Lung Cancer 128 (2019) 152–157

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

Genetic alterations of driver genes as independent prognostic factors for disease-free survival in patients with resected non-small cell lung cancer

Akira Ono[a,*,1], Mitsuhiro Isaka[b,1,2], Masakuni Serizawa[c,1,3], Katsuhiro Omae[d], Hideaki Kojima[b], Kazuhisa Nakashima[a], Shota Omori[a], Kazushige Wakuda[a], Hirotsugu Kenmotsu[a], Tateaki Naito[a], Haruyasu Murakami[a], Kenichi Urakami[e], Takeshi Nagashima[f], Takashi Sugino[g], Masatoshi Kusuhara[h], Toshiaki Takahashi[a], Ken Yamaguchi[i], Yasuhisa Ohde[b]

[a] Division of Thoracic Oncology, Shizuoka Cancer Center, Japan
[b] Division of Thoracic Surgery, Shizuoka Cancer Center, Japan
[c] Drug Discovery and Development Division, Shizuoka Cancer Center Research Institute, Japan
[d] School of Public Health, Graduate School of Medicine, Kyoto University, Japan
[e] Cancer Diagnostics Division, Shizuoka Cancer Center Research Institute, Japan
[f] SRL Inc, Tokyo, Japan
[g] Division of Diagnostic Pathology, Shizuoka Cancer Center, Japan
[h] Region Resources Division, Shizuoka Cancer Center Research Institute, Japan
[i] Shizuoka Cancer Center, Japan

ARTICLE INFO

Keywords:
NSCLC
Driver gene alterations
Disease-free survival
Prognostic factor

ABSTRACT

Objectives: This study assessed the associations between the molecular signatures and clinical information in non-small cell lung cancer (NSCLC) patients with postoperative disease-free survival (p-dfs) to identify novel prognostic factors, focusing on associations with driver gene alterations.

Materials and methods: Between February 2014 and September 2015, 242 patients with NSCLC, including 192 patients with adenocarcinoma (Ad) and 50 patients with squamous cell carcinoma (Sq), underwent surgery and were enrolled in this study. Surgically resected tissues were subjected to whole exome sequencing. Mt detected in 138 cancer-related genes were evaluated as driver mutations. A multivariate analysis using the multi-state model was used to establish the associations between co-variables and p-dfs.

Results: Postoperative recurrence (p-rec) was observed in 49 (20.2%) and 19 (7.9%) patients with Ad and Sq, respectively. The median (range) follow-up period for all the censored cases was 2.5 (2.0–3.5) years. The characteristics of the patients with postoperative recurrence were as follows: median age (range), 71 (50–87) years; male, 38 (56%); smoker, 51 (75%); p-stage (I/II/III), 30 (44%)/19 (28%)/19 (28%); histological type (Ad/Sq), 49 (72%)/19 (28%); adjuvant chemotherapy (yes/no), 30 (44%)/38 (56%); and driver gene alteration (presence/absence), 65 (96%)/3 (4%). In univariate analyses, age ($< 70/\geqq 70$ years), smoking history (yes/no), p-stage (I, II/III), histological type (Ad/Sq), and driver mutation (presence/absence) were favorable prognostic factors ($P = .017$, $P = .048$, $P = .0002$, $P = .006$, $P = .029$, respectively). A multivariate analysis also revealed a significant association between the driver mutation status and p-dfs ($P = .046$; odds ratio [OR], 2.86; 95% confidence interval [CI], 1.02–8.08), when adjusted according to histological type ($P = .10$), smoking status ($P = .09$), gender ($P = .51$), age ($P = .008$) and p-stage ($P = .00003$).

Conclusion: The driver mutation status may be an independent prognostic factor of p-dfs in NSCLC.

* Corresponding author at: Division of Thoracic Oncology, Shizuoka Cancer Center, 1007, Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka, 411-8777, Japan.
E-mail addresses: a.ono@scchr.jp (A. Ono), mi.isaka@scchr.jp (M. Isaka), m.serizawa@scchr.jp (M. Serizawa).
[1] These authors equally contributed to this study.
[2] Division of Thoracic Surgery, Shizuoka Cancer Center, 1007, Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan.
[3] Drug Discovery and Development Division, Shizuoka Cancer Center Research Institute, 1007, Shimonagakubo, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8777, Japan.

https://doi.org/10.1016/j.lungcan.2018.12.005
Received 5 October 2018; Received in revised form 30 November 2018; Accepted 4 December 2018

1. Introduction

Driver genes give a selective growth advantage to tumor cells, leading to uncontrolled cell growth and proliferation [1]. Oncogene aberrations, such as EGFR mutations, ALK fusions, BRAF mutations, RET fusions, and ROS1 fusions, have recently been revealed as targetable driver alterations for molecular-targeted agents in patients with advanced non-small cell lung cancer (NSCLC). Through the development of next-generation sequencing (NGS), the application of NGS clinical sequencing, such as Foundation CDx, could promote precision medicine. In evaluating the tumor mutation burden (TMB), the total number of mutations identified using NGS was strongly correlated with the total exome mutation number [2]. However, whole-exome sequencing (WES) remains a proven method for analyzing genetic alterations in adequate specimens of NSCLC [3,4].

