Clinical outcome of epidermal growth factor receptor-tyrosine kinase inhibitors therapy for patients with overlapping kirsten rat sarcoma 2 viral oncogene homolog and epidermal growth factor receptor gene mutations

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Abstract
Background: Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) is the second most common mutated gene following epidermal growth factor receptor (EGFR) mutation in Chinese lung adenocarcinoma (LADC) patients. Investigating the clinical characteristics and outcomes of patients with co-existing KRAS and EGFR mutations can provide significant information for suitable therapies.

Methods: We retrospectively investigated 2106 LADC patients who had undergone EGFR and KRAS mutation tests at the Peking University Cancer Hospital. Only advanced LADC patients who carried KRAS and/or EGFR mutations, received EGFR-tyrosine kinase inhibitors (TKIs) and/or chemotherapy, and had completed follow-up analysis were analyzed further. KRAS and EGFR mutations were tested by denaturing high-performance liquid chromatography.

Results: A KRAS mutation was detected in 123 out of 2106 LADC patients (5.8%) and 38 (1.8%) had a concurrent EGFR mutation. Seventy-two of 123 patients were advanced cases, which were divided into two sub-groups according to EGFR mutation status: overlapping KRAS and EGFR mutations (n = 24) and KRAS mutation alone (n = 48). Clinical characteristics of the two subgroups were similar. A greater ratio of patients with double mutations received EGFR-TKIs compared to KRAS mutation alone (75% vs. 43.8%, P = 0.012), and obtained a better objective response rate (38.9% vs. 9.5%, P = 0.027) and longer progression-free survival (8.0 vs. 1.5 months, P = 0.028) following EGFR-TKIs therapy. However, these differences were not observed in patients treated with platinum-based chemotherapy.

Conclusions: Overlapping KRAS and EGFR mutations occurred in 1.8% of Chinese LADC patients studied. The co-presence of EGFR mutations could predict a clinical benefit from EGFR-TKIs treatment for patients with KRAS mutations.

Introduction
Non-small cell lung cancer (NSCLC) has been well recognized as a diverse disease based on the identification of serial driver genes and the existence of intra-tumor genetic heterogeneity.1,2 Recently, sub-clonal populations have been identified within single biopsy specimens of naïve-treatment lung cancer patients.3–5 Yang et al. reported the co-existence of epidermal growth factor receptor (EGFR) mutations with anaplastic lymphoma kinase (ALK) fusion in treatment-naive NSCLC tumors.6 Several studies (including our previous studies) have also shown that T790M may co-exist with the EGFR mutation in cancer cells or tumor tissue samples before EGFR-tyrosine kinase inhibitors (TKIs) treatment.7,8 Increasing evidence has indicated that the presence of sub-clones in EGFR-mutated tumors may influence the therapeutic efficacy of EGFR-TKIs.5,9,10

The Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) mutation is the most common gene aberrance in Caucasian NSCLC patients, and the second most common somatic mutation following EGFR mutation in Chinese patients with lung adenocarcinoma (LADC). However, despite 40 years of
research, the prognostic and predictive roles of \textit{KRAS} mutations with respect to EGFR-TKIs treatment and chemotherapy have been being controversial because of inconsistent results reported between trials and meta-analyses.\textsuperscript{11} Several studies have shown that \textit{KRAS} mutations can be a negative predictor for EGFR-TKIs therapy.\textsuperscript{12,13} However, a retrospective study using a random-matching method based on tumor node metastasis (TNM) stage, histology, and \textit{KRAS}/EGFR status displayed that \textit{KRAS} mutation is a poor prognostic factor, but is not an independent predictor of response to EGFR-TKIs or chemotherapy in patients with lung cancer.\textsuperscript{14} A recent pooled analysis of 1543 patients from four studies further indicated that neither \textit{KRAS} wild-type nor codon 12 mutations had any predictive value to adjuvant chemotherapy.\textsuperscript{15} \textit{KRAS} mutations cannot be recommended for selecting patients with NSCLC for adjuvant chemotherapy.\textsuperscript{16}

