



NOTCH1 and CREBBP co-mutations negatively affect the benefit of adjuvant therapy in completely resected EGFR-mutated NSCLC: translational research of phase III IMPACT study

Satoshi Ikeda¹ , Masahiro Tsuboi², Kazuko Sakai³, Toshihiro Misumi⁴, Hiroaki Akamatsu⁵, Hiroyasu Shoda⁶, Noriaki Sakakura⁷, Atsushi Nakamura⁸, Yasuhisa Ohde⁹, Hidetoshi Hayashi¹⁰, Kyoichi Okishio¹¹, Morihito Okada¹², Ichiro Yoshino¹³, Jiro Okami¹⁴, Kazuhisa Takahashi¹⁵, Norihiko Ikeda¹⁶, Masayuki Tanahashi¹⁷, Yuichi Tambo¹⁸, Haruhiro Saito¹⁹, Shinichi Toyooka²⁰, Hidetoshi Inokawa²¹, Toyofumi Chen-Yoshikawa²², Toshihide Yokoyama²³, Tatsuro Okamoto²⁴, Noriko Yanagitani²⁵, Masahide Oki²⁶, Makoto Takahama²⁷, Kenji Sawa²⁸, Hirohito Tada²⁹, Kazuhiko Nakagawa¹⁰, Tetsuya Mitsudomi³⁰ and Kazuto Nishio³ 

- 1 Department of Respiratory Medicine, Kanagawa Cardiovascular and Respiratory Center, Yokohama, Japan
- 2 Division of Thoracic Surgery, National Cancer Center Hospital East, Kashiwa, Japan
- 3 Department of Genome Biology, Kindai University Faculty of Medicine, Osaka-Sayama, Japan
- 4 Department of Data Science, National Cancer Center Hospital East, Kashiwa, Japan
- 5 Internal Medicine III, Wakayama Medical University, Japan
- 6 Department of Respiratory Medicine, Hiroshima Citizens Hospital, Japan
- 7 Department of Thoracic Surgery, Aichi Cancer Center Hospital, Nagoya, Japan
- 8 Department of Pulmonary Medicine, Sendai Kousei Hospital, Japan
- 9 Division of Thoracic Surgery, Shizuoka Cancer Center, Sunto-gun, Japan
- 10 Department of Medical Oncology, Kindai University Faculty of Medicine, Osaka-Sayama, Japan
- 11 Department of Thoracic Oncology, National Hospital Organization Kinki-Chuo Chest Medical Center, Sakai, Japan
- 12 Department of Surgical Oncology, Hiroshima University, Japan
- 13 Department of General Thoracic Surgery, Chiba University Graduate School of Medicine, Japan
- 14 Department of General Thoracic Surgery, Osaka International Cancer Institute, Japan
- 15 Department of Respiratory Medicine, Juntendo University Graduate School of Medicine, Bunkyo-ku, Japan
- 16 Department of Surgery, Tokyo Medical University, Shinjuku-ku, Japan
- 17 Division of Thoracic Surgery, Respiratory Disease Center, Seirei Mikatahara General Hospital, Hamamatsu, Japan
- 18 Department of Respiratory Medicine, Kanazawa University Hospital, Japan
- 19 Department of Thoracic Oncology, Kanagawa Cancer Center, Yokohama, Japan
- 20 Department of General Thoracic Surgery, Okayama University Graduate School of Medicine, Japan
- 21 Department of Thoracic Surgery, Yamaguchi-Ube Medical Center, Japan
- 22 Department of Thoracic Surgery, Nagoya University Graduate School of Medicine, Japan
- 23 Department of Respiratory Medicine, Kurashiki Central Hospital, Japan
- 24 Department of Thoracic Oncology, National Hospital Organization Kyushu Cancer Center, Fukuoka, Japan
- 25 Department of Thoracic Medical Oncology, Cancer Institute Hospital of Japanese Foundation for Cancer Research, Koto-ku, Japan
- 26 Department of Respiratory Medicine, National Hospital Organization Nagoya Medical Center, Japan
- 27 Department of General Thoracic Surgery, Osaka City General Hospital, Japan
- 28 Department of Clinical Oncology, Osaka Metropolitan University Graduate School of Medicine, Japan
- 29 Department of Thoracic Surgery, Suita Tokushukai Hospital, Japan
- 30 Division of Thoracic Surgery, Kindai University Faculty of Medicine, Osaka-Sayama, Japan

Abbreviations

cis/vin, cisplatin plus vinorelbine; CREBBP, cAMP response element binding protein; CSMD3, CUB and Sushi multiple domains 3; DCC, deleted in colorectal cancer; DFS, disease-free survival; EGFR, epidermal growth factor receptor; FFPE, formalin-fixed, paraffin-embedded; HR, hazard ratio; KMT, lysine (K)-specific methyltransferase; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; OS, overall survival; PPS, per protocol set; SYNE1, spectrin repeat containing nuclear envelope protein 1; TKI, tyrosine kinase inhibitor; TMB, tumor mutation burden; TP53, tumor protein p53.

