RESEARCH ARTICLE

Predictive and Prognostic Biomarkers for Patients Treated with Anti-EGFR Agents in Lung Cancer: A Systemic Review and Meta-Analysis

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Abstract

Background: Several studies have investigated predictive and prognostic biomarkers for patients treated with anti-epidermal growth factor receptor (EGFR) agents in lung cancer. However, the conclusion is controversial. Materials and Methods: A meta-analysis was conducted to evaluate the associations of mutant K-ras, PIK3CA and PTEN deficiency with the efficacy of anti-EGFR agents in lung cancer. The primary endpoint was objective response rate (ORR). The secondary endpoints were overall survival (OS) and progression-free survival (PFS). Results: A total of 61 studies were included in the final meta-analysis. The result showed that K-ras mutation was a good predictor for ORR (RR=0.42, 95% CI, 0.33-0.55, *p*=0.000) and an effective prognostic marker for OS (HR=1.37, 95% CI, 1.15-1.65, *p*=0.001) and PFS (HR=1.33, 95% CI, 1.05-1.69, *p*=0.019). However, PTEN deficiency or PIK3CA mutation did not show any significance predictive value for ORR (PTEN, RR=0.82, 95% CI, 0.56-1.19, *p*=0.286; PIK3CA, RR=1.08, 95% CI, 0.17-6.66, P=0.938). And PTEN deficiency or expression of PIK3CA did not show significance prognostic value for OS (PTEN, HR=0.88, 95% CI, 0.31-2.46,P=0.805; PIK3CA, HR=0.79, 95% CI: 0.23-2.68, P=0.706). Conclusions: Our meta-analysis showed that K-ras mutation may be an effective predictor in lung cancer patients treated with anti-EGFR agents. Whereas, the predictive and prognostic value of PTEN deficiency and PIK3CA mutation need to be further investigated.

Keywords: Hung cancer - K-ras mutation - loss of PTEN - PIK3CA mutation - anti-EGFR agents

Asian Pac J Cancer Prev, **16 (11)**, 4759-4768

Introduction

Lung cancer is the leading cause of cancer-related death in both sexes worldwide (Siegel et al., 2013). Although the therapeutic options have made great progress for these patients, the overall outcome remains poor. Since the standard chemotherapy for non-small-cell lung cancer (NSCLC), platinum-based doublet chemotherapy, has reached an efficacy plateau, anti-epidermal growth factor receptor (EGFR) agents became a standard optional for advanced lung cancer (Dingemans et al., 2011; Guan et al., 2013). As most kinds of targeted agents, the clinicians need to find effective biomarkers to identify appreciate patients. Although EGFR mutation has been shown as a promising biomarker for lung cancer patients treated with anti-EGFR agents, there exists 20% patients with EGFR mutations do not respond to anti-EGFR agents and 10% patients without EGFR mutations response to anti-EGFR-agents (Hirsch et al., 2007; Zucali et al., 2008). To identify patients who may benefit from the treatment of anti-EGFR agents, convenient predictors urgently need to be identified.

K-ras plays a vital role in the downstream signaling network of EGFR and the mutations are common at codon12, codon13 of exon2 (Qi et al., 2012; Tong et al., 2012; Pan et al., 2013; Fang et al., 2014). Since EGFR and K-ras function sequentially in MAPK signaling pathway, it is redundant to activate both gene mutations (Eberhard et al., 2005). Several studies have investigated the association of K-ras mutation and therapeutic responses to anti-EGFR agents. However, the conclusion is controversial (Hirsch et al., 2007; Khambata-Ford et al., 2010; Sun et al., 2012).

PIK3CA mutation and PTEN deficiency happen in the PI3K/PTEN/AKT pathway (Ludovini et al., 2012). PIK3CA mutations corresponding somatic mutations mainly are missense mutations clustering in exons 9 and 20 that encode a part of the helical and kinase domains, respectively (Chaft et al., 2012). PTEN, as a non-redundant lipid phosphatase, can prevent tumorigenesis and regulate a plethora of cellular processes (Endoh et al., 2006; Fidler et al., 2011; Ludovini et al., 2012). It has been reported that high expression of PTEN has longer survival in NSCLC patients, but the responsiveness to anti-EGFR agents is unclear (Endoh et al., 2006).

To elucidate the relationships between mutation of K-ras, PIK3CA and PTEN with outcomes of anti-EGFR

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agents in lung cancer patients, we performed the meta-analysis with up-to-date data.

Materials and Methods

Publication search

We carried out a comprehensive systematic search of PubMed, Cochrane databases and EMBASE (up to September 08, 2014). The following search key words were used: "erlotinib", "gefitinib", "lapatinib", "afatinib", "TKI", "Tryrosine-kinase inhibition", "monoclonal antibodies", "MoAb", "cetuximab", "panitumumab", "biomarker", "K-ras", "PTEN", "PIK3CA", "lung cancer", "non-small cell lung cancer", "NSCLC". There are no restrictions on the types of studies and only publications published by English were included. The bibliographies of eligible studies were searched by hand for other relevant articles.

Inclusion criteria

The inclusion criteria to obtain eligible studies: *i*) evaluating the K-ras mutations, loss of PTEN expression, PIK3CA mutations status and response to anti-EGFR agents in lung cancer; *ii*) sufficient data on ORR, OS, PFS stratified by corresponding mutation status; *iii*) studies with full text articles; *iv*)when the same author or group reported results obtained from the same patient population in several publications, only the most recent report or most informative one was included; Studies included in our analysis are assessed by two reviewers. When it came to discrepancies, they decided to include or exclude studies after joint review.

Statistical analysis

The primary endpoint was ORR and the secondary endpoints were OS and PFS. The ORR was defined as the sum of partial response (PR) and complete response (CR). We conducted heterogeneity analysis to find an appropriate calculation model. Heterogeneity assumption was assessed by the I^2 statistic. An $I^2 \geq 50\%$ indicated a lack of between-study heterogeneity (JP et al., 2003) and directed the analysis to be conducted in a fixed-effects model. Meta-regression was performed to find the source of heterogeneity. Sensitivity analyses were conducted by removing one study each time (Yang et al., 2013). Potential publication bias was evaluated by Egger's funnel plots and if the funnel plot showed asymmetry, it suggested a possible publication bias. And $p \leq 0.05$ used to assess the heterogeneity suggested statistically significantly bias in two-tailed level. All the statistical tests were performed with Stata 12.0 software.

Results

Studies characteristics

A total of 61 (Eberhard et al., 2005; Fujimoto et al., 2005; Pao et al., 2005; Endoh et al., 2006; Giaccone et al., 2006; Han et al., 2006; Hirsch et al., 2006; Cappuzzo et al., 2007; Hirsch et al., 2007; Ichihara et al., 2007; Jackman et al., 2007; Loprevite et al., 2007; Massarelli et al., 2007; van Zandwijk et al., 2007; Chang et al., 2008; D'Addario et al., 2008; Felip et al., 2008; Kalikaki et al., 2008; Miller et al., 2008; Sasaki et al., 2008; Schneider et al., 2008; Wu et al., 2008; Zucali et al., 2008; Boldrini et al., 2009; Jackman et al., 2009; Lara-Guerra et al., 2009; M et al., 2009; Marchetti et al., 2009; Pesek et al., 2009; Schittenhelm et al., 2009; Douillard et al., 2010; Khambata-Ford et al., 2010; Lind et al., 2010; Price et al., 2010; Ready et al., 2010; Tiseo et al., 2010; Wang et al., 2010; ZHU Yu-jia et al., 2010; Brugger et al., 2011; Dahabreh et al., 2011; Dingemans et al., 2011; Fidler et al., 2011; Hirsch et al., 2011; Ludovini et al., 2011; O'Byrne et al., 2011; Sequist et al., 2011; Spigel et al., 2011; Zhao et al., 2011; Cadranel et al., 2012; Ludovini et al., 2012; Metro et al., 2012; Milella et al., 2012; Murray et al., 2012; Ramalingam et al., 2012; Sun et al., 2012; Guan et al., 2013; Johnson et al., 2013; Karampeazis et al., 2013; Kerner et al., 2013; Kim et al., 2013; Tsao et al., 2013) were included (shown in Figure 1). Among the 63 studies, 60 (Eberhard et al., 2005; Fujimoto et al., 2005; Pao et al., 2005; Giaccone et al., 2006; Han et al., 2006; Hirsch et al., 2006; Cappuzzo et al., 2007; Hirsch et al., 2007; Ichihara et al., 2007; Jackman et al., 2007; Loprevite et al., 2007; Massarelli et al., 2007; van Zandwijk et al., 2007; Chang et al., 2008; D'Addario et al., 2008; Felip et al., 2008; Kalikaki et al., 2008; Miller et al., 2008; Sasaki et al., 2008; Schneider et al., 2008; Wu et al., 2008; Zucali et al., 2008; Boldrini et al., 2009; Jackman et al., 2009; Lara-Guerra et al., 2009; M et al., 2009; Marchetti et al., 2009; Pesek et al., 2009; Schittenhelm et al., 2009; Douillard et al., 2010; Khambata-Ford et al., 2010; Lind et al., 2010; Price et al., 2010; Ready et al., 2010; Tiseo et al., 2010; Wang et al., 2010; ZHU Yu-jia et al., 2010; Brugger et al., 2011; Dahabreh et al., 2011; Dingemans et al., 2011; Hirsch et al., 2011; Kim et al., 2011; Ludovini et al., 2011; O'Byrne et al., 2011; Sequist et al., 2011; Spigel et al., 2011; Zhao et al., 2011; Cadranel et al., 2012; Ludovini et al., 2012; Metro et al., 2012; Milella et al., 2012; Murray et al., 2012; Ramalingam et al., 2012; Sun et al., 2012; Guan et al., 2013; Johnson et al., 2013; Karampeazis et al., 2013; Kerner et al., 2013; Kim et al., 2013; Tsao et al., 2013), 4 (Endoh et al., 2006; Fidler et al., 2011; Ludovini et al., 2011; Ludovini et al., 2012), 4 (Endoh et al., 2006; Han et al., 2006; Fidler et al., 2011; O'Byrne et al., 2011)studies were accessible to evaluate the predictive values of K-ras mutation, PIK3CA mutation and PTEN deficiency, respectively, in lung cancer patients treated with anti-EGFR agents. The basic characteristics of the enrolled studies were listed in Table 1.

The median age ranged from 56 to 75 years, the percent of female from 17.3% to 83.8%, and the percent of non-smoker from 4.1% to 97.3%. Most patients were received erlotinib and (or) gefitinib. In 46 studies known response criteria, the response to treatment was evaluated mostly according to RECIST or WHO criteria.

Predictive and prognostic value of K-ras mutation

Forty eight studies (Eberhard et al., 2005; Fujimoto et al., 2005; Pao et al., 2005; Endoh et al., 2006; Giaccone et al., 2006; Han et al., 2006; Hirsch et al., 2006; Cappuzzo et al., 2007; Hirsch et al., 2007; Ichihara et al., 2007; Jackman et al., 2007; Loprevite et al., 2007; Massarelli et

Table 1.1 Main Characteristics of Included Studies

Study	N	Age,mean&range	F	NS	S
Yujia Zhu 2010	79	60.9,35-83y	29	40	39
Qiong Zhao 2011	33	58,34-69y	16	19	14
Fred R.Hisch 2006	1692	NA	553	375	1317
Wolfram Brugger2011	889	60, 30-83y	231	151	738
ChunChieh Wu 2008	237	61.8,28-84y	100	90	147
MILOS PESEK 2009	360	65.8,36-85y	83	55	305
Shuhang Wang 2010	273	NA	115	138	135
Lecia V.Sequist 2011	167	63,23-89y	67	35	132
William Pao 2005	60	NA	NA	NA	NA
Suresh.Ramalingam2013	94	62.0,27-85	38	19	75
Suresh.Ramalingam2013	94	60.0,24-82y	39	18	78
Vincent A. Miller 2008	101	66, ,32-85y	66	25	76
Hidefumi Sasaki 2008	27	NA	13	14	13
Marcus M.Schittenhelm2009	23	NA	NA	NA	NA
Katharine A.Price 2010	62	66,40–86y	31	0	62
Samuel Murray 2012	59	NA	NA	NA	NA
Antonio Marchetti 2009	83	65,43-80y	35	29	54
V. Ludovini 2012	166	60.2, 25.6-84y	75	61	105
Joline S.W.Lind 2010	50	60, 41-78y	22	11	39
Humberto Lara Guerra 2009	36	65,38-81y	18	6	30
Seung Tae Kim 2013	57	64, 28–84y	22	25	32
Gerald S.M.A.Kerner 2013	442	NA	NA	NA	NA
Athanasios Karampeazis 2013	166	65, 37-83y	31	29	137
A Kalikaki 2008	25	55,41-70y	3	3	22
Melissa Ljohnson 2014	1036	65,25-92y	NA	NA	NA
David M.Jackman 2007	80	75,70-91y	40	8	72
David M.Jackman 2009	223	69, 26-91y	153	73	150
Shuji Ichihara 2007	98	NA	36	37	61
Fred R.Hirsch 2011	143	NA	NA	8%	92%
Sae-Won Han 2006	69	59, 30-82y	30	35	34
Ji-lin Guan 2013	273	59	101	137	136
Giuseppe Giaccone 2006	53	60, 30-80y	31	NA	NA
David A.Eberhard 2005	274	NA	NA	NA	NA
Jean-Yves Douillard 2010	275	NA	84	31	244
Giulio Metro 2012	67	60,39–84y	30	22	45
Neal Ready 2010	60	66, 41-86y	16	6	51
Chang-Qi Zhu 2008	206	NA	71	47	142 17
Kenneth J O'Byrne 2011	557	NA	172	121	435
Laura Boldrini 2009	411	65.7,37-88y	176	52	165
A.-M.C.Dingemans 2011	47	59, 34-40y	24	8	39
MJ Fidler 2011	82	NA	44	12	70
Hideki Endoh 2006	78	61.9,39–80y	34	33	45
Marcello Tiseo 2010	91	67,40-85y	35	20	71
Claus-Peter Schneider 2008	393	65,31-90y	161	96	296
Michele Milella 2012	118	NA	NA	NA	NA
Ondrej Fiala 2013	448	NA	NA	NA	NA
Jacques Cadranel 2012	552	NA	168	94	424
Jong-Mu Sun 2013	484	NA	205	232	252
F.R.Hirsch 2007	204	NA	88	42	162
Enriqueta Felip 2008	83	56,35-78y	23	11	72
Nobukazu Fujimoto 2005	NA	NA	NA	NA	NA
N. van Zandwijk 2006	NA	NA	NA	NA	NA
Erminia Massarelli 2007	70	59.2	41	17	53
David R.Spigel 2011	168	65,45-83y	29	NA	NA
P.A.Zucali 2008	51	NA	17	9	41
John Wen-Cheng Chang 2008	182	NA	77	NA	NA
Federico Cappuzzo 2006	37	NA	31	36	1
Maura Loprevite 2007	58	NA	19	11	NA
G.D'Addario 2007	63	NA	NA	NA	NA
Marileila Varella-Garcia 2009	44	60.9,38-79y	21	23	21
Shirin Khambata-Ford 2010	676	64,37-87y	NA	28	NA

1. F:female 2. S: smoking former or current 3. N:never smoking 4. G:gefitinib 5. E erlotinib 6. CRT: Chemoradiotherapy 7. CT Chemotherapy 8. Cet Cetuximab 9. Bev Bevacizumab 10. So Sorafenib 11. I Icotinib 12. D Dacomitinib 13. Ga Ganetespib 14. Mat Matuzumab 15. Pac: Paclitaxel 16. Eve: Everolimus 17. EHGSM: enzymatic heteroduplex gene scanning method 18. CGCE: cycling-gradient capillary electrophoresis,exon2 codons12&13 19. BRSA: bidirectional sequence analysis 20. BDAS: bidirectional automatic sequencing 21. RECIST: Response Evaluation Criteria in Solid Tumour 22. WHO: World Health Organization criteria

Table 1.2. Main Characteristics of Included Studies

Study	Stage III/IV	Pathologic Type	ethnicity	Treatment protocols	Response criteria	Methods for Kras mutations
Yujia, 2010	IIIB/IV	NSCLC(ADC,SCC,ADSQC)	A	E	RECIST	PCR
Qiong, 2011	III/IV	NSCLC(ADC,SCC,not clear)	NA	I	RECIST	PCR+DS, exon1 (codons12&13)
Fred, 2006	NA	ADC and others	A	G	WHO	DS+ARMS,exon2 (codon12/13)
Wolfram, 2011	IIIB/IV	ADC/BAC,SCC and other	A,W	E	RECIST	PCR,exons2-3 (codons12 /13/61)
Chun, 2008	I/IV	ADC,SCC and other	A	E and/or G	RECIST	PCR+DS, exon1&2
MILOS, 2009	I/IV	NSCLC(ADC,SCC)	NA	E and/or G	NA	CGCE,exon2 (codons12&13)
Shuhang, 2010	IIIB/IV	NSCLC(ADC,SCC)	A	E and/or G	RECIST	PCR,exon2 (codons12&13)
Lecia, 2011	IIIB/IV	NSCLC(ADC,SCC,BAC)	W	E	RECIST	DS allele-specific PCR kit
William, 2005	NA	NA	NA	E and/or G	RECIST	PCR,exon2
Suresh, 2013	NA	NSCLC(ADC and others)	A,W	E	RECIST	NA
Suresh, 2013	NA	NSCLC(ADC and others)	A,W	D	RECIST	DS,exon2
Vincent, 2008	IIIB/IV	ADC and BAC	NA	E	RECIST	DS,exon2
Hidefumi, 2008	NA	NA	A	G	RECIST	DS
Marcus, 2009	NA	NA	NA	Mat&/or Pac	RECIST	exon2
Katharine, 2010	IIIB/IV	NSCLC(ADC,SCC)	NA	G& Eve	RECIST	PCR-RFLP,exon2 (codons12/13)
Samuel, 2012	IIIB/IV	NA	NA	E	RECIST	PCR+SD,exon2(codon12)
Antonio, 2009	IIIB/IV	NA	NA	E and/or G	who	PCR+SD,exon2 (codons12&13)
V. Ludovini 2012	IIIB/IV	NSCLC(ADC,SCC,BAC,LC)	NA	E and/or G	RECIST	PCR+SD,exon1&2
Joline, 2010	IIIB/IV	NSCLC(ADC,SCC,LC)	W,B,A	E & So	RECIST	SD,exon2
Humberto, 2009	NA	ADC,SCC,LC	A	G	RECIST	PCR+SD,exon2 (codons12&13)
Seung, 2013	IIIB/IV	ADC,SCC	NA	NA	NA	BRSA,exon2(codon12/13)
Gerald, 2013	NA	NSCLC, Carcinoid, saliva glands	NA	NA	RECIST	pyrosequencing,exon2(codon12,13,61)
Athanasios, 2013	IIIB/IV	SCC and non SCC	NA	E	RECIST	PRC+DS,exon1
A Kalikaki, 2008	IIIB/IV	ADC and others	NA	NA	NA	DS,exon2(codon12&13)
Melissa, 2014	IV	NA	NA	NA	NA	DS+WAVE-HS,exon2&3
David, 2007	IIIB/IV	ADC, SCC, BAC and others	NA	E	RECIST	DS,exons1&2
David, 2009	NA	ADC, ADC with BAC and others	W,B,A	E and/or G	RECIST	PCR+DS,exon2
Shuji, 2007	NA	ADC and others	A	G	WHO	exon2 (codons12&13)
Fred, 2011	NA	NA	NA	E	RECIST	DS,exon2
Sae, 2006	IIIB/IV	ADC, BAC, SCC and others	NA	G	WHO	DS,exon2(codon12&13&59&61)
Ji-lin, 2013	I/IV	ADC, SCC, SC, LC, others	NA	NA	NA	PCR,exon1&2
Giuseppe, 2006	IIIB/IV	ADC, BAC, SCC, LC, others	NA	E	NA	PCR+DS,exon2
David, 2005	NA	NA	NA	E	RECIST	PRC+DS,exon2 (codons12&13)
Jean, 2010	NA	ADC and others	A	G	RECIST	PCR,exon2&3
Giulio, 2012	IIIB/IV	NSCLC	NA	E and/or G	NA	EHGSM,exon2
Neal, 2010	III	NSCLC(ADC, SCC, LC)	W,B	CRT& G	NA	PCR,exon2
Chang, 2008	NA	ADC and others	A	E	NA	PCR,exon2 (codons12&13)
Kenneth, 2011	IIIB/IV	ADC,SCC and others	A,W	CT&Cet	NA	RFLP analysis,exon2 (codons12&13)
Laura, 2009	NA	NA	NA	E and/or G	WHO	PCR,exon1
Dingemans, 2011	IIIB/IV	ADC and others	NA	Bev and E	RECIST	NA
MJ Fidler, 2011	NA	ADC and others	NA	G	NA	DS,exon2(codons12&13&61)
Hideki, 2006	I/IV	ADC,SCC,LC,ADSQC	NA	G	RECIST	PCR,exon2
Marcello, 2010	IIIB/IV	ADC and others	NA	G	RECIST	PCR,exon2&3
Claus, 2008	IIIB/IV	ADC,SCC and others	W	E	NA	DS
Michele, 2012	IIIB/IV	NA	NA	E and/or G	RECIST	PCR,exon1
Ondrej, 2013	NA	NA	NA	E and/or G	NA	PCR,exon2
Jacques, 2012	I/IV	ADC,SCC and others	NA	E	NA	PCR+DS,exon2&3
Jong, 2013	NA	ADC,SCC and others	NA	E and/or G	RECIST	PCR,exon2
Hirsch, 2007	IIIB/IV	ADC,SCC,BAC,LC and others	NA	G	RECIST	PCR,exon2&3
Enriqueta, 2008	NA	ADC,SCC,LC and others	NA	E	NA	DS,exon2(codons12&13&61)
Nobukazu, 2005	NA	NA	NA	G	NA	PCR,exon2(codons12)
Zandwijk, 2006	NA	NA	NA	E and/or G	RECIST	PCR,exon1
Erminia, 2007	IIIB/IV	NSCLC(ADC,SCC and others)	A,W	E and/or G	WHO	Genzyme Genetics Assay
David, 2011	NA	SCC and others	NA	E	RECIST	exons1&2
Zucali, 2008	IIIB/IV	ADC and others	NA	G	RECIST	DS,exon2
John, 2008	NA	NA	NA	E	RECIST	DS,exon2
Federico, 2006	NA	NA	W	G	RECIST	DS,exon2
Maura, 2007	NA	NA	W	G	RECIST	DS,exon2
Addario, 2007	NA	NA	NA	G	WHO	PCR,exon2
Marileila, 2009	I/IV	ADC and others	NA	G	RECIST	exon2 (codons12&13)
Shirin, 2010	NA	SCC and others	W,B,A	Cet	NA	bidirectional sequence analysis,exon2

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al., 2007; van Zandwijk et al., 2007; Chang et al., 2008; D'Addario et al., 2008; Felip et al., 2008; Kalikaki et al., 2008; Miller et al., 2008; Sasaki et al., 2008; Schneider et al., 2008; Wu et al., 2008; Zhu et al., 2008; Zucali et al., 2008; Boldrini et al., 2009; Jackman et al., 2009; Lara-Guerra et al., 2009; M et al., 2009; Marchetti et al., 2009; Schittenhelm et al., 2009; Douillard et al., 2010; Khambata-Ford et al., 2010; Lind et al., 2010; Price et al., 2010; Tiseo et al., 2010; Wang et al., 2010; ZHU Yu-jia et al., 2010; Dingemans et al., 2011; Hirsch et al., 2011; O'Byrne et al., 2011; Zhao et al., 2011; Ludovini et al., 2012; Murray et al., 2012; Sun et al., 2012; Guan et al., 2013; Karampeazis et al., 2013; Kim et al., 2013; Socinski et al., 2013) presented data on the relationship between K-ras mutation with ORR were summarized and their overall RR was 0.42 (95%CI: 0.33-0.55, P=0.000, heterogeneity test p=0.664, I^2=0.0%). Among them the RR of studies used with anti-EGFR monoclonal antibodies(Anti-EGFR MoAbs) was 0.93(95%CI: 0.62-1.38, P=0.923, heterogeneity test p=0.705, I^2=0.0%) and the RR of studies used with only EGFR-TKIs was 0.31 (95%CI: 0.22-0.44, P=0.928, heterogeneity test p=0.000, I^2=0.0%) as shown in Figure 2. In the forty eight studies, the ORRs of mutant K-ras and wild-type K-ras patients were 7.01 %(37/528) and 36.54 %(764/2091), respectively. The overall RR indicated that patients with K-ras mutation had a lower ORR and worse response than wild-type K-ras patients. Furthermore, EGFR-TKIs were more sensitive than anti-EGFR MoAbs to K-ras gene status.

Eleven studies (Endoh et al., 2006; Ichihara et al., 2007; Felip et al., 2008; Schneider et al., 2008; Marchetti et al., 2009; O'Byrne et al., 2011; Sequist et al., 2011; Cadranel et al., 2012; Ludovini et al., 2012; Milella et al., 2012; Johnson et al., 2013) showed the correlation between OS and the K-ras status. Available HRs from the 11 studies were combined to get the pooled HR, shown in Figure 3A. The pooled HR was 1.37(95%CI: 1.15-1.65, p=0.001;heterogeneity test p=0.008, I^2=58.1%). The pooled HR indicated that patients with K-ras mutation had a shorter OS than did wild-type K-ras patients.

In the meantime, based on eight studies (Felip et al., 2008; Schneider et al., 2008; Marchetti et al., 2009; O'Byrne et al., 2011; Sequist et al., 2011; Cadranel et al., 2012; Metro et al., 2012; Milella et al., 2012), the pooled HR for PFS was 1.33 (95%CI: 1.05-1.69, P=0.019 heterogeneity test P=0.10, I^2=61.9%), shown in Figure 3B. The result suggested that patients with K-ras mutation had a shorter PFS than did wild-type K-ras patients.

Predictive and prognostic value of PIK3CA mutation

The four eligible studies (Endoh et al., 2006; Giaccone et al., 2006; Fidler et al., 2011; Ludovini et al., 2012) on PIK3CA mutation were incorporated into the meta-analysis. Three studies (Giaccone et al., 2006; Fidler et al., 2011; Ludovini et al., 2012) showed the relationship between PIK3CA mutation with ORRs were summarized and their overall RR was 1.08 (95%CI: 0.17-6.66 P=0.938; heterogeneity: p=0.020, I^2=74.6%) and the result was shown in Figure 4A. The result suggested that the relationship between patients with PIK3CA mutation and ORR was insignificant

Three studies (Endoh et al., 2006; Fidler et al., 2011; Ludovini et al., 2012) showed the correlation between OS and the PIK3CA status. Two of them (Endoh et al., 2006; Fidler et al., 2011) focused on the OS of high versus low PIK3CA expression patients and the pooled HR (Figure .4B)was 0.79(95%CI: 0.23-2.68 p=0.706; heterogeneity test p=0.041, I^2=76.1%). The result did not show

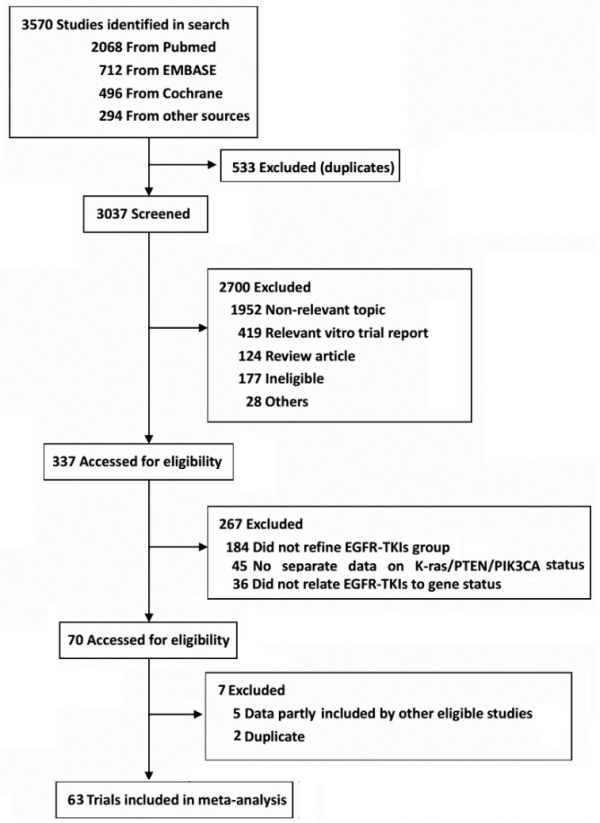

Figure 1. The Flow Chart of Study Selection

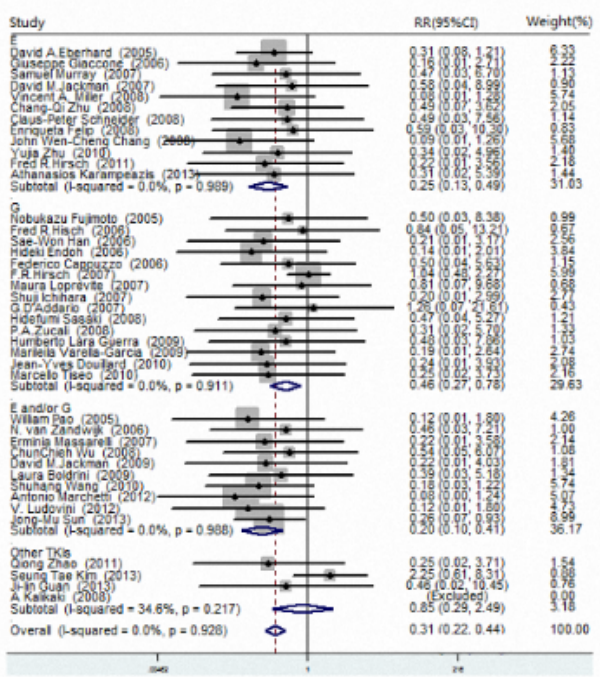

Figure 2. The Relationship between K-ras Mutation with ORR in Lung Cancer Patients Treated with EGFR-TKIs

significantly relationship between PIK3CA status with OS. In another way, the rest one study (Ludovini et al., 2012) focused on the OS of mutant PIK3CA versus wild-type PIK3CA patients. The HR was 3.35(95%CI: 1.34-8.36, P=0.01). The result showed that mutant PIK3CA patients had a shorter OS.

Predictive value of expression of PTEN

The four eligible studies (Endoh et al., 2006; Han et al., 2006; Fidler et al., 2011; O'Byrne et al., 2011) on expression of PTEN were incorporated into the meta-analysis. Of three studies (Endoh et al., 2006; Han et al., 2006; O'Byrne et al., 2011), the result did not show significant relationship between expression of PTEN with ORR (RR=0.82, 95%CI: 0.56-1.19, p=0.286; heterogeneity: p=0.373, I^2=0.0%, Figure 4C).

Three studies (Endoh et al., 2006; Fidler et al., 2011; O'Byrne et al., 2011) showed the correlation between OS and the PTEN expression. The result did not show significant relationship between expression of PTEN with OS (HR=0.88; 95%CI, 0.31-2.46 P=0.805; heterogeneity, p=0.030, I^2=71.6%, Figure 4D). The result on PFS of two studies (Fidler et al., 2011; O'Byrne et al., 2011) did not show the expression of PTEN was significantly related to the PFS (HR=1.06, 95%CI: 0.57-1.97,P=0.857; heterogeneity test p=0.035, I^2=77.4%, Figure .4E).

Combined multiple biomarkers on the clinical outcomes

Three articles (Endoh et al., 2006; Fidler et al., 2011; Ludovini et al., 2012) studied the predictive and prognostic values of the combination biomarkers. V. Ludovini et al. (2012) reported that patients with the combination of KRAS, PIK3CA, MET, and non-sensitizing EGFR mutations had worse OS. MJ Fidler et al (Fidler et al., 2011) investigated and found that PFS and OS were significantly shorter in patients who meet CEN7<4 copies per cell, PTEN loss, PIK3CA gain and EGFR wild-type. Additionally, high levels of PIK3CA gain and PTEN loss

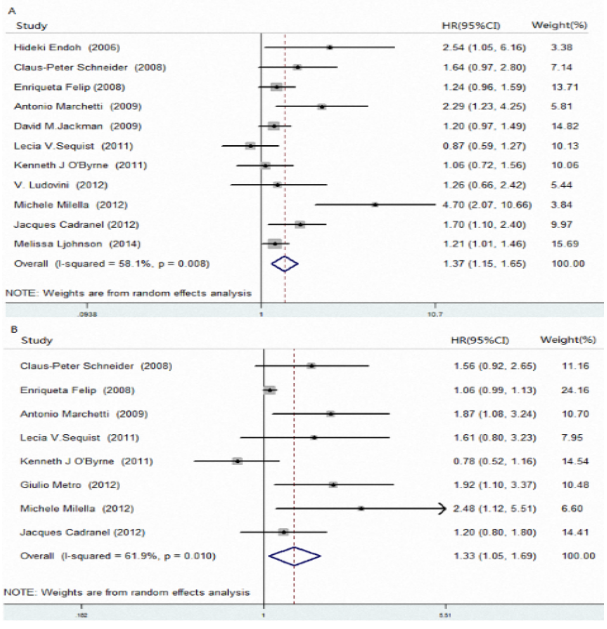

Figure 3. The Relationships between K-ras Mutation with OS (A) and PFS (B) in Lung Cancer Patients Treated with Anti-EGFR Agents

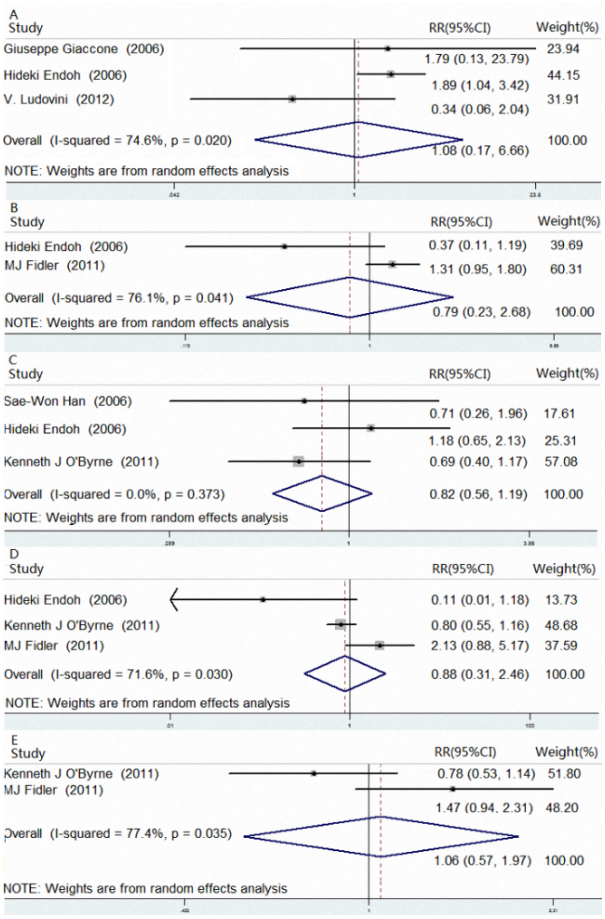

Figure 4. The Relationship between PIK3CA Mutations with ORR (A), the Relationship between PIK3CA Expression with OS (B), and the Relationships between Loss of PTEN with ORR (C), OS (D) and PFS (E) in Lung Cancer Patients Treated with anti-EGFR Agents

had strongly significantly shorter PFS and OS. Hideki Endoh et al. (2006) found that PTEN expression and PIK3CA mutation did not correlate with the response to gefitinib, but high expressions of PIK3CA and PTEN were associated with prolonged survival. Although these articles showed that the predictive and prognostic powers of the combined markers were stronger than single markers, it lack of sufficient data to conduct meta-analysis.

Discussion

In our meta-analysis, we reviewed the literature systematically on the predictive and prognostic values of K-ras mutation, PIK3CA mutation and loss of PTEN in lung cancer patients treated with anti-EGFR agents. 63 studies were brought into in the final meta-analysis.

Of the 48 studies, K-ras mutation showed significantly predictive value of anti-EGFR agents in lung cancer patients. The ORRs of mutant K-ras and wild-type K-ras suggested the former probably lack of sensitivity to anti-EGFR agents. Furthermore, we analyzed the sensitivity of EGFR-TKIs and anti-EGFR MoAbs separately. The results showed that mutant K-ras patients were insensitive to EGFR-TKIs not anti-EGFR MoAbs. But it was noteworthy that the studies referred with anti-EGFR MoAbs also used other agents three chemotherapy and one

EGFR-TKIs. The conclusion about anti-EGFR MoAbs might be affected by chemotherapy and could not display the real curative effect on mutant K-ras patients.

Eleven studies presented data on the association between K-ras mutation with OS, and eight studies on PFS. The results suggested that K-ras mutation possessed the prognostic value when lung cancer patients used with anti-EGFR agents. It was found that mutant K-ras patients had a significantly shorter OS and shorter PFS than wild-type K-ras patients. It indicated that K-ras mutant patients had poor survival benefits than wild-type K-ras patients. According to the incompatible relation between K-ras mutation with EGFR-mutation, K-ras mutation may be a valid predictive and prognostic biomarker of anti-EGFR agents to complement EGFR mutations to select insensitive patients to anti-EGFR agents.

In the four studies, the ORRs of mutant PIK3CA and wild-type PIK3CA lung cancer patients were about 50.00%(3/6) and 74.75%(74/99), respectively. The analysis incorporated with corresponding RR indicated that the predictive value of PIK3CA mutation was insignificant. Only 1 study referred to OS of PIK3CA mutations. In that study, the relevant results statistically significantly showed that mutant PIK3CA patients had a shorter OS.

Two studies focused on the relationship between the expression of PIK3CA with OS, but the result did not show significantly prognostic value of PIK3CA expression. There was one study on PFS of PIK3CA expression which showed us low expression of PIK3CA patients had a shorter PFS.

Three studies showed that the ORRs of high and low expression of PTEN patients were about 43.48%(30/69) and 53.15%(59/111), respectively. The results indicated the predictive value of loss of PTEN was insignificant. In another way, three and two studies concentrated on OS and PFS of loss of PTEN respectively, the results both showed the insignificant prognostic value of loss of PTEN in lung cancer treated with anti-EGFR agents.

Although our meta-analysis showed PTEN and PIK3CA had no significantly association with outcomes of anti EGFR-agents, but in the studies focused on the predictive and prognostic values of the combination biomarkers, their conclusions suggested PTEN, PIK3CA, EMT and so on still had the promising predictive and prognostic values.

EGFR mutation plays a vital role in predicting the outcomes of anti-EGFR agents in lung cancer patients. It reported that patients with EGFR mutations had better response to anti-EGFR agents than wild-type EGFR patients in lung cancer (Brugger et al., 2011; Johnson et al., 2013; Kerner et al., 2013; Kim et al., 2013). However, there still exists 10% patients without EGFR mutations response to anti-EGFR agents (Hirsch et al., 2007). K-ras, PTEN and PIK3CA as important downstream signaling molecules of EGFR network were studied in predicting outcomes of anti-EGFR agents recently.

Chen Mao et al. (2010) summarized 22 studies from 2005 to 2009 to evaluate the association between K-ras mutation and resistance to the treatment of anti-EGFR agents in patients with NSCLC. They found that K-ras mutation may be a negative predictive biomarker. The report was very deep and consultative to the clinical usefulness of anti-EGFR agents. However after 2009, more large sample relevant studies emerged up. Based on this, we collected and reintegrated the relevant data systematically to conduct the analysis comprehensively. The analysis consisted of predictive and prognostic value. We perfected the conclusion about the relationship between ORR with K-ras mutation. Additionally, the associations between OS/PFS and K-ras mutant patients were further analyzed to explore the survival benefits.

Since PIK3CA and PTEN regulate phosphorylation of Akt, it is rational to hypothesize that alteration of these genes might influence the response with anti-EGFR agents (Endoh et al., 2006; Giaccone et al., 2006; Fidler et al., 2011; Ludovini et al., 2012). Recently, several articles reported that PIK3CA mutation and loss of PTEN might have the association with resistance to anti-EGFR agents in lung cancer patients but the conclusions were controversial. To the best of our knowledge, there did not have one meta-analysis focused on these two genes. We analyzed how PIK3CA expression, PIK3CA mutation and loss of PTEN affected the outcomes of anti-EGFR agents.

Our results did not show PTEN and PIK3CA had significant predictive or prognostic values, but some reasons may lead to the conclusion. It was difficult to determine the cutoff line of PTEN expression. The studies involved in PTEN had different classification to be divided into groups (Endoh et al., 2006; Fidler et al., 2011). It may resulted in discrepancies of combining and analyzing data. De Roock W et al (De Roock et al., 2010) investigated the relationship between PIK3CA mutation and the outcomes of colorectal cancer patients treated with anti-EGFR agents, and found it was exon20 exon9 showed significantly shorter PFS than did those wild-type for this exon. Zu-Yao Yang et al (Yang et al., 2013) conducted the relevant colorectal meta-analysis also showed that PIK3CA exon20 mutation was significantly associated with worse PFS, OS, and ORR of wild-type K-ras colorectal cancer patients treated with anti-EGFR agents. It may give us hints that PIK3CA exon20 and exon9 should be analyzed separately. Unfortunately, the data collected did not compare PIK3CA exon20 and exon9, but it was a research orientation to further study.

We collected the trials estimated the predictive and prognostic values of combination of biomarkers. The studies all suggested that the combination biomarkers showed stronger predictive power. Due to the types of combination biomarkers were various, it was impossible to integrate to conduct meta-analysis.

There were several limitations should be taken into consideration in our meta-analysis: the present meta-analysis was not based on individual patient data; heterogeneity existed in the trial design; there is no subgroup analysis about ethnicity, smoking status or gender since the data is not sufficient; the predictive and prognostic value of combination of the three biomarkers were not estimate. However, the combination biomarkers to predict the outcomes of anti-EGFR agents may be a enlightening idea.

In conclusion, our meta-analysis showed that K-ras

mutation may be an effective predictor in lung cancer patients treated with anti-EGFR agents. Whereas, the predictive and prognostic value of PTEN deficiency and PIK3CA mutation need to be further investigated..

Acknowledgements

Prof. Du had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Prof. Liu had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis .

Miss. Wang: contributed to the study design, definition of the inclusion and exclusion criteria, data analysis and interpretation, and drafting and revision the manuscript.

Dr Qu: contributed to the study design, definition of the inclusion and exclusion criteria, data analysis and interpretation, and drafting and revision the manuscript.

Dr Chang: contributed to the study design, definition of the inclusion and exclusion criteria, data analysis and interpretation, and drafting and revision the manuscript.

Mr. Wang: contributed to the data interpretation and review of the manuscript.

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