Predictive Biomarkers of the Efficacy of Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Treating Advanced Non-small Cell Lung Cancer: A Systematic Review of Randomized Controlled Trials

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Abstract (in English)

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Objective:

Despite the many new progresses in chemotherapy, the prognosis of advanced non-small cell lung cancer (NSCLC) remains poor. The introduction of epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) seems to offer new promises for advanced NSCLC patients. However, EGFR TKIs have a limited overall efficacy, clear adverse events and large costs. It has become particularly appealing to identify, through new biomarkers, patients who are more likely to benefit from the treatment so that the treatment can be more personalized and effective.

EGFR mutations, *EGFR* gene copy number gain, EGFR protein expression and *KRAS* mutations were indicated as potential predictive biomarkers for the efficacy of the treatment in single-arm studies that compared survival of treated patients with and without a biomarker. However, such comparisons are flawed and the appropriate study design to evaluate the value of a biomarker in predicting efficacy which is known as interaction in epidemiology is the randomized controlled trial with stratified analysis that compared the efficacy of EGFR TKIs between patients with and without the biomarker.

As trials in this field are usually small in sample size and insufficiently powered for drawing a robust conclusion, we conducted this systematic review to summarize the evidence from all relevant randomized controlled trials that have data for investigating the interaction between EGFR TKIs and the 4 biomarkers.

Methods:

PubMed, EMBASE, the Cochrane Library, Chinese Biomedical Literature Database (in Chinese), Wanfang Data (in Chinese), the abstracts of conferences of the American Society of Clinical Oncology and European Society of Medical Oncology, the reference list of relevant original studies, systematic reviews and meta-analyses, guidelines, consensus, and expert opinions were searched up to June 2012.

Eligible studies had to be non-duplicate, extractable studies meeting all the following criteria: 1) Population: patients with advanced NSCLC; 2) Intervention: EGFR TKIs alone or EGFR TKIs plus other treatments; 3) Control: placebo, no treatment, or chemotherapy, with or without the baseline treatments in the intervention arm; 4) Outcome: progression-free survival and/or overall survival; 5) Study design: randomized controlled trial; 6) Subgroup analyses were conducted according to the status of one or more of the 4 biomarkers.

Data on patients' characteristics, treatment protocols, outcomes, biomarker analysis and methodological quality were extracted by two researchers independently. Within a study, we defined the measure of the value of a biomarker in predicting efficacy or biomarker-treatment interaction as the hazard ratio in patients with the biomarker

relative to that in those without the marker. The ratio of hazard ratios from relevant studies was then combined by using the random-effect model.

Heterogeneity among studies was assessed by the Cochran' *Q* test and I^2. Sensitivity analyses were conducted to examine the impact of factors such as methodological quality on the results. Begg's funnel plots and Egger's tests were used to examine the possibility of publication bias.

Results:

Eighteen studies were included. The number of patients available for analyses on different biomarkers varied from 1,763 to 3,246. Data on the methodological quality of included studies are generally under-reported. Some studies seemed to have important biases. EGFR TKIs are in general effective in increasing progression-free and overall survival as compared with placebo although the effect size is smaller for overall survival than for progression free survival. EGFR TKIs are comparable to chemotherapy in their effect in prolonging both progression-free and overall survival, except in *EGFR* mutation group in which EGFR TKIs seem much more effective than chemotherapy in prolonging progression-free survival.

Importantly, for progression-free survival, the summary ratio of hazard ratios was 0.37 (95% confidence interval [CI]: 0.22-0.60, $P < 0.0001$) for *EGFR* mutations (versus wild-type), 0.72 (95% CI: 0.52-0.99, $P = 0.04$) for *EGFR* gene copy number gain (versus no gain), 0.99 (95% CI: 0.78-1.26, $P = 0.93$) for EGFR protein expression (versus negative), and 1.35 (95% CI: 1.02-1.80, $P = 0.04$) for *KRAS* mutations (versus wild-type), indicating interaction may exist between EGFR TKIs and *EGFR* mutation, *EGFR* gene copy number and *KRAS* mutations. For overall

survival, the summary ratio of hazard ratios for *EGFR* mutations, *EGFR* gene copy number gain, EGFR protein expression and *KRAS* mutations was 0.84 (95% CI: 0.64-1.11, $P = 0.22$), 0.92 (95% CI: 0.69-1.23, $P = 0.57$), 0.86 (95% CI: 0.70-1.05, $P = 0.14$) and 1.37 (95% CI: 0.89-2.10, $P = 0.15$), respectively.

In general, the results on *EGFR* gene copy number gain, *KRAS* mutations and EGFR protein expression were less certain than those on *EGFR* mutations in terms of statistical significance, consistency and robustness, and the results on overall survival were less certain than those on progression-free survival. Publication bias did not seem present in the study.

Conclusions:

EGFR mutations and possibly *EGFR*-GCN and *KRAS* mutations can help identify who are more likely to benefit from EGFR TKIs treatment. However, it is not clear whether the interaction with *EGFR*-GCN and *KRAS* mutations are independent or obtained through their relation with *EGFR* mutations. Furthermore, in *EGFR* wild-type patients, given that chemotherapy is cheaper and of fewer side effects, chemotherapy seems clearly a better choice than EGFR TKIs.

Our findings provided the most comprehensive evidence for the recommendations of current guidelines. Although the predictive value of the other 3 biomarkers in wild-type *EGFR* patients may be worth further investigation, we suggest that multivariate analyses are explored in future studies of biomarker-treatment interactions.

Key words: non-small cell lung cancer, epidermal growth factor receptor tyrosine kinase inhibitors, gefitinib, erlotinib, systematic review, meta-analysis, randomized controlled trial, evidence based medicine

Abstract (in Chinese)
摘要（中文）

表皮生长因子受体酪氨酸激酶抑制剂治疗晚期非小细胞肺癌的疗效预测生物标志物：随机对照试验的系统综述

目的：

尽管过去几十年癌症的化疗取得了很大进步，但晚期非小细胞肺癌的预后仍然较差。表皮生长因子受体酪氨酸激酶抑制剂（epidermal growth factor receptor tyrosine kinase inhibitors，EGFR TKIs）给晚期非小细胞肺癌的患者带来了新的希望。然而，EGFR TKIs 的总体效果有限，且不良反应较多，价格也较昂贵。如果能找到 EGFR TKIs 的疗效预测因子，则该治疗就可以只给予那些最有可能从中获益的人，从而提高成本效果，并使治疗变得更加个体化。

已有单组研究在接受 EGFR TKIs 治疗的患者中对有或没有某个标志物的人的预后进行了比较，发现 *EGFR* 基因突变、*EGFR* 基因拷贝数增加、EGFR 蛋白表达和 *KRAS* 基因突变这 4 个生物标志物可能能够预测 EGFR TKIs 的疗效。然而，此类研究的方法学是有缺陷的。要确定以上生物标志物是否有预测作用，应该在评估 EGFR TKIs 疗效的随机对照试验中作亚组分析，对该治疗在有某个生物标志物及没有某个生物标志物的患者中的疗效进行比较，检测治疗与生物标记物的交互作用。

但是，现有的随机对照试验通常样本量较小，统计效能不足，难以从中得到确定的结论。因此，我们做了一个随机对照试验的系统综述，以总结现有的最佳证据，对 EGFR TKIs 与上述 4 个生物标志物的交互作用进行评估。

方法：

我们检索了 PubMed，EMBASE，考科蓝图书馆，中国生物医学文献数据库（中文），万方数据库（中文），美国临床肿瘤学会和欧洲肿瘤学会的会议摘要，以及相关原始研究、系统综述与 Meta 分析、临床指南、共识及专家意见

的参考文献。检索时间截至 2012 年 6 月。合格研究为非重复、提供了具体数据且符合下列所有条件的研究：1）研究对象：晚期非小细胞肺癌患者；2）干预措施：EGFR TKIs 单药治疗或联合其他药物治疗；3）对照措施：安慰剂对照，空白对照或化疗，或者它们任一种加上干预组的基线治疗；4）结局指标：无进展生存期和/或总生存期；5）研究设计：随机对照试验；6）根据上述任一种或多种生物标志物的状态作了亚组分析。

两名研究者平行独立地从合格研究中提取了患者特征、治疗方案、结局、生物标志物分析和方法学质量等方面的资料。对每一个研究，我们都根据生物标志物阳性亚组的风险比（hazard ratio）和阴性亚组的风险比计算了一个风险比之比（ratio of hazard ratios）来测量该标志物对疗效的预测能力或者说治疗与该生物标志物的交互作用。然后，采用随机效应模型对来自不同研究的风险比之比进行 Meta 分析；采用 Cochran *Q* 检验和 I^2 评估研究间的异质性；通过敏感性分析考察原始研究的方法学质量等因素对结果的影响；采用 Begg 漏斗图和 Egger 检验来检测发表偏倚存在的可能性。

结果：

共有 18 个合格研究入选。可用于各个生物标志物分析的患者数量从 1763 到 3246 不等。原始研究普遍对关于方法学质量的信息报告得不够充分；有的研究可能存在重要偏倚。与安慰剂相比，EGFR TKIs 可以有效延长无进展生存期和总生存期，但对总生存期的效果相对较小。除了在 *EGFR* 基因突变的患者中 EGFR TKIs 延长无进展生存期的效果明显好于化疗外，其它情形下，不管是无进展生存期还是总生存期，EGFR TKIs 与化疗的效果均相当。

以无进展生存期为结局的风险比之比，在 *EGFR* 基因突变状态不同的亚组间（野生型亚组为参照）为 0.37（95% 置信区间 [CI]：0.22-0.60，$P < 0.0001$），*EGFR* 基因拷贝数状态不同的亚组间（未增加的亚组为参照）为 0.72（95% CI：0.52-0.99，$P = 0.04$），EGFR 蛋白表达状态不同的亚组间（无表达的亚组为参照）为 0.99（95% CI：0.78-1.26，$P = 0.93$），*KRAS* 基因突变状态不同的亚组间（野生型亚组为参照）为 1.35（95% CI：1.02-1.80，$P = 0.04$）。这些结果

提示 EGFR TKIs 治疗与 *EGFR* 基因突变，*EGFR* 基因拷贝数及 *KRAS* 基因突变之间可能存在交互作用。以总生存期为结局的风险比之比，在 *EGFR* 基因突变、*EGFR* 基因拷贝数、EGFR 蛋白表达及 *KRAS* 基因突变状态不同的亚组间分别为 0.84（95% CI：0.64-1.11，*P* = 0.22）、0.92（95% CI：0.69-1.23，*P* = 0.57）、0.86（95% CI：0.70-1.05，*P* = 0.14）和 1.37（95% CI：0.89-2.10，*P* = 0.15）。

就统计学显著性、异质性和稳定性而言，关于其它 3 个生物标志物的结果不如 *EGFR* 基因突变的相关结果确定，关于总生存期的结果不如无进展生存期的相关结果确定。没有证据表明本研究中存在发表偏倚。

结论：

EGFR 基因突变可用于确定哪些患者更有可能从 EGFR TKIs 治疗中获益。*EGFR* 基因拷贝数增加和 *KRAS* 基因突变可能也有类似用途，但它们与治疗的交互作用是独立存在的还是由于它们与 *EGFR* 基因突变的相关性而获得的，目前尚不清楚。在 *EGFR* 野生型的患者中，选择化疗似乎比 EGFR TKIs 更好，因为它的副作用相对较少，且更为便宜。

本研究的结果为当前的临床指南提供了全面的证据支持。其它 3 个标志物在 *EGFR* 野生型患者中的预测价值可能还值得进一步的探讨，但我们更建议未来的研究在探讨治疗与生物标志物的交互作用时进行多因素分析。

关键词：非小细胞肺癌，表皮生长因子受体酪氨酸激酶抑制剂，吉非替尼，厄洛替尼，系统综述，Meta 分析，随机对照试验，循证医学

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1.1 The disease burden of advanced NSCLC

Lung cancer is the most common cancer worldwide, in terms of both incidence and mortality. In 2008, it was estimated that there were 1.61 million of new cases, representing 12.7% of all new cancer patients[1]. Deaths due to lung cancer were estimated to be 1.38 million per year, accounting for 18.2% of the world's total cancer deaths[1]. In Hong Kong, there are over 4400 incident cases and 3800 deaths of lung cancer each year. Compared with patients with other solid tumors, significantly more lung cancer patients reported poor quality of life and severe symptoms.

There are 4 major pathological types of lung cancer: adenocarcinoma, squamous cell carcinoma, small cell carcinoma and large cell carcinoma[2]. The types other than small cell cancer are often collectively referred to as non-small cell lung cancer (NSCLC), which accounts for approximately 85% to 90% of all lung cancer cases[3]. The majority of patients with NSCLC have advanced cancer at diagnosis, which is considered incurable and unsuitable for surgery, but could benefit from chemotherapy[2].

1.2 Basic concepts in the chemotherapy of advanced NSCLC

1.2.1 Lines of treatment

Initial chemotherapy given after the diagnosis of advanced cancer is referred to as the 1st-line treatment. The standard 1st-line chemotherapy for advanced NSCLC is a platinum compound, either carboplatin or cisplatin, combined with a second cytotoxic or anti-folate agent, usually paclitaxel, docetaxel, gemcitabine, vindesine, vinorelbine or pemetrexed[4]. Recently, platinum-based chemotherapy in combination with bevacizumab or cetuximab has also been used as 1st-line treatment for advanced non-squamous NSCLC[5].

If the 1st-line treatment is effective (i.e. the disease is controlled according to well-established criteria), patients may or may not be given maintenance treatment with drug(s) used in the 1st-line treatment or drugs that have not been used previously. Maintenance therapy is aimed to help maintain the efficacy of the 1st-line treatment so that the remission already achieved can last for a longer time. The commonly used agents for maintenance treatment, which are usually used independently, include pemetrexed, gemcitabine, docetaxel, bevazicumab and cetuximab.

If the 1st-line treatment is ineffective (i.e. cancer continues to progress during or shortly after the 1st-line treatment), or induces unacceptable side effects, or cancer progresses again after a period of remission achieved by the 1st-line treatment,

patients may be given a new treatment, which is called the 2nd-line treatment. Similar to the maintenance treatment, the established 2nd-line treatments for advanced NSCLC normally use a single drug, either pemetrexed or docetaxel. However, in most cases cancer would progress again, regardless the effectiveness of the 2nd-line treatment, sooner or later as long as patients remain alive. Thus, patients may further receive 3rd-line or higher treatment, although it is less common. Figure 1 shows the flow from diagnosis to death of advanced NSCLC.

1.2.2 Measures of treatment efficacy

The least time-consuming way to roughly examine the effects of non-surgical treatments of cancer such as chemotherapy is to compare the tumor's longest diameter or the square (or "product") of the diameter before and after the treatment by review of radiologic images[6]. A decrease in the diameter or its square greater than a pre-specified cut-off value suggests that tumor has 'responded' to the treatment, while an increase in the parameter exceeding the cut-off value indicates 'tumor progression'. The efficacy of treatment is then assessed by the comparison of response rates in the treatment and control groups. The most commonly used criteria for assessing tumor response to a treatment is the Response Evaluation Criteria in

Solid Tumors, which uses the diameter of tumor, followed by the World Health Organization criteria, which uses the square of the diameter[6].

However, tumor response is merely a surrogate outcome, which is often not a good indicator of patient-concerned benefit[7-10]. In theory, the golden standard for measuring the efficacy of cancer treatment is overall survival, which is defined as the time elapsed from initiation of the treatment to death of any cause[11]. Overall survival is of unquestionable importance to patients, can be accurately measured, and addresses both efficacy and safety at the same time. Nevertheless, overall survival as a primary efficacy outcome in the evaluation of cancer treatment is potentially confounded by the crossover to other treatments (in a controlled study), use of post-study treatments, and deaths of causes other than the cancer under investigation[11].

Another frequently used measure in evaluating the efficacy of cancer treatment is progression-free survival, which is defined as the time elapsed from initiation of the treatment to the progression of cancer or death of any cause, whichever earlier[12,13]. Compared with overall survival, progression-free survival is “purer” in that it directly measures the effects of treatment on cancer growth, and is not confounded by such factors as subsequent post-study treatments[11].

Although the improvement in progression-free survival does not always translate into overall survival benefits[14], it does help reduce patients' suffering from the growing cancer and the adverse events of treatments they would otherwise receive, and thus enhances the quality of life. In addition, if each of the sequentially used treatments is able to improve the progression-free survival in a small but clinically significant magnitude, it is possible that these treatments would collectively contribute to a prolonged overall survival[11]. Therefore, progression-free survival as an efficacy outcome measure has its unique value.

1.3 Limited efficacy of traditional chemotherapies for advanced NSCLC

In the late 1980s and 1990s, various chemotherapeutic agents were developed for treating advanced NSCLC as mentioned above. Although their superiority over best supportive care has been well established, conventional chemotherapies seem to have reached a "plateau" of efficacy[15]. For example, the response rate, median progression-free survival and median overall survival of advanced NSCLC patients treated by standard 1st-line chemotherapy remain to be only about 20%, 4-6 months and 8-10 months, respectively[5,15-17]. The 5-year survival rate of advanced NSCLC is only 3.5%[3].

1.4 Rationale of EGFR TKIs for treating advanced NSCLC

Against the above background, epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs), including gefitinib and erlotinib (marketed as Iressa and Tarceva respectively), were introduced to the treatment of advanced NSCLC[18,19]. EGFR is a transmembrane protein that consists of an extracelluar ligand-binding domain, a transmembrane segment, an intracellular tyrosine kinase domain and a regulatory C-terminal segment (Figure 2)[20-22]. In more than a half of patients with advanced NSCLC, EGFR is activated and could result in the generation of signals that are crucial for cell proliferation, angiogenesis induction and metastasis formation of cancer. EGFR TKIs were designed to competitively bind to the tyrosine kinase domain of EGFR (Figure 2), block the signaling of the activated pathways and thus inhibit the proliferation and metastasis of cancer[20].

1.5 Clinical use and efficacy of EGFR TKIs in advanced NSCLC

Dozens of randomized controlled trials have evaluated the efficacy of EGFR TKIs in unselected patients with advanced NSCLC. Based on the results of early trials, gefitinib was first approved in Japan in 2002 and then in the USA in 2003 for the treatment of advanced NSCLC after failure of other treatment options, including both

platinum-based and docetaxel chemotherapies[23]. As of now, gefitinib has been marketed in nearly 70 countries (www.iressa.com)[3]. Erlotinib was initially approved in 2004 for 2nd- or 3rd-line treatment of advanced NSCLC following failure of prior chemotherapy, and later approved as maintenance treatment[19]. The use of EGFR TKIs in other settings, e.g. erlotinib as 1st-line treatment and gefitinib as 1st-line or maintenance treatment, has also been investigated in randomized trials.

A number of meta-analyses[24-31] have been conducted to synthesize the results of these trials, as summarized in Table 1. Briefly, when EGFR TKIs were compared with placebo, the hazard ratios (HRs) varied from 0.87 (95% confidence interval [CI]: 0.76-0.99) in 1st-line treatment to 0.61 (95% CI: 0.51-0.73) in 2nd-line treatment for progression-free survival, and from 1.01 (95% CI: 0.96-1.13) in 1st-line treatment to 0.70 (95% CI: 0.58-0.84) in 2nd-line treatment for overall survival (Table 1). When EGFR TKIs were compared with standard chemotherapy, the HRs generally approximated to 1 (the null effect), except for erlotinib as 1st-line treatment when the HRs favored standard chemotherapy (HR = 1.55 [95% CI: 1.24-1.93] for progression-free survival, HR = 1.39 [95% CI: 0.99-1.94] for overall survival) (Table 1).

To our knowledge, evidence that directly compared gefitinib and erlotinib is mainly from cohort studies[32-39], with only one randomized controlled trial available[33]. Both studies in which gefitinib and erlotinib arms had similar baseline (achieved either by randomization or by multivariate adjustment) and those in which the comparability between the two arms was unclear consistently showed that the two agents had comparable efficacy[33-36,38,39]. In the studies where gefitinib and erlotinib arms were incomparable in baseline characteristics which all favored the gefitinib arm, as expected, gefitinib was found to be superior to erlotinib[37]. Overall, current evidence does not suggest that gefitinib and erlotinib differ in efficacy.

1.6 Predicting those who may respond to EGFR TKIs treatment

As with traditional chemotherapy for cancer, EGFR TKIs are associated with considerable adverse events[10,19]. For example, in maintenance treatment erlotinib induces an excessive occurrence of rash in 43.4%, diarrhea in 15.8%, and a variety of other adverse events in 3%-6% of treated patients[19]. In 2nd-line treatment, adverse events are even more frequent, with an excessive rate of 58% of rash, 36% of diarrhea, and 4%-14% of other various events[19]. EGFR TKIs also constitute a heavy economic burden to their recipients. In the United States, erlotinib and gefitinib cost about $4,000 and $1,800 per month, respectively[3]. In Hong Kong, the monthly cost

is about HK$15,000 for either of them[40]. In view of these problems, it is important that the treatment is given only to those who are likely to benefit from it.

As demonstrated above (Table 1), in unselected patients with advanced NSCLC, the overall efficacy EGFR TKIs is better than that of placebo, but is quite limited in the 1st-line setting (Table 1). Even in maintenance or 2nd-line setting, the absolute benefit provided by EGFR TKIs is still small, with a prolonged median progression-free survival of 0.4 to 2.2 months and a prolonged median overall survival of 0.5 to 3.3 months[10,41-43]. This is probably due to the fact that only 10%-20% of the patients respond to the treatment[44,45]. This also means that 80%-90% of treated patients may suffer from adverse events and bear a huge cost without any benefit in return. Compared with standard chemotherapy, EGFR TKIs generally have similar overall efficacy in unselected patients, except that erlotinib was found inferior as the 1st-line treatment. Again, EGFR TKIs appear to be much better than standard chemotherapy in some patients (Table 1).

For example, in a randomized trial by Lilenbaum et al[46], where EGFR TKI (erlotinib) was compared with standard chemotherapy (carboplatin plus paclitaxel) as 1st-line treatment for advanced NSCLC, the median overall survival was 6.5 months in the

EGFR TKI arm and 9.7 months in the chemotherapy arm, respectively (HR = 1.73 [95% CI: 1.09-2.73]). This suggested that the overall efficacy of EGFR TKI in unselected patients was inferior to that of standard chemotherapy. Surprisingly, for a subgroup of patients who experienced progression, did not tolerate, or refused further chemotherapy in the chemotherapy arm and were thus crossed over to erlotinib, the median survival was 14.9 months, much longer that the median overall survival of either treatment arm. This indicated that EGFR TKI might be exceptionally better than chemotherapy in some patients.

The above evidence and other similar data clearly point to a need for distinguishing patients who may respond to EGFR TKIs from those who may not, in order to improve the efficacy of the therapy. If patients who are most likely to benefit from the treatment can be identified beforehand through use of predictive markers, optimal treatment can be decided. It is at the heart of so-called personalized (or individualized) treatment to identify and treat those who are most likely to respond.

1.7 Potential predictive markers, with focus on topical biomarkers

The exploratory analyses of early trials and subsequent large cohort studies that assessed the efficacy of EGFR TKIs frequently showed that patients with the

following clinical and/or pathological characteristics were more likely to benefit from the treatment: Asian or East Asian (versus other ethnicities), female (versus male), a never- or light-smokers (versus an ever- or heavy-smokers) and adenocarcinoma (versus other histological types)[10,44,45,47-51]. In the meantime, since EGFR TKIs are targeted at the EGFR signaling pathway, molecular alterations closely related to this pathway, especially *EGFR* mutations, *EGFR* gene copy number (GCN) gain, EGFR protein expression and *KRAS* mutations, are also been considerably investigated as potential predictive biomarkers for the efficacy of the treatment, and are postulated to be the genetic basis underlying the impact of the abovementioned clinical and/or pathological characteristics on cancer treatment outcomes[52-62]. Sections 1.7.1-1.7.4 below provide a brief description of the epidemiology, biological effects and clinical significance of the four biomarkers.

1.7.1 *EGFR* mutations

In NSCLC, the rate of *EGFR* mutations varies considerably according to ethnicity, and is generally 15%-20% in North Americans and Europeans[63-65], 20%-30% in Latin Americans[66], and 40%-60% in Asians[41,52,67,68]. Studies have also shown consistently that *EGFR* mutations are more frequent in females, never- or light-smokers, and adenocarcinoma patients[52,55,65-67,69-71].

EGFR mutations mainly occur in exons 18 to 21 that encode the tyrosine kinase domain of EGFR protein which, as stated above, is the target of EGFR TKIs. *EGFR* gene without mutations is called wild-type *EGFR*[72]. Common methods for detecting *EGFR* mutations include direct sequencing, denaturing high-performance liquid chromatography, and amplification refractory mutation system[73]. Although there is no standard method for detecting *EGFR* mutations in lung cancer specimens, direct sequencing has the highest sensitivity and is the most widely used among over 10 methods available.

The EGFR protein encoded by mutant *EGFR* gene selectively transduces the signals on which the NSCLC cells depend for surviving. This makes the cancer cells with mutant *EGFR* 100-fold more sensitive than those with wild-type *EGFR* to the inhibition of survival signals by EGFR TKIs, a phenomenon often referred to as "oncogene addiction"[74,75].

1.7.2 *EGFR*-GCN gain

The prevalence of *EGFR*-GCN gain in advanced NSCLC varies from 7% to 70%, with an average of about 35% in European or North American patients and 50% or

higher in East Asians[68,76]. *EGFR*-GCN gain seems also more frequent in females than males and in adenocarcinoma patients than those of other histological types[77] and is strongly associated with *EGFR* mutations, with a rate of 50%-80% in patients who harbor *EGFR* mutations and 20%-25% in those who have wild-type *EGFR*[55,77,78].

Some researchers proposed that the presence of *EGFR*-GCN gain might also be indicative of "oncogene addiction", which means that the tumor is highly dependent on the "abnormal" gene for proliferation and/or survival and thus is more sensitive to the treatment with EGFR TKIs[79,80]. The most commonly used technique to quantify *EGFR*-GCN is fluorescence in situ hybridization and chromogenic in situ hybridization[81,82].

1.7.3 EGFR protein expression

As a transmembrane protein and one of the 4 members of ERBB receptor family, EGFR is also called HER-1 or ERBB1. The expression of EGFR protein is seen in 50%-90% of NSCLC[83]. There seems to be no obvious relationship between EGFR protein expression and clinical or pathological characteristics such as sex, smoking status and tumor histology[58]. At the cellular level, the binding of EGFR to epidermal growth factor or other similar factors could result in the dimerization of EGFR. The

tyrosine kinase domain of EGFR is then phosphorylated and results in the generation of signals that are involved in the proliferation, angiogenesis, metastasis and survival of the cancer cells[21,22]. As EGFR TKIs are targeted at EGFR, the expression status of EGFR protein has been hypothesized to be able to affect the efficacy of the treatment. EGFR protein expression status is almost universally analyzed by immunohistochemistry[83].

1.7.4 *KRAS* mutations

KRAS, which stands for v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, is a member of the *ras* gene family. *KRAS* mutations occur in around 15%-20% of unselected patients with advanced NSCLC[51,61,64,66,84]. Similar to *EGFR* mutations, *KRAS* mutations are more frequent in adenocarcinoma (20%-30%) than in squamous-cell carcinoma (around 7%)[85]. However, unlike *EGFR* mutations, *KRAS* mutations are more frequent in smokers than never-smokers and in Americans than Asians (30% versus 10%)[66,86,87].

