Biological and Clinical Features in Predicting Efficacy of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors: A Systematic Review and Meta-analysis

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Abstract. *Background: Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs), developed for patients with advanced non-small cell lung cancer (NSCLC), give modest results similar to those with chemotherapy. There is evidence of a greater survival benefit from TKIs in patients with certain molecular and clinical features, but results are conflicting. To assess the role of these factors in predicting TKI efficacy, a pooled analysis was performed on data from randomized trials in NSCLC. Materials and Methods: An electronic search of all randomized trials comparing the efficacy or activity of TKIs and a pooled analysis were performed. The hazard ratio (HR) with 95% confidence interval (CI) was calculated for each level of the factors and an interaction test was used to detect differences in treatment effect related to the different levels. Results: Of ten randomized trials identified, five were considered for analysis. Smoking was shown to be the only clinical factor to have a predictive effect (non smokers: overall survival (OS) HR 0.53, 95% CI 0.42-0.67; smokers: HR 0.91, 95% CI 0.81-1.02; p-value for interaction <0.001). A negative predictive value was suggested for K-ras mutations (K-ras$^+$: HR 1.97 95% CI 1.16-3.33; K-ras$^-$: HR 0.79, 95% CI 0.59-1.05; p-value for interaction 0.003). Conclusion: At the present time, none of the biological features which have been evaluated in patients who have undergone therapy using TKIs is proven to be of predictive value; only K-ras mutations and smoking habits can be considered as a possible criteria for selection. Results of prospective randomized trials on biological markers are awaited.*

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Non-small cell lung cancer (NSCLC) is the most common cause of cancer deaths worldwide (1). The majority of patients have advanced disease at diagnosis and chemotherapy with third-generation platinum-based doublets is the standard of care for these patients (2). In patients progressing after first-line chemotherapy, pemetrexed and docetaxel are the referral chemotherapic treatments, but their efficacy is limited and a survival plateau has been reached with cytotoxic agents (3, 4).

Erlotinib and gefitinib are tyrosine kinase inhibitors (TKIs) that compete with the ATP-binding sites in the intracellular domain of the epidermal growth factor receptor (EGFR), inhibiting phosphorylation of the receptor and therefore blocking downstream signalling. They have been licensed for refractory NSCLC, erlotinib worldwide and gefitinib in Asian countries (5, 6), but results are modest, similar to those with second-line chemotherapy (7, 8). No clinical benefit has been demonstrated when EGFR TKIs are combined with chemotherapy as first-line treatment of advanced NSCLC (9, 10).

Since all available drugs have been developed in unselected populations of NSCLC patients, it is important to identify those patients who are most likely to obtain clinical benefit from specific treatments. In recent years, the biological differences and the clinical features of patients with NSCLC have been amply characterized, with a view to identify subsets of patients who may derive the most benefit from TKIs. This has led to the development of various markers, addressing particular biological features (EGFR pathway characteristics, *K-ras* mutations and *c-met* amplification) for selection for TKI therapy. This research, however, has not been so vigorous on cytotoxic agents, creating a scarcity of knowledge in this field.

In 2004, Lynch (11) *et al*. identified mutations in the *EGFR* gene in the tumours of patients with NSCLC. They correlated the response to gefitinib in these patients with mutations. Multiple clinical trials indicated that patients with these activating mutations have higher response rates to TKIs than those without them (11-23). Many investigators have become convinced that *EGFR* mutation status might be a reliable criterion for selection for therapy with TKIs. Moreover, EGFR protein expression assessed by immunohistochemistry (IHC) (24-30) and *Her-1/EGFR* gene copy number have now been linked to likelihood of response and extended survival with TKIs (25, 26, 29-35). In addition, negative predictive markers, specifically *K-ras* mutations, were investigated and Eberhard *et al*., in the TRIBUTE study, suggested its predictive role in identifying non-responders (34). A similar result was found in the BR.21 study (35).

Unfortunately, analyses of data from the pivotal trials in this field have given contradictory results, and a pooled analysis of the best available knowledge is needed. This research sought to review all the published evidence in the literature to quantify the role of biological and clinical markers in the prediction of TKIs efficacy.

Materials and Methods

A systematic review of all randomized trials published either as full papers in peer-reviewed journals or presented at the American Society of Clinical Oncology (ASCO), European Cancer Conference (ECCO), European Society for Medical Oncology (ESMO), or International Association for the Study of Lung Cancer (IASLC) Congresses up to March 2008 was performed. Analysis was carried out on all patients with advanced NSCLC who were treated with erlotinib or gefitinib, either alone or in trials against placebo, or combined with chemotherapy compared to chemotherapy alone, in which subgroup analysis was performed. Data were independently selected and reviewed by two members of the Fatebenefratelli and Ophthalmic Oncological Hospital Department and by two statisticians of the Oncology Department of the Mario Negri Institute in Milan (Italy). The evidence in this systematic review primarily comprises randomized controlled trial data.

Literature search strategy. MEDLINE, EMBASE, CANCERLIT and the Cochrane Library databases were searched. The search strategy followed the algorithm used by Feld (36). The subject headings "lung cancer", "carcinoma, non-small cell lung", "lung neoplasm", "non-small cell lung cancer", "lung carcinogenesis", "lung adenocarcinoma", "lung alveolus cell carcinoma", "lung squamous cell carcinoma", "erlotinib", "gefitinib" and "epidermal growth factor receptor" were combined with each of the following phrases used as text words: "non-small cell lung", "Iressa", "gefitinib", "ZD1839", "Tarceva", "erlotinib", "EGFR antagonists", "OSI774", "*EGFR* mutations", "*EGFR* copy number", "*K-ras* mutations", "EGFR expression", "predictive factors" and "EGFR-tyrosine kinase". These terms were then combined with the search terms for the following publication types and study designs: practice guidelines, systematic reviews, meta-analyses, randomized controlled trials, clinical trials, phase II clinical trials, phase III clinical trials and cohort analyses. In addition, conference proceedings of the ASCO, ECCO, IASLC and ESMO were manually searched for abstracts of relevant trials. Pilot trials, dose-escalation trials or case series, letters and editorials reporting trial outcomes were excluded.

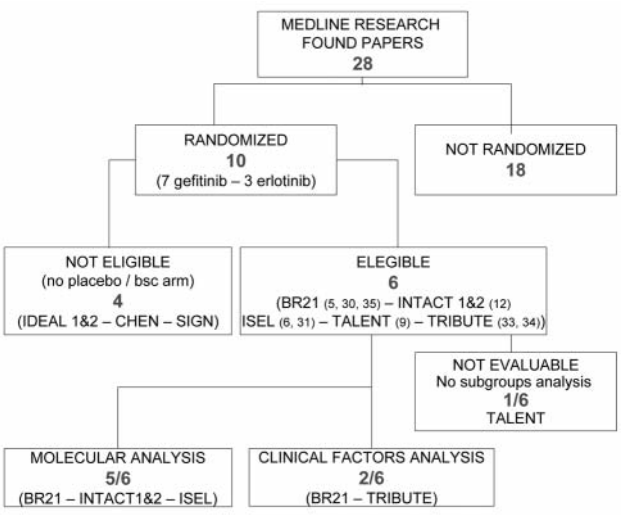

Figure 1. *Meta-analysis literature strategy.*

The quality of study design and reporting results was evaluated according to CONSORT guidelines (37).