Keywords

adjuvant therapy; early-stage; epidermal growth factor receptor; non-small cell lung cancer; tyrosine kinase inhibitor

Correspondence

S. Ikeda, Department of Respiratory Medicine, Kanagawa Cardiovascular and Respiratory Center, 6-16-1 Tomioka-higashi, Kanazawa-ku, Yokohama, Kanagawa 236-0051, Japan
Tel: +81 45 701 9581
E-mail: ikeda.0880f@kanagawa-pho.jp, isatoshi0112@gmail.com

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The phase III IMPACT study (UMIN000044738) compared adjuvant gefitinib with cisplatin plus vinorelbine (cis/vin) in completely resected *epidermal growth factor receptor (EGFR)*-mutated non-small cell lung cancer (NSCLC). Although the primary endpoint of disease-free survival (DFS) was not met, we searched for molecular predictors of adjuvant gefitinib efficacy. Of 234 patients enrolled in the IMPACT study, 202 patients were analyzed for 409 cancer-related gene mutations and tumor mutation burden using resected lung cancer specimens. Frequent somatic mutations included *tumor protein p53 (TP53)*; 58.4%), *CUB and Sushi multiple domains 3 (CSMD3)*; 11.8%), and *NOTCH1* (9.9%). Multivariate analysis showed that *NOTCH1* co-mutation was a significant poor prognostic factor for overall survival (OS) in the gefitinib group and *cAMP response element binding protein (CREBBP)* co-mutation for DFS and OS in the cis/vin group. In patients with *NOTCH1* co-mutations, gefitinib group had a shorter OS than cis/vin group (Hazard ratio 5.49, 95% CI 1.07–28.00), with a significant interaction (P for interaction = 0.039). In patients with *CREBBP* co-mutations, the gefitinib group had a longer DFS than the cis/vin group, with a significant interaction (P for interaction = 0.058). In completely resected *EGFR*-mutated NSCLC, *NOTCH1* and *CREBBP* mutations might predict poor outcome in patients treated with gefitinib and cis/vin, respectively.

1. Introduction

Although patients who underwent complete resection of non-small cell lung cancer (NSCLC) are expected to have favorable survival outcome, 5-year disease-free survival (DFS) and overall survival (OS) for completely resected NSCLC were low: 46–50% and 56–64% for pathological stage IIA–IIB, and 28% and 48% for stage IIIA, respectively [1]. Since the recurrence often occurs as distant metastases, cisplatin-based adjuvant chemotherapy has long been used as a standard-of-care. However, an increase in 5-year survival rates is only 5–10% [2,3].

For advanced NSCLC harboring *epidermal growth factor receptor (EGFR)* mutations, EGFR-tyrosine kinase inhibitors (TKIs) have demonstrated higher efficacy than platinum-based chemotherapy and are now the standard of care for the first-line therapy [4–9]. Thus, attempting to apply EGFR-TKIs as adjuvant therapy after complete resection of *EGFR*-mutated NSCLC is logical. The ADJUVANT/CTONG1104 study compared gefitinib with cisplatin/vinorelbine (cis/vin) for stage II–III *EGFR*-mutated NSCLC and showed a significant prolongation of DFS by gefitinib (hazard ratio [HR] 0.56, $P = 0.001$) but not OS [10,11]. We recently reported that, in a similarly designed WJOG6410L/IMPACT study, gefitinib failed to significantly prolong DFS and OS [12]. However, DFS curves

of these two studies showed a similar trend: the gefitinib group was superior during the first 4 years, then the two curves crossed, and this initial superiority was not translated into an OS benefit. Meanwhile, the placebo-controlled randomized phase III ADAURA study, launched later than the IMPACT and ADJUVANT/CTONG1104 trials, demonstrated that osimertinib for 3 years provided statistically significant and clinically meaningful DFS and OS benefits, as well as a reduction in central nervous system recurrence [13,14]. Based on these results, osimertinib has been approved by various national and regional regulatory agencies. However, the 4-year DFS rate of adjuvant osimertinib for *EGFR*-mutated stage II–III NSCLC is only 70%, and the presence of common *EGFR* mutations alone is still not a sufficient biomarker for adjuvant EGFR-TKI.

To date, in advanced *EGFR*-mutated NSCLC, co-occurring mutations and/or amplifications in the *tumor protein p53 (TP53)*, *HER2*, *MET*, *CDK4/6* gene, etc., have been reported to negatively affect the survival outcome of the patients treated with EGFR-TKIs [15–23]. We hypothesized that in the adjuvant setting, the benefits of gefitinib might be clinically meaningful by selecting patients who have specific molecular biomarkers among those with *EGFR* mutations. With these backgrounds, we decided to search for molecular predictors of efficacy using tumor specimens from the phase III IMPACT study.

2. Materials and methods

2.1. Clinical specimens

The eligibility criteria of this IMPACT-TR study included (a) enrollment in the IMPACT study and (b) availability of surgically resected lung cancer tissue specimens. The registration period was from April 2021 to June 2022. Formalin-fixed, paraffin-embedded (FFPE) specimens of surgically resected tumors (10 slides of 4–5 μm thickness) were available in 211 of the 234 patients enrolled in the IMPACT study. These specimens were subjected to histologic examination and those that contained sufficient tumor cells (at least 10%) were used for nucleic acid extraction. DNA was isolated from tissues using the GeneRead DNA FFPE Kit (Qiagen, Valencia, CA, USA). The quality and quantity of nucleic acids were verified using a NanoDrop 2000 instrument and PicoGreen dsDNA Reagent (Thermo Scientific, Wilmington, DE, USA).

2.2. Next-generation sequencing (NGS)

Co-existing somatic mutation profiling and tumor mutation burden (TMB) estimation were performed using the OncoPrint Tumor Mutation Load Assay (Thermo Fisher Scientific, Wilmington, DE, USA) in accordance with the manufacturer's recommended protocol. The assay covers 1.65 Mb across 409 oncogenes relevant across major cancer types (full list of the genes is available at <https://www.thermofisher.com/order/catalog/product/A37909>). Sequencing was performed using IonS5™ XL platform (Thermo Fisher Scientific). Sequencing data were analyzed with Ion Torrent Suite and Ion Reporter (Thermo Fisher Scientific). In order to evaluate the quality of the sequencing, on-target alignment rate > 90%, mean depth > 500, and deamination ≤ 10 were set as the reference values. Samples that deviated from the reference values for any of the items were considered unassessable and subsequently excluded from further investigations in this study.

