J Med Sci 2010;30(5):189-198
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Efficacy of Cetuximab on Wild-type and Mutant *KRAS* in Colorectal Cancer: Systematic Review and Meta-Analysis

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Background: Cetuximab, an IgG1 chimeric monoclonal antibody against epidermal growth factor receptor (EGFR), has activity against colorectal cancers. This meta-analysis was performed to compare the efficacy of cetuximab on wild-type and mutant *KRAS* in advanced colorectal cancers. **Methods:** A total of 2,875 patients from 18 clinical trials were available for analysis. The efficacy data included overall response rate, progression-free survival, and overall survival. **Results:** The overall response rate of patients with wild-type *KRAS* is significantly higher than that of patients with mutant *KRAS* (OR= 5.01, 95% CI, 3.89 to 6.45). The same is true for progression-free survival (HR= 0.46, 95% CI, 0.37 to 0.58) and overall survival (HR= 0.51, 95% CI, 0.38 to 0.69). In patients with mutant *KRAS*, the overall survival is worse for cetuximab treatment than that for non-cetuximab treatment (OR=0.67, 95% CI, 0.47 to 0.95). **Conclusions:** Patients with wild-type *KRAS* treated with cetuximab have significantly increased overall response rate, progression-free survival, and overall survival. However, patients with mutant *KRAS* treated with cetuximab not only showed no significant difference in progression-free survival, they also had decreased overall response rate.

Key words: cetuximab, colorectal cancer, *KRAS*, meta-analysis

INTRODUCTION

Colorectal cancer (CRC) is the third most common form of cancer and the second leading cause of cancer-related death in the Western world.[1] In Taiwan, the incidence of CRC has risen to become the most common cancer.[2] The initial treatment of CRC is surgical resection, but about 40-60% of patients will have disease recurrence or metastasis.[3] Chemotherapy is indicated for the treatment of patients with advanced disease to extend survival and control symptoms.[4] Cytotoxic agents such as irinotecan, capecitabine, leucovorin, fluorouracil, and oxaliplatin have increased the median survival.[5-7] These agents have been widely used in first-line therapy in clinical treatment. Latest advances have led to the development of agents that specifically inhibit tumor growth, such as epidermal growth factor receptor (EGFR) inhibitor.[8] EGFR is the cell-surface transmembrane receptor and binding of its specific ligands leads to autophosphorylation of receptor tyrosine kinase and consequential activation of signal transduction pathways involved in regulating cellular proliferation, migration, adhesion, and invasion.[9] Mutations that lead to overexpression of EGFR have been associated with many tumors, including CRC and lung cancer.[10]

Cetuximab is an IgG1 chimeric monoclonal antibody that binds to the extracellular domain of EGFR, inhibits EGF binding and receptor auto-phosphorylation, thus blocking ligand-induced receptor signaling and regulating tumor growth.[8,11] Significant improvements have been made in both response rate and survival of patients with colorectal cancer treated with cetuximab.[12-14] Mutations of *KRAS*, a small G protein functions downstream of EGFR-induced cell signaling, occur in over 30% of CRCs, the majority (~82%) of reported mutations are in codons 12 and 13.[15] These mutations lead to constitutive activation of the *RAS*, and subsequent signaling events are unregulated and independent of EGFR control.[16]

Clinical trials comparing the efficacy of cetuximab on wild-type *KRAS* and mutant *KRAS* in advanced colorectal cancers have been conducted.[17-19] In 2008, Linardou

Received: April 16, 2010; Revised: July 13, 2010; Accepted: July 23, 2010
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et al.[20] reported a meta-analysis of five clinical trials of cetuximab administered for colorectal cancer.[19,21-24] Their results showed that the overall response rate of patients with wild-type *KRAS* is significantly higher than that of patients with mutant *KRAS*. However, meta-analysis of progression-free survival and overall survival were not performed in their studies. To date, meta-analysis with a greater power of using statistical comparisons to detect efficacy of cetuximab on mutant *KRAS* has not been reported.

In the past two years, there were 13 relevant clinical trials published.[17-18,25-35] Here, we integrated these trials with those in the report of Linardou *et al*.[19,21-24] for meta-analysis, and then compared not only the overall response rate, but also progression-free survival and overall survival for CRC patients with wild-type and mutant *KRAS* treated with and without cetuximab.

