously raised by the investigators from the University of California at Davis. An EGFR TKI therapy results in a G1 phase cell cycle arrest and makes the activity of the G2/M phase-specific chemotherapy suboptimal. Based on this hypothesis a pharmacodynamic separation between the chemotherapy and the EGFR TKI would be more optimal and this hypothesis is today studied in prospective clinical trials.

Data from three phase II clinical studies were recently presented with EGFR FISH as a predictive marker for gefitinib monotherapy in chemonaive patients. In the INSTEP study, 201 chemonaive NSCLC patients with poor performance status (PS 2–3) were randomized to gefitinib versus placebo. Consistently with previous observations, a subset analysis from patients with available tumor biopsies demonstrated that FISH positive patients had a HR = 0.44 for survival compared with HR = 1.02 in the FISH negative patient category. In the INVITE study, 196 chemonaive NSCLC patients ≥70 years were randomized to gefitinib versus placebo. In this trial, the HR for PFS in the FISH positive patients was 3.13 compared with 0.93 in the FISH negative category. These results, together with the results of previously discussed INTERST trial, indicate that EGFR FISH does not seem to predict who should be treated with EGFR TKIs versus who should be treated with chemotherapy, either in the first or second-line setting (Table 1). More data on this important issue are urgently needed.

Gefitinib was also tested in a phase II clinical trial (ONCOBEll study) involving 42 untreated NSCLC patients with at least two of the following criteria: never-smoking history, EGFR FISH positivity, or phospho-Akt positivity by immunohistochemistry. Patients who were EGFR FISH positive had higher response rate (68% versus 9%), longer median time-to-progression (7.6 versus 2.7 months) and a trend to longer survival as compared with EGFR FISH negative patients. Although these results are encouraging, small patient numbers, multiple selection criteria and lack of control group do not allow us to assess predictive value of EGFR FISH based on this trial.
Association Between EGFR FISH and Outcome with Anti-EGFR Monoclonal Antibodies

Although most of the studies performed in NSCLC patients with EGFR antagonists used orally available small-molecule TKI inhibitors, anti-EGFR monoclonal antibodies have shown promising results in phase II trials, but no predictive marker for outcome and selection of patients has so far been identified. Several new compounds are currently actively investigated and most data are available for cetuximab. The Southwest Oncology Group presented recently preliminary results from the phase II study 0342, in which the patients were randomized in between chemotherapy and cetuximab given either concomitantly or sequentially. EGFR FISH analysis was done in biopsies from the subset of patients who participated in this study. A doubling of PFS from 3 to 6 months and of median overall survival from 7 to 15 months was seen for FISH-positive and negative patients, respectively. Thus, these data indicate that EGFR FISH might also be strongly associated with outcome after the cetuximab therapy. Interestingly, the best outcome in the FISH positive patients was seen in the concurrent arm with a median survival of 16 months compared with 7 months in the FISH negative group. Thus, the concurrent therapy with monoclonal antibody and chemotherapy seems to give the expected synergistic effect in the FISH positive patients, in contrast to the combination of chemotherapy and EGFR TKIs, where antagonistic effect is observed.

Two large prospective randomized studies with cetuximab in patients with advanced NSCLC have just been reported. The BMS 099 study comparing taxane/carboplatin with or without cetuximab was performed in unselected NSCLC patients and did not meet the primary end point of superiority in overall survival in the experimental arm. The other study, the FLEX trial (first-line treatment for patients with EGFR-expressing advanced NSCLC), compared cisplatin/vinorelbine with or without cetuximab in EGFR immunohistochemistry positive patients. Preliminary announcement of the results from the latter study showed significantly better survival by adding cetuximab, however, full report from this trial is awaited. In light of contradictory results of two studies described above, selection of patients for cetuximab therapy based on molecular criteria seems to be crucial. Studies with other anti-EGFR antibodies in NSCLC did not report on EGFR FISH status.

Methodological Considerations

EGFR gene copy number may be heterogeneous within different areas of the same tumor and between the primary and metastatic site, influencing the result of the FISH analysis. Clinical significance of tumor heterogeneity with regard to sensitivity to EGFR TKIs is presently unknown. Other techniques of gene copy number assessment include quantitative PCR (qPCR) and chromogenic in situ hybridization (CISH). In the former method, copy number of the gene of interest is quantified in individual tumor cells, whereas qPCR technologies assess gene copy number in a pool of cells, which may also contain inflammatory and stromal components. Tumor microdissection may help to ensure that the assessment is carried out in areas abundant in tumor cells, but this procedure significantly increases the assay cost. In qPCR technique, quantification of the reference gene copy number presents additional challenge because of the possibility of its deletion or amplification in tumor cells. CISH technique implements an enzymatic reaction to detect the DNA probe hybridized to the gene of interest. The main advantage of this technology is the use of light instead of fluorescent microscope enabling the reader to score the signals in histologic sections. Data on CISH gene copy number evaluation and sensitivity to EGFR TKIs are sparse. In a group of 44 NSCLC patients treated with gefitinib or erlotinib, EGFR gene copy number by CISH did not associate with response rate. A comparison study between CISH and FISH is currently ongoing.

In summary, retrospective studies have shown a significant survival benefit in pretreated NSCLC patients with high EGFR gene copy number evaluated by FISH, as demonstrated in several studies involving almost 700 patients. Several prospective clinical studies with patient selection based on FISH or combination of FISH and other biomarkers are currently underway in the adjuvant, first and second-line setting. The value of EGFR FISH for the prediction of clinical outcome to EGFR TKIs compared with first or second-line chemotherapy is not yet established. Ongoing large prospective adjuvant studies with EGFR TKIs (i.e., the RADIANT study) in selected patients based on EGFR expression will further shed light to the use of EGFR FISH for selection of NSCLC patients to adjuvant therapy. The association of EGFR FISH and outcome of NSCLC patients treated with chemotherapy and anti-EGFR monoclonal antibodies is compelling and should be further prospectively studied.

REFERENCES


tocytologic (IHC) and chromogenic in situ hybridization (CISH) as predictors of sensitivity to erlotinib and gefitinib in patients (pts) with NSCLC. *J Clin Oncol* 2005;23(Suppl 16S):7031.