2.3. Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics Review Board of National Cancer Center Hospital East on February 22, 2021 (approval number: 2020-401), and the Institutional Review Board or Ethics Committee of 23 participating facilities in Japan. This study was registered in the University

Hospital Medical Information Network Clinical Trials Registry on July 2, 2021 (registry number: UMIN000044738). Written informed consent was obtained for patients who were still being followed up. Patients who had died or were no longer being followed up were opted out according to the criteria of each participating facilities.

2.4. Statistical analysis

Of the patients enrolled in this IMPACT-TR study, those who fell within the Per Protocol Set (PPS) population as defined in the WJOG6410L/IMPACT study (excluding patients not receiving protocol treatment and ineligible patients from the ITT population) and successfully performed each of the measures were analyzed.

To explore whether co-existing somatic mutations and TMB are associated with DFS and OS in completely resected *EGFR*-mutated NSCLC who received adjuvant gefitinib, the following analyses were performed in the gefitinib group; (a) The detected co-existing somatic mutations with a prevalence of $\geq 5\%$ were considered as candidate biomarkers. TMB was categorized into TMB high and TMB low groups, with the median value as a cut-off. (b) Univariate and multivariate analyses using the Cox proportional hazards model were performed to analyze the correlation between individual parameters and DFS or OS, and HRs and their 95% confidence intervals were calculated. Kaplan–Meier curves were used to estimate survival, and the log-rank test was used to compare DFS and OS between groups. The same analyses described above were also performed in the cis/vin group.

Subsequently, we compared DFS and OS between the gefitinib and cis/vin groups among patient subgroups according to the biomarkers. The interaction of each biomarker was tested using the Cox proportional hazards model and *P* for interaction was calculated, with a *P*-value of < 0.1 indicating a significant interaction between treatment and marker. Because *P* values for interactions have low statistical power and are unlikely to reach statistical significance, 0.1 or 0.2 is generally used as the significance level [24].

When comparing between groups, Fisher's exact test was used for nominal variables. The statistical significance level for all tests (excluding tests of interaction for each biomarker) was set at 5% (*P* = 0.05). JMP (ver. 14.0; SAS Institute Inc., Cary, NC, USA) and GRAPHPAD PRISM software (ver. 8; GraphPad Software Inc., San Diego, CA, USA) were used for the statistical analysis.

3. Results

3.1. Sample availability and clinical characteristics

Of the collected 211 specimens, 202 from patients who fell within the PPS population were analyzed (Fig. 1). Of these 202 patients, there were no differences in baseline patient characteristics, 5-year DFS, or 5-year OS between the gefitinib group ($N = 101$) and the cis/vin group ($N = 101$) (Table 1). The success rates of somatic mutation analysis and TMB analysis were 79.7% and 68.3%, respectively. Significant differences were not observed in patient characteristics among those successfully analyzed by somatic mutation analysis ($N = 161$) and TMB analysis ($N = 138$) (Table 1).

3.2. Profile of NGS

Co-existing somatic mutations with a prevalence of $\geq 5\%$ and the TMB values are shown in Fig. 2 and Table S1. The most frequent co-existing somatic mutation was *TP53* (58.4%), followed by *CUB* and *Sushi multiple domains 3 (CSMD3)*, 11.8%), *NOTCH1* (9.9%) and *Spectrin Repeat Containing Nuclear Envelope Protein 1 (SYNE1)*, 9.9%). Co-mutation of *cAMP response element binding protein (CREBBP)* was found in 5%. The median TMB was 6.67 mutations-Mb⁻¹. There were no differences in the frequencies of various coexisting somatic mutations or TMB between the gefitinib and cis/vin group (Table S1).

3.3. Correlation of individual parameters with clinical outcomes

In the gefitinib group, univariate and multivariate analyses revealed no statistically significant predictors of DFS. However, *NOTCH1* mutation (HR 4.18, 95% CI 1.65–10.61, $P = 0.003$), *Lysine (K)-specific methyltransferase (KMT) 2C* mutation (HR 2.91, 95% CI 1.09–7.82, $P = 0.034$) and *deleted in colorectal cancer (DCC)* mutation (HR 3.72, 95% CI 1.11–12.50, $P = 0.034$) were associated with shorter OS (Table S2). In multivariate analysis, no statistically significant predictors of DFS were found (Fig. 3A), but *NOTCH1* mutation (HR 5.90, 95% CI 1.85–18.82, $P = 0.003$) was associated with shorter OS (Fig. 3B). Figure S1 shows the Kaplan–Meier curves for DFS and OS compared with and without *NOTCH1* mutation in each treatment arms.

In the cis/vin group, multivariate analysis showed that *CREBBP* mutation was significantly associated with shorter DFS (HR 6.47, 95% CI 1.56–26.87, $P = 0.010$) and shorter OS (HR 7.51, 95% CI 1.06–53.33, $P = 0.044$) (Table S2, Fig. 3C,D). Figure S2 shows the Kaplan–Meier curves for DFS and OS compared with and without *CREBBP* mutation in each treatment arms.

TP53 co-mutation and TMB were not associated with DFS or OS in either treatment group.

3.4. Interaction between molecular biomarkers and treatment effect on DFS and OS

The interaction of DFS and OS by treatment groups and biomarkers are shown in Fig. 4 and Table S3.

