Impact of KRAS mutation on response and outcome of patients with stage III non-squamous non-small cell lung cancer

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Key words
Biomarkers, chemoradiotherapy, KRAS, non-small cell lung cancer, relapse

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Lung cancer remains the leading cause of cancer-related deaths worldwide.1) Non-small cell lung cancer (NSCLC) accounts for 80% of all lung cancer cases, and approximately 30% of patients with NSCLC present with stage III disease.2,3 For patients with a good performance status and adequate organ function, combined chemotherapy plus radiotherapy (RT) is the standard of care.4,5 Combined platinum-containing chemotherapy with concurrent radiotherapy (CRT) has been reported to offer a median survival time of approximately 20 months.6–10

The Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation is one of the most frequently observed somatic mutations in NSCLC, particularly non-squamous NSCLC. Hotspot KRAS mutations induce the irreversible and continuous activation of RAS-dependent downstream signals.11) The impact of KRAS mutations in NSCLC was reported over 20 years ago as being associated with a poor prognosis.12) Since then, the clinical significance of the KRAS mutation has been widely studied.13–16 Since, however, the results of studies have not been consistent, probably because of the heterogeneity of patients included in the analyses. Thus, association studies for KRAS mutations should be performed in a cohort of patients with a defined progressive status who are receiving a standard therapy.

In the present study, the prevalence of KRAS mutations and their impact on the therapeutic responses and outcomes were examined in a patient cohort with stage III non-squamous NSCLC. All the patients received definitive CRT at a single hospital. The impact of KRAS mutation on the therapeutic responses and outcomes was examined.

Materials and Methods

 Patients. Between January 2001 and December 2010, a total of 528 NSCLC patients received CRT at the National Cancer Center Hospital, Japan. Under an institutional review board-approved protocol, we reviewed the medical records of these patients.
patients (approval number: 2012-187). During the review, we identified 274 patients with unresectable stage III non-squamous NSCLC. We excluded patients with epidermal growth factor receptor (EGFR)-activating mutations because we had observed a characteristic effect of EGFR mutation on the pattern of recurrence and patient outcome among patients with stage III non-squamous NSCLC.\(^{(17)}\)

The following data regarding the pretreatment patient characteristics were collected: patient age, sex, Eastern Cooperative Oncology Group (ECOG) performance status (PS) and smoking history. The tumor characteristics were noted, including the histology and TNM stage, according to the sixth edition of the Union for International Cancer Control. T and N staging was based on computed tomography (CT) findings, \(^{[18F]}\)fluorodeoxyglucose PET findings, and a pathological diagnosis of N2 based on the results of invasive procedures, if applicable. Data on the treatment characteristics, including the radiation dose, the timing of RT (concurrent or sequential), the chemotherapy regimens, the number of chemotherapy cycles and the treatment after disease relapse were also collected.

**KRAS mutational analysis.** Screening for KRAS mutations in exon 2 (codon 12 and 13) was performed using cytological specimens or paraffin-embedded tumor specimens and a high-resolution melting analysis, as previously described.\(^{(18)}\) All the KRAS mutational statuses were determined using tumor specimens obtained at the time of first diagnosis. For tumors with an unknown KRAS mutational status, we analyzed specimens obtained before the initial treatment for the purpose of this study. All tumor specimens were checked by HE stain for tumor content before analyses.