The Shizuoka Cancer Center launched "Project HOPE (High-Tech-Omics-based Patient Evaluation)" as a first prospective pan-cancer molecular profiling study in Japan in January 2014 to promote personalized medicine. The purpose of this research program is to identify the cancer characteristics of individual patients using multi-omics-based analyses across all types of tumors. By May 2018, WES had been completed for over 4600 patients with all types of tumors.

Although complete surgical resection with curative intent is the standard treatment for early-stage NSCLC, the five-year survival rates range from about 70% for pathologic stage IA disease to about 20% for pathologic stage IIIA disease [5]. Overall, nearly 50% of patients develop recurrence despite curative resection [6,7]. Disease-free survival is a valid surrogate endpoint for overall survival in studies performed in adjuvant settings involving patients who have undergone resections for NSCLC [8].

TMB using WES is not a significant prognostic factor for disease-free survival in patients with stage I to III NSCLC [9]. However, the association of driver gene alterations with patient outcomes has not been clearly established. None of the previously reported studies have suggested driver gene alterations identified using WES as a possible prognostic factor in patients with surgically resected NSCLC. Therefore, we hypothesized that the presence or absence of driver gene alterations could be a prognostic factor for disease-free survival in patients with resected NSCLC. In the present study, we investigated the prognostic implications of driver gene alterations in patients with resected NSCLC.

2. Materials and methods

2.1. Patients

Between February 2014 and September 2015, 242 patients with NSCLC underwent surgery and were analyzed in this study. We conducted a retrospective review of prospectively collected data from 192 patients with adenocarcinoma (Ad) and 50 patients with squamous cell carcinoma (Sq) using the patient database for Project HOPE. In the present study, cases with surgically resected primary non-small cell lung cancers were analyzed using data obtained according to the Project HOPE protocol. Briefly, we assessed genetic alterations in surgical tumor specimens using whole exome sequencing (WES) performed with the Ion torrent proton platform (Thermo Fisher Scientific). We sequenced the whole exome to an average effective coverage of ×123. Gene alterations detected in 138 cancer-related genes listed in Vogelstein et al. [1] were evaluated as driver Gene alterations. Project HOPE was conducted in accordance with the "Ethical Guidelines for Human Genome and Genetic Analysis Research in Japan," which were revised in 2013 (The Revised Ethical Guidelines for Human Genome/Gene Analysis Research in Japan. The Japanese Government 2013 URL: http://www.lifescience.mext.go.jp/files/pdf/n1115_01.pdf). We obtained consent from each of the patients prior to their participation in this study. This study was approved by the Institutional Review Board of the Shizuoka Cancer Center, Japan (Approval No. 25-33). All the patients were staged based on the International Association for the Study of Lung Cancer (IASLC) TNM (tumor-node-metastasis) classification, 7th edition [10].

2.2. Samples

Tumor tissue samples with sizes corresponding to weights of ≥ 0.1 g were dissected from surgical specimens, along with samples of surrounding normal tissue. The areas from which the tumor samples were dissected were visually assessed as containing a tumor content of ≥ 50%. For the DNA analysis, tumor and normal tissues were immediately frozen in liquid nitrogen before DNA extraction. For the RNA analysis, tissue samples were submerged in RNAlater solution (Thermo Fisher Scientific), minced, and stored overnight at 4℃ before RNA extraction. In addition, whole blood was collected as a control for WES.

