**EGFR gene copy number as a predictive biomarker for patients receiving tyrosine kinase inhibitor treatment: a systematic review and meta-analysis in non-small-cell lung cancer**

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**Introduction:** We conducted a systematic review and meta-analysis to assess epidermal growth factor receptor (EGFR) gene copy number as a potential biomarker of survival for patients with advanced non-small-cell lung cancer (NSCLC) receiving single-agent treatment with EGFR tyrosine kinase inhibitors (TKIs).

**Methods:** We systematically identified articles investigating EGFR gene copy number by fluoroscent or chromogenic in situ hybridization in patients with advanced or recurrent NSCLC treated with the TKIs erlotinib or gefitinib, (last search: 31 June 2009). Eligible studies had to report on overall survival (OS), progression-free survival (PFS) or time-to-progression (TTP), stratified by EGFR gene copy number. Summary hazard ratios (HRs) were calculated using random-effects models.

**Results:** Among 255 identified studies, 20 (1689 patients, 594 with increased gene copy number), 10 (822 patients, 290 with increased gene copy number) and 5 (294 patients, 129 with increased gene copy number) were eligible for the OS, PFS and TTP meta-analyses, respectively. Increased EGFR gene copy number was associated with increased OS (HR = 0.77; 95% CI 0.66–0.89; P = 0.001), PFS (HR = 0.60; 95% CI 0.46–0.79; P < 0.001) and TTP (HR = 0.50; 95% CI 0.28–0.91; P = 0.02). Among predominantly white populations, increased EGFR gene copy number was strongly associated with improved survival (HR = 0.70; 95% CI 0.59–0.82; P < 0.001), whereas it did not influence survival in East Asians (HR = 1.11; 95% CI 0.82–1.50; P = 0.50). This difference was statistically significant (P = 0.02).

**Conclusion:** Among TKI-treated patients, increased EGFR gene copy number appears to be associated with improved survival outcomes. The effect on OS appears to be limited to patients of non-Asian descent.

**Key words:** amplitification, CISH, epidermal growth factor receptor, FISH, gene copy number, lung cancer

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The epidermal growth factor receptor (EGFR), one of the earliest identified oncopines, has been implicated in processes such as cell proliferation, resistance to apoptosis, angiogenesis and cell motility [1]. It is highly expressed in non-small-cell lung cancer (NSCLC), with the highest expression being observed in the histological subtype of squamous cell carcinoma [2]. Increased EGFR expression, both at the messenger RNA and protein levels, has been correlated with adverse disease characteristics such as advanced stage at diagnosis and resistance to treatment [1, 3]. It has also been suggested to be a prognostic factor associated with reduced survival [2, 4]. The central role of EGFR in lung cancer carcinogenesis provided the rationale for the development of drugs that would target the receptor, abrogate receptor signaling and thus inhibit cancer growth [5, 6]. More than three decades of clinical investigation have now culminated into the clinical availability of several EGFR-targeted agents, which fall in two classes: anti-EGFR antibodies, such as cetuximab (Erbitux®; Imclone LLC, New York, NY) and panitumumab (Vectibix®; Immunex Corporation, Thousand Oaks, CA); and tyrosine kinase inhibitors (TKIs) erlotinib (Tarceva®; OSI-Pharmaceuticals, New York, NY) and gefitinib (Iressa®; AstraZeneca, Macclesfield, UK).

During the early clinical development of the EGFR TKIs, one of the central questions was the relationship between the target (EGFR protein) expression and treatment effectiveness. Based on this reasoning, there was debate over whether enrollment in clinical trials should be limited to patients with tumors demonstrating increased EGFR expression. Evidence
from colorectal cancer led to some confusion over the target-presence principle [7, 8]; however, in NSCLC, initial studies indicated improved response rates in patients expressing (overexpressing) EGFR [9, 10]. After the first clinical results became available, the observation that certain patients demonstrated rapid objective tumor responses led to the identification of EGFR gene copy number gain [11, 12] and somatic EGFR mutations [13–15] as candidate biomarkers of response and survival. Compared with PCR-based methods, scoring EGFR gene copy number is fairly assessor dependent. In an attempt to standardize EGFR gene copy number in NSCLC, and to overcome differences between EGFR in NSCLC and the routinely assessed cErbB2 gene in breast cancer [11, 16], an alternative scoring system was proposed by Cappuzzo et al. [11]: the Colorado Classification System (CCS) is fairly well acknowledged and routinely used for the assessment of EGFR gene copy number analysis in NSCLC.

