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Clinical outcomes in non-small-cell lung cancer patients with *EGFR* mutations: pooled analysis

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Abstract

Non-small-cell lung cancer (NSCLC) with mutations in the epidermal growth factor receptor (EGFR) is a distinct subgroup of NSCLCs that is particularly responsive to EGFR tyrosine-kinase inhibitors (TKIs). A weighted pooled analysis of available studies was performed to evaluate clinical outcome in patients with *EGFR*-mutated NSCLC who were treated with chemotherapy or EGFR TKIs. Median progression-free survival (PFS) times were pooled from prospective or retrospective studies that evaluated chemotherapy or single-agent EGFR TKIs (erlotinib or gefitinib) in patients with NSCLC and *EGFR* mutations. Among the studies identified for inclusion in the analysis, 12 evaluated erlotinib (365 patients), 39 evaluated gefitinib (1069 patients) and 9 evaluated chemotherapy (375 patients). Across all studies, the most common *EGFR* mutations were deletions in exon 19 and the L858R substitution in exon 21. In the weighted pooled analysis, the overall median PFS was 13.2 months with erlotinib, 9.8 months with gefitinib and 5.9 months with chemotherapy. Using a two-sided permutation, erlotinib and gefitinib produced a longer median PFS *versus* chemotherapy, both individually ($P = 0.000$ and $P = 0.002$, respectively) and as a combined group (EGFR TKI *versus* chemotherapy, $P = 0.000$). EGFR TKIs appear to be the most effective treatment for patients with advanced *EGFR*-mutant NSCLC. Ongoing prospective trials comparing the efficacy of first-line chemotherapy and EGFR TKIs in *EGFR*-mutant disease should provide further insight into the most appropriate way to treat this specific group of patients.

Keywords: adenocarcinoma • chemotherapy • epidermal growth factor receptor • erlotinib • gefitinib • lung cancer • mutation • tyrosine kinase inhibitor

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Introduction

Molecular techniques have allowed us to redefine the ways in which we describe cancer. In the lung cancer setting, it is becoming increasingly apparent that non-small-cell lung cancer (NSCLC) may not only be subgrouped according to tumour histology but also by the aberrant coding, expression or activation of various proteins. The best studied example of this is the subgroup of tumours with mutations in the gene encoding the epidermal growth factor receptor (EGFR). These mutations may generate a distinct molecular pathway in the pathogenesis of NSCLC, responsible for the aetiology seen in this group of patients, and appear to confer sensitivity to the EGFR tyrosine-kinase inhibitors (TKIs) erlotinib [1] and gefitinib [1–3].

Erlotinib and gefitinib have been extensively investigated in unselected patients with NSCLC. In the phase III BR.21 study, which was performed in advanced NSCLC patients who had failed previous chemotherapy, erlotinib produced a significant overall survival (OS) benefit relative to placebo in the whole study population ($P < 0.001$), as well as significant improvements in progression-free survival (PFS), overall response rate, disease control rate, tumour-related symptoms and patient quality of life [4, 5]. In contrast, the pivotal phase III placebo-controlled study of gefitinib in previously treated advanced NSCLC patients, Iressa Survival Evaluation in Lung Cancer (ISEL), failed to demonstrate a statistically significant improvement in OS with gefitinib in the overall study population [6]. Second-line gefitinib was shown to be non-inferior to docetaxel in a multi-national study Iressa Non-Small-Cell Lung Cancer Trial Evaluating Response and Survival Against Taxotere (INTEREST) [7], but not in a similarly designed trial performed in Japanese patients (V15–32) [8]. A recent meta-analysis including data from INTEREST, V15–32 and two other (open-label) studies of gefitinib *versus* docetaxel (second-line indication of gefitinib in NSCLC [SIGN] [9] and Iressa as Second-Line Therapy in Advanced NSCLC-Asia [ISTANA] [10]) concluded that the efficacy of gefitinib was similar to that of docetaxel in previously treated advanced NSCLC (hazard ratio [HR] for OS 1.03, 95% confidence interval [C.I.] 0.93–1.13) [11]. As two of the studies included in this meta-analysis were conducted exclusively in Asia, 43% of the 2257 patients were of Asian ethnicity.

Biomarker analyses in the BR.21, ISEL, INTEREST and sequential Tarceva in UN resectable NSCLC (SATURN) studies [12–15] have indicated that patients with *EGFR* mutations have a pronounced response to EGFR TKIs; however, the relatively low rate of mutations and the limited availability of tumour samples from randomized trials make it difficult to conduct large-scale investigations of possible associations between *EGFR* mutations and therapeutic outcome. For this reason, we have performed an in-depth literature review to examine the evidence surrounding *EGFR*-mutated NSCLC and have then conducted a pooled analysis of available studies to evaluate clinical outcome in patients with *EGFR*-mutated NSCLC, who have been treated with chemotherapy or EGFR TKIs.

Literature review

An in-depth review of the published literature was undertaken. The PubMed database was searched on June 11, 2009 using the search string: ((lung[title] OR NSCLC[title] OR adenocarcinoma[title]) AND ('epidermal growth factor receptor' [title] OR EGFR[title]) AND (mutation*)) AND English[language]. A total of 499 papers were identified. Titles were scanned for relevant papers that add to the body of evidence describing *EGFR*-mutated NSCLC for inclusion in the literature review. Reports from the 2009 Annual Meeting of the American Society of Clinical Oncology (ASCO) were also included in the review in order to accommodate the latest information in the field. Reference lists in key reviews were also scanned to identify any other papers of interest. Because of space restrictions, only key or summary papers have been cited in this literature review.

Molecular biology of *EGFR* mutations

EGFR is a 170-kDa member of the ErbB or human epidermal receptor (HER) family of tyrosine kinase (TK) growth factor receptors. Following binding of specific ligands, the transmembrane receptor homodimerizes with another EGFR protein or heterodimerizes with related proteins (primarily HER2/ErbB2). Once the dimer is formed, the intracellular enzymatic subunit of one EGFR phosphorylates several tyrosines of the other protein. This leads to the recruitment of additional intracellular signalling molecules, which begin a cascade that activates specific cellular growth and differentiation pathways [16]. *EGFR* has also been identified as a cellular proto-oncogene with close sequence homology to the viral oncogene *v-erb-B* [17] and is expressed in a variety of solid tumours, including lung cancer [18]. Although the mechanisms by which EGFR contributes to a malignant phenotype have not been fully elucidated, it is clear that tumourigenic pathways such as the ras-raf-MEK-ERK pathway and the PI3K/AKT pathway are mediated by EGFR activation, leading to tumour progression, proliferation, evasion from apoptosis, angiogenesis, invasion and metastatic spread [19–21].

Mutations in the region of the *EGFR* gene that encodes the TK domain of the receptor were first reported in patients with NSCLC in 2004 [2, 3]. Paez and colleagues amplified and sequenced the *EGFR* gene from 119 unselected patients with NSCLC. Somatic deletion and missense mutations were identified in 16 of the samples. When *EGFR* was sequenced in five patients who had responded to gefitinib and four patients who had progressed on gefitinib, mutations were found in all of the responders but none of those with disease progression. Lynch's group published a report on the same day that revealed somatic heterozygous mutations clustered around the adenosine triphosphate (ATP)-binding pocket of the TK domain in eight of nine patients with primary NSCLC who had responded to gefitinib, while no mutations were found in tumour cells from patients who had not responded to gefitinib. Furthermore, *in vitro* studies showed that mutant receptors were functional, more responsive to endogenous ligands, and

