Med Oncol (2015) 32:61
DOI 10.1007/s12032-015-0489-y

ORIGINAL PAPER

The efficacy of first-line chemotherapy is associated with KRAS mutation status in patients with advanced non-small cell lung cancer: a meta-analysis

Yaxiong Zhang · Wenfeng Fang · Yue Yan · Mengyao Wang · Shiyang Kang · Jin Sheng · Jianhua Zhan · Nan Chen · Shaodong Hong · Yunpeng Yang · Yuxiang Ma · Dacheng He · Tao Qin · Ting Zhou · Yanna Tang · Xiaobo He · Wenhua Liang · Li Zhang

Received: 26 December 2014 / Accepted: 23 January 2015 / Published online: 8 February 2015

Abstract Non-small cell lung cancer (NSCLC) patients harboring KRAS mutation were associated with worse prognosis and lower response to epidermal growth factor receptor (EGFR) target therapy than those with wild-type tumors. However, whether the underlying biological differences are associated with the efficacy of cytotoxic chemotherapy in advanced NSCLC patients remained controversial. We searched electronic databases for eligible literatures. The primary outcomes were objective response rate (ORR), 6-month and 1-year progression-free survival (PFS) rate. The pooled odds ratio (OR) was calculated using random-effect model. Subgroup analyses stratified by literature type, mutation analysis method, therapeutic regimen, patient origin, and EGFR mutation status in KRAS wild-type patients were proposed. Heterogeneity and publication bias were quantitatively evaluated. A total of ten studies involving 1,677 advanced NSCLC patients with known KRAS mutation status who had received first-line chemotherapy were included. KRAS mutants had lower ORR than wild-type patients (25.1 vs. 34.4 %) significantly (OR 0.67, 95 % CI 0.50–0.88, $P = 0.004$). Additionally, patients with KRAS mutation had numerically lower 6-month (51.0 vs. 56.8 %) and 1-year (10.3 vs. 13.3 %) PFS rate than wild-type patients, but there was no significant difference between the two groups (OR 0.75, 95 % CI 0.54–1.04, $P = 0.08$; OR 0.75, 95 % CI 0.47–1.21, $P = 0.25$). Results of the subgroup analyses were almost concordant with the overall ones. This comprehensive analysis revealed that advanced NSCLC patients with KRAS mutations had significantly lower ORR and potentially lower 6-month/1-year PFS rate compared with wild-type patients after first-line chemotherapy.

Keywords NSCLC · KRAS mutation · First-line chemotherapy · Meta-analysis

Abbreviations

NSCLC	Non-small cell lung cancer
EGFR	Epidermal growth factor receptor
ORR	Objective response rate
PFS	Progression-free survival
OR	Odds ratio
EML4	Echinoderm microtubule-associated protein-like 4
ALK	Anaplastic lymphoma kinase
EGFR-TKI	Tyrosine kinase inhibitors
EGFR	
ACT	Adjuvant chemotherapy

Yaxiong Zhang, Wenfeng Fang, Yue Yan and Mengyao Wang have contributed equally to this work.

Y. Zhang · W. Fang · Y. Yan · M. Wang · S. Kang · J. Sheng · J. Zhan · N. Chen · S. Hong · Y. Yang · Y. Ma · D. He · T. Qin · T. Zhou · Y. Tang · X. He
State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Sun Yat-sen University Cancer Center, Guangzhou, China

Y. Zhang · M. Wang · S. Kang · D. He
Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China

W. Liang (✉) · L. Zhang (✉)
Department of Medical Oncology, Sun Yat-sen University Cancer Center, State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, 651 Dongfeng Road East, Guangzhou 510060, Guangdong, People's Republic of China
e-mail: liangwh1987@163.com

L. Zhang
e-mail: zhangli6@mail.sysu.edu.cn

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) being the predominant form of the disease [1, 2]. The majority of patients are diagnosed at advanced stages for which few treatment options remain [3]. Although the effect of platinum-based doublet chemotherapy was insufficient, it was still a standard first-line treatment for advanced NSCLC in these years [4–7]. Additionally, NSCLC could be caused by the accumulation of genetic alterations, among which, the most common are Kirsten rat sarcoma viral oncogene (KRAS) mutations (22 %), epidermal growth factor receptor (EGFR) mutations (17 %), and anaplastic lymphoma kinase (ALK) rearrangement (7 %) [8]. EGFR tyrosine kinase inhibitors (EGFR-TKIs) were considered to be the first-line therapy for advanced NSCLC patients harboring EGFR exon 19 deletions and exon 21 L858R mutations [9–12]. Gene translocation involving ALK and echinoderm microtubule-associated protein-like 4 (EML4) of NSCLC revealed an extraordinary response to crizotinib [13, 14]. However, neither targeting drugs nor cytotoxic chemotherapy showed obvious curative effect in patients with KRAS mutation [15].

KRAS mutations, most frequently occur on exon 2 and exon 3, affect genes which encode protein harboring single amino acid substitutions primarily at residues G12, G13, or Q61 [16, 17]. The high prevalence of KRAS mutation made it an appealing biomarker for investigation, but its guidance for clinical medication was limited. NSCLC patients harboring KRAS mutation were associated with worse prognosis and lower response to EGFR target therapy including EGFR-TKIs and anti-EGFR monoclonal antibodies than those with wild-type tumors [18–24]. Nevertheless, the effect of KRAS mutation status on front-line chemotherapy response in advanced NSCLC was ambiguous. Several studies have found that chemo-naive patients with KRAS mutation had lower objective response rate (ORR) and/or shorter progression-free survival (PFS) following first-line treatment with conventional chemotherapy compared with wild-type patients [25, 26], although this has not been shown in all reports [27, 28].

Therefore, whether KRAS mutation status is associated with responsiveness to front-line chemotherapy in advanced NSCLC is still not clear. A comprehensive analysis of the various outcomes is warranted. Thus, we sought to perform a meta-analysis incorporating all available evidences to evaluate the clinical outcome according to the KRAS mutation status in patients with advanced NSCLC treated with front-line conventional chemotherapy.