**Efficacy analysis.** Tumor responses were classified according to the Response Evaluation Criteria for Solid Tumors (RECIST), version 1.1. In compliance with the protocols of clinical trials or clinical practice, all the patients were regularly followed up every 1 to 2 months in the outpatient department. Regular work-ups were performed every 3 to 6 months within the first year after the completion of CRT and were subsequently performed every 6 months. Regular systemic work-ups included chest X-ray, chest and abdominal CT, brain CT or MRI and PET examinations, as needed. Relapse-free survival (RFS) was defined as the time from the first day of chemotherapy to the detection of the earliest signs of disease progression using CT or MRI, or death from any cause. The time to local relapse (TTLR) and the time to distant relapse (TTDR) were defined as the time from the first day of chemotherapy until the detection of the earliest signs of disease progression within and outside of the field of radiotherapy, respectively. Overall survival was defined as the time from the first day of chemotherapy until the last day on which the patient was confirmed to be alive or dead from any cause, and survival post-progression (SPP) was calculated by subtracting the RFS from the OS, as previously described.\(^{(19)}\)

**Statistical analysis.** Differences between covariates among NSCLC patients with KRAS mutations and those with wild-type KRAS were analyzed using the Fisher exact test and the \(\chi^2\)-test. Clinical outcomes were analyzed using the Kaplan–Meier method, and the log-rank test was used to compare survival according to the mutational status. To investigate the association between TTLR and factors related to the patient characteristics, the Cox proportional hazards model was used. The following potential factors were investigated: KRAS mutational status, age, clinical stage (IIIA vs IIIB), and timing of RT (concurrent vs sequential). Differences with probability values of <0.05 were considered to be statistically significant. All the analyses were performed using STATA, ver. 12.0 (Stata, College Station, TX, USA).

**Results**

**KRAS mutation in the study cohort.** Among the 274 patients for whom a KRAS mutational analysis was planned, 134 patients including 16 patients with EGFR-activating mutations

| Table 1. Patient characteristics | Mutated KRAS | Wild-type KRAS | P-value† | Total |
|---------------------------------|-------------|---------------|----------|-------|
| Number of patients (%)          | 16 (13)     | 103 (87)      |          | 119   |
| Age (median range)              | 60 (41–74)  | 61 (33–76)    | 0.521    | 61 (33–76) |
| Sex (male/female)               | 12/4 (75/25)| 85/18 (83/17)| 0.337    | 97/22 (82/18) |
| ECOG performance status         | 1/15 (6/94) | 25/78 (24/76)| 0.090    | 26/93 (22/78) |
| Smoking status (pack-year)      | 1/15 (6/94) | 25/78 (24/76)| 0.090    | 26/93 (22/78) |
| Median (range)                  | 28 (0–84)   | 42 (0–150)    | 0.064    | 40 (0–150) |
| Never/former—current (%)        | 3/13 (19/81)| 14/89 (14/86)| 0.409    | 17/102 (14/86) |
| Clinical stage                  | 10/6 (63/37)| 55/48 (53/47)| 0.343    | 65/54 (55/45) |
| IIIA—IIIB (%)                   | 11/5 (69/31)| 86/17 (84/17)| 0.143    | 97/22 (82/18) |
| Histology                       |             |               |          |       |
| Adenocarcinoma/NOS (%)          |             |               |          |       |
| Type of radiotherapy            |             |               |          |       |
| Concurrent/sequential (%)       | 13/3 (81/19)| 91/12 (88/12)| 0.325    | 104/15 (87/13) |
| Radiotherapy dose (Gy)          |             |               |          |       |
| Median (range)                  | 50 (60–60)  | 60 (52–78)    | 0.979    | 60 (52–78) |

†For differences between mutated KRAS and wild-type KRAS. ECOG, Eastern Clinical Oncology Group; NOS, not otherwise specified.
were excluded from the present study either because a tumor specimen was not available (47 cases) or the specimen was insufficient for the analysis (87 cases; Suppl. Fig. S1). In addition, 21 patients with EGFR-activating mutations were excluded because of the distinct response patterns and patient outcomes that have been observed among this population. (17)

The remaining 119 patients were subjected to the KRAS mutation screening: 16 patients (13%) had KRAS mutations, while 103 patients (87%) had wild-type KRAS.

**KRAS mutation and patient baseline characteristics.** The baseline patient characteristics are shown in Table 1. Among the 119 patients for whom a KRAS mutational analysis was performed, the median age was 61 years (range: 33–76 years); 97 patients (82%) were male, and 65 patients (55%) had clinical stage IIIA disease. No significant differences in age, sex, ECOG-PS, smoking status, clinical stage, histology, type of radiotherapy or radiotherapy dose were observed between the patients with KRAS mutations and those with wild-type KRAS. Patients with KRAS mutations had a marginally lighter smoking habit than those with wild-type KRAS, but the difference was not statistically significant (median smoking status of patients with KRAS mutations versus patients with wild-type KRAS: 28 vs 42 pack-year, \( P = 0.064 \)).

**KRAS mutation and therapeutic response.** Of the 119 patients who were analyzed, 104 (87%) had received concurrent CRT and 15 (13%) had received sequential CRT (Table S1). All the patients received platinum-containing chemotherapy regimens. The most frequently used chemotherapy regimens were cisplatin plus vinorelbine in the concurrent CRT group (86%) and carboplatin plus paclitaxel in the sequential CRT group (60%). In a phase I trial, 7 patients received nedaplatin plus paclitaxel, and, in line with a phase II trial, 5 patients received gefitinib. (6,20) These patients were included in the analysis because their survival results compared favorably to that of standard chemoradiotherapy for stage III NSCLC. The median radiation dose was 60 Gy (range, 52–78 Gy). There were 28 patients who received radiation doses of 60 Gy (66 Gy: 13 patients, 72 Gy: 13 patients, 78 Gy: 2 patients) and all these patients were included in a phase I dose-escalation trial reported previously. (9)

Patients with KRAS mutations had a lower ORR and a higher progressive disease (PD) rate than those with wild-type KRAS (Table 2, patients with KRAS mutations versus those with wild-type KRAS: 63% vs 81% for ORR, 19% vs 4% for PD).

**KRAS mutation and local/distant relapses.** A total of 96 patients (81%, 96/119) relapsed; the relapsed cases consisted of 13 patients with KRAS mutations and 83 patients with wild-type KRAS (Table 3). The frequency of local relapse was lower among the patients with KRAS mutations than among those with wild-type KRAS (8% vs 23%).

Patients with KRAS mutations tended to have a shorter median TTDR than those with wild-type KRAS (Suppl. Fig. S2a, S2b).

### Table 2. Response

| Mutated KRAS | Wild-type KRAS |
|--------------|---------------|
| Number of patients | 16 | 103 |
| Objective response rate | 10 (63%) | 83 (81%) |
| Complete response | 0 (0%) | 6 (6%) |
| Partial response | 10 (63%) | 77 (75%) |
| Stable disease | 3 (19%) | 15 (15%) |
| Progressive disease | 3 (19%) | 4 (4%) |
| Not evaluable | 0 (0%) | 1 (1%) |

### Table 3. Type of first relapse

| Mutated KRAS | Wild-type KRAS |
|--------------|---------------|
| Number of relapses | 13 | 83 |
| Local relapses | 1 (8%) | 19 (23%) |
| Mixed relapse | 3 (23%) | 16 (19%) |
| Distant relapses | 9 (69%) | 48 (58%) |
| Brain only | 6 (46%) | 10 (12%) |
| With brain | 0 (0%) | 9 (11%) |
| Without brain | 3 (23%) | 29 (35%) |

Local relapses are defined as radiologic recurrences within the range of radiation field. Distant relapses are defined as recurrences outside of the radiation field.
patients with KRAS mutations vs those with wild-type KRAS: 6.3 vs 13.0 months for median TTDR, \(P = 0.0865\), while the TTLR was similar for both groups (Suppl. Fig. S2b).

As a post-relapse treatment, 23% of the patients with KRAS mutations and 36% of the patients with wild-type KRAS received cytotoxic chemotherapy; this difference was not statistically significant (\(P = 0.278\)). Some of the patients (those treated before 2004) received EGFR-TKI as a second-line therapy (Table 4).