2.3. DNA extraction and whole exome sequencing

DNA was extracted from tissue samples using a QIAamp Kit (Qiagen) according to the manufacturer's instructions and subjected to WES using the Ion Proton System (Thermo Fisher Scientific). Whole exome sequence and variant calling were performed using the Ion Proton AmpliSeq Exome kit and the Ion Torrent server, as previously reported [11]. Briefly, 100 ng of DNA was amplified as follows: 99℃, 2 min; 95℃, 15 s, 10 cycles of 60℃, 16 min, and a final hold at 10℃. The incorporated primer sequences were partially digested using FuPa reagent (Thermo Fisher Scientific). Ion Torrent Proton adapters were ligated to the amplicons at 22℃ for 30 min followed by 10 min incubation at 72℃, and the library was purified using Agencourt AMPure XT beads (Thermo Fisher Scientific). The library was quantified using qPCR, and 7 pM of library DNA was sequenced using the Ion Torrent Proton Sequencer with a PI chip V2 according to the manufacturer's protocol (Thermo Fisher Scientific). Torrent Suite software (ver.4.4) was used to convert raw binary data into sequence reads that were mapped to the reference human genome (hg19 assembly, UCSC). Somatic mutations were identified by comparing data from the tumor and corresponding blood samples. Single-nucleotide variants (SNVs) with quality scores < 30, frequency < 10%, or a depth of coverage < 20 were discarded. SNVs of the total exonic mutations for each sequenced tumor included nonsynonymous, synonymous, and indels/frameshift mutations. We sequenced the whole exome to an average effective coverage of ×123.

2.4. RNA extraction and fusion analysis

Total RNA was extracted from approximately 10 mg of minced tissue samples using the miRNeasy Mini Kit (Qiagen), according to the manufacturer's instructions. Total RNA was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Fusion gene data were analyzed using the Ion Reporter server. The Ion AmpliSeq RNA fusion workflow (Thermo Fisher Scientific) was used to detect fusion transcripts targeted by the HOPE fusion panel [11].

2.5. Statistical methods

The prognostic impact of adjuvant chemotherapy in patients with completely resected early-stage NSCLC has been previously reported [12–15]. We investigated transitions, including the time of resection, adjuvant chemotherapy, disease recurrence, or death using a multivariate "multi-state" model. This model enabled us to evaluate the effects of the covariates for each transition taking into account the clinical course after resection. Each patient was classified into one of three states in a multi-state model: (1) transition from surgery to adjuvant chemotherapy, (2) transition from adjuvant chemotherapy to recurrence or death including patients who died of other causes, and (3) transition from surgery to recurrence or death resulting from any cause

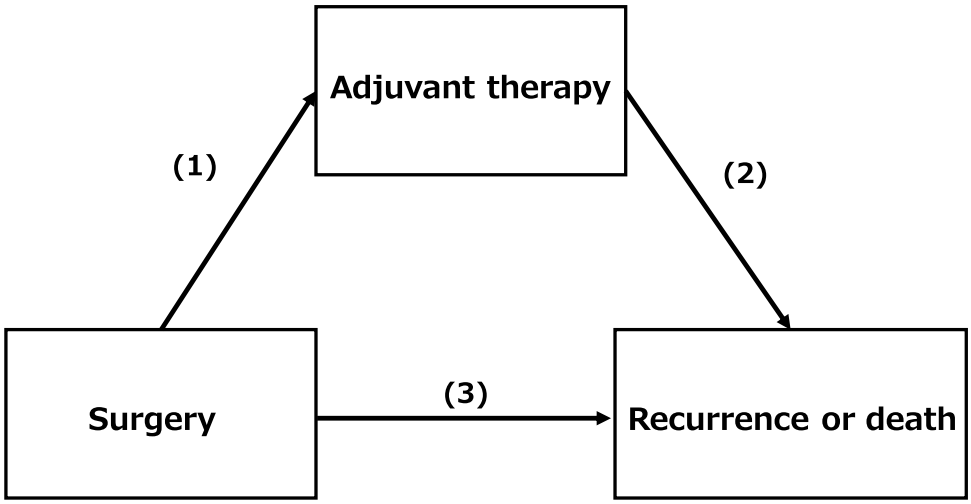

Fig. 1. Resected NSCLC relapse-free survival as a multi-state model. Arrow (1) indicates the transition from surgery to adjuvant chemotherapy. Arrow (2) indicates the transition from adjuvant chemotherapy to recurrence or death, including patients who died from other causes. Arrow (3) indicates the transition from surgery to recurrence or death resulting from any cause.

(Fig. 1). A recurrence was defined as any recurrence including both locoregional and distant recurrence. Recurrence at the surgical resection margin, ipsilateral hilum, and ipsilateral or contralateral mediastinum was considered a locoregional recurrence. All other sites of failure, including the supraclavicular fossa, contralateral hilum, and ipsilateral lobe of the lung, were considered distant recurrences.

Survival were estimated using the Kaplan-Meier method. Relapse-free survival (RFS) was defined as the time from the day of surgery until disease relapse or death resulting from any cause, whichever occurred first. Prognostic factors among baseline covariates were identified using univariate log-rank tests and a multivariate, multi-state model. The prognostic significances of all the variables were measured by calculating the adjusted hazard ratio (HR) with 95% confidence interval (95% CI). *P* values < 0.05 were considered to be indicative of statistical significance. All the statistical analyses were performed using R package, version 3.4.3 for Mac.