In a recent systematic review, we demonstrated that the sensitivity and specificity of EGFR gene copy number for predicting complete or partial response to EGFR inhibitors is relatively low compared with the presence of somatic EGFR mutations [17]. However, survival is generally regarded as a more relevant outcome both by patients and their treating physicians. With this in mind, we present the results of a systematic review and meta-analysis of published studies evaluating EGFR gene copy number as a predictive biomarker of survival for patients with advanced NSCLC receiving treatment with single-agent EGFR TKIs.

**methods**

**study eligibility and identification**

We performed systematic computerized searches of the PubMed (Medline), and SCOPUS databases (from inception to 31 June 2009) and the Cochrane library (Issue 1, 2009) to identify all published articles reporting on EGFR TKIs for advanced NSCLC using a previously described search strategy [18]. We also hand searched journals known to publish data relevant to our topic, the reference lists of all retrieved articles and those of relevant review articles were also cross-referenced. Experts in the field were contacted to broaden the yield of our search. We also drew upon a regularly updated online database of published peer-reviewed evidence on somatic EGFR mutations (http://www.somaticmutations-EGFR.info/) maintained by our group [18]. Eligible studies were those that reported or allowed the calculation of hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) comparing overall survival (OS), progression-free survival (PFS) or time-to-progression (TTP) stratified by EGFR gene copy number, for patients receiving monotherapy with either erlotinib or gefitinib. Whenever multiple reports pertained to overlapping groups of patients, we retained only the report with the longest follow-up (largest number of events) to avoid duplication of information. All study designs were considered potentially eligible as long as they provided adequate survival information, whether they were single or multiple arm trials, randomized or nonrandomized, prospective or retrospective. Studies examining EGFR-targeted agents in combination with any other cytotoxic or targeted agent were excluded from the meta-analysis. Case series, defined as studies reporting on 15 or fewer patients, were excluded. Our literature search was limited to published studies, however, when datasets were incomplete for required data corresponding authors were contacted on a planned schedule of timed follow-up and a dataset closure (details available upon request).

Information on one outcome was added following data supplementation and a further six publications were confirmed as either overlapping or updated.

**data extraction**

The following information was recorded from each recovered article: first author, journal and year of publication, number of patients screened, number of patients with increased EGFR gene copy number, number of patients treated with a TKI, specific TKI used, treatment schedules and line of treatment, clinicopathological and demographic data (smoking history, histology, gender, ethnicity), median OS, PFS and TTP of patients by EGFR gene copy number status. The specific methods of gene copy number determination were recorded (fluorescent in situ hybridization (FISH) and chromogenic in situ hybridization (CISH)), as were the methods for gene copy number scoring (CCS or others). Also, we classified studies by line of treatment (first-line or higher). When studies were conducted in mixed treatment settings, we operationally defined studies where at least 80% of patients had received previous chemotherapy as ‘second-line’ studies. Finally, we extracted HRs and their variance for the relevant survival outcomes comparing patients with increased and not increased EGFR gene copy number receiving treatment with either erlotinib or gefitinib. The HR is the most appropriate metric for time-to-event outcomes, such as such as survival and TTP [19, 20]. Unadjusted HRs were preferred over multivariable adjusted ones. When the HR and/or its variance were not provided by the eligible studies, we used the methods developed by Parmar et al. [20] to calculate them. If only the variance of the HR was unavailable, we calculated it using the log-rank $P$ value. When neither HR nor variance was available, we estimated the variance as $(T_1 + T_2)^2/(E_1 + E_2) \times T_1 \times T_2$, where $E_1$ and $E_2$ are the number of events and $T_1$ and $T_2$ the number of randomly assigned patients in each arm, and then estimated the log(RR), such that it would have the $P$ value of the log-rank test. When $P$ values were unavailable, the HR was approximated by the ratio of median survivals [21]. Two authors (IJD and SM) performed data extraction independently and discrepancies were resolved by consensus including a third author (HL).