Materials and methods

Literature search

All relevant articles were retrieved by searching PubMed, Embase, and the Central Registry of Controlled Trials of the Cochrane Library using a combination of the terms "KRAS," "Kirsten rat sarcoma viral oncogene," "mutation," "Lung," "non-small cell lung cancer," "NSCLC," and "chemotherapy." An additional search through Google Scholar and a manual search through reference lists of relevant reviews were additionally performed. Two authors (ZY and WM) carried out the search independently. No restriction by language or year was set in the search.

Inclusion and exclusion criteria

Eligible studies should meet the following criteria: (i) Random control trials (RCTs), prospective studies or retrospective studies which investigate or report a subset of chemo-naïve patients with local advanced or metastatic (IIIB or IV) NSCLC using cytotoxic chemotherapy without combination of other targeting drugs (e.g., anti-EGFR monoclonal antibodies); (ii) prior neoadjuvant or adjuvant chemotherapy in patients with recurrence after surgery was permitted if it had elapsed from last administration to relapse at least 6 months; (iii) KRAS mutation analysis was performed on available tumor tissue samples instead of circulating free DNA in serum in first-line chemotherapy treatment cohort; (iv) at least one of the primary outcomes was available. Studies failed to meet the inclusion criteria will be excluded.

Outcomes measures, data extraction, and quality assessment

Primary outcomes for this meta-analysis were ORR, namely partial response (PR) plus complete response (CR), 6-month PFS rate, and 1-year PFS rate. The data collection and assessment of methodological quality followed the QUORUM and the Cochrane Collaboration guidelines (http://www.cochrane.de). The data on study type, treatment regimens, KRAS mutation features, ORR, and PFS rate were extracted by two investigators (KS and FW) independently. Figures were electronically digitized, and Kaplan–Meier curves were downloaded by appropriate software (Engauge Digitizer, ver 2.12, Mark Mitchell 2002, free software down loaded from http://sourceforge.net). Two reviewers (YY and WX) used the Jadad scale to assess the quality of included RCTs and a modified Newcastle–Ottawa scale to assess other studies. Discrepancies were discussed by all investigators to reach consensus.

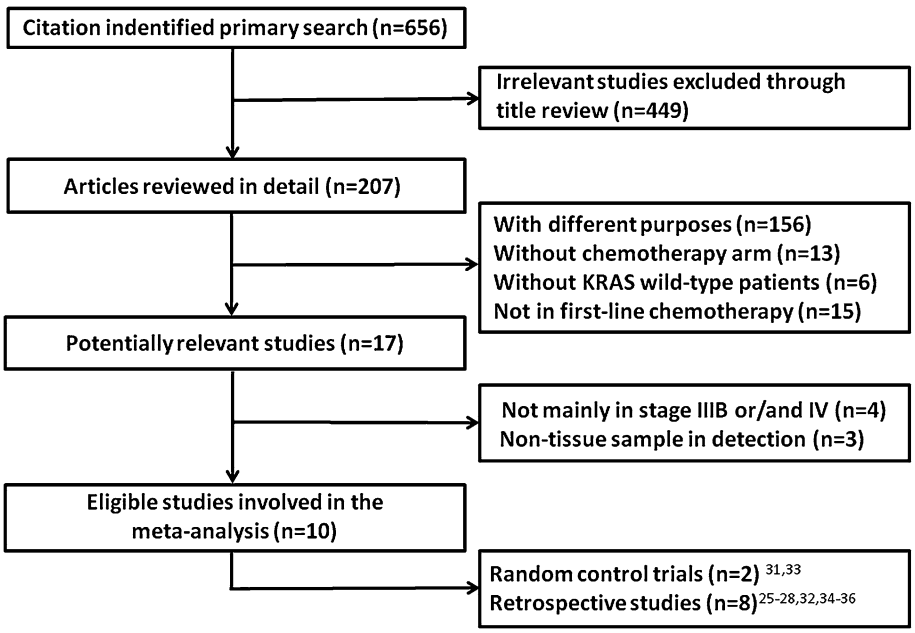

Fig. 1 Profile summarizing the trial flow

Statistical analysis

In consideration of any potential heterogeneity, we conducted this meta-analysis with a random-effect model. The results were reported as pooled odds radios (ORs) with the corresponding 95 % confidence interval (CI). Subgroup and sensitivity analysis were stratified for literature type, KRAS mutation analysis method, therapeutic regimen, patient origin, and EGFR mutation status in KRAS wild-type patients. An OR >1 reflected a better ORR or PFS rate in the KRAS mutant arm. Statistical heterogeneity across studies was assessed with a forest plot and the inconsistency statistic (I^2). Statistical significance was considered at $P < 0.05$. All calculations were performed using REVIEW MANAGER (version 5.0 for Windows; the Cochrane Collaboration, Oxford, UK).

Publication bias

An extensive search strategy was made to minimize the potential for publication bias. Graphical funnel plots were generated to visually assess a publication bias. The statistical methods to detect funnel plot asymmetry were the rank correlation test of Begg and Mazumdar and the regression asymmetry test of Egger [29, 30].

Results

Eligible studies

We identified 656 records according to the search strategy and focused on 17 potentially relevant studies [25–28, 31–43]. However, seven articles were not eligible for mutation detection in non-tissue sample [37–39] or involving patients at early stage (I to IIIA) [40–43]. We finally included ten studies [25–28, 31–36] (two RCTs and eight retrospective studies) involving 1,677 advanced NSCLC patients who had been tested for KRAS mutations in first-line chemotherapy treatment cohort. Figure 1 summarizes the flow chart. Among these studies, chemotherapy regimens were platinum-based doublets at standard dose, namely cisplatin/carboplatin plus one of the third-generation agents (including gemcitabine, paclitaxel, docetaxel, vinorelbine, and pemetrexed), or some non-platinum-based regimens. Regimens were not specific in four retrospective studies [25, 32, 35, 36] so that they were excluded in subgroup analysis stratified for therapeutic regimen. Detecting approaches for KRAS mutation included direct sequencing, nested polymerase chain reaction (PCR), real-time PCR (RT–PCR), which were also a subgrouping factor. Additionally, we considered time to progression (TTP) as PFS in studies by Eberhard [31] and Lee [25]. Table 1 summarizes the characteristics of all involved studies.

Objective response rate

According to all the literature with available data on 1,677 patients, KRAS mutants had lower ORR than wild-type patients (25.1 vs. 34.4 %) significantly (OR 0.67, 95 % CI, 0.50–0.88; $P = 0.004$; heterogeneity: $\chi^2 = 9.12$, $P = 0.43$, $I^2 = 1$ %; Fig. 2a). Subgroup analysis showed that data from retrospective studies (OR 0.67, 95 % CI, 0.49–0.92; $P = 0.01$; heterogeneity: $\chi^2 = 7.73$, $P = 0.36$, $I^2 = 9$ %) and those using PCR methods in mutation detecting (OR 0.58, 95 % CI, 0.37–0.89; $P = 0.01$;

Table 1 KRAS mutation as a predictive marker of first-line chemotherapy in advanced NSCLC

Lead author (year)	Origin of patients	Study category (phase)	Therapeutic regimen	Evaluable cases for KRAS mutation	KRAS mutation analysis method	KRAS exon identified as mutant (codon)	KRAS mutation status	ORR (%)	Six-month PFS rate[a] (%)	One-year PFS rate[a] (%)
KRAS (+) versus KRAS (−)&EGFR (−)										
Camidge [34]	USA	Retrospective	Pemetrexed + cisplatin/ carboplatin	18	Direct sequencing	2 (NA)	Positive	6/10 (60.0)	NA	NA
							Negative	1/8 (12.5)	NA	NA
Metro (2013)	Europe	Retrospective	Platinum-based regimen	204	Nested PCR	2,3 (12,13,61)	Positive	21/77 (27.3)	37/77 (48.1)	14/77 (18.2)
							Negative	54/127 (42.5)	73/127 (57.5)	31/127 (24.4)
Sun [26]	Korea	Retrospective	Pemetrexed + cisplatin/ carboplatin	152	Direct sequencing	2,3 (12,13,61)	Positive	4/16 (25.0)	NA	NA
							Negative	52/136 (38.2)	NA	NA
			Gemcitabine + cisplatin/ carboplatin	238			Positive	4/18 (22.2)	NA	NA
							Negative	88/220 (40.0)	NA	NA
			Taxane + cisplatin/ carboplatin	52			Positive	2/5 (40.0)	NA	NA
							Negative	23/47 (23.0)	NA	NA
KRAS (+) versus KRAS (−) & EGFR (NA)										
Eberhard [31]	USA	RCT (III)	Paclitaxel 200 mg/m^2 BSA, d1, q3w + carboplatin (AUC=6), d1, q3w×6 cycles	133	Nested PCR	2 (12,13)	Positive	7/30 (23.3)	12/30 (40.0)	3/30 (10.0)
							Negative	27/103 (26.2)	41/103 (39.8)	14/103 (13.6)
Khambata-Ford [33]	USA	RCT(III)	Paclitaxel 225mg/m^2 BSA or docetaxel 75mg/m^2 BSA,d1,q3w + carboplatin (AUC = 6) d1,q3w×6 cycles	104	Direct sequencing	2 (NA)	Positive	2/22 (9.1)	7/22 (31.8)	0/22 (-)
							Negative	21/82 (25.6)	30/82 (36.6)	7/82 (8.53)
Lee [25]	Korea	Retrospective	Platinum-based regimen	60	Direct sequencing	2 (12,13)	Positive	2/8 (25.0)	3/8 (37.5)	0/8 (-)
							Negative	20/52 (38.5)	28/52 (53.8)	10/52 (19.2)

Table 1 continued

Lead author (year)	Origin of patients	Study category (phase)	Therapeutic regimen	Evaluable cases for KRAS mutation	KRAS mutation analysis method	KRAS exon identified as mutant (codon)	KRAS mutation status	ORR (%)	Six-month PFS rate[a] (%)	One-year PFS rate[a] (%)
Kalikaki [32]	Greece	Retrospective	Platinum-based regimen	80	Direct sequencing	2 (12,13)	Positive	5/17 (29.4)	NA	NA
							Negative	19/63 (30.2)	NA	NA
			Non-platinum-based regimen	50			Positive	2/11 (18.2)	NA	NA
							Negative	8/39 (20.5)	NA	NA
O'Byrne [27]	International	Retrospective	Cisplatin + vinorelbine	196	RT–PCR	2 (12,13)	Positive	8/37 (21.6)	NA	NA
							Negative	54/159 (28.3)	NA	NA
Dong[28]	China	Retrospective	Gemcitabine + cisplatin	81	Direct sequencing	2 (12,13)	Positive	8/22 (36.4)	21/22 (95.5)	1/22 (4.55)
							Negative	18/59 (30.5)	48/59 (81.4)	3/59 (5.09)
			Docetaxel + cisplatin	77			Positive	7/22 (31.8)	20/22 (90.9)	2/22 (9.09)
							Negative	21/55 (38.2)	49/55 (89.1)	4/59 (6.87)
			Vinorelbine + cisplatin	71			Positive	6/20 (30.0)	16/20 (80.0)	4/20 (20.0)
							Negative	20/51 (39.2)	46/51 (90.2)	5/51 (9.80)
Mellema [35]	Netherlands	Retrospective	Platinum-based regimen	161	Direct sequencing	2,3 (12,13,61)	Positive	10/60 (16.7)	17/60 (28.3)	3/60 (5.00)
							Negative	22/101 (21.7)	43/101 (42.6)	10/101 (9.90)