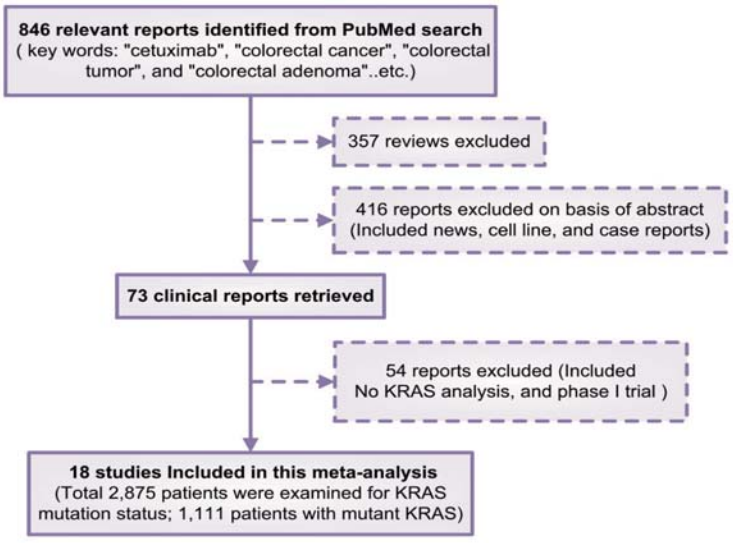

Fig. 1 Flow chart of the meta-analysis.

METHODS

Literature search

The PubMed was employed to search systematically for all articles published from May 1996 to December 2009, which included the following terms in their titles, or abstracts: “cetuximab”, “colorectal neoplasm”, “colorectal tumor”, “colorectal adenoma”, “colorectal cancer”, “colorectal carcinoma”, “colorectal disease”, “colorectal polyps”, “colonic”, “sigmoid neoplasms”, “rectal neoplasms”, and “anus neoplasms”. From these studies, we obtained relevant data concerning number of patients, baseline characteristics, treatment strategies, and study results including treatment efficiency.

Selection of studies

The reference lists of all candidate articles of this study were examined manually. Reviews, comments, guidelines, news, and case reports were excluded. Citations selected from this preliminary search were subsequently screened for eligibility as clinical trials according to the following criteria: (1) patients with metastatic (or advanced) colorectal cancer; (2) patients treated with cetuximab versus without cetuximab and not confounded by additional agents or interventions (i.e. cetuximab is the only difference in treatment between the combined chemotherapy, control and experimental groups); and (3) *KRAS* gene mutations analyzed in tumors of patients.

Data extraction

We recorded the following information from each article: first author, journal and year of publication, number of patients screened, number of patients with *KRAS* mutations, age, gender, regimens used, overall response rate, progression-free survival, and median overall survival.

Data analysis

Meta-analysis was performed on all clinical trials comparing the efficacy of cetuximab on wild-type and mutant *KRAS* in advanced colorectal cancers. The outcomes used were (1) overall response rate, defined as the sum of partial and complete response rates according to the Response Evaluation Criteria in Solid Tumors (RECIST);[36] (2) progression-free survival, defined as the time from randomization until the first documented progression or death from any cause; (3) overall survival, defined as the time from randomization until death from any cause, censoring patients who had not died at the date last known alive. These trials were pooled and subjected to fixed-effects or random-effects meta-analysis using Review Manager V. 5.0.20. All reported *P* values were two-sided. $I^2=[(Q - df)/Q] \times 100\%$, where Q is the chi-square statistic for heterogeneity and *df* denoting its degrees of freedom is a statistic for quantifying inconsistency among the study results. If P values were less than 0.1, the assumption of homogeneity was deemed invalid, and the random-effects model was reported.[37] Publication bias was assessed through visual inspection of funnel plots of outcomes.[38] Pooled results are expressed as odds ratio (OR) and hazard ratio (HR) with 95% confidence