3. Results

A flow-diagram of the patients included in the analysis is shown in Fig. 2. The characteristics of the 242 patients were as follows: median age, 70 years (range: 39–87 years); male/female, 151 (62%)/91 (38%); smokers/never-smokers, 170 (70%)/72 (30%); pathological stage (p-stage) I/II/III, 164 (68%)/50 (21%)/28 (11%); adenocarcinoma/squamous cell carcinoma, 192 (79%)/50 (21%); adjuvant chemotherapy (yes/no), 78 (32%)/164 (68%); and driver gene alteration (presence/absence), 202 (83%)/40 (17%) (Table 1).

The median (range) follow-up period of all the censored cases was 2.5 (2.0–3.5) years. The median relapse-free survival time was 2.3 (0.05–3.5) years. Postoperative recurrence was observed in 68 (28%) patients. The respective background characteristics for the recurrence (rec) and non-recurrence (non-rec) groups were as follows: median age (range), 71 (50–87) and 74 (39–87) years; male, 56% and 65%; smoker, 75% and 68%; pathological stage (p-stage) (I/II/III), 44%/28%/28% and 77%/18%/5%; histological type (Ad/Sq), 72%/28% and 82%/18%; adjuvant chemotherapy (yes/no), 44%/56% and 28%/72%; driver gene alteration (presence/absence), 96%/4% and 79%/21%. Overall, 204 (84%) patients had a lobectomy, 6 (3%) had a

Table 1
Patients' characteristics.

Characteristics	n	%
Gender		
Male	151	62
Female	91	38
Median age (range) years	70 (39-87)	
Smoking status		
Former/current smoker	170	70
Never smoker	72	30
Pathological stage		
Stage I	164	68
Stage II	50	21
Stage III	28	11
Histological type		
Adenocarcinoma	192	79
Squamous cell carcinoma	50	21
Adjuvant therapy		
Yes	78	32
No	164	68
Driver gene alteration		
Presence	202	83
Absence	40	17

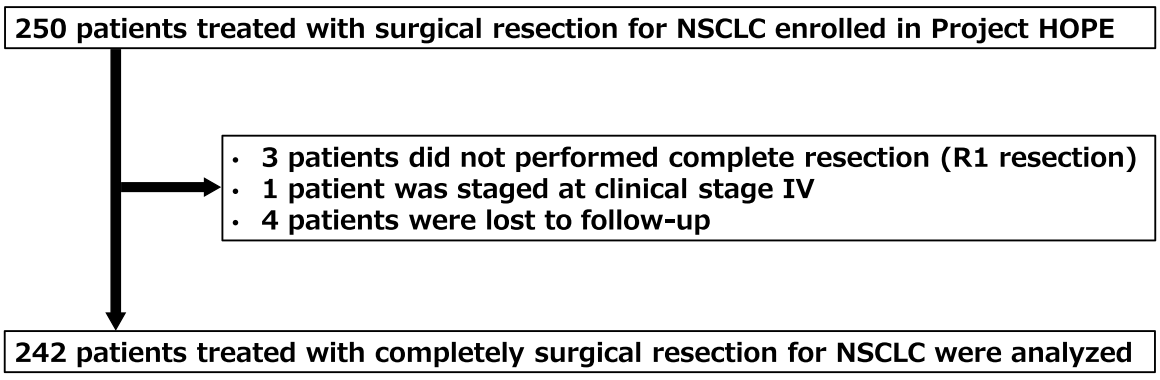

Fig. 2. Flow diagram showing the patients included in the analysis.