**KRAS mutation and survival.** Of the 119 patients who were analyzed, 82 (69%) had died by the end of the median follow-up period of 29 months (range, 3–140 months). No statistically significant differences in the 2-year relapse-free rate (patients with KRAS mutations vs those with wild-type KRAS: 18.8% vs 33.6%, \(P = 0.204\)) and the 5-year survival rate (14.6% vs 35.3%, \(P = 0.149\)) were seen according to the KRAS mutational status (Suppl. Table S2). We observed a tendency toward a shorter median RFS (Fig. 1a, 6.1 vs 10.9 months, \(P = 0.083\)) and a statistically significant shorter median SPP (Fig. 1b, 2.5 vs 7.3 months, \(P = 0.028\)) and OS (Fig. 1c, 15.1 vs 29.1 months, \(P = 0.022\)).

In a univariate analysis, the KRAS mutational status exhibited statistically significant associations with OS (\(P = 0.025\)) and SPP (\(P = 0.031\)), but not with RFS (\(P = 0.087\); Table 5). In a multivariate analysis, the KRAS mutation was more strongly associated with OS (\(P = 0.042\)) and SPP (0.035) than with age, clinical stage or timing of radiotherapy (Table 5).

**Discussion**

In this study, we demonstrated that KRAS mutation acts as a negative prognostic factor in patients with stage III non-squamous NSCLC receiving definitive CRT. A marginally weaker clinical effect in terms of RR and RFS was also observed in patients with KRAS mutation, compared with those with wild-type KRAS. These results suggest a therapeutically resistant phenotype of KRAS-mutated tumors. Patients with KRAS mutations had fewer local relapses and more brain metastases after CRT. In addition, these patients experienced a shorter TTDR than those with wild-type KRAS.

Reports describing the association between the KRAS mutation and the clinical effect of radiotherapy have been limited. In particular, its association with chemoradiotherapeutic effects in stage III NSCLC has been unclear. Broermann et al. analyzed KRAS exon 2, codon 12 mutations in 28 patients who underwent tumor resection after neoadjuvant treatment with two cycles of chemotherapy (ifosfamide, carboplatin and etoposide) and subsequent twice-daily radiotherapy (45 Gy) with concurrent carboplatin and vindesine. In their study, KRAS mutation was found to be a negative predictive and prognostic factor. Hallaqvist et al. analyzed 66 cases from two phase II studies of chemoradiotherapy for KRAS exon 2 mutation and showed that the KRAS mutation was a negative prognostic factor. In contrast, Ready et al. report an analysis of the KRAS exon 2 mutation in a clinical trial evaluating the effect of the

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**Table 4. Second-line treatment**

| Number of relapses | Mutated KRAS | Wild-type KRAS |
|--------------------|--------------|----------------|
| CT                 | 13 (23%)     | 83 (36%)       |
| Docetaxel          | 2 (67%)      | 25 (83%)       |
| Pemetrexed         | 0 (0%)       | 2 (7%)         |
| TS-1               | 0 (0%)       | 2 (7%)         |
| CBDC + PtX         | 1 (33%)      | 0 (0%)         |
| Investigational drug| 0 (0%)       | 1 (3%)         |
| EGFR-TKI           | 2 (15%)      | 11 (13%)       |
| Supportive care    | 8 (62%)      | 42 (51%)       |

