Is FISH floating or still swimming in the lung cancer ocean?

The epidermal growth factor receptor (EGFR) is a crucial factor in the development and growth of several human malignancies, including non-small-cell lung cancer (NSCLC). The main strategies aimed at inhibiting the EGFR pathway include agents directed against the extracellular domain of the receptor, such as monoclonal antibodies, and small molecules interfering with the tyrosine kinase activity of the intracellular domain, the tyrosine kinase inhibitors (TKIs). From the first clinical studies, it became clear that both strategies only seemed effective in a fraction of NSCLC patients, highlighting the need for patient preselection if these agents were to be optimally used. While no reliable biomarker has been yet identified for anti-EGFR monoclonal antibodies [1, 2], the presence of activating EGFR mutations and increased EGFR gene copy number detected by FISH emerged early on as the two most relevant predictors for EGFR-TKI sensitivity [3–5].

Potentially predictive biomarkers may predict for a higher likelihood of response, longer survival from the treatment, or both. Dahabreh and collaborators [6, 7] have recently published two meta-analyses on this subject. When analyzing the predictive value of EGFR mutation and copy number gain (CNG) for response to EGFR-TKIs, the authors concluded that EGFR mutation testing was both more sensitive and more specific than CNG [6]. In the current issue, these authors presented the first meta-analysis exploring the role of EGFR–CNG as a predictor of survival in NSCLC patients treated with EGFR-TKIs [7]. All patients were treated with the EGFR-TKIs erlotinib or gefitinib as monotherapy. EGFR CNG was evaluated using different methods and with different scoring systems, with the majority using FISH and the Colorado Classification System [5, 8]. Overall survival (OS), the primary end point of the study, was significantly associated with EGFR CNG as was progression-free survival (PFS) and time to progression. The association with survival benefit was detected in studies dominated by Caucasian patients but not in those dominated by East Asian patients.

Taken at face value, the work of Dahabreh et al. concluded for evidence that the EGFR FISH is still swimming along smoothly as a predictive biomarker of OS from TKIs, especially in Caucasian populations. Yet the field of EGFR biomarkers in NSCLC has evolved significantly since many of the studies in these meta-analyses were conducted. EGFR mutations have recently emerged as the most dominant marker of benefit from EGFR-TKIs. We also know that they do not exist in isolation, and to interpret any biomarker study, it is now essential to understand the true interplay between EGFR mutations and CNG in NSCLC.

Since their identification in 2004, activating EGFR gene mutations have emerged as the most relevant predictor of response to gefitinib [3, 4] or erlotinib [9]. Several retrospective and prospective studies confirmed that patients carrying activating EGFR mutations in their tumors were particularly sensitive to EGFR-TKIs, with response rates of up to 90% and with longer PFS and OS when compared with historical chemotherapy-treated controls [10–19]. These findings represented the rationale for conducting large phase III trials comparing an EGFR-TKI versus standard chemotherapy in the first-line setting in variously selected patient populations. At present, data from five large studies have been presented or fully published (IPASS, First-SIGNAL, WJTOG 3405, NEJ002, and OPTIMAL; Table 1). In the first two trials (IPASS and First-SIGNAL), patient selection was based on clinical characteristics [22, 23]. In such studies, Asian patients (exclusively Korean in the First-SIGNAL) with adenocarcinoma histology, who were only (First-SIGNAL) or mainly (IPASS) never smokers, were randomly assigned to gefitinib or standard chemotherapy (carboplatin plus paclitaxel in IPASS and cisplatin plus gemcitabine in First-SIGNAL). Biological tissue suitable for molecular analysis was available in only 31%–36% of patients, but the frequency of EGFR mutations in these Asian samples was sufficiently high (44%–60%) to still allow relevant assessments of the importance of this biomarker to be made. In both trials, PFS benefit favoring gefitinib was confined to patients with EGFR mutations, while in EGFR wild-type patients, the risk of progression was significantly lower in patients treated with chemotherapy. Similar results were observed in three additional trials conducted in patients selected by the presence of proven sensitizing EGFR mutations [24–26]. The WJTOG 3405 and NEJ002 trials randomly assigned chemonaive NSCLC patients harboring an EGFR mutation to gefitinib or platinum-based chemotherapy (carboplatin plus paclitaxel in NEJ002 and cisplatin plus docetaxel in WJTOG 3405). Both trials demonstrated that in NSCLC patients with an EGFR mutation, gefitinib produced a higher response rate and longer PFS than standard chemotherapy. More recently, Chinese investigators compared erlotinib with a combination of carboplatin and gemcitabine in chemonaive, metastatic NSCLC with activating EGFR mutation [26]. Similar to the gefitinib studies, this study demonstrated that erlotinib was superior to chemotherapy in terms of both response rate and PFS. However, no statistically significant survival benefit for the use of the EGFR-TKI in EGFR mutated patients has been reported in any of the above-mentioned studies. Nor, for that matter, in the known EGFR wild-type...
populations (again a subgroup of the overall study population) in either IPASS or First-SIGNAL [23, 31].

Two additional studies investigated the efficacy of erlotinib as maintenance therapy in NSCLC patients with metastatic disease not progressing after standard chemotherapy [32, 33]. In the SATURN trial, NSCLC patients not progressing after four cycles of platinum-based chemotherapy were randomized to erlotinib or placebo [32]. The study met its primary and secondary end points, showing that patients receiving erlotinib had a significant reduction in the risk of progression [hazard ratio (HR) 0.71] and death (HR 0.81). The highest PFS benefit was observed in patients with EGFR mutations, although a PFS improvement was also detected in the EGFR wild-type population. Interestingly, OS was significantly prolonged in the known EGFR wild-type population but not in the known EGFR mutation-positive population. In the ATLAS trial, metastatic NSCLC patients not progressing after four cycles of platinum-based chemotherapy plus bevacizumab were randomly assigned to bevacizumab plus placebo or erlotinib [33]. The results of the ATLAS trial confirmed that the addition of erlotinib significantly reduced the risk of progression (HR 0.72), with the highest benefit observed in the EGFR mutated population. No statistically significant OS differences were observed in the ATLAS study in either the overall population (HR 0.92) or known EGFR mutant (HR 0.46) or wild-type patients (HR 0.86).

In the second-line setting, a recent meta-analysis [34] including data coming from four randomized trials (INTEREST, V15-32, SIGN, and ISTANA) confirmed that gefitinib appeared to be no different from docetaxel in unselected, pretreated NSCLC [27–30, 34]. In the largest of these studies, the INTEREST trial, biological information on EGFR mutations was available from 20% of the study population [27]. In these patients, the frequency of EGFR mutations was 15%, far lower than in studies predominantly conducted in Asia, shrinking the relevant dataset available for analyses. Nevertheless, in this subgroup it was possible to show that gefitinib produced a significantly higher response rate and longer PFS than docetaxel in patients with EGFR mutations [35]. The HR for OS in the EGFR mutants was 0.83 but failed to reach statistical significance.

Why do we have so much data in both front- and second-line settings showing that patients with EGFR mutations benefit more from EGFR-TKI than from standard chemotherapy in terms of response and PFS but not in terms of survival? There are several potential explanations for the observed lack of statistically significant survival benefit in EGFR mutated patients. Many of these studies can be criticized for expecting statistical significance in the same end point that they originally sized their entire study for when analyzing only a small fraction of the treated patients. Moreover, only a proportion of the analyzed patients (varying by geography) expressed the biomarker of interest. Consequently, the recurring presence of nonstatistically significant trends must be seen as something other than a simple coincidence. For example, the HRs for OS from the EGFR-TKI compared with chemotherapy in IPASS, First-SIGNAL, and INTEREST were 0.78, 0.82, and 0.83, respectively. However, among the studies such as WJTOG 3405