pneumonectomy, 10 (4%) had a segmentectomy, and 22 (9%) had a wedge resection. Seventy-eight (32%) of the patients received a curative resection with adjuvant chemotherapy, while 164 (68%) of the patients received a curative resection without adjuvant chemotherapy. Thirty-six (46%) patients received platinum-based chemotherapy, 40 (51%) patients received UFT, and 2 (3%) patients received an investigational drug. Thirty-one patients who received adjuvant chemotherapy developed a recurrence or died. Of these 31 patients, 1 died from a treatment-related cause after receiving adjuvant chemotherapy. Forty-nine patients who did not receive adjuvant chemotherapy developed a recurrence or died. Of these 49 patients, 11 died without having developed a recurrence (6 died from other malignancies, 2 died from other diseases, 2 died from treatment-related causes, and 1 died from postoperative pneumonitis). Locoregional recurrence occurred in 13 patients (5.4% of the entire population, and 19% of the patients with any type of recurrence). Distant recurrence was identified in 55 patients (22.7% of the entire population and 81% of those with any type of recurrence). Each variable was tested first in a univariate analysis. The presence of a driver gene alteration (HR: 3.13; 95% CI: 1.12–8.71; P = 0.028), an elderly age (≧70 years; HR: 2.41; 95% CI: 1.17–4.98; P = 0.016), smoking habit (HR: 2.14; 95% CI: 1.00–4.57; P = 0.048), p-stage III (HR: 3.88; 95% CI: 1.92–7.82; P = 0.0001), and a squamous histology (HR: 2.25; 95% CI: 1.25–4.03; P = 0.006) were significant prognostic risk factors. In the multivariate analysis, the presence of a driver gene alteration (HR: 2.86; 95% CI: 1.02–8.08; P = 0.046), an elderly age (≧70 years; HR: 2.72; 95% CI: 1.29–5.77; P = 0.0087), and p-stage III (HR: 4.70; 95% CI: 2.27–9.71; P = 0.00002) were significant prognostic risk factors for RFS (Table 2). In this study, we estimated the risk score using the multivariate "multi-state" model, which was calculated as follows: exp {(driver gene alteration × 1.05) + (age × 1.00) + (sex × -0.26) + (smoking status × 0.83) + (p-stage × 1.55) + (histological type × 0.53)}. To identify the best prognostic risk score for 2-year survival, a receiver operating characteristic (ROC) curve was plotted. Since sensitivity plus "1 minus specificity" was maximized at a score of 8.1, this was considered to be the optimal cutoff value for the detection of 2-year survival. The relapse-free survival rates of patients with resected NSCLC stratified according to a cutoff score of 8.1 are shown in Fig. 3. The prognosis was more favorable in the group with risk score < 8.1 (n = 126) than in the group with risk score ≥ 8.1 (n = 116) (MST: NA vs. 1008 days, P < .0001) (Fig. 3).

Table 2
Multi-state model results. Prognostic factors with respect to parameter estimates related each transition in multivariate analysis.

Variables	Transition (1)		Transition (2)		Transition (3)	
No. at risk	242		78		242	
No. of events	0		31		49	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age						
< 70	1		1		1	
≥70	0.24 (0.14-0.41)	< .0001	1.17 (0.49-2.82)	.72	2.72 (1.29-5.77)	.0087
Gender						
Female	1		1		1	
Male	0.80 (0.45-1.43)	.45	0.37 (0.15-0.93)	.033	0.77 (0.36-1.66)	.50
Smoking status						
Never	1		1		1	
Former/Current	0.92 (0.50-1.71)	.80	1.71 (0.63-4.62)	.28	2.28 (0.88-5.93)	.09
Pathological stage						
I/II	1		1		1	
III	2.08 (1.22-3.54)	.007	2.54 (1.20-5.37)	.015	4.70 (2.27-9.71)	.0002
Histological type						
Adenocarcinoma	1		1		1	
Squamous cell carcinoma	0.79 (0.40-1.55)	.48	2.99 (1.07-8.37)	.037	1.69 (0.89-3.22)	.10
Driver gene alteration						
Absence	1		1		1	
Presence	1.38 (0.69-2.79)	.36	2.09 (0.49-8.98)	.322	2.86 (1.02-8.08)	.046

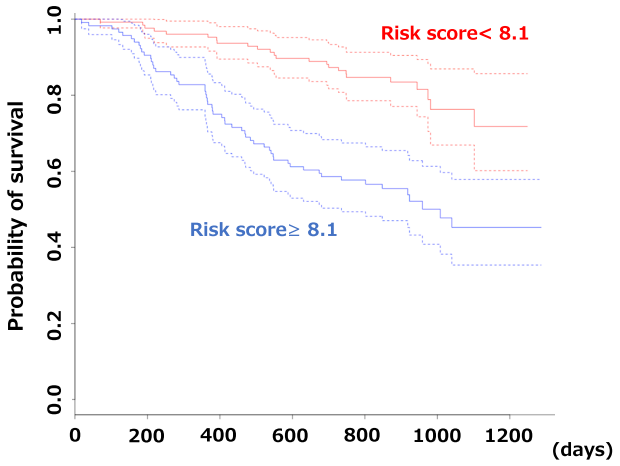

Fig. 3. Kaplan-Meier curves for progression-free survival in the two groups, stratified according to a cutoff score of 8.1.

4. Discussion

In the present study, we demonstrated that the presence of a driver gene alteration is a potential independent prognostic factor for disease-free survival in patients with resected NSCLC.