AUC area under the concentration time curve; *BSA* body-surface area; *EGFR* epidermal growth factor receptor; *KRAS* Kirsten rat sarcoma viral oncogene; *NA* not available; *NSCLC* non-small cell lung cancer; *ORR* objective response rate; *PCR* polymerase chain reaction; *PFS* progression-free survival; *RCT* random control trial; *RT–PCR* real-time PCR

[a] We considered time to progression (TTP) as PFS in studies of Eberhard and Lee

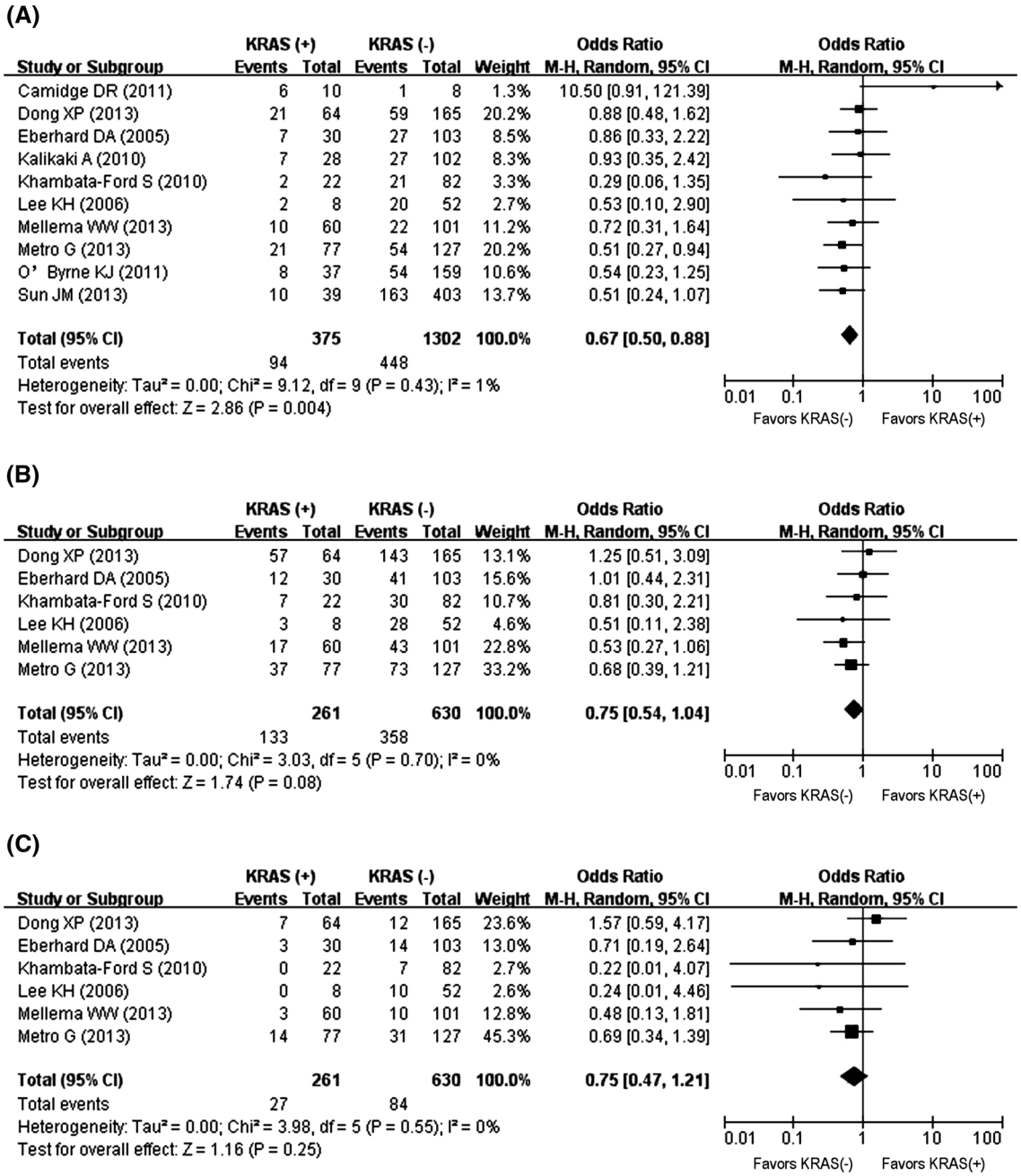

Fig. 2 Meta-analysis on following outcomes among advanced NSCLC patients receiving first-line chemotherapy according to KRAS mutation status (**a** ORR; **b** 6-month PFS rate; **c** 1-year PFS rate) *CI* confidence interval; *NSCLC* non-small cell lung cancer; *ORR* objective response rate; *PFS* progression-free survival

heterogeneity: $\chi^2 = 0.86$, $P = 0.65$, $I^2 = 0$ %) had similar tendency of significantly inferior ORR in KRAS mutants, while no significant difference in terms of ORR between the mutant group and wild-type group if stratified by therapeutic regimen (pemetrexed-based vs. gemcitabine-based vs. taxane-based vs. vinorelbine-based regimens and carboplatin-based vs. cisplatin-based regimens), patient origin (Asia vs. Non-Asia area), and EGFR mutation status in KRAS wild-type patients (EGFR mutation negative vs. not available for EGFR mutation) (Table 2).

Six-month and one-year PFS rate

Data on PFS rate were available only in 891 patients enrolled in six trials. Patients with positive KRAS mutation had lower pooled 6-month (51.0 vs. 56.8 %) and 1-year

Table 2 Subgroup analysis on objective response rate among advanced NSCLC patients receiving first-line chemotherapy according to KRAS mutation status

Categories of included studies	Number of included studies	Objective response rate (event/total)		Test of heterogeneity			Test of effect size	
		KRAS mutation positive	KRAS mutation negative	χ^2	*P* value	I^2 (%)	OR (95 % CI)	*P* value
Total	10	94/375	448/1,302	9.12	0.43	1	0.67 (0.50–0.88)	0.004
Literature type								
Random control trial	2	9/52	48/185	1.40	0.24	29	0.59 (0.21–1.63)	0.31
Retrospective study	8	85/323	400/1,117	7.73	0.36	9	0.67 (0.49–0.92)	0.01
KRAS mutation analysis method								
Direct sequencing	7	58/231	313/913	7.57	0.27	21	0.73 (0.48–1.11)	0.14
PCR[a]	3	36/144	135/389	0.86	0.65	0	0.58 (0.37–0.89)	0.01
Therapeutic regimen								
Pemetrexed with platinum	2	10/26	53/144	4.62	0.03	78	1.95 (0.11–35.27)	0.65
Gemcitabine with platinum	2	12/40	106/279	2.01	0.16	50	0.77 (0.26–2.29)	0.64
Taxane with platinum	4	18/79	92/287	1.46	0.69	0	0.68 (0.37–1.25)	0.21
Vinorelbine with platinum	2	14/57	74/210	0.09	0.76	0	0.58 (0.30–1.14)	0.11
Therapeutic regimen								
Carboplatin-based regimen	2	9/52	48/185	1.40	0.24	29	0.59 (0.21–1.63)	0.31
Cisplatin-based regimen	2	29/101	113/324	0.85	0.36	0	0.74 (0.45–1.22)	0.24
Patient origin								
Asia	3	33/111	242/620	1.33	0.51	0	0.69 (0.44–1.09)	0.11
Non-Asia area	7	61/264	206/682	7.75	0.26	23	0.67 (0.44–1.01)	0.06
EGFR mutation status in KRAS wild-type patients								
KRAS (−)&EGFR(−)	3	37/126	218/538	5.72	0.06	65	0.72 (0.28–1.86)	0.50
KRAS (−)&EGFR(NA)	7	57/249	230/764	2.73	0.84	0	0.73 (0.52–1.03)	0.07

CI confidence interval; *EGFR* epidermal growth factor receptor; *KRAS* Kirsten rat sarcoma viral oncogene; *NA* not available; *NSCLC* non-small cell lung cancer; *OR* odds radio; *PCR* polymerase chain reaction; *RT–PCR* real-time PCR

[a] PCR methods included nested PCR or RT–PCR

(10.3 vs. 13.3 %) PFS rate than wild-type patients, but there was no significant difference between the two groups (6-month PFS rate: OR 0.75, 95 % CI, 0.54–1.04; $P = 0.08$; heterogeneity: $\chi^2 = 3.03$, $P = 0.70$, $I^2 = 0$ %; Fig. 2b and 1-year PFS rate: OR 0.75, 95 % CI, 0.47–1.21; $P = 0.25$; heterogeneity: $\chi^2 = 3.98$, $P = 0.55$, $I^2 = 0$ %; Fig. 2c). Subgroup analysis stratified by literature type, mutation analysis method, and patient origin consistently revealed no significant difference between the mutant group and wild-type group (Tables 3, 4).

Assessment of heterogeneity and publication bias

As described above, the statistical heterogeneity was moderate. In addition, sensitivity analysis by leaving any study out did not alter the tendency of the general results. There was no publication bias for outcome measures, with asymmetrical appearance on funnel plot analysis (Fig. 3) and all *P* values >0.05 in Begg's test and Egger's test.

Discussion

Previous pooled analysis of the prognostic and predictive effects of KRAS mutation status in early-stage resected NSCLC after cisplatin-based adjuvant chemotherapy (ACT) revealed that there was no significant difference in overall survival (OS) and disease-free survival (DFS) based on KRAS. Moreover, patients with tumors harboring codon 12 mutations seemed to derive no benefit from ACT, whereas the presence of codon 13 mutations was associated with significantly worse OS and DFS with ACT [44]. However, whether KRAS mutation could predict the efficacy of first-line chemotherapy in advanced NSCLC was controversial based on previous small-size reports. A meta-analysis that could incorporate all available results, including subgroup data from RCTs as well, was a good way to address our concerns.

In the current study, we found that ORR was significantly higher in wild-type patients than KRAS mutants after first-line chemotherapy. In addition, similar results

Table 3 Subgroup analysis on 6-month PFS rate among advanced NSCLC patients receiving first-line chemotherapy according to KRAS mutation status

Categories of included studies	Number of included studies	Six-month PFS rate (event/total)		Test of heterogeneity			Test of association	
		KRAS mutation positive	KRAS mutation negative	χ^2	*P* value	I^2 (%)	OR (95 % CI)	*P* value
Total	6	133/261	358/630	3.03	0.70	0	0.75 (0.54–1.04)	0.08
Literature type								
Random control trial	2	19/52	71/185	0.11	0.74	0	0.92 (0.49–1.75)	0.80
Retrospective study	4	114/209	287/445	2.35	0.50	0	0.69 (0.47–1.02)	0.06
KRAS mutation analysis method								
Direct sequencing	4	84/154	244/400	2.41	0.49	0	0.72 (0.46–1.14)	0.16
PCR[a]	2	49/107	114/230	0.57	0.45	0	0.77 (0.48–1.24)	0.29
Patient origin								
Asia	2	60/72	171/217	0.96	0.33	0	1.00 (0.46–2.17)	0.99
Non-Asia area	4	73/189	187/413	1.43	0.70	0	0.70 (0.49–1.01)	0.06

CI confidence interval; *EGFR* epidermal growth factor receptor; *KRAS* Kirsten rat sarcoma viral oncogene; *NSCLC* non-small cell lung cancer; *OR* odds radio; *PCR* polymerase chain reaction; *PFS* progression-free survival

[a] PCR method was nested PCR

Table 4 Subgroup analysis on 1-year PFS rate among advanced NSCLC patients receiving first-line chemotherapy according to KRAS mutation status

Categories of included studies	Number of included studies	One-year PFS rate (event/total)		Test of heterogeneity			Test of association	
		KRAS mutation positive	KRAS mutation negative	χ^2	*P* value	I^2 (%)	OR (95 % CI)	*P* value
Total	6	27/261	84/630	3.98	0.55	0	0.75 (0.47–1.21)	0.25
Literature type								
Random control trial	2	3/52	21/185	0.52	0.47	0	0.58 (0.17–1.93)	0.37
Retrospective study	4	24/209	63/445	3.23	0.36	7	0.79 (0.46–1.38)	0.41
KRAS mutation analysis method								
Direct sequencing	4	10/154	39/400	3.88	0.27	23	0.74 (0.29–1.88)	0.53
PCR[a]	2	17/107	45/230	0.00	0.97	0	0.69 (0.37–1.29)	0.25
Patient origin								
Asia	2	7/72	22/217	1.51	0.22	34	1.01 (0.20–5.02)	0.99
Non-Asia area	4	20/189	62/413	0.75	0.86	0	0.62 (0.36–1.08)	0.09

CI confidence interval; *EGFR* epidermal growth factor receptor; *KRAS* Kirsten rat sarcoma viral oncogene; *NSCLC* non-small cell lung cancer; *OR* odds radio; *PCR* polymerase chain reaction; *PFS* progression-free survival

[a] PCR method was nested PCR

were found for PFS but the difference did not reach significance. An interesting in vitro study showed different sensitivity for specific chemotherapy among wild-type and KRAS-overexpressing clones from the human NSCLC cell line [45]. Nevertheless, no significant difference was found in ORR if stratified for therapeutic regimen as above research [45] in subgroup analysis. Therefore, more efforts should be made to evaluate the curative effect of specific chemotherapy in NSCLC patients harboring different types of KRAS mutation as well as those in wild type.

Subgroup analysis revealed significant difference in terms of ORR between the mutant group and wild-type group in data from retrospective studies and those using PCR methods in mutation detecting. The above results admit of following interpretations. Firstly, the sample size was too small in RCT cohort to get the significant results. Secondly, the commercial real-time PCR kit could provide greater sensitivity than direct sequencing to detect KRAS mutations so that it might avoid loss of positive detection to some degree [46]. As a result, the lower efficacy of chemotherapy in KRAS-mutated chemo-naïve patients with advanced NSCLC could be demonstrated obviously in PCR cohort.

Notably, this is the first study to comprehensively answer the impact of KRAS mutation on chemotherapy in

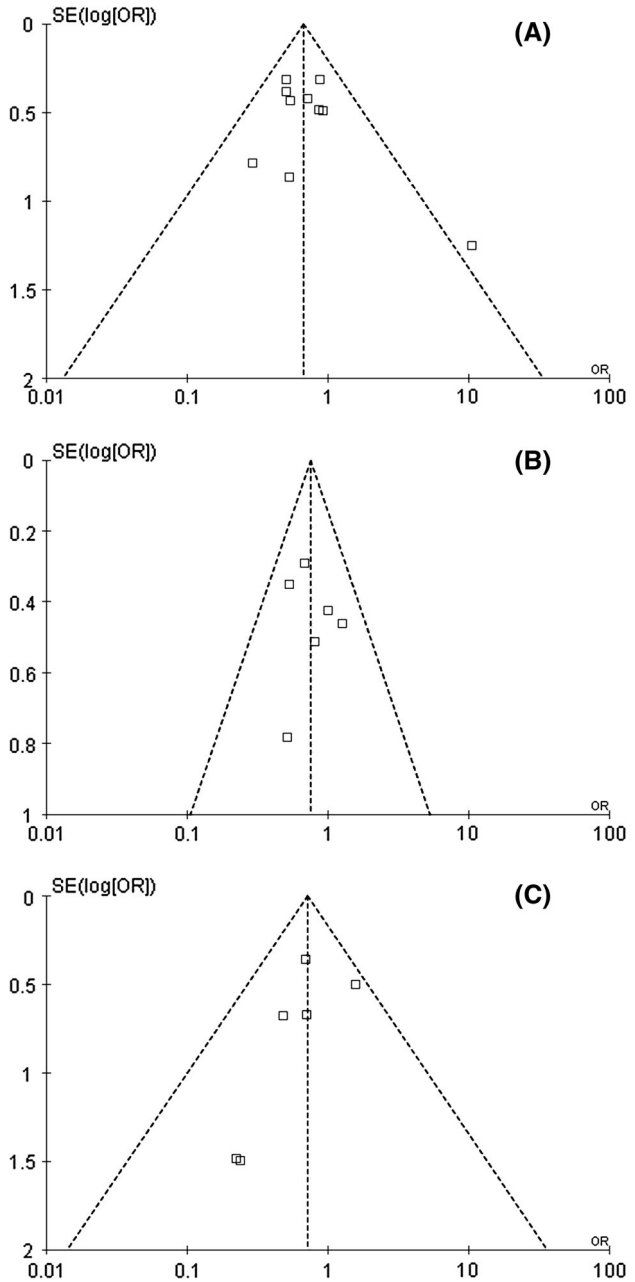

Fig. 3 Funnel plots of SE by Log OR (**a** ORR; **b** 6-month PFS rate; **c** 1-year PFS rate). *OR* odds ratios; *ORR* objective response rate; *PFS* progression-free survival; *SE* standard error

advanced NSCLC, which only focused on first-line chemotherapy without combination of other target treatments in order to minimize the crossover effects. However, there existed several limitations. First, our meta-analysis was based on subgroup data extracted from RCTs and retrospective studies, which somehow compromised the evidence level. Besides, more than half of included studies could not exclude the occurrence of EGFR mutation in KRAS wild-type cohort. Additionally, KRAS exons identified as mutant were heterogeneous among included articles, but we were unable to assess whether 2 or 3 exon alterations, as well as different codons' mutations, had different impact on chemotherapy. Finally, we cannot differentiate the respective impact of KRAS mutation on cell cycle-specific agents (third-generation agents) and non-specific antineoplastic agents (platinum).

Nonetheless, regardless of above limitations, this comprehensive analysis confirmed the association between KRAS mutation status and ORR in advanced NSCLC. The result led to an important hint that the response to chemotherapy in KRAS-mutated patients was worse than what we acknowledged. The combination of chemotherapy and target therapy might be superior toward KRAS mutants in ongoing clinical trials compared with chemotherapy alone. Encouragingly, a randomized phase II study reported selumetinib, a selective inhibitor of mitogen-activated protein kinase kinase (MEK), in combination with docetaxel had a significant improvement in ORR and PFS compared with docetaxel and placebo in the second-line treatment of advanced NSCLC patients with KRAS mutation [47]. Similar combination therapy in chemo-naïve KRAS mutant patients with NSCLC is warranted in further studies.

In conclusion, this meta-analysis showed that advanced NSCLC patients with KRAS mutations had significantly lower ORR and potentially lower 6-month/1-year PFS rate compared with wild-type patients after first-line chemotherapy.

Acknowledgments This study was supported by the following funds: (1) National High Technology Research and Development Program of China (Grant No. 2012AA02A502). (2) Innovative drug R&D center based on real-time high-throughput cell-based screening platform and large capacity compound library (Grant No. 2013ZX09401003-002). (3) National Natural Science Funds of China (Grant No. 81372502). (4) Wu Jieping Medical Foundation Project (Grant No. 320.6750.131). All the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest The authors have declared no conflicts of interest.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. Global cancer statistics. CA Cancer J Clin. 2011;61:69–90.
2. Ettinger DS, Akerley W, Bepler G, Blum MG, Chang A, et al. Non–small cell lung cancer. J Natl Compr Canc Netw. 2010;8:740–801.
3. Wakelee H, Belani CP. Optimizing first-line treatment options for patients with advanced NSCLC. Oncologist. 2005;10:1–10.
4. Scagliotti GV, De Marinis F, Rinaldi M, Crinò L, Gridelli C, et al. Phase III randomized trial comparing three platinum-based doublets in advanced non–small-cell lung cancer. J Clin Oncol. 2002;20:4285–91.

5. Pfister DG, Johnson DH, Azzoli CG, Sause W, Smith TJ, et al. American society of clinical oncology treatment of unresectable non–small-cell lung cancer guideline: update 2003. J Clin Oncol. 2004;22:330–53.
6. Pallis AG, Georgoulias V. Is there a standard regimen for first-line treatment of advanced/metastatic Non–Small-Cell Lung Cancer? what has meta-analyses contributed to today's standard of care. Lung Cancer. 2012;75:269–74.
7. Sörenson S, Glimelius B, Nygren P. A systematic overview of chemotherapy effects in non–small cell lung cancer. Acta Oncol. 2001;40:327–39.
8. Kris MG, Johnson BE, Kwiatkowski DJ, Iafrate AJ, Wistuba II, et al. Identification of driver mutations in tumor specimens from 1,000 patients with lung adenocarcinoma: the NCI's lung cancer mutation consortium (LCMC). J Clin Oncol. 2011;29:CRA7506.
9. Rossi A, Pasquale R, Esposito C, Normanno N. Should epidermal growth factor receptor tyrosine kinase inhibitors be considered ideal drugs for the treatment of selected advanced non–small cell lung cancer patients? Cancer Treat Rev. 2013;39:489–97.
10. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, et al. Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non–small-cell lung cancer (OPTIMAL, CTONG-0802): a multicentre, open-label, randomised, phase 3 study. Lancet Oncol. 2011;12:735–42.
11. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. N Engl J Med. 2004;350:2129–39.
12. Keedy VL, Temin S, Somerfield MR, Beasley MB, Johnson DH, et al. American society of clinical oncology provisional clinical opinion: epidermal growth factor receptor (EGFR) mutation testing for patients with advanced non–small-cell lung cancer considering first-line EGFR tyrosine kinase inhibitor therapy. J Clin Oncol. 2011;29:2121–7.
13. Cufer T, Ovcaricek T, O'Brien ME. Systemic therapy of advanced non–small cell lung cancer: major-developments of the last 5-years. Eur J Cancer. 2013;49:1216–25.
14. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non–small-cell lung cancer. N Engl J Med. 2010;363:1693–703.
15. Roberts PJ, Stinchcombe TE, Der CJ, Socinski MA. Personalized medicine in non–small-cell lung cancer: Is KRAS a useful marker in selecting patients for epidermal growth factor receptor-targeted therapy? J Clin Oncol. 2010;28:4769–77.
16. Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer Res. 2002;62:6997–7000.
17. Karachaliou N, Mayo C, Costa C, Magrí I, Gimenez-Capitan A, et al. KRAS mutations in lung cancer. Clin Lung Cancer. 2013;14:205–14.
18. Roberts PJ, Stinchcombe TE. KRAS mutation: Should we test for it, and does it matter? J Clin Oncol. 2013;31:1112–21.
19. Mascaux C, Iannino N, Martin B, Paesmans M, Berghmans T, et al. The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. Br J Cancer. 2005;92:131–9.
20. Slebos RJ, Kibbelaar RE, Dalesio O, Kooistra A, Stam J, et al. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med. 1990;323:561–5.
21. Meng D, Yuan M, Li X, Chen L, Yang J, et al. Prognostic value of K-RAS mutations in patients with non–small cell lung cancer: a systematic review with meta-analysis. Lung Cancer. 2013;81:1–10.
22. Linardou H, Dahabreh IJ, Kanaloupiti D, Siannis F, Bafaloukos D, et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non–small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol. 2008;9:962–72.
23. Mao C, Qiu LX, Liao RY, Du FB, Ding H, et al. KRAS mutations and resistance to EGFR-TKIs treatment in patients with non–small cell lung cancer: a meta-analysis of 22 studies. Lung Cancer. 2010;69:272–8.
24. Maus MKH, Grimminger PP, Mack PC, Astrow SH, Stephens C, et al. KRAS mutations in non–small-cell lung cancer and colorectal cancer: implications for EGFR-targeted therapies. Lung Cancer. 2014;83:163–7.
25. Lee KH, Han SW, Hwang PG, Oh DY, Kim DW, et al. Epidermal growth factor receptor mutations and response to chemotherapy in patients with non–small-cell lung cancer. Jpn J Clin Oncol. 2006;36:344–50.
26. Sun JM, Hwang DW, Ahn JS, Ahn MJ, Park K. Prognostic and predictive value of KRAS mutations in advanced non–small cell lung cancer. PLoS One. 2013;8:e64816.
27. O'Byrne KJ, Gatzemeier U, Bondarenko I, Barrios C, Eschbach C, et al. Molecular biomarkers in non–small-cell lung cancer: a retrospective analysis of data from the phase 3 FLEX study. Lancet Oncol. 2011;12:795–805.
28. Dong X, Zhao X, Hao Y, Wei Y, Yin Q, et al. Response to first-line chemotherapy in patients with non–small-cell lung cancer according to epidermal growth factor receptor and K-RAS mutation status. Clin lung Cancer. 2013;14:680–7.
29. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ. 1997;315:629–34.
30. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics. 1994;50:1088–101.
31. Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non–small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol. 2005;23:5900–9.
32. Kalikaki A, Koutsopoulos A, Hatzidaki D, Trypaki M, Kontopodis E, et al. Clinical outcome of patients with non–small cell lung cancer receiving front-line chemotherapy according to EGFR and K-RAS mutation status. Lung Cancer. 2010;69:110–5.
33. Khambata-Ford S, Harbison CT, Hart LL, Awad M, Xu LA, et al. Analysis of potential predictive markers of cetuximab benefit in BMS099, a phase III study of cetuximab and first-line taxane/carboplatin in advanced non–small-cell lung cancer. J Clin Oncol. 2010;28:918–27.
34. Camidge DR, Kono SA, Lu X, Okuyama S, Barón AE, et al. Anaplastic lymphoma kinase gene rearrangements in non–small cell lung cancer are associated with prolonged progression-free survival on pemetrexed. J Thorac Oncol. 2011;6:774.
35. Mellema WW, Dingemans AMC, Thunnissen E, Snijders PJ, Derks J, et al. KRAS mutations in advanced nonsquamous non–small-cell lung cancer patients treated with first-line platinum-based chemotherapy have no predictive value. J Thorac Oncol. 2013;8:1190–5.
36. Metro G, Chiari R, Bennati C, Cenci M, Ricciuti B, et al. Clinical outcome with platinum-based chemotherapy in patients with advanced nonsquamous EGFR wild type non–small-cell lung cancer segregated according to KRAS mutation status. Clin Lung Cancer. 2014;15:86–92.
37. Kim ST, Sung JS, Jo UH, Park KH, Shin SW, et al. Can mutations of EGFR and KRAS in serum be predictive and prognostic markers in patients with advanced non–small cell lung cancer (NSCLC)? Med Oncol. 2013;30:1–10.
38. Nygaard AD, Garm Spindler KL, Pallisgaard N, Andersen RF, Jakobsen A. The prognostic value of KRAS mutated plasma

DNA in advanced non–small cell lung cancer. Lung Cancer. 2013;79:312–7.
39. Camps C, Jantus-Lewintre E, Cabrera A, Blasco A, et al. The identification of KRAS mutations at condon 12 in plasma DNA is not a prognostic factor in advanced non–small cell lung cancer patients. Lung Cancer. 2011;72:365–9.
40. Guan J, Zhong WZ, An SJ, Yang JJ, Su J, et al. KRAS mutation in patients with lung cancer: a predictor for poor prognosis but not for EGFR-TKIs or chemotherapy. Ann Surg Oncol. 2013; 20:1381–8.
41. Wang S, An T, Duan J, Zhang L, Wu M, et al. Alterations in EGFR and related genes following neo-adjuvant chemotherapy in Chinese patients with non–small cell lung cancer. PLoS One. 2013;8:e51021.
42. Villaruz LC, Socinski MA, Cunningham DE, Chiosea SI, et al. The prognostic and predictive value of kras oncogene substitutions in lung adenocarcinoma. Cancer. 2013;119:2268–74.
43. Macerelli M, Caramella C, Faivre L, Besse B, Planchard D, et al. Does KRAS mutational status predict chemoresistance in advanced non–small cell lung cancer (NSCLC)? Lung Cancer. 2014;83:383–8.
44. Shepherd FA, Domerg C, Hainaut P, Jänne PA, Pignon JP, et al. Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non–small-cell lung cancer in four trials of adjuvant chemotherapy. J Clin Oncol. 2013;31:2173–81.
45. Garassino MC, Marabese M, Rusconi P, Rulli E, Martelli O, et al. Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non–small-cell lung cancer. Ann Oncol. 2011;22:235–7.
46. Angulo B, García-García E, Martínez R, Suárez-Gauthier A, et al. A commercial real-time PCR kit provides greater sensitivity than direct sequencing to detect KRAS mutations: a morphology-based approach in colorectal carcinoma. J Mol Diagn. 2010; 12:292–9.
47. Janne PA, Shaw AT, Pereira JR, Jeannin G, Vansteenkiste J, et al. Phase II double-blind, randomized study of selumetinib (SEL) plus docetaxel (DOC) versus DOC plus placebo as second-line treatment for advanced KRAS mutant non–small cell lung cancer (NSCLC). J Clin Oncol 2012; 30:480s (suppl 15; abstr 7503).