The following features were considered for analysis: sex, smoking habit, race, histology, EGFR expression, *EGFR* mutations, *EGFR* copy number and *K-ras* mutations.

Endpoints considered for analysis were progression-free (PFS) and overall survival (OS). From each trial and from each factor hazard ratios (HR), p and confidence intervals (CI) were extracted. A pooled analysis was performed using Parmar's method (38) to estimate the size effect. Tests were carried out for treatment differences in subgroups. The predictive effects of factor were analysed by a test for interaction between each factor and treatment and $p<0.05$ was considered statistically significant. Heterogeneity explained by inconsistency across study results was measured with the I^2 statistic (39).

Results are depicted in all figures as conventional meta-analysis forest plots, where HR lower than 1.00 means fewer events in the subset analysed. SCHARP, a software from the Meta Analysis Group of the MRC Clinical Trials Unit, was used to produce forest plot figures and compute interaction tests and log-rank tests for each subgroup of patients (40).

Results

Quality assessment. Of 28 published reports from 1996 up to March 2008, 18 were excluded because they were not randomized controlled trials. Of the ten remaining, four trials were not included because they did not compare TKIs to placebo, therefore only six were eligible and in one article it was not possible to calculate the log HR and its variance for subgroups identified by the investigated factors.

Thus, five trials (12, 30, 31, 33-35) were analyzed in this meta-analysis (Figure 1).

Table I. *Characteristics of clinical trials and patients included in meta-analysis.*

	Study design	No. patients	Clinical factors	EGFR expression IHC	*EGFR* copy number (FISH)	19-21 *EGFR* mutations	*KRAS* mutations
BR21 (5, 30, 35)	A: Erlotinib	A: 488	▲	▲	▲	▲	▲
	B: Placebo	B: 243					
Tribute (33, 34)	A: Carbo-tax + erlotinib	A: 539		▲	▲	▲	▲
	B: Carbo-tax	B: 540					
ISEL (6, 31)	A: Gefitinib	A: 1129	▲	▲	▲		
	B: Placebo	B: 563					
	A: Cis-gem + gefitinib	A: 730					
INTACT1 (12)	B: Cis-gem	B: 363			▲	▲	
INTACT2 (12)	A: Carbo-tax + gefitinib	A: 690					
	B: Carbo-tax	B: 347					
		% pts	100%	31%	19.3%	14.7%	33%

Carbo, carboplatin; tax, taxol; gem, gemcitabine; IHC, Immunohistochemistry; FISH, fluorescence *in situ* hybridization.

All studies included in the analysis reported the protocol compliance. A full description of patients excluded (if any) from the analysis and the type of statistical analysis was provided in all studies; all trials were multicenter. The median follow-up of patients in the trials ranged from 6 to 12 months and the survivals from 4.7 to 11 months.

Figure 1 demonstrates the results of the search strategy and Table I reports the data available for each trial and the percentage of patients with available factors in each. Basically, all patients were assessed for clinical factors, but only a small percentage was available for molecular factors, ranging from 14% of data from patients in trials testing *EGFR* mutations to 33% for *K-ras* mutational analysis.

Effect of treatment on the overall population. For each level of each factor, the HR and CI were computed in terms of PFS and OS and the interaction was tested to identify the predictive effect. The pooled results are presented in Figures 2 and 3.

Overall, there was a 3% mortality reduction and a 10% reduction in event rate. However, results differed when comparing second-line therapies and first-line therapies, in which TKIs were added to chemotherapy. In first-line chemotherapy, there was a consistent lack of effect, while second-line therapy promised an 18% reduction in mortality and a 24% reduction in event rate with a greater effect for erlotinib (test for heterogeneity between studies, p=0.04 for OS and p=0.007 for PFS).

The results of the predictive factors in terms of OS and PFS are reported in Figures 4-7.

Predictive effect of biological factors

EGFR expression. There was a significant effect in OS in patients with positive EGFR immunohistochemical expression (IHC), but no interaction for the predictive value of this factor was demonstrated (χ^2 for interaction: 3.197 p=0.074). No predictive effect was demonstrated in analysis of PFS (χ^2 for interaction: 1.906 p=0.167) but only one study was available.

EGFR amplification. There was a positive effect of TKIs on OS and PFS in patients whose tumours had a high *EGFR* gene copy number. A significant predictive effect was seen only for PFS (χ^2 for interaction: 7.904 p=0.005). However, the inconsistency of results in the subsets with high *EGFR* copy number was high, as expressed by the I^2 statistic (77.5%).

EGFR mutations. *EGFR* mutations were analysed in only 14.7% of randomized subjects in these studies.

No predictive effect of 19,21 *EGFR* mutations was seen either in OS or PFS. Inconsistency of results was evident in PFS analysis of patients without *EGFR* mutations (I^2=81.7, p=0.019).

K-Ras mutations. *K-ras* was analysed in 33% of randomized subjects in these studies. Patients with *K-ras* mutations had a greater relative risk of death (HR 1.97, 95% CI. 1.16-3.33), while patients without had a 21% lower risk. The test for interaction (p=0.003) found a significant predictive effect of *K-ras* mutations in patients treated with TKIs. The effect on PFS was similar in magnitude, but only one study was available and no significant effect of interaction was detected.

Histology. Patients were grouped according to adenocarcinoma histotype. TKIs had similar effects in the two subgroups (OS, non-squamous: HR 0.87; 95% CI: 0.77-0.98; squamous: HR. 0.77; 95% CI: 0.66-0.88) and no evidence of a predictive effect was detected (p=0.193). It was not possible to evaluate the effect in terms of PFS.