Previous studies have assessed the prognostic role of driver gene alterations detected using target sequencing in patients with stage IV or recurrent lung adenocarcinoma [16,17]. Another report also revealed the prognostic role of driver gene alterations detected using target sequencing in patients with NSCLC [18]; an analysis of disease stage in the aforementioned report showed that stage IV or postoperative recurrence accounted for the majority of NSCLC cases (78%).

Remains the proven method for measuring tumor mutation burden [2]. In this study, we analyzed the molecular profile using WES to determine the underlying relationship between driver gene alterations and RFS in patients with surgically resected NSCLC.

In the multivariate analysis, an advanced stage at diagnosis (stage III: HR: 2.08; 95% CI: 1.22–3.54) was associated with a significantly higher transition rate from surgery to adjuvant chemotherapy. Elderly subjects (≥ 70 years: HR: 0.24; 95% CI: 0.14-0.41) had a lower transition rate from surgery to adjuvant chemotherapy. These findings were consistent with our routine clinical practice. We studied whether the presence of driver gene alterations at the time of the primary diagnosis was a prognostic factor associated with the transition rate from surgery to RFS. The presence of a driver gene alteration was an independent significant prognostic risk factor for a transition from surgery to RFS (HR: 2.86; 95% CI: 1.02–8.08; P = 0.046). Other prognostic risk factors for RFS were an elderly age at diagnosis (≥ 70 years: HR: 2.72; 95% CI: 1.29–5.77; P = 0.0087) and an advanced stage at diagnosis (stage III: HR: 4.70; 95% CI: 2.27–9.71; P = 0.00002). An elderly age (≥ 70 years) and an advanced stage are important prognostic risk factors for the occurrence of RFS in resected NSCLC patients [19]. In this study, we hypothesized that the presence or absence of a driver gene alteration is a prognostic factor for disease-free survival in patients with resected

NSCLC. Our findings supported our hypothesis. We consider that the status of driver gene alterations is, along with conventional prognostic factors such as age and p-stage, a statistically significant prognostic factor in patients with surgically resected NSCLC, since statistically significant P values < 0.05 were achieved.

In this study, a high proportion (81%) of the recurrences were distant recurrences. A study of the recurrence type as a prognostic factor in patients with resected NSCLC showed that no significant difference in OS was seen between patients with local recurrence and those with distant recurrence [20].

Adjuvant chemotherapy is now the standard of care for the treatment of resected pathological stage I [12,21] and stage II/III NSCLC [13–15]. The application of adjuvant chemotherapy was reviewed during a thoracic oncological conference at our institution that included thoracic surgeons, medical oncologists, and radiation oncologists. In this study, the prognostic impact of the presence of driver gene alterations on RFS in resected NSCLC was investigated using a multi-state model because the effect of a prognostic factor could have been underestimated by an increase in the risk of an intermediate event occurrence, which itself influenced the outcome independently. An accurate assessment of the anatomic extent of the disease, as expressed by the TMN status, is currently the most important factor in tumor staging and prognostication. A favorable five-year survival outcome has been reported for about 80% of patients with pathologic stage I disease and about 50% of patients with pathologic stage II disease according to the IASLC TNM classification, 7th edition [10].

In this study, 182 (75%) patients underwent lymph node dissection (selective nodal or complete nodal dissection), and 60 (25%) patients did not undergo lymph node dissection. In a retrospective study, no significant difference in the 5-year OS was seen between patients with selective dissection and those with complete dissection among patients with clinical stage I-II resected NSCLC [22]. However, whether selective dissection and complete dissection are correlated with survival among patients with resected stage I-II NSCLC remains unknown. This question is being investigated in a prospective trial [23]. The presence or absence of lymph node dissection was not included as a co-variate in the analysis of multicollinearity among the variables.

The present study had several limitations. First, the study was performed as a retrospective review of prospectively collected data and had a relatively small sample size; thus, the selection of patients with heterogeneous characteristics resulted in an inherent selection bias in our analysis. We estimated tumor purity using WES data and the previously reported PurBayes method [24]. The median percentage tumor purity was 25.9% (range: 15.5%–99.9%). There may have been a correlation between the low tumor purity and the false-negative rate. Second, not all of the specimens were analyzed in a Clinical Laboratory Improvement Amendments (CLIA) laboratory. Third, the follow-up time was insufficient to analyze the overall survival data. Fourth, WES has some drawbacks, as it is expensive, time-consuming, and has a relatively low sensitivity. We sequenced the whole exome to an average effective coverage of $\times 123$. Meanwhile, alternative target NGS platforms have been developed, such as hybrid capture-based NGS [25], digital sequencing [26], and CAPP-Seq [27]. These next-generation methods enable sequencing with a high coverage depth ($\times 500$-$\times 8000$). A mutation burden analysis using WES in plasma samples is often challenging. The development of new treatment strategies, such as the application of neo-adjuvant or adjuvant therapy, based on plasma DNA findings may require more sensitive analysis methods. In the future, molecular profile assessments of resected lung cancer based on clinical sequencing may help to guide the application of adjuvant therapy. This needs to be addressed in a future study.