Studies have shown that *KRAS* and *EGFR* mutations are often mutually exclusive, which means that patients harboring one usually do not harbor the other[62,67,88]. The majority of *KRAS* mutations (90%) occur in codons 12 and 13[89-91]. Similar to *EGFR*

mutations, common methods used to detect *KRAS* mutations include direct sequencing, denaturing high-performance liquid chromatography, and amplification refractory mutation system.

KRAS gene encodes KRAS protein. In the EGFR signaling pathway, KRAS protein can be activated by upstream stimulation resulting from the binding of EGFR to epidermal growth factor or other similar factors (Figure 2), and become inactivated after it transduces the signal to downstream effectors. However, if *KRAS* gene is mutated, KRAS protein would lose the ability to become inactivated after transducing the signal, and thus the downstream mitogen-activated protein kinase pathways may be continuously activated. This may enable the pathways to remain unaffected by the blockage of the upstream signal with EGFR TKIs and continue to result in autonomous growth and differentiation of cells[92].

1.8 Evaluation of the four potential predictive biomarkers

1.8.1 Limitation of previous studies

A major problem with many existing studies that set out to assess the predictive value of the aforementioned biomarkers is that their conclusions on whether a biomarker was “predictive” or not were drawn from single-arm (i.e. EGFR TKIs

treated arm) studies by comparing the clinical outcomes of biomarker-positive patients with biomarker-negative ones[76]. Admittedly, evidence from such studies is a preliminary scrutinizing of potential important predictive biomarkers, and can be hypothesis-generating. If a biomarker is associated with better treatment outcomes in single-arm studies, it has a potential for being a predictive marker. However, it is likely that such a biomarker is only a general prognostic marker[93]. Thus, such evidence alone is insufficient to produce a firm conclusion on whether the biomarker has a predictive value for efficacy. To illustrate this point, I will explain below what an efficacy predictive biomarker is, and how it differs from a prognostic marker.

1.8.2 Predictive versus prognostic biomarkers

Consider the hypothetical example below (Figure 3(A)). In a randomized controlled trial to evaluate the efficacy of treatment A, participants are randomized into two groups, with one group receiving treatment A and the other treatment B. Treatment B serves as the control group and, for simplicity, is placebo or no treatment in this example. In each treatment group, some patients harbor the biomarker of interest, which is *EGFR* mutations in this example, while the rest do not. Subgroup analysis can be conducted according to the biomarker status, in which treatments A and B are compared separately in the mutant *EGFR* subgroup and in the wild-type *EGFR*

subgroup. If the effect measure is hazard ratio (treatment A versus treatment B), a hazard ratio <1 favors treatment A and a hazard ratio >1 means the opposite.

If *EGFR* mutations are predictive of the efficacy of treatment A, treatment A would be associated with better outcomes in the mutant *EGFR* subgroup (HR_{B+} <1, say, = 0.5) and at the same time less strongly associated (say, HR_{B-} = 0.9), not associated (say, HR_{B-} = 1), or adversely associated (say, HR_{B-} = 1.5) with the outcomes in the wild-type *EGFR* subgroup[94,95]. Conversely, for *EGFR* mutations to be predictive of the resistance to treatment A, treatment A should be adversely associated with the treatment outcomes in the mutant *EGFR* subgroup (say, HR_{B+} = 1.5), and at the same time is less strongly associated (say, HR_{B-} = 1.1), not associated (say, HR_{B-} = 1), or associated with better outcomes (say, HR_{B-} = 0.5) in the wild-type *EGFR* subgroup.

In epidemiology, the relation between treatment and *EGFR* mutations status in this example is called interaction or effect modification in which *EGFR* mutations are called the effect modifier, and the treatment effect varies according to the effect modifier[96,97]. If confirmed as a predictive biomarker, *EGFR* mutations can be used to facilitate selecting the recipients who are more likely to benefit from treatment A. For example, if the treatment is effective in those with *EGFR* mutations and

ineffective in those with wild-type *EGFR*, then only patients with *EGFR* mutations should be given the treatment. It should be noted that other markers may be used together with *EGFR* mutations to achieve a greater predictive power.

However, if treatment A is (statistically) equally associated with the outcomes in both subgroups, then *EGFR* mutations cannot be used to select patients who are more likely to benefit from the treatment, as the patients with or without this biomarker are not different from each other in terms of benefiting from or resistance to the treatment. In this case, there is no interaction between the treatment and *EGFR* mutations status, and *EGFR* mutations are only a general prognostic biomarker, which means that with this biomarker patients have consistently better or worse prognosis than do those without this biomarker, whether they are treated or not[94,96].

Therefore, to evaluate the predictive value is essentially to test for interaction. Now, it is easier for us to understand why the association of a biomarker with the outcome in the intervention group alone cannot be necessarily taken as an indicator of a "predictive" ability – because there may well be the same association in the control group, in which case the biomarker only has a general prognostic role.

1.8.3 The best study design to evaluate predictive biomarkers

As discussed above, to evaluate the predictive value of a biomarker is essentially to test for the treatment—biomarker interaction. The interaction is best evaluated by the randomized controlled trial that assesses the efficacy of treatment with subgroup analysis according to the biomarker status (Figure 3(A)). Alternatively, the interaction can be equally evaluated in a randomized trial with a design shown in Figure 3(B). The difference between Figures 3(A) and 3(B) is that the latter conducts stratified randomization based on biomarker status. However, in terms of statistical comparison, the two designs are equally valid.

Of note, the design shown in Figure 3(B) is rarely adopted in practice, mainly for three reasons[98,99].

1) Stratified randomization helps improve the balance of biomarker status in the overall comparison (i.e. all patients receiving treatment A versus all patients receiving treatment B). However, the balance of treatments A and B within each subgroup is more relevant in evaluating the treatment—biomarker interaction, and stratified randomization performs no better than Figure 3(A) in this aspect.
2) The potential predictive biomarkers for a treatment are often not found until some initial trials with subgroup analyses according to biomarker status have

been completed. It is impossible for these trials to conduct retrospective stratified randomization according to biomarker status.

3) It takes some time (usually 10 to 18 days[100,101]) from requesting tumor samples to the availability of the results of biomarker analysis, which means that in order to conduct stratified randomization, patients who badly need a treatment would have to wait till the results on biomarker status are available. This often makes it unethical and infeasible to conduct stratified randomization.

We have not seen any randomized controlled trials about the treatment of advanced NSCLC with EGFR TKIs that were conducted in the approach shown in Figure 3(B).

The types of control used in trials are also worth mentioning. In the above example (Figure 3(A)), the control treatment, i.e. treatment B, is placebo or no treatment, because the trial aims to evaluate the absolute efficacy of treatment A. However, nowadays, more and more trials use existing effective treatments as control with an aim to evaluate the relative or comparative efficacy of new treatments and inform decision on which is better. Accordingly, in evaluating predictive biomarkers, the control treatment can also be an existing effective treatment, which is mostly chemotherapy in the case of advanced NSCLC.

The appropriateness of using chemotherapy as control in trials to evaluate predictive biomarkers relies on an assumption that the efficacy of chemotherapy does not vary with status of the biomarkers, which we deemed valid in the present systematic review, for two reasons. First, chemotherapy is not biologically targeted at the signaling pathway where the 4 biomarkers of our interest take effect, thus its efficacy is unlikely to be affected by biomarker status. Second, currently available, empirical studies found no significant interaction between chemotherapy and the biomarkers. In other words, there is no strong evidence that the efficacy of chemotherapy would vary with biomarker status[102-105].

Thus, both placebo- and chemotherapy-controlled trials were considered relevant and valid in this review (see below). While placebo-controlled trials are better for demonstrating the maximum predictive power of a biomarker, chemotherapy-controlled trials would add evidence to further support the interaction if indeed present.

1.9 The need for a comprehensive summary of current best evidence

To achieve personalized treatment of advanced NSCLC, a number of randomized controlled trials that can be used to test for the interaction between EGFR TKIs treatment and the abovementioned four biomarkers are available[43,84,106,107]. However, individually these trials provide no straightforward answer to the question whether the investigated biomarkers are qualified for clinical use. On one hand, this is because the results about treatment—biomarker interaction from single trials are often statistically insignificant, preventing a firm conclusion to be drawn. On the other hand, the results from different trials were not always consistent.

Take *EGFR* mutations for predicting the overall survival benefit from EGFR TKIs as an example. Bell et al[108] compared EGFR TKI (gefitinib) plus platinum-based doublet (i.e. the standard chemotherapy) with placebo plus platinum-based doublet as first-line treatment of advanced NSCLC. The HR (EGFR TKI versus control, the same below) for death was 1.77 (95% CI: 0.50-6.23) in the mutant *EGFR* subgroup and 0.91 (95% CI: 0.67-1.23) in the wild-type *EGFR* subgroup. There seems an interaction that those with *EGFR* mutations would benefit less from EGFR TKI, although the result is statistically insignificant (p for interaction = 0.84).

Zhu et al[84] compared EGFR TKI (erlotinib) alone with placebo alone as second-line treatment of advanced NSCLC. The HR for death was 0.55 (95% CI: 0.25-1.19) in the mutant *EGFR* subgroup and 0.74 (95% CI: 0.52-1.05) in the wild-type *EGFR* subgroup. There seems an interaction that those with *EGFR* mutations would benefit more from EGFR TKI, although the result is statistically insignificant (*p* for interaction = 0.25).

Brugger et al[43] compared EGFR TKI (erlotinib) alone with placebo alone as maintenance treatment of advanced NSCLC. The HR for death was 0.83 (95% CI: 0.34-2.02) in the mutant *EGFR* subgroup and 0.77 (95% CI: 0.61-0.97) in the wild-type *EGFR* subgroup. There seems no interaction between treatment and *EGFR* mutations in the benefit from EGFR TKI (*p* for interaction = 0.56).

The results in these studies were not consistent. Of note, all of the tests for interaction and almost all within-subgroup HRs from these studies were statistically insignificant. Thus, it remains uncertain whether the insignificant and inconsistent interaction was a true difference in the effects of *EGFR* mutation status, a result of the play of chance, or due to other factors. However, as discussed above, to conduct a new trial to specifically address these problems would be both resource- and time-

consuming and thus unethical before these trials are summarized. But a systematic review of existing trials with meta-analysis of the treatment—biomarker interaction is lacking.

1.10 Objectives of the present study

The present study was aimed to summarize the current best evidence and examine the predictive value of *EGFR* mutations, *EGFR*-GCN gain, EGFR protein expression and *KRAS* mutations in the treatment of advanced NSCLC with EGFR TKIs. Specifically, the objectives were two-fold, as justified in Section 1.8.3: (1) to examine the predictive value of the four biomarkers under the circumstance that EGFR TKIs were compared with placebo, and (2) to examine the predictive value of the four biomarkers under the circumstance that EGFR TKIs were compared with chemotherapy. Our null hypothesis is that one of the four biomarkers can predict who are more likely to respond to EGFR TKIs treatment, while the alternative hypothesis is that some or all of these biomarkers have that predictive power.

Chapter 2 Materials and Methods

2.1 Study design

2.2 Eligibility criteria for original studies

2.3 Literature search

2.4 Study selection

2.5 Data collection

2.6 Statistical analyses

2.1 Study design

The present study is a systematic review with meta-analysis of data from randomized controlled trials that assessed the efficacy of EGFR TKIs with subgroup analysis according to biomarker status. To evaluate the predictive value of each biomarker, the treatment—biomarker interaction was assessed. The flow of the study is shown in Figure 4 and elaborated below.

2.2 Eligibility criteria for original studies

To be eligible for the present systematic review, the original studies had to meet all the following criteria:

1) **P**opulation: advanced NSCLC patients, with cancer at stage IIIB or IV or "locally advanced, metastatic or recurrent";
2) **I**ntervention: EGFR TKIs alone or EGFR TKIs plus other treatments;
3) **C**ontrol: placebo, no treatment, or chemotherapy, with or without the baseline treatments in the intervention arm;
4) **O**utcome: progression-free survival, overall survival, or both;
5) **S**tudy design: randomized controlled trial;
6) Subgroup analyses were conducted according to the status of at least one of the following biomarkers: *EGFR* mutations, *EGFR*-GCN gain, EGFR protein

expression and *KRAS* mutations. In other words, the comparison of EGFR TKIs versus control was made in biomarker-positive (e.g. mutant *EGFR*) patients and in biomarker-negative (e.g. wild-type *EGFR*) patients, separately.

Although randomized controlled trials with prospective or pre-planned subgroup analysis according to biomarker status are desirable in the evaluation of predictive biomarkers[98,99,109], such trials are not always available, because the predictive value of biomarkers may have not been recognized until the first trials assessing the EGFR TKIs efficacy were completed. Retrospective or *post hoc* subgroup analyses using archived tumor tissues from previously completed trials can be more readily conducted than new trials to specifically address the same question. Moreover, if well conducted, retrospective subgroup analyses can also produce high-level evidence and achieve similar validity of prospective analyses[98]. Thus, in the present systematic review, both prospective and retrospective subgroup analyses according to biomarker status were considered eligible.

2.3 Literature search

A comprehensive search strategy was constructed by discussion and consensus among three researchers (the present PhD candidate, a research staff who had a lot of experience in literature search and thesis supervisor).

2.3.1 Search of electronic databases

We performed a systematic literature search in five electronic databases:

1) PubMed;

2) EMBASE;

3) The Cochrane Library;

4) Chinese Biomedical Literature Database (in Chinese);

5) Wanfang Data (in Chinese)

Each of the databases was searched from its inception to the search date (initial search: 28 October 2011; updated search: 26 May 2012), limited to "human studies" where possible, with no restrictions on the time or language of publication. The search strategy and history were described in detail in Appendix 1. Briefly, the following three groups of search terms were used, which were about the disease, the treatment and the biomarkers of interest, respectively.

1) Group 1: Carcinoma, non-small-cell lung [Mesh]; lung non small cell cancer /(in EMBASE); non-small-cell lung cancer; non-small cell lung cancer; non-small-cell lung carcinoma; non-small cell lung carcinoma; NSCLC.
2) Group 2: gefitinib [Mesh]; erlotinib [Mesh]; gefitinib/(in EMBASE); erlotinib /(in EMBASE); tyrosine kinase inhibitor; TKI; gefitinib; Iressa; erlotinib; Tarceva.
3) Group 3: Receptor, epidermal growth factor [Mesh]; genes, erbB-1[Mesh]; EGFR protein, human [Mesh]; genes, erbB; [Mesh]; genes, ras [Mesh]; epidermal growth factor receptor/(in EMBASE); epidermal growth factor receptor; EGF receptor; EGFR; KRAS; K-RAS; RAS.

2.3.2 Search of conference proceedings

The conference abstracts of American Society of Clinical Oncology and European Society of Medical Oncology were reviewed online via their official websites to identify additional studies. In particular, we reviewed the abstracts of the following conferences:

(1) Conferences held by American Society of Clinical Oncology (ASCO)

1) 2011 ASCO Annual Meeting

2) 2010 Molecular Markers

3) 2010 ASCO Annual Meeting

4) 2009 ASCO Annual Meeting

5) 2008 Molecular Markers

6) 2008 ASCO Annual Meeting

7) 2007 ASCO Annual Meeting

(2) Conferences held by European Society of Medical Oncology (ESMO)

1) 2011 The European Multidisciplinary Cancer Congress

2) 2011 9th International Symposium on Targeted Anticancer Therapies (TAT)

3) 2011 European Multidisciplinary Conference in Thoracic Oncology (EMCTO)

4) 2010 35th ESMO Congress, Milan

5) 2010 8th International Symposium on Targeted Anticancer Therapies

6) 2010 2nd European Lung Cancer Conference (ELCC)

7) 2009 European Multidisciplinary Conference in Thoracic Oncology

8) 2009 7th International Symposium on Targeted Anticancer Therapies

9) 2009 ECCO 15 and 34th ESMO Multidisciplinary Congress

10) 2008 ESMO Conference Lugano (ECLU)

11) 2008 33rd ESMO Congress, Stockholm

12) 2008 1st European Lung Cancer Conference

13) 2007 ESMO Conference Lugano (ECLU)

14) 2007 EIS on Chest Tumors

15) 2006 31st ESMO Congress, Istanbul

16) 2004 29th ESMO Congress, Vienna

17) 2002 27th ESMO Congress, Nice

2.3.3 Other searches

We also scrutinized the reference lists of highly relevant publications, which in particular include:

1) Studies that met the inclusion criteria as stated above; and
2) Reviews (including systematic reviews and meta-analyses), guidelines, consensus and expert opinions about the treatment of advanced NSCLC with EGFR TKIs, especially those with reference to predictive or prognostic biomarkers.

This part was done after screening the retrieved references from electronic databases and conference proceedings and relevant publications were identified.

2.4 Study selection

First, the title and abstract of retrieved references were screened to judge for their relevance. Then, the full text of the studies potentially fulfilling the inclusion criteria were obtained for detailed examination. Studies that met all of the inclusion criteria were considered eligible and included with the following exceptions.

1) When the same population was used in more than one study addressing the same question, only the study with the largest sample size or the study with most relevant information was included for the present analysis, while the others were excluded as “duplicates”. However, where appropriate, we used these “duplicates” as a supplementary source of information on clinical and methodological characteristics of the included studies.
2) Studies without extractable data (e.g. it was clearly stated that relevant analysis had been conducted, but provided no detailed data, and the data was still not available after contact of authors) were excluded from meta-analyses, but were carefully reviewed to see if there was possibility that their results could affect our overall conclusion.

The eligibility of each "potentially eligible" study was assessed independently by two researchers. In case of disagreement, a third expert on systematic review was consulted for final decision.

2.5 Data collection

2.5.1 Data extraction

The following data were extracted from each eligible study:

1) Bibliographic information: first author, publication year, etc;
2) Patients' characteristics: number of patients, age, gender, ethnicity, smoking status, stage of cancer, etc.
3) Treatment protocols: dose, frequency and duration of treatment, etc.
4) Biomarker analysis: testing method, percentage of positive findings, etc.
5) Main results stratified by biomarker status: median progression-free survival in EGFR TKIs treated and biomarker-positive patients, median progression-free survival in biomarker-positive control patients, the hazard ratio for comparison of the two, etc.
6) Information related to the methodological characteristics of study for quality assessment: whether the treatment and control groups were comparable in each

subgroup defined by biomarker status, whether the biomarker analyses were blinded to those who assessed the outcome, etc.

A detailed data extraction form is shown in Appendix 2. Data was extracted by 2 researchers independently. Any disagreements between the two were resolved by discussion with reference to the original papers or, when deemed necessary, by a third reviewer if disagreement persisted. Authors were contacted if deemed necessary via email to clarify the ambiguities in reported methods or results and to seek additional data not included in the published report.

2.5.2 Transformation and estimation based on reported data

It is a frequently encountered problem that data reported by the original studies do not directly match the need of a systematic review or cannot be used directly for meta-analysis. Under this circumstance, transformation and/or estimation are needed in order to obtain the required data. Specifically, in the present study, transformation and estimation were conducted in the following circumstances.

1) The original study did not report the median progression-free survival, overall survival, and/or hazard ratio explicitly, but provided the relevant survival curves. In this case, figures containing survival curves were enlarged and printed out.

Then a scale was applied to measure the curves to obtain the numerical values of interest. Hazard ratio was then estimated according to the method recommended by the Cochrane Handbook for Systematic Reviews of Interventions[110-112].

2) The original study reported hazard ratio and *P* value but not 95% CI of the hazard ratio. In this case, the 95% CI was calculated according to the method recommended by the Cochrane Handbook for Systematic Reviews of Interventions. In particular, 95% CI = exp (B±1.96×standard error), where B is ln(HR), and standard error is the absolute value of ln(HR) divided by the absolute value of *Z* score corresponding to the reported *P* value under standard normal distribution. Using data from studies with available HRs, 95% CIs and *P* values, this method was proved to be valid.

3) The intervention and control groups of the hazard ratio reported in the original study needed to be swapped when included in the analysis of our systematic review. For example, in the present systematic review, the hazard ratio is based on the comparison of EGFR TKIs versus control, with control as the reference group. However, in the original study, the reported hazard ratio was based on control versus EGFR TKIs, with EGFR TKIs as the reference group. In this case, we used the formulas in Table 2 to transform the hazard ratio.

2.6 Statistical analyses

2.6.1 Main analyses

The primary and secondary clinical outcomes of interest in the present systematic review were progression-free survival and overall survival, respectively. The efficacy of treatment in terms of progression-free survival or overall survival was measured by HR with 95% CI. Progression-free survival was selected as the primary clinical outcome of interest because it is more representative of the "pure" efficacy of treatment. By contrast, the results on overall survival are vulnerable to confounding by possible cross-over of patients during study and the imbalance of post-study treatments between different arms.

As stated above, whether or not a biomarker has a predictive value should be determined according to the treatment—biomarker interaction. If the interaction is statistically significant — in other words, the treatment efficacy differ significantly across the two subgroups defined by biomarker status — then the biomarker has a predictive value. Otherwise, it will be uncertain whether the biomarker is predictive or not. To illustrate how we evaluated the predictive value of a specific biomarker for the treatment efficacy in terms of a specific outcome, we take *EGFR* mutations (the biomarker) and progression-free survival (the outcome) as an example.

Step 1: Meta-analysis to estimate the efficacy of EGFR TKIs

Using the HRs for progression-free survival, stratified by *EGFR* mutation status, we conducted meta-analyses with the random-effect model to obtain a summary HR for mutant *EGFR* subgroup and wild-type *EGFR* subgroup, separately, as the estimate of the efficacy of EGFR TKIs in the two subgroups. As the control group might receive placebo or chemotherapy, and the efficacy of EGFR TKIs as compared with different types of control is probably different, we conducted separate meta-analyses of studies that compared EGFR TKIs with placebo and those that compared EGFR TKIs with chemotherapy. Thus, four meta-analyses were conducted to estimate the efficacy of EGFR TKIs in terms of progression-free survival, which were as follows.

1) EGFR TKIs vs placebo in mutant *EGFR* subgroup (summary HR_1)
2) EGFR TKIs vs placebo in wild-type *EGFR* subgroup (summary HR_2)
3) EGFR TKIs vs chemotherapy in mutant *EGFR* subgroup (summary HR_3)
4) EGFR TKIs vs chemotherapy in wild-type *EGFR* subgroup (summary HR_4)

As there were two outcomes and four biomarkers (Figure 4), a total of 32 meta-analyses (4×2×4) were conducted to estimate the efficacy of EGFR TKIs under different circumstances.

Step 2: Calculation of the interaction term

To assess the treatment—*EGFR* mutations interaction, one method is to compare the abovementioned HRs, say, HR_1 vs HR_2 or HR_3 vs HR_4[113]. This method first combines subgroup treatment effects across trials to obtain a summary estimate and then compares different subgroups' summary estimates, which is flawed for several reasons. First, as the mutant *EGFR* subgroup and wild-type *EGFR* subgroup in each trial are recruited and managed within the confines of a single protocol, they are more similar to each other than to those from other trials in various characteristics or covariates. However, this kind of similarity or correlation is not accounted for by the abovementioned method which takes each subgroup as an independent dataset in the meta-analysis, leading to the loss of efficiency and inappropriate standard errors[114]. Second, in combining subgroup treatment effects, heterogeneity in treatment effects across trials is usually ignored. Random-effect model could partly address this problem but seems not enough. Thus, it has been recommended that this method should be avoided[114]. A second method is to compare the summary HR_1 and HR_2 that are derived from different sets of studies, which in essence is "indirect" comparison and weak in scientific rigor.

A more appropriate way is to compare the two HRs (i.e. test for interaction) within the same study first, and then combine through meta-analysis the results from different studies[114]. This is similar to the following common practice in trials: to estimate the average before-after change in blood pressure of a group of patients, the change in blood pressure of each patient should be calculated first, and then the average change can be estimated by pooling the data of all patients.

In the present systematic review, we did it in this third method. First, based on the HR with 95% CI in the mutant *EGFR* subgroup and that in the wild-type subgroup from the same trial, we calculated a ratio of the two HRs with 95% CI (Table 3)[115]. The ratio is then the measure of the treatment—*EGFR* mutations interaction[115]. A ratio of HRs equal to 1 suggests no interaction, i.e. *EGFR* mutated and wild-type patients benefit from EGFR TKIs to the same extent[116].

A ratio of HRs statistically significantly different from 1 suggests the presence of treatment—*EGFR* mutations interaction. If it is smaller than 1, it suggests *EGFR* mutated patients benefit more from EGFR TKIs than *EGFR* wild-type patients, whereas a ratio of HRs greater than 1 suggests the opposite[116]. The further away the ratio of HRs is from 1, the stronger the interaction is.

As mentioned in Section 1.8.3, there is no strong evidence that the efficacy of chemotherapy would vary with the status of biomarkers of our interest. Thus, in calculating the ratio of HRs, the effect of control treatment in mutant *EGFR* subgroup and that in wild-type *EGFR* subgroup is offset (Figure 5), and the ratio of HRs in chemotherapy-controlled trials is mainly determined by the effect of EGFR TKIs treatment, similar to the situation in placebo-controlled trials. This suggests that the types of control matter little for analyses on ratio of HRs under the condition we assumed. Thus, the ratios of HRs from trials of different controls were expected to be homogeneous, which make it justifiable to combine all of them.

Step 3: Meta-analysis of interaction terms

After the ratios of HRs were obtained from all relevant trials, including both placebo-controlled and chemotherapy-controlled ones, they were combined by using a random-effect model to produce a summary estimate of the treatment—*EGFR* mutations interaction[117,118]. This approach has been employed by previous studies[116]. Within each meta-analysis, studies using different controls were first combined separately in a stratified approach to see whether they provide similar results. If the summary ratio of HRs based on placebo-controlled trials and that based on

chemotherapy-controlled trials were indeed similar, the result of meta-analysis combining all trials was preferred, as it was based on more studies and had higher statistical power. If the summary ratios of HRs from trials of different controls did differ, say, in an opposite direction, the combined results should be interpreted with caution.

In evaluating the interaction between treatment and other biomarkers, we used the same methods as described above. These methods were also employed in evaluating the treatment—biomarker interaction on overall survival. Thus, for two outcomes and four biomarkers, a total of eight meta-analyses (1×2×4) (Figure 4) were implied. In doing these meta-analyses, statistical heterogeneity among studies was assessed by the Cochran's *Q*-test and the I^2 statistic[118-120]. A *P* value ≤ 0.10 for the *Q*-test or an I^2 > 50% was considered suggestive of substantial between-study heterogeneity.

2.6.2 Additional analyses

2.6.2.1 Meta-regression analysis

In case of substantial heterogeneity, meta-regression analyses were conducted to investigate whether heterogeneity could be explained by important clinical and/or pathological factors, which in particular included:

1) EGFR TKI used: gefitinib versus erlotinib

2) Treatment modality: monotherapy versus combination therapy

3) Line of treatment: 1st versus maintenance versus ≥2nd

4) Comparator: chemotherapy versus placebo

5) Ethnicity: "more" versus "less" Asian, with median percentage of Asian as the cut-off point (the same for the following factors 6 to 8)

6) Sex: more versus less female

7) Smoking history: more versus less never-smokers

8) Cancer histology: more versus less adenocarcinoma

First, one factor each time was included in a univariate meta-regression analysis. Then, if the number of studies was sufficiently large, say 5~10 times the number of factors, all eight factors would be put into the model for mutivarate meta-regression analyses. A P value ≤ 0.10 indicated that the examined factor could be a major source of heterogeneity. Then the meta-analysis was stratified by this factor. If no such factor was found, only one meta-analysis was conducted with the random-effect model without stratified analysis.