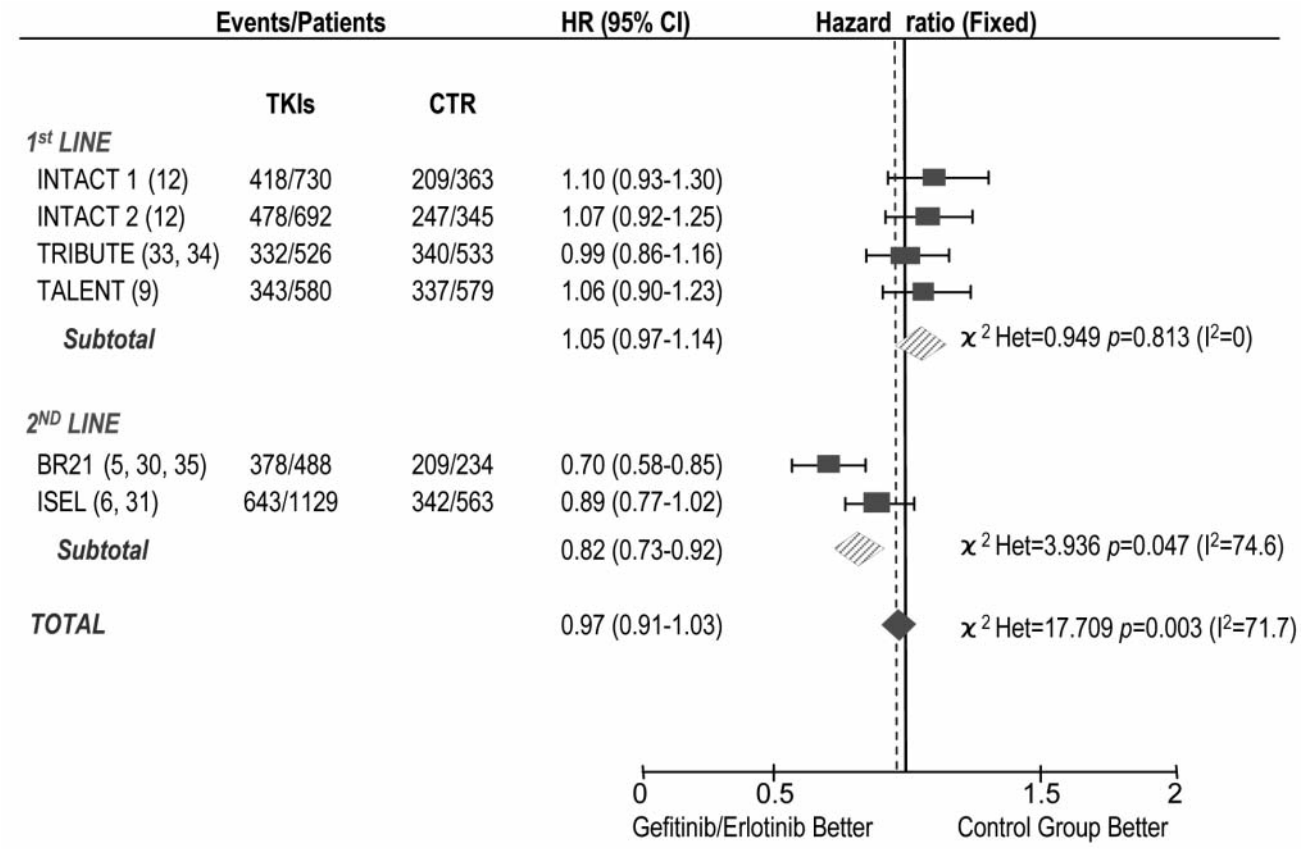

Figure 2. *Annotated forest plot for meta-analysis survival of patients treated with TKIs. The graph shows hazard ratios (with 95% confidence intervals) for mortality among studies of patients allocated to TKIs or control group (CTR). Summary measures are calculated using the fixed-effects model. For each factor, size of squares is directly proportional to amount of information available.*

Predictive effect of clinical factors

Race. A modest effect of TKIs was seen, in terms of survival in patients classified as "Asians" and "non-Asians", though Asians seemed to benefit more in OS, with an interaction close to significance (OS, Asian: HR 0.64; 95% CI: 0.49-0.63; non-Asian: HR 0.85; 95% CI: 0.77-0.93. p=0.046). A similar effect was observed for PFS, but only one study was available for this analysis.

Gender. From the data retrieved, information was available only from the BR.21 trial which evaluated the efficacy of erlotinib against placebo. The treatment effect was similar in males and females (OS, males: HR 0.80; 95% CI: 0.65-0.98; females: HR 0.80; 95% CI: 0.59-1.08) and no evidence of predictive effect was detected (p=1.00).

Smoking. A strong predictive effect of smoking habit was found for PFS and OS. The effect was stronger in non-smokers (OS non-smokers: HR 0.53, 95% CI. 0.42-0.67; smokers: HR 0.91, 95% CI.0.81-1.02, p=0.002). For PFS, data were available only for one study and were similar in magnitude.

Discussion

Selection of patients for treatment is a major challenge of paramount importance since it may permit a better cure rate, better use of drugs and the avoidance of cost of drugs and toxicity. Aware that NSCLC clinical trials have been conducted mainly in unselected patient populations, without any selection criteria and giving poor quality results, a noteworthy body of studies has assessed the role of clinical and biological variables as selection criteria. These have focused mainly on TKIs, without studying the same value in relation to chemotherapy. Most are based on retrospective series or *post hoc* analyses of prospective studies, yielding contradictory results and creating confusion.

This meta-analysis attempted to group all the evidence derived from randomized trials which are the best source of unbiased information for this type of analysis. However, the results are dependent on the subgroup-analysis in each trial, and at least for biological factors, it is not possible to rule out selection bias. It also cannot be excluded that lack of information on certain characteristics in some trials reflected

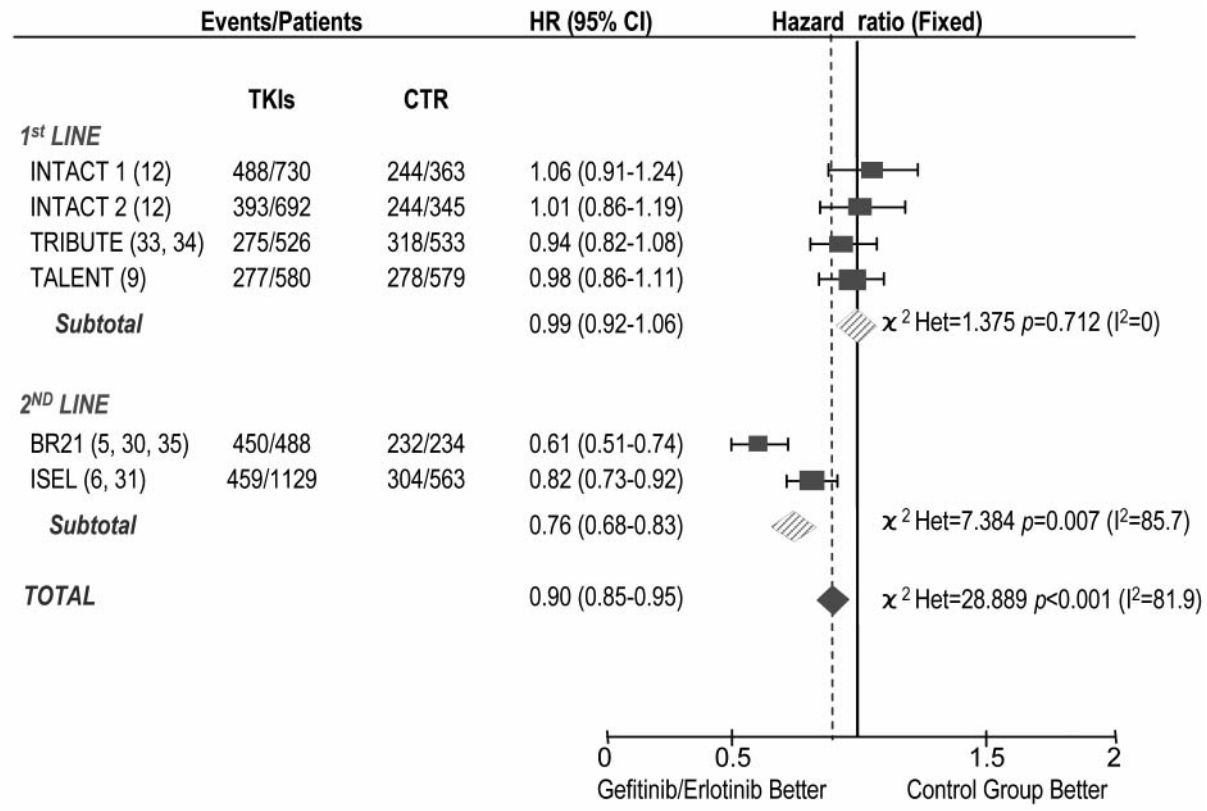

Figure 3. *Annotated forest plot for meta-analysis progression of patients treated with TKIs. The graph shows hazard ratios (with 95% confidence intervals) for progression among studies of patients allocated to TKIs or control group (CTR). Summary measures are calculated using the fixed-effects model. For each factor, size of squares is directly proportional to amount of information available.*

information selection, leading to publication bias. Nevertheless, at present it probably represents the best effort to systematize current knowledge.