Table 1 Baseline characteristics of included Patients

Author	Year	Patients included in analysis, n	KRAS mutation positive, n	Age	Male (%)	Treatment
Moroni[19]	2005	21	5	67	84.6	Ce; Ir + Ce;
Benvenuti[17]	2007	23	6	65	73.9	Ce; Ir + Ce
Di Fiore[22]	2007	59	22	NA	NA	Ir + Ce; Ox + Ce
Frattin[23]	2007	27	10	66	66.7	Ir + Ce; CAPOX + Ce
Cappuzzo[18]	2008	80	42	63.2	63.5	Ce; Ir + Ce;
De Roock[21]	2008	108	42	60	61.9	Ce; Ir + Ce;
Lievre[24]	2008	114	36	59.2	49.4	Ir + Ce; FOLFIRI + Ce
Karapetis[25]	2008	394	164	62.3	94.7	BSC + Ce; Ce
Lurje[26]	2008	130	42	NA	49.2	Ce
Bibeau[27]	2009	64	27	60	67	Ir + Ce
Garm Spindler[29]	2009	64	22	61	52	Ir + Ce
Bokemeyer[28]	2009	233	99	59.5	52.5	FOLFOX-4 +Ce; FOLFOX-4
Loupakis[30]	2009	138	51	62	59	Ir + Ce
Prenen[31]	2009	199	77	61	60	Ce; Ir + Ce
Sohn[32]	2009	66	27	58	60.6	Ir + Ce
Tol[33]	2009	520	206	62	68.5	Ca + Ox +Be + Ce; Ca + Ox + Be
Van Cutsem[34]	2009	540	192	61	60.5	FOLFIRI + Ce; FOLFIRI
Yen[35]	2009	95	41	66	57.9	FOLFOX + Ce; FOLFIRI+ Ce

Abbreviations: Be, bevacizumab; Ca, capecitabine; Ce, cetuximab; Ir: irinotecan; Ox, oxaliplatin; CAPOX, capecitabine+ oxaliplatin; FOLFIRI, leucovorin + fluorouracil + irinotecan; FOLFOX-4, leucovorin + oxaliplatin + fluorouracil

intervals (CI). Odds ratio > 1 reflects a favorable outcome in response rate of wild-type *KRAS* to cetuximab therapy, or hazard ratio < 1 reflects a favorable outcome in survival of wild-type *KRAS* to non-cetuximab therapy. The results are presented as Forest plots with point estimates and 95% CI for each trial and the entire study.

RESULTS

Description of Included Trials

Our initial literature search identified 846 articles, 828 of them were excluded as they were not relevant to the study. Figure 1 is the flow chart of the meta-analysis in this study. A total of 2,875 patients from 18 clinical studies were available for analysis, including 1,764 patients with wild-type *KRAS* and 1,111 patients with mutant *KRAS*. Table 1 summarizes the baseline characteristics of patients. The prior treatment included best supportive care (BSC), bevacizumab, capecitabine, irinotecan, capecitabine + oxaliplatin (CAPOX), leucovorin + fluorouracil + irinotecan (FOLFIRI), and leucovorin + oxaliplatin + fluorouracil (FOLFOX-4). Their average age ranged from 22 to 89 years; the percentage of male patients ranged from 49.2% to 84.6%; and the total number of patients in each trial varied from 21 to 520.