2.6.2.2 Sensitivity analysis

Sensitivity analyses were conducted to test the robustness of the results by modifying the inclusion criteria in three ways:

1) Trials in which biomarker studies were not pre-planned were excluded;
2) Trials in which intervention and control arms had incomparable baseline characteristics such as the proportions of female patients, never-smokers and adenocarcinoma between the subgroups defined by biomarker status were excluded;
3) Trials in which there were significant cross-over of patients or imbalance of post-study treatments between treatment arms were excluded.

2.6.2.3 Assessment of publication bias

Begg's funnel plots were used to visually and Egger's tests statistically assess the possibility of publication bias if a meta-analysis included 10 studies or more[121]. Symmetrical funnel plots indicate that publication bias is less likely to exist. However, asymmetrical funnel plots do not necessarily mean there is publication bias, as it might well be a result of some other causes, especially when there is significant heterogeneity among studies[122,123]. Thus, in the present systematic review,

asymmetrical funnel plots were considered only suggestive of publication bias rather than a definitive evidence for the bias.

All analyses were performed with RevMan 5.0 or Stata 11.0.

Chapter 3 Results

3.1 Results of literature search and study selection

3.2 Methodological characteristics of included studies

3.3 Predictive value of *EGFR* mutations

3.4 Predictive value of *EGFR*-GCN gain

3.5 Predictive value of EGFR protein expression

3.6 Predictive value of *KRAS* mutations

3.1 Results of literature search and study selection

The flow of study selection is shown in Figure 6. For literature search on *EGFR* mutation status, *EGFR*-GCN and EGFR protein expression status (Figure 6A), 4,014 references were identified from the five electronic databases, with 1,131 of them being duplicates. Among the 2,883 unique references, 2,827 were excluded due to various reasons through screening of titles and abstracts, and the remaining 56 were subject to a review of full texts. In the meantime, screening of titles and abstracts indicated that 11 review papers on biomarkers, 18 review papers on the efficacy of EGFR TKIs, two drug labels, one paper of expert opinion and one clinical guideline/recommendation were regarded as highly relevant to the present systematic review. Thus, their reference lists were scrutinized.

After the above procedures were completed, 41 studies (including one identified from the reference lists of relevant papers) from electronic databases and 14 from the abstracts of ASCO and ESMO conferences were considered potentially eligible. Careful review of the 55 reports led to further exclusion of 37 studies. Thirty-three were duplicates and 4 did not provide any detailed data needed for this review. Thus, 18 studies were considered eligible and included in final analyses[41,43,61,68,84,106-108,124-

[133]. The data of two studies, Johnson et al[132,134] and Lee et al[133,135], was obtained from more than one source.

For literature search and selection of studies on *KRAS* mutations, the process was similar (Figure 6B). Six eligible studies[43,61,84,107,127,132] were identified, but all of them have been included in the 18 abovementioned studies for EGFR alterations. There were no further findings from the references of the eligible studies. Authors of five studies[61,68,130,136,137] were contacted for data needed to judge the eligibility of a study or to conduct meta-analysis. Only one of them replied with useful data, which were the hazard ratios in mutant *KRAS* and wild-type *KRAS* subgroups[61].

3.2 Methodological characteristics of included studies

The 18 included studies were mainly based on the following trials: ATLAS (AVF 3671 g)[132,134], First-SIGNAL[133,135], INFORM (C-TONG 0804)[41], INSTEP[106], INTACT[108], INTEREST[127], INVITE[126], IPASS[128], ISEL[130], NCIC CTG BR.21[84,125], SATURN (BO18192)[43], STEPAN[124], TITAN[107], TRIBUTE[61,129,131] and V-15-32[68]. The information on the methodological characteristics of these studies was summarized in Table 4.

Twelve studies were pre-planned analyses to examine the predictive value of biomarkers[41,43,68,106,107,124-128,132,133], while the others retrospectively analyzed the data from completed randomized controlled trials. Patients with available biomarker testing results and thus included in our analyses accounted for 12%-86%[68,107] (in most cases, 20%-50%) of the total trial population, and were reported as similar to (or "representative of") the original population in nine (50%) studies[41,43,61,106-108,128,131,132].

Three studies provided information on the comparability between intervention and control arms in biomarker-positive and biomarker-negative patients, respectively[41,107,131]. The comparability was achieved in one of them[41]. In the other two[107,131], the predictive value of biomarkers might have been biased due to the imbalance between intervention and control arms.

Eleven studies (61%) were based on double-blind trials[41,43,61,84,106,108,125,129-132], with the rest being open-label trials. Eight studies (44%) clearly stated that their biomarker analyses were blinded to treatment allocation and clinical outcomes[43,61,84,125,127,128,130,131]. The cross-over of treatment or use of post-study treatments in the parent trial was significant in nine (50%) studies[41,43,68,107,124,126-

[128,133]. Three studies clearly showed that the HRs they reported were obtained by multivariate analyses[41,128,130].

Overall, information on the methodological characteristics of included studies is limited. The available data indicated that some studies might suffer from important bias. Yet, for most of them, it was difficult to tell whether the bias indeed existed and, if yes, to which direction and what extent.

3.3 Predictive value of *EGFR* mutations

3.3.1 Basic characteristics of included studies

Twelve studies were included in the evaluation of the predictive value of *EGFR* mutations[41,43,61,68,84,107,108,124,127,128,132,133]. The basic characteristics of these studies are summarized in Table 5. As shown in this table, the number of patients included from each study varied from 57 to 437, with a total of 2,714. Five studies were conducted in Asians only. The rate of *EGFR* mutations in the five studies ranged from 38% to 60%, while it was between 7% and 17% in studies that were conducted in other populations consisting mainly of Caucasians. Totally, there were 610 patients (22.5%) with *EGFR* mutations. The testing of *EGFR* mutations was limited

to exons 18-21 in almost all studies and was mostly done by direct sequencing (7 studies, 58%) and Amplification Refractory Mutation System (3 studies, 25%).

The median age reported in these studies varied from 55 to 77 years. The proportion of female patients was under 50% (19%-48%) in all studies except Fukuoka 2011 and Lee 2009, where it was 77% and 89%, respectively. A similar trend was seen for smoking history and cancer histology. Specifically, the percentage of never-smokers in Fukuoka 2011 and Lee 2009 was 93% and 100%, respectively, compared to 9% to 49% in other studies, and the proportion of patients with adenocarcinoma in the two studies was both 100%, compared to 46% to 82% in other studies. The proportion of patients with a performance status score of 0 to 1, who were considered as "fit" patients[138], varied between 67% and 100% in the included studies.

Five, 3 and 4 studies were conducted in the 1st-line, maintenance and 2nd-line settings, respectively. Gefitinib was used in one half of the included studies and erlotinib in the others. Three studies compared EGFR TKIs alone with placebo alone, 3 studies EGFR TKIs plus other treatments with placebo plus other identical treatments, 4 studies EGFR TKIs alone with single-agent chemotherapy and 2 studies EGFR TKIs

alone with combination chemotherapy. The outcome assessment criteria reported were uniformly Response Evaluation Criteria in Solid Tumors.

3.3.2 Efficacy of EGFR TKIs and treatment—*EGFR* mutations interaction: progression-free survival

Table 6 summarizes the median progression-free survival of intervention and control arms and corresponding HRs, stratified by *EGFR* mutation status.

When EGFR TKIs were compared with placebo (5 studies), the HRs ranged from 0.10 (95% CI: 0.04-0.25) to 0.55 (95% CI: 0.19-1.60) in mutant *EGFR* subgroups, and 0.73 (95% CI: 0.53-1.01) to 1.25 (95% CI: 0.94-1.66) in wild-type *EGFR* subgroups (Table 6). The summary HR for the two subgroups was 0.29 (95% CI: 0.15-0.55) and 0.88 (95% CI: 0.72-1.07), respectively (Figure 7, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (6 studies), the HRs ranged from 0.16 (95% CI: 0.05-0.49) to 0.71 (95% CI: 0.13-3.97) in mutant *EGFR* subgroups, and 0.15 (95% CI: 0.04-0.57) to 2.85 (95% CI: 2.05-3.98) in wild-type *EGFR* subgroups (Table 6). The summary HR for the two subgroups was 0.47 (95%

CI: 0.37-0.61) and 1.09 (95% CI: 0.65-1.82), respectively (Figure 7, the lower-left and lower-middle panels).

To test for the treatment—*EGFR* mutations interaction, a ratio of HRs was calculated within each study based on the data in Table 6. The ratio of HRs from different studies is presented in Figure 7 (the right panel). The I^2 and P value for heterogeneity test were 64% and 0.002, respectively, suggesting presence of heterogeneity. The summary ratio of HRs was 0.37 (95% CI: 0.22-0.60, $P < 0.0001$), which indicated a strong interaction between the treatment and *EGFR* mutations. The results from placebo-controlled trials and chemotherapy-controlled ones were consistent, both statistically significant (Figure 7, the right panel).

3.3.3 Efficacy of EGFR TKIs and treatment—*EGFR* mutations interaction: overall survival

Table 7 summarizes the median overall survival of intervention and control arms and corresponding HRs, stratified by *EGFR* mutation status.

When EGFR TKIs were compared with placebo (5 studies), the HRs ranged from 0.46 (95% CI: 0.21-1.02) to 1.77 (95% CI: 0.50-6.23) in mutant *EGFR* subgroups,

and 0.74 (95% CI: 0.52-1.05) to 1.10 (95% CI: 0.77-1.56) in wild-type *EGFR* subgroups (Table 7). The summary HR for the two subgroups was 0.68 (95% CI: 0.44-1.04) and 0.85 (95% CI: 0.75-0.97), respectively (Figure 8, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (5 studies), the HRs ranged from 0.82 (95% CI: 0.35-1.92) to 1.80 (95% CI: 0.10-32.97) in mutant *EGFR* subgroups, and 0.59 (95% CI: 0.24-1.44) to 1.20 (95% CI: 0.57-2.52) in wild-type *EGFR* subgroups (Table 7). The summary HR for the two subgroups was 0.97 (95% CI: 0.76-1.24) and 1.01 (95% CI: 0.85-1.20), respectively (Figure 8, the lower-left and lower-middle panels).

To test for the treatment—*EGFR* mutations interaction, a ratio of HRs was calculated within each study based on the data in Table 7. The ratio of HRs from different studies is presented in Figure 8 (the right panel). The I^2 and P value for heterogeneity test were 0% and 0.90, respectively, suggesting no obvious heterogeneity. The summary ratio of HRs was 0.84 (95% CI: 0.64-1.11, P = 0.22), which did not support strongly an interaction between the treatment and *EGFR* mutation. The

results from placebo-controlled trials and chemotherapy-controlled ones were consistent, although both statistically insignificant (Figure 8, the right panel).

3.3.4 Additional analyses

Due to the limited number of studies, only univariate meta-regression analyses were conducted to investigate the heterogeneity observed in Figure 7 (the right panel). The *P* value of significance test for each factor ranged from 0.393 to 0.962, which did not suggest that any of the pre-specified factors could explain the heterogeneity. This further supports our analyses that combined placebo-controlled and chemotherapy-controlled studies. In the sensitivity analyses, the conclusion on the predictive value of *EGFR* mutations did not change, although the numerical values of ratios of HRs and their 95% CIs were not exactly the same (Table 8).

The funnel plots based on the data presented in Figure 7 (the right panel) and Figure 8 (the right panel) are shown in Figure 9 and Figure 10, respectively. Visually no apparent asymmetry was observed in Figure 10, and there is no evidence to suggest presence of publication bias (Egger's test: $P = 0.248$). Figure 9 was found to be asymmetrical (Egger's test: $P = 0.041$) and skewed to the right side. If the asymmetry was truly resulting from publication bias, it means that the studies with

larger standard errors (which are often “small” studies) and ratios of HRs closer to 1, i.e. the studies on the lower-right part of Figure 9, were less likely to be published. It is not the case here. Thus, there is no evidence for publication bias. If Figure 9 was symmetrical, there would be more studies present on its lower-left part or less studies on its lower-right part, in which case the summary ratio of HRs in Figure 7 would become even further away from 1, supporting the conclusion on treatment-*EGFR* mutations interaction rather than undermining it.

3.4 Predictive value of *EGFR*-GCN gain

3.4.1 Basic characteristics of included studies

Twelve studies were included for the evaluation of the predictive value of *EGFR*-GCN gain[43,68,84,106-108,126-128,130-132]. The basic characteristics of these studies are summarized Table 9. As shown in this table, the number of patients included from each study varied from 60 to 488, with a total of 3,246. Two studies were conducted in Asians only. The rates of *EGFR*-GCN gain in the two studies were 61% and 70%, respectively, while it was between 7% and 48% in the studies that were conducted in other populations consisting mainly of Caucasians. In total, there were 1,299 patients (40.0%) with *EGFR*-GCN gain. *EGFR*-GCN was analyzed by fluorescence in situ hybridization technique in all except one study.

The median age reported in these studies varied from 59 to 75 years. The proportion of female patients was under 50%, ranging from 24% to 48%, in all but one study (Fukuoka 2011) in which females accounted for 77% of all patients. A similar pattern was seen for smoking history. The percentage of never-smokers in the study of Fukuoka 2011 was 92%, compared to 8% to 32% in other studies. The proportion of patients with adenocarcinoma ranged from 37% to 100%. The proportion of patients with a performance status score of 0 to 1, who were considered as "fit" patients[138], were mostly between 62% and 100%.

Five, 2 and 5 studies were conducted in the 1st-line, maintenance and 2nd-line settings, respectively. Gefitinib and erlotinib were used in 7 and 5 studies, respectively. Two trials compared EGFR TKIs alone with placebo alone, 5 trials EGFR TKIs plus other treatments with placebo plus other identical treatments, 4 trials EGFR TKIs alone with single-agent chemotherapy and 1 trial EGFR TKIs alone with combination chemotherapy. The outcome assessment criteria reported were uniformly Response Evaluation Criteria in Solid Tumors.

3.4.2 Efficacy of EGFR TKIs and treatment—*EGFR*-GCN interaction: progression-free survival

Table 10 summarizes the median progression-free survival of intervention and control arms and corresponding HRs, stratified by *EGFR*-GCN status.

When EGFR TKIs were compared with placebo (5 studies), the HRs ranged from 0.29 (95% CI: 0.11-0.75) to 0.83 (95% CI: 0.32-2.17) in the subgroups with *EGFR*-GCN gain, and 0.74 (95% CI: 0.38-1.45) to 1.42 (95% CI: 0.95-2.14) in the subgroups without *EGFR*-GCN gain (Table 10). The summary HR for the two subgroups was 0.64 (95% CI: 0.52-0.79) and 0.97 (95% CI: 0.74-1.27), respectively (Figure 11, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (5 studies), the HRs ranged from 0.66 (95% CI: 0.50-0.88) to 3.13 (95% CI: 1.45-6.76) in the subgroups with *EGFR*-GCN gain, and 0.45 (95% CI: 0.14-1.41) to 1.46 (95% CI: 1.00-2.11) in the subgroups without *EGFR*-GCN gain (Table 10). The summary HR for the two subgroups was 0.96 (95% CI: 0.65-1.42) and 1.20 (95% CI: 0.96-1.49), respectively (Figure 11, the lower-left and lower-middle panels).

To test for the treatment—*EGFR*-GCN interaction, a ratio of HRs was calculated within each study based on the data in Table 10. The ratio of HRs from different studies is presented in Figure 11 (the right panel). The I^2 and P value for heterogeneity test were 57% and 0.01, respectively, suggesting presence of heterogeneity. The summary ratio of HRs was 0.72 (95% CI: 0.52-0.99, P = 0.04), which indicated an interaction between the treatment and *EGFR*-GCN. The results from placebo-controlled trials and chemotherapy-controlled ones were in the same direction, although statistically insignificant in the latter case (Figure 11, the right panel).

3.4.3 Efficacy of EGFR TKIs and treatment—*EGFR*-GCN interaction: overall survival

Table 11 summarizes the median overall survival of intervention and control arms and corresponding HRs, stratified by *EGFR*-GCN status.

When EGFR TKIs were compared with placebo (7 studies), the HRs ranged from 0.43 (95% CI: 0.23-0.78) to 2.03 (95% CI: 0.67-6.13) in the subgroups with *EGFR*-GCN gain, and 0.77 (95% CI: 0.58-1.03) to 1.24 (95% CI: 0.84-1.82) in the subgroups without *EGFR*-GCN gain (Table 11). The summary HR for the two

subgroups was 0.81 (95% CI: 0.57-1.17) and 0.98 (95% CI: 0.86-1.12), respectively (Figure 12, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (4 studies), the HRs ranged from 0.73 (95% CI: 0.48-1.11) to 2.88 (95% CI: 1.21-6.83) in the subgroups with *EGFR*-GCN gain, and 0.79 (95% CI: 0.46-1.37) to 1.30 (95% CI: 0.92-1.85) in the subgroups without *EGFR*-GCN gain (Table 11). The summary HR for the two subgroups was 1.08 (95% CI: 0.77-1.51) and 1.06 (95% CI: 0.87-1.29), respectively (Figure 12, the lower-left and lower-middle panels).

To test for the treatment—*EGFR*-GCN interaction, a ratio of HRs was calculated within each study based on the data in Table 11. The ratio of HRs from different studies is presented in Figure 12 (the right panel). The I^2 and P value for heterogeneity test were 54% and 0.02, respectively, suggesting presence of heterogeneity. The summary ratio of HRs was 0.92 (95% CI: 0.69-1.23, $P = 0.57$), which did not support an interaction between the treatment and *EGFR*-GCN. The results from placebo-controlled trials and chemotherapy-controlled ones were inconsistent in terms of direction, although both were statistically insignificant (Figure 12, the right panel).

3.4.4 Additional analyses

Due to the limited number of studies, only univariate meta-regression analyses were conducted to investigate the heterogeneity observed in Figure 11 (the right panel) and Figure 12 (the right panel). The *P* value of significance test for each factor ranged from 0.119 to 0.961 and 0.197 to 0.782 for Figure 11 (the right panel) and Figure 12 (the right panel), respectively, which did not suggest that any of the pre-specified factors could strongly explain the heterogeneity. Thus, we combined the placebo-controlled and chemotherapy-controlled studies in the analyses, because the type of control did not affect the results of our meta-analyses. In the pre-planned sensitivity analyses, the conclusions on the predictive value of *EGFR*-GCN gain did not change, although the numerical values of the ratios of HRs and their 95% CIs were slightly altered (Table 12). Funnel plots based on the data presented in Figure 11 (the right panel) and Figure 12 (the right panel) are shown in Figure 13 and Figure 14, respectively. Visually no apparent asymmetry was observed, and there is no evidence to suggest presence of publication bias (Egger's test: $P = 0.487$ for Figure 13 and $P = 0.981$ for Figure 14).

3.5 Predictive value of EGFR protein expression

3.5.1 Basic characteristics of included studies

Eight studies were included for the evaluation of the predictive value of EGFR protein expression[43,107,125,127-130,132]. The basic characteristics of these studies are summarized in Table 13. The number of patients included in each study varied from 258 to 742, with a total of 3,156. One study was conducted in Asians only. The rate of EGFR protein expression in this study was 73%. In other studies where the proportions of Asians ranged from 3% to 16%, the rate of EGFR protein expression varied from 49% to 84%. In total, there were 2,269 patients (71.9%) with EGFR protein expression. The EGFR protein expression status was determined by immunohistochemistry in all included studies.

The median age reported in these studies varied from 59 to 63 years. The proportion of females was under 50%, ranging from 24% to 48%, in all but one study (Fukuoka 2011) in which females accounted for 78% of all patients. A similar trend was seen for smoking history and cancer histology. Specifically, the percentage of never-smokers in the study by Fukuoka 2011 was 92%, compared to 11% to 22% in other studies; and the proportion of patients with adenocarcinoma in the study was 100%, compared to 44% to 82% in other studies. The proportion of patients with a

performance status score of 0 to 1, who were considered as "fit" patients[138], varied between 62% and 100% in the included studies.

Two, 2 and 4 studies were conducted in the 1st-line, maintenance and 2nd-line settings, respectively. Gefitinib and erlotinib were used in 3 and 5 studies, respectively. Two studies compared EGFR TKIs alone with placebo alone, 3 EGFR TKIs plus other treatments with placebo plus other identical treatments, 2 EGFR TKIs alone with single-agent chemotherapy and 1 EGFR TKIs alone with combination chemotherapy. The outcome assessment criteria were uniformly Response Evaluation Criteria in Solid Tumors.

3.5.2 Efficacy of EGFR TKIs and treatment—EGFR protein expression interaction: progression-free survival

Table 14 summarizes the median progression-free survival of intervention and control arms and corresponding HRs, stratified by EGFR protein expression status.

When EGFR TKIs were compared with placebo (2 studies), the HRs ranged from 0.69 (95% CI: 0.58-0.82) to 0.92 (95% CI: 0.64-1.32) in the subgroups with EGFR protein expression, and 0.77 (95% CI: 0.51-1.14) to 1.00 (95% CI: 0.55-1.82) in the

subgroups without EGFR protein expression (Table 14). The summary HR for the two subgroups was 0.76 (95% CI: 0.58-0.99) and 0.84 (95% CI: 0.60-1.17), respectively (Figure 15, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (three studies), the HRs ranged from 0.73 (95% CI: 0.55-0.96) to 1.29 (95% CI: 0.98-1.70) in the subgroups with EGFR protein expression, and 0.90 (95% CI: 0.53-1.52) to 1.02 (95% CI: 0.61-1.69) in the subgroups without EGFR protein expression (Table 14). The summary HR for the two subgroups was 1.06 (95% CI: 0.74-1.52) and 0.96 (95% CI: 0.73-1.27), respectively (Figure 15, the lower-left and lower-middle panels).

To test for the treatment—EGFR protein expression interaction, a ratio of HRs was calculated within each study based on the data in Table 14. The ratio of HRs from different studies is presented in Figure 15 (the right panel). The I^2 and P value for heterogeneity test were 0% and 0.48, respectively, suggesting no obvious heterogeneity. The summary ratio of HRs was 0.99 (95% CI: 0.78-1.26, P = 0.93), which did not support an interaction between the treatment and EGFR protein expression. The results from placebo-controlled trials and chemotherapy-controlled

ones were inconsistent in terms of direction, although both were statistically insignificant (Figure 15, the right panel).

3.5.3 Efficacy of EGFR TKIs and treatment—EGFR protein expression interaction: overall survival

Table 15 summarizes the median overall survival of intervention and control arms and corresponding HRs, stratified by EGFR protein expression status.

When EGFR TKIs were compared with placebo (four studies), the HRs ranged from 0.68 (95% CI: 0.50-0.90) to 1.00 (95% CI: 0.69-1.45) in the subgroups with EGFR protein expression, and 0.91 (95% CI: 0.59-1.38) to 1.57 (95% CI: 0.86-2.87) in the subgroups without EGFR protein expression (Table 15). The summary HR for the two subgroups was 0.78 (95% CI: 0.68-0.89) and 1.02 (95% CI: 0.82-1.27), respectively (Figure 16, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (3 studies), the HRs ranged from 0.94 (95% CI: 0.72-1.21) to 1.05 (95% CI: 0.80-1.37) in the subgroups with EGFR protein expression, and 0.95 (95% CI: 0.55-1.62) to 1.09 (95% CI: 0.70-1.70) in the subgroups without EGFR protein expression (Table 15). The summary HR for

the two subgroups was 0.99 (95% CI: 0.85-1.16) and 1.02 (95% CI: 0.78-1.33), respectively (Figure 16, the lower-left and lower-middle panels).

To test for the treatment—EGFR protein expression interaction, a ratio of HRs was calculated within each study based on the data in Table 15. The ratio of HRs in different studies is presented in Figure 16 (the right panel). The I^2 and P value for heterogeneity test were 0% and 0.68, respectively, suggesting no clear heterogeneity. The summary ratio of HRs was 0.86 (95% CI: 0.70-1.05, P = 0.14), which did not support strongly an interaction between the treatment and EGFR protein expression. The summary results of placebo-controlled trials and chemotherapy-controlled ones were in the same direction and both were statistically insignificant (Figure 16, the right panel).

3.5.4 Additional analyses

As the heterogeneity test for Figure 15 (the right panel) and Figure 16 (the right panel) was both statistically insignificant, no meta-regression analyses were conducted. In the pre-planned sensitivity analyses, the conclusions on the predictive value of EGFR protein expression did not change, although the numerical values of the ratios of HRs and their 95% CIs were slightly altered (Table 16). Begg's funnel

plot and Egger's test were not conducted for Figure 15 (the right panel) and Figure 16 (the right panel) as the two meta-analyses included less than 10 studies.

3.6 Predictive value of *KRAS* mutations

3.6.1 Basic characteristics of included studies

Six studies were included for the evaluation of the predictive value of *KRAS* mutations[43,61,84,107,127,132]. The basic characteristics of these studies are summarized in Table 17. The number of patients included from each study varied from 195 to 493, with a total of 1,763. The proportions of Asians in the included studies ranged from 2% to 14%, and the rate of *KRAS* mutations ranged from 15% to 28%. In total, there were 352 patients (20.0%) with *KRAS* mutations. In 4 of the 6 studies, the *KRAS* mutation status was determined by direct sequencing, while in the remaining 2 it was determined by denaturing high-performance liquid chromatography or amplification refractory mutation system.

The median age reported in these studies varied from 58 to 65 years. The proportion of females patients and never-smokers ranged from 22% to 48% and 9% to 23%, respectively. The proportion of patients with adenocarcinoma was around 50% in all studies, except one (Johnson 2009) in which it was 82%. The proportion of patients

with a performance status score of 0 to 1, who were considered as "fit" patients[138], varied between 66% and 100% in the included studies.

One, 2 and 3 studies were conducted in the 1st-line, maintenance and 2nd-line settings, respectively. Gefitinib and erlotinib were used in 1 and 5 studies, respectively. Two studies compared EGFR TKIs alone with placebo alone, 2 EGFR TKIs plus other treatments with placebo plus other identical treatments, and 2 EGFR TKIs alone with single-agent chemotherapy. No study compared EGFR TKIs alone with combination chemotherapy. The outcome assessment criteria were uniformly Response Evaluation Criteria in Solid Tumors.

3.6.2 Efficacy of EGFR TKIs and treatment—*KRAS* mutations interaction: progression-free survival

Table 18 summarizes the median progression-free survival of intervention and control arms and corresponding HRs, stratified by *KRAS* mutation status.

When EGFR TKIs were compared with placebo (three studies), the HRs ranged from 0.77 (95% CI: 0.50-1.19) to 1.90 (95% CI: 1.10-3.60) in mutant *KRAS* subgroup, and 0.67 (95% CI: 0.49-0.91) to 0.93 (95% CI: 0.67-1.29) in wild-type *KRAS* subgroup

(Table 18). The summary HR for the two subgroups was 1.07 (95% CI: 0.64-1.79) and 0.74 (95% CI: 0.62-0.89), respectively (Figure 17, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (two studies), the HRs ranged from 1.16 (95% CI: 0.56-2.41) to 1.90 (95% CI: 0.89-4.05) in mutant *KRAS* subgroup, and 1.00 (95% CI: 0.71-1.41) to 1.23 (95% CI: 0.90-1.68) in wild-type *KRAS* subgroup (Table 18). The summary HR for the two subgroups was 1.47 (95% CI: 0.87-2.49) and 1.12 (95% CI: 0.89-1.41), respectively (Figure 17, the lower-left and lower-middle panels).