Three major findings are reflected in this meta-analysis. First, evaluation of the treatment effect on the overall population allowed confirmation that the addition of erlotinib and gefitinib to first-line chemotherapy does not improve outcome; this issue is widely discussed and explanations for the negative results included mainly the possible antagonism between TKIs and chemotherapy and that no three-drug cytotoxic regimen has proven better as compared to a two-drug regimen (41). From these results, erlotinib and gefitinib act differently in the second-line treatment setting and this difference is statistically significant. The question of whether gefitinib is less efficacious than erlotinib was previously answered on the basis of the difference in clinical characteristics of patients enrolled (42); in particular, the higher proportion of patients who were chemotherapy refractory might have contributed to the negative results of the ISEL trial comparing gefitinib to placebo (6). In the Authors' opinion, this cannot explain the different effects of the two drugs, evident not only in the overall population, but in every subgroup analysis, including the number of previous lines of chemotherapy; the reason for this different behavior may be manifold and it may be mainly biological, pharmacological or both (43). Only a hypothetical direct comparison should remove this doubt and select the best drug also for the toxicity profile.

Second, among clinical features, only smoking habit showed a strong predictive value: non-smokers or patients who had smoked fewer than 100 cigarettes in their life had a greater chance of response to TKIs with a 47% reduction in the relative risk of death. Contrary to widespread belief, sex, histology and race did not seem to have any predictive effect. In addition, none of the biological features positively selected patients for therapy with TKIs. *EGFR* mutations and EGFR expression failed as selection criteria. *EGFR* copy number detected by FISH had a significant predictive role in terms of PFS, but there was wide heterogeneity among studies and the OS advantage was not confirmed, rendering the interpretation of its role ambiguous.

Third, recent results of the INTEREST (7) and INVITE (44) trials, comparing chemotherapy *versus* TKIs, further complicated interpretation, since the treatment effect in

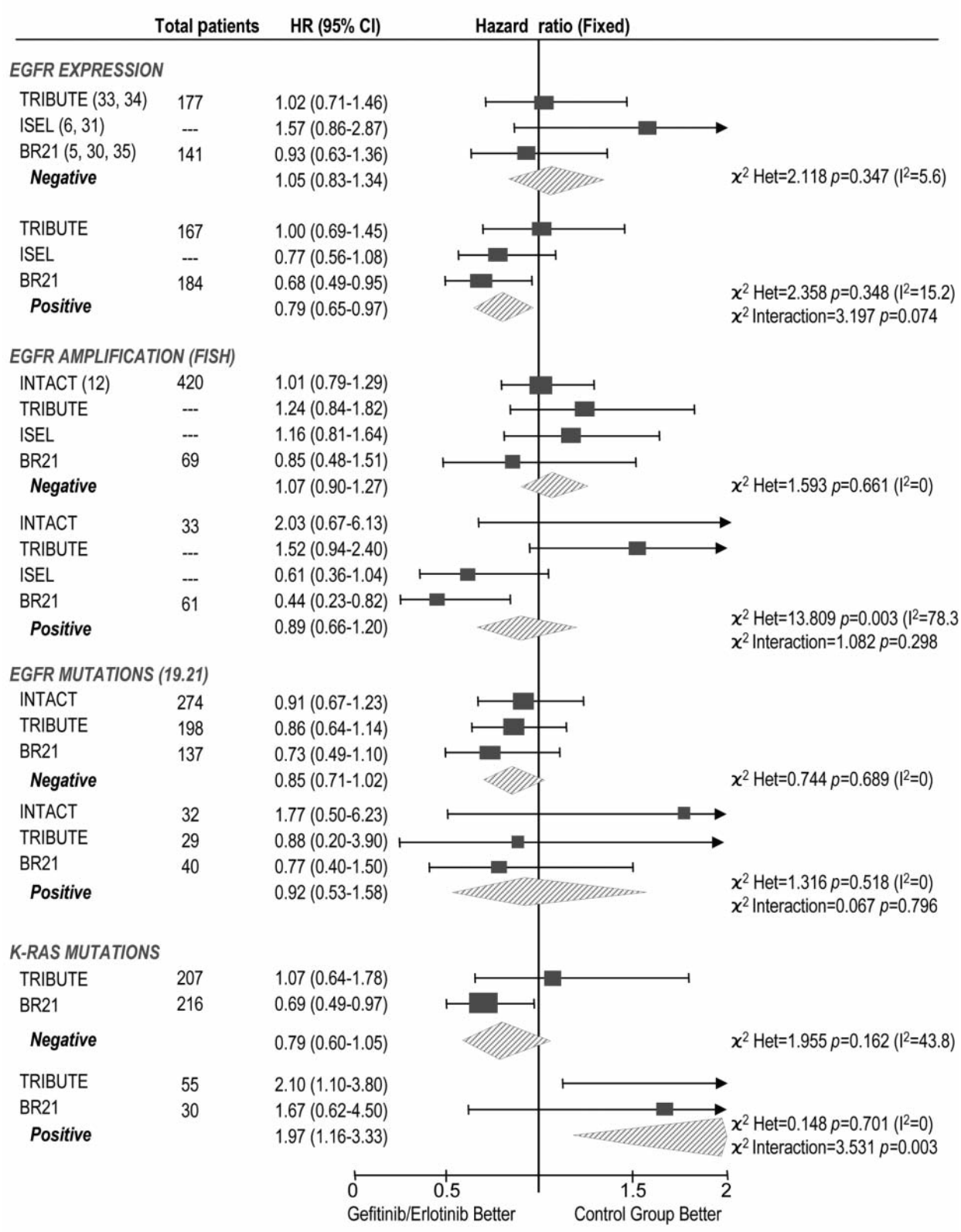

Figure 4. *Annotated forest plot for overall survival according to biological characteristics. The graph shows hazard ratios (with 95% confidence intervals) for mortality among subgroups of patients. Summary measures are calculated using the fixed-effects model. For each factor, size of squares is directly proportional to amount of information available.*