Efficacy of cetuximab on wild-type *KRAS* versus mutant *KRAS*

Overall response rate

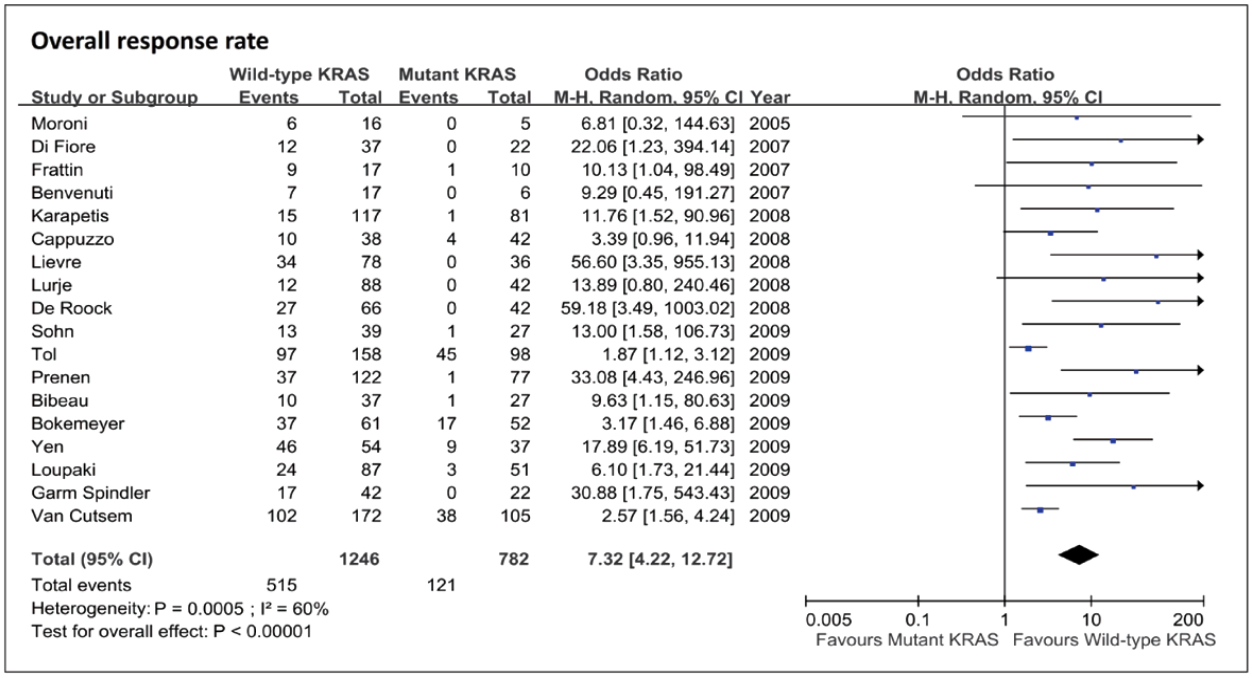

Fig. 2 Meta-analysis of overall response rate for *KRAS* wild-type and *KRAS* mutated patients treated with cetuximab. *KRAS* wild-type patients had a statistically significant improvement in overall response rate compared with *KRAS* mutated patients. Odds ratios were calculated using a random-effects model.

All the 18 clinical studies[17-19,21-35] included in our meta-analysis reported data of overall response rate. The overall response rate for patients with wild-type *KRAS* was 41.3% (515/1,246), while that for patients with mutant *KRAS* was 15.5% (121/782). As can be seen, among patients treated with cetuximab, the response rate for those with wild-type *KRAS* was significantly higher than that for those with mutant *KRAS* (OR= 7.32, 95% CI: 4.22 to 12.72, heterogeneity $P = 0.0005$, Fig. 2).

Progression-free survival

Three eligible trials[24,28,31] contained data on progression-free survival of 334 patients with wild-type *KRAS* and 212 patients with mutant *KRAS*. Patients with wild-type *KRAS* had a statistically significant improvement in progression-free survival compared with their counterparts with mutant *KRAS* (HR = 0.44, 95% CI: 0.30 to 0.63, heterogeneity $P = 0.10$, Fig. 3A).

Overall survival

Three eligible trials[21,24,32] contained data on overall survival of 183 patients with wild-type *KRAS* and 105 patients with mutant *KRAS*. Patients with wild-type *KRAS* had a statistically significant improvement in overall survival compared with their counterparts with mutant *KRAS* (HR = 0.51, 95% CI: 0.38 to 0.69, heterogeneity $P = 0.41$, Fig. 3B).

Outcomes of patients with mutant *KRAS* treated with versus without cetuximab

Overall response rate

Data of overall response rate were available from four studies.[25,28,33-34] The overall response rate for patients with mutant *KRAS* treated with cetuximab was 30.1% (101/336), while that for those treated without cetuximab was 37.5% (122/325). As can be seen, among patients with mutant *KRAS*, the response rate for those with cetuximab treatment is lower than that for those without cetuximab treatment (OR = 0.67, 95% CI: 0.47 to 0.95, heterogeneity $P = 0.54$, Fig. 4A).

Progression-free survival

Three eligible trials[25,28,34] contained data on progression-free survival of 712 patients treated with cetuximab and 455 patients treated without cetuximab. There was no significant difference in progression-free survival between patients with mutant *KRAS* treated with and without cetuximab (HR = 1.19, 95% CI: 0.85 to 1.66, heterogeneity $P = 0.12$, Fig. 4B).