To test for the treatment—*KRAS* mutations interaction, a ratio of HRs was calculated within each study based on the data in Table 18. The ratio of HRs from different studies is presented in Figure 17 (the right panel). The I^2 and P value for heterogeneity test were 0% and 0.47, respectively, suggesting no clear heterogeneity. The summary ratio of HRs was 1.35 (95% CI: 1.02-1.80, $P = 0.04$), which indicated an interaction between the treatment and *KRAS* mutation. The summary results of placebo-controlled trials and chemotherapy-controlled ones were in the same direction but both were statistically insignificant (Figure 17, the right panel).

3.6.3 Efficacy of EGFR TKIs and treatment—*KRAS* mutations interaction: overall survival

Table 19 summarizes in the median overall survival of intervention and control arms and corresponding HRs, stratified by *KRAS* mutation status.

When EGFR TKIs were compared with placebo (4 studies), the HRs ranged from 0.79 (95% CI: 0.48-1.32) to 2.10 (95% CI: 1.10-3.80) in mutant *KRAS* subgroup, and 0.69 (95% CI: 0.49-0.97) to 1.05 (95% CI: 0.73-1.50) in wild-type *KRAS* subgroup (Table 19). The summary HR for the two subgroups was 1.14 (95% CI: 0.69-1.89) and 0.83 (95% CI: 0.71-0.97), respectively (Figure 18, the upper-left and upper-middle panels).

When EGFR TKIs were compared with chemotherapy (two studies), the HRs ranged from 0.81 (95% CI: 0.44-1.49) to 2.20 (95% CI: 0.96-5.06) in mutant *KRAS* subgroup, and 0.69 (95% CI: 0.49-0.99) to 1.03 (95% CI: 0.77-1.37) in wild-type *KRAS* subgroup (Table 19). The summary HR for the two subgroups was 1.28 (95% CI: 0.48-3.40) and 0.85 (95% CI: 0.58-1.26), respectively (Figure 18, the lower-left and lower-middle panels).

To test for the treatment—*KRAS* mutations interaction, a ratio of HRs was calculated within each study based on the data in Table 19. The ratio of HRs from different studies is presented in Figure 18 (the right panel). The I^2 and P value for heterogeneity test were 53% and 0.06, respectively, suggesting presence of heterogeneity. The summary ratio of HRs was 1.37 (95% CI: 0.89-2.10, P = 0.15), which did not support strongly an interaction between the treatment and *KRAS* mutation. The summary results of placebo-controlled trials and chemotherapy-controlled ones were in the same direction but both were statistically insignificant (Figure 18, the right panel).

3.6.4 Additional analyses

Due to the limited number of studies, only univariate meta-regression analyses were conducted to investigate the heterogeneity observed in Figure 18. The P value of significance test for each factor ranged from 0.305 to 0.963, which did not suggest that any of the pre-specified factors could explain the heterogeneity. Thus, we combined placebo-controlled and chemotherapy-controlled studies in these analyses. In the pre-planned sensitivity analyses, the conclusions on the predictive value of *KRAS* mutations did not change, although the numerical values of the ratios of HRs

and their 95% CIs were slightly altered (Table 20). Begg's funnel plot and Egger's test were not conducted for Figure 17 (the right panel) and Figure 18 (the right panel) as the two meta-analyses included less than 10 studies.

Chapter 4 Discussion

4.1 Summary of the main findings

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4.3 Interpretation and implication of the results on *EGFR*-GCN gain

4.4 Interpretation and implication of the results on *KRAS* mutations

4.5 Interpretation and implication of the results on EGFR protein expression

4.6 Implications for future research

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4.9 Summary

4.1 Summary of the main findings

The present systematic review summarized comprehensively the evidence from existing randomized controlled trials to assess the value of *EGFR* mutations, *EGFR*-GCN gain, EGFR protein expression and *KRAS* mutations in predicting the treatment effect of EGFR TKIs in advanced NSCLC. The results of meta-analyses are summarized in Table 21 and the main points reiterated as follows.

First, EGFR TKIs are in general effective in increasing progression-free and overall survival as compared with placebo although the effect size is smaller for overall survival than for progression free survival. This is consistent in all the groups defined by the 4 biomarkers and with findings from other meta-analyses (Table 1). The observation that the effect size is quantitatively less consistent in all the groups by the 4 biomarkers can be partly explained by the fact that not all the patients from original trials that compared EGFR TKIs and placebo were included in our comparison of the efficacy between biomarker-positive and -negative subgroups. As a result, the selection process may make a difference among studies and the sample size is relatively small in each group.

Second, there is good evidence that EGFR TKIs are comparable to chemotherapy in their effect in prolonging both progression-free and overall survival, except in *EGFR* mutation group in which EGFR TKIs seem much more effective than chemotherapy in prolonging progression-free survival.

Third, the above results are not the primary objective of this study as a more appropriate method to answer the above questions should not exclude trials that did not perform subgroup analyses according to biomarkers. The following is the primary and unique findings of this study. There is convincing evidence that the efficacy of EGFR TKIs differs considerably according to *EGFR* mutation status in prolonging progression-free survival and to a lesser degree and with some uncertainty in prolonging overall survival, suggesting the existence of a treatment-*EGFR* mutations interaction. A similar pattern was also found for the relation (or more adequately the interaction) of EGFR TKIs with the status of *EGFR*-GCN and with *KRAS* mutation status, although the interaction is less strong and there remains some uncertainty in particular in overall survival. There seems also some inconsistent evidence for a weak interaction between EGFR TKIs and EGFR protein expression, which is least certain among the 4 biomarkers.

Fourth, we would like to re-emphasize that another important finding of this study is that EGFR TKIs and chemotherapy are similarly effective in prolonging both progression-free and overall survival regardless the biomarker status of patients except in *EGFR* mutant patients in which EGFR TKIs seem much more effective than chemotherapy in prolonging progression-free survival.

4.2 Interpretation and implication of the results on *EGFR* mutations

EGFR mutations are clearly predictive of the progression-free survival benefit from EGFR TKIs and can be used to identify those who are more likely to benefit from EGFR TKIs treatment and avoid the treatment in those who may not respond to the treatment so that they can be exempted from adverse events and expenses. These findings lend strong support for the ASCO provisional clinical opinion and the National Comprehensive Cancer Network Guidelines for Treatment of Non-Small Cell Lung Cancer[5,135]. Both suggested testing *EGFR* mutation status to assist clinical decision-making for advanced NSCLC patients in considering EGFR TKIs therapy. EGFR mutations in making such decisions seems more useful in Asian patients with advanced NSCLC, as they are more frequent in Asian populations (38%-60%) than in others (7-17%)[41,84,107,128].

The present systematic review, however, does not support strongly that *EGFR* mutations are predictive of the overall survival benefit from EGFR TKIs. The reasons for this seem uncertain, although there seem to be some explanations. First, the effect of EGFR TKIs in prolonging overall survival is in general small and inconsistent if any (Table 1). As a result, the interaction between EGFR mutations and EGFR TKIs, if any, would also be small. Thus, this review may not have a sufficient statistical power to identify a small interaction. Second, cross-over of treatment during the trial and post-study treatments used, may have further diluted the benefit in overall survival and the size of the interaction.

As EGFR TKIs are comparable to chemotherapy in their effect in prolonging both progression-free and overall survival in patients harboring wild-type *EGFR*, the choice of treatment for these patients should be determined by factors other than efficacy such as adverse effects and costs. As mentioned in the Background of this thesis, EGFR TKIs are associated with significantly more adverse events as compared with chemotherapy and the cost per quality adjusted life year gained from EGFR TKIs treatment is also generally higher than that from chemotherapy except in mutant *EGFR* patients[3,139]. Thus, chemotherapy seems on the whole a better option for wild-type *EGFR* patients.

4.3 Interpretation and implication of the results on *EGFR*-GCN gain

Although *EGFR*-GCN gain seems also predictive of the efficacy of EGFR TKIs, the evidence is weaker than that for *EGFR* mutations. For example, one of the studies[126] showed an opposite conclusion that the efficacy of EGFR TKIs was inferior in patients with *EGFR*-GCN gain and similar in those without this biomarker as compared with vinorelbine (chemotherapy) (Figure 11 and Figure 12). Importantly, the clinical application of *EGFR*-GCN seems very limited for the reason below. Studies found that people harboring *EGFR*-GCN gain overlapped substantively with those harboring *EGFR* mutations. For example, the percentage of *EGFR*-GCN gain in those with mutant *EGFR* was shown to be 72% (28/39) in one study[127] and 70% (14/20) in another[130]. Thus, it is likely that the predictive power of *EGFR*-GCN gain is obtained through its overlapping with *EGFR* mutations. This assumption seems to be supported by our observation that the predictive power of *EGFR*-GCN gain is smaller than that of *EGFR* mutations. Thus *EGFR*-GCN gain may become superfluous if *EGFR* mutations are tested and used.

4.4 Interpretation and implication of the results on *KRAS* mutations

Different from *EGFR* mutations and *EGFR*-GCN gain, *KRAS* mutations are likely to be predictive of the efficacy of EGFR TKIs in an opposite manner. In other words, patients with *KRAS* mutations are less likely to benefit from EGFR TKIs than *KRAS* wild-type patients. As *EGFR* mutant patients are almost exclusively *KRAS* wild-type [67,84,88,140], the predictive value of *KRAS* mutations thus may well be fully a result of their inverse relation with *EGFR* mutations. This assumption seems to be supported by the observation that the predictive power of *KRAS* mutations is much lower than *EGFR* mutations. As a result, *KRAS* mutation status may become completely superfluous in *EGFR* mutant patients.

Would *KRAS* mutation status have a role in predicting the efficacy of EGFR TKIs in *EGFR* wild-type patients? If we assume that the predictive power of *KRAS* mutation status is completely obtained from its inverse relation with *EGFR* mutation status, *KRAS* mutation status would have no predictive power in *EGFR* wild-type patients. To address this question empirically, we would need trials to compare the efficacy of EGFR TKIs treatment between *KRAS* mutant and wild-type patients in *EGFR* wild-type patients. Such empirical evidence is however lacking.

4.5 Interpretation and implication of the results on EGFR protein expression

Our study showed the role of EGFR protein expression in predicting the efficacy of EGFR TKIs was weakest and also least certain among the 4 biomarkers. This was unexpected at the early years of use of EGFR TKIs, because EGFR protein is the target of the treatment and as anticipated naturally, should have an impact on the treatment efficacy. It is likely that EGFR protein expression indeed does not predict the efficacy of EGFR TKIs. However, several possible reasons might have prevented us from finding a relation between EGFR protein expression and the efficacy of EGFR TKIs.

First, the sensitivity of immunohistochemistry assay may be low. Although standardized immunohistochemistry assay kit has been available, the procedures prior to the assay are less consistent in terms of the quality of reagents, the fixative used in the storage period, and so on[141]. These factors may affect the accuracy of the assay results.

Secondly, there may be intratumor heterogeneity of EGFR expression status in advanced NSCLC. In colorectal cancer and head and neck cancer, the distribution of EGFR was reported to be heterogeneous within tumor samples, and its expression was increased at the invading edge[142]. This means that the results of

immunohistochemistry assay may depend on the part of the tumor used for the testing. As a result, information bias may exist in some studies.

Last but not least, the lack of predictive power of EGFR protein might have to do with its genetic status. It is possible that EGFR TKIs are discriminately targeted at the proteins that are encoded by mutated *EGFR* gene or the *EGFR* gene with copy number gain, rather than indiscriminately targeted at all EGFR proteins[141]. If this is true, the predictive power of EGFR protein expression would have well been diluted in these studies.

4.6 Implications for future research

Findings of this study also point to a few directions for future research. First, our results showed that in *EGFR* mutant patients, the predictive value of *EGFR*-GCN gain and *KRAS* mutations might well be explained by the effect of *EGFR* mutations, implying that *EGFR*-GCN gain and *KRAS* mutations might not have an independent predictive value. However, there may exist an independent predictive value of *EGFR*-GCN gain and *KRAS* mutations in *EGFR* wild-type patients but no data from subgroup analyses are currently available. Thus it is not certain whether these two markers would be able to predict the efficacy of EGFR TKIs in *EGFR* wild-type

patients. Such analyses will provide evidence for the independent value, if any, of the two biomarkers and can be used to identify who are more likely to benefit from the treatment in *EGFR* wild-type patents. However, given the wide spread recommendation of EGFR TKIs for *EGFR* mutant patients only, *EGFR* wild-type patients would rarely be given EGFR TKIs. This will make the subgroup analyses almost impossible except in early studies before *EGFR* mutation was widely used as an efficacy predictive biomarker.

Even in early studies a more powerful method can be used in any future analyses. It is multivariate analyses. In a multivariate analysis, all possible predictive factors can be put in one regression model and the effects of these factors are thus "combined" to produce a predictive tool that can be much more powerful than any single predictive factor. Such multivariate prediction models have been widely used in predicting the future risk of stroke and myocardial infarction[143] and other areas but rarely used in predicting who are more likely to benefit from EGFR TKIs.

The second possible research question is chemotherapy-biomarker interaction. In the analyses of this study (Sections 1.8.3 and 2.6.1), we assumed that efficacy of chemotherapy do not vary with biomarker status, namely, there was no

chemotherapy-biomarker interaction. Under this assumption, the ratios of HRs from placebo-controlled trials should be similar to those from chemotherapy-controlled trials (Figure 5). As shown in Table 21, the two are indeed similar with *EGFR* mutations and *KRAS* mutations but a statistically insignificant difference was observed with *EGFR*-GCN gain and EGFR protein expression.

This could be explained by a possibility that efficacy of chemotherapy was slightly greater than expected in biomarker-positive patients and/or smaller than expected in biomarker-negative patients, suggesting there might be a chemotherapy-biomarker interaction (Figure 5(B)). This seems to be supported by a few small studies that showed that *EGFR* mutations, *EGFR*-GCN and *KRAS* mutations appeared to be able to modify the effect of chemotherapy[103,104]. More evidence is needed to further confirm this hypothesis.

The third implication is about the conduct and reporting of biomarker studies based on randomized controlled trials. In most of the trials we included, patients with available biomarker testing results accounted for only 20%-50% of the total trial populations. With the majority of the original trial population excluded, the treatment and control arms within a subgroup defined by biomarker status could be

incomparable. This has been shown in the studies we included. As a result, selection bias and confounding bias may well exist in these studies. We thus suggest that in future studies, multivariate analyses be used to control for confounding and the possibility of selection bias be acknowledged.

4.7 Strengths

This study has several strengths. First, it summarized data from randomized controlled trials, which is the best study design for assessing the efficacy of predictive biomarkers[98,109]. Second, we strictly followed the existing guidelines for conducting systematic reviews and obtained a comprehensive set of data on 4 topical biomarkers in this field and 2 most important clinical outcomes. We obtained not only data the original papers explicitly reported, but also estimates with indirect data and data obtained from contact with authors. Although all these procedures are clearly required by guidelines and seem not worth mentioning, in practice many systematic reviewers often failed in these aspects[76,144,145].

The third strength is that we clearly defined and estimated an interaction term (i.e. ratio of HRs) according to the theories and formulas widely suggested in epidemiological textbooks. However many previous studies just compared the

prognosis (e.g., progression free survival and overall survival) of patients treated with EGFR TKIs between biomarker positive and negative patients. This is a flawed approach as an observed difference may only suggest that the biomarker be related to prognosis but not necessarily to efficacy.

Finally, many previous studies compared the pooled efficacy of the treatment in biomarker positive patients with that in biomarker negative patients. This is an indirect comparison which may be biased by difference between studies. Instead, we estimated the interaction term within each study and then pooled the interaction term among studies. This is direct comparison and can provide better evidence than indirect comparisons.

4.8 Limitations

The findings of this study should be interpreted with some caution. First, some original studies might have suffered from biases. For example, in a few studies[131], the intervention and control arms in biomarker-positive and/or biomarker-negative subgroups were incomparable in baseline characteristics, but the HR was estimated without control of potential confounding effects. Second, substantial between-study heterogeneity existed in some of our meta-analyses and cannot not be satisfactorily

explained by such pre-specified factors as EGFR TKI used, line of treatment, and ethnicity composition of patients. This may make our simple interpretation of the result less valid. Third, test for interaction generally requires a much larger sample size than for test for statistical significance of HR itself. In some of our meta-analyses, the results showed a consistent trend but were not statistically significant, probably due to insufficient sample size. This issue limited our interpretation and inference in certain areas.

Fourth, the exclusion of four studies that were potentially eligible but lacked detailed data suitable for our meta-analyses might have raised concern about selection bias (Figure 6(A)). However, further examination revealed that they were either too small to materially change our combined results or reported data supportive of our conclusions[93,136,137,146]. For example, in the study of Lee et al[136], only 11 of the 311 patients harbored *EGFR* mutations, making it unlikely to conduct a meaningful analysis of the treatment—*EGFR* mutations interaction within the study. Therefore, exclusion of the studies is unlikely to have biased our results. Lastly, new evidence on the predictive value of these biomarkers may have emerged since the time of our literature search which is 26 May 2012. To assess the impact of this issue, we did a

quick, updated search on 20 January 2014 and identified one new eligible study (n=71). The results of this study are consistent with our conclusions.

4.9 Summary

In summary, the present systematic review evaluated 4 biomarkers for their predictive value in the EGFR TKIs treatment of advanced NSCLC. We draw from this study 2 important conclusions for clinical decision making regarding the use of EGFR TKIs for treating advanced NSCLC. First, *EGFR* mutations and possibly *EGFR*-GCN and *KRAS* mutations can help determine what patients are more likely to benefit from EGFR TKIs treatment. Second, given the fact that chemotherapy is cheaper and of fewer side effects, it is generally a clear choice except in *EGFR* mutant patients in which EGFR TKIs is a better option.

These conclusions lend strong support for current guidelines about *EGFR* mutations testing. In the future, it may be worthwhile to identify markers that can identify what wild-type *EGFR* patients are more likely to benefit from EGFR TKIs treatment. Also, there is room for improving the conduct and reporting of biomarker studies nested in randomized controlled trials.

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Table 1. The efficacy of EGFR TKIs in advanced NSCLC[24-31]

Line of treatment	Comparison	Hazard ratio: progression-free survival	Hazard ratio: overall survival
1st line	EGFR TKIs vs. placebo	0.87 (0.76-0.99)	1.01 (0.96-1.13)
	EGFR TKIs vs. chemotherapy	1.03 (0.91-1.15)[a] 1.55 (1.24-1.93)[b]	1.05 (0.94-1.17)[a] 1.39 (0.99-1.94)[b]
Maintenance	EGFR TKIs vs. placebo	0.71 (0.60-0.83)	0.90 (0.83-0.98)
	EGFR TKIs vs. chemotherapy	Not available	Not available
≥2nd line	EGFR TKIs vs. placebo	0.61 (0.51–0.73)	0.70 (0.58–0.84)
	EGFR TKIs vs. chemotherapy	1.01 (0.99–1.02)	1.00 (0.98–1.02)

[a] for gefitinib; [b] for erlotinib.

Table 2. Transformation of hazard ratio

Suppose:	Example:
1. Comparator vs. EGFR TKIs:	HR_1 (95%CI: LL_1-UL_1):
HR_1 (95%CI: LL_1-UL_1)	0.525 (95%CI: 0.343-0.803)
2. EGFR TKIs vs. comparator:	
HR_2 (95%CI: LL_2-UL_2)	
Then:	Then:
$HR_2=1/HR_1$	$HR_2=1/0.525=1.905$
$LL_2=\exp[\ln HR_2-(\ln HR_1-\ln LL_1)]$	$LL_2=\exp[\ln(1/0.525)-(\ln 0.525-\ln 0.343)]=1.244$
$UL_2=\exp[\ln HR_2+(\ln UL_1-\ln HR_1)]$	$UL_2=\exp[\ln(1/0.525)+(\ln 0.803-\ln 0.525]=2.913$

Table 3. Calculation of the interaction term: an example[a]

Step	Measure	Mutant *EGFR* subgroup	Wild-type *EGFR* subgroup
1	HR	0.16	1.24
2	log HR[a]	-1.8326 (E_1)	0.2151 (E_2)
3	95% CI for HR	0.05 to 0.49	0.94 to 1.64
4	95% CI for log HR[b]	-2.9957 to -0.7133	-0.0619 to 0.4947
5	Width of CI	2.2824	0.5566
6	SE[=width/(2×1.96)]	0.5822	0.1420
Difference between log hazard ratios:			
7	d[=E_1-E_2]	-1.8326 – 0.2151 = -2.0477	
8	SE(d)	$(0.5822^2 + 0.1420^2)^{1/2}$ = 0.5993	
9	CI(d)	-2.0477 ± 1.96 × 0.5993, or -3.2223 to -0.8731	
10	Test of interaction	Z = -2.0477/ 0.5993 = -3.4168 (p = 0.00006)	
Ratio of hazard ratios:			
11	Ratio of HR=exp(d)	exp(-2.0477) = 0.13	
12	CI(Ratio of HR)	exp(-3.2223) to exp(-0.8731), or 0.04 to 0.42	

Abbreviations: HR = hazard ratio; CI = confidence interval; SE = standard error

[a] in this table, HR and 95% CI in steps 1 and 3, respectively, are reported by original studies, while all others are calculated on the basis of them.

[b] the natural logarithm of hazard ratio.

Table 4. Methodological characteristics of eligible studies (to be continued)

Study	Design	Percentage of the parent RCT population used for biomarker study	Representative of the parent RCT population?	Intervention and control arms comparable?		Blinding of treatment allocation in the parent RCT	Biomarker analysis blinded to treatment allocation and outcome?	Cross-over of treatment or use of post-study treatments in the parent RCT	HR analysis: multivariate or univariate?
				B+ subgroup	B- subgroup				
Bell 2005[108]	Retro	15% (EGFR MT) 21% (EGFR GCN)	Yes	UC	UC	Double-blind	UC	UC	UC
Brugger 2011[43]	Pro	49% (EGFR MT) 55% (EGFR GCN) 83% (EGFR IHC) 55% (KRAS MT)	Yes	UC	UC	Double-blind	Yes	Significant	UC
Eberhard 2005[61]	Retro	21% (EGFR MT) 24% (KRAS MT)	Yes	UC	UC	Double-blind	Yes	UC	UC
Johnson 2009[132,134]	Pro	47% (EGFR MT) 26% (EGFR GCN) 35% (EGFR IHC) 45% (KRAS MT)	Yes	UC	UC	Double-blind	UC	UC	UC
Zhang 2012[41]	Pro	27% (EGFR MT)	Yes	Yes	Yes	Double-blind	UC	Significant	Multivariate
Zhu 2008[84]	Retro	28% (EGFR MT) 22% (EGFR GCN) 28% (KRAS MT)	No	UC	UC	Double-blind	Yes	Minor	Univariate
Chen 2012[124]	Pro	53% (EGFR MT)	UC	UC	UC	Open-label	UC	Significant	UC
Ciuleanu 2012[107]	Pro	38% (EGFR MT) 60% (EGFR GCN) 86% (EGFR IHC) 46% (KRAS MT)	Yes	For EGFR GCN+: yes; For EGFR MT+: no (may favor TKI arm); For KRAS MT+: no	For KRAS MT-: yes; For EGFR MT-: no (may disfavor TKI arm); For GCN-: no (may disfavor TKI arm)	Open-label	UC	Significant	UC

Table 4. Methodological characteristics of eligible studies (to be continued)

Study	Design	Percentage of the parent RCT population used for biomarker study	Representative of the parent RCT population?	Intervention and control arms comparable?		Blinding of treatment allocation in the parent RCT	Biomarker analysis blinded to treatment allocation and outcome?	Cross-over of treatment or use of post-study treatments in the parent RCT	HR analysis: multivariate or univariate?
				B+ subgroup	B- subgroup				
Douillard 2010[127]	Pro	20% (EGFR MT) 26% (EGFR GCN) 26% (EGFR IHC) 19% (KRAS MT)	No	UC	UC	Open-label	Yes	Significant	Univariate
Fukuoka 2011[128]	Pro	36% (EGFR MT) 33% (EGFR GCN) 30% (EGFR IHC)	Yes	UC	UC	Open-label	Yes	Significant[a]	Multivariate
Lee 2009[133,135]	Pro	31% (EGFR MT)	UC	UC	UC	Open-label	UC	Significant	UC
Maruyama 2008[68]	Pro	12% (EGFR MT) 12% (EGFR GCN)	No	UC	UC	Open-label	UC	Significant	Univariate
Goss 2009[106]	Pro	42% (EGFR GCN)	Yes	UC	UC	Double-blind	UC	Minor	Univariate
Hirsch 2006[130]	Retro	22% (EGFR GCN) 22% (EGFR IHC)	No	UC	UC	Double-blind	Yes	Negligible	Multivariate
Hirsch 2008[131]	Retro	23% (EGFR GCN)	Yes	No	No (may favor TKI arm)	Double-blind	Yes	UC	Univariate
Crino 2008[126]	Pro	81% (EGFR GCN)	UC	UC	UC	Open-label	UC	Significant	UC
Clark 2006[125]	Pro	44% (EGFR IHC)	No	UC	UC	Double-blind	Yes	Minor	Univariate
Herbst 2005[129]	Retro	32% (EGFR IHC)	UC	UC	UC	Double-blind	UC	UC	UC

Abbreviations: RCT = randomized controlled trial; B+ = biomarker positive; B- = biomarker negative; HR = hazard ratio; Retro = retrospective analysis of completed RCT; MT = mutation analysis; GCN = gene copy number analysis; UC = unclear; Pro = prospective or pre-planned biomarker analysis of RCT; IHC = immunohistochemistry analysis; TKI: EGFR tyrosine kinase inhibitors. Magnitude of cross-over: 0-5%, negligible; 5-10%, minor; >10%, significant.

