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

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The frequency and clinical profile of patients with stage III non-small cell lung cancer harboring *KRAS* mutations have not yet been well documented. Here, we analyzed hotspot *KRAS* mutations using high-resolution melting analyses in tumor specimens from patients who received chemoradiotherapy between January 2001 and December 2010 at the National Cancer Center Hospital. The associations between the presence of *KRAS* mutations and the response rate, relapse-free survival, first relapse sites, survival post-progression and overall survival were investigated. A total of 274 non-squamous non-small cell lung cancer patients received chemoradiotherapy at our hospital. After excluding 121 patients for whom tumor specimens were not available and 34 patients with *EGFR* mutations, the remaining 119 patients were included in the analysis. *KRAS* mutations were found at a frequency of 13%. Patients with *KRAS* mutations had a shorter median relapse-free survival (6.1 *vs* 10.9 months) and a lower response rate (63% *vs* 81%). As for the first relapse site, patients with KRAS mutations had fewer local relapses (8% *vs* 23%) and more brain metastases (46% *vs* 12%). After disease progression, patients with *KRAS* mutations had a significantly shorter median survival post-progression (2.5 *vs* 7.3 months, $P = 0.028$) and median overall survival (15.1 *vs* 29.1 months, $P = 0.022$). Our results suggested that *KRAS* mutation could be associated with a reduced efficacy of chemoradiotherapy and a shortened survival time.

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 Kirstein 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)zx; 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 (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 χ^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				
0/1 (%)	1/15 (6/94)	25/78 (24/76)	0.090	26/93 (22/78)
Smoking status (pack-year)				
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				
IIIA/IIIB (%)	10/6 (63/37)	55/48 (53/47)	0.343	65/54 (55/45)
Histology				
Adenocarcinoma/NOS (%)	11/5 (69/31)	86/17 (84/17)	0.143	97/22 (82/18)
Type of radiotherapy				
Concurrent/sequential (%)	13/3 (81/19)	91/12 (88/12)	0.325	104/15 (87/13)
Radiotherapy dose (Gy)				
Median (range)	60 (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,

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.