In conclusion, the presence of a driver gene alteration might be an independent prognostic factor. Driver gene alterations might be useful as a routine demographic variable with prognostic value in patients with resected NSCLC. However, even in this era of personalized cancer medicine incorporating NGS, conventional prognostic factors remain better than WES analysis for determining appropriate therapeutic strategies for patients with surgically resected NSCLC. We plan to test the hypothesis that the application of driver gene alteration assessments, along with p-stage and age, might enable the identification of candidates for adjuvant chemotherapy in a future prospective study.

Declaration of conflicting interests

Akira Ono has received honoraria from Chugai Pharma, MSD, Taiho Pharmaceutical, Ono Pharmaceutical, Boehringer Ingelheim and Novartis and research funding to our institution from Chugai Pharma, and Taiho Pharmaceutical. Kazuhisa Nakashima has received honoraria from Ono Pharmaceutical, Taiho Pharmaceutical, Mochida Pharmaceutical and Eli Lilly Japan. Shota Omori has received honoraria from Ono Pharmaceutical. Kazushige Wakuda has received honoraria from Taiho Pharmaceutical, Boehringer Ingelheim and Ono Pharmaceutical. Hirotsugu Kenmotsu has received research funding from AstraZeneca, Boehringer Ingelheim and honoraria from AstraZeneca, Boehringer Ingelheim, Ono Pharmaceutical, Eli Lilly Japan, Chugai Parma, Taiho Pharmaceutical, Bristol-Myers Squibb, and Kyowa Hakko Kirin. Tateaki Naito has received honoraria from Ono Pharmaceutical. Haruyasu Murakami has received honoraria from Nippon Boehringer Ingelheim, Pfizer, Chugai Pharma, Taiho Pharmaceutical, AstraZeneca, Lilly Japan, Ono Pharmaceutical, Bristol-Myers Squibb Japan, Novartis, and Astellas Pharma. Toshiaki Takahashi has received honoraria from Eli Lilly Japan, AstraZeneca, Chugai Pharma, Boehringer Ingelheim, Pfizer Japan and Ono Pharmaceutical and research funding from Takeda Pharmaceuticals, Ono Pharmaceutical, AstraZeneca, Eli Lilly Japan, Chugai Pharma, Pfizer, Taiho Pharmaceutical and MSD. For the remaining authors no conflict interested are declared.

Acknowledgements

No financial support was obtained for this study.

References

[1] B. Vogelstein, N. Papadopoulos, V.E. Velculescu, et al., Cancer genome landscapes, Science 339 (2013) 1546–1558.
[2] D.B. Johnson, G.M. Frampton, M.J. Rioth, et al., Targeted next generation sequencing identifies markers of response to PD-1 blockade, Cancer Immunol. Res. 4 (959) (2016).
[3] Cancer Genome Atlas Research Network, Comprehensive genomic characterization of squamous cell lung cancers, Nature 489 (519) (2012).
[4] Cancer Genome Atlas Research Network, Comprehensive molecular profiling of lung adenocarcinoma, Nature 511 (543) (2014).
[5] P. Goldstraw, J. Crowley, K. Chansky, et al., International association for the study of lung Cancer international staging committee; participating institutions. The IASLC lung Cancer Staging project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM classification of malignant tumours, J. Thorac. Oncol. 2 (706) (2007).
[6] K. Al-Kattan, E. Sepsas, S.W. Fountain, et al., Disease recurrence after resection for stage I lung cancer, Eur. J. Cardiothorac. Surg. 12 (380) (1997).
[7] P.C. Hoffman, A.M. Mauer, E.E. Vokes, Lung cancer, Lancet 355 (479) (2000).
[8] A. Mauguen, J.P. Pignon, S. Burdett, Surrogate Lung Project Collaborative Group, et al., Surrogate endpoints for overall survival in chemotherapy and radiotherapy trials in operable and locally advanced lung cancer: a re-analysis of meta-analyses of individual patients' data, Lancet Oncol. 14 (619) (2013).
[9] Y. Owada-Ozaki, S. Muto, H. Takagi, et al., Prognostic impact of tumor mutation burden in patients with completely resected non-small cell lung Cancer: brief report, J. Thorac. Oncol. 13 (2018) 1217–1221.
[10] P. Goldstraw, K. Chansky, J. Crowley, et al., International association for the study of lung Cancer staging and prognostic factors committee, advisory boards, and participating institutions; international association for the study of lung Cancer staging and prognostic factors committee advisory boards and participating institutions. The IASLC lung Cancer Staging project: proposals for revision of the TNM stage groupings in the forthcoming (Eighth) edition of the TNM classification for lung Cancer, J. Thorac. Oncol. 11 (39) (2015).
[11] K. Urakami, Y. Shimoda, K. Ohshima, et al., Next generation sequencing approach for detecting 491 fusion genes from human cancer, Biomed. Res. 37 (2016) 51.
[12] H. Kato, Y. Ichinose, M. Ohta, Japan Lung Cancer research Group on Postsurgical Adjuvant Chemotherapy, et al., A randomized trial of adjuvant chemotherapy with uracil-tegafur for adenocarcinoma of the lung, N. Engl. J. Med. 350 (1713) (2004).