Given that \textit{EGFR} and \textit{KRAS} are the two most common driver genes in Chinese lung adenocarcinoma, it is crucial to investigate their association with each other and clinical characteristics, especially as the inhibitors that target \textit{KRAS} and its downstream pathway will be incorporated into clinical practice in the near future.\textsuperscript{17–20} \textit{KRAS} and \textit{EGFR} mutations were reported to be mutually exclusive in lung cancer.\textsuperscript{21} However, Gumerlock \textit{et al}. reported four patients with both \textit{KRAS} and \textit{EGFR} mutations at the American Society of Clinical Oncology annual meeting in 2005.\textsuperscript{22} Our previous study showed coexisting \textit{KRAS} and \textit{EGFR} mutations in five out of 273 patients with lung adenocarcinoma.\textsuperscript{23} In 2014, Li \textit{et al}. reported that 30 out of 5125 Chinese patients with NSCLC concurrently harbored \textit{EGFR} and \textit{KRAS} mutations.\textsuperscript{24}

Because of the low incidence of patients manifesting these double mutations, to date there are no reports comparing clinical characteristics and responses to EGFR-TKIs or chemotherapy for patients harboring \textit{KRAS} mutations with or without \textit{EGFR} mutations. Here, we analyzed the clinical significance of double mutations of advanced LADC with respect to EGFR-TKIs and chemotherapy.

**Materials and methods**

**Study population**

All patients included in this retrospective study were diagnosed and treated at the Peking University Cancer Hospital between 1 January 2004 and 31 December 2013. A total of 2106 LADC patients who underwent \textit{EGFR} and \textit{KRAS} mutation tests were screened and the analysis focused on patients who met the following criteria: (i) harboring a \textit{KRAS} mutation with/without \textit{EGFR} mutational status; (ii) received EGFR-TKIs and/or chemotherapy; and (iii) completed follow-up analysis. For all patients, laboratory data was obtained and recorded independently, and blinded from clinical review until final analyses.

The institutional review board of the Peking University Cancer Hospital approved the study. All patients provided written informed consent for the procurement of tumor specimens.

**Mutational analysis**

Epidermal growth factor receptor and \textit{KRAS} mutations were assessed by denaturing high-performance liquid chromatography (DHPLC) based on polymerase chain reaction, which detects \textit{EGFR} exon 19 and exon 21, and \textit{KRAS} exon 2, as described previously.\textsuperscript{24–25} In patients with mutated sub-types that could not be determined by DHPLC, the amplification-refractory mutation system was used for re-analysis.

**Data collection**

We collected clinical variables for all patients from the database, including age, gender, Eastern Cooperative Oncology Group (ECOG) performance status (PS), TNM stage, and smoking status (smoker or non-smoker). A non-smoker was defined as a patient who smoked less than 100 cigarettes in a lifetime. The patients’ treatment histories were recorded, including whether they had received EGFR-TKIs (gefitinib, erlotinib or icotinib) and/or platinum-based doublet chemotherapy.\textsuperscript{26} Patients with unknown treatment histories were excluded from therapeutic analyses.

**Statistical analysis**

Patient demographics (excluding age), clinical characteristics, treatment histories, and responses to treatments were compared using the chi-square test. The student’s \textit{t}-test was used for age comparison. Up to 16 May 2014, the follow-up time of patients who were still alive was calculated from the date of the first treatment to the last available follow-up date. Overall survival (OS) was defined as the date of diagnosis of advanced lung adenocarcinoma to the date of death or the last available follow-up. Progression-free survival (PFS) was defined as the time from initial treatment to the time of disease progression or the date of last follow-up. OS and PFS for EGFR-TKIs and chemotherapy were estimated using the Kaplan–Meier method and were compared across groups using the log-rank test. Cox regression univariate analysis was used to evaluate every variable to PFS and OS. The statistically significant variables in univariate analysis, age and gender were used in the proportional hazard model for multivariate analysis. SSPS 2.0 was used for statistics (IBM Corp., Armonk, NY, USA). \textit{P}<0.05 was defined as statistically significant in regard to differences.
Results

Clinical characteristics

Among the 2106 LADC patients who underwent EGFR and KRAS analysis, 123 (5.8%) had KRAS mutations, including 38 patients (38/2106, 1.8%) harboring both EGFR and KRAS mutations. Most of the KRAS-mutated patients were diagnosed with stage IIIIB and IV disease (72 of 123, 58.5%). Of the 72 patients with locally advanced and advanced LADC, the median age was 56 years (inter-quartile range, 11); 48 cases presented KRAS mutations alone, and 24 carried overlapping KRAS and EGFR mutations. In patients with overlapping KRAS and EGFR mutations, there were more non-smokers (62.5%) compared to those with KRAS mutations alone (52.1%), but the difference did not reach statistical significance (Table 1).