[a] not only in the overall population, but also in *EGFR* mutant patients and *EGFR* wild-type patients, respectively

Table 5. The basic characteristics of eligible studies for *EGFR* mutations

Study	*N*	*EGFR* MT No. (%)	Asian (%)	Age (years)	Female (%)	Never-smoker (%)	Adenocarcinoma (%)	PS 0-1 (%)	Line	Intervention vs. control	Response criteria	Mutation testing method
Bell 2005[108]	312	32(10)	2	35% ≥65	30	12[a]	53	90[a]	1st	G+PAC+CAR or G+CIS+GEM vs. PLB+PAC+CAR or PLB+CIS+GEM	RECIST	Ex18-21 DS
Brugger 2011[43]	437	49(11)	9	60(30-83)	25	17	46[b]	100	M	E vs. PLB	RECIST	Ex18-21 DS
Eberhard 2005[61]	228	29(12)	3[a]	63(23-82)	44	9	46	100	1st	E+PAC+CAR followed by E vs. PLB+PAC+CAR followed by PLB	RECIST	Ex18-21 DS
Johnson 2009[132,134]	347	52(15)	12[a]	NA	48[a]	17[a]	82[a]	NA	M	E+BEV vs. PLB+BEV	NA	Ex18-21 DHPLC
Zhang 2012[41]	79	30(38)	100	55(31-75)	41	49	68	97	M	G vs. PLB	RECIST	Ex18-21 ARMS
Zhu 2008[84]	204	34(17)	6	56% ≥60	36	23	52	67	≥2nd	E vs. PLB	RECIST	Ex19&21 DS
Chen 2012[124]	60	24(40)	100	77(70-90)[a]	19[a]	21[a]	65[a]	77[a]	1st	E vs. VIN	RECIST	Ex18-21 DS
Ciuleanu 2012[107]	160	11(7)	13	59(22-80)	26	17	46	83	≥2nd	E vs. DOC or PEM	RECIST	Ex18-21 DS
Douillard 2010[127]	297	44(15)	16	60(20-84)[a]	31	17	57	89	≥2nd	G vs. DOC	RECIST	Ex18-21 ARMS
Fukuoka 2011[128]	437	261(60)	100	25% ≥65	77	93	100[b]	92	1st	G vs. PAC+CAR	RECIST	Ex18-21 ARMS
Lee 2009[133,135]	96	42(44)	100	57(19-74)[a]	89[a]	100	100	91[a]	1st	G vs. CIS+GEM	NA	NA
Maruyama 2008[68]	57	31(54)	100	44% ≥65[a]	38[a]	32[a]	78[a]	96[a]	≥2nd	G vs. DOC	RECIST	Ex18-21 DS

Abbreviations: *N* = the number of patients included for meta-analyses; *EGFR* MT = patients with *EGFR* mutations; PS = performance status score as defined by Eastern Cooperative Oncology Group or World Health Organization; G = gefitinib; PAC = paclitaxel; CAR = carboplatin; CIS = cisplatin; GEM = gemcitabine; PLB = placebo; RECIST = Response Evaluation Criteria in Solid Tumors; Ex = exon; DS = direct sequencing; M = maintenance treatment; E = erlotinib; NA = not available; BEV = bevacizumab; DHPLC = denaturing high-performance liquid chromatography; ARMS, amplification refractory mutation system; VIN = vinorelbine; DOC = docetaxel; PEM = pemetrexed.

[a] estimated according to the baseline characteristics of the total population in the original randomized controlled trial. [b] including both adenocarcinoma and bronchoalveolar.

Table 6. Median progression-free survival (months) of intervention and control arms and corresponding hazard ratios, stratified by *EGFR* mutation status

Study	Mutant *EGFR* subgroup			Wild-type *EGFR* subgroup		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Bell 2005[108]	NA	6.7	0.55(0.19-1.60)	5.5	4.5	0.73(0.53-1.01)
Brugger 2011[43]	10.4	3.0	0.10(0.04-0.25)	2.8	2.1	0.78(0.63-0.96)
Eberhard 2005[61]	12.5	6.6	0.49(0.20-1.20)	4.6[a]	5.4[a]	1.25(0.94-1.66)[a]
Johnson 2009[132,134]	NA	NA	0.44(0.22-0.86)	NA	NA	0.85(0.64-1.13)
Zhang 2012[41]	16.6	2.8	0.17(0.07-0.42)	2.7	1.5	0.86(0.48-1.51)
Chen 2012[124]	8.4	4.0	0.70(0.22-2.19)[a]	3.8	1.5	0.50(0.26-0.93)[a]
Ciuleanu 2012[107]	8.4[a]	10.1[a]	0.71(0.13-3.97)	1.4[a]	2.1[a]	1.25(0.88-1.78)
Douillard 2010[127]	7.0	4.1	0.16(0.05-0.49)	1.7	2.6	1.24(0.94-1.64)
Fukuoka 2011[128]	9.7[a]	6.2[a]	0.48(0.36-0.64)	1.6[a]	5.8[a]	2.85(2.05-3.98)
Lee 2009[133,135]	8.4	6.7	0.62(0.31-1.22)	2.1	6.4	1.52(0.88-2.62)
Maruyama 2008[68]	NA	NA	0.33(0.11-0.97)[b]	NA	NA	0.15(0.04-0.57)[c]

Abbreviations: HR = hazard ratio; CI, confidence interval; NA = not available.

[a] estimated from the published survival curves.

[b] hazard ratio for biomarker-positive vs. biomarker-negative patients in intervention group.

[c] hazard ratio for biomarker-positive vs. biomarker-negative patients in control group.

Table 7. Median overall survival (months) of intervention and control arms and corresponding hazard ratios, stratified by *EGFR* mutation status

Study	Mutant *EGFR* subgroup			Wild-type *EGFR* subgroup		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Bell 2005[108]	14.6	19.4	1.77(0.50-6.23)	9.3	9.2	0.91(0.67-1.23)
Brugger 2011[43]	NA	22.5[a]	0.83(0.34-2.02)	11.0[a]	10.0[a]	0.77(0.61-0.97)
Eberhard 2005[61]	NA	NA	0.88(0.20-3.90)	9.5[a]	11.1[a]	1.10(0.77-1.56)[a]
Johnson 2009[132,134]	NA	NA	0.46(0.21-1.02)	NA	NA	0.86(0.65-1.15)
Zhu 2008[84]	10.9	8.3	0.55(0.25-1.19)	7.9	3.3	0.74(0.52-1.05)
Chen 2012[124]	22.7	29.9	1.80(0.10-32.97)[a]	6.9	4.4	0.59(0.24-1.44)[a]
Ciuleanu 2012[107]	19.3	NA	1.19(0.12-11.49)	6.6	4.4	0.85(0.59-1.22)
Douillard 2010[127]	14.2	16.6	0.83(0.41-1.67)	6.4	6.0	1.02(0.78-1.33)
Fukuoka 2011[128]	21.6	21.9	1.00(0.76-1.33)	11.2	12.7	1.18(0.86-1.63)
Lee 2009[133,135]	30.6	26.5	0.82(0.35-1.92)	18.4	23.3	1.20(0.57-2.52)

Abbreviations: HR = hazard ratio; CI, confidence interval; NA = not available.

[a] estimated from the published survival curves.

Table 8. Sensitivity analyses for treatment-*EGFR* mutations interaction measured by ratio of hazard ratios

Clinical outcome	Progression-free survival	Overall survival
Main meta-analysis	0.37(0.22-0.60)	0.84(0.64-1.11)
Sensitivity analysis A	0.34(0.19-0.60)	0.82(0.61-1.11)
Sensitivity analysis B	0.36(0.21-0.60)	0.84(0.63-1.10)
Sensitivity analysis C	0.51(0.30-0.85)	0.77(0.46-1.29)

Sensitivity analysis A: Studies with *post hoc* subgroup analysis according to *EGFR* mutation status were excluded. Sensitivity analysis B: Studies with incomparable intervention and control arms in either *EGFR* subgroup were excluded. Sensitivity analysis C: Studies with significant cross-over of treatment or imbalance of post-study treatments between treatment arms were excluded.

Table 9. The basic characteristics of eligible studies for *EGFR* gene copy number gain

Study	*N*	GCN+ No. (%)	Asian (%)	Age (years)	Female (%)	Never-smoker (%)	Adenocarcinoma (%)	PS 0-1 (%)	Line	Intervention vs. control	Response criteria	GCN analysis method
Bell 2005[108]	453	33(7)	3	36% ≥65	35	13	49	90[a]	1st	G+PAC+CAR or G+CIS+GEM vs. PLB+PAC+CAR or PLB+CIS+GEM	RECIST	Quantitative real-time PCR
Brugger 2011[43]	488	232(48)	16	61(30-83)	27	19	48[b]	100	M	E vs. PLB	RECIST	FISH
Goss 2009[106]	84	32(38)	3[a]	75(42-90) [a]	39[a]	9[a]	37[a]	0	1st	G+BSC vs. PLB+BSC	RECIST	FISH
Hirsch 2006[130]	370	114(31)	3	62(28-90) [a]	32	13	42	62	≥2nd	G+BSC vs. PLB+BSC	RECIST	FISH
Hirsch 2008[131]	245	100(41)	3	65(24-82)	43	8	60	100	1st	E+PAC+CAR followed by E vs. PLB+PAC+CAR followed by PLB	RECIST	FISH
Johnson 2009[132,134]	196	87(44)	12[a]	NA	48[a]	17[a]	82[a]	NA	M	E+BEV vs. PLB+BEV	NA	FISH
Zhu 2008[84]	159	61(38)	6	59% ≥60	37	24	53	69	≥2nd	E vs. PLB	RECIST	FISH
Ciuleanu 2012[107]	253	121(48)	12	59(22-80)	24	18	47	82	≥2nd	E vs. DOC or PEM	RECIST	FISH
Crino 2008[126]	158	54(34)	16[a]	74(70-89) [a]	24[a]	14[a]	40[a]	80[a]	1st	G vs. VIN	RECIST	FISH
Douillard 2010[127]	374	174(47)	14	60(20-84) [a]	31	16	55	90	≥2nd	G vs. DOC	RECIST	FISH
Fukuoka 2011[128]	406	249(61)	100	25% ≥65	77	92	100[b]	92	1st	G vs. PAC+CAR	RECIST	FISH
Maruyama 2008[68]	60	42(70)	100	44% ≥65[a]	38[a]	32[a]	78[a]	96[a]	≥2nd	G vs. DOC	RECIST	FISH

Abbreviations: *N* = the number of patients included for meta-analyses; GCN+ = patients with gene copy number gain; PS = performance status score as defined by Eastern Cooperative Oncology Group or World Health Organization; G = gefitinib; PAC = paclitaxel; CAR = carboplatin; CIS = cisplatin; GEM = gemcitabine; PLB = placebo; RECIST = Response Evaluation Criteria in Solid Tumors criteria; PCR = polymerase chain reaction; M = maintenance treatment; E = erlotinib; FISH = fluorescence in situ hybridization; BSC = best supportive care; NA = not available; BEV = bevacizumab; DOC = docetaxel; PEM = pemetrexed; VIN = vinorelbine.

[a] estimated according to the baseline characteristics of the total population in the original randomized controlled trial.

[b] including both adenocarcinoma and bronchoalveolar.

Table 10. Median progression-free survival (months) of intervention and control arms and corresponding hazard ratios, stratified by *EGFR* gene copy number status

Study	Subgroup with GCN+			Subgroup without GCN+		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Bell 2005[108]	6.9	7.3	0.83(0.32-2.18)	4.8	4.6	0.77(0.60-1.00)
Brugger 2011[43]	3.7	2.7	0.68(0.51-0.90)	2.8	2.7	0.81(0.62-1.07)
Goss 2009[106]	3.3[a]	1.5[a]	0.29(0.11-0.73)	1.3[a]	1.3[a]	0.74(0.38-1.45)
Hirsch 2008[131]	6.3	5.8	0.59(0.35-0.99)	4.6	6.0	1.42(0.95-2.14)
Johnson 2009[132,134]	NA	NA	0.66(0.39-1.13)	NA	NA	1.40(0.86-2.28)
Ciuleanu 2012[107]	NA	NA	0.93(0.63-1.38)	NA	NA	1.46(1.00-2.11)
Crino 2008[126]	2.6[a]	4.0[a]	3.13(1.45-6.76)	2.8[a]	2.8[a]	0.93(0.59-1.46)
Douillard 2010[127]	2.5	2.8	0.84(0.59-1.19)	2.1	2.8	1.30(0.93-1.83)
Fukuoka 2011[128]	NA	NA	0.66(0.50-0.88)	NA	NA	1.24(0.87-1.76)
Maruyama 2008[68]	NA	NA	0.75(0.28-1.98)[b]	NA	NA	0.45(0.14-1.41)[c]

Abbreviations: GCN+ = *EGFR* gene copy number gain; HR = hazard ratio; CI, confidence interval; NA = not available.

[a] estimated from the published survival curves.

[b] hazard ratio for biomarker-positive vs. biomarker-negative patients in intervention group.

[c] hazard ratio for biomarker-positive vs. biomarker-negative patients in control group.

Table 11. Median overall survival (months) of intervention and control arms and corresponding hazard ratios, stratified by *EGFR* gene copy number status

Study	Subgroup with GCN+			Subgroup without GCN+		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Bell 2005[108]	11.5	>20	2.03(0.67-6.13)	8.8	10.2	1.01(0.79-1.29)
Brugger 2011[43]	12.0[a]	13.9[a]	0.96(0.71-1.30)	13.2[a]	10.6[a]	0.77(0.58-1.03)
Goss 2009[106]	3.5[a]	2.7	0.44(0.17-1.12)	2.5[a]	2.5[a]	1.02(0.56-1.88)
Hirsch 2006[130]	8.3	4.5	0.61(0.36-1.04)	4.3	6.2	1.16(0.81-1.64)
Hirsch 2008[131]	12.6	14.3	1.52(0.94-2.46)	9.5	12.4	1.24(0.84-1.82)
Johnson 2009[132,134]	NA	NA	0.74(0.42-1.29)	NA	NA	1.03(0.64-1.67)
Zhu 2008[84]	10.5	3.1	0.43(0.23-0.78)	6.4	4.7	0.80(0.49-1.29)
Ciuleanu 2012[107]	6.4	5.5	0.73(0.48-1.11)	5.3	5.8	1.17(0.80-1.72)
Crino 2008[126]	4.0[a]	11.0[a]	2.88(1.21-6.83)	6.9[a]	5.6[a]	0.79(0.46-1.37)
Douillard 2010[127]	8.4	7.5	1.09(0.78-1.51)	6.4	7.7	0.93(0.68-1.26)
Fukuoka 2011[128]	NA	NA	1.03(0.78-1.37)	NA	NA	1.30(0.92-1.85)

Abbreviations: GCN+ = *EGFR* gene copy number gain; HR = hazard ratio; CI, confidence interval; NA = not available.

[a] estimated from the published survival curves.

Table 12. Sensitivity analyses for treatment-*EGFR* gene copy number gain interaction measured by ratio of hazard ratios

Clinical outcome	Progression-free survival	Overall survival
Main meta-analysis	0.72 (0.52-0.99)	0.92 (0.69-1.23)
Sensitivity analysis A	0.74(0.52-1.07)	0.96(0.68-1.36)
Sensitivity analysis B	0.80(0.54-1.18)	0.93(0.66-1.32)
Sensitivity analysis C	0.51(0.34-0.76)	0.76(0.50-1.15)

Sensitivity analysis A: Studies with *post hoc* subgroup analysis according to *EGFR* gene copy number status were excluded. Sensitivity analysis B: Studies with incomparable intervention and control arms in either *EGFR* subgroup were excluded. Sensitivity analysis C: Studies with significant cross-over of treatment or imbalance of post-study treatments between treatment arms were excluded.

Table 13. The basic characteristics of eligible studies for EGFR protein expression

Study	*N*	EGFR+ No. (%)	Asian (%)	Age (years)	Female (%)	Never-smoker (%)	Adenocarcinoma (%)	PS 0-1 (%)	Line	Intervention vs. control	Response criteria	EGFR analysis method
Brugger 2011[108]	742	621(84)	16	60(30-83)	27	18	46	100	M	E vs. PLB	RECIST	IHC
Clark 2006[125]	325	184(57)	6	61	35	22	50	62	≥2nd	E vs. PLB	RECIST	IHC
Herbst 2005[129]	344	167(49)	3	63(24-84) [a]	39	11[a]	61[a]	100	1st	E+PAC+CAR followed by E vs. PLB+PAC+CAR followed by PLB	RECIST	IHC
Hirsch 2006[130]	379	264(70)	6[a]	62(28-90) [a]	32[a]	14	44	62	≥2nd	G+BSC vs. PLB+BSC	RECIST	IHC
Johnson 2009[132,134]	258	191(74)	12[a]	NA	48[a]	17[a]	82[a]	NA	M	E+BEV vs. PLB+BEV	NA	IHC
Ciuleanu 2012[107]	363	292(80)	13[a]	59(22-80) [a]	24[a]	17[a]	50[a]	80[a]	≥2nd	E vs. DOC or PEM	RECIST	IHC
Douillard 2010[127]	380	284(75)	15	60(20-84) [a]	33	18	54	89	≥2nd	G vs. DOC	RECIST	IHC
Fukuoka 2011[128]	365	266(73)	100	28% ≥65	78	92	100	92	1st	G vs. PAC+CAR	RECIST	IHC

Abbreviations: *N* = the number of patients included for meta-analyses; EGFR+ = patients with EGFR protein expression; PS = performance status score as defined by Eastern Cooperative Oncology Group or World Health Organization; M = maintenance treatment; E = erlotinib; PLB = placebo; RECIST = Response Evaluation Criteria in Solid Tumors criteria; IHC = immunohistochemistry; PAC = paclitaxel; CAR = carboplatin; G = gefitinib; BSC = best supportive care; NA = not available; BEV = bevacizumab; DOC = docetaxel; PEM = pemetrexed.

[a] estimated according to the baseline characteristics of the total population in the original randomized controlled trial.

Table 14. Median progression-free survival (months) of intervention and control arms and corresponding hazard ratios, stratified by EGFR protein expression status

Study	Subgroup with EGFR protein expression			Subgroup without EGFR protein expression		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Brugger 2011[108]	2.9	2.6	0.69(0.58-0.82)	2.6	2.1	0.77(0.51-1.14)
Johnson 2009[132,134]	NA	NA	0.92(0.64-1.32)	NA	NA	1.00(0.55-1.82)
Ciuleanu 2012[107]	NA	NA	1.26(0.98-1.61)	NA	NA	1.02(0.61-1.69)
Douillard 2010[127]	1.6	2.8	1.29(0.98-1.70)	2.9	3.0	0.90(0.53-1.52)
Fukuoka 2011[128]	NA	NA	0.73(0.55-0.96)	NA	NA	0.97(0.64-1.48)

Abbreviations: HR = hazard ratio; CI, confidence interval; NA = not available.

Table 15. Median overall survival (months) of intervention and control arms and corresponding hazard ratios, stratified by EGFR protein expression status

Study	Subgroup with EGFR protein expression			Subgroup without EGFR protein expression		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Brugger 2011[108]	12.7[a]	10.5[a]	0.77(0.64-0.93)	10.7[a]	10.7[a]	0.91(0.59-1.38)
Clark 2006[125]	NA	NA	0.68(0.50-0.90)	NA	NA	0.93(0.60-1.40)
Herbst 2005[129]	NA	NA	1.00(0.69-1.45)	NA	NA	1.02(0.71-1.46)
Hirsch 2006[130]	5.5	4.6	0.77(0.56-1.08)	4.2	NA	1.57(0.86-2.87)
Ciuleanu 2012[107]	5.6	5.5	0.94(0.72-1.21)	5.4	6.7	0.95(0.55-1.62)
Douillard 2010[127]	7.9	6.5	1.00(0.77-1.29)	7.5	9.2	1.00(0.65-1.55)
Fukuoka 2011[128]	NA	NA	1.05(0.80-1.37)	NA	NA	1.09(0.70-1.70)

Abbreviations: HR = hazard ratio; CI, confidence interval; NA = not available.

[a] estimated from the published survival curves.

Table 16. Sensitivity analyses for treatment-EGFR protein expression interaction measured by ratio of hazard ratios

Clinical outcome	Progression-free survival	Overall survival
Main meta-analysis	0.99 (0.78-1.26)	0.86 (0.70-1.05)
Sensitivity analysis A	0.99(0.78-1.26)	0.89(0.71-1.12)
Sensitivity analysis B	0.94(0.72-1.23)	0.85(0.68-1.05)
Sensitivity analysis C	0.92(0.46-1.85)	0.74(0.52-1.07)

Sensitivity analysis A: Studies with *post hoc* subgroup analysis according to EGFR protein expression status were excluded. Sensitivity analysis B: Studies with incomparable intervention and control arms in either EGFR subgroup were excluded. Sensitivity analysis C: Studies with significant cross-over of treatment or imbalance of post-study treatments between treatment arms were excluded.

Table 17. The basic characteristics of eligible studies for *KRAS* mutations

Study	*N*	*KRAS* MT No. (%)	Asian (%)	Age (years)	Female (%)	Never-smoker (%)	Adenocarcinoma (%)	PS 0-1 (%)	Line	Intervention vs. control	Response criteria	Mutation testing method
Brugger 2011[43]	493	90(18)	10	60(30-83)	24	17	46[a]	100	M	E vs. PLB	RECIST	Ex2-3 DS
Eberhard 2005[61]	262	55(21)	3	65(24-82)	42	9	46	100	1st	E+PAC+CAR vs. PLB+PAC+CAR	RECIST	Ex2 DS
Johnson 2009[132,134]	332	93(28)	12[b]	NA	48[b]	17[b]	82[b]	NA	M	E+BEV vs. PLB+BEV	NA	Ex2-3 DHPLC
Zhu 2008[84]	206	30(15)	7	57% ≥60	34	23	54	66	≥2nd	E vs. PLB	RECIST	Ex2 DS
Ciuleanu 2012[107]	195	35(18)	14	58(22-80)	22	16	45	84	≥2nd	E vs. DOC or PEM	RECIST	Ex2-3 DS
Douillard 2010[127]	275	49(18)	2	60(20-84)[b]	31	11	52	89	≥2nd	G vs. DOC	RECIST	Ex2 ARMS

Abbreviations: *N* = the number of patients included for meta-analyses; *KRAS* MT = patients with *KRAS* mutations; PS = performance status score as defined by Eastern Cooperative Oncology Group or World Health Organization; M = maintenance treatment; E = erlotinib; PLB = placebo; RECIST = Response Evaluation Criteria in Solid Tumors criteria; Ex = exon; DS = direct sequencing; PAC = paclitaxel; CAR = carboplatin; NA = not available; BEV = bevacizumab; DHPLC = denaturing high-performance liquid chromatography; DOC = docetaxel; PEM = pemetrexed; G = gefitinib; ARMS, amplification refractory mutation system.

[a] including both adenocarcinoma and bronchoalveolar.

[b] estimated according to the baseline characteristics of the total population in the original randomized controlled trial.

Table 18. Median progression-free survival (months) of intervention and control arms and corresponding hazard ratios, stratified by *KRAS* mutation status

Study	Mutant *KRAS* subgroup			Wild-type *KRAS* subgroup		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Brugger 2011[43]	2.2	1.5	0.77(0.50-1.19)	2.9	2.7	0.70(0.57-0.87)
Eberhard 2005[61]	3.4	6.0	1.90(1.10-3.60)	5.3	5.4	0.93(0.67-1.29)[a]
Johnson 2009[132,134]	NA	NA	0.93(0.55-1.56)	NA	NA	0.67(0.49-0.91)
Ciuleanu 2012[107]	NA	NA	1.90(0.89-4.05)	NA	NA	1.00(0.71-1.41)
Douillard 2010[127]	1.4	1.5	1.16(0.56-2.41)	2.6	3.3	1.23(0.90-1.68)

Abbreviations: HR = hazard ratio; CI, confidence interval; NA = not available.

[a] obtained by contact with investigators.

Table 19. Median overall survival (months) of intervention and control arms and corresponding hazard ratios, stratified by *KRAS* mutation status

Study	Mutant *KRAS* subgroup			Wild-type *KRAS* subgroup		
	Intervention	Control	HR (95% CI)	Intervention	Control	HR (95% CI)
Brugger 2011[43]	9.4[a]	9.6[a]	0.79(0.49-1.27)	12.6[a]	11.2[a]	0.86(0.68-1.08)
Eberhard 2005[61]	4.4	13.5	2.10(1.10-3.80)	12.1	11.3	1.05(0.73-1.50)[b]
Johnson 2009[132,134]	NA	NA	0.79(0.48-1.32)	NA	NA	0.75(0.54-1.03)
Zhu 2008[84]	3.7	7.0	1.67(0.62-4.50)	7.5	3.4	0.69(0.49-0.97)
Ciuleanu 2012[107]	2.9	6.4	2.20(0.96-5.06)	7.8	4.5	0.69(0.49-0.99)
Douillard 2010[127]	7.8	4.2	0.81(0.44-1.49)	7.5	6.3	1.03(0.77-1.37)

Abbreviations: HR = hazard ratio; CI, confidence interval; NA = not available.

[a] estimated from the published survival curves.

[b] obtained by contact with investigators.

Table 20. Sensitivity analyses for treatment-*KRAS* mutations interaction measured by ratio of hazard ratios

Clinical outcome	Progression-free survival	Overall survival
Main meta-analysis	1.35(1.02-1.80)	1.37(0.89-2.10)
Sensitivity analysis A	1.24(0.90-1.70)	1.15(0.70-1.88)
Sensitivity analysis B	1.29(0.95-1.75)	1.18(0.81-1.72)
Sensitivity analysis C	1.65(1.05-2.59)	1.56(0.94-2.59)

Sensitivity analysis A: Studies with *post hoc* subgroup analysis according to *KRAS* mutation status were excluded. Sensitivity analysis B: Studies with incomparable intervention and control arms in either *KRAS* subgroup were excluded. Sensitivity analysis C: Studies with significant cross-over of treatment or imbalance of post-study treatments between treatment arms were excluded.

Table 21. Summary of the main results

Biomarker, outcome and comparison	Trials	*N*	Hazard ratio (95% CI): EGFR TKIs vs control			Ratio of hazard ratios (HR_{B+}/ HR_{B-})
			in biomarker-positive patients (HR_{B+})	in biomarker-negative patients (HR_{B-})	overall	
1. *EGFR* mutation						
- Progression-free survival						
EGFR TKIs vs placebo	5	1403	0.29 (0.15-0.55)	0.88 (0.72-1.07)	0.69 (0.53-0.90)	0.34 (0.19-0.61)
EGFR TKIs vs chemotherapy	6	1107	0.47 (0.37-0.61)	1.09 (0.65-1.82)	0.68 (0.38-1.22)	0.42 (0.18-0.98)
Combined	11	2510	NA	NA	NA	0.37 (0.22-0.60)
- Overall survival						
EGFR TKIs vs placebo	5	1528	0.68 (0.44-1.04)	0.85 (0.75-0.97)	0.84 (0.71-0.98)	0.83 (0.53-1.31)
EGFR TKIs vs chemotherapy	5	1050	0.97 (0.76-1.24)	1.01 (0.85-1.20)	0.99 (0.86-1.14)	0.85 (0.60-1.20)
Combined	10	2578	NA	NA	NA	0.84 (0.64-1.11)
2. *EGFR* gene copy number						
- Progression-free survival						
EGFR TKIs vs placebo	5	1466	0.64 (0.52-0.79)	0.97 (0.74-1.27)	0.76 (0.65-0.88)	0.62 (0.42-0.90)
EGFR TKIs vs chemotherapy	5	1251	0.96 (0.65-1.42)	1.20 (0.96-1.49)	1.02 (0.79-1.31)	0.89 (0.51-1.56)
Combined	10	2717	NA	NA	NA	0.72 (0.52-0.99)
- Overall survival						
EGFR TKIs vs placebo	7	1995	0.81 (0.57-1.17)	0.98 (0.86-1.12)	0.95 (0.78-1.15)	0.85 (0.58-1.24)
EGFR TKIs vs chemotherapy	4	1191	1.08 (0.77-1.51)	1.06 (0.87-1.29)	1.05 (0.91-1.22)	1.06 (0.62-1.79)
Combined	11	3186	NA	NA	NA	0.92 (0.69-1.23)

3. EGFR protein expression						
- Progression-free survival						
EGFR TKIs vs placebo	2	1000	0.76 (0.58-0.99)	0.84 (0.60-1.17)	0.79 (0.59-1.04)	0.90 (0.62-1.31)
EGFR TKIs vs chemotherapy	3	1108	1.06 (0.74-1.52)	0.96 (0.73-1.27)	1.04 (0.80-1.36)	1.07 (0.72-1.59)
Combined	5	2108	NA	NA	NA	0.99 (0.78-1.26)
- Overall survival						
EGFR TKIs vs placebo	4	1790	0.78 (0.68-0.89)	1.02 (0.82-1.27)	0.84 (0.74-0.96)	0.78 (0.60-1.02)
EGFR TKIs vs chemotherapy	3	1108	0.99 (0.85-1.16)	1.02 (0.78-1.33)	1.00 (0.88-1.14)	0.98 (0.72-1.34)
Combined	7	2898	NA	NA	NA	0.86 (0.70-1.05)
4. *KRAS* mutation						
- Progression-free survival						
EGFR TKIs vs placebo	3	1087	1.07 (0.64-1.79)	0.74 (0.62-0.89)	0.75 (0.62-0.91)	1.37 (0.97-1.93)
EGFR TKIs vs chemotherapy	2	470	1.47 (0.87-2.49)	1.12 (0.89-1.41)	1.23 (0.95-1.59)	1.32 (0.67-2.63)
Combined	5	1557	NA	NA	NA	1.35 (1.02-1.80)
- Overall survival						
EGFR TKIs vs placebo	4	1293	1.14 (0.69-1.89)	0.83 (0.71-0.97)	0.85 (0.72-0.99)	1.31 (0.85-2.02)
EGFR TKIs vs chemotherapy	2	470	1.28 (0.48-3.40)	0.85 (0.58-1.26)	1.00 (0.77-1.29)	1.53 (0.39-6.03)
Combined	6	1763	NA	NA	NA	1.37 (0.89-2.10)

Abbreviations: *N* = sample size; EGFR TKI = epidermal growth factor receptor tyrosine kinase inhibitor; HR = hazard ratio; B+ = biomarker-positive; B- = biomarker-negative; NA = not applicable.