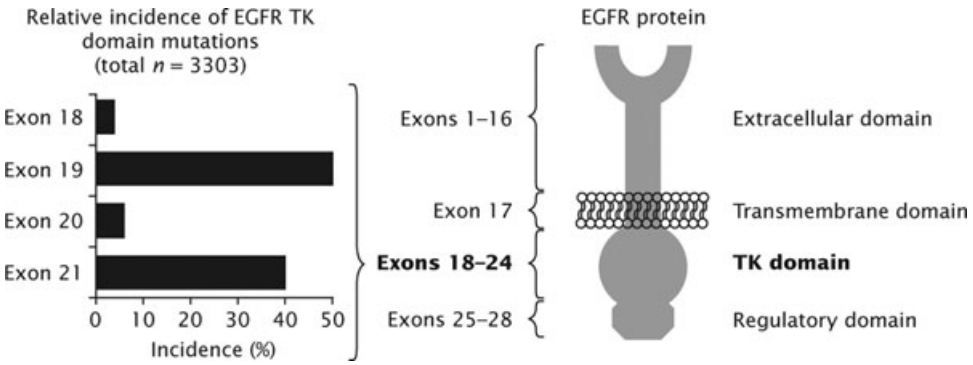

Fig. 1 Sites of common activating mutations in exons 18–21 of the epidermal growth factor receptor gene [22, 23].

more sensitive to gefitinib than wild-type receptors. In a similar fashion, Pao *et al.* sequenced exons 18–24 of the *EGFR* gene from seven patients with bronchioloalveolar carcinoma (BAC) who had responded to erlotinib in a phase II trial, five of whom were shown to possess mutations [1]. In contrast, analysis of tumour tissue from 10 patients in the trial who did not respond to erlotinib did not reveal any *EGFR* mutations.

The discovery of somatic *EGFR* mutations in some patients with NSCLC was a very significant breakthrough in the understanding of this disease. Further molecular studies performed by a number of research groups have shown that *EGFR* mutations occur almost exclusively within exons 18–21 of the gene, which encodes the amino lobe and part of the carboxy lobe of the receptor (Fig. 1). Murray and colleagues have compiled an extensive database of published literature, containing data from over 12,000 patients, that has identified 254 independent somatic mutations in this region of the gene [22]. The most common mutations involve point mutations in exon 18, deletions and/or insertions in exon 19, insertions/duplications and point mutations in exon 20 and point mutations in exon 21. One mutation in exon 20 (T790M) appears to confer resistance to EGFR TKIs, although this is very rare among EGFR TKI-naïve patients [22]. Other mutations, occurring in the region of the *EGFR* gene encoding the TK domain, appear to confer sensitivity to EGFR TKIs. The best characterized of these are deletions in exon 19 around the ATP-binding cleft of the receptor (particularly E746-A750del) and a missense mutation in exon 21 (L858R) (Fig. 1); in this review we focus on these mutations.

Mutations in the *EGFR* gene may lead to the stimulation of oncogenic pathways. For example, NIH-3T3 cells expressing EGFR with L858R and G719S mutations underwent oncogenic transformation, even when no ligand was present [24]. Furthermore, injection of the clonal, transformed NIH-3T3 fibroblasts into immunocompromised mice led to the formation of tumours. In contrast, no transformation or tumour growth occurred when similar experiments were performed with wild-type EGFR. Ji and colleagues have provided further evidence to support the oncogenic potential of mutated EGFR: they created bitransgenic mice with inducible expression of two common EGFR mutants seen in human lung cancer. Both transgenic lines developed lung adenocarcinoma after sustained mutant EGFR expression, with tumour maintenance dependent on the continued expression of the mutated proteins [25]. Similar observations in mutant EGFR transgenic mice were reported by Politi's group [26].

The mechanism by which mutated EGFR induces an oncogenic state has not been fully elucidated. The mutated receptors may be constitutively activated, take longer to de-phosphorylate following ligand activation, or lead to the aberrant phosphorylation of other proteins [2, 27–34]. Experiments with the kinase domain of EGFR have shown that the L858R mutated form is a faster enzyme than the wild-type form. Specifically, the turnover number k_{cat} is increased by an order of magnitude and the mutation seems to disrupt autoinhibitory interactions so that the enzyme is 'locked' in the constitutively active conformation [35]. Mutated EGFR may also preferentially form heterodimers with different proteins to wild-type EGFR; for example, mutated EGFR is associated with increased expression of HER3 (ErbB3) [36] and, unlike wild-type EGFR, may preferentially bind with this protein [37]. These aberrant activities may generate sustained activation of intracellular pathways and promotion of a cancer phenotype. It has been shown that mutated EGFR is a potent activator of particular downstream pathways, including ras-raf-MEK-ERK, PI3K/AKT, STAT3 and STAT5 [36, 38–41]. The ras-raf-MEK-ERK pathway is particularly involved in cell proliferation and the PI3K/AKT pathway is particularly involved in cell survival. Indeed, EGFR-mutant tumours appear to be strongly dependent on activation of the PI3K/AKT pathway for survival, leading to the suggestion that mutated EGFR is an 'addicting oncogene' – in other words, tumours with EGFR mutations may actually require hyperactivated EGFR for survival [25, 26, 42]. This has implications for therapy, as blockade of EGFR may be necessary in such patients to re-establish apoptotic signalling and undermine tumour maintenance or growth.

Clinical characteristics of NSCLC patients with *EGFR* mutations

NSCLC patients with specific clinical features appear to have a greater likelihood of having a mutated form of *EGFR*. Analysis of 14 studies involving 2880 patients showed *EGFR* mutations occur more frequently in women than men (38% *versus* 10%), Asian than non-Asian patients (32% *versus* 7%), never-smokers than current or former smokers (47% *versus* 7%) and patients with

adenocarcinoma histology than non-adenocarcinoma histology (30% *versus* 2%) [43]. Furthermore, *EGFR* mutations have been largely associated with specific adenocarcinoma histologies: BAC, invasive adenocarcinomas with prominent lepidic growth and papillary adenocarcinomas [44–52]. In particular, *EGFR* mutations may be more common in the macropapillary subset of lung adenocarcinomas, a particularly aggressive lung cancer type [44, 53, 54].

While *EGFR* mutations appear more frequently in patients with certain clinical characteristics, no characteristics have been identified that are sufficient or necessary for such mutations to occur. This can be demonstrated by analysis of the clinical characteristics of patients enrolled in a recent trial performed by the Spanish Lung Cancer Group: while all patients had advanced NSCLC with *EGFR* mutations, a notable portion were male (30%), former smokers (26%) or had non-adenocarcinoma/BAC histology (9%) [55]. This suggests that it is not appropriate to rule the possibility of an *EGFR* mutation in or out on the grounds of gender, ethnicity, histology or smoking status. Therefore, in the future, diagnoses will need to determine not only the type of disease and histology of tumours, but also their molecular characteristics, including *EGFR* mutation status. As a consequence, the role of pathologists is likely to evolve to incorporate relevant molecular techniques.