Subsequent analyses focused on the 72 patients who were diagnosed with advanced LADC harboring KRAS mutations. A total of 39 patients received EGFR-TKIs therapy, including 18 with double mutations and 21 with a single mutation, most of which (69.2%) were second-line therapies or beyond. Of the 21 patients with a single KRAS mutation who received EGFR-TKIs as first-line therapy, one was enrolled in an IPASS clinical trial, one refused chemotherapy, and three other patients could not tolerate the toxicity of chemotherapy. Of the total 72 patients, 65 received chemotherapy and 32 patients had both EGFR-TKIs treatment and chemotherapy. Patients with overlapping KRAS and EGFR mutations were significantly more likely to receive EGFR-TKIs treatment compared with patients harboring KRAS mutations alone (75% vs. 43.8%; P = 0.012), including seven cases who were treated with first-line EGFR-TKIs and 11 cases treated with second-line or beyond. However, no differences were observed between these two subgroups of patients for those selected to receive platinum-based doublet chemotherapies (83.3% vs. 93.8%; P = 0.325) (Table 1).

Association of overlapping Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) and epidermal growth factor receptor (EGFR) mutations with EGFR-tyrosine kinase inhibitors therapy

On 16 May 2014, 49 out of the 72 patients (68.1%) had died, 15 patients were still alive, and eight patients did not maintain follow-up. The median follow-up was 18 months (inter-quartile range 19.75 months).

We analyzed ORR and PFS in the 39 patients treated with EGFR-TKIs. The ORR and median PFS were 23.1% and 5.5 months (95% confidence interval [CI], 0.40–10.60 months), respectively. For patients whose tumors carried both KRAS and EGFR mutations (n = 18), the ORR and median PFS was significantly longer after EGFR-TKIs treatment compared to those with KRAS mutations alone (n = 21) (ORR 38.9% vs. 9.5%, P = 0.027; median PFS, 8 months, 95% CI, 1.76–14.24 vs. 1.5 months, 95% CI, 0.60–2.40 months, P = 0.028) (Table 2 and Fig 1).

Overall survival was also analyzed according to genotype. The median OS for the 39 patients who had received EGFR-TKIs treatment was 27 months (95% CI, 23.07–30.93 months). The median OS for patients whose tumors had overlapping KRAS and EGFR mutations was longer (29.5 months, 95% CI, 5.79–53.21 months) compared with patients carrying KRAS mutations alone (25 months; 95% CI, 21.09–28.91 months), but there was no significant difference (P = 0.084).

Association of overlapping KRAS and EGFR mutations with chemotherapy

We then analyzed PFS in the platinum-based doublet chemotherapy population. The ORR and median PFS for the 65 patients who received platinum-based doublet chemotherapy were 23.1% and four months (95% CI, 2.61–5.39 months), respectively. For patients who harbored both KRAS and EGFR mutations, the ORR and median PFS were 30% and 4.5 months (95% CI, 2.49–6.51 months), respectively, and were comparable to those without EGFR mutations (ORR 20%; median PFS 3 months, 95% CI, 1.60–4.40 months).

Table 1 The clinical characteristics of patients with advanced adenocarcinoma harboring KRAS mutation

| Characteristic       | KRAS     | KRAS & EGFR | P-value |
|----------------------|----------|-------------|---------|
| Age, median (QR)     | 56 (15.75)| 56.5 (11)   | 0.537** |
| Gender               |          |             |         |
| Male                 | 32 (66.7)| 13 (54.2)   | 0.302** |
| Female               | 16 (33.3)| 41 (45.8)   |         |
| PS                   |          |             |         |
| 0–1                  | 42 (87.5)| 24 (100)    | 0.344** |
| 2–3                  | 4 (8.3)  | 0           |         |
| Unknown              | 2 (4.2)  | 0           |         |
| Smoking              |          |             | 0.578** |
| Smoker               | 20 (41.7)| 9 (37.5)    |         |
| Non-smoker           | 25 (52.1)| 15 (62.5)   |         |
| Unknown              | 3 (6.3)  | 0           |         |
| EGFR-TKIs            | 21 (43.8)| 18 (75)     | 0.012** |
| Chemotherapy         | 45 (93.8)| 20 (83.3)   | 0.325** |