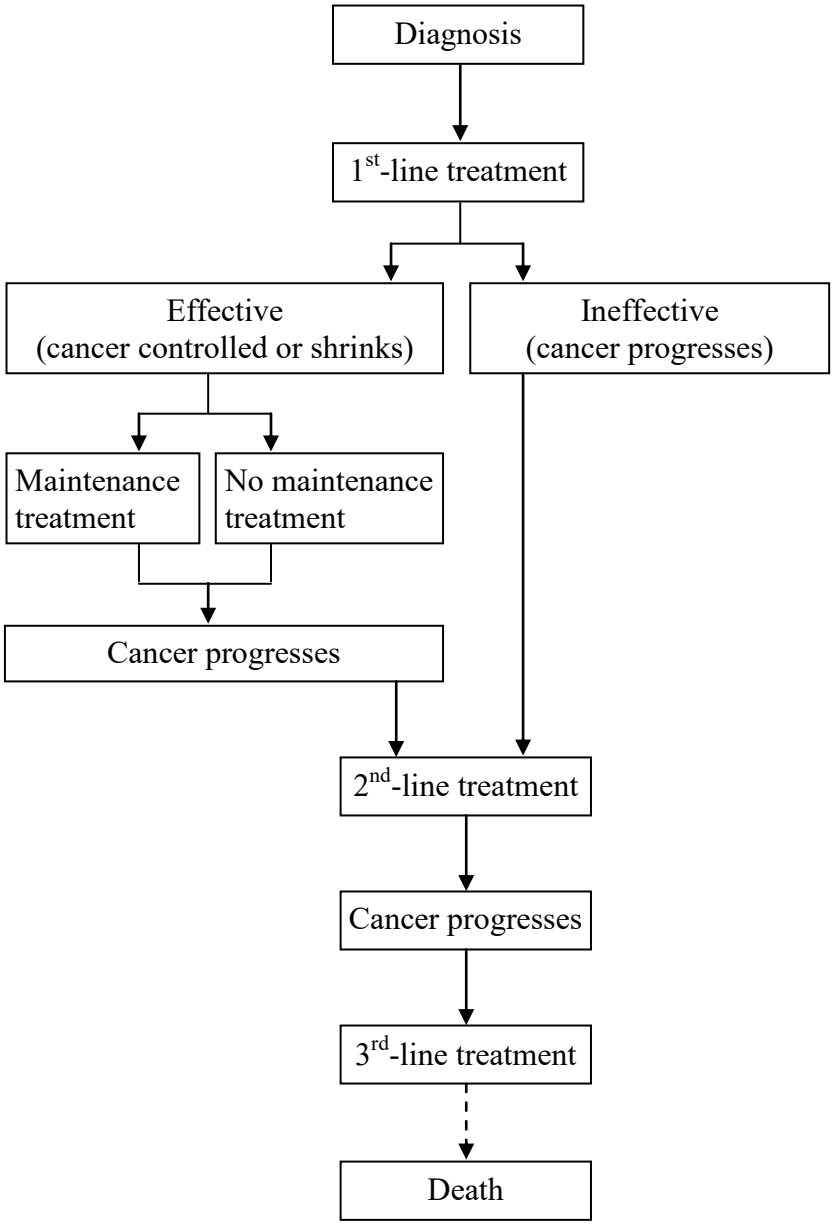


Figure 1. Lines of treatment from diagnosis to death in advanced NSCLC patients

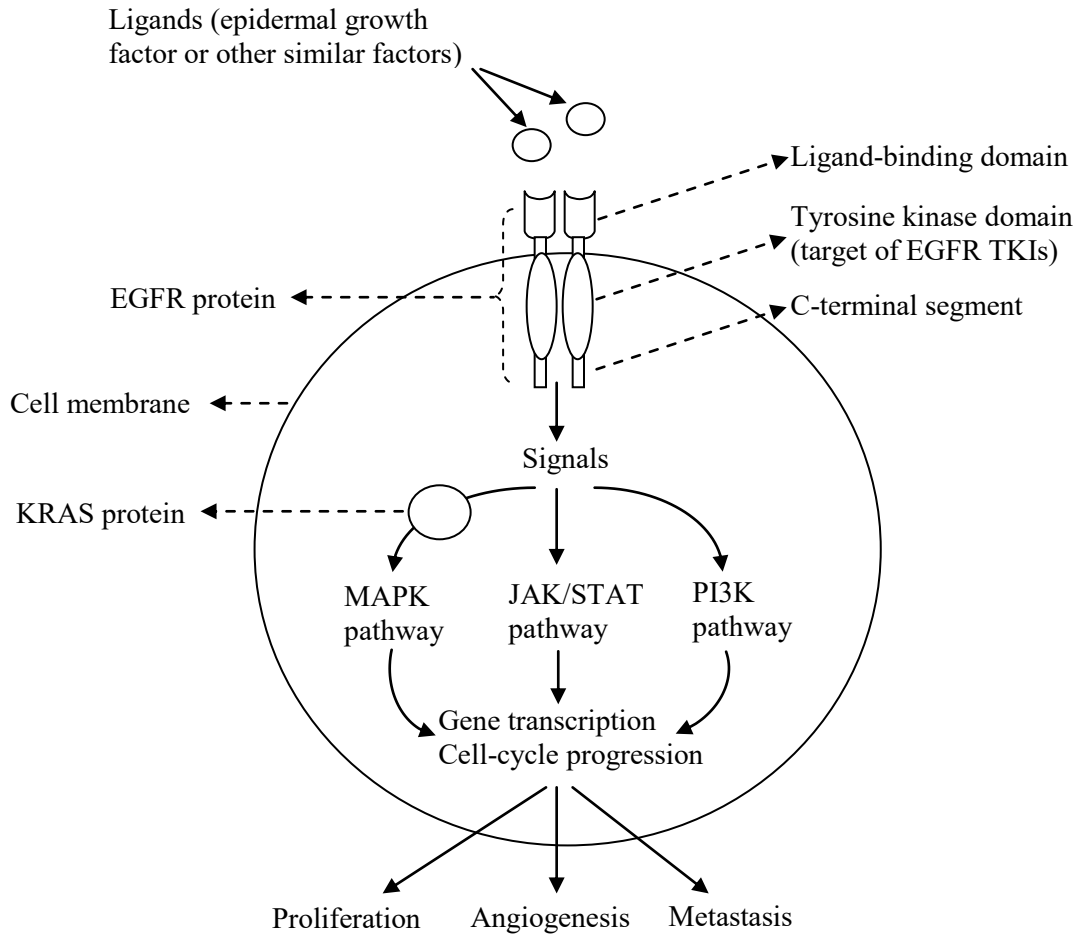
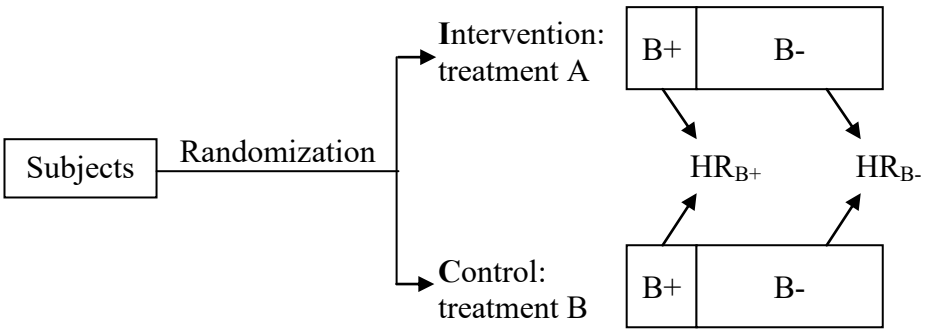
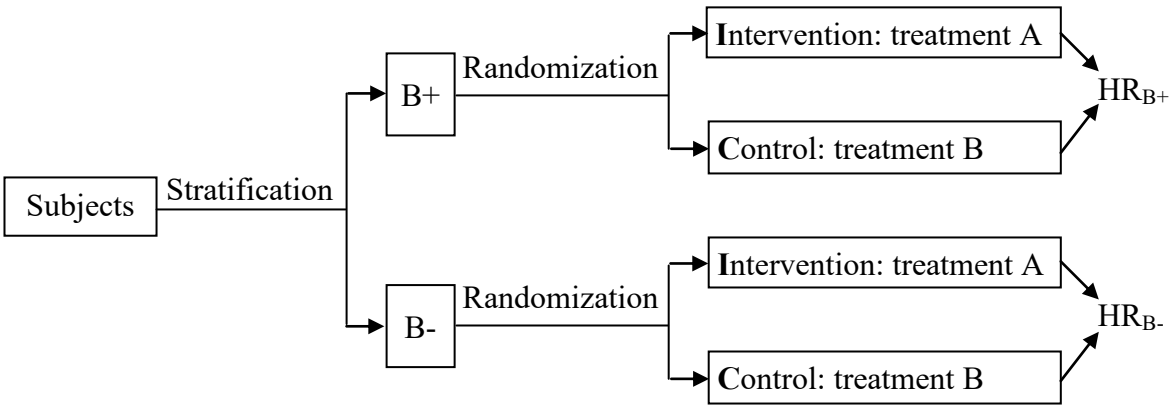


Figure 2. The EGFR and downstream signaling pathways

EGFR TKI = epidermal growth factor receptor tyrosine kinase inhibitor; MAPK = the mitogen-activated protein kinase; JAK/STAT = Janus kinase and signal transducer and activator of transcription; PI3K = phosphatidylinositol 3-kinase.



(A) Subgroup analysis approach

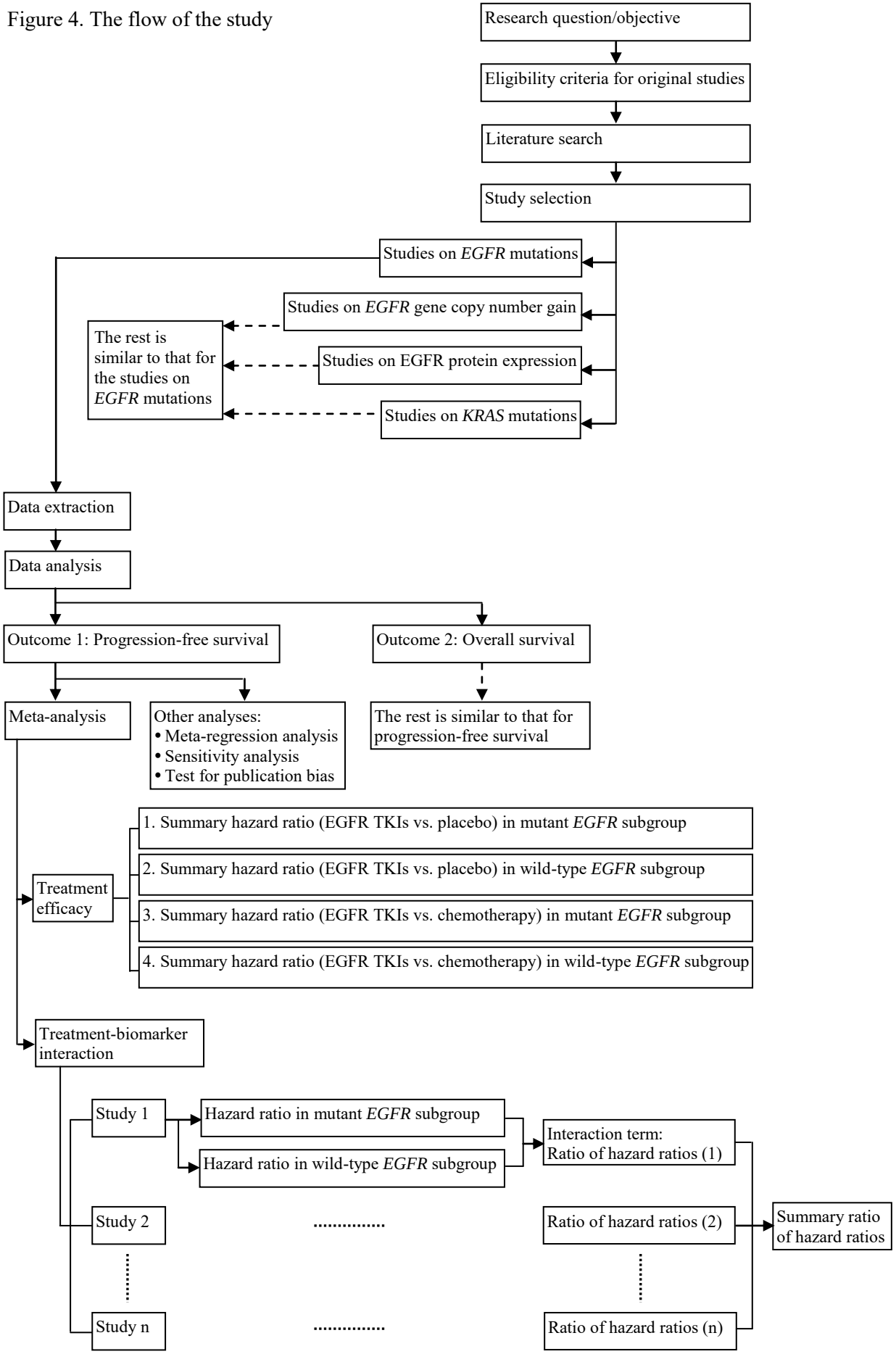


(B) Stratified randomization approach

Figure 3. The method to evaluate predictive biomarkers

B+ = biomarker positive; B- = biomarker negative; HR_{B+} = hazard ratio (intervention vs. control) in biomarker-positive patients; HR_{B-} = hazard ratio (intervention vs. control) in biomarker-negative patients. The treatment-biomarker interaction can be evaluated by comparing HR_{B+} with HR_{B-}.

Figure 4. The flow of the study



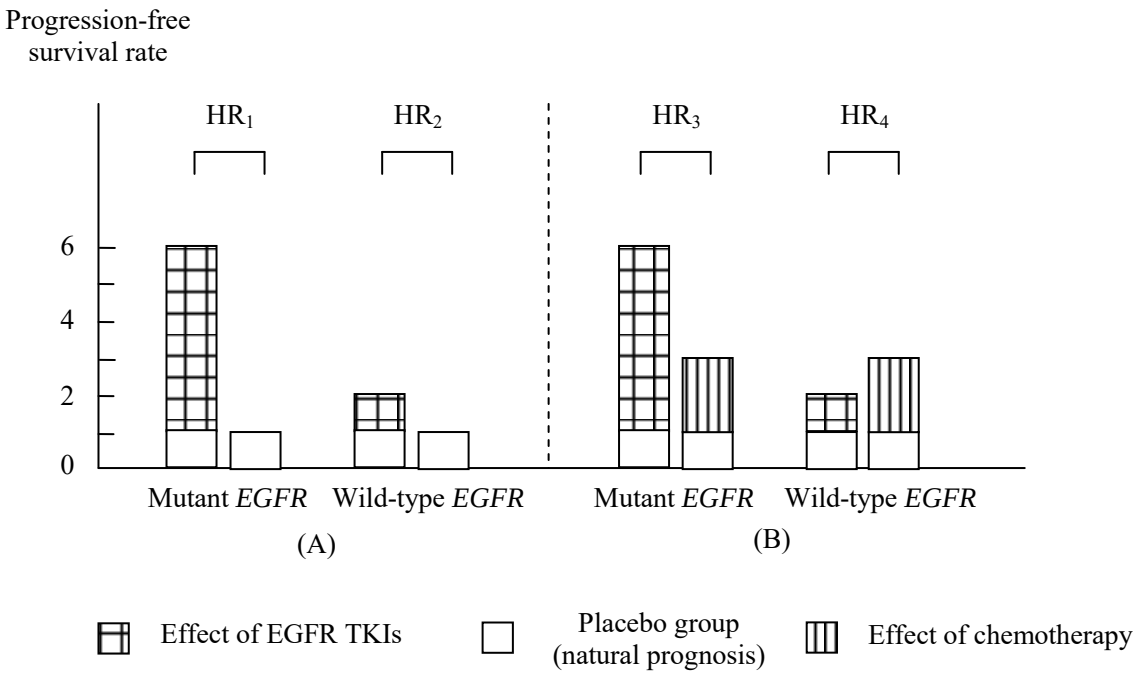


Figure 5. A hypothetical example of treatment-*EGFR* mutations interaction measured by ratio of HRs in placebo-controlled trials (A) and in chemotherapy-controlled trials (B).

In (A), the ratio of HRs = HR_1/HR_2 = (1/6) / (1/2) = 0.33; in (B), the ratio of HRs = HR_3/HR_4 = (1/2) / (3/2) = 0.33. Effects of control treatments were offset in calculating the ratios and, as a result, the two ratios were equal to each other.

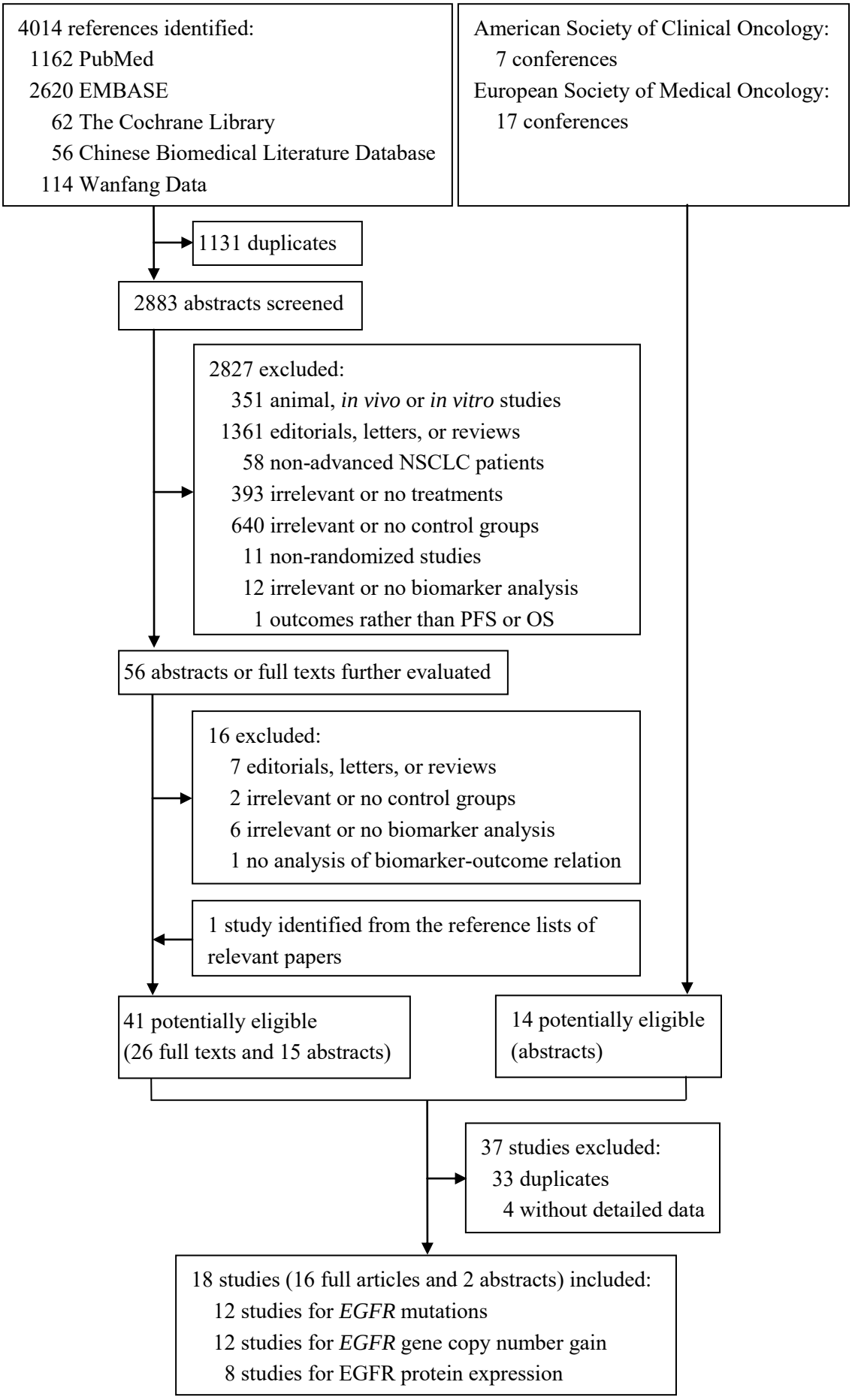


Figure 6(A). Flow chart of study selection (for EGFR alterations)

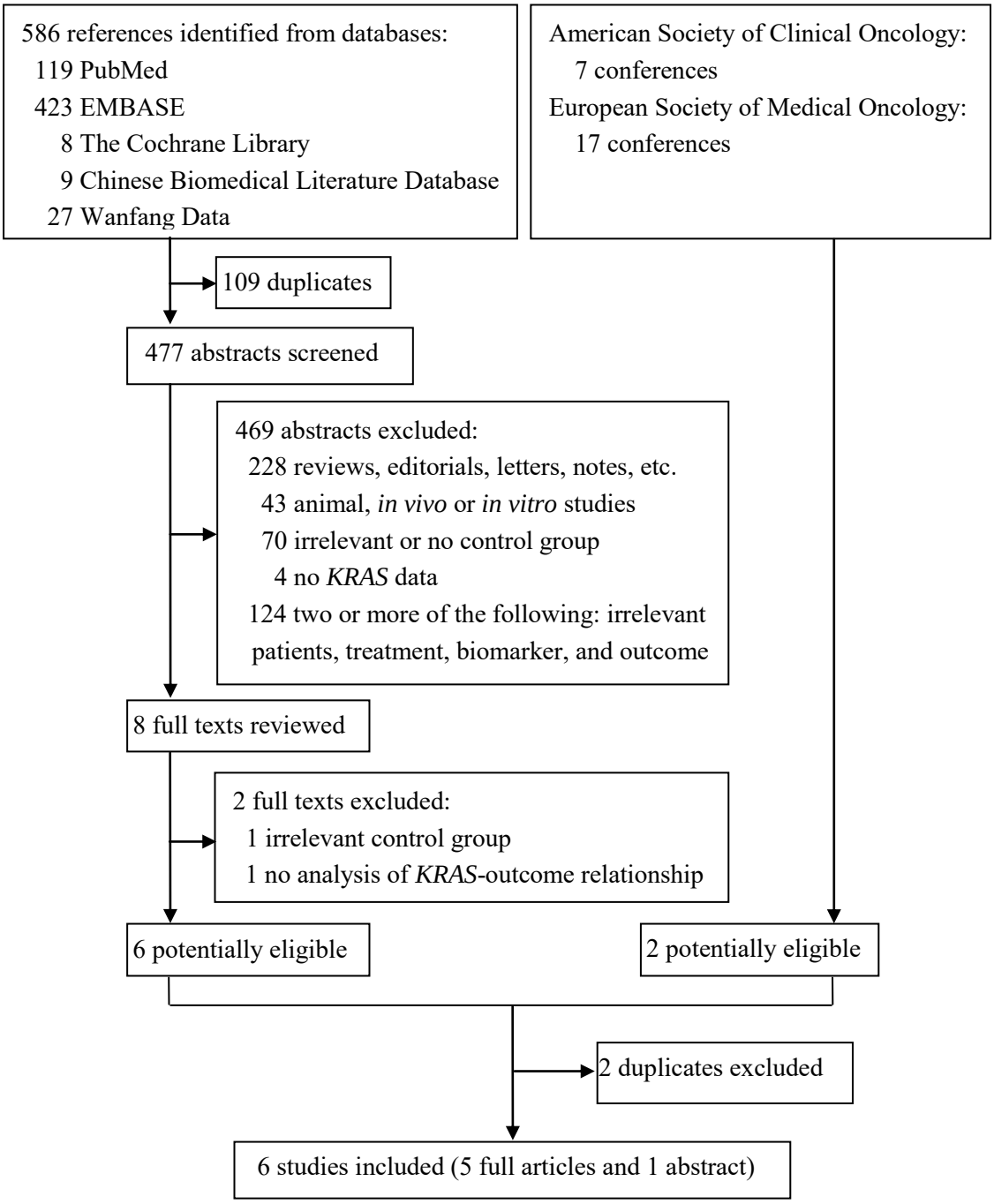


Figure 6(B). Flow chart of study selection (for *KRAS* mutations)

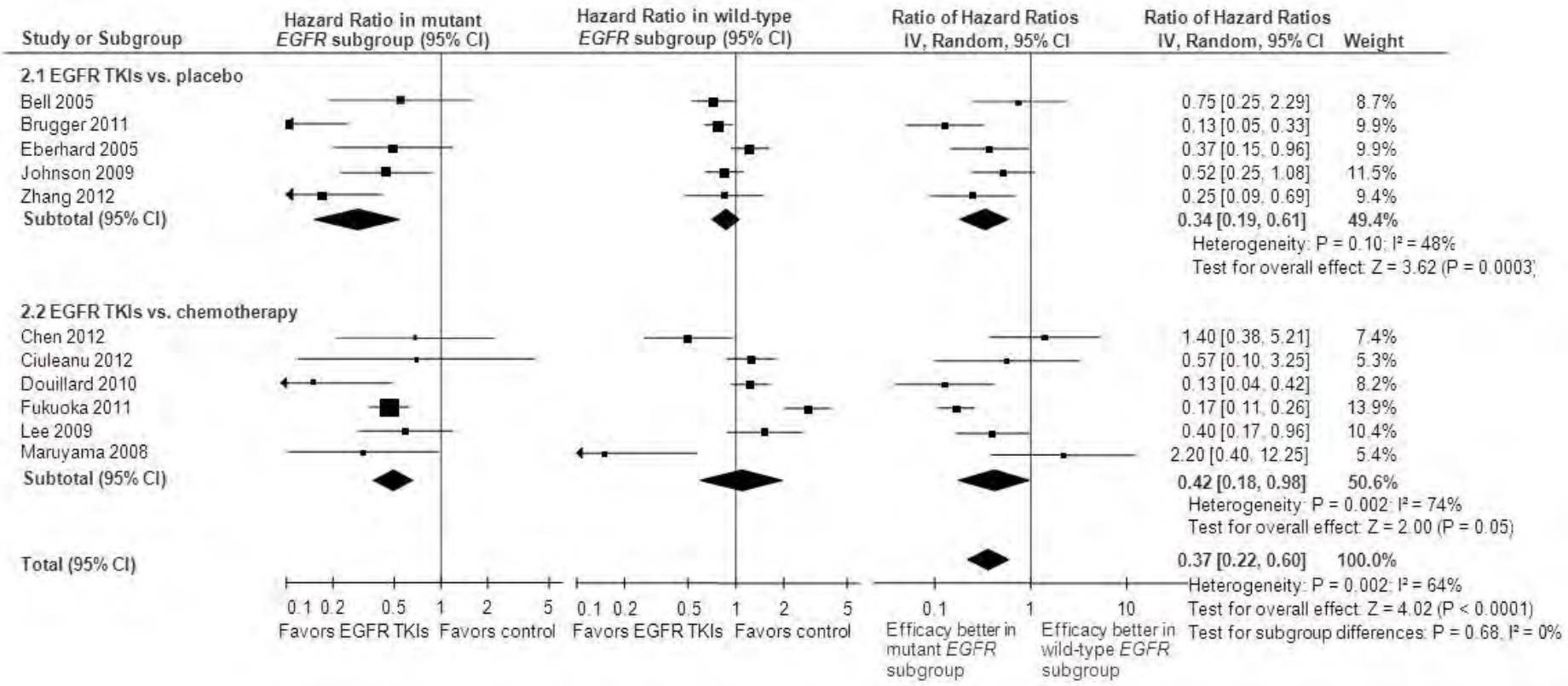


Figure 7. The interaction between EGFR TKIs treatment and *EGFR* mutations in terms of progression-free survival

For each study, the ratio of hazard ratios is calculated as the hazard ratio in the mutant *EGFR* subgroup relative to that in the wild-type *EGFR* subgroup.

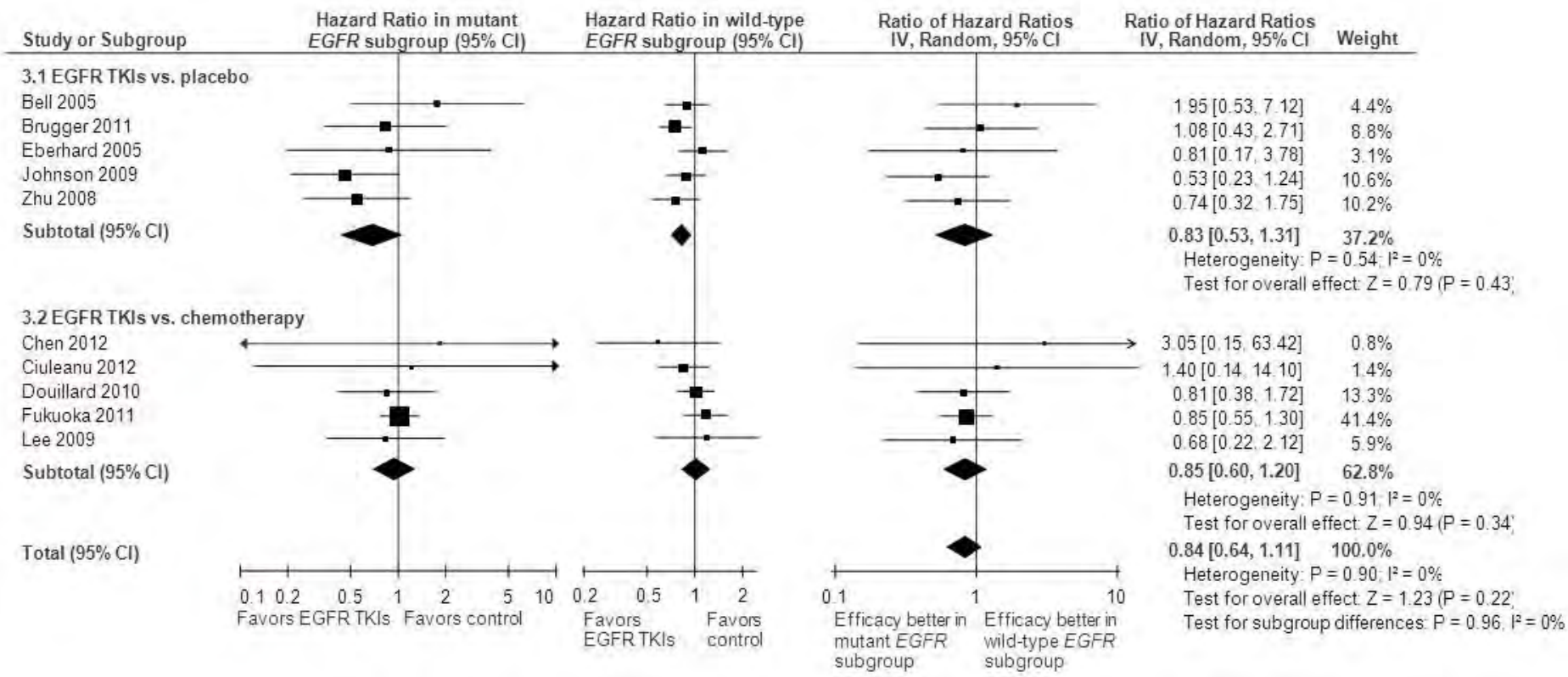


Figure 8. The interaction between EGFR TKIs treatment and *EGFR* mutations in terms of overall survival

For each study, the ratio of hazard ratios is calculated as the hazard ratio in the mutant *EGFR* subgroup relative to that in the wild-type *EGFR* subgroup.

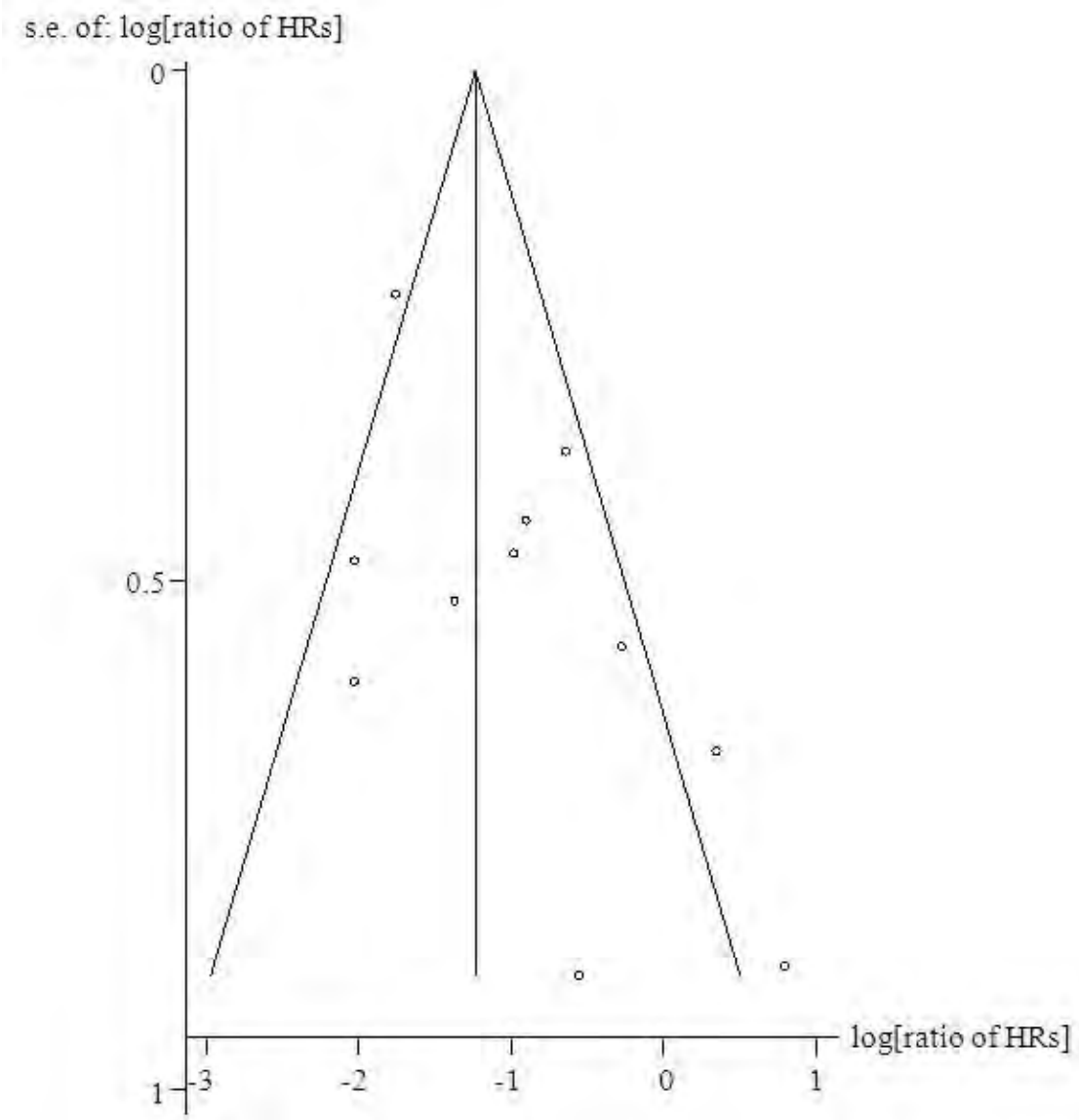


Figure 9. Funnel plot constructed on the basis of studies for treatment-*EGFR* mutations interaction in terms of progression-free survival

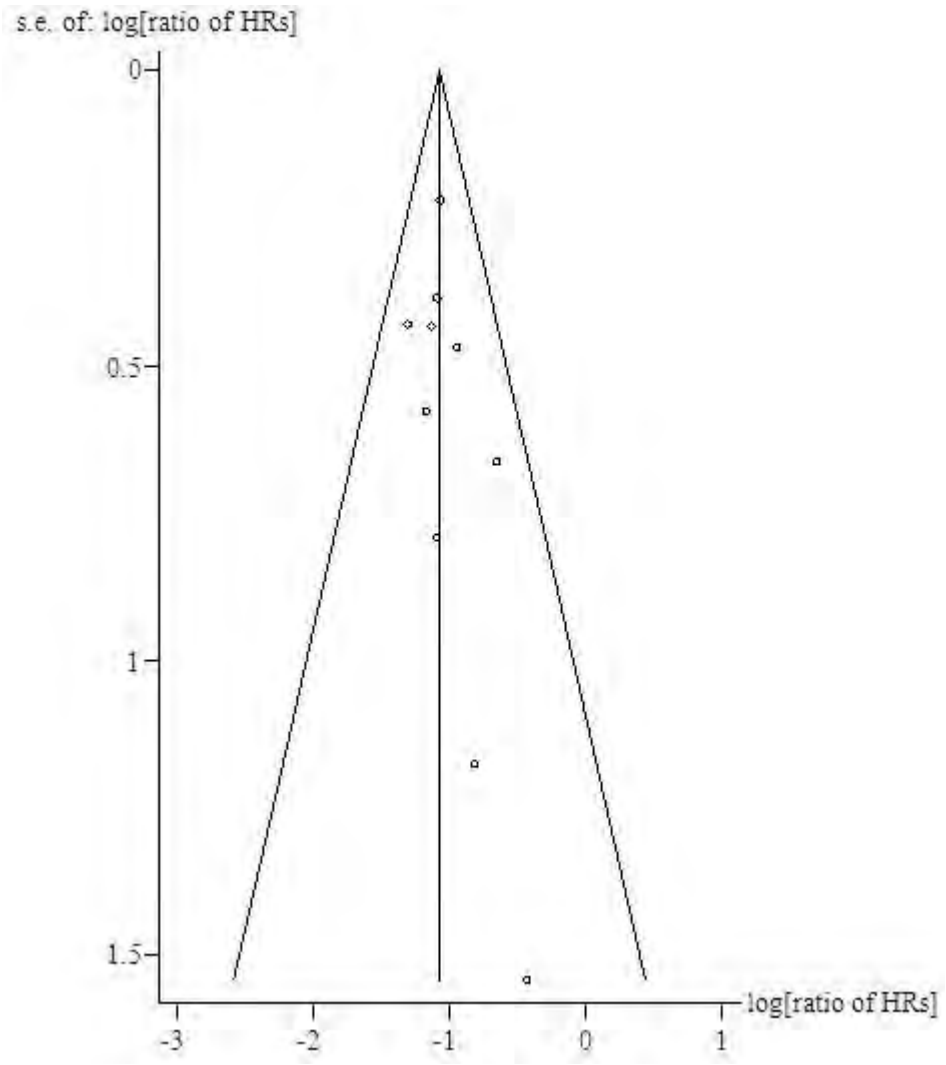


Figure 10. Funnel plot constructed on the basis of studies for treatment-*EGFR* mutations interaction in terms of overall survival

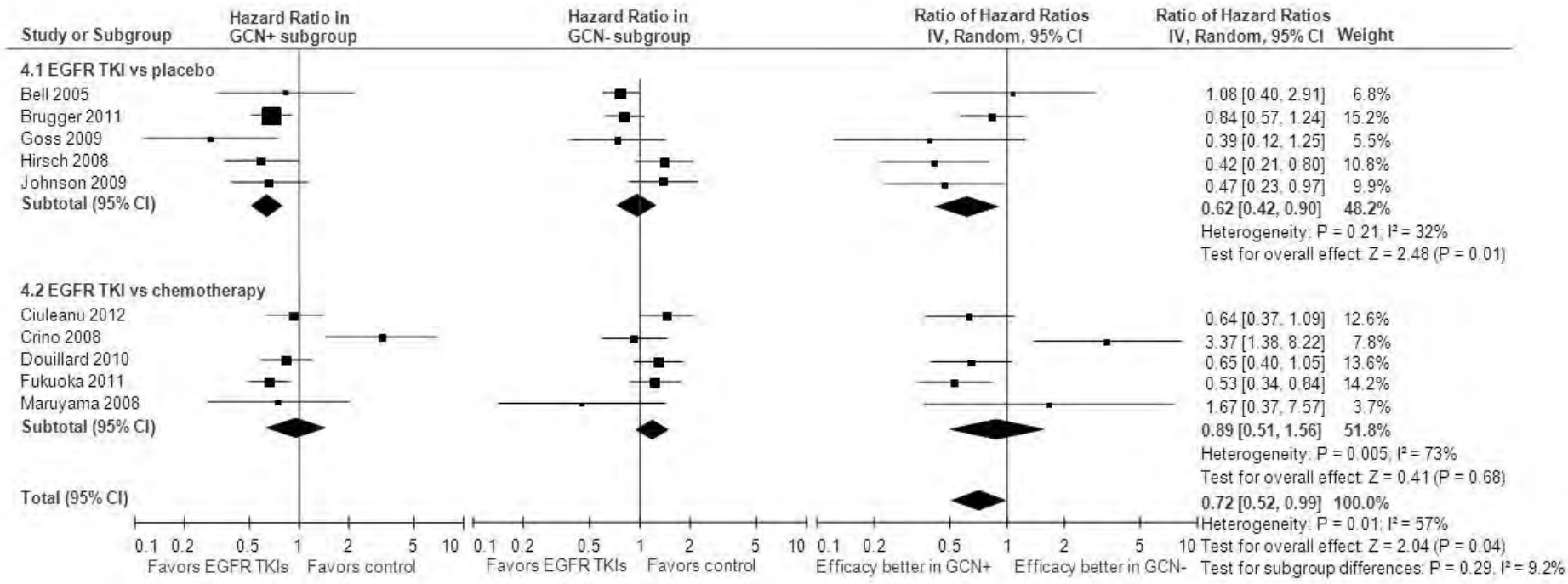


Figure 11. The interaction between EGFR TKIs treatment and *EGFR* gene copy number gain in terms of progression-free survival

GCN+ = with *EGFR* gene copy number gain; GCN- = without *EGFR* gene copy number gain. For each study, the ratio of hazard ratios is calculated as the hazard ratio in the GCN+ subgroup relative to that in the GCN- subgroup.

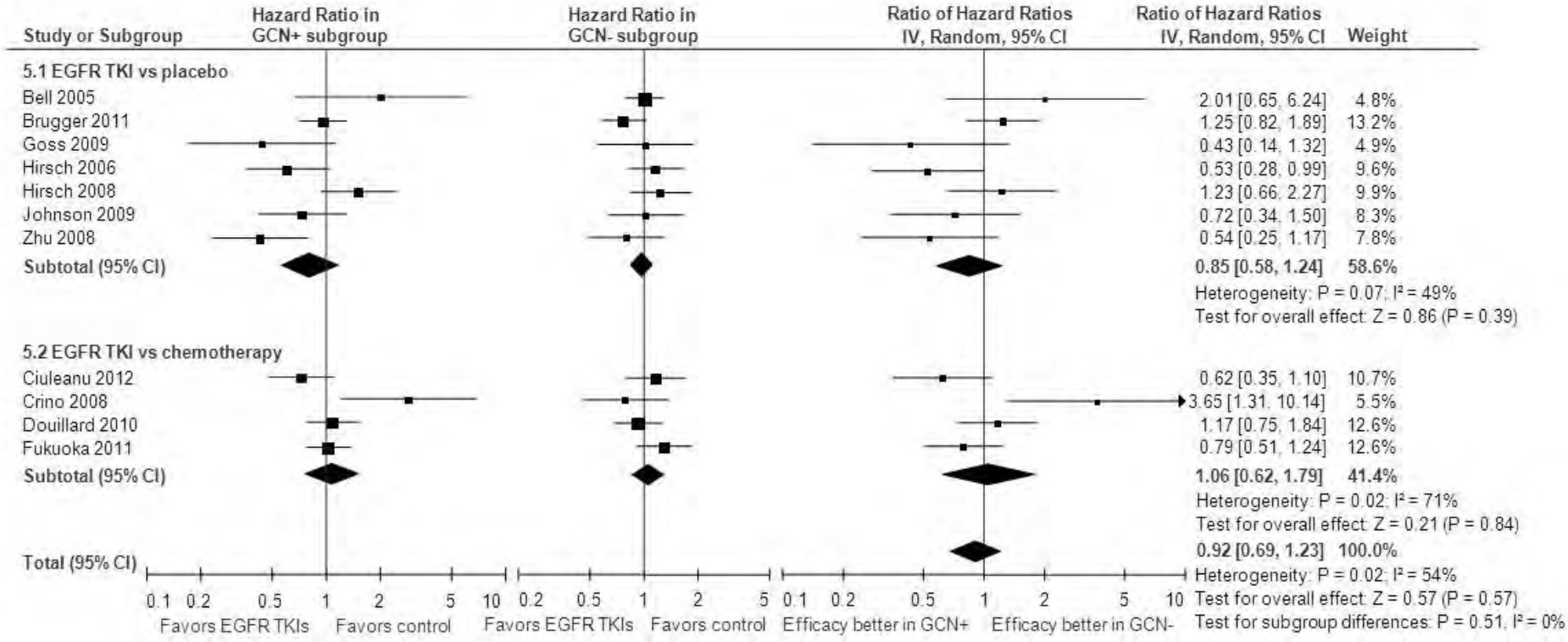


Figure 12. The interaction between EGFR TKIs treatment and *EGFR* gene copy number gain in terms of overall survival

GCN+ = with *EGFR* gene copy number gain; GCN- = without *EGFR* gene copy number gain. For each study, the ratio of hazard ratios is calculated as the hazard ratio in the GCN+ subgroup relative to that in the GCN- subgroup.

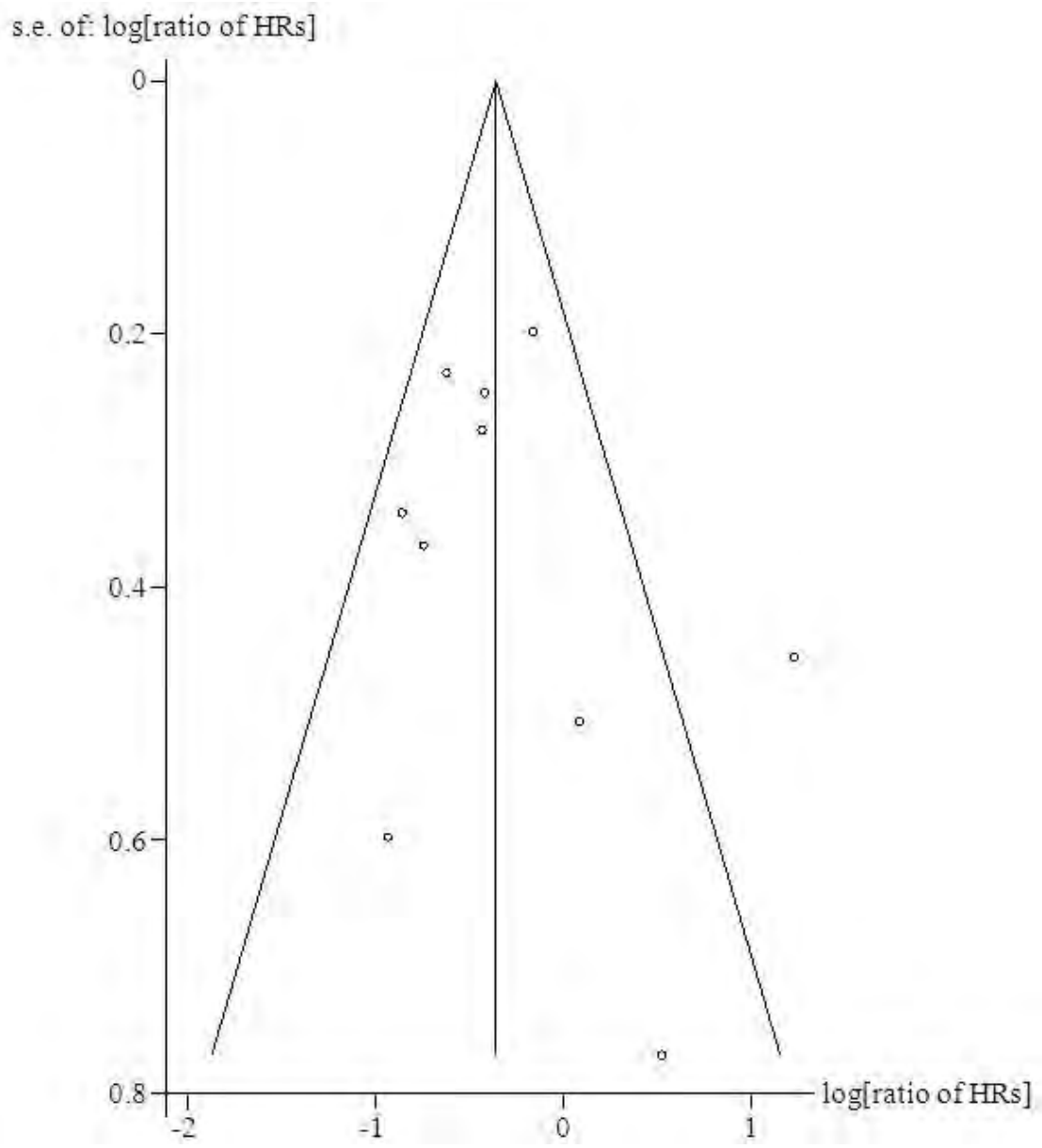


Figure 13. Funnel plot constructed on the basis of studies for treatment-*EGFR* gene copy number gain interaction in terms of progression-free survival

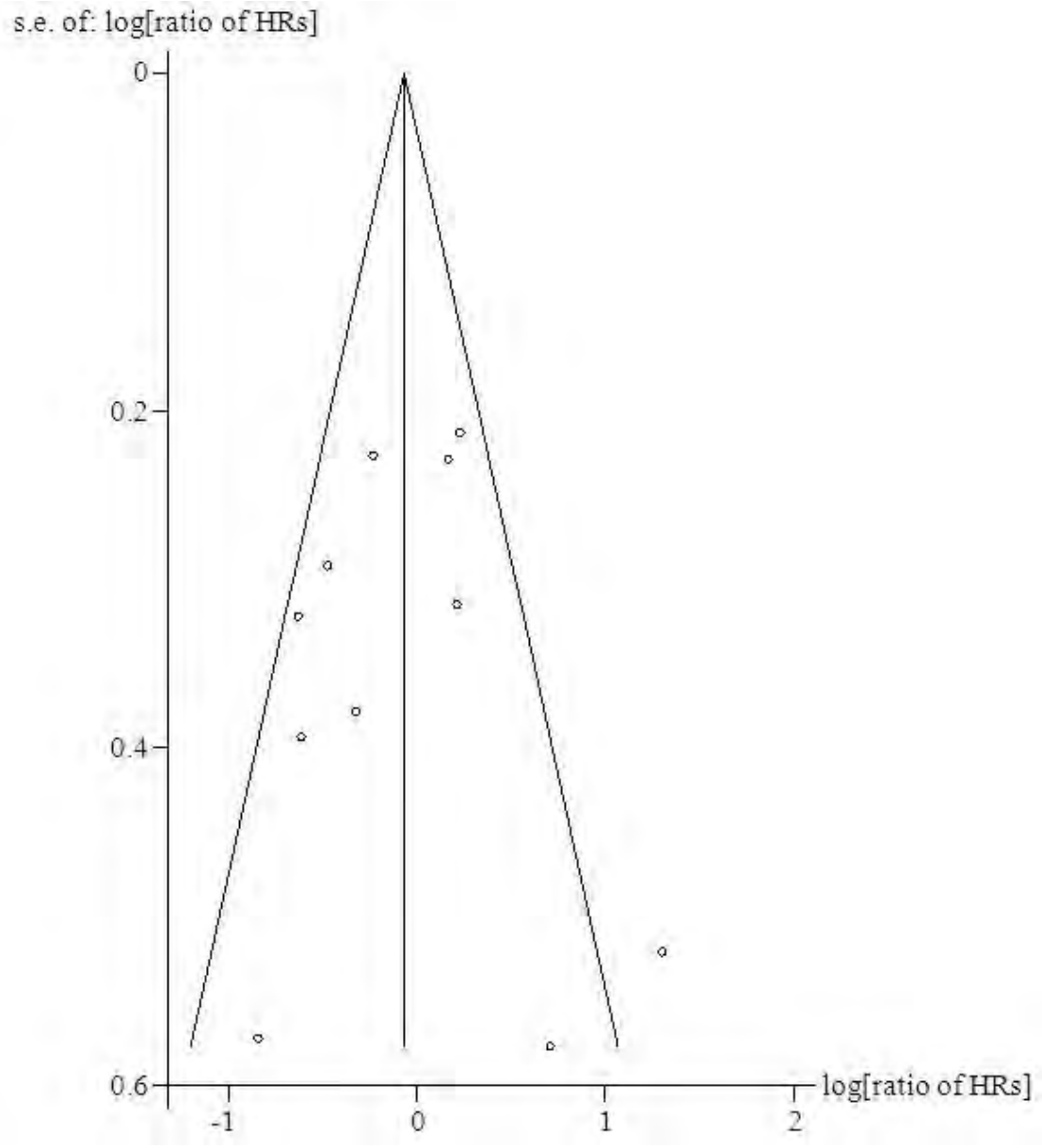


Figure 14. Funnel plot constructed on the basis of studies for treatment-*EGFR* gene copy number gain interaction in terms of overall survival

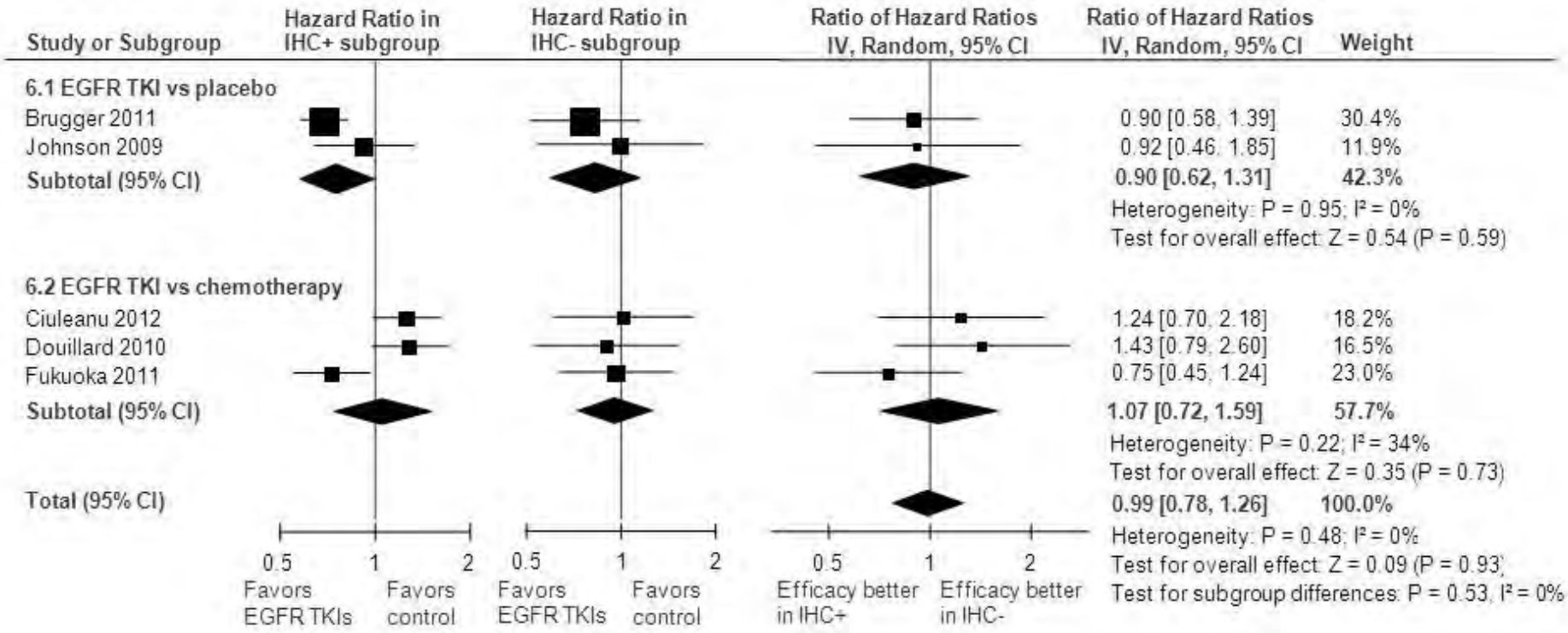


Figure 15. The interaction between EGFR TKIs treatment and EGFR protein expression in terms of progression-free survival

IHC+ = immunohistochemistry positive, i.e. with EGFR protein expression; IHC- = immunohistochemistry negative, i.e. without EGFR protein expression.