Genetic characteristics of EGFR-mutated tumours

While it is not yet known why certain patients are susceptible to the development of *EGFR* mutations, *EGFR*-mutated tumours may have distinct genetic characteristics from tumours expressing wild-type EGFR. Several studies have indicated that there is a strong correlation between *EGFR* mutations and increased *EGFR* gene copy number, as determined by fluorescence *in situ* hybridization (FISH) or chromogenic *in situ* hybridization [56–58]. Further to this, a Japanese group used laser-capture microdissection and array comparative genomic hybridization to show that adenocarcinomas with *EGFR* mutations have significantly more genetic copy number alterations than wild-type tumours ($P = 0.01$) [59]. This study focused on 800 chromosomal loci containing cancer-related genes and discovered 58 loci that showed significant differences in the frequency of copy number alterations between *EGFR* wild-type and mutated tumours, including amplification of the mutated *EGFR* gene itself. When a supervised hierarchical clustering technique was used to classify tumours according to 46 selected loci, two distinct groups were formed: one containing wild-type tumours only and one containing primarily *EGFR*-mutated tumours. Another recent study showed a distinct pattern of alteration in tumour samples from patients with *EGFR* mutations compared with patients with wild-type *EGFR* and *KRAS* mutations [60]. In this analysis, genome-wide single nucleotide polymorphism assay screening for allelic imbalance in patients with adenocarcinoma revealed one region on chromosome 14 (14q21.3) and three regions on chromosome 7 (7p21.3-p21.2, 7p21.3 and 7p21.2–7p15.3) that were significantly amplified in *EGFR*-mutated tumours compared with wild-type tumours. *EGFR*-mutated tumours also showed homozygous deletions at *CDKN2A*, which encodes the tumour suppressor cyclin-dependent kinase inhibitor 2A, and loss of heterozygosity at *RB1*, the gene encoding the tumour-suppressing retinoblastoma protein. Patients with *EGFR* mutations have also been shown to have a distinct pattern of methylation of several tumour suppressor genes compared with patients with *KRAS* mutations, which may affect the function of the tumour suppressor genes [61]. Further adding to the hypothesis that *EGFR*-mutated NSCLC may be a distinct subtype of disease is the results of a recent integrated genomic analysis of lung adenocarcinoma [62]. In this study, *EGFR*-mutant tumours were clustered according to genomic copy number alterations into two particular classifications, different from the clusters that *KRAS*-mutant tumours fell into. This non-random pattern of copy number alterations associated with different mutations suggests that distinct oncogenic pathways may be differentiated by co-ordinated genetic alterations. Furthermore, this analysis showed that *EGFR*-mutant adenocarcinomas are associated with underexpression of DUSP4, a protein involved in negative feedback of EGFR signalling. This underexpression may further perpetuate EGFR hyperactivity in *EGFR*-mutant disease. In contrast, *KRAS*-mutant tumours are associated with overexpression of DUSP4.

Interestingly, there appears little association between the presence of *EGFR* mutations and mutations seen in other NSCLCs [63] including *BRCA* mutations [62, 64], *KRAS* mutations [48, 50, 62, 65], *BRAF* mutations [62], *HER2* mutations [62], *LKB1* mutations [62], *p16* homozygous deletions [66] and *p53* mutations [65]. *EGFR* mutations may therefore be clonal events with high oncogenic potential that reduces the likelihood of further mutations and leads to oncogenic addiction of cancer cells. This suggests that *EGFR*-mutated NSCLC has a different genetic basis to other forms of NSCLC and this may have implications for treatment.

Prognostic significance of NSCLC with *EGFR* mutations

The prognostic significance of *EGFR* mutations in NSCLC, independent of other features, is not clear. However, the available data suggest that any prognostic impact of *EGFR* mutations is likely to be small. In a large cohort of Japanese patients with lung adenocarcinoma who had undergone potentially curative pulmonary resection, the presence of an *EGFR* mutation was associated with longer survival than the absence of a mutation in a univariate analysis; however, the significance of this association was lost in multivariate analysis [65]. Likewise, in a series of unselected patients with NSCLC from Japan, Taiwan, USA and Australia, OS was not significantly longer in patients with *EGFR* mutations compared with those without mutations, although there was a trend for decreased survival in patients with exon 19 deletions and increased survival in patients with L858R mutations [46]. Analysis of the recent Iressa Pan-ASia Study (IPASS), which compared first-line gefitinib with carboplatin/paclitaxel in East Asian patients with adenocarcinoma who were never-smokers or light ex-smokers, found that there were no major differences in OS among patients with or without *EGFR* mutations in the chemotherapy group [67].

These observations are further validated by a biomarker analysis of the recently completed phase III SATURN study that evaluated erlotinib as maintenance therapy in patients with NSCLC treated with first-line platinum-containing chemotherapy. In the group of patients who received four cycles of chemotherapy followed by placebo, the presence of an *EGFR* mutation did not influence PFS (HR 0.78; 95% C.I. 0.52−1.22) [15].

How do structural changes in EGFR lead to greater sensitivity to EGFR TKIs?

Seminal studies by Paez and Lynch's groups showed that NSCLC cell lines expressing EGFR with a L858R missense mutation are more sensitive to gefitinib than wild-type receptors [2, 3], a finding that has also been demonstrated by other groups [24, 33, 35]. Analyses of the kinetic parameters of wild-type EGFR and the L858R and del747–753 mutants have revealed that the mutant forms bind ATP less tightly than the wild-type enzyme [35, 68]. Since erlotinib and gefitinib bind to the same pocket as ATP, it may be expected that they are affected in a similar manner to ATP by mutations in this domain. Surprisingly, however, the opposite is the case: depending on the assay, erlotinib and gefitinib bind similarly [69] or even more tightly [35, 68] to the mutant forms of EGFR. It is this unique property that allows the TKIs to target the mutated EGFR so effectively. While both the L858R mutation and del747−753 are highly sensitive to both erlotinib and gefitinib [70], some *in vitro* studies have found that EGFR with exon 19 deletions or L858R, G719S, V742A or R766C substitutions are more sensitive to erlotinib [70, 71]. As previously discussed, *EGFR* mutations may be associated with *EGFR* amplification [56–58, 72]. Such gains in gene copy number may also be related to EGFR TKI sensitivity [73], and this highlights the need to consider the contribution of patients with *EGFR* mutation-positive disease when analysing other potential biomarkers.

In the clinical setting, NSCLC patients with *EGFR* mutations have shown dramatic responses to EGFR TKIs. In IPASS, gefitinib produced a response rate of 71% among 132 Asian patients with *EGFR* mutations [67]. In a Spanish study involving 197 evaluable patients with *EGFR* mutations, erlotinib produced a response rate of 71% (including 12% complete response), with a further 20% achieving stable disease [55]. The recent phase III SATURN study has given particular insight into the benefits of erlotinib in *EGFR*-mutant disease [15]. In this placebo-controlled study where erlotinib was used as maintenance therapy following first-line platinum-based chemotherapy, patients with both wild-type *EGFR* and mutated *EGFR* derived a significant PFS benefit from erlotinib; however, the impact of erlotinib on PFS was particularly profound in *EGFR*-mutated patients (HR 0.10; 95% C.I. 0.04−0.25). Some studies have found that patients with exon 19 deletions may have a better response to EGFR TKIs than those with L858R mutations [55, 74, 75], although this has not been shown in all reports [76, 77].

Taken together, the evidence suggests that NSCLC with mutations in the TK domain of the EGFR is a distinct subgroup of NSCLCs. This appears to be a disease characterized by 'oncogene addiction', with tumour cells dependent on hyperactivated EGFR for survival, and is more common in, but not restricted to, Asians, non-smokers, women and patients with adenocarcinoma histology. Patients with *EGFR*-mutated disease appear to have distinct genetic characteristics, different from patients with wild-type *EGFR*.

In the pre-biological era, survival outcome in patients with *EGFR* mutations appears to be similar to those with wild-type disease. However, the advent of EGFR TKIs has potentially changed the prognosis of patients with *EGFR*-mutant NSCLC. A pooled analysis of available studies has been performed to evaluate clinical outcome in patients with *EGFR*-mutated NSCLC who have been treated with chemotherapy or EGFR TKIs.

Methods

Selection criteria

All prospective or retrospective studies were eligible for the study pool if they evaluated chemotherapy or single-agent EGFR TKIs (erlotinib or gefitinib) in patients with NSCLC and *EGFR* mutations. Among the studies identified for inclusion, a variety of techniques were used to determine the *EGFR* mutation status of tumours; the methods used in individual reports were not critically assessed as part of this analysis.

Search strategy

The medical literature was reviewed to identify appropriate clinical studies for pooled analysis inclusion. The Datastar Web search engine was used to search Medline, Biosis previews and Embase (excluding reviews, and non-English language) on June 11, 2009 using the search string ('epidermal growth factor' OR EGFR) AND (lung OR NSCLC) AND (mutation OR mutations). Searches were limited to studies published in 2004 or later (given that EGFR TK domain mutations were first identified in NSCLC in 2004). Non-English language papers and reviews were excluded. Search results were initially filtered by scanning titles. The abstracts of papers of potential relevance were reviewed and papers that were clearly relevant were selected for further analysis. Studies presented at the 2008 and 2009 ASCO meetings were also searched to ensure that the most up-to-date data were included in the analysis.