[13] J.P. Pignon, H. Tribodet, G.V. Scagliotti, LACE Collaborative Group, et al., Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group, J. Clin. Oncol. 26 (3552) (2008).
[14] J.Y. Douillard, H. Tribodet, D. Aubert, LACE Collaborative Group, et al., Adjuvant cisplatin and vinorelbine for completely resected non-small cell lung cancer: subgroup analysis of the lung adjuvant cisplatin evaluation, J. Thorac. Oncol. 5 (220) (2010).
[15] NSCLC Meta-analyses Collaborative Group, R. Arriagada, A. Auperin, S. Burdett, J.P. Higgins, D.H. Johnson, T. Le Chevalier, C. Le Pechoux, M.K. Parmar, J.P. Pignon, R.L. Souhami, R.J. Stephens, L.A. Stewart, J.F. Tierney, H. Tribodet, J. van Meerbeeck, Adjuvant chemotherapy, with or without postoperative radiotherapy, in operable non-small-cell lung cancer: two meta-analyses of individual patient data, Lancet 375 (2010) 1267.
[16] M.G. Kris, B.E. Johnson, L.D. Berry, et al., Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs, JAMA 311 (1998) (2014).
[17] D.L. Aisner, L.M. Sholl, L.D. Berry, et al., The impact of smoking and TP53 mutations in lung adenocarcinoma patients with targetable mutations-the lung cancer mutation consortium (LCMC2), Clin. Cancer Res. 24 (1038) (2018).
[18] M. Takeda, K. Sakai, M. Terashima, et al., Clinical application of amplicon-based next-generation sequencing to therapeutic decision making in lung cancer. Clinical application of amplicon-based next-generation sequencing to therapeutic decision making in lung cancer, Ann. Oncol. 26 (2477) (2015).
[19] H. Asamura, T. Goya, Y. Koshiishi, et al., A Japanese lung cancer registry study: prognosis of 13,010 resected lung cancers, J. Thorac. Oncol. 3 (46) (2008).
[20] M. Isaka, H. Kojima, S. Takahashi, et al., Risk factors for local recurrence after lobectomy and lymph node dissection in patients with non-small cell lung cancer: implications for adjuvant therapy, Lung Cancer 115 (28) (2018).
[21] C. Hamada, F. Tanaka, M. Ohta, et al., Meta-analysis of postoperative adjuvant chemotherapy with tegafur-uracil in non-small-cell lung cancer, J. Clin. Oncol. 23 (4999) (2005).
[22] T. Maniwa, T. Okumura, M. Isaka, et al., Recurrence of mediastinal node cancer after lobe-specific systematic nodal dissection for non-small-cell lung cancer, Eur. J. Cardiothorac. Surg. 44 (2013) e59.
[23] T. Hishida, H. Saji, S.I. Watanabe, et al., A randomized Phase III trial of lobe-specific vs. Systematic nodal dissection for clinical Stage I-II non-small cell lung cancer (JCOG1413), Jpn. J. Clin. Oncol. 48 (2018) 190–194.
[24] N.B. Larson, B.L. Fridley, PurBayes: estimating tumor cellularity and subclonality in next-generation sequencing data, Bioinformatics 29 (2013) 1888–1889.
[25] G.M. Frampton, A. Fichtenholtz, G.A. Otto, et al., Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing, Nat. Biotechnol. 31 (1023) (2013).
[26] R.B. Lanman, S.A. Mortimer, O.A. Zill, et al., Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA, PLoS One 10 (2015) e0140712.
[27] A.M. Newman, A.F. Lovejoy, D.M. Klass, et al., Integrated digital error suppression for improved detection of circulating tumor DNA, Nat. Biotechnol. 34 (547) (2016).