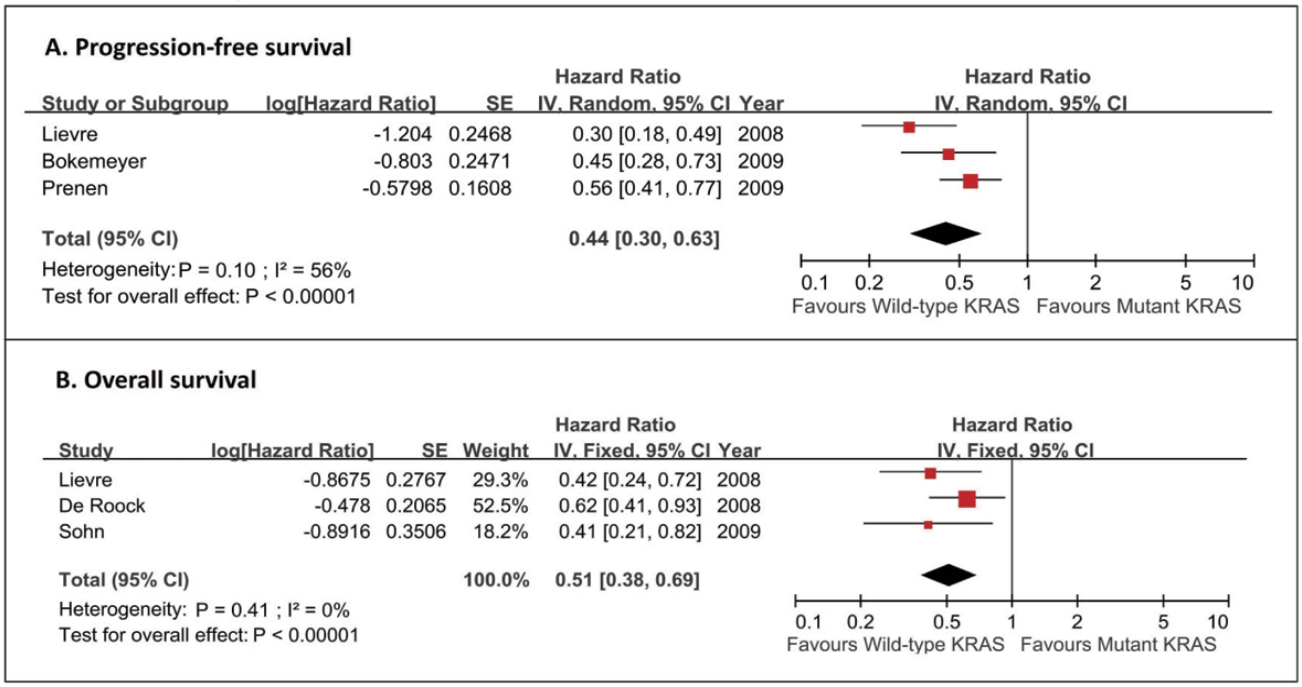

Fig. 3 Meta-analysis of survival for *KRAS* wild-type and *KRAS* mutated patients treated with cetuximab. *KRAS* wild-type patients had a statistically significant improvement in progression-free survival (A) and overall survival (B) compared with *KRAS* mutated patients. Hazard ratios were calculated using the random-effects (A) and fixed-effects models (B).