Data extraction and synthesis

PFS (or time to progression [TTP]) was chosen as the most appropriate end-point to compare between studies. Data from all eligible publications were extracted by three individuals and tabulated in an Excel spreadsheet. Entries from one individual were

validated by at least one of the other two individuals. Predefined exclusion codes were assigned for studies that did not include the minimal necessary information: PFS or TTP values for the group with *EGFR* mutations and the associated sample size. Furthermore, studies that were performed in the maintenance or adjuvant treatment settings or involved sequential administration of multiple EGFR TKIs were excluded. To avoid inclusion of duplicate data, papers were checked for overlap in authorship, institutions and reported recruitment dates. For trials that were published in more than one paper, data from the most recent publication was used, with prior publications used to verify data.

Statistical analysis

The majority of published papers included in this analysis did not report individual patient data, and so only high-level information could be extracted for analysis. The statistical approach therefore required a number of simplifying assumptions to be made, as detailed below.

The main focus of the analysis was to obtain an estimate of the pooled median PFS by a weighted average of the single study medians. Median PFS estimates, $\hat{\mu}_1$, $\hat{\mu}_{2,\ldots}$, $\hat{\mu}_k$, obtained in each eligible study, with group sizes N_1, $N_{2,\ldots}$, N_k, were summed to yield N_{all}. The pooled median PFS was then estimated as the group-size weighted average, as follows:

$$\hat{\mu}_{all} = \frac{1}{N_{all}} \sum_i N_i \cdot \hat{\mu}_i$$

In one study, where PFS information was given only as the percentage of progression-free survivors at a specified time-point [78], an estimate of median PFS was calculated through the simplifying assumption that PFS times were exponentially distributed, as described by the survival function $S_{exp}(t) = \exp\{-\lambda t\}$, where λ is the constant hazard rate. A further study reported only mean PFS [79]: in this case, the pertaining median $\hat{\mu}$ was also estimated through the exponential assumption leading to $\hat{\mu} = \ln(2) \cdot \text{mean}$. The influence of these assumptions on the overall pooled median PFS was checked in a sensitivity analysis.

C.I.s could not be calculated based only on high level median PFS data. Therefore the exponential distribution was used to estimate surrogate 'accuracy intervals'. The observed median PFS was considered as an approximate (maximum likelihood) estimate of the median of an exponential distribution. Based on this, a 90% accuracy interval for single studies and 95% accuracy interval for pooled median estimates were calculated. This approximative method does not provide a true C.I. and censoring could not be accounted for, although this may not be a strong deficiency for the PFS end-point. Therefore, the interval reported from this analysis is referred to as an 'accuracy interval' as its size reflects the accuracy of the relevant estimate.

Permutation testing was performed to allow the comparison of median pooled PFS for each specific therapy [80]. Random permutations across studies were generated for 1000 iterations to test the null hypothesis that the difference in median PFS between treatments was zero (*i.e.* that the treatment effects were identical). This statistical test is not biased but is based solely on the selected study pool, and cannot be extrapolated to any study pool.

The potential for publication bias in reported median PFS values was assessed using funnel plots, with the appropriate accuracy intervals.

Sensitivity analysis

Sensitivity analyses were performed to test the robustness of the calculated accuracy intervals. This was achieved using a re-sampling technique known as 'bootstrap' [80]. As with permutation testing, the results of this analysis can only be applied to the selected study and cannot be extrapolated to a wider pool.

Results

Figure 2 summarizes the process of identifying studies eligible for inclusion in our analysis. We reviewed the full text from 175 published studies and meeting abstracts identified. A total of 54 studies met the criteria for inclusion (Table 1). Of these, 12 evaluated erlotinib (365 patients), 39 evaluated gefitinib (1069 patients) and 9 evaluated chemotherapy (375 patients) in *EGFR*-mutant NSCLC (some studies reported data for more than one regimen).

Study characteristics

As expected, the majority of studies evaluating chemotherapy in patients with *EGFR* mutations were conducted in the first-line treatment setting (Table 1). Many studies involving EGFR TKIs did not report the specific line of treatment for patients with *EGFR* mutations, although this information was generally presented for the overall population. It is, therefore, only possible to estimate the proportion of patients included in this analysis who received an EGFR TKI as first-line treatment. The estimated proportion of patients in this analysis who received first-line treatment with erlotinib, gefitinib and chemotherapy was 57%, 57%, and 95%, respectively. For chemotherapy, three studies were prospective analyses performed as part of phase III trials, while the remaining studies were retrospective or phase II trials; these studies included a cross-section of East Asian and Caucasian patients. For EGFR TKIs, the majority of studies were retrospectively analysed cohorts, often involving patients from a single ethnic group (Table 1). Across all studies, the most common *EGFR* mutations were deletions in exon 19 and the L858R substitution in exon 21.

PFS analysis

For patients treated in any line of therapy, median PFS ranged from 8.6–15.8 months in patients treated with erlotinib,

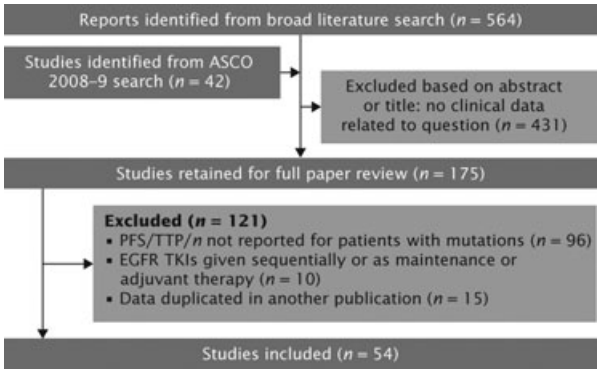

Fig. 2 Flow diagram showing citations retrieved from literature searches and number of trials included in analysis.

3−16 months in patients treated with gefitinib and 4−8.4 months in patients treated with chemotherapy (Fig. 3). In the weighted, pooled analysis, shown in Fig. 4, the overall median PFS was 13.2 months with erlotinib, 9.8 months with gefitinib, and 5.9 months with chemotherapy. As discussed previously, it was not possible to assess outcome according to line of therapy, as many reports did not provide data on this aspect specifically for patients with *EGFR* mutations. In order to estimate the effect of treatments in the first-line setting, an analysis was performed that included only studies where 90% or more of the included patients (regardless of *EGFR* mutation status) received the treatment in question as first-line therapy. This analysis showed similar pooled median PFS in the first-line setting to that in the overall analysis for all lines of therapy (Table 2).

Permutation testing was performed to determine whether there was any difference between outcome with each treatment strategy in this study pool. Using a two-sided permutation, erlotinib and gefitinib produced a longer median PFS compared with chemotherapy, both individually ($P = 0.000$ and $P = 0.002$, respectively) and as a combined group (EGFR TKI *versus* chemotherapy, $P = 0.000$). The permutation *P*-value for the comparison of erlotinib versus gefitinib was 0.005 in this study pool.

Sensitivity analysis

A bootstrap analysis using 1000 runs for the estimated pooled median PFS found similar pooled median values and 95% accuracy intervals to the estimated pooled median values and 95% accuracy intervals in the original analysis; bootstrap median PFS and 95% accuracy intervals: erlotinib 13.2 (11.1–13.8) months; gefitinib 9.8 (8.9–10.8) months; chemotherapy 5.9 (5.0–6.8) months.

Publication bias

Potential publication bias was assessed using funnel plots with PFS or TTP as the outcome. The funnel plots were symmetrical for each of the treatment groups (Fig. 5A–C), indicating a lack of publication bias.