**data synthesis**

We used the HR and corresponding CI extracted from each study to assess between-study heterogeneity using the $Q$ statistic and inconsistency using the $I^2$ index [22]. Heterogeneity was considered significant at the $P < 0.1$ level. Summary HRs with their 95% CI were calculated using an inverse variance method. We fitted a random-effects model since between-study heterogeneity was anticipated [23]. For the meta-analysis, OS was defined as the primary outcome and PFS and TTP as secondary outcomes. Small-study effects were evaluated using the Begg-Mazumdar and Egger tests [24, 25].

Subgroup analyses were performed to evaluate the effect of ethnicity (East Asian versus white), method of EGFR gene copy number determination (FISH versus CISH), gene copy number scoring criteria (CCS versus ‘other’), the specific EGFR TKI used (gefitinib versus erlotinib) and line of treatment (>80% versus <80% second-line) on the predictive value of EGFR gene copy number. A test for interaction was used for comparisons between subgroups. In addition, we used multivariable random-effects meta-regression to assess whether the effect of participant ethnicity on the predictive ability of EGFR gene copy number was independent of line of treatment [26, 27].

Statistical analyses were conducted with Stata (version SE/10; StataCorp, College Station, TX). $P$ values for all comparisons were two-tailed and statistical significance was defined as $P < 0.05$ for all tests except those for heterogeneity.

**ethics and funding source**

This was a literature-based study and as such no ethics approval was required. There was no funding source associated with the study design,
collection, analysis and interpretation of the data or writing of the report. All authors had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

results

eligible studies

Our initial search yielded 255 studies concerning EGFR-targeted TKI treatment in NSCLC that were assessed in full text. As indicated in the search flow diagram (Figure 1), 22 studies reported at least one of the outcomes of interest and were finally included in the meta-analysis [12, 28–48]. The search flow diagram is summarized in Figure 1 and the characteristics of eligible studies are summarized in Table 1.

Nineteen of the studies employed FISH, two employed CISH and one employed both methods (Table 1). Gene copy number was scored/assessed according to CCS in 18 of the studies, 1 study used a modification of these criteria and another 3 used different scoring schemes. Eleven studies were retrospective and 11 were prospective. All eligible studies were small, with sample sizes ranging from 27 to 257 patients (median size = 63 patients, mean size = 83 patients, standard deviation = 60). Overall, the eligible studies reported on 1821 patients of whom 666 (37%) were characterized as having increased EGFR gene copy number. The frequency increased EGFR gene copy number ranged from 22% to 70%. Fifteen of the studies were conducted in European or North American populations with mixed but mostly white participants (1460 patients, 487 with increased gene copy number; 33%) whereas seven were conducted in East Asian populations (361 patients, 179 with increased gene copy number; 50%).

evidence synthesis

Regarding OS, 20 studies involving 1725 patients (599 with increased gene copy number, 35%) contributed data for the meta-analysis. There was limited between-study heterogeneity (P = 0.1; I² = 30%) and increased gene copy number was significantly associated with improved OS among patients treated with EGFR TKIs, HR = 0.77; 95% CI 0.66–0.89; P = 0.001) (Figure 2). For PFS, 10 studies involving 822 patients (290 with increased gene copy number; 35%) contributed data for the meta-analysis. Moderate between-study heterogeneity was observed (P = 0.03; I² = 51%) and increased EGFR gene copy number was significantly associated with improved PFS (HR = 0.60; 95% CI 0.46–0.79; P < 0.001). Finally, for TTP, only five studies (294 patients, 129 with increased gene copy number, 44%) provided information to be included in the meta-analysis. There was significant between-study heterogeneity (P = 0.002; I² = 77%) and we found a significant TTP benefit for patients with increased EGFR gene copy number (HR = 0.50; 95% CI 0.28–0.91; P = 0.02).