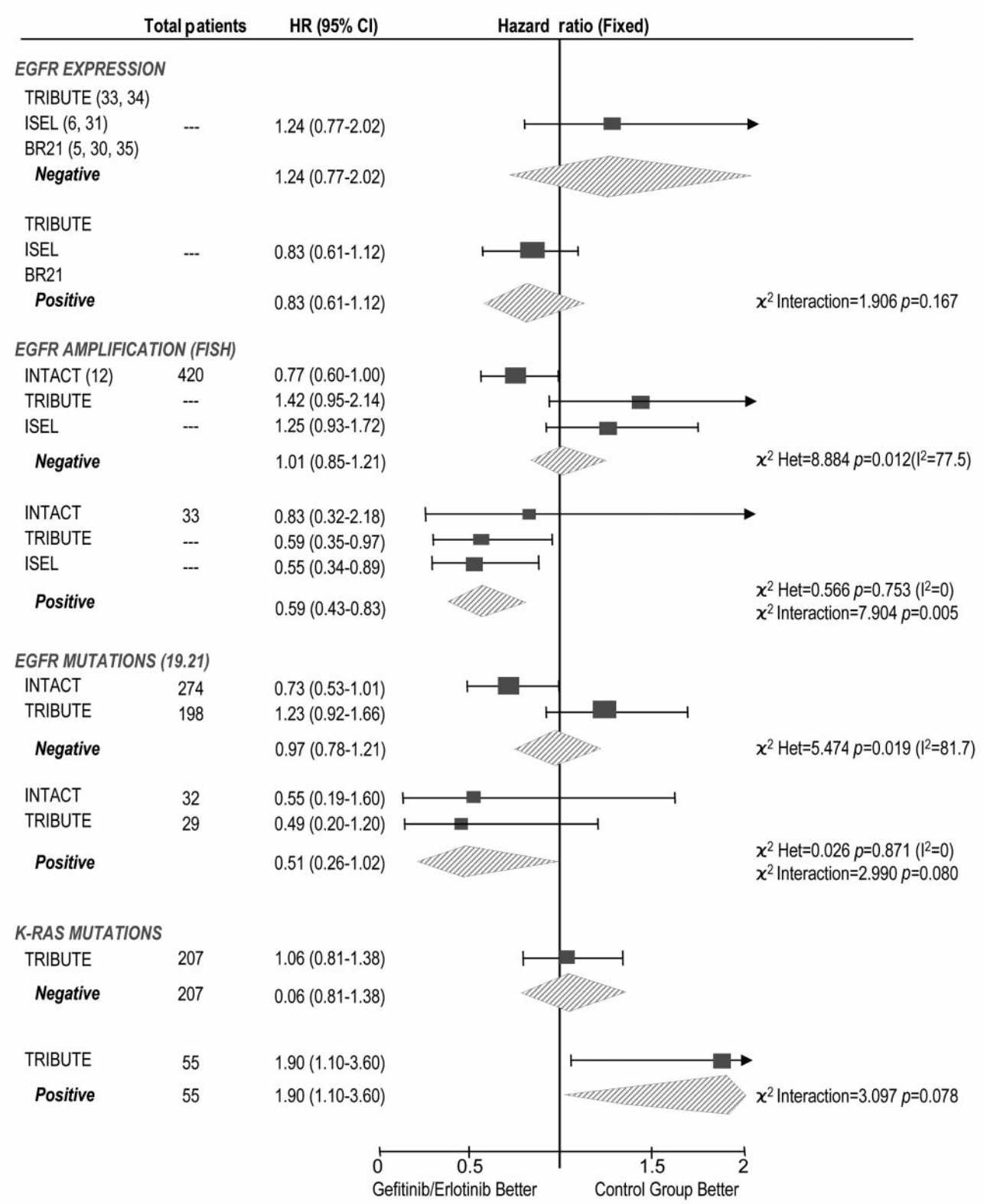

Figure 5. *Annotated forest plot for progression-free survival according to biological characteristics. The graph shows hazard ratios (with 95% confidence intervals) for progression among subgroups of patients. Summary measures are calculated using the fixed-effects model. For each factor, size of squares is directly proportional to amount of information available.*

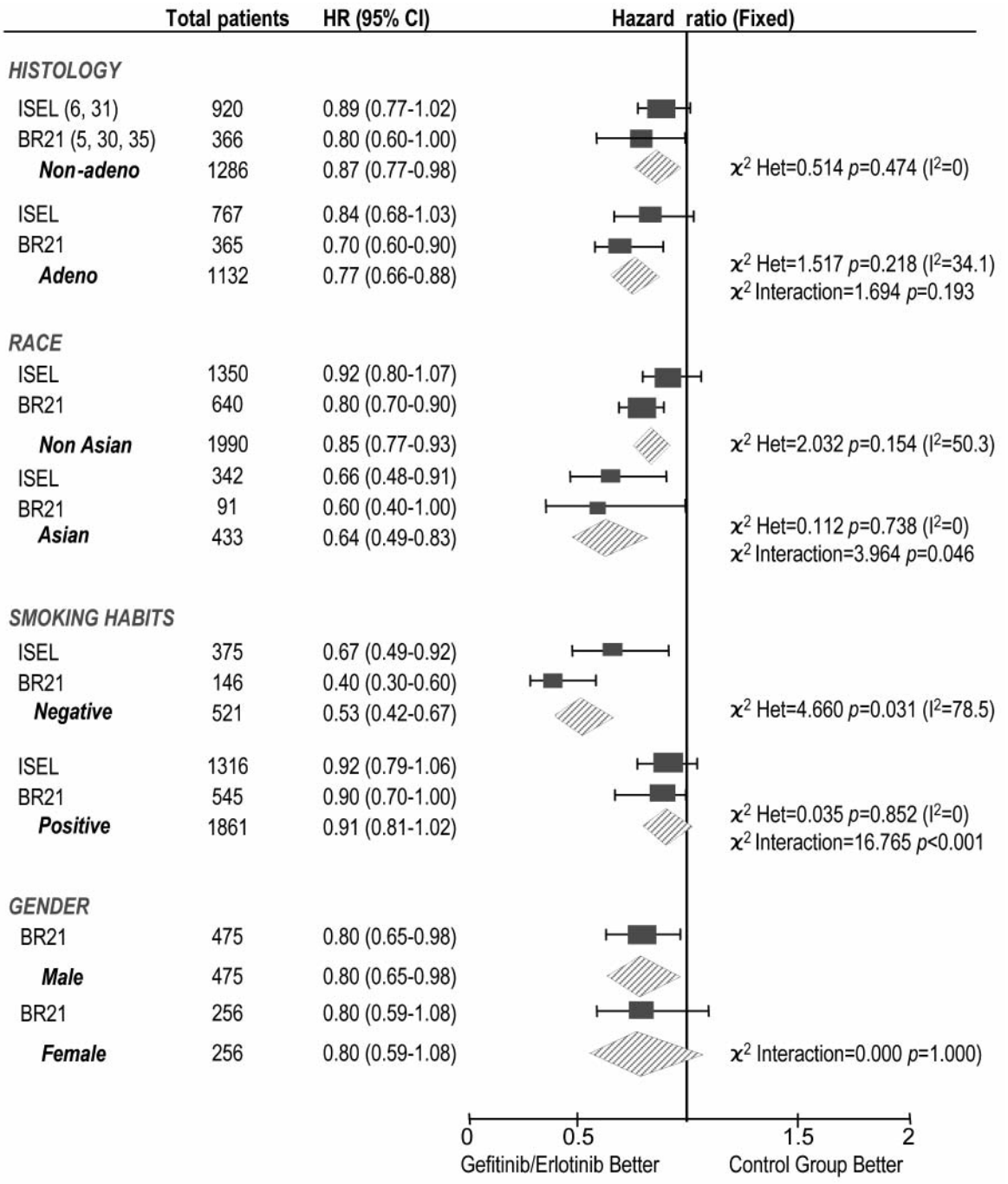

Figure 6. *Annotated forest plot for overall survival according to clinical characteristics. The graph shows hazard ratios (with 95% confidence intervals) for mortality among subgroups of patients. Summary measures are calculated using the fixed-effects model. For each factor, size of squares is directly proportional to amount of information available.*