**Table 5. Univariate/multivariate analysis, Cox proportional hazard model**

|                  | RFS         | OS          | SPP         |
|------------------|-------------|-------------|-------------|
| **Univariate KRAS** |             |             |             |
| Mt vs W/T Age (years) | 1.67 (0.93–3.00) | 1.98 (1.09–3.59) | 1.94 (1.06–3.53) |
| 65 vs <65 Clinical stage | 0.89 (0.59–1.35) | 0.67 (0.43–1.05) | 0.63 (0.40–1.00) |
| Stage IIIA vs IIIB Radiotherapy | 1.04 (0.69–1.55) | 0.94 (0.61–1.47) | 0.89 (0.57–1.39) |
| Seq vs Conc | 0.77 (0.43–1.35) | 0.64 (0.35–1.19) | 0.75 (0.39–1.42) |
| **Multivariate KRAS** |             |             |             |
| Mt vs W/T Age (years) | 1.69 (0.93–3.06) | 1.87 (1.02–3.42) | 1.98 (1.05–3.73) |
| 65 vs <65 Clinical stage | 0.86 (0.57–1.31) | 0.69 (0.44–1.08) | 0.63 (0.39–1.01) |
| Stage III A vs IIIB Radiotherapy | 1.09 (0.72–1.65) | 0.98 (0.62–1.54) | 0.88 (0.56–1.39) |
| Seq vs Conc | 0.78 (0.44–1.39) | 0.71 (0.37–1.33) | 1.01 (0.49–2.05) |

Conc, concurrent; Mt, mutation; OS, overall survival; W/T, wild-type; RFS, relapse-free survival; Seq, sequential; SPP, survival post-progression.
addition of gefitinib, an EGFR-TKI, to sequential or concurrent CRT in stage III NSCLC. In their study, no obvious correlation was seen between the KRAS mutation and the RFS or OS of the 45 patients who were analyzed.\(^{23}\) All three of these studies had limitations, such as the inclusion of a relatively small number of subjects, the uniformity of the therapeutic strategy, or the inclusion of squamous cell carcinoma. In the present study, we analyzed stage III non-squamous NSCLC cases that were consecutively collected over a 10-year period, and all the patients were treated according to defined CRT protocols at a single hospital. Thus, the present results should help to understand the impact of KRAS mutation on the prediction of CRT response and on the prognosis of patients.

We also analyzed the relapse patterns after CRT and found that patients with KRAS mutations experience early distant relapses, especially in the brain, more frequently than patients with wild-type KRAS. Johung \textit{et al.} (2013) report differences in the intracranial relapse pattern after gamma-knife surgery for brain metastasis, depending on the EGFR mutation, ALK translocation, or KRAS mutation status.\(^{24}\) In patients with KRAS mutation, the time to distant-brain recurrence tended to be shorter than that of patients with EGFR mutation or ALK translocation.

Because the findings of the present study showed that patients with KRAS mutations had a shorter RFS, OS and SPP, KRAS-mutated tumors may possess a radio-resistant phenotype and might not be responsive to chemotherapy for distant metastasis control. As for the fewer local relapses in patients with KRAS mutations that we observed in the present study, we could not find reasonable molecular mechanisms which elucidate this phenomenon. Because the present study included only 16 patients with KRAS mutations, these results should be evaluated in future studies.

The present study has several limitations. First, our report is based on a retrospective study. Although we tried to collect tumor samples for diagnosis from all the patients in this study cohort, we could not analyze the KRAS mutational status in 121 patients. Furthermore, the patients did not necessarily have the same follow-up periods, although all the patients were regularly followed up every 1 to 2 months in the outpatient department and underwent work-ups every 3 to 6 months within the first year after the end of CRT, and were subsequently examined every 6 months using X-ray, CT, MRI and/or PET-CT. Second, we conducted the KRAS mutational analysis focusing on exon 2, which contains approximately 90% of all KRAS mutations in non-squamous NSCLC (data from the Catalogue of Somatic Mutations in Cancer database [COSMIC]). The impact of other minor KRAS mutations remains unknown.

In summary, our results suggest that KRAS mutations could be associated with the reduced efficacy of definitive CRT and a shortened survival time in patients with stage III non-squamous NSCLC.

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Disclosure Statement

The authors have no conflict of interest to declare.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Fig. S1. Consort diagram.

Fig. S2. (a) Time to distant relapse. (b) Time to local relapse.

Table S1. Treatment.

Table S2. Survival analysis.