subgroup analysis and assessment of bias

The results of subgroup analysis are presented in Table 2. Increased EGFR gene copy number was statistically significantly associated with increased OS in studies of mixed (predominantly white) populations (HR = 0.70; 95% CI 0.59–0.82; P < 0.001) but not East Asian populations (HR = 1.11; 95% CI 0.82–1.50; P = 0.50). The difference between the

Figure 1. Search strategy and study eligibility flow chart. Abstracts screening was followed by full text data extraction (N = 767). Of 41 potentially eligible articles, 5 performed gene copy number analysis by quantitative PCR (Q-PCR), 9 publications had been subsequently updated one study pertained to combination treatment (TKI + another agent). Data were not extractable only from four studies.
### Table 1. Characteristics of studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Author (year) reference</th>
<th>Patients (N)</th>
<th>Ethnicity</th>
<th>Gender (M/F)</th>
<th>Adenocarcinoma histology, n (%)</th>
<th>Increased EGFR gene copy number, n (%)</th>
<th>Detection method</th>
<th>Score criteria</th>
<th>TKI</th>
<th>Study design</th>
<th>EGFR mutation frequency (%)</th>
<th>Outcomes reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hirsch (SWOG) (2005) [35]</td>
<td>81</td>
<td>Mixed</td>
<td>40/41</td>
<td>81 (100)</td>
<td>26 (32)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Prospective</td>
<td>NR</td>
<td>OS, PFS</td>
</tr>
<tr>
<td>Han (2006) [34]</td>
<td>66</td>
<td>East Asian</td>
<td>NR</td>
<td>31 (47)</td>
<td>18 (22)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>18</td>
<td>OS, PFS</td>
</tr>
<tr>
<td>Hirsch (ISEL) (2006) [36]</td>
<td>257</td>
<td>Mixed (predominantly white)</td>
<td>NR</td>
<td>78 (30)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Prospective</td>
<td>12</td>
<td>OS</td>
<td></td>
</tr>
<tr>
<td>Buckingham (2007) [28]</td>
<td>81</td>
<td>Mixed (predominantly white)</td>
<td>37/34</td>
<td>56 (69)</td>
<td>18 (22)</td>
<td>FISH</td>
<td>Othera</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>67</td>
<td>OS, PFS</td>
</tr>
<tr>
<td>Cappuzzo (ONCOBEL) (2007) [29]</td>
<td>36</td>
<td>Mixed (predominantly white)</td>
<td>10/26</td>
<td>25 (70)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Prospective</td>
<td>38</td>
<td>TTP</td>
<td></td>
</tr>
<tr>
<td>Massarelli (2007) [39]</td>
<td>59</td>
<td>Mixed (predominantly white)</td>
<td>33/26</td>
<td>39 (66)</td>
<td>32 (54)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>29</td>
<td>OS, TTP</td>
</tr>
<tr>
<td>Soh (2007) [42]</td>
<td>74</td>
<td>East Asian</td>
<td>50/24</td>
<td>60 (81)</td>
<td>31 (42)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>11</td>
<td>OS, PFS</td>
</tr>