For each study, the ratio of hazard ratios is calculated as the hazard ratio in the IHC+ subgroup relative to that in the IHC- subgroup.

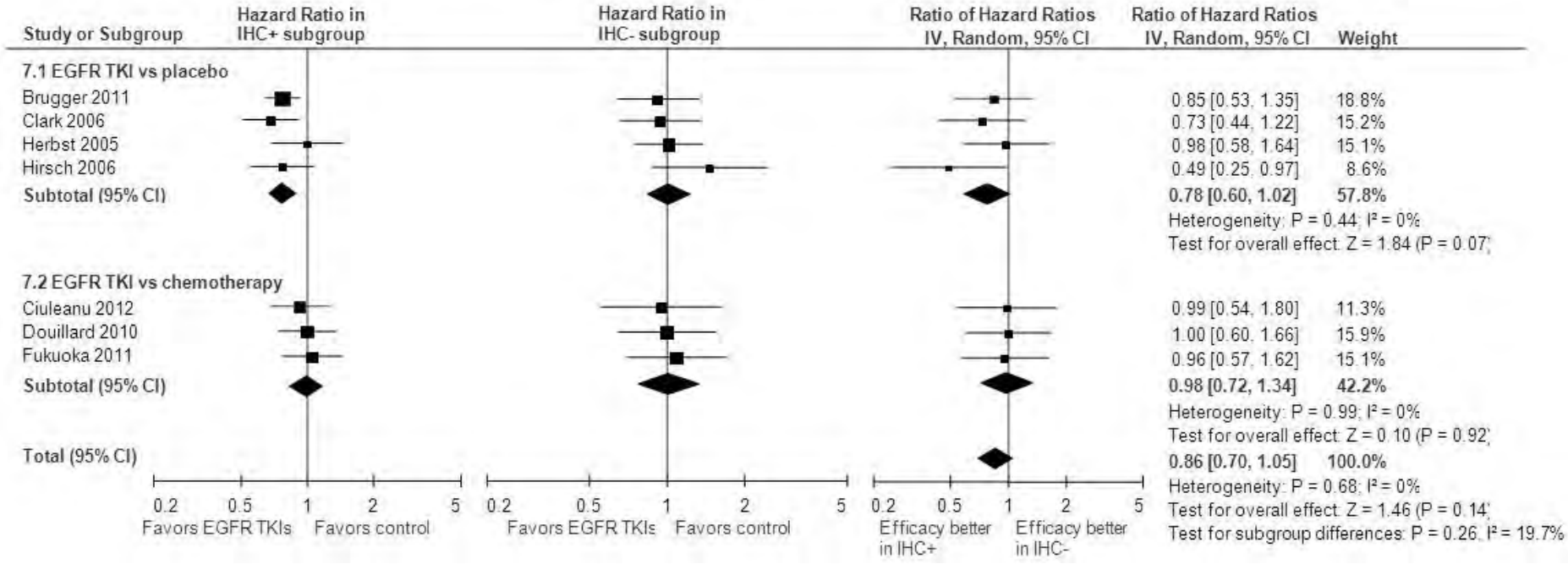


Figure 16. The interaction between EGFR TKIs treatment and EGFR protein expression in terms of overall survival

IHC+ = immunohistochemistry positive, i.e. with EGFR protein expression; IHC- = immunohistochemistry negative, i.e. without EGFR protein expression.

For each study, the ratio of hazard ratios is calculated as the hazard ratio in the IHC+ subgroup relative to that in the IHC- subgroup.

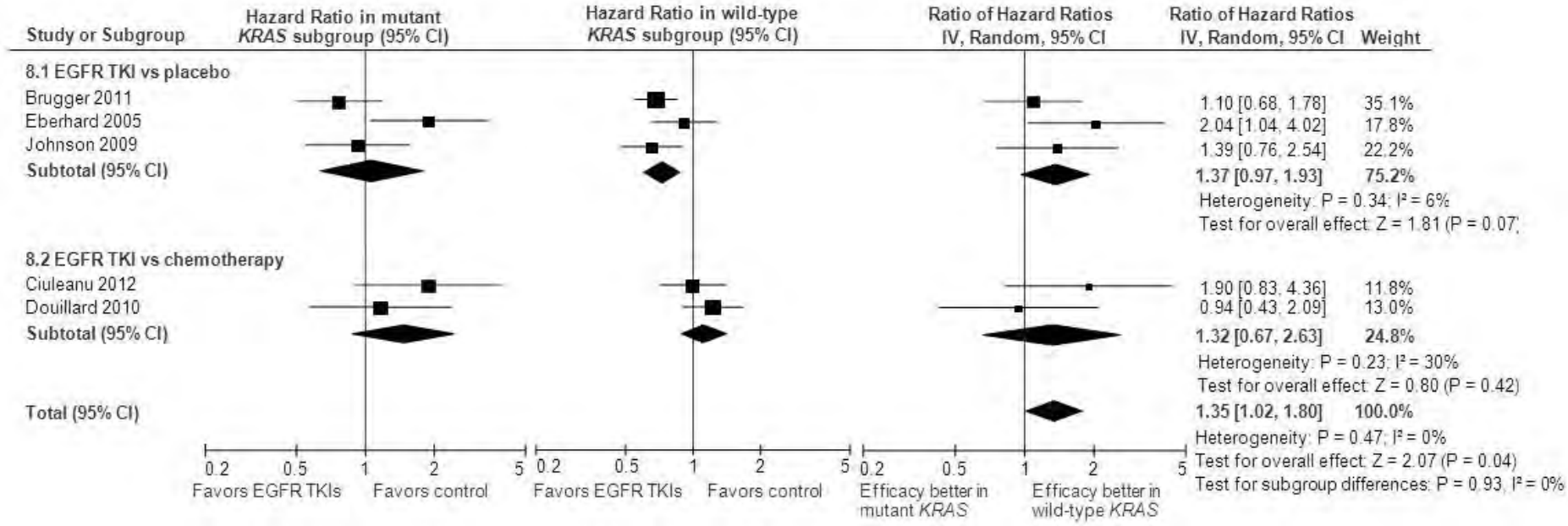


Figure 17. The interaction between EGFR TKIs treatment and *KRAS* mutations in terms of progression-free survival

For each study, the ratio of hazard ratios is calculated as the hazard ratio in the mutant *KRAS* subgroup relative to that in the wild-type *KRAS* subgroup.

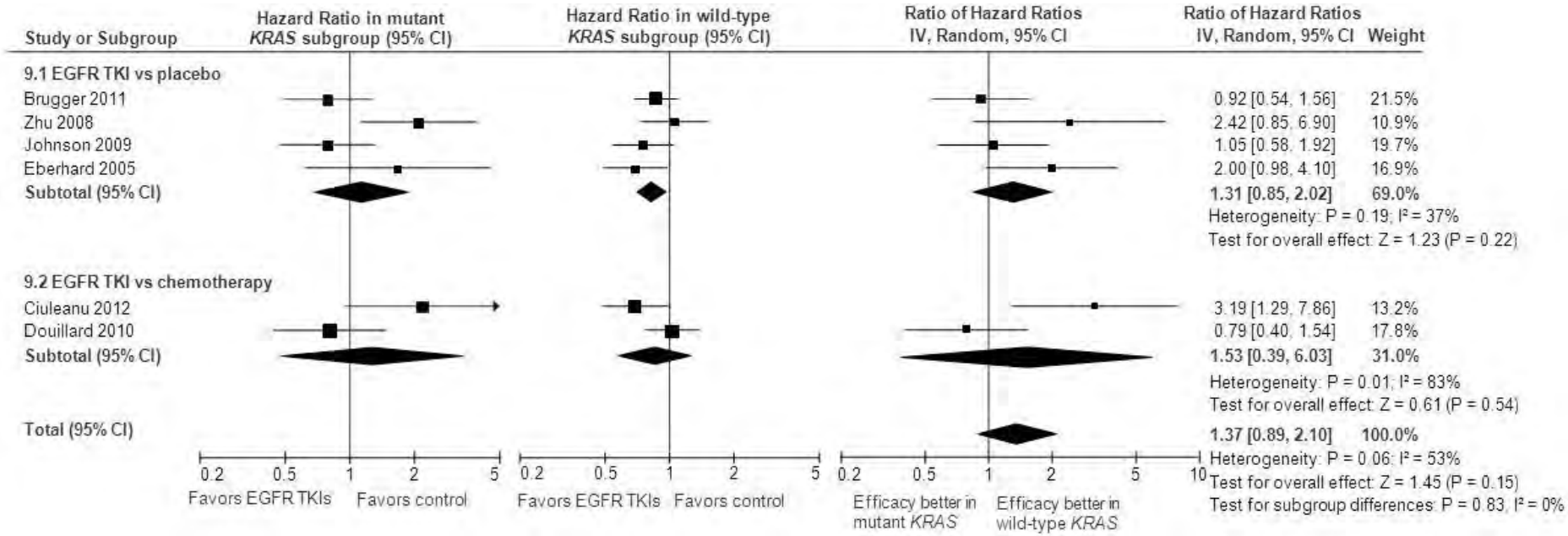


Figure 18. The interaction between EGFR TKIs treatment and *KRAS* mutations in terms of overall survival

For each study, the ratio of hazard ratios is calculated as the hazard ratio in the mutant *KRAS* subgroup relative to that in the wild-type *KRAS* subgroup.

Appendix 1: Detailed search strategy and history

Part 1: PubMed search history for EGFR alterations

Search	Most Recent Queries	Result
#11	Search #8 not (#9 or #10)	1162
#10	Search (#1 or #2) and (#3 or #4 or #5) and (#6 or #7) Limits: Animals	212
#9	Search (#1 or #2) and (#3 or #4 or #5) and (#6 or #7) Limits: Editorial, Letter, Review	588
#8	Search (#1 or #2) and (#3 or #4 or #5) and (#6 or #7)	1884
#7	Search "epidermal growth factor receptor" or "EGF receptor" or EGFR	35867
#6	Search "Genes, erbB-1"[Mesh] OR "EGFR protein, human" [Supplementary Concept] OR "Genes, erbB"[Mesh]	4296
#5	Search "tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva	10745
#4	Search "erlotinib" [Supplementary Concept]	1399
#3	Search "gefitinib" [Supplementary Concept]	2343
#2	Search "Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC	31142
#1	Search "Carcinoma, Non-Small-Cell Lung"[Mesh]	24199

Part 2: PubMed search history for *KRAS* mutations

Search	Most Recent Queries	Result
#11	Search #8 not (#9 or #10)	119
#10	Search (#1 or #2) and (#3 or #4 or #5) and (#6 or #7) Limits: Animals	17
#9	Search (#1 or #2) and (#3 or #4 or #5) and (#6 or #7) Limits: Editorial, Letter, Review	50
#8	Search (#1 or #2) and (#3 or #4 or #5) and (#6 or #7)	182
#7	Search KRAS or K-RAS or RAS	45235
#6	Search "Genes, ras"[Mesh]	10746
#5	Search "tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva	10745
#4	Search "erlotinib" [Supplementary Concept]	1399
#3	Search "gefitinib" [Supplementary Concept]	2343
#2	Search "Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC	31142
#1	Search "Carcinoma, Non-Small-Cell Lung"[Mesh]	24199

Part 3: EMBASE search history for EGFR alterations

# ▲	Searches	Results
1	lung non small cell cancer/	39111
2	("Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC).af.	31467
3	gefitinib/	11303
4	erlotinib/	10006
5	("tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva).af.	29844
6	epidermal growth factor receptor/	30666
7	("epidermal growth factor receptor" or "EGF receptor" or EGFR).af.	60287
8	1 or 2	44028
9	3 or 4 or 5	29844
10	6 or 7	60287
11	8 and 9 and 10	4469
12	limit 11 to (editorial or letter or "review")	1680
13	limit 11 to animals	65
14	limit 11 to animal studies	144

15	12 or 13 or 14	1849
16	(((lung non small cell cancer or ("Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC)) and (gefitinib or erlotinib or ("tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva)) and (epidermal growth factor receptor or ("epidermal growth factor receptor" or "EGF receptor" or EGFR))) not 15).af.	2620

Part 4: EMBASE search history for *KRAS* mutations

# ▲	Searches	Results
1	lung non small cell cancer/	39111
2	("Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC).af.	31467
3	gefitinib/	11303
4	erlotinib/	10006
5	("tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva).af.	29844
6	oncogene ras/	8132
7	(KRAS or "K-RAS" or RAS).af.	56384
8	1 or 2	44028
9	3 or 4 or 5	29844
10	6 or 7	56384
11	8 and 9 and 10	789
12	limit 11 to (editorial or letter or "review")	344
13	limit 11 to animals	11
14	limit 11 to animal studies	14
15	12 or 13 or 14	366

16	(((lung non small cell cancer or ("Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC)) and (gefitinib or erlotinib or ("tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva)) and (oncogene ras or (KRAS or "K-RAS" or RAS))) not 15).af.	423

Part 5: The Cochrane Library search history for EGFR alterations

ID	Search	Hits
#1	MeSH descriptor Carcinoma, Non-Small-Cell Lung explode all trees	1811
#2	"Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC in Clinical Trials	3038
#3	"tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva in Clinical Trials	276
#4	MeSH descriptor Receptor, Epidermal Growth Factor explode all trees	223
#5	MeSH descriptor Genes, erbB-1 explode all trees	9
#6	"epidermal growth factor receptor" or "EGF receptor" or EGFR in Clinical Trials	535
#7	((#1 OR #2) AND #3 AND (#4 OR #5 OR #6))	62

Part 6: The Cochrane Library search history for *KRAS* mutations

ID	Search	Hits
#1	MeSH descriptor Carcinoma, Non-Small-Cell Lung explode all trees	1811
#2	"Non-Small-Cell Lung Cancer" or "Non-Small Cell Lung Cancer" or "Non-Small-Cell Lung Carcinoma" or "Non-Small Cell Lung Carcinoma" or NSCLC in Clinical Trials	3038
#3	"tyrosine kinase inhibitor" or TKI or gefitinib or Iressa or erlotinib or Tarceva in Clinical Trials	276
#4	MeSH descriptor Genes, ras explode all trees	36
#5	(KRAS or K-RAS or RAS) in Clinical Trials	429
#6	((#1 OR #2) AND #3 AND (#4 OR #5))	8

Part 7: Chinese Biomedical Literature Database search history for EGFR alterations

序号	命中文献数	检索表达式
27	56	#19 not #26 -限定:-
26	30	#25 or #24 or #23 or #22 or #21 or #20 -限定:-
25	1	#18 and #7 and #6 -限定:体外研究
24	2	#18 and #7 and #6 -限定:动物
23	1	#18 and #7 and #6 -限定:病例报告
22	1	#18 and #7 and #6 -限定:译文
21	0	#18 and #7 and #6 -限定:讲座
20	26	#18 and #7 and #6 -限定:综述
19	86	#18 and #7 and #6 -限定:-
18	3536	#17 or #9 -限定:-
17	3508	#16 and #15 -限定:-
16	4766	#14 or #8 -限定:-
15	441672	#13 or #12 -限定:-
14	4766	#11 and #10 -限定:-
13	287189	全部字段:基因 -限定:-
12	296161	全部字段:表达 -限定:-
11	125175	全部字段:受体 -限定:-
10	8471	全部字段:表皮生长因子 -限定:-

9	191	主题词:基因, erbB-1/全部树/全部副主题词 -限定:-
8	0	主题词:受体, 表皮生长因子/全部树/全部副主题词 -限定:-
7	1044	全部字段:Erlotinib or Tarceva or 伊诺替尼 or 厄洛替尼 or 埃罗替尼 or 特罗凯 or 它赛瓦 or Gefitinib or Iressa or 吉非替尼 or 易瑞沙 -限定:-
6	16740	#5 or #1 -限定:-
5	16740	#4 and #3 and #2 -限定:-
4	514484	全部字段:癌 -限定:-
3	411034	全部字段:肺 -限定:-
2	16756	全部字段:非小细胞 -限定:-
1	14671	主题词:癌, 非小细胞肺/全部树/全部副主题词 -限定:-

Part 8: Chinese Biomedical Literature Database search history for *KRAS* mutations

序号	命中文献数	检索表达式
21	9	#13 not #20 -限定:-
20	4	#19 or #18 or #17 or #16 or #15 or #14 -限定:-
19	0	#12 and #7 and #6 -限定:体外研究
18	1	#12 and #7 and #6 -限定:动物
17	0	#12 and #7 and #6 -限定:病例报告
16	0	#12 and #7 and #6 -限定:译文
15	0	#12 and #7 and #6 -限定:讲座
14	3	#12 and #7 and #6 -限定:综述
13	13	#12 and #7 and #6 -限定:-
12	1342	#11 and #10 -限定:-
11	6632	#9 or #8 -限定:-
10	39896	全部字段:突变 -限定:-
9	6632	全部字段:KRAS or K-RAS or RAS -限定:-
8	2503	主题词:基因, ras/全部树/全部副主题词 -限定:-
7	1044	全部字段:Erlotinib or Tarceva or 伊诺替尼 or 厄洛替尼 or 埃罗替尼 or 特罗凯 or 它赛瓦 or Gefitinib or Iressa or 吉非替尼 or 易瑞沙 -限定:-
6	16740	#5 or #1 -限定:-
5	16740	#4 and #3 and #2 -限定:-

4	514484	全部字段:癌 -限定:-
3	411034	全部字段:肺 -限定:-
2	16756	全部字段:非小细胞 -限定:-
1	14671	主题词:癌, 非小细胞肺/全部树/全部副主题词 -限定:-

Part 9: Wanfang Data search history for EGFR alterations

(非小细胞 AND 肺 AND 癌) AND (Erlotinib OR Tarceva OR 伊诺替尼 OR 厄洛替尼 OR 埃罗替尼 OR 特罗凯 OR 它赛瓦 OR Gefitinib OR Iressa OR 吉非替尼 OR 易瑞沙) AND (表皮生长因子 AND 受体) AND (表达 OR 基因)

Part 10: Wanfang Data search history for *KRAS* mutations

(非小细胞 AND 肺 AND 癌) AND (Erlotinib OR Tarceva OR 伊诺替尼 OR 厄洛替尼 OR 埃罗替尼 OR 特罗凯 OR 它赛瓦 OR Gefitinib OR Iressa OR 吉非替尼 OR 易瑞沙) AND (KRAS OR “K-RAS” OR RAS) AND 突变

Appendix 2: Data Extraction Form

Date.....Reviewer: .....

Study Title.....

1. Bibliographic Information	
1.1 First author	
1.2 Year of publication	
1.3 Country of study	
1.4 Type of publication	Jrnl / Abstr / Other

2. Patients' characteristics	
2.1 Age (median and range)	
2.2 Sex (percentage of female)	
2.3 Ethnicity (percentage of Asian)	
2.4 Smoking status (percentage of never- or light-smokers)	
2.5 Eastern Cooperative Oncology Group or World Health Organization performance status score	
2.6 Stage	
2.7 Histology (percentage of adenocarcinoma)	

3. Treatment protocols: Intervention arm	
3.1 Line of treatment	
3.2 Drug(s) used	
3.3 Dose of drug(s)	
3.4 Frequency of administration	
3.5 Duration of treatment	
3.6 Regimens of prior chemotherapy, if any	
3.7 Prior surgery	Yes / No / Unclear
3.8 Prior radiotherapy	Yes / No / Unclear

4. Treatment protocols: Control arm	
4.1 Line of treatment	
4.2 Drug(s) used	
4.3 Dose of drug(s)	
4.4 Frequency of administration	
4.5 Duration of treatment	
4.6 Regimens of prior chemotherapy, if any	
4.7 Prior surgery	Yes / No / Unclear
4.8 Prior radiotherapy	Yes / No / Unclear

5. Biomarker analysis	
5.1 Types of tissue samples (e.g., Primary tumour /Metastasis tumour)	
5.2 Method for detecting *EGRF* mutations	
5.3 Exons of *EGRF* gene tested	
5.4 Rate of *EGRF* mutations	
5.5 Method for quantifying *EGFR* gene copy number	
5.6 Criteria for *EGFR* gene copy number gain	
5.7 Rate of *EGFR* gene copy number gain	
5.8 Method for detecting EGFR protein expression	
5.9 Criteria for EGFR protein expression	
5.10 Rate of EGFR protein expression	
5.11 Method for detecting *KRAS* mutations	
5.12 Exons of *KRAS* gene tested	
5.13 Rate of *KRAS* mutations	

6. Information related to the methodological characteristics	
6.1 Was the biomarker study prospective or retrospective?	
6.2 What was the percentage that the biomarker population accounted for the parent RCT population?	
6.3 Was the biomarker population representative of the parent trial population?	Yes / No / Unclear
6.4 Were the treatment and control arms comparable in the biomarker-positive population?	Yes / No / Unclear
6.5 Were the treatment and control arms comparable in the biomarker- negative population?	Yes / No / Unclear

6.6 Was the biomarker analysis blinded to treatment allocation and outcome?	Yes / No / Unclear
6.7 What was the magnitude of the cross-over of treatment and use of post-study therapy?	Negligible/ Moderate/ Significant
6.8 Was the hazard ratio analysis univariate or multivariate?	

7. Outcomes reported	
7.1 Progression-free survival	Yes / No
7.2 Overall survival	Yes / No
7.3 Response criteria	

8. Main results for mutant *EGFR* subgroup				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

9. Main results for wild-type *EGFR* subgroup				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

10. Main results for the subgroup with *EGFR* gene copy number gain				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

11. Main results for the subgroup without *EGFR* gene copy number gain				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

12. Main results for the subgroup with EGFR protein expression				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

13. Main results for the subgroup without EGFR protein expression				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

14. Main results for mutant *KRAS* subgroup				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

15. Main results for wild-type *KRAS* subgroup				
Outcome	Intervention arm	Control arm	Crude HR, 95% CI & *p*	Adjusted HR, 95% CI & *p*
Progression-free survival				
Overall survival				

Bibliography

Education Background

1. Doctor of Philosophy in Public Health, The Chinese University of Hong Kong, Hong Kong SAR, China, 2014 (GPA: 3.83/4.0)
2. Master of Medicine, Peking University, Beijing, China, 2010 (GPA: 3.67/4.0)
3. Bachelor of Medicine, Peking University, Beijing, China, 2008 (GPA: 3.34/4.0)

Research Interests

1. Evidence-based medicine
2. Systematic review and meta-analysis
3. Clinical research on predictive biomarkers in cancer treatment

Publications during PhD study

Summary

- Total: 24 (article: 19; correspondence/comment: 4; book chapter: 1)
- 1st-author: 12; 2nd- or 3rd-author: 12

Part 1: manuscripts published/accepted

1. **Yang ZY**, Wu XY, Huang YF, Di MY, Zheng DY, Chen JZ, Ding H, Mao C, Tang JL. Promising biomarkers for predicting the outcomes of patients with *KRAS* wild-type metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: A systematic review with meta-analysis. *Int J Cancer*. 2013;133:1914-25. [IF=6.198]
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9. **Yang ZY**, Yuan JY, Di MY, Zheng DY, Chen JZ, Ding H, Wu XY, Huang YF, Mao C, Tang JL. Gemcitabine plus erlotinib for advanced pancreatic cancer: a systematic review with meta-analysis. *PLoS One*. 2013;8:e57528. [IF=3.730]
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11. **Yang ZY**, Mao C, Tang JL. Apixaban versus enoxaparin in medically ill patients. *N Engl J Med.* 2012;366:767. [Correspondence/comment] [IF=51.658]
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Part 2: manuscripts under review (as of 21 January 2014)

19. **Yang ZY**, Di MY, Zheng DY, Mao C, Tang JL. Phosphorylated Akt as a prognostic biomarker in breast cancer: A meta-analysis of 35 studies with 10,094 patients. *Int J Cancer*. [IF=6.198]
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metastases: A systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev.* [IF=4.559]

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