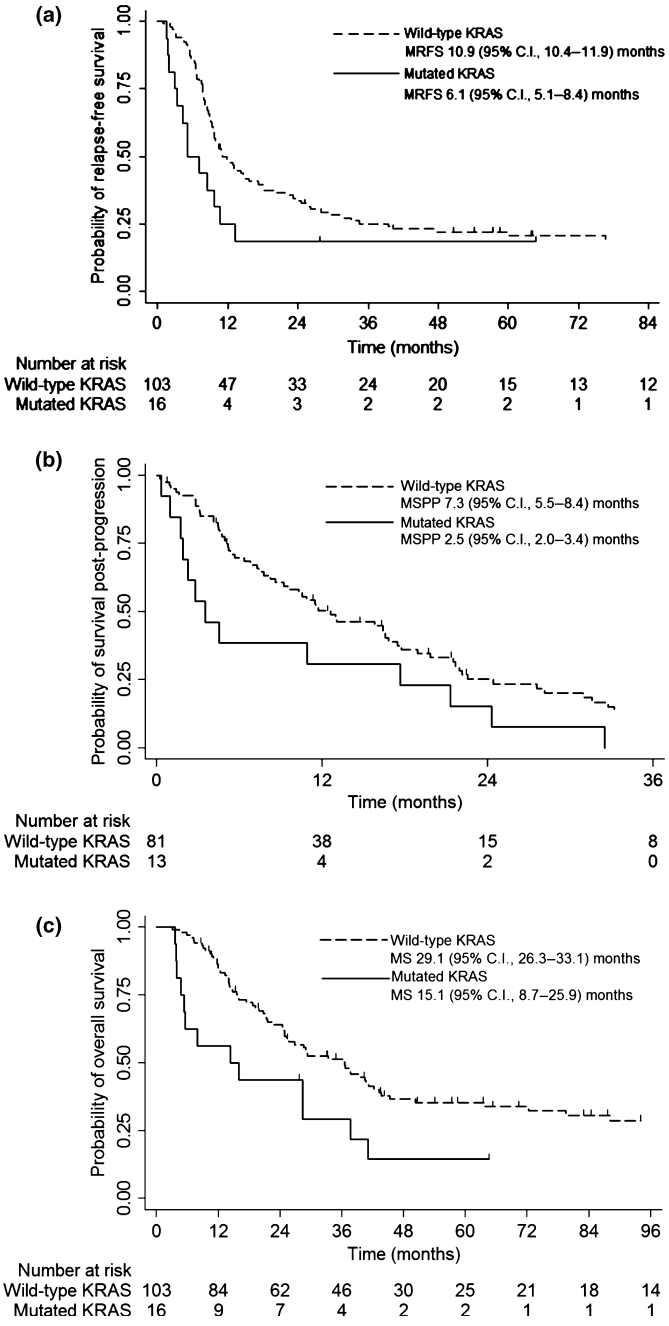

Fig. 1. Kaplan–Meier survival analyses for relapse-free survival (RFS) (a), overall survival (OS) (b), and survival post-progression (SPP) (c).

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 ($P = 0.035$) than with age, clinical stage or timing of radiotherapy (Table 5).

Table 4. Second-line treatment

	Mutated *KRAS*	Wild-type *KRAS*
Number of relapses	13	83
Cytotoxic chemotherapy	3 (23%)	30 (36%)
Docetaxel	2 (67%)	25 (83%)
Pemetrexed	0 (0%)	2 (7%)
TS-1	0 (0%)	2 (7%)
CBDCA + PTx	1 (33%)	0 (0%)
Investigational drug	0 (0%)	1 (3%)
EGFR-TKI	2 (15%)	11 (13%)
Supportive care	8 (62%)	42 (51%)

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.[21] In their study, *KRAS* mutation was found to be a negative predictive and prognostic factor. Hallaqvist *et al.*[22] 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

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

	RFS			OS			SPP		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Univariate *KRAS*									
Mt *vs* W/T	1.67	0.93–3.00	0.087	1.98	1.09–3.59	0.025	1.94	1.06–3.53	0.031
Age (years)									
65 *vs* <65	0.89	0.59–1.35	0.581	0.67	0.43–1.05	0.080	0.63	0.40–1.00	0.051
Clinical stage									
Stage IIIA *vs* IIIB	1.04	0.69–1.55	0.867	0.94	0.61–1.47	0.798	0.89	0.57–1.39	0.606
Radiotherapy									
Seq *vs* Conc	0.77	0.43–1.35	0.358	0.64	0.35–1.19	0.158	0.75	0.39–1.42	0.369
Multivariate *KRAS*									
Mt *vs* W/T	1.69	0.93–3.06	0.083	1.87	1.02–3.42	0.042	1.98	1.05–3.73	0.035
Age (years)									
65 *vs* <65	0.86	0.57–1.31	0.488	0.69	0.44–1.08	0.103	0.63	0.39–1.01	0.055
Clinical stage									
Stage III A *vs* IIIB	1.09	0.72–1.65	0.695	0.98	0.62–1.54	0.929	0.88	0.56–1.39	0.589
Radiotherapy									
Seq *vs* Conc	0.78	0.44–1.39	0.403	0.71	0.37–1.33	0.283	1.01	0.49–2.05	0.989

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 *et al.* (2013) report differences in the intracranial relapse pattern after gamma-knife surgery for brain metastases 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, SPP and OS, *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.

References

1 GLOBOCAN WHO. *Cancer Fact Sheets Lung Cancer*. Switzerland: World Health Organization, 2012. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx
2 Blackstock AW, Govindan R. Definitive chemoradiation for the treatment of locally advanced non small-cell lung cancer. *J Clin Oncol* 2007; **25**: 4146–52.
3 Govindan R, Bogart J, Vokes EE. Locally advanced non-small cell lung cancer: the past, present, and future. *J Thorac Oncol* 2008; **3**: 917–28.
4 Ramnath N, Dilling TJ, Harris LJ *et al.* Treatment of stage III non-small cell lung cancer: diagnosis and management of lung cancer, 3rd edn: American College of Chest Physicians evidence-based clinical practice guidelines. *Chest* 2013; **143**: e314S–40S.
5 NCCN. *NCCN Clinical Practice Guidelines in Oncology, Non-Small Cell Lung Cancer*. Washington, PA: National Comprehensive Cancer Network, 2014. http://www.nccn.org/professionals/physician_gls/f_guidelines.asp
6 Sekine I, Nokihara H, Sumi M *et al.* Docetaxel consolidation therapy following cisplatin, vinorelbine, and concurrent thoracic radiotherapy in patients with unresectable stage III non-small cell lung cancer. *J Thorac Oncol* 2006; **1**: 810–5.
7 Segawa Y, Kiura K, Takigawa N *et al.* Phase III trial comparing docetaxel and cisplatin combination chemotherapy with mitomycin, vindesine, and cisplatin combination chemotherapy with concurrent thoracic radiotherapy in locally advanced non-small-cell lung cancer: OLCSG 0007. *J Clin Oncol* 2010; **28**: 3299–306.
8 Yamamoto N, Nakagawa K, Nishimura Y *et al.* Phase III study comparing second- and third-generation regimens with concurrent thoracic radiotherapy in patients with unresectable stage III non-small-cell lung cancer: West Japan Thoracic Oncology Group WJTOG0105. *J Clin Oncol* 2010; **28**: 3739–45.
9 Sekine I, Sumi M, Ito Y *et al.* Phase I study of concurrent high-dose three-dimensional conformal radiotherapy with chemotherapy using cisplatin and vinorelbine for unresectable stage III non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2012; **82**: 953–9.
10 Horinouchi H, Sekine I, Sumi M *et al.* Long-term results of concurrent chemoradiotherapy using cisplatin and vinorelbine for stage III non-small-cell lung cancer. *Cancer Sci* 2013; **104**: 93–7.
11 Karachaliou N, Mayo C, Costa C *et al.* KRAS mutations in lung cancer. *Clin Lung Cancer* 2013; **14**: 205–14.
12 Slebos RJ, Kibbelaar RE, Dalesio O *et al.* K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. *N Engl J Med* 1990; **323**: 561–5.
13 Schiller JH, Adak S, Feins RH *et al.* Lack of prognostic significance of p53 and K-ras mutations in primary resected non-small-cell lung cancer on E4592: a Laboratory Ancillary Study on an Eastern Cooperative Oncology Group Prospective Randomized Trial of Postoperative Adjuvant Therapy. *J Clin Oncol* 2001; **19**: 448–57.
14 Mascaux C, Iannino N, Martin B *et al.* The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer* 2005; **92**: 131–9.
15 Tsao MS, Aviel-Ronen S, Ding K *et al.* Prognostic and predictive importance of p53 and RAS for adjuvant chemotherapy in non small-cell lung cancer. *J Clin Oncol* 2007; **25**: 5240–7.
16 Shepherd FA, Domerg C, Hainaut P *et al.* Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. *J Clin Oncol* 2013; **31**: 2173–81.
17 Yagishita S, Horinouchi H, Katsui Taniyama T *et al.* Epidermal Growth factor receptor mutation is associated with longer local control after definitive chemoradiotherapy in patients with stage III nonsquamous non-small-cell lung cancer. *Int J Radiat Oncol Biol Phys* 2015; **91**: 140–8.
18 Akiyoshi K, Yamada Y, Honma Y *et al.* KRAS mutations in patients with colorectal cancer as detected by high-resolution melting analysis and direct sequencing. *Anticancer Res* 2013; **33**: 2129–34.
19 Broglio KR, Berry DA. Detecting an overall survival benefit that is derived from progression-free survival. *J Natl Cancer Inst* 2009; **101**: 1642–9.
20 Niho S, Ohe Y, Ishikura S *et al.* Induction chemotherapy followed by gefitinib and concurrent thoracic radiotherapy for unresectable locally advanced

adenocarcinoma of the lung: a multicenter feasibility study (JCOG 0402). *Ann Oncol* 2012; **23**: 2253–8.
21 Broermann P, Junker K, Brandt BH *et al.* Trimodality treatment in Stage III nonsmall cell lung carcinoma: prognostic impact of K-ras mutations after neoadjuvant therapy. *Cancer* 2002; **94**: 2055–62.
22 Hallqvist A, Enlund F, Andersson C *et al.* Mutated KRAS is an independent negative prognostic factor for survival in NSCLC stage III disease treated with high-dose radiotherapy. *Lung Cancer Int* 2012; **2012**: 1–6.
23 Ready N, Janne PA, Bogart J *et al.* Chemoradiotherapy and gefitinib in stage III non-small cell lung cancer with epidermal growth factor receptor and KRAS mutation analysis: cancer and leukemia group B (CALEB) 30106, a CALGB-stratified phase II trial. *J Thorac Oncol* 2010; **5**: 1382–90.
24 Johung KL, Yao X, Li F *et al.* A clinical model for identifying radiosensitive tumor genotypes in non-small cell lung cancer. *Clin Cancer Res* 2013; **19**: 5523–32.

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.