*P-value was estimated by t-test; **P-value was estimated by chi-square test. Age, reported in years; chemotherapy, platinum-based doublet chemotherapy; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors treatment; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog mutation; KRAS & EGFR, KRAS mutation coexisting with EGFR mutation; N, number of patients; QR, inter-quartile range; PS, performance status.
months) \( (P = 0.829) \). The median OS for the total 65 patients who accepted platinum-based doublet chemotherapy was 23 months (95% CI, 19.28–26.72 months). The median OS for the double-mutated patients was similar to that of patients with \( \text{KRAS} \) mutations alone (24 months, 95% CI, 19.64–28.36 vs. 23 months, 95% CI, 13.24–32.76, \( P = 0.122 \)).

### Univariate and multivariate analyses

Finally, we evaluated each clinical and genetic variable, including gender, age, PS, smoking status, and \( \text{KRAS} \) and \( \text{EGFR} \) mutations, to determine their impact on survival outcomes. In univariate Cox regression analysis, gender and PS (0–1/2–3) were associated with OS (hazard ratio [HR] 0.467, 95% CI, 0.25–0.88; \( P = 0.018 \) and HR 0.159, 95% CI, 0.04–0.59; \( P = 0.006 \), respectively); however, only \( \text{EGFR} \) mutation was associated with PFS (HR 0.497, 95% CI, 0.26–0.97; \( P = 0.040 \)) in \( \text{EGFR-TKIs} \) treated patients (Table 3).

Notably, in univariate analysis, none of these factors (age, smoking, PS, \( \text{EGFR} \) mutation) were observed to have a significant association with PFS in patients treated with platinum-based doublet chemotherapy (Table 3).

Multivariate Cox regression models were used to assess the predictive effect on OS of each clinical parameter (age,
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Table 3 Clinical variables and EGFR mutation associated with PFS in chemotherapy or TKIs treatment and OS: Univariate analysis

| Variables | PFS (chemotherapy) N = 65 | PFS (EGFR-TKI) N = 39 | OS (All Patients) N = 72 |
|-----------|---------------------------|------------------------|--------------------------|
| Age (≤56/ > 56) | 0.695 | 0.41–1.19 | 0.184 | 1.426 | 0.74–2.74 | 0.287 | 0.711 | 0.40–1.26 | 0.244 |
| Gender (female/male) | 0.915 | 0.52–1.61 | 0.757 | 0.736 | 0.38–1.42 | 0.363 | 0.467 | 0.25–0.88 | 0.018 |
| PS (0/1) | 0.359 | 0.11–1.18 | 0.091 | 0.247 | 0.03–1.95 | 0.185 | 0.159 | 0.04–0.59 | 0.006 |
| Smoking (non-smoker/smoker) | 1.297 | 0.75–2.25 | 0.355 | 0.857 | 0.42–1.77 | 0.676 | 0.626 | 0.35–1.12 | 0.115 |
| Group (KRAS & EGFR/KRAS) | 0.943 | 0.53–1.66 | 0.839 | 0.497 | 0.26–0.97 | 0.040 | 0.570 | 0.309–1.05 | 0.072 |

P-value estimated by univariate cox regression analysis. Bold type indicates P < 0.05. Age, reported in years; chemotherapy, platinum-based doublet chemotherapy; CI, confidence interval; EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors treatment; HR, hazard ratio; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog mutation; KRAS & EGFR, KRAS mutation coexisting with EGFR mutation; N, number of patients; OS, overall survival; PFS, progression-free survival; PS, performance status.

Discussion

Coexisting EGFR and KRAS mutations have been reported by several investigators in a minority of the NSCLC population, although previous reports have indicated that these two genes were mutually exclusive. From 2005 to 2014, there were 12 case reports involving 60 patients with overlapping EGFR and KRAS mutations, and 25 cases who underwent EGFR-TKIs treatment. Seven patients presented a positive response with partial or complete remission, while others did not benefit from EGFR-TKIs treatment. However, the number of patients in these reports was too small to make any relevant analysis.