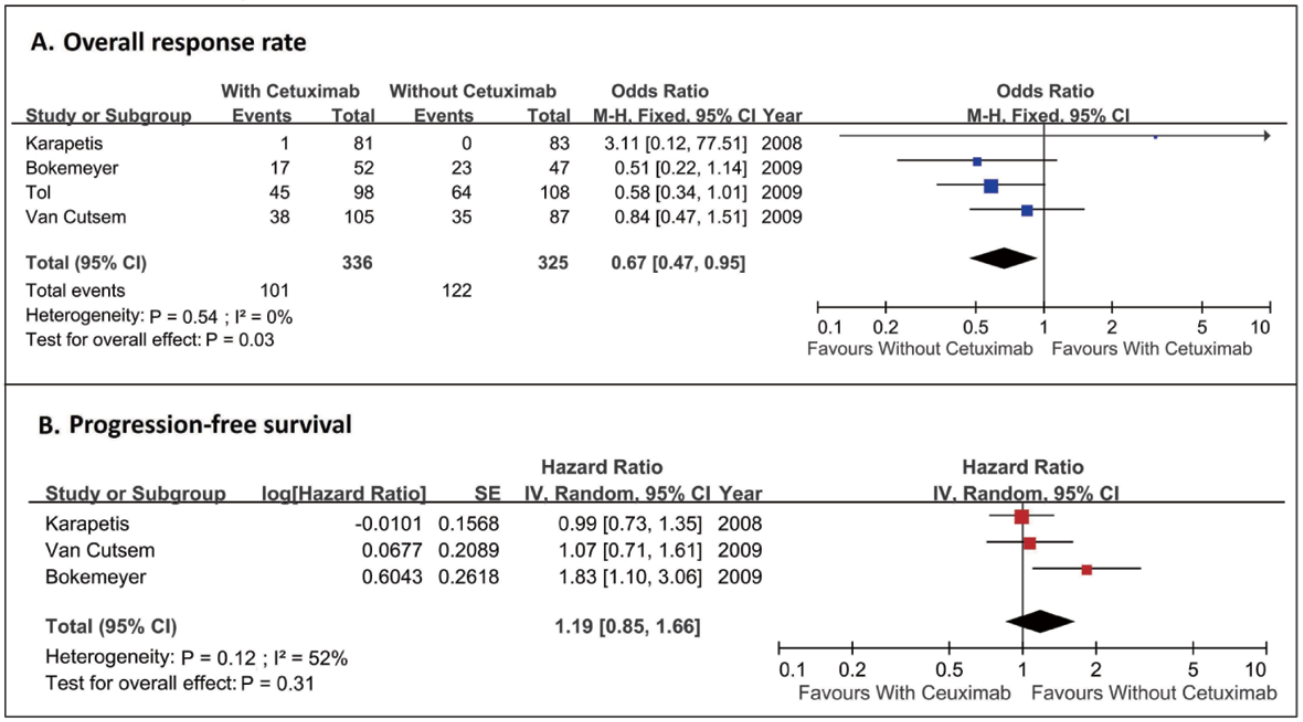

Fig. 4 Meta-analysis of overall response rate and progression-free survival for *KRAS* mutated patients treated with and without cetuximab. *KRAS* mutated patients had significantly lower overall response rate in the cetuximab group than in the non-cetuximab group (A), and there was no significant difference in progression-free survival (B). Odds ratios and Hazard ratios were calculated using the fixed-effects and random-effects models, respectively.