subgroups defined by clinical and molecular characteristics, showed no factor that could help in selecting chemotherapy or TKIs. As a consequence, the supposed predictive effect of molecular and clinical factors suggested by this meta-analysis and by noncontrolled data may not be selective only for TKIs. This consideration is particularly valid for exon 19,21 *EGFR* mutations. Patients with mutations treated with TKIs have dramatic and lasting responses, but the predictive role has still to be demonstrated. According to this meta-analysis, these mutations have a prognostic

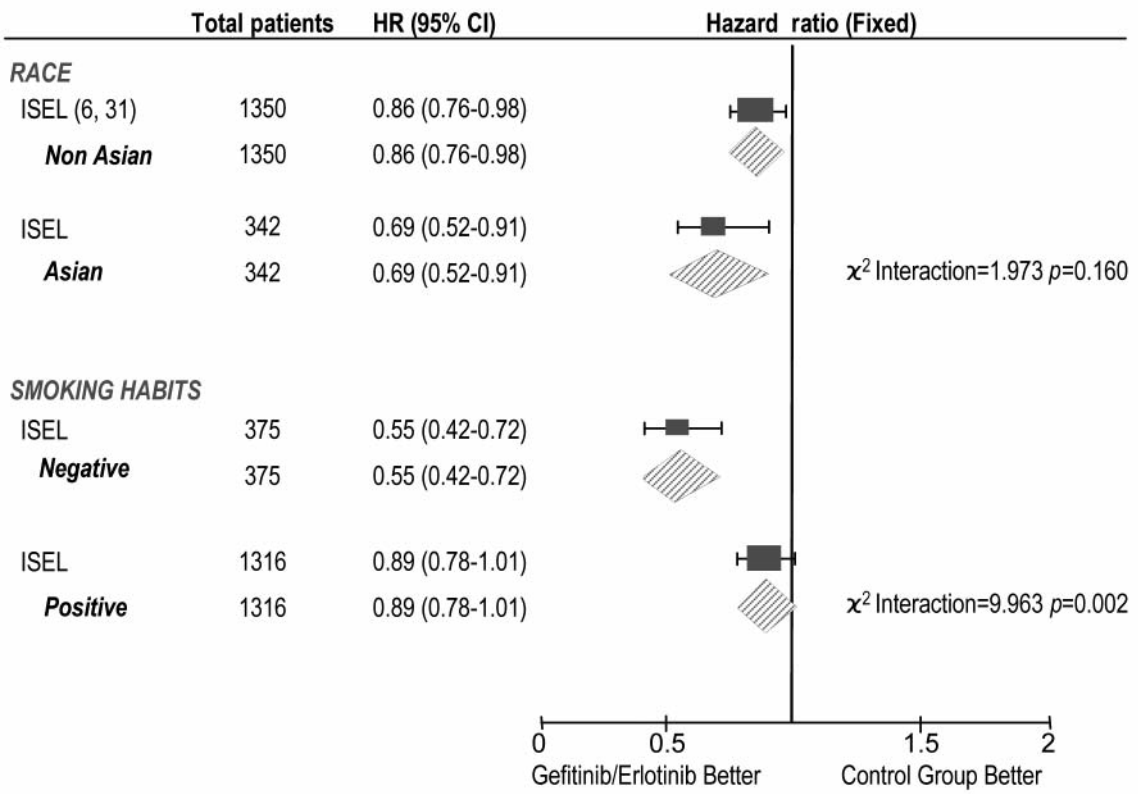

Figure 7. *Annotated forest plot for progression-free survival according to clinical characteristics. The graph shows hazard ratios (with 95% confidence intervals) for progression among subgroups of patients. Summary measures are calculated using the fixed-effects model. For each factor, size of squares is directly proportional to amount of information available.*

rather than a predictive role in terms of OS. It is possible that *EGFR* mutations select a population with a particular prognosis and response to more agents, not only TKIs. A large Spanish trial is now trying to show whether erlotinib as first-line treatment in patients with *EGFR* mutations is superior to conventional platinum based chemotherapy. The same considerations hold for *K-ras*. *K-ras* is very promising for distinguishing non- esponders to TKIs. At present, from these results this seems the only biological variable that may select patients with different responses to TKI; since *K-ras* mutations are more frequent in smokers and are rare in non-smokers, this creates the hypothesis of a different mechanism of carcinogenesis in smokers in which *K-ras* is implicated. However, the same INTEREST trial failed to demonstrate a different effect in this subgroup, so *K-ras* might select a population of non-responders to any treatment.

From the research methodology point of view, these findings highlight the fact that so far no proper research has been carried out on predictive factors for TKIs, and prospective randomized trials focused on these markers are needed.

In conclusion, only smoking habits and *K-ras* mutations can currently be considered of interest. The combination of clinical and molecular predictors remains to be defined. *EGFR* and *K-ras* abnormalities open up a fascinating window that invites future investigation on the mechanisms of acquired resistance which might potentially improve the effectiveness of treatment. TAILOR (NCTG00637910), large Italian trial, supported by the Italian regulatory agency (AIFA) is now in progress specifically powered to identify prognostic and predictive values of all these factors in order to select patients for TKI and chemotherapy. A large American intergroup trial N0723 is also ongoing with similar goals. The sponsored TITAN trial, a direct comparison in second-line of chemotherapy setting with erlotinib, and SATURN, a first-line maintenance trial of erlotinib *versus* placebo, will provide important information for prospectively identifying the patients most likely to respond to TKIs.

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References

1 Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T and Thun MJ: Cancer Statistics. CA Cancer J Clin *58*: 71-96, 2008.

2 Schiller JH, Harrington D and Belani CP: Comparison of four chemotherapy regimens for advanced NSCLC. N Engl J Med *346*: 92-98, 2002.

3 Shepherd FA, Dancey J, Ramlau R, Mattson K, Gralla R, O'Rourke M, Levitan N, Gressot L, Vincent M, Burkes R, Coughlin S, Kim Y and Berille J: Prospective randomized trial of docetaxel *versus* best supportive care in patients with non–small-cell lung cancer previously treated with platinum-based chemotherapy. J Clin Oncol *18*: 2095-2103, 2000.