Discussion

The principal finding of this pooled analysis is that patients with *EGFR*-mutated NSCLC appear to have a longer PFS when treated with erlotinib (13.2 months) or gefitinib (9.8 months) than with cytotoxic chemotherapy (5.9 months).

Permutation testing of the studies included in our analysis found that both erlotinib and gefitinib were associated with a significantly longer PFS than chemotherapy. This analysis should be interpreted in the light of permutation testing, which is based solely on the selected study pool and cannot be extrapolated to any study pool.

This analysis adds to our understanding of the place of EGFR TKIs in the treatment of patients with NSCLC. At present, only four studies have directly compared EGFR TKI monotherapy with cytotoxic chemotherapy [7, 8, 67, 108] and only one of these [108] has been adequately powered to detect any difference in outcome according to *EGFR* mutation status. In this phase III study, chemonaïve patients with *EGFR* mutations were randomized to gefitinib or carboplatin plus paclitaxel. The full results of the study are not yet available; however, a preliminary analysis performed in 198 patients found that gefitinib was associated with significantly longer PFS than chemotherapy. This supports the findings of other analyses of phase III comparative studies. In the INTEREST study [7], which compared second-line gefitinib with second-line docetaxel, retrospective analysis found that PFS was significantly longer in the *EGFR*-mutated patients treated with gefitinib than docetaxel [14]. However, in the V15–32 study, which performed a similar comparison between gefitinib and docetaxel in Japanese patients, there were no significant differences in PFS between treatment groups in *EGFR*-mutation-positive patients, although actual median PFS values were not published [8]. The low number of patients with *EGFR* mutations in this study should also be noted. Analysis of IPASS, which compared first-line gefitinib with carboplatin/paclitaxel in East Asian patients with adenocarcinoma who were never-smokers or light ex-smokers, found that *EGFR* mutations were a strong driver of PFS in the gefitinib group: median PFS in *EGFR* mutation-positive patients was longer with gefitinib than chemotherapy (HR 0.48: 95% C.I. 0.36–0.64; $P < 0.0001$); however, in *EGFR* mutation-negative patients, PFS was longer with chemotherapy than gefitinib (HR 2.85: 95% C.I. 2.05–3.98; $P < 0.0001$) [67]. This is consistent with the preclinical findings of Gandhi and colleagues that EGFR wild-type cell lines are resistant to gefitinib [73].

The results of this study are consistent with a smaller analysis of gefitinib studies recently undertaken [129]. This combined analysis of individual patient data from seven trials conducted in Japan, involving 148 patients, found that median PFS was 9.7 months with gefitinib in *EGFR*-mutated patients treated in the

Table 1 Characteristics of included studies for the pooled studies that evaluated the effects of chemotherapy or EGFR TKIs in patients with *EGFR*-mutant NSCLC

Study	Design	Patients	Treatment	PFS/TTP/TTF
Erlotinib				
Ahn *et al.* [81]	Prospective	$n = 24$: Korean; ≥1 prior treatment; exon 19 deletion ($n = 17$); L858R ($n = 5$); exon 20 mutation ($n = 1$); exon 18 mutation and exon 19 deletion ($n = 1$)	Erlotinib 150 mg/day	TTP: 8.6 months
Amann *et al.* [82]	Ph II, single-arm	$n = 3$: chemo-naïve	Erlotinib 150 mg/day	PFS: 13.1 months
Hirsch *et al.* [83]	Ph II; randomized comparison with erlotinib intercalated with carboplatin/ paclitaxel	$n = 13$: chemo-naïve; EGFR mutation	Erlotinib 150 mg/day	PFS: 11.04 months
Jackman *et al.* [84]	Ph II, single-arm	$n = 9$: primarily white; chemo-naïve; ≥70 years; exon 19 deletion ($n = 3$); L858R ($n = 5$); L861Q and exon 19 deletion ($n = 1$)	Erlotinib 150 mg/day	TTP: 13 months
Jackman *et al.* [85]	Ph II, single-arm	$n = 33$: female; chemo-naïve; adenocarcinoma; EGFR mutations	Erlotinib 150 mg/day	TTP: 12.6 months
Massuti *et al.* [55]	Prospective	$n = 217$: Spanish; 0−2 prior treatments; exon 19 deletion ($n = 134$); L858R ($n = 83$)	Erlotinib 150 mg/day	PFS: 14 months
Miller *et al.* [86]	Ph II, single-arm	$n = 18$: BAC and adenocarcinoma, BAC subtype; 0−1 prior treatments; exon 19 or 21 EGFR mutation	Erlotinib 150 mg/day	PFS: 13 months
Pirker *et al.* [87]	Prospective (TRUST study)	$n = 12$: primarily white; chemo-naïve or previously treated; exon 19 deletion ($n = 7$); L858R ($n = 5$)	Erlotinib 150 mg/day	PFS: 405 days
Riely *et al.* [75]	Retrospective	$n = 12$: primarily white; chemo-naïve or previously treated; exon 19 deletion ($n = 8$); L858R ($n = 4$)	Erlotinib 150 mg/day	PFS: 12 months
Rosell *et al.* [88]	Prospective, ph II	$n = 12$: Spanish; non-squamous cell carcinoma; exon 19 or 21 EGFR mutation	Erlotinib 150 mg/day	PFS: 13 months
Schneider *et al.* [89]	Prospective (TRUST study)	$n = 6$: German; chemo-naïve or previously treated; exon 19 deletion ($n = 2$); L858R ($n = 4$)	Erlotinib 150 mg/day	PFS: 12.4 months
Zhou *et al.* [90]	Retrospective	$n = 6$: Chinese; ≥1 previous treatment; EGFR mutations	Erlotinib 150 mg/day	TTP: 15.8 months
Gefitinib				
Asahina *et al.* [76]	Ph II, single-arm	$n = 16$: Japanese; chemo-naïve; exon 19 deletion ($n = 13$); L858R; ($n = 3$)	Gefitinib 250 mg/day	PFS: 8.9 months
Bell *et al.* [91]	Retrospective (ph II IDEAL studies)	$n = 14$: ≥1 previous treatment; exon 19 deletion ($n = 11$); L858R ($n = 2$); InsG771 ($n = 1$)	Gefitinib 250 or 500 mg/day	TTP: 3.8 months
Buckingham *et al.* [92]	Retrospective	$n = 17$: ≥1 previous treatment; EGFR mutation	Gefitinib 250 mg/day	PFS: 13.6 months