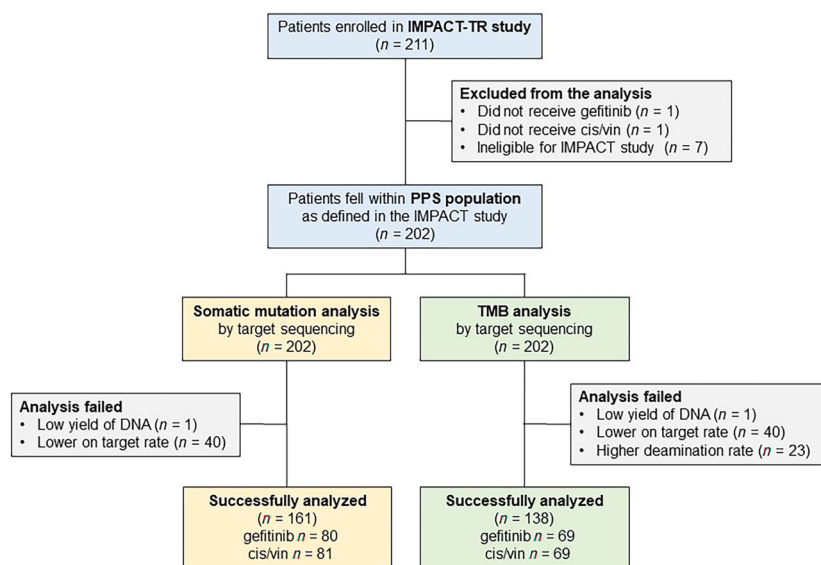


Fig. 1. Patient disposition. Of the collected 211 specimens, 202 from patients who fell within the PPS population were analyzed. The success rates of somatic mutation analysis and TMB analysis were 79.7% and 68.3%, respectively.

Table 1. Characteristics of patients successfully analyzed in each test. ECOG, Eastern Cooperative Oncology Group; PS, performance status.

	All analyzed patients (N = 202)				Successfully analyzed for co-existing somatic mutations (N = 161)				Successfully analyzed for tumor mutation burden (N = 138)			
	Gefitinib group (N = 101)		Cis/vin group (N = 101)		Gefitinib group (N = 80)		Cis/vin group (N = 81)		Gefitinib group (N = 69)		Cis/vin group (N = 69)	
	N	%	N	%	N	%	N	%	N	%	N	%
Age												
< 65	54	53.5	50	49.5	39	48.8	39	48.1	33	47.8	33	47.8
≥ 65	47	46.5	51	50.5	41	51.3	42	51.9	36	52.2	36	52.2
Gender												
M	40	39.6	39	38.6	32	40.0	32	39.5	27	39.1	28	40.6
F	61	60.4	62	61.4	48	60.0	49	60.5	42	60.9	41	59.4
Stage												
IIA	32	31.7	34	33.7	27	33.8	32	39.5	20	29.0	30	43.5
IIB	5	5.0	3	3.0	4	5.0	3	3.7	4	5.8	1	1.4
IIIA	61	60.4	62	61.4	46	57.5	44	54.3	42	60.9	37	53.6
IIIB	3	3.0	2	2.0	3	3.8	2	2.5	3	4.3	1	1.4
ECOG PS												
0	83	82.2	78	77.2	66	82.5	60	74.1	56	81.2	50	72.5
1	18	17.8	23	22.8	14	17.5	21	25.9	13	18.8	19	27.5
Smoking history												
Former	41	40.6	36	35.6	34	42.5	28	34.6	28	40.6	25	36.2
Never	60	59.4	65	64.4	46	57.5	53	65.4	41	59.4	44	63.8
EGFR												
19del	57	56.4	51	50.0	47	58.8	40	48.8	42	60.9	37	52.9
L858R	44	43.6	51	50.0	33	41.3	42	51.2	27	39.1	33	47.1
	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
5-Year DFS	32.3	23.8–42.1	31.4	23.0–41.3	33.3	23.8–44.5	34.3	24.6–45.5	32.8	22.7–44.9	36.1	25.4–48.3
5-Year OS	77.6	68.3–84.7	75.2	65.6–82.8	76.6	65.9–84.8	76.8	66.1–84.9	78.8	67.3–87.0	75.7	63.9–84.5

Candidate biomarkers were selected based on the results of univariate and multivariate analyses described in the previous section (Fig. 3 and Table S2). In addition, *TP53* and TMB, which had been considered promising from previous reports and focused on in this study, were also added as candidate biomarkers. For the interaction test in this study, a *P*-value of < 0.1 was considered to indicate an interaction between treatment and marker.

The interaction between *NOTCH1* co-mutation and treatment effect on OS was significant (*P* for interaction = 0.039) (Fig. 4B). The gefitinib group had a shorter OS than the cis/vin group (median 55.3 months vs Not Reached; HR 5.49, 95% CI 1.07–28.00) in patients with *NOTCH1* mutation, but no such difference was observed in patients without *NOTCH1* mutation (Fig. 5B). The interaction between *NOTCH1* co-mutation and DFS was not significant (Figs 4A and 5A).

The interaction between *CREBBP* co-mutation and treatment effect on DFS was also significant (*P* for interaction = 0.058) (Fig. 4A). The gefitinib group had

a longer DFS than the cis/vin group in patients with *CREBBP* mutation (median 53.8 months vs 12.0 months; HR Not Evaluable), but no such difference was observed in patients without *CREBBP* mutation (Fig. 5C). The interaction between *CREBBP* mutation and OS was not significant (Figs 4B and 5D).