### Table 1. Randomized trials with EGFR-TKI single agent versus chemotherapy

<table>
<thead>
<tr>
<th>Reference</th>
<th>Phase</th>
<th>Line</th>
<th>Drug</th>
<th>RR</th>
<th>PFS</th>
<th>OS</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>INTEREST</td>
<td>II</td>
<td>Gefitinib versus vinorelbine</td>
<td>3.1</td>
<td>0.03</td>
<td>1.19</td>
<td>8.1</td>
<td></td>
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<tr>
<td>Lilenbaum</td>
<td>III</td>
<td>Erlotinib versus CB</td>
<td>4.0</td>
<td>0.13</td>
<td>1.9</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>IPASS</td>
<td>III</td>
<td>Gefitinib versus CB</td>
<td>43.0</td>
<td>&lt;0.001</td>
<td>0.74</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td>Lee</td>
<td>III</td>
<td>Gefitinib versus GC</td>
<td>53.3</td>
<td>0.15</td>
<td>0.04</td>
<td>21.3</td>
<td></td>
</tr>
<tr>
<td>Maemondo</td>
<td>III</td>
<td>Gefitinib versus CB</td>
<td>73.7</td>
<td>&lt;0.001</td>
<td>0.30</td>
<td>30.5</td>
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</tr>
<tr>
<td>WJTOG</td>
<td>III</td>
<td>Gefitinib versus DC</td>
<td>56.3</td>
<td>5.4</td>
<td>&lt;0.001</td>
<td>0.48</td>
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</tr>
<tr>
<td>OPTIMAL</td>
<td>III</td>
<td>Erlotinib versus CG</td>
<td>83.3</td>
<td>&lt;0.0001</td>
<td>0.16</td>
<td>NR</td>
<td></td>
</tr>
<tr>
<td>INTEREST</td>
<td>II</td>
<td>Gefitinib versus docetaxel</td>
<td>9.1</td>
<td>0.27</td>
<td>0.47</td>
<td>7.6</td>
<td></td>
</tr>
<tr>
<td>V15-32</td>
<td>II</td>
<td>Gefitinib versus docetaxel</td>
<td>22.5</td>
<td>2.0</td>
<td>0.33</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>SIGN</td>
<td>II</td>
<td>Gefitinib versus docetaxel</td>
<td>13.2</td>
<td>3.4</td>
<td>0.94</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>ISTANA</td>
<td>II</td>
<td>Gefitinib versus docetaxel</td>
<td>28.1</td>
<td>0.0007</td>
<td>0.044</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

EGFR, epidermal growth factor receptor; TKI, tyrosine kinase inhibitor; RR, response rate; PFS, progression-free survival; OS, overall survival; HR, hazard ratio; NR, not reported; NS, not significant; CB, carboplatin–paclitaxel; CG, carboplatin–gemcitabine; GC, gemcitabine–cisplatin; DC, docetaxel–cisplatin.
and and SATURN, in which 43% of patients had biological material available for analysis, other explanations have to be considered. Most notable among these is the potential confounding effect of salvage chemotherapy and post-study EGFR-directed therapies. We know that EGFR mutant cancers tend to be more sensitive to some chemotherapies than EGFR wild-type patients [22] and that the benefit from EGFR-TKIs in EGFR mutant patients in terms of PFS seems to be independent of line of therapy [19]. It is only in the earliest studies, when routine access to EGFR-TKIs and other chemotherapies were far less available, that the survival benefit of a TKI in EGFR mutations can be reasonably assessed independently of the effect of post-study treatments. In the Canadian BR21, a placebo-controlled trial comparing erlotinib versus placebo in second- and third-line settings [36], biomarker information on EGFR mutation status was available in only 24% of the study patients and in a population in which EGFR mutations are relatively rare compared with an East Asian-based study. These limitations could explain why, in the small dataset available for molecular analyses, erlotinib did not improve OS in either EGFR mutant patients or, for that matter, in the subgroup of known EGFR wild-type patients at a statistically significant level [37]. However, the HRs were 0.55 (P = 0.12) for EGFR mutant patients and 0.74 (P = 0.09) for wild-type patients, suggesting that EGFR mutants may indeed have a greater trend toward a discernible survival benefit when salvage therapies are more restricted.

Therefore, the only statistically significant data to date relating to a beneficial effect of EGFR-TKIs on OS in either a known EGFR wild-type or mutant population come from the SATURN study. In this study, in which a significant proportion of patients had material available for biological analyses, the data appear to show that EGFR-TKIs only improve OS in EGFR wild-type patients. Can this be so, or do all these other lines of evidence suggest that erlotinib does not improve OS in either EGFR mutant patients or, for that matter, in the subgroup of known EGFR wild-type patients at a statistically significant level [37]? The HRs were 0.55 (P = 0.12) for EGFR mutant patients and 0.74 (P = 0.09) for wild-type patients, suggesting that EGFR mutants may indeed have a greater trend toward a discernible survival benefit when salvage therapies are more restricted.

While the above represents a pragmatic synthesis of available data, the puzzle of proven EGFR-TKI activity in the EGFR wild-type population remains to be addressed. EGFR ‘wild-type’ is clearly not a uniform entity, any more than ‘non-small-cell lung cancer’ is, as both terms really define what a disease is not, as opposed to what it is. We know that, unless otherwise specified, the EGFR wild-type lung cancer population must contain ALK gene rearranged, KRAS mutant, and a whole host of other molecular subpopulations with different oncogenic drivers that are still being discovered. Consequently, a diffuse consistent benefit of EGFR-TKIs seems unlikely in the EGFR wild-type population. As, in general, this population has few therapeutic options following first-line therapy, defining additional biomarkers that could be used together with EGFR mutation testing to help identify the group of patients deriving even minimal survival benefit from an EGFR-TKI therapy is vitally important. In this context, EGFR–FISH might be the best biomarker to start with.

EGFR CNG determined by FISH was detected by our group as a relevant marker for patient selection at the same time of the EGFR mutation discovery [5]. Using the Colorado scoring system described in our first study and others [5, 8], ~30%–50% of NSCLC are EGFR FISH positive. The relevance of EGFR CNG for EGFR-TKI sensitivity has been confirmed in several studies, as shown in Tables 2–4. Table 2 summarizes retrospective and prospective single-arm studies with EGFR-TKIs. EGFR FISH-positive patients had a response rate ranging from 26% to 72%, with a median PFS and OS up to 9 and 18 months, respectively [5, 10, 38–45]. In the absence of studies directly comparing EGFR FISH with mutation, these data suggest that, while both may be useful, EGFR mutation is better than FISH for detection of the most highly sensitive population (by response and PFS) and data from the first meta-analysis of Dahabreh et al. [6] support this conclusion.

Several randomized phase II and III trials have evaluated the role of EGFR CNG for selection of patients for EGFR-TKI therapy [20, 27, 28, 33, 40, 46–51]. Those included two phase II randomized studies (INVITE and INSTEP), three phase III trials comparing gefitinib or erlotinib versus placebo (ISEL, BR21, and SATURN), and six phase III studies comparing EGFR-TKI alone (IPASS, INTEREST, and V15-32) or EGFR-TKI plus chemotherapy (INTACT 1, INTACT 2, and TRIBUTE) versus chemotherapy alone. In the INTACT 1 and 2 trials, EGFR CNG was evaluated by RT-PCR, whereas in all others, FISH was the method of choice. Studies including chemotherapy (INVITE, INTACT 1 and 2, TRIBUTE, IPASS, SATURN, ATLAS, INTEREST, V15-32, SIGN, and ISTANA) provided discordant results (Table 3) likely because of the confounding effects of chemotherapy and post-study therapies. Only three published studies (INSTEP, BR21, and ISEL) were not biased by chemotherapy or post-study therapies (Table 4). In all these three studies, EGFR FISH-positive patients receiving EGFR-TKIs had longer survival than EGFR FISH-positive patients receiving placebo, which contrast with the multiple negative studies looking for an effect of EGFR-TKIs on survival in EGFR mutant patients described above. Overall, these data and the analyses of Dahabreh et al. suggest that although EGFR–CNG by FISH is less sensitive than EGFR mutation for identifying responders, it...
may capture more patients who will ultimately derive a survival benefit from the TKI.

However, this view is out of time with current knowledge. Given our growing understanding about the significance of EGFR mutations, it is critical to analyze CNG data in direct comparison with EGFR mutation status. In the IPASS study, while initially FISH positivity was associated with increased benefit from gefitinib compared with chemotherapy, this

Table 2. Retrospective and prospective single-arm studies of EGFR CNG and outcome to EGFR-TKIs

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study</th>
<th>N</th>
<th>Method</th>
<th>Line</th>
<th>Drug</th>
<th>EGFR (%)</th>
<th>RR (%)</th>
<th>P</th>
<th>PFS (months)</th>
<th>P</th>
<th>OS (months)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cappuzzo [5]</td>
<td>R</td>
<td>102</td>
<td>FISH</td>
<td>2+</td>
<td>Gefitinib +</td>
<td>32.4</td>
<td>36.0</td>
<td>&lt;0.001</td>
<td>9.0</td>
<td>&lt;0.001</td>
<td>18.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Hirsch [38]</td>
<td>R</td>
<td>81</td>
<td>FISH</td>
<td>1+</td>
<td>Gefitinib +</td>
<td>32.0</td>
<td>26.0</td>
<td>0.08</td>
<td>9.0</td>
<td>0.07</td>
<td>NR</td>
<td>0.04</td>
</tr>
<tr>
<td>Varella-Garciaa [39]</td>
<td>R</td>
<td>44</td>
<td>FISH</td>
<td>1+</td>
<td>Gefitinib +</td>
<td>66.0</td>
<td>65.0</td>
<td>0.07</td>
<td>5.6</td>
<td>0.72</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Takano [10]</td>
<td>R</td>
<td>66</td>
<td>PCR</td>
<td>1+</td>
<td>Gefitinib +</td>
<td>44.0</td>
<td>72.0</td>
<td>0.005</td>
<td>9.4</td>
<td>0.03</td>
<td>16.4</td>
<td>0.3</td>
</tr>
<tr>
<td>IDEAL 1 and 2 [40]</td>
<td>R</td>
<td>86</td>
<td>PCR</td>
<td>2+</td>
<td>Gefitinib +</td>
<td>56.0</td>
<td>38.0</td>
<td>2.6</td>
<td>15.7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ahn [41]</td>
<td>P</td>
<td>88</td>
<td>PCR</td>
<td>1+</td>
<td>Erlotinib +</td>
<td>40.9</td>
<td>41.7</td>
<td>0.01</td>
<td>5.8</td>
<td>&gt;0.001</td>
<td>NR</td>
<td>0.01</td>
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<tr>
<td>Cappuzzo [42]</td>
<td>P</td>
<td>35</td>
<td>FISH</td>
<td>1+</td>
<td>Gefitinib +</td>
<td>69.4</td>
<td>68.0</td>
<td>&lt;0.001</td>
<td>7.6</td>
<td>0.02</td>
<td>NR</td>
<td>0.3</td>
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<tr>
<td>Felip [43]</td>
<td>P</td>
<td>57</td>
<td>FISH</td>
<td>2+</td>
<td>Erlotinib +</td>
<td>26.0</td>
<td>33.0</td>
<td>&lt;0.001</td>
<td>4.5</td>
<td>0.002</td>
<td>7.5</td>
<td>0.2</td>
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<tr>
<td>Tiseo [44]</td>
<td>P</td>
<td>54</td>
<td>FISH</td>
<td>2+</td>
<td>Gefitinib +</td>
<td>22.0</td>
<td>50.0</td>
<td>–</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sequistb [45]</td>
<td>P</td>
<td>29</td>
<td>FISH</td>
<td>1</td>
<td>Gefitinib +</td>
<td>76.0</td>
<td>50.0</td>
<td>1.0</td>
<td>–</td>
<td>NS</td>
<td>–</td>
<td>NS</td>
</tr>
</tbody>
</table>

aJapanese population.
bAll patients had EGFR mutation.

N, number of patients evaluated for EGFR gene copy number; EGFR, epidermal growth factor receptor; CNG, copy number gain; TKIs, tyrosine kinase inhibitors; RR, response rate; PFS, progression-free survival; OS, overall survival; R, retrospective; P, prospective; NR, not reached; NS, not significant.

Table 3. EGFR CNG and outcome to EGFR-TKIs in phase II and III randomized trials with chemotherapy arm

<table>
<thead>
<tr>
<th>Reference</th>
<th>Phase</th>
<th>N</th>
<th>Method</th>
<th>Line</th>
<th>Drug</th>
<th>EGFR (%)</th>
<th>RR (%)</th>
<th>P</th>
<th>PFS (months)</th>
<th>HR</th>
<th>OS (months)</th>
<th>HR</th>
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</thead>
<tbody>
<tr>
<td>INVITE [20]</td>
<td>II</td>
<td>158</td>
<td>FISH</td>
<td>I</td>
<td>Gefitinib versus vinorelbine</td>
<td>+34.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.13</td>
<td>–</td>
<td>2.88</td>
</tr>
<tr>
<td>INTACT 1 and 2 [40]</td>
<td>III</td>
<td>453</td>
<td>PCR</td>
<td>I</td>
<td>Gefitinib + CT versus CT</td>
<td>+7.0</td>
<td>29.0 versus 15.0</td>
<td>0.3</td>
<td>6.9 versus 7.3</td>
<td>0.83</td>
<td>11.5 versus NR</td>
<td>2.03</td>
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<tr>
<td>TRIBUTE [46]</td>
<td>III</td>
<td>245</td>
<td>FISH</td>
<td>I</td>
<td>Erlotinib + CT versus CT</td>
<td>+40.8</td>
<td>11.6 versus 29.8</td>
<td>0.049</td>
<td>6.3 versus 5.8</td>
<td>0.59</td>
<td>12.6 versus 14.3</td>
<td>1.52</td>
</tr>
<tr>
<td>IPASS [47]</td>
<td>III</td>
<td>406</td>
<td>FISH</td>
<td>I</td>
<td>Gefitinib versus CT</td>
<td>+61.0</td>
<td>58.9 versus 44.8</td>
<td>0.02</td>
<td>–</td>
<td>0.66</td>
<td>–</td>
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<tr>
<td>ATLASa [33]</td>
<td>III</td>
<td>196</td>
<td>FISH</td>
<td>I</td>
<td>Erlotinib + B versus B</td>
<td>+44.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.66</td>
<td>–</td>
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<tr>
<td>INTEREST [27]</td>
<td>III</td>
<td>374</td>
<td>FISH</td>
<td>II</td>
<td>Gefitinib versus docetaxel</td>
<td>+46.5</td>
<td>13.0 versus 7.4</td>
<td>0.03</td>
<td>2.5 versus 2.8</td>
<td>0.84</td>
<td>8.4 versus 7.5</td>
<td>1.09</td>
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<tr>
<td>V15.32 [28]</td>
<td>III</td>
<td>60</td>
<td>FISH</td>
<td>II</td>
<td>Gefitinib versus docetaxel</td>
<td>+70.0</td>
<td>46.0 versus 33.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3</td>
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</table>

aFirst-line maintenance therapy. 1. Interaction test for PFS was statistically significant, with a P value of 0.007. 2. Interaction test for PFS was statistically significant, with a P value of 0.043. 3. EGFR FISH-positive patients appeared to have better PFS than EGFR FISH-negative patients on both treatments (gefitinib-positive versus gefitinib-negative HR, 0.75; docetaxel HR, 0.45).

N, number of patients evaluated for EGFR gene copy number; EGFR, epidermal growth factor receptor; CNG, copy number gain; TKI, tyrosine kinase inhibitor; RR, response rate; PFS, progression-free survival; HR, hazard ratio; OS, overall survival differences between EGFR-TKI versus control arm in EGFR+ and EGFR−, respectively; CT, chemotherapy; B, bevacizumab; NR, not reached.
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disclosure

The authors have declared no conflicts of interest.

references

7. Dahabreh I, Linardou H, Kosmidis P et al. EGFR gene copy number as a predictive biomarker for patients receiving tyrosine kinase inhibitor treatment:

Table 4. EGFR CNG and outcome to EGFR-TKIs in phase II and III randomized trials with placebo control

<table>
<thead>
<tr>
<th>Reference</th>
<th>Phase</th>
<th>N</th>
<th>Method</th>
<th>Line</th>
<th>Drug</th>
<th>EGFR (%)</th>
<th>RR (%)</th>
<th>P</th>
<th>PPS (months)</th>
<th>HR</th>
<th>OS (months)</th>
<th>HR</th>
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<tr>
<td>INSTEP [48]</td>
<td>II</td>
<td>84</td>
<td>FISH</td>
<td>I</td>
<td>Gefitinib versus P</td>
<td>+38.0</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.26</td>
<td>0.47</td>
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<td></td>
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<tr>
<td>ISEL [49]</td>
<td>III</td>
<td>159</td>
<td>FISH</td>
<td>II/III</td>
<td>Erlotinib versus P</td>
<td>+38.0</td>
<td>16.4 versus 3.0</td>
<td>–</td>
<td>–</td>
<td>8.3 versus 4.5</td>
<td>0.61</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BR21 [50]</td>
<td>III</td>
<td>159</td>
<td>FISH</td>
<td>II/III</td>
<td>Erlotinib versus P</td>
<td>+38.0</td>
<td>21.0 versus 5.0</td>
<td>0.02</td>
<td>–</td>
<td>10.5 versus 3.1</td>
<td>0.43</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>SATURN [51]</td>
<td>III</td>
<td>488</td>
<td>FISH</td>
<td>I</td>
<td>Erlotinib versus P</td>
<td>+48.0</td>
<td>–</td>
<td>–</td>
<td>6.8</td>
<td>–</td>
<td>–</td>
<td>0.96</td>
<td></td>
</tr>
</tbody>
</table>

*First-line maintenance therapy. 1. The interaction test was statistically significant, with a P value of 0.045. 2. The positive predictive value of EGFR FISH+ status was confirmed in the multivariable model (P value 0.005).

N, number of patients evaluated for EGFR gene copy number; EGFR, epidermal growth factor receptor; CNG, copy number gain; TKI, tyrosine kinase inhibitor; RR, response rate; PFS, progression-free survival; HR, hazard ratio; OS, overall survival differences between EGFR-TKI versus control arm in EGFR+ and EGFR−, respectively; P, placebo.

It is therefore clear that it is impossible to dissect the contribution of one biomarker from another unless both biomarkers are evaluated in combination. In many of the studies used in the meta-analysis of Dahabreh et al., this information was not available and often one of the tests was not done. Sometimes, this was by design and sometimes, given that the technology for mutation screening often requires more tissue than for FISH analysis, it was just not feasible. A key point that has to be considered before benefit from FISH is completely dismissed is that the prevalence of EGFR mutations in East Asians is very high. Certainly, the association of CNG and mutation has also been observed in Caucasian studies [5, 42] but at a lesser extent, suggesting that in Western countries there is a consistently higher proportion of EGFR wild-type patients with EGFR CNG. Why is this CNG present when it is not associated with an EGFR mutation? Although we have reservations about the broad conclusions of the current meta-analysis of Dahabreh et al., the differential effect of CNG on survival in Asian and Caucasian populations is certainly intriguing, given their differential overlap with the EGFR mutant population. If we are to explore whether EGFR CNG can indeed help to predict for benefit from EGFR-TKIs in an EGFR wild-type population, we may need to go back to basics. We may need to explore the role of EGFR-TKIs in the wild-type Caucasian population compared with placebo or chemotherapy in later lines of therapy, and we may need to look in gene amplified and high polysomy subsets separately.

In conclusion, recent findings have made waters turbulent for the FISH assay. The FISH is still swimming but perhaps it needs to use a different style from that in which it first started. The significance of the association between CNG and EGFR mutations must be considered plus the small but proven benefit of the EGFR-TKIs in the wild-type population. The meta-analysis of Dahabreh et al. has a major caveat around the lack of information on EGFR mutation status and salvage therapies that potentially affect OS in the different studies. Nevertheless, it should generate sufficient debate to warrant further investigation of EGFR CNG for selection of EGFR wild-type NSCLC patients for EGFR-TKIs. EGFR wild-type patients represent a large and heterogenous group of patients including individuals harboring genetic aberrations largely unknown and with modest effective therapies available. EGFR FISH testing may still be the best possible option to explore in EGFR wild-type NSCLC patients while waiting for new genomic discoveries and specific, effective therapies.

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