Continued

Table 1 Continued

Study	Design	Patients	Treatment	PFS/TTP/TTF
Cappuzzo *et al.* [93]	Prospective, Ph II, single-arm	$n = 21$: Italian; chemo-naïve or previously treated; never smokers and *EGFR* FISH$^+$ or p-AKT$^+$, or any smoking history and both *EGFR* FISH- and p-AKT$^-$; exon 19 deletion ($n = 13$); exon 21 mutation ($n = 4$); exon 19 and 21 mutation ($n = 3$); exon 19 and 20 mutation ($n = 1$)	Gefitinib 250 mg/day	TTP: 7.1 months
Chou *et al.* [94]	Retrospective	$n = 33$: Taiwanese; prior platinum therapy; exon 18 substitution ($n = 4$); exon 19 deletion ($n = 11$); exon 20 substitution or deletion ($n = 4$); exon 21 substitution ($n = 12$); $\geq$1 mutation ($n = 2$)	Gefitinib 250 mg/day	PFS: 7.6 months
Cortes-Funes *et al.* [95]	Retrospective	$n = 10$: Spanish; $\geq$1 previous treatment; exon 19 deletion ($n = 8$); L858R ($n = 2$)	Gefitinib 250 mg/day	TTP: 12.3 months
D'Addario *et al.* [96]	Ph II, single-arm	$n = 4$: Swiss; chemo-naïve; exon 19 deletion ($n = 2$); L858R ($n = 2$)	Gefitinib 250 mg/day	TTP: 7.5 months
Dongiovanni *et al.* [97]	Retrospective	$n = 9$: Italian; chemo-naïve or previously treated; exon 19 deletion ($n = 8$); L858R ($n = 1$)	Gefitinib 250 mg/day	TTP: 14.9 months
Douillard *et al.* [14]	Ph III INTEREST study; randomized, comparison with docetaxel	$n = 19$: primarily white; prior platinum chemotherapy; EGFR mutation	Gefitinib 250 mg/day	PFS: 7.0 months
Fukuoka *et al.* [98]	Ph III IPASS; randomized comparison with carboplatin/ paclitaxel	$n = 132$: East-Asian; adenocarcinoma; never-smokers; chemo-naïve; EGFR mutation	Gefitinib 250 mg/day	PFS: 9.5 months
Han *et al.* [99]	Retrospective	$n = 21$: Korean; previously treated; exon 19 deletion ($n = 12$); L858R ($n = 6$); G719A ($n = 3$)	Gefitinib 250 mg/day	TTP: 13.8 months
Hirsch *et al.* [100]	Pooled analysis of [101] and [102]	$n = 43$: Italian or US; chemo-naïve or previously treated; exon 21 mutations ($n = 31$); exon 19 deletions ($n = 11$); mutations in exons 19 and 21 ($n = 1$)	Gefitinib 250 or 500 mg/day	PFS: 3 months
Hong *et al.* [103]	Prospective, Ph II, single-arm	$n = 3$: Korean; exon 19 deletion ($n = 2$); L858R ($n = 1$)	Gefitinib 250 mg/day	PFS: 5.8 months
Ichihara *et al.* [104]	Retrospective	$n = 30$: Japanese; chemo-naïve and previously treated; exon 19 deletion ($n = 16$); L858R ($n = 14$)	Gefitinib 250 mg/day	PFS: 11.3 months
Inoue *et al.* [77]	Ph II, non-randomized comparison with standard chemotherapy	$n = 16$: Japanese; chemo-naïve; exon 19 deletion ($n = 9$); L858R ($n = 7$)	Gefitinib 250 mg/day	PFS: 9.7 months
Inoue *et al.* [105]	Ph II, single-arm	$n = 29$: Japanese; chemo-naïve; poor performance status; exon 19 deletion ($n = 18$); L858R ($n = 10$), L861Q ($n = 1$)	Gefitinib 250 mg/day	PFS: 6.5 months
Kim *et al.* [106]	Retrospective	$n = 6$: Korean; $\geq$1 previous treatment; exon 19 deletion ($n = 5$); L858R ($n = 1$)	Gefitinib 250 mg/day	TTP: 12.6 months

Continued

Table 1 Continued

Study	Design	Patients	Treatment	PFS/TTP/TTF
Kim *et al.* [78]	Prospective, ph II, single-arm	$n = 45$: Korean; chemo-naïve; adenocarcinoma; exon 19 deletion ($n = 29$); L858R ($n = 15$); L861Q ($n = 1$)	Gefitinib 250 mg/day	PFS at 6 months: 75%
Kimura *et al.* [107]	Prospective, single-arm	$n = 9$: Japanese; chemo-naïve and previously treated; exon 19 deletion ($n = 4$); L858R ($n = 4$); V689L ($n = 1$)	Gefitinib 250 mg/day	PFS: 6.4 months
Kobayashi *et al.* [108]	Ph III; randomized comparison with carboplatin/paclitaxel	$n = 98$: chemo-naïve; EGFR mutation	Gefitinib 250 mg/day	PFS: 10.4 months
Koyama *et al.* [79]	Retrospective	$n = 18$: Japanese; chemo-naïve or previous treatment; G719C ($n = 2$); G719C and W731R ($n = 1$); P733S ($n = 1$); exon 19 deletion ($n = 6$); V738–I744 ins ($n = 2$); S768C ($n = 1$); T790M ($n = 1$); Q812R ($n = 1$); V843I ($n = 1$); L858R ($n = 2$)	Gefitinib 250 mg/day	Mean TTP: 13.7 months
Massarelli *et al.* [109]	Retrospective	$n = 7$: Asian or Caucasian; chemo-naïve or previous treatment; exon 19 deletion ($n = 6$); G719A ($n = 1$)	Gefitinib 250 mg/day	TTP: 9.3 months
Oshita *et al.* [110]	Retrospective	$n = 11$: Japanese; $\geq$1 previous treatment; EGFR mutation	Gefitinib 250 mg/day	PFS: 16 months
Pallis *et al.* [111]	Retrospective	$n = 11$: Greek; $\geq$1 previous treatment; exon 19 deletion ($n = 6$); L858R ($n = 3$); G719D ($n = 1$); E746V ($n = 1$)	Gefitinib 250 mg/day	TTP: 14.7 months
Riely *et al.* [75]	Retrospective	$n = 22$: primarily white; chemo-naïve or previously treated; exon 19 deletion ($n = 15$); L858R ($n = 7$)	Gefitinib 250 mg/day	PFS: 12 months
Sequist *et al.* [112]	Ph II, single-arm	$n = 31$: primarily non-Asian; chemo-naïve; exon 19 deletion ($n = 17$); L858R ($n = 8$); atypical mutation ($n = 6$)	Gefitinib 250 mg/day	PFS: 9.2 months
Shao *et al.* [113]	Ph II, single-arm	$n = 51$: Taiwanese; chemo-naïve; EGFR mutation	Gefitinib 250 mg/day	PFS: 8.8 months
Shoji *et al.* [114]	Retrospective	$n = 20$; Japanese; chemo-naïve and previously treated; exon 19 deletion ($n = 10$); L858R ($n = 8$); E709A and G719S ($n = 1$); L858R and Y725Y ($n = 1$)	Gefitinib 250 mg/day	PFS: 14 months
Sugio *et al.* [115]	Ph II, single-arm	$n = 19$: Japanese; exon 19 deletion ($n = 7$); L858R ($n = 10$); exon 19 deletion and L858R ($n = 1$); exon 19 deletion and G796A ($n = 1$)	Gefitinib 250 mg/day	PFS: 7.1 months
Sunaga *et al.* [116]	Ph II, single-arm	$n = 21$: Japanese; chemo-naïve or previously treated; exon 19 deletion ($n = 17$); L858R ($n = 4$)	Gefitinib 250 mg/day	PFS: 12.9 months
Sutani *et al.* [117]	Ph II, single-arm	$n = 27$: Japanese; 0−1 previous treatments; exon 19 deletion; L858R, L861Q	Gefitinib 250 mg/day	TTP: 9.4 months

Continued

Table 1 Continued

Study	Design	Patients	Treatment	PFS/TTP/TTF
Takano *et al.* [118]	Retrospective	$n = 85$: Japanese; chemo-naïve or previously treated; exon 19 deletion ($n = 49$); L858R ($n = 36$)	Gefitinib 250 mg/day	PFS: 9.2 months
Tamura *et al.* [119]	Ph II, single-arm	$n = 28$: Japanese; 0−2 previous treatments; exon 19 deletion ($n = 14$); L858R ($n = 14$)	Gefitinib 250 mg/day	PFS: 11.5 months
Varella-Garcia *et al.* [120]	Retrospective	$n = 27$: Japanese; chemo-naïve or previously treated; EGFR mutations	Gefitinib 250 mg/day	TTP: 10.2 months
Wu *et al.* [121]	Retrospective	$n = 16$: Taiwanese; chemo-naïve or previously treated; EGFR mutation	Gefitinib 250 mg/day	PFS: 8.1 months
Wu *et al.* [122]	Retrospective	$n = 32$: Chinese; ≥1 previous treatment; EGFR mutation	Gefitinib 250 mg/day	PFS: 8 months
Xu *et al.* [123]	Retrospective	$n = 32$: Chinese; chemo-naïve and previous treatment; exon 19 deletion ($n = 11$); exon 19–not deletion ($n = 6$); L858R ($n = 6$); exon 18 mutation ($n = 6$); exon 20 mutation ($n = 2$); exon 23 mutation ($n = 1$)	Gefitinib 250 mg/day	TTP: 15 months
Yoshida *et al.* [124]	Prospective	$n = 21$: Japanese; chemo-naïve and previously treated; exon 19 deletion ($n = 8$); L858 ($n = 13$)	Gefitinib 250 mg/day	PFS: 7.7 months
Zhang *et al.* [125]	Retrospective	$n = 12$: Chinese; ≥1 previous treatment; exon 19 deletion ($n = 4$); L858 ($n = 8$)	Gefitinib 250 mg/day	PFS: 10 months
Chemotherapy				
Bell *et al.* [91]	Retrospective (ph III INTACT studies; randomized comparison with gefitinib)	$n = 9$: primarily white; chemo-naïve; exon 19 deletion; L858R; other mutations	Paclitaxel/carboplatin or gemcitabine/cisplatin	PFS: 6.7 months
Douillard *et al.* [14]	Ph III INTEREST study; randomized, comparison with gefitinib	$n = 19$; primarily white; prior platinum chemotherapy; EGFR mutation	Docetaxel	PFS: 4.1 months
Eberhard *et al.* [126]	Retrospective (ph III TRIBUTE study; randomized comparison with erlotinib plus carboplatin/ paclitaxel)	$n = 14$: primarily white; chemo-naïve; exon 19 deletion; L858R; other mutations	Carboplatin/paclitaxel	TTP: 6.6 months
Fukuoka *et al.* [98]	Prospective, ph III IPASS; randomized comparison with gefitinib	$n = 129$: East-Asian; adenocarcinoma; never-smokers; chemo-naïve; EGFR mutation	Carboplatin/paclitaxel	PFS: 6.3 months
Inoue *et al.* [77]	Ph II, non-randomized comparison with gefitinib	$n = 9$: Japanese; chemo-naïve; exon 19 deletions ($n = 8$); L858R ($n = 1$)	Standard chemotherapy	PFS: 7.6 months
Kobayashi *et al.* [108]	Prospective, ph III; randomized comparison with gefitinib	$n = 100$: chemo-naïve; EGFR mutation	Carboplatin/paclitaxel	PFS: 5.5 months
Lee *et al.* [127]	Retrospective	$n = 14$: Korean; chemo-naïve; patients receiving platinum-based chemotherapy; EGFR mutation	Platinum-based chemotherapy	TTP: 8 months paclitaxel, 9.7 months; gemcitabine, 7.4 months

Continued

Table 1 Continued

Study	Design	Patients	Treatment	PFS/TTP/TTF
Tambo *et al.* [128]	Retrospective	$n = 26$: Japanese; chemo-naïve; EGFR mutations	Chemotherapy	PFS: 8.4 months
Wu *et al.* [122]	Retrospective	$n = 55$: Chinese; chemo-naïve; exon 19 deletion ($n = 32$); L858R ($n = 21$); exon 19 deletion and L858R ($n = 2$)	Chemotherapy	PFS: 4 months

Ph = phase.

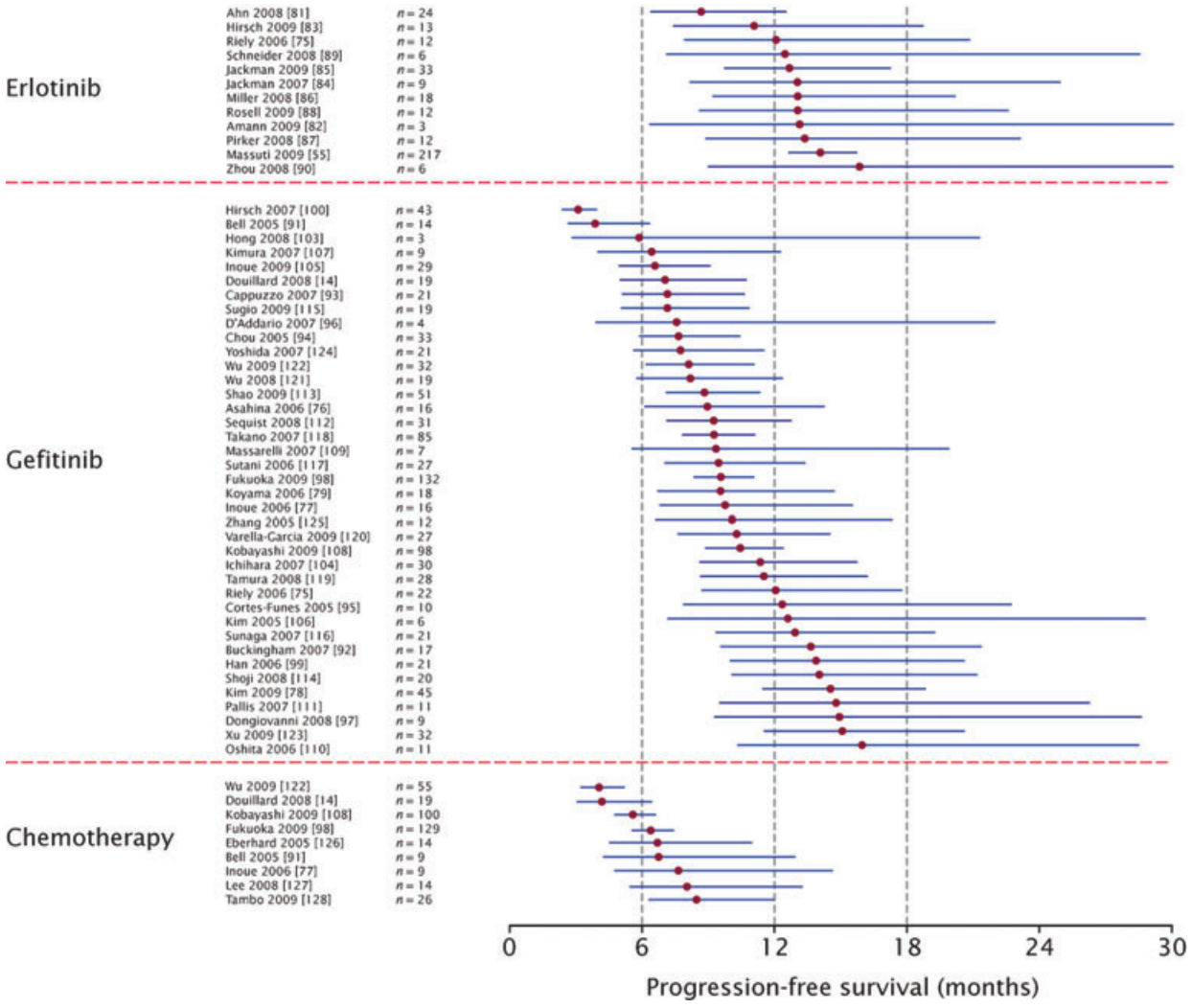

Fig. 3 Forest plot showing analysis of median pooled PFS or TTP and 90% accuracy intervals during treatment with single-agent erlotinib, single-agent gefitinib or chemotherapy, in patients with *EGFR*-mutant NSCLC.

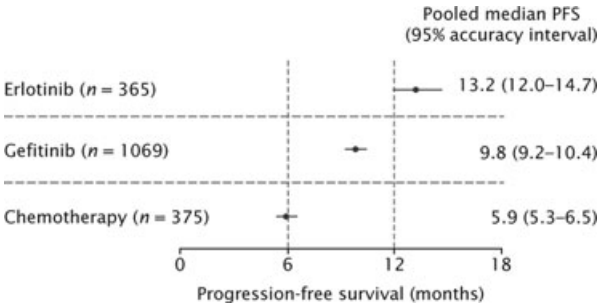

Fig. 4 Forest plot showing pooled analysis of median PFS or TTP and 95% accuracy intervals during treatment with single-agent erlotinib, single-agent gefitinib or chemotherapy, in patients with *EGFR*-mutant NSCLC.