4. Discussion

Few reports have investigated biomarkers predicting the efficacy of adjuvant therapy and the risk of post-operative recurrence in patients with completely resected *EGFR*-mutated NSCLC, particularly in conjunction with data from interventional studies. The biomarker analysis of ADJUVANT/CTONG1104 study reported that the MINERVA score, which is calculated using five potential biomarkers of DFS (*RBI* alteration, *NKX2-1* copy number gain, *CDK4* copy number gain, *TP53* exon4/5 missense alterations, and *MYC* copy number gain) identified by comprehensive genomic profiling, could be used to categorize resected

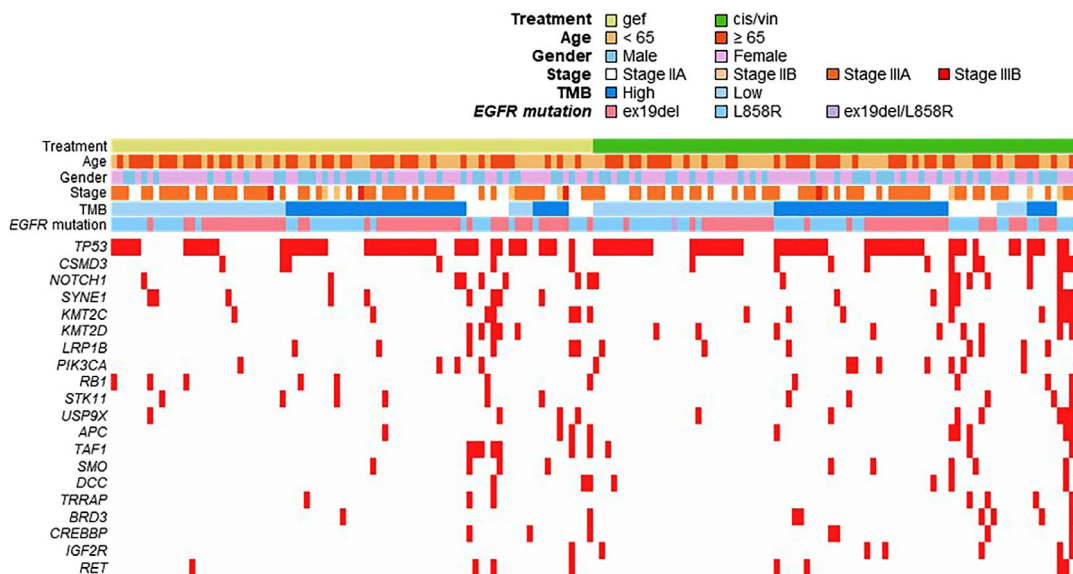


Fig. 2. Profile of next-generation sequencing. The detected co-existing somatic mutations with a prevalence of $\geq 5\%$ and the TMB status. APC, adenomatous polyposis coli; BRD3, bromodomain containing 3; IGF2R, insulin like growth factor 2 receptor; LRP1B, LDL receptor related protein 1B; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; RB1, RB transcriptional corepressor 1; RET, ret proto-oncogene; SMO, smoothened, frizzled class receptor; STK11, serine/threonine kinase 11; TAF1, TATA-Box binding protein associated factor 1; TRRAP, transformation/transcription domain-associated protein; USP9X, ubiquitin specific peptidase 9 X-linked.

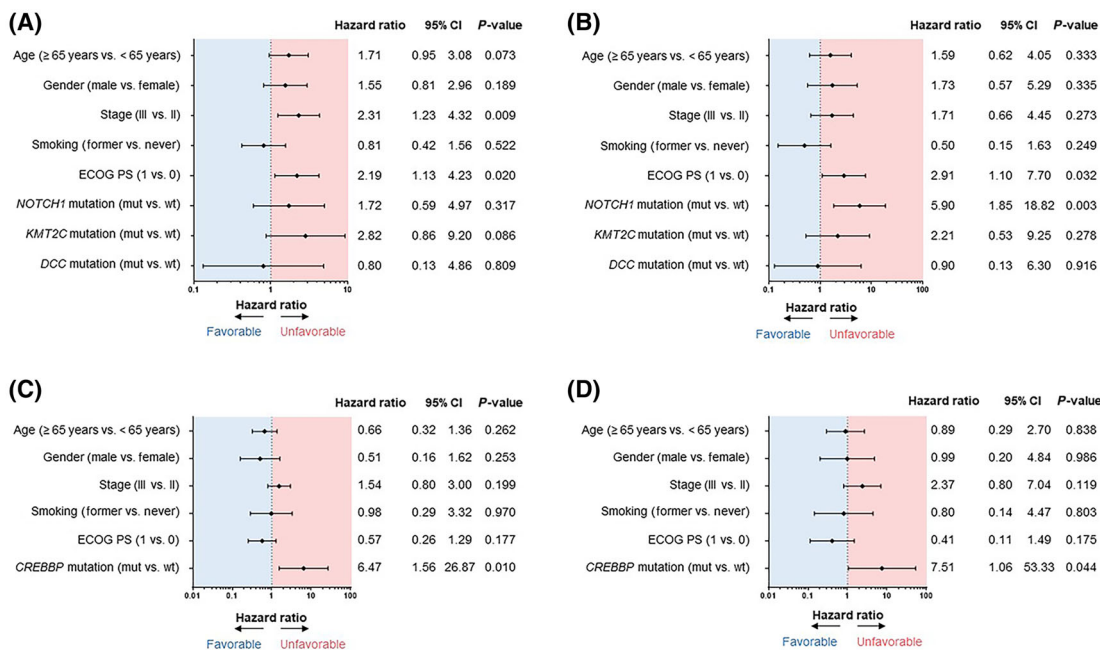


Fig. 3. Multivariate analysis using Cox proportional hazards model. Univariate and multivariate analyses using the Cox proportional hazards model were performed to analyze the correlation between individual parameters and DFS/OS, and HRs and their 95% confidence intervals were calculated. Forest plots represent the results of multivariate analysis using the Cox proportional hazards model for each treatment group. DFS (A) and OS (B) in the gefitinib group; DFS (C) and OS (D) in the cis/vin group. ECOG, Eastern Cooperative Oncology Group; PS, performance status.