4 Hanna N, Shepherd F, Fossella F, Pereira JR, De Marinis F, von Pawel J, Gatzemeier U, Tsao TC, Pless M, Muller T, Lim HL, Desch C, Szondy K, Gervais R, Shaharyar, Manegold C, Paul S, Paoletti P, Einhorn L and Bunn PA Jr: Randomized phase III trial of pemetrexed *versus* docetaxel in pts with non-small cell lung cancer previously treated with chemotherapy. J Clin Oncol *122(9)*: 1589-1597, 2004.

5 Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabárbara P and Seymour L: Erlotinib in previously treated non-small cell lung cancer. N Engl J Med *353(2)*: 123-132, 2005.

6 Thatcher N, Chang A, Parikh P, Rodrigues Pereira J, Ciuleanu T, von Pawel J, Thongprasert S, Tan EH, Pemberton K, Archer V and Carroll K: Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small cell lung cancer: results from a randomized, placebo-controlled, multicentre study (Iressa™ Survival Evaluation in Lung Cancer). Lancet *366(9496)*: 1527-1537, 2005.

7 Kim ES, Hirsh V, Mok T, Socinski MA, Gervais R, Wu YL, Li LY, Watkins CL, Sellers MV, Lowe ES, Sun Y, Liao ML, Osterlind K, Reck M, Armour AA, Shepherd FA, Lippman SM and Douillard JY: Gefitinib *versus* docetaxel in previously treated non-small cell lung cancer (INTEREST): a randomised phase III trial. Lancet *22;372(9652)*: 1809-1818, 2008.

8 Maruyama R, Nishiwaki Y, Tamura T, Yamamoto N, Tsuboi M, Nakagawa K, Shinkai T, Negoro S, Imamura F, Eguchi K, Takeda K, Inoue A, Tomii K, Harada M, Masuda N, Jiang H, Itoh Y, Ichinose Y, Saijo N and Fukuoka M: Phase III study, V-15-32, of gefitinib *versus* docetaxel in previously treated Japanese patients with non-small cell lung cancer. J Clin Oncol *10;26(26)*: 4244-4252, 2008.

9 Gatzemeier U, Pluzanska A, Szczesna A, Kaukel E, Roubec J, De Rosa F, Milanowski J, Karnicka-Mlodkowski H, Pesek M, Serwatowski P, Ramlau R, Janaskova T, Vansteenkiste J, Strausz J, Manikhas GM and Von Pawel J: Phase III study of erlotinib in combination with cisplatin and gemcitabine in advanced non-small cell lung cancer: the Tarceva™ Lung Cancer Investigation Trial (TALENT). J Clin Oncol *25*: 1545-1552, 2007.

10 Herbst RS, Preger D, Hermann R, Fehrenbacher L, Johnson BE, Sandler A, Kris MG, Tran HT, Klein P, Li X, Ramies D, Johnson DH, Miller VA: TRIBUTE Investigator Group *et al*: TRIBUTE: a phase III trial of erlotinib hydrochloride (OSI-774) combined with carboplatin and paclitaxel chemotherapy in advanced non-small cell lung cancer. J Clin Oncol *23*: 5892-5899, 2005.

11 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J and Haber DA: Activating mutations in the *epidermal growth factor receptor* underlying responsiveness of non-small cell lung cancer to gefitinib. N Engl J Med *350*: 2129-2139, 2004.

12 Bell DW, Lynch TJ, Haserlat SM, Harris PL, Okimoto RA, Brannigan BW, Sgroi DC, Muir B, Riemenschneider MJ, Iacona RB, Krebs AD, Johnson DH, Giaccone G, Herbst RS, Manegold C, Fukuoka M, Kris MG, Baselga J, Ochs JS and Haber DA: *Epidermal growth factor receptor* mutations and gene amplification in non-small cell lung cancer: Molecular analysis of the IDEAL/INTACT gefitinib trials. J Clin Oncol *23*: 8081-8092, 2005.

13 Janne PA, Engelman JA and Johnson BE: Epidermal growth factor receptor mutations in non-small cell lung cancer: Implications for treatment and tumor biology. J Clin Oncol *23*: 3227-3234, 2005.

14 Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T and Mitsudomi T: Mutations of the epidermal growth factor receptor gene in lung cancer: Biological and clinical implications. Cancer Res *64*: 8919-8923, 2004.

15 Mitsudomi T, Kosaka T, Endoh H, Horio Y, Hida T, Mori S, Hatooka S, Shinoda M, Takahashi T and Yatabe Y: Mutations of the *epidermal growth factor receptor* gene predict prolonged survival after gefitinib treatment in patients with non-small cell lung cancer with postoperative recurrence. J Clin Oncol *23*: 2513-2520, 2005.

16 Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, Herman P, Kaye FJ, Lindeman N, Boggon TJ, Naoki K, Sasaki H, Fujii Y, Eck MJ, Sellers WR, Johnson BE and Meyerson M: *EGFR* mutations in lung cancer: Correlation with clinical response to gefitinib therapy. Science *304*: 1497-1500, 2004.

17 Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, Singh B, Heelan R, Rusch V, Fulton L, Mardis E, Kupfer D, Wilson R, Kris M and Varmus H: *EGF receptor* gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci USA *101*: 13306-13311, 2004.

18 Riely GJ, Pao W, Pham D, Li AR, Rizvi N, Venkatraman ES, Zakowski MF, Kris MG, Ladanyi M and Miller VA: Clinical course of patients with non-small cell lung cancer and *epidermal growth factor receptor* exon 19 and exon 20 mutations treated with gefitinib or erlotinib. Clin Cancer Res *12*: 839-844, 2006.

21 Sequist LV, Joshi VA, Janne PA, Muzikansky A, Fidias P, Meyerson M, Haber DA, Kucherlapati R, Johnson BE and Lynch TJ: Response to treatment and survival of patients with non-small cell lung cancer undergoing somatic *EGFR* mutation testing. Oncologist *12*: 90-98, 2007.

22 Tokumo M, Toyooka S, Kiura K, Shigematsu H, Tomii K, Aoe M, Ichimura K, Tsuda T, Yano M, Tsukuda K, Tabata M, Ueoka H, Tanimoto M, Date H, Gazdar AF and Shimizu N: The relationship between *epidermal growth factor receptor* mutations and clinicopathologic features in non-small cell lung cancers. Clin Cancer Res *11*: 1167-1173, 2005.

23 Chou TY, Chiu CH, Li LH, Hsiao CY, Tzen CY, Chang KT, Chen YM, Perng RP, Tsai SF and Tsai CM: Mutation in the tyrosine kinase domain of *epidermal growth factor receptor* is a predictive and prognostic factor for gefitinib treatment in patients with non-small cell lung cancer. Clin Cancer Res *11*: 3750-3757, 2005.

24 Bailey R, Kris M, Wolf M, Kay A, Averbuch S, Askaa J and Janas M: Gefitinib (Iressa, ZD1839) monotherapy for pretreated advanced non-small cell lung cancer in IDEAL 1 and 2: Tumor response is not clinically predictable from tumor EGFR membrane staining alone. Lung Cancer *41*: S71 (abstr O-242), 2004.