<tr>
<td>Sone (2007) [43]</td>
<td>54</td>
<td>East Asian</td>
<td>NR</td>
<td>NR</td>
<td>26 (48)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>11</td>
<td>OS, TTP</td>
</tr>
<tr>
<td>Crino (2008) [31]</td>
<td>77</td>
<td>Mixed (predominantly white)</td>
<td>NR</td>
<td>30 (39)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Prospective</td>
<td>54</td>
<td>OS, PFS</td>
<td></td>
</tr>
<tr>
<td>Dongiovanni (2008) [47]</td>
<td>43</td>
<td>White</td>
<td>NR</td>
<td>NR</td>
<td>13 (30)</td>
<td>FISHb/CISH</td>
<td>Otherb</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>56</td>
<td>OS, TTP</td>
</tr>
<tr>
<td>Felip (2008) [32]</td>
<td>57</td>
<td>White</td>
<td>NR</td>
<td>NR</td>
<td>15 (26)</td>
<td>FISH</td>
<td>CCS</td>
<td>Erlotinib</td>
<td>Prospective</td>
<td>6</td>
<td>OS</td>
</tr>
<tr>
<td>Kim (2008) [37]</td>
<td>185</td>
<td>Mixed</td>
<td>NR</td>
<td>NR</td>
<td>85 (46)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Prospective</td>
<td>67</td>
<td>OS</td>
</tr>
<tr>
<td>Maruyama (2008) [38]</td>
<td>60</td>
<td>East Asian</td>
<td>NR</td>
<td>NR</td>
<td>42 (70)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Prospective</td>
<td>17</td>
<td>PFS</td>
</tr>
<tr>
<td>Miller (2008) [46]</td>
<td>76</td>
<td>Mixed (predominantly white)</td>
<td>NR</td>
<td>24 (32)</td>
<td>CISH</td>
<td>Otherc</td>
<td>Erlotinib</td>
<td>Prospective</td>
<td>NR</td>
<td>OS, PFS</td>
<td></td>
</tr>
<tr>
<td>Sasaki (2008) [49]</td>
<td>27</td>
<td>East Asian</td>
<td>14/13</td>
<td>22 (81)</td>
<td>12 (44)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>61</td>
<td>OS</td>
</tr>
<tr>
<td>Schneider (2008) [41]</td>
<td>206</td>
<td>Mixed (predominantly white)</td>
<td>NR</td>
<td>49 (24)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Erlotinib</td>
<td>Prospective</td>
<td>29</td>
<td>OS, PFS</td>
</tr>
<tr>
<td>Wen-Cheng Chang (2008)</td>
<td>36</td>
<td>East Asian</td>
<td>16/20</td>
<td>33 (92)</td>
<td>22 (61)</td>
<td>CISH</td>
<td>Modified CCSd</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>18</td>
<td>OS</td>
</tr>
<tr>
<td>Zhu (2008) [45]</td>
<td>102</td>
<td>Mixed (predominantly white)</td>
<td>NR</td>
<td>35 (34)</td>
<td>FISH</td>
<td>CCS</td>
<td>Erlotinib</td>
<td>Prospective</td>
<td>10</td>
<td>OS</td>
<td></td>
</tr>
<tr>
<td>Goss (2009) [33]</td>
<td>44</td>
<td>Mixed (predominantly white)</td>
<td>NR</td>
<td>NR</td>
<td>12 (27)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Prospective</td>
<td>21</td>
<td>OS, PFS</td>
</tr>
<tr>
<td>Tiseo (2009) [48]</td>
<td>54</td>
<td>White</td>
<td>NR</td>
<td>NR</td>
<td>12 (22)</td>
<td>FISH</td>
<td>CCS</td>
<td>Gefitinib</td>
<td>Retrospective</td>
<td>15</td>
<td>OS</td>
</tr>
</tbody>
</table>