Table 2 Pooled median PFS and 95% accuracy intervals for single-agent erlotinib, single-agent gefitinib or chemotherapy, in patients with *EGFR*-mutant NSCLC with chemotherapy: all lines of therapy, and studies in which ≥90% of patients received treatment in the first-line setting

Single-agent erlotinib	
All lines of therapy (n = 365)	13.2 (12.0−14.7)
Predominantly first-line (n = 70)	12.5 (10.0−16.0)
Single-agent gefitinib	
All patients (n = 1069)	9.8 (9.2−10.4)
Predominantly first-line (n = 520)	9.9 (9.0−10.9)
Chemotherapy	
All patients (n = 375)	5.9 (5.3−6.5)
Predominantly first-line (n = 359)	6.0 (5.4−6.7)

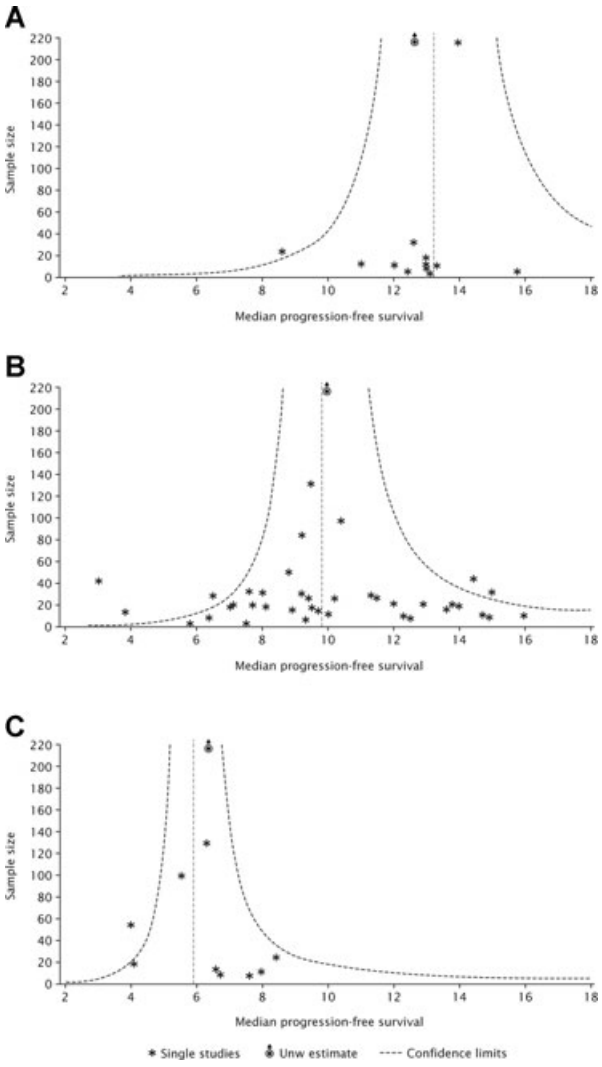

Fig. 5 Funnel plots using PFS or TTP as an outcome for (**A**) erlotinib; (**B**) gefitinib and (**C**) chemotherapy.

first- or subsequent-line setting. This is consistent with the 9.8 months seen with gefitinib in our review.

Our analysis is broad in scope, including patients of a number of different ethnicities in different clinical settings. While clinical characteristics, such as female gender, non-smoking history, adenocarcinoma histology and Asian ethnicity, have previously been used to guide which patients should be selected for EGFR TKI therapy, it is evident that the benefits of treatment are not restricted to these patient subgroups. Indeed, this supports the previous findings of the erlotinib BR.21 study [4, 130] and SATURN study [15], which showed that erlotinib is effective in a proportion of patients in all clinical and biomarker subgroups. Furthermore, given the impressive response to EGFR TKIs in patients with *EGFR* mutations, it is becoming more apparent that it is inappropriate to use clinical characteristics alone as a surrogate for mutation testing. This has been further emphasized by IPASS, which included the best possible group for mutation prediction (Asian patients with adenocarcinoma who were predominantly female and had never been smokers); however, only 60% of these patients were mutation-positive. As previously stated, those patients who were mutation-positive had a better outcome with gefitinib, while those who were mutation-negative achieved better results on cytotoxic chemotherapy. This highlights the critical need to determine *EGFR* mutation status before making clinical decisions regarding the use of first-line EGFR TKIs.

In our study, PFS was chosen as the most appropriate end-point to pool. OS was not evaluated, as data are often immature at the time of trial publication and median duration of OS may be influenced by subsequent therapies. While most studies report response data, this end-point does not share the reputation and weight of PFS, particularly given that treatment with EGFR TKIs has been shown to prolong PFS but does not necessarily lead to

an objective response measured according to standard criteria. Furthermore, concentrating on the single end-point of PFS allows avoidance of the well-known multiplicity trap.

To evaluate the sensitivity of our analysis, a bootstrap test was performed. This showed very similar findings to the weighted analysis, providing confidence in the validity of the main analysis.

As with any analysis, there are several limitations with our study. As many of the included studies were retrospective in nature with the inherent potential for bias, it is possible that this bias would have also affected our pooled analysis. No quality analysis of the included studies was undertaken; therefore, it is not possible to determine the quality of the data that were actually included. Furthermore, while PFS was considered the most appropriate end-point to pool to provide the best estimate of efficacy, it must be noted that PFS is not assessed in the same way in all studies and is likely to be influenced by the frequency and timings of tumour measurement. Consequently, PFS values from prospective trials are likely to be more accurate than those from retrospective trials because of the stipulation for regular assessment using pre-specified criteria. Similarly, different methods, with different sensitivities, were used between studies to identify patients with *EGFR* mutations.

Nevertheless, the findings of this study are relevant as we continue to learn how best to tailor treatment for patients with NSCLC. The median PFS achieved with EGFR TKIs in this setting is dramatic, reaching a median of 13.2 months with erlotinib and 9.8 months with gefitinib. This is particularly notable, given that standard first-line platinum-based chemotherapy offers PFS in the range of 3 to 5 months in the general NSCLC population [131]. Indeed, the response of some *EGFR*-mutant patients with poor performance status to EGFR TKIs has been described as a 'Lazarus response' – the returning to life after resuscitation has been given up [132]. By identifying patients with *EGFR* mutations and treating them with EGFR TKIs rather than chemotherapy, it is not only likely that they will have a superior outcome but also that they will not be subject to the debilitating toxicities associated with cytotoxic agents. Ideally, all patients who present with NSCLC should be tested for *EGFR* mutations, given that these are not restricted to a group of patients with certain clinical characteristics. The feasibility of this will be greatly improved when non-invasive tests (such as serum-based assays) become available.

Phase III trials are currently underway that will prospectively evaluate EGFR TKIs as first-line therapy in patients with *EGFR*-mutant disease. For example, the European Trial of Tarceva *versus* Chemotherapy (EURTAC; NCT00446225) is evaluating erlotinib *versus* platinum doublet chemotherapy, with a targeted accrual of 173 patients and a primary end-point of PFS. The phase III West Japan Thoracic Oncology Group study 3405 is assessing gefitinib *versus* cisplatin/docetaxel, also with PFS as the primary end-point. The outcome of these studies will be essential in further helping to determine the impact of EGFR TKIs in the treatment of patients with *EGFR*-mutant NSCLC.

Conclusions

This extensive review of the literature has shown that NSCLC associated with *EGFR* mutations presents as a distinct disease that is dependent on hyperactivated EGFR for survival. Because of this, it is not surprising that blockade of EGFR TK activity appears to be the most effective treatment for this subgroup of NSCLC. Ongoing trials that are prospectively comparing the efficacy of chemotherapy and EGFR TKIs as first-line therapy in *EGFR*-mutant disease should provide further insight into the most appropriate way to treat NSCLC in patients with *EGFR* mutations.

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