25 Cappuzzo F, Hirsch FR, Rossi E, Bartolini S, Ceresoli GL, Bemis L, Haney J, Witta S, Danenberg K, Domenichini I, Ludovini V, Magrini E, Gregorc V, Doglioni C, Sidoni A, Tonato M, Franklin WA, Crino L, Bunn PA Jr and Varella-Garcia M: *Epidermal growth factor receptor* gene and protein and gefitinib sensitivity in non-small-cell lung cancer. J Natl Cancer Inst *97*: 643-655, 2005.

26 Miller VA, Zakowski M, Riely GJ, Pao W, Ladanyi M, Tsao AS, Sandler A, Herbst R, Kris MG and Johnson DH: *EGFR* mutation and copy number, EGFR protein expression and *KRAS* mutation as predictors of outcome with erlotinib in bronchioloalveolar cell carcinoma (BAC): Results of a prospective phase II trial. J Clin Oncol *24*: 364s, (abstr 7003), 2006.

27 Parra HS, Cavina R, Latteri F, Zucali PA, Campagnoli E, Morenghi E, Grimaldi GC, Roncalli M and Santoro A: Analysis of epidermal growth factor receptor expression as a predictive factor for response to gefitinib (Iressa, ZD1839) in non-small cell lung cancer. Br J Cancer *91*: 208-212, 2004.

28 Perez-Soler R, Chachoua A, Hammond LA, Rowinsky EK, Huberman M, Karp D, Rigas J, Clark GM, Santabárbara P and Bonomi P: Determinants of tumor response and survival with erlotinib in patients with non-small cell lung cancer. J Clin Oncol *22*: 3238-3247, 2004.

29 Toschi L, Metro G, Magrini E, Bartolini S, Ligorio C, Finocchiaro G, Pession A, Cancellieri A, Tallini G and Cappuzzo Fl: EGFR, HER2, and phospho-Akt are not predictive factors for response to first-line chemotherapy in patients with advanced non-small cell lung cancer (NSCLC). J Clin Oncol *24*: 391s (abstr 7111), 2006.

30 Tsao MS, Sakurada A, Cutz JC, Zhu CQ, Kamel-Reid S, Squire J, Lorimer I, Zhang T, Liu N, Daneshmand M, Marrano P, da Cunha Santos G, Lagarde A, Richardson F, Seymour L, Whitehead M, Ding K, Pater J and Shepherd FA: Erlotinib in lung cancer: Molecular and clinical predictors of outcome. N Engl J Med *353*: 133-144, 2005.

31 Hirsch FR, Varella-Garcia M, Bunn P, Franklin WA, Dziadziuszko R, Thatcher N, Chang A, Parikh P, Pereira JR, Ciuleanu T, von Pawel J, Watkins C, Flannery A, Ellison G, Donald E, Knight L, Parums D, Botwood N and Holloway B: Molecular predictors of outcome with gefitinib in a phase III placebo-controlled study in advanced non-small cell lung cancer study. J Clin Oncol *31(24)*: 5034-5042, 2006.

32 Felip E, Rojo F, Reck M, Heller A, Klughammer B, Sala G, Cedres S, Peralta S, Maacke H, Foernzler D, Parera M, Möcks J, Saura C, Gatzemeier U and Baselga J: A phase II pharmacodynamic study of erlotinib in patients with advanced non-small cell lung cancer previously treated with platinum-based chemotherapy. Clin Cancer Res *14(12)*: 3867-74, 2008.

33 Hirsch FR, Varella-Garcia M and Bunn PA: Fluorescence *in situ* hybridization (FISH) subgroup analysis of TRIBUTE, a phase III trial of erlotinib plus carboplatin and paclitaxel in NSCLC. J Clin Oncol *25*: 18s (abstr 7570), 2007.

34 Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, Ince WL, Jänne PA, Januario T, Johnson DH, Klein P, Miller VA, Ostland MA, Ramies DA, Sebisanovic D, Stinson JA, Zhang YR, Seshagiri S and Hillan KJ: Mutations in the *epidermal growth factor receptor* and in *KRAS* are predictive and prognostic indicators in patients with non-small cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol *23*: 5900-5909, 2005.

35 Shepherd FA, Ding K, Sakurada A, Da Cunha Santos G, Zhu C, Seymour L, Whitehead M, Kamel-Reid S, Squire J and Tsao MSl: Updated molecular analyses of the epidermal growth factor receptor (*EGFR*) gene and codon 12 and 13 of the *k-ras* gene in non-small cell lung cancer patients treated with erlotinib in National Cancer Institute. J Clin Oncol *25*: 18s (abstr 7571), 2007.

36 Feld R, Sridhar SS, Shepherd, Mackay JA, Evans WK and the Lung Cancer Disease Site Group: Use of the Epidermal Growth Factor Receptor Inhibitors, Gefitinib (Iressa®) and Erlotinib (Tarceva®), in the Treatment of Non-small Cell Lung Cancer: A Clinical Practice Guideline. A Quality Initiative of the Program in Evidence-based Care (PEBC), Cancer Care Ontario (CCO) Evidence-based Series #7-9: Section 1.

37 Begg C, Cho M, Eastwood S, Horton R, Moher D, Olkin I, Pitkin R, Rennie D, Schulz KF, Simel D and Stroup DF: Improving the quality of reporting of randomized controlled trials. The CONSORT statement. JAMA *276*: 637-639, 1996.

38 Parmar MK, Torri V and Stewart L: Extracting summary statistics to perform meta-analyses of the published literature for survival endpoints. Stat Med *17(24)*: 2815-2834, 1998.

39 Higgins JPT and Thompson SG: Quantifying heterogeneity in a meta-analysis. Stat Med *21*: 1539-1558, 2002.

40 Tierney J and Tinazzi A: A demonstration and guide to SCHARP, a software application for the analysis and plotting of individual patient data (IPD) meta-analysis. Cochrane 2006 - Dublin, Workshop W43.

41 Sridhar SS, Seymour L and Shepherd FA: Inhibitors of epidermal growth factor receptors: a review of clinical research with a focus on non-small cell lung cancer. Lancet Oncol *4*: 397-406, 2003.

42 Blackhall F, Ranson M and Thatcher N: Where next for gefitinib in patients with lung cancer? Lancet Oncol *7*: 499-507, 2006.

43 Siegel-Lakhai WS, Beijnen JH and Schellens JHM: Current knowledge and future directions of the selective epidermal growth factor receptor inhibitors erlotinib (Tarceva™) and gefitinib (Iressa™). Oncologist *10*: 579-589, 2005.

44 Crinò L, Cappuzzo F, Zatloukal P, Reck M, Pesek M, Thompson JC, Ford HE, Hirsch FR, Varella-Garcia M, Ghiorghiu S, Duffield EL, Armour AA, Speake G and Cullen M: Gefitinib *versus* vinorelbine in chemotherapy-naive elderly patients with advanced non-small cell lung cancer (INVITE): a randomized, phase II study. J Clin Oncol *26(26)*: 4253-4260, 2008.

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