**FISH scoring criteria:** CCS: Patients are classified into six strata with ascending number of copies of the EGFR gene per cell according to the frequency of tumor cells with specific number of copies of the EGFR gene and chromosome 7 centromere: (i) disomy (≤2 copies in >90% of cells); (ii) low trisomy (2 copies in 40% of cells, three copies in 10%–40% of the cells, 4 copies in <10% of cells); (iii) high trisomy (5 copies in ≥40% of cells, three copies in 40% of cells, ≥4 copies in <10% of cells); (iv) low polysomy (≥4 copies in 10%–40% of cells); (v) high polysomy (≥4 copies in ≥40% of cells); and (vi) gene amplification (defined by presence of tight EGFR gene clusters and a ratio of EGFR gene to chromosome of ≥2 or ≥4 copies of EGFR per cell in ≥10% of analyzed cells). FISH positivity is defined as high polysomy or gene amplification. Mutation frequency is the percentage of EGFR mutation positive patients in the same report from which we extracted amplification data, but the populations assessed for mutation and amplification are not always identical (due to sample availability). Percentages have been rounded to the nearest integer. 

*a East Asian’ denotes studies where >90% of participants were of East Asian descent; ‘white’ denotes studies where >90% of participants were of Caucasian ethnic descent; all other studies were classified as mixed (with the qualification ‘predominantly white’ when >50% of patients were of Caucasian descent).

b One study (Wen-Cheng Chang et al.) modified the high polysomy criterion of Cappuzzo et al. from four to five copies, as four copies was very easy to be confused with two partially overlapping disomy signals of two nuclei, as encountered when using CISH.

c This study reported negative cases as those with balanced disomy (1.6–2.0) and for gene and chromosome; and FISH-positive were those with balanced trisomy (2.2–3.0) and balanced polysomy (3.1–4.4) for gene and chromosome when at least 10 cells showed genomic gain.

d This study, using CISH as the detection method, scored at least 30 nuclei (with a light microscope using a ×40 objective) and considered a test positive for increased gene copy number when the average number of gene copies was >5 per nucleus.
the study's primary outcome, i.e. OS (Begg's test analysis. Finally, there was no evidence of small-study effects for a limited number of studies precluded meaningful subgroup evaluating both gefitinib and erlotinib therapy. For PFS, in all studies of predominantly white patients and in studies statistical significance was achieved in favor of patients with between subgroup differences (Table 2). For OS, nominal studies for meta-analysis, we could not detect any significance evaluated, and for both outcomes with sufficient published calculations are shown by diamonds. Values lower than one indicate that gene copy number have improved survival compared to patients with no increase in EGFR gene copy number.

two estimates was statistically significant (P = 0.02) (Figure 2). When adjusted for line of treatment, the interaction of ethnicity with the predictive ability of EGFR gene copy number remained statistically significant (P = 0.03). For all other subgroups evaluated, and for both outcomes with sufficient published studies for meta-analysis, we could not detect any significance between subgroup differences (Table 2). For OS, nominal statistical significance was achieved in favor of patients with increased EGFR gene copy number in prospective studies, in studies of predominantly white patients and in studies evaluating both gefitinib and erlotinib therapy. For PFS, in all subgroups investigated, a clear benefit in favor of patients with increased EGFR gene copy number was observed. For TTP, the limited number of studies precluded meaningful subgroup analysis. Finally, there was no evidence of small-study effects for the study’s primary outcome, i.e. OS (Begg’s test P value = 0.77; Egger’s test P value = 0.70).

discussion

Increased EGFR gene copy number has been proposed as a candidate biomarker for predicting response and/or survival benefit for advanced lung cancer patients receiving treatment with TKIs. In fact, several authors have proposed that increased EGFR gene copy number, compared with somatic EGFR mutations, may be a better marker for identifying a subgroup of patients with the potential of gaining a survival benefit from EGFR TKIs [49]. We have reviewed the published literature on EGFR gene copy number and identified 22 independent studies assessing its utility for predicting survival benefit. A meta-analysis of these studies confirms that increased EGFR gene copy number is indeed associated with a moderate OS benefit and a substantial PFS benefit from EGFR TKI treatment. Interestingly, the predictive value of EGFR gene copy number on OS appears to be limited to studies of predominantly white populations.

Several authors have commented that statistically significant effects on PFS are insufficient for obtaining reliable evidence of important clinical benefit on OS [50]. Yet, the magnitude and statistical significance of the effect on PFS detected by this meta-analysis, along with the congruent effects on OS and TTP, suggest that increased EGFR gene copy number is associated with improved survival outcomes compared with wild-type EGFR among patients treated with TKIs. Furthermore, PFS has been considered a direct measure of the effect of treatment on the tumor burden, is sensitive to cytostatic as well as cytotoxic mechanisms of TKIs and incorporates the clinically relevant event of death [50]. In addition, several lines of empirical evidence link benefits in PFS to benefits in OS, both in NSCLC and other solid malignancies [51–53].