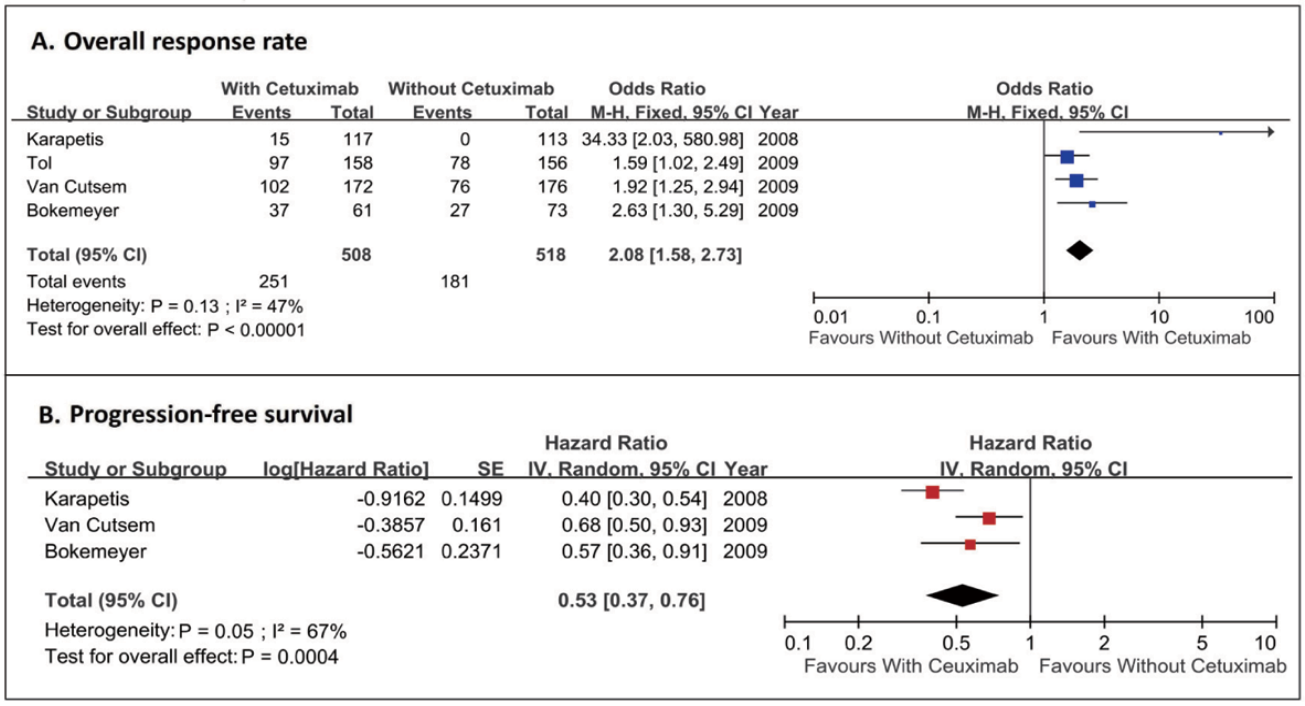

Fig. 5 Meta-analysis of overall response rate and progression-free survival for *KRAS* wild-type patients treated with and without cetuximab. Overall response rate (A) and progression-free survival (B) of *KRAS* wild-type patients treated with cetuximab were significantly higher than that of patients treated without cetuximab. Odds ratios and Hazard ratios were calculated using the fixed-effects and random-effects models, respectively.

Outcomes of patients with wild-type *KRAS* treated with versus without cetuximab

Overall response rate

Data of overall response rate data were available from four studies.[25,28,33-34] The overall response rate for patients treated with cetuximab was 49.4% (251/508), while that for patients treated without cetuximab was 34.9% (181/518). As can be seen, among patients with wild-type *KRAS*, cetuximab treatment achieved a statistically significant improvement in response rate compared with non-cetuximab treatment (OR = 2.08, 95% CI: 1.58 to 2.73, heterogeneity $P = 0.13$, Fig. 5A).

Progression-free survival

Three eligible trials[25,28,34] contained data on progression-free survival of 712 patients treated with cetuximab and 455 patients treated without cetuximab. Among patients with wild-type *KRAS*, cetuximab treatment achieved a statistically significant improvement in progression-free survival compared with non-cetuximab treatment (HR = 0.53, 95% CI: 0.37 to 0.76, heterogeneity $P = 0.05$, Fig. 5B).

Assessment of publication bias

In our study, publication bias was assessed through visual inspection of the funnel plots of outcomes. Visual inspection of the funnel plots showed no substantial evidence of publication bias, except the one shown Fig. 6, which included studies for the overall response rate of patients with wild-type and mutant *KRAS* treated with cetuximab. This plot tended to cluster toward the right, suggesting the possibility of publication bias. However, this might just reflect what the American Society of Clinical Oncology (ASCO) has recently confirmed;[39] that is, patients with mutant *KRAS* showed no response to cetuximab treatment.

DISCUSSION

The meta-analysis of Liu et al.[13] has shown that overall response rate of CRC patients in the cetuximab group (40.0%) is higher with statistical significance, compared with that in the non-cetuximab group (34.5%). Only 5.5% of CRC patients benefit from the treatment of cetuximab, suggesting that a reliable molecule or biomarker is needed to identify patients suitable for cetuximab treatment.

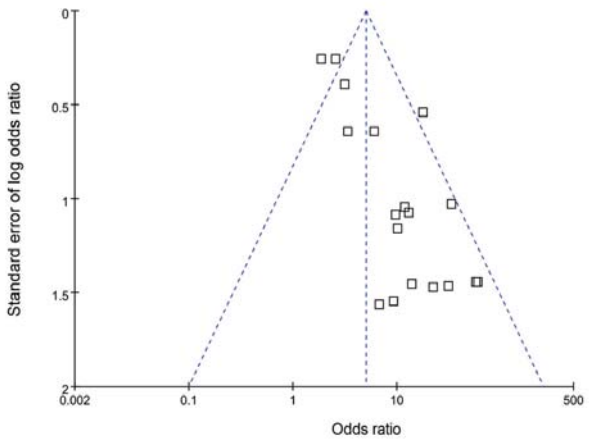

Fig. 6 Funnel plot of the 18 included studies for the overall response rate of *KRAS* wild-type and *KRAS* mutated patients treated with cetuximab.

The study of Di Fiore *et al*.[22] has confirmed the predictive value of *KRAS* mutation in metastatic CRC patients treated with cetuximab. Linardou *et al*.[3] have also proven that the overall response rate of cetuximab-treated patients with wild-type *KRAS* is better than those with mutant *KRAS*.