In our study, we analyzed the data of 38 (38/2106, 1.8%) lung adenocarcinoma patients with overlapping KRAS and EGFR mutations, which, to the best of our knowledge, is the largest cohort to date. We analyzed the clinical outcomes of 24 advanced adenocarcinoma patients with co-existing EGFR and KRAS mutations and 48 patients with KRAS mutations alone who had received EGFR-TKIs treatment or/platinum-based doublet chemotherapy. The results showed that more patients with double mutations received EGFR-TKIs treatment, and obtained a better response with longer PFS and OS compared with those carrying KRAS mutations alone. However, these differences were not observed in patients treated with platinum-based doublet chemotherapy between KRAS-mutated patients with or without EGFR mutations.

Our study showed that ORR, PFS, and OS in patients with co-existing KRAS and EGFR mutations after EGFR-TKIs treatment were superior to those with KRAS mutations alone. Interestingly, the median PFS and OS (8 and 29.5 months, respectively) in this subgroup were similar to the results of a serial prospective clinical studies in which EGFR-mutated patients received EGFR-TKIs therapy (PFS 9.2–13.1 months, OS 19.3–30.9 months), but ORR (38.9%) was inferior to the results of these studies. A possible reason for the lower ORR might be that most patients in this subgroup received EGFR-TKIs as second-line or further therapy. Several clinical trials have shown that EGFR-TKIs as second or third-line therapy presented a response of 30–60% in EGFR-mutated patients, which may be attributed to the dynamic alteration of EGFR mutations after chemotherapy in heterogeneous tumors. Further investigations are, therefore, needed.

Multivariate analysis revealed that gender and PS status were independent prognostic factors in patients with overlapping KRAS and EGFR mutations, which is consistent with the historical data observed in NSCLC. For the specific genotype of patients with overlapping KRAS and EGFR mutations, EGFR mutation, but not KRAS mutation, was associated with an efficient response to EGFR-TKIs therapy, suggesting that EGFR mutations are more effective in predicting a clinical benefit from EGFR-TKIs treatment in this genotype of patients with concurrent KRAS and EGFR mutations.

Table 4 Clinical variables and EGFR mutations associated with overall survival: Multivariate analysis

| Variable | HR | 95% CI | P |
|----------|----|--------|---|
| PS (0/1) | 0.18 | 0.05–0.67 | 0.01 |
| Gender (female/male) | 0.515 | 0.27–0.97 | 0.04 |

P-value estimated by Cox-regression. Multivariate analysis by Cox regression, included age (ages≤56/ > 56), gender (female/male), performance status (PS) (0–1/2–3), smoking (non-smoker/smoker), and group (KRAS & EGFR/KRAS). CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; KRAS, Kirsten rat sarcoma 2 viral oncogene homolog mutation; KRAS & EGFR, KRAS mutation coexisting with EGFR mutation.
Despite initial studies reporting KRAS as a potential predictive marker to chemotherapy resistance, these studies were small and frequently did not have untreated control arms. Several randomized clinical trials involving adjuvant platinum-based chemotherapy versus untreated control arms in completely postoperative NSCLC analyzed the impact of KRAS mutation on chemotherapy, and negative results were observed. The present study has shown that patients with co-existing KRAS and EGFR mutations had a similar PFS and ORR following platinum-based doublet chemotherapy to those harboring KRAS mutations alone. Thus, neither EGFR nor KRAS mutations predicts longer PFS in patients with NSCLC receiving platinum-based doublet chemotherapy.

Limitations of this study included small sample size and the retrospective nature, with a large span of therapeutic time. In addition, a portion of these patients were treated from January 2004 to December 2008, during which time an EGFR mutation was not identified as a strong predictor for EGFR-TKIs therapy. Patients with certain clinicopathological characteristics, such as women, non-smokers, and adenocarcinoma, were thought to be a population favorable to EGFR-TKIs therapy. This is the main reason why patients with KRAS mutations received EGFR-TKIs therapy. In addition, enrollment in a clinical trial (IPASS) or intolerance of chemotherapy suggested that patients with a single EGFR mutation should receive EGFR-TKIs treatment.

Conclusions

Our results indicate that EGFR and KRAS mutations can co-exist in LADC tumors. Furthermore, the co-existing EGFR mutation in KRAS-mutated patients is a predictive factor for a better response and prolonged PFS following EGFR-TKIs treatment. However, this is not the case for platinum-based doublet chemotherapy in advanced LADC patients.

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Disclosure

No authors report any conflict of interest.

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