For OS, we found a statistically significant interaction between ethnicity and the predictive ability of EGFR gene copy number, which appears to be independent of line of treatment. Increased gene copy number was associated with a 30% improvement in OS among studies enrolling predominantly white individuals but did not appear to have an effect in studies enrolling individuals of East Asian descent. Considering the number of studies is limited and adjustment for potential confounders is not possible given the aggregate nature of the data, this difference should be interpreted with caution [54]. Even so, it is a hypothesis-generating observation that may have grounding in the biology of the disease [55–57].

We have previously shown that the incidence of somatic EGFR mutations is indeed different between whites and Asians, wherein individuals of East Asian decent tend to have a higher incidence of mutations [18]. Other authors have also indicated that the frequency of KRAS mutations (which are mutually exclusive with somatic EGFR mutations) is much lower in East Asians compared with whites [55]. It is not clear if these differences reflect differences in the underlying population genetics, are the product of differences in tobacco use patterns or a combination of these factors [56, 57]. Evidence suggests that increased gene copy number and EGFR mutations often coexist [36]. This appears to fall in line with data from the studies included in this meta-analysis where the East Asian populations had a higher proportion of increased gene copy number compared with predominantly white populations (50% versus 31%). Alternatively, differences in the constitutional genetic background between populations may account for the ‘ethnicity’ effect [58]. However, it is obvious from the limited data that currently exists, that it will be some time before we have a more robust understanding of the nature of what constitutes an ever-expanding list of candidate
predictive biomarkers in NSCLC, let alone the influence of individuals’ constitutional genetic make-up or its interaction with somatic genetic aberrations. No other between-subgroup difference was observed for OS. Regarding PFS, all subgroups indicated a significant benefit in favor of patients with increased **EGFR** gene copy number compared with patients with no increase, under treatment with TKIs. This may be taken to suggest that timing (line of treatment) of TKI use or differences in treatment strategies following TKI failure account for the ethnicity–biomarker effect observed for OS.

This study provides the first comprehensive assessment of the predictive value of **EGFR** gene copy number among patients receiving TKI monotherapy. In view of the small sample sizes of published studies and the relatively small magnitude of effects for OS, meta-analysis appears to be the only method to increase statistical power and investigate study level covariates that may influence the predictive ability of **EGFR** gene copy number. Our results indicate that among patients treated with EGFR TKIs, those with increased gene copy number have improved OS, PFS and TTP. We caution that these results do not imply that treatment choice should be based on **EGFR** gene copy number; such analysis would require randomized controlled trials of TKIs versus alternative treatments (or placebo) and the investigation of treatment-by-biomarker interactions or trials directly comparing testing and no-testing strategies. It should be noted that such studies are costly and alternative designs may be more appealing, such as ‘prospective-retrospective’ studies [59, 60], as exemplified by the biomarker analyses of the ISEL and BR.21 studies [36, 45].

Several limitations need to be taken into account when interpreting our results, primarily the unavailability of individual patient data that would allow correction for potential confounding factors such as, age, gender, smoking habits, lung cancer histology, or additional genetic aberrations (somatic **EGFR** or **KRAS** mutations). Given that literature-based approaches are unlikely to yield adequate information for

Table 2. Subgroup analyses for overall and progression-free survival for treatment with EGFR tyrosine kinase inhibitors, comparing patients with increased versus not increased EGFR gene copy number. Subgroup analysis was performed when at least two studies were in each subgroup