To evaluate the efficacy of cetuximab in patients with mutant *KRAS* in terms of overall response rate and progression-free survival, we have systematically integrated currently available clinical trial data[17-19,21-35] containing *KRAS* mutation status in CRC patients treated with cetuximab. In addition to previous meta-analysis[20] of overall response rate for cetuximab-treated patients with wild-type *KRAS* and mutant *KRAS*, we have also included progression-free survival and overall survival in this study. Our study combined 18 clinical trial data[17-19,21-35] that included a total of 2,875 patients, comprising 1,111 with mutant *KRAS* and 1764 with wild-type *KRAS*.

This study confirmed that patients having wild-type *KRAS* treated with cetuximab had significantly higher overall response rate (OR= 2.08, 95% CI: 1.58 to 2.73) and progression-free survival (HR= 0.52, 95% CI: 0.43 to 0.63) than those treated without cetuximab. In addition, our results showed that patients with wild-type *KRAS* had a statistically significant improvement in overall response rate (OR= 5.01, 95% CI: 3.89 to 6.45) compared with patients with mutant *KRAS*. As for survival, the results showed that patients with wild-type *KRAS* had significantly higher progression-free survival (HR= 0.46, 95% CI: 0.37 to 0.58) and overall survival (HR= 0.51, 95% CI: 0.38 to 0.69) compared with patients with mutant *KRAS*. These data confirmed that *KRAS* mutation status could serve as a reliable biomarker in predicting the efficacy of cetuximab treatment in CRC patients. Thus, it is wise to test *KRAS* mutation in colorectal cancer patients before initiating cetuximab therapy.

Unexpectedly, among CRC patients with mutant *KRAS* in this study, the cetuximab group showed a lower overall response rate than the non-cetuximab group (OR = 0.67, 95%CI: 0.47 to 0.95). In contrast, there was no significant difference in progression-free survival (HR = 1.14, 95% CI: 0.91 to 1.42). This result is consistent with the latest meta-analysis of the effect of *KRAS* mutation on cetuximab-based therapy for CRC (OR= 0.67, 95% CI: 0.50 to 0.90).[40] Thus, we recommend that CRC patients with mutant *KRAS* should not be treated with cetuximab, regardless whether it is financed by insurance or at their own expense.

The molecular mechanism by which CRC patients with mutant *KRAS* show a lower overall response rate to cetuximab treatment remains unclear. One possible explanation for this is that colorectal tumor cells with *KRAS* mutation are less sensitive to cetuximab-combined chemotherapy than chemotherapy alone. The recent study of de Bruijn *et al*. has shown that *KRAS* mutation sensitizes colorectal tumor cells to chemotherapy by p53-dependent induction of Noxa. Noxa suppression does not affect tumor growth *per se*, but reduces strongly the response of these tumors to chemotherapy.[41] Moreover, it has been shown that cetuximab in culture medium decreases expression of human HIF1A protein[42] and HIF1A is involved in activity of promoter fragment from Noxa gene.[43] Thus, it is possible that cetuximab desensitize the tumor cells with *KRAS* mutation to cetuximab-combined chemotherapy by decreasing the expression of HIF1A. HIF1A induces the expression of Noxa gene. The HIF1A-mediated suppression of Noxa by cetuximab may lead to a lower overall response rate in the cetuximab-combined chemotherapy.

As mentioned previously, CRC patients having wild-type *KRAS* treated with cetuximab showed a significant increase in overall response rate and progression-free survival. However, there are also some evidences that patients with wild-type *KRAS* do not respond to cetuximab. This suggests that besides *KRAS* mutation, there are other factors that affect the efficacy of cetuximab in CRC patients with wild-type *KRAS*.

There are also recent evidences indicating that mutations in B-type Raf kinase,[21] PI3K genes,[44] and EGFR gene copy numbers[45] are also significantly associated with the efficacy of cetuximab in CRC patients. Further

research is required to investigate the role of these genes in predicting the efficacy of cetuximab therapy in CRC patients when cetuximab or EGFR-targeted therapy is considered.

CONCLUSIONS

Patients having mutant *KRAS* treated with cetuximab show significant decrease in overall response rate but no significant difference in progression-free survival. However, patients having wild-type *KRAS* treated with cetuximab show significant increase in both progression-free survival and overall survival.

ACKNOWLEDGEMENTS

This study was supported by grants from the National Defense Medical Center (DOD 97-T11-05 and DOD98-14-08-021), Taipei, Taiwan, ROC.

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