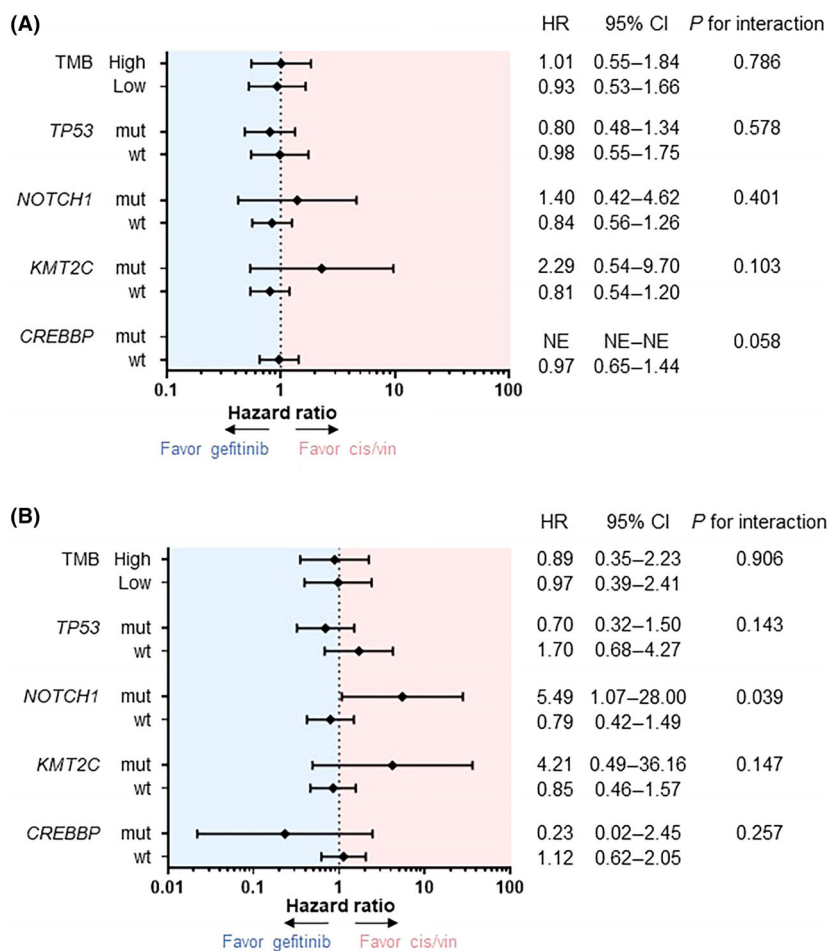


Fig. 4. Subgroup analysis and interaction test. Forest plots showing comparison of DFS (A) and OS (B) between gefitinib and cis/vin groups in patient subgroups according to representative biomarkers, and results of interaction tests. The interaction of each biomarker was tested using the Cox proportional hazards model and *P* for interaction was calculated, with a *P*-value of < 0.1 indicating a possible interaction between treatment and marker. NE, not evaluable.

EGFR-mutated NSCLC into three subgroups by efficacy of adjuvant therapy (highly TKI-preferable, TKI-preferable, and chemotherapy-preferable groups) [25]. However, due to the complexity of this scoring system and the small sample sizes, the results of the study did not provide a definitive biomarker.

This IMPACT-TR study demonstrated two important findings. First, *NOTCH1* co-mutation can serve as a poor prognostic factor for OS in the gefitinib group while probably predicting a poor response to adjuvant *EGFR*-TKI. Second, *CREBBP* co-mutation may be a biomarker for predicting a poor response to adjuvant platinum-based chemotherapy for completely resected *EGFR*-mutated NSCLC.

NOTCH1 is a gene encoding a transmembrane protein that plays an important role in tumorigenesis, and *NOTCH1* mutations are frequently detected in colon adenocarcinoma, lung adenocarcinoma, and breast invasive ductal carcinoma [26]. *NOTCH1* has been reported to be associated with acquired resistance to gefitinib by triggering epithelial-mesenchymal transition, both *in vivo*

and *in vitro* [27–29]. Since there was a significant interaction between *NOTCH1* co-mutation and treatment effect on OS in this IMPACT-TR study, *NOTCH1* co-mutation may predict the efficacy of adjuvant gefitinib rather than simply being a prognostic factor in postoperative *EGFR*-mutated NSCLC. In fact, the biomarker analysis of the ADJUVANT/CTONG1104 study also demonstrated that *NOTCH1* co-mutation tended to be associated with shorter DFS of gefitinib (HR 2.48, 95% CI 1.12–5.47), although it was detected less frequently at 5–6% [25]. The analysis of DFS did not show statistical significance in our study, but a trend was observed in the multivariate analysis of the gefitinib group, suggesting an association between *NOTCH1* co-mutation and shorter DFS (HR 1.72, 95% CI 0.59–4.97, *P* = 0.317). A subgroup analysis of patients with *NOTCH1* co-mutation also revealed a trend toward shorter DFS in the gefitinib group (HR 1.40, 95% CI 0.42–4.62, *P* for interaction = 0.401). Since the number of *NOTCH1* co-mutation positive samples in both IMPACT-TR and ADJUVANT/CTONG1104 studies was small, further studies

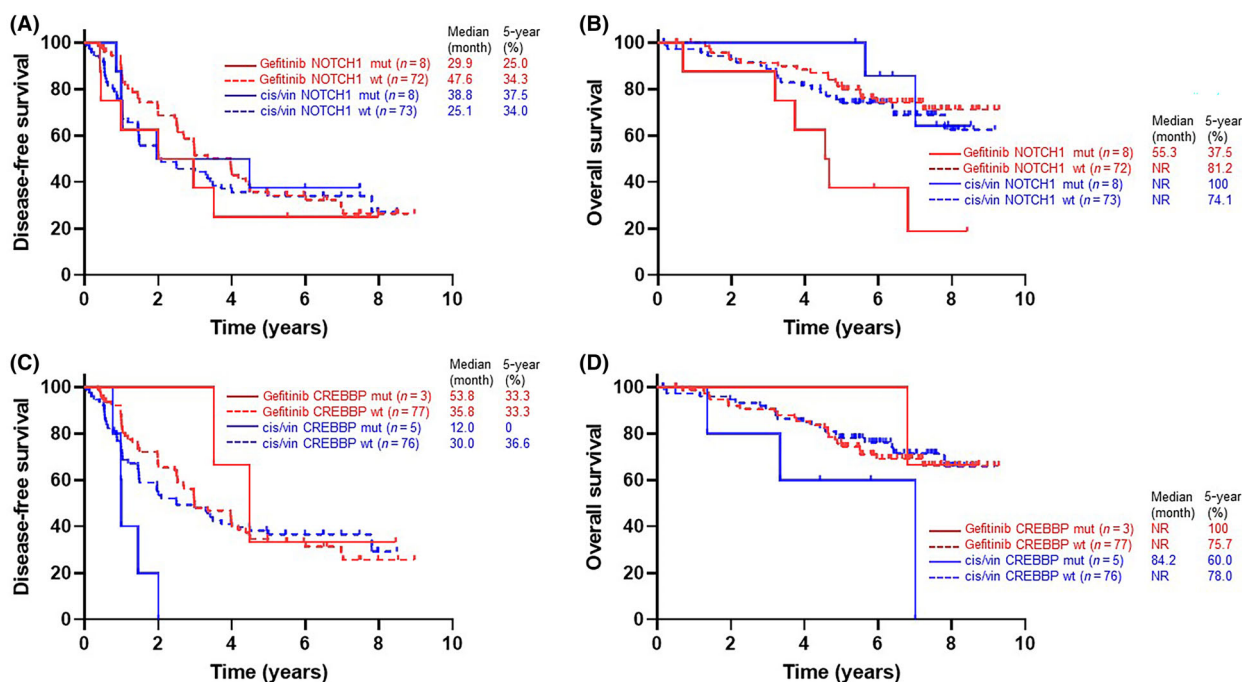


Fig. 5. Kaplan–Meier curve with and without *NOTCH1* and *CREBBP* mutations. Kaplan–Meier curves for DFS (A) and OS (B), divided into four groups according to treatment (gefitinib [red] or cis/vin [blue]) and with [solid line] or without [dashed line] *NOTCH1* mutation. For *CREBBP* mutation, Kaplan–Meier curves for DFS (C) and OS (D) are also shown in the same way, divided into four groups. NR, not reached.

exploring a larger number of cases are warranted. However, the 5-year DFS rate with gefitinib in patients without *NOTCH1* co-mutation is only 34.3% (Fig. 5), which is far below the data for adjuvant osimertinib in the ADAURA study. Therefore, it may be difficult to substitute gefitinib for osimertinib in the adjuvant setting, even in patients without *NOTCH1* co-mutation. Furthermore, it is also necessary to verify whether *NOTCH1* co-mutation can be a biomarker for osimertinib, the current standard for adjuvant EGFR-TKI. As for the ADAURA study, data exploring biomarkers such as co-mutations are not yet available. The *NOTCH1* pathway has also been reported to be associated with osimertinib resistance, suggesting that *NOTCH1* co-mutation could be a promising biomarker candidate [30]. To note, the frequency of *NOTCH1* co-mutation in this study was skewed at 4.6% (4/87) for exon 19 deletion and 16.0% (12/75) for L858R. A subgroup analysis of the IMPACT study showed that patients with L858R had a poor response to adjuvant gefitinib, with a HR of 1.164 (95% CI 0.739–1.833) for DFS [12], which may have been partly due to this high frequency of *NOTCH1* co-mutations. No such trend has been reported in previous reports, and confirmation in a larger number of cases is also warranted.

Meanwhile, *CREBBP* co-mutation was not only significantly associated with shorter DFS and OS in multivariate analysis in the cis/vin group, but also showed a significant interaction in subgroup analysis of DFS in this study. These results suggest that *CREBBP* co-mutation may be a predictor of adjuvant cis/vin efficacy for *EGFR*-mutated NSCLC. *CREBBP* mutations are observed in various types of cancers including lung adenocarcinoma and are involved in carcinogenesis and progression [23]. In addition, it has been suggested that *CREBBP* mutation may lead to chemotherapy resistance via impaired histone acetylation and corresponding gene dysregulation, not only in hematologic tumors such as lymphoma and acute lymphoblastic leukemia [31–33], but also in various solid tumors such as breast cancer and small-cell lung cancer [34,35]. Although osimertinib is undoubtedly the key drug for postoperative adjuvant therapy in *EGFR*-mutated NSCLC, platinum-based chemotherapy prior to osimertinib remains the standard based on the ADAURA study design. However, the efficacy of adjuvant osimertinib is consistent with or without prior chemotherapy according to a subgroup analysis of the ADAURA study [29,36]. Therefore, if there are significant toxicity concerns with adjuvant chemotherapy or the patient does not wish to

receive it, the presence of *CREBBP* co-mutation may support the decision to omit platinum-based chemotherapy and start with osimertinib.

TP53 co-mutation can negatively affect the efficacy of EGFR-TKI in advanced NSCLC and was one of the potential biomarkers found in the biomarker analysis of the ADJUVANT/CTONG1104 study. Although it was detected in 58.4% of the patients in this IMPACT-TR study, no significant association with DFS or OS was found. There are several subtypes of *TP53* mutations, and the ADJUVANT/CTONG1104 study found only *TP53* exon4/5 missense alterations as potential biomarker for adjuvant gefitinib. Because the archival surgically resected specimens used in this IMPACT-TR study are old, the *TP53* mutation subtypes might not have been accurately evaluated. Among the other potential biomarkers found in the ADJUVANT/CTONG1104 study, *RBI* mutation was also detected in 6.8% of the patients in this study, but showed no usefulness as a biomarker. Moreover, although higher TMB has been reported to be associated with better prognosis in patients with resected NSCLC [37] and was of interest in this study, no significant association with DFS/OS was demonstrated for *EGFR*-mutated NSCLC in this study.

This study has some limitations. First, the success rates of NGS analysis were low (< 80%), probably attributed to the enrollment period for the IMPACT study (September 2011 to December 2015); this means that the archival surgically resected specimens used in this study were old. Second, the multiplicity of comparisons should be considered because analysis was performed on many co-mutations for the number of enrolled patients. In this study, we initially planned to narrow down the candidate factors considering false discovery rate and to conduct a two-step test by dividing into test and validation sets, but we abandoned the plan mainly because of the low frequency of each co-mutation. Third, the number of patients with *NOTCH1* and *CREBBP* mutations in this study was small. Therefore, this study is only an exploratory biomarker study, and further validation using a larger number of patients is warranted.

5. Conclusion

In patients with completely resected *EGFR*-mutated NSCLC, *NOTCH1* mutation was identified as a poor prognostic factor for OS in the gefitinib group and may serve as a predictor of a poor response to gefitinib. *CREBBP* mutation was identified as a poor prognostic factor for DFS and OS in the cis/vin group and may serve as a predictor of a poor response to cis/vin.

Further studies in a larger number of patients, as well as validation of the utility of these biomarkers in patients receiving adjuvant osimertinib are warranted.

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Conflict of interest

Ikeda S received honoraria from AstraZeneca, Bristol Myers Squibb, Ono, Taiho, Chugai, Boehringer Ingelheim, Eli Lilly, Takeda, and Pfizer; research funding from AstraZeneca and Chugai; and took on a consulting or advisory roles for AstraZeneca, Chugai and Daiichi Sankyo. Tsuboi M received honoraria from AstraZeneca, Bristol Myers Squibb, Ono, Taiho, Chugai, Eli Lilly, Johnson & Johnson, Novartis, MSD, Medtronic and Daiichi Sankyo; research funding from AstraZeneca, Boehringer Ingelheim, Merck, Ono, Bristol Myers Squibb, Novartis, MirXES Japan and BMG KK; and took on a consulting or advisory roles for AstraZeneca, Chugai, MSD and Novartis. Sakai K received honoraria from Chugai, Hitachi, Life Technologies, Takeda and Yodosha Co., Ltd. Misumi T has no relationships to disclose. Akamatsu H received honoraria from AstraZeneca, Eli Lilly, Pfizer, MSD, Bristol Myers Squibb, Ono, Taiho, Chugai, Boehringer Ingelheim, Amgen, Nippon Kayaku. Co. Ltd., Novartis and Takeda; research funding from Amgen and Chugai; and took on a consulting or advisory roles for Amgen, Janssen, and Sandoz K.K. Shoda H has no relationships to disclose. Sakakura N has no relationships to disclose. Nakamura A received honoraria from AstraZeneca, Chugai, Eli Lilly, Kyowa Kirin, Taiho, Novartis, Thermo Fisher, and MSD. Ohde Y has no relationships to disclose. Hayashi H received honoraria from AstraZeneca, Eli Lilly, Ono, Bristol Myers Squibb, Taiho, Boehringer Ingelheim, Chugai, Pfizer, MSD, Novartis and Merck Serono; research funding from MSD, Daiichi Sankyo, Taiho, Chugai, SYNEOS HEALTH CLINICAL, Japan Clinical Research Operations, AstraZeneca, IQVIA Services Japan, Covance Japan, Takeda, GlaxoSmithKline, Sanofi, EPS Corporation, Novartis, Medical Research Support, Bristol

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Author contributions

SI contributed to conceptualization, methodology, investigation, visualization, project administration, funding acquisition, and writing (original draft). MTsuboi contributed to conceptualization, supervision, project administration, funding acquisition, and writing (review and editing). KSakai contributed to methodology, formal analysis, visualization, and writing (review and editing). TMisumi contributed to methodology, formal analysis, data curation, and writing (review and editing). HA contributed to methodology, investigation, and writing (review and editing). HShoda, NS, AN, YO, HH, KO, MOkada, IY, JO, KT, NI, MTanahashi, YT, HSaito, ST, HI, TC-Y, TY, TO, NY, MOki, MTakahama, KSawa, and HT

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Data accessibility

All data generated or analyzed during this study are included in this published article.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Kaplan–Meier curve of each treatment arms with and without *NOTCH1* mutation.

Fig. S2. Kaplan–Meier curve of each treatment arms with and without *CERBBP* mutation.

Table S1. Profile of next-generation sequencing.

Table S2. Univariate analysis using Cox proportional hazards model.

Table S3. Subgroup analysis and interaction test.