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Overall survival</th>
<th>Progression-free survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of studies, heterogeneity ($P_Q; I^2$)</td>
<td>HR (95% CI); $P$ value</td>
</tr>
<tr>
<td></td>
<td>Number of studies, heterogeneity ($P_Q; I^2$)</td>
<td>HR (95% CI); $P$ value</td>
</tr>
<tr>
<td>All studies</td>
<td>20 (0.1; 30)</td>
<td>0.77 (0.66–0.89); 0.001</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>14 (0.19; 24)</td>
<td>0.70 (0.59–0.82); 0.001</td>
</tr>
<tr>
<td>East Asian</td>
<td>6 (0.72; 0)</td>
<td>1.11 (0.82–1.50); 0.50</td>
</tr>
<tr>
<td>Tyrosine kinase inhibitor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gefitinib</td>
<td>16 (0.04; 42)</td>
<td>0.79 (0.65–0.96); 0.02</td>
</tr>
<tr>
<td>Erlotinib</td>
<td>4 (0.85; 0)</td>
<td>0.70 (0.55–0.89); 0.004</td>
</tr>
<tr>
<td>Study design</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prospective</td>
<td>9 (0.29; 17)</td>
<td>0.74 (0.62–0.89); 0.001</td>
</tr>
<tr>
<td>Retrospective</td>
<td>11 (0.07; 42)</td>
<td>0.81 (0.62–1.06); 0.12</td>
</tr>
<tr>
<td>Gene copy number scoring system</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCS</td>
<td>15 (0.16; 27)</td>
<td>0.82 (0.70–0.97); 0.02</td>
</tr>
<tr>
<td>Other scoring system</td>
<td>5 (0.59; 30)</td>
<td>0.54 (0.39–0.75); 0.001</td>
</tr>
<tr>
<td>Gene copy number assessment method$^a$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FISH</td>
<td>17 (0.13; 29)</td>
<td>0.79 (0.67–0.92); 0.003</td>
</tr>
<tr>
<td>CISH</td>
<td>2 (0.67; 0)</td>
<td>0.75 (0.39–1.44); 0.39</td>
</tr>
<tr>
<td>Line of treatment$^b$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;80% second-line or higher</td>
<td>7 (0.04; 56)</td>
<td>0.95 (0.59–1.52); 0.83</td>
</tr>
<tr>
<td>280% second-line or higher</td>
<td>12 (0.43; 1)</td>
<td>0.73 (0.63–0.83); 0.001</td>
</tr>
</tbody>
</table>

Subgroup analysis was not performed for TTP as only three studies provided information for this outcome.

$^a$One study [47] used both CISH and FISH and was excluded from the subgroup analysis by different gene copy number assessment methods.

$^b$One study [30] did not report line of treatment and was excluded from the subgroup analysis.

CCS, Colorado Classification System; CI, confidence interval; CISH, chromogenic in situ hybridization; FISH, fluorescent in situ hybridization; HR, hazard ratio; NA, not applicable.
such an analysis, we have long been advocating a global consortium to address these issues [18, 61]. We were unable to disentangle the predictive effect of EGFR gene copy number from that of EGFR mutations because of the lack of published relevant data and the potential for bias that could result from the use of group level data on mutational frequency [62]. Similarly, we were unable to differentiate between EGFR amplification and chromosome 7 polysomy since separate survival estimates were not available from the primary studies. Our analysis has included data from several pivotal prospective trials, such as INTEREST, ISEL, BR.21, INVITE, ONCOBELL, SWOG S0126, and V-15-32. Furthermore, the differences observed based on ethnicity will need further investigation because of the relatively limited number of Asian studies. It should also be noted that tissue availability has been a limiting factor in most EGFR inhibitor prospective trials. For example, tissue was available for EGFR copy number assessment from only 159 (102 erlotinib treated) of the 731 patients who were randomized in the BR.21 study [45]; similar experiences have also been noted for other clinical studies [61, 63, 64]. Limited tissue availability once again highlights the potential of using evidence synthesis methodology to attain increased statistical power in such situations.

In conclusion, this systematic review provides evidence that EGFR gene copy number is a predictive marker for survival among patients receiving TKI monotherapy for advanced or recurrent NSCLC. The predictive effect of EGFR gene copy number appears to be significantly stronger among North American and Eastern European populations (predominantly white) compared to East Asian populations. Further research should potentially focus on investigating the underlying causes of this discrepancy (be they genetic, environmental or simply a statistical artifact of the published literature) and the incorporation of additional genetic biomarkers in predicting patient outcomes.

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**disclosure**

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