A retrospective study of the efficacy of combined EGFR-TKI plus VEGF inhibitor/cytotoxic therapy vs. EGFR-TKI monotherapy for PD-L1-positive EGFR-mutant non-small cell lung cancer: North Japan Lung Cancer Study Group 2202

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Abstract. The present multicenter study was performed to compare the efficacy of epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI) monotherapy with that of combined EGFR-TKI plus vascular endothelial growth factor receptor (VEGF) inhibitor/cytotoxic therapy in patients with programmed death-ligand 1 (PD-L1)-positive EGFR-mutant non-small cell lung cancer (NSCLC). Data from patients with PD-L1-positive EGFR-mutant NSCLC were collected from 12 institutes. Survival in patients treated with first- and second-generation EGFR-TKIs, osimertinib (third-generation EGFR-TKI), and combined EGFR-TKI plus VEGF inhibitor/cytotoxic therapy was analyzed by multiple regression analysis with adjustments for sex, performance status, EGFR mutation status, PD-L1 expression level, and the presence or absence of brain metastasis using a Cox proportional hazards model. Data from a total of 263 patients were analyzed, including 111 (42.2%) patients who had received monotherapy with a first- or second-generation EGFR-TKI, 132 (50.2%) patients who had received osimertinib monotherapy, and 20 (7.6%) patients who had received combined EGFR-TKI plus VEGF inhibitor/cytotoxic therapy (hereafter referred to as combined therapy). Multiple regression analysis using the Cox proportional hazards model showed that the hazard ratio (95% confidence interval) for progression-free survival was 0.73 (0.54-1.00) in the patients who had received osimertinib monotherapy and 0.47 (0.25-0.90) in patients who had received combined therapy. The hazard ratio for overall survival was 0.98 (0.65-1.48) in the patients who had received osimertinib monotherapy and 0.52 (0.21-1.31) in patients who had received combined therapy. In conclusion, combined therapy was associated with a significant reduction in the risk of progression compared with first- and second-generation EGFR-TKI monotherapy, and therefore, may be promising for the treatment of patients of NSCLC.

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Introduction

Non-small cell lung cancer (NSCLC) is a leading cause of cancer-related mortality worldwide (1), and the advanced disease is difficult to cure. Although systemic therapy with cytotoxic agents was a standard therapy for advanced NSCLC, the identification of driver gene mutation and development of kinase inhibitors has improved the prognosis of patients with driver gene mutated NSCLC. In the subset of patients with

NSCLC harboring EGFR mutations, it has been reported that treatment with epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKI) conferred a longer progression-free survival (PFS) than treatment with cytotoxic agents (2-4). A longer overall survival (OS) was also reported in patients who received EGFR-TKI therapy than in those who did not, suggesting an OS benefit of EGFR-TKIs in patients with EGFR-mutant NSCLC (5).

Furthermore, it has been shown that osimertinib, a third-generation EGFR-TKI, is effective in overcoming the acquired resistance to EGFR-TKI therapy that is associated with the EGFR exon 20 T790M mutation (6), which is a major mechanism underlying resistance to first- and second-generation EGFR-TKIs (7). Osimertinib also yielded a longer PFS and OS than first-generation EGFR-TKIs in previously untreated patients with EGFR-mutant NSCLC (8,9). Therefore, osimertinib is an important therapeutic alternative for patients with EGFR-mutant NSCLC.

Programmed death-ligand 1 (PD-L1) is an immune checkpoint molecule, and the interaction between PD-1 and PD-L1 inactivates T-cell immunity (10). The PD-L1 expression level in tumors has been correlated with the efficacy of immune checkpoint inhibitor therapy in patients with nonsquamous cell NSCLC (11). In addition, PD-L1 expression is also expected to be a biomarker of the efficacy of EGFR-TKIs in patients with EGFR-mutant NSCLC. PFS after initiation of treatment with first- and second-generation EGFR-TKIs was reported to be shorter in cases of EGFR-mutant NSCLC showing increased tumor PD-L1 expression (12-16). Subsequently, increased tumor expression of PD-L1 was shown to be associated with a shorter PFS after osimertinib therapy (17-20). Furthermore, tumor PD-L1 expression has been associated with a lower detection rate of secondary EGFR exon 20 T790M mutation developing after first- and second-generation EGFR-TKI therapy (12-14), which resulted in a shorter duration of treatment with EGFR-TKIs in patients with PD-L1-positive EGFR-mutant NSCLC (21).

Based on these reports, determining the most appropriate treatment strategy for PD-L1-positive EGFR-mutant NSCLC patients may be a clinical issue. Previous clinical trials have shown that combined EGFR-TKI plus vascular endothelial growth factor (VEGF) inhibitor (22,23) or cytotoxic agent (24,25) therapy conferred a longer PFS than treatment with first-generation EGFR-TKI alone. It has also been reported that tumor cell growth and survival may be relatively less dependent on EGFR signaling in cases of PD-L1-positive EGFR-mutant NSCLC, and it may be advisable to recommend the aforementioned combined therapy (14).

The present retrospective study was conducted to analyze survival after the initiation of treatment with first- or second-generation EGFR-TKI monotherapy, osimertinib monotherapy, and combined therapy in patients with PD-L1-positive EGFR-mutant NSCLC.

Patients and methods

Patients. Data from patients with PD-L1-positive EGFR-mutant NSCLC who had received EGFR-TKI monotherapy or combined EGFR-TKI plus VEGF inhibitor/cytotoxic therapy (hereafter referred to as combined therapy) at one of the 12 participating institutions were retrospectively analyzed. The patient inclusion criteria were established as follows: i) patients who had been cytologically or histopathologically diagnosed with NSCLC; ii) patients with tumors confirmed as harboring common EGFR mutations in clinical practice; iii) patients in whom tumor PD-L1 positivity was confirmed using the 22C3 antibody, with a tumor proportion score (TPS) of ≥1%; and iv) patients who had received EGFR-TKI therapy, including EGFR-TKI monotherapy or combined therapy, between January 2015 and June 2021. The exclusion criteria were determined as follows: i) NSCLC patients with tumors confirmed as harboring uncommon EGFR mutations; ii) patients for whom information on tumor PD-L1 expression was unavailable; and iii) patients who had received treatment with EGFR-TKIs prior to the study period. The present study was conducted following the principles outlined in the Declaration of Helsinki and Ethical Guidelines for Medical and Biological Research Involving Human Subjects (Ministry of Health, Labour and Welfare, Japan). The need to obtain informed consent from the patients was waived under approval from the Ethics Committee, University of Toyama (approval number: R2022070), and we disclosed information about the study to the patients.

Statistical analysis. The endpoints of the present study were PFS, OS, and duration of treatment with EGFR-TKIs. PFS was calculated from the day of treatment initiation until the day on which any progressive disease was confirmed according to the Response Evaluation Criteria in Solid Tumors version 1.1, clinical judgment of progression, or death from any cause was confirmed, and PFS was censored at the last visit without any events. After the discontinuation of EGFR-TKI therapy because of adverse events, if subsequent therapy was initiated before any event was confirmed, PFS was censored on the initiation day of the subsequent treatment. However, any switch between first- and second-generation EGFR-TKIs was not considered a change in treatment. OS was calculated from the initiation day of EGFR-TKI therapy until the day on which death was confirmed, and OS was censored at the last visit prior to death. The duration of treatment with EGFR-TKIs was defined as the sum of the PFS after the initial EGFR-TKI therapy and subsequent osimertinib therapy after acquiring the secondary T790M mutation. The association between the treatment option and risk of progression or death was analyzed using the Cox proportional hazards model, with sex, performance status (PS), EGFR mutation status, PD-L1 expression level, and the presence or absence of brain metastasis set as independent variables. Kaplan-Meier curves were plotted to evaluate PFS and OS and analyzed by the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed using the JMP statistical software package version 15.0.0 (SAS, Cary, NC, USA).

Results

Table I shows the patient characteristics and the therapeutic regimens. Data from a total of 263 patients with PD-L1-positive EGFR-mutant NSCLC were included. The majority of the NSCLC patients (249, 94.7%) were diagnosed with adenocarcinoma. Tumor PD-L1 expression was evaluated

Table I. Patient characteristics and therapeutic regimens (n=263).

Characteristic	Value
Median age, years (range)	71 (29-92)
Age, n (%)	
<75 years	175 (66.5)
≥75 years	88 (33.5)
Sex, n (%)	
Male	92 (35.0)
Female	171 (65.0)
PS, n (%)	
0-1	231 (87.8)
≥2	32 (12.2)
Histology, n (%)	
Adenocarcinoma	249 (94.7)
Others	14 (5.3)
EGFR, n (%)	
del 19	132 (50.2)
L858R	131 (49.8)
PD-L1, n (%)	
1-49%	182 (69.2)
≥50%	81 (30.8)
Brain metastasis, n (%)	
Yes	90 (34.2)
No	173 (65.8)
Therapeutic regimen, n (%)	
Gefitinib	50 (19.0)
Erlotinib	16 (6.1)
Afatinib	45 (17.1)
Osimertinib	132 (50.2)
Erlotinib + bevacizumab	6 (2.3)
Erlotinib + ramucirumab	1 (0.4)
Gefitinib + platinum doublet	9 (3.4)
Osimertinib + platinum doublet	4 (1.5)

EGFR, epidermal growth factor receptor; PD-L1, programmed death ligand-1; PS, performance status.

Table II. Analysis using the Cox proportional hazards model for PFS after the initiation of EGFR-TKI therapy.

Characteristic	HR	95% CI	P-value
Sex			
Male	1.16	0.86-1.58	0.328
Female	1.00		
PS			
0-1	0.68	0.42-1.08	0.105
≥2	1.00		
EGFR			
L858R	1.39	1.03-1.88	0.030
del 19	1.00		
PD-L1			
1-49%	0.81	0.59-1.12	0.206
≥50%	1.00		
Brain metastasis			
No	0.79	0.57-1.09	0.151
Yes	1.00		
EGFR-TKI therapy			
First/second generation	1.00		
Osimertinib	0.73	0.54-1.00	0.053
Combination therapy	0.47	0.25-0.90	0.024

CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; PD-L1, programmed death ligand-1; PFS, progression-free survival; PS, performance status.

Fig. 1 shows the Kaplan-Meier curves for PFS, duration of treatment with EGFR-TKIs, and OS according to the EGFR-TKI received. Among the three treatments, combined therapy was associated with the longest PFS (P=0.009, log-rank test), duration of treatment with EGFR-TKIs (P=0.169, log-rank test) and OS (P=0.292, log-rank test). Osimertinib monotherapy was associated with a longer PFS than first- or second-generation EGFR-TKI monotherapy but with a similar duration of treatment with EGFR-TKIs and OS to those with first- or second-generation EGFR-TKI monotherapy.

Table II shows an analysis of the PFS conducted using the Cox proportional hazards model. Combined therapy was associated with a significant reduction in the risk of disease progression compared with first- and second-generation EGFR-TKI monotherapy, suggesting a PFS benefit. Table III shows an analysis of the OS conducted using the Cox proportional hazards model. Neither osimertinib monotherapy nor combined therapy was associated with any significant reduction in the risk of death compared with first- and second-generation EGFR-TKI monotherapy. In contrast, the EGFR mutation status was associated with both PFS and OS.

Fig. 2 shows the Kaplan-Meier curve for PFS according to the tumor PD-L1 expression level and EGFR mutation status. Although the log-rank test showed no significant difference in PFS, patients with a PD-L1 TPS of ≥50% and tumors harboring the exon 21 L858R point mutation showed the shortest PFS

using tissue specimens in 262 cases and a pleural cell block in 1 patient. The TPS was determined to be 1-49% in 182 (69.2%) patients and ≥50% in 81 (30.8%) patients. Brain metastases were detected in 90 (34.2%) patients at the time of treatment initiation. Of these patients, 40 received local therapy for brain metastases, which involved surgery, whole-brain irradiation, or stereotactic radiotherapy. Of the 263 patients, 111 (42.2%), 132 (50.2%), and 20 (7.6%) received first- or second-generation EGFR-TKI monotherapy, osimertinib monotherapy, and combined therapy, respectively. Disease progression was confirmed in 89, 81, and 11 patients who had received first- or second-generation EGFR-TKI monotherapy, osimertinib monotherapy, and combined therapy, respectively. Secondary T790M mutation was detected in 35 patients, all of whom were subsequently treated with osimertinib.
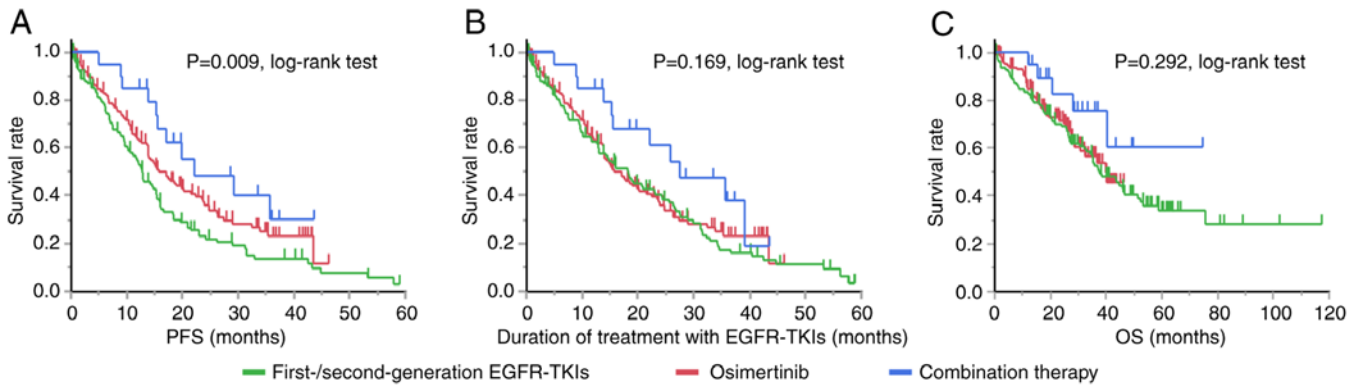
Figure 1. Kaplan-Meier curves for (A) PFS, (B) duration of treatment with EGFR-TKIs and (C) OS according to the therapy received. EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; OS, overall survival; PFS, progression-free survival.
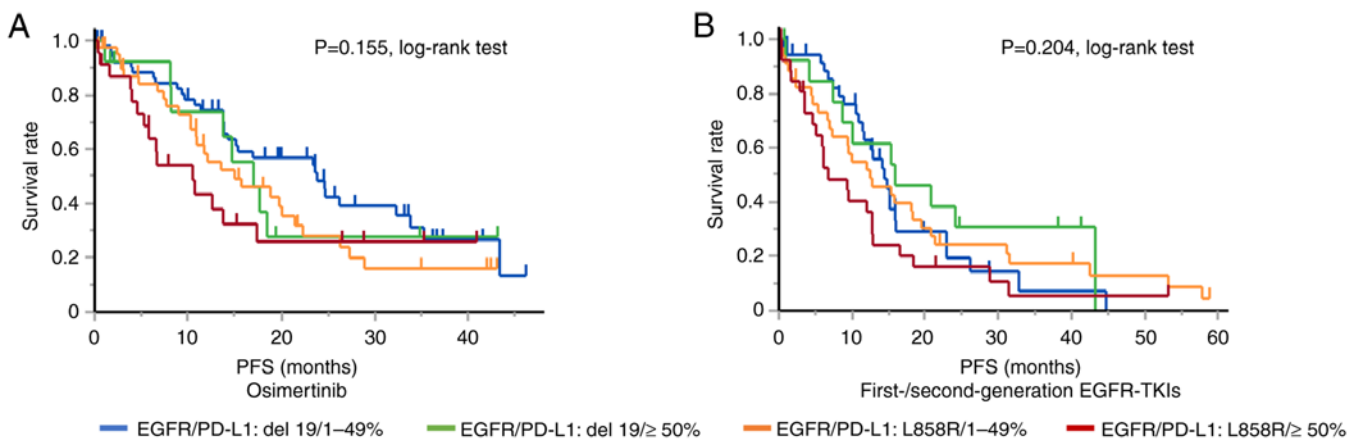

Figure 2. Kaplan-Meier curves for PFS according to the PD-L1 expression level and EGFR mutation status in patients treated with (A) osimertinib or (B) first- and second-generation EGFR-TKIs. EGFR-TKIs, epidermal growth factor receptor-tyrosine kinase inhibitors; PD-L1, programmed death ligand-1; PFS, progression-free survival.

after both osimertinib therapy (10.5 months) and first- or second-generation EGFR-TKI monotherapy (6.8 months).

Discussion

Increased tumor expression of PD-L1, evaluated using the 22C3 antibody, has been associated with poor efficacy of EGFR-TKI monotherapy, independent of the clinical background, including the EGFR mutation status (12-20). The present study showed that combined therapy was significantly associated with a lower risk of disease progression than first- and second-generation EGFR-TKI therapy and was associated with the longest PFS, duration of treatment with EGFR-TKIs, and OS among the treatments used in patients with PD-L1-positive EGFR-mutant NSCLC.

A previous phase III clinical trial showed that osimertinib monotherapy was associated with a longer PFS (8) and OS (9) than first-generation EGFR-TKI monotherapy (median, 18.9 vs. 10.2 months and 38.6 vs. 31.8 months, respectively). Regarding second-generation EGFR-TKIs, a network meta-analysis suggested that osimertinib monotherapy might confer a longer PFS than second-generation EGFR-TKI monotherapy (afatinib or dacomitinib), while an observational study using propensity score analysis showed a similar time

to discontinuation of EGFR-TKIs between osimertinib and afatinib (20.5 vs. 18.6 months, respectively) (26). It is difficult to compare the findings of the present study with these previous reports because we considered patients treated with first- and second-generation EGFR-TKIs as a single group, but the multivariate analysis showed no significant reduction in the risk of disease progression or death, and the Kaplan-Meier curves showed a similar duration of treatment with EGFR-TKIs and OS between patients who received osimertinib and those who received first- or second-generation EGFR-TKI monotherapy.

Previous clinical trials have demonstrated the PFS benefit of combined therapy regimens, including erlotinib plus VEGF inhibitors and gefitinib plus cytotoxic agents, compared with first-generation EGFR-TKI monotherapy. The NEJ026 trial, a phase 3 trial, showed that PFS was significantly longer in combination therapy with bevacizumab plus erlotinib compared with erlotinib monotherapy (16.9 months vs. 13.3 months, respectively) (22). Similarly, the Relay trial, another phase 3 trial, demonstrated that PFS was significantly longer in the combination therapy with ramucirumab plus erlotinib than that in the erlotinib monotherapy (19.4 months vs. 12.4 months, respectively) (23).

Additionally, VEGF plays a major role in angiogenesis, and VEGF inhibitors exert several functions, including the

Table III. Analysis using the Cox proportional hazards model for OS after the initiation of EGFR-TKI therapy.

Characteristic	HR	95% CI	P-value
Sex			
Male	1.31	0.89-1.92	0.174
Female	1.00		
PS			
0-1	0.53	0.30-0.92	0.025
≥2	1.00		
EGFR			
L858R	1.88	1.28-2.77	0.001
del 19	1.00		
PD-L1			
1-49%	1.24	0.80-1.90	0.334
≥50%	1.00		
Brain metastasis			
No	0.86	0.58-1.29	0.469
Yes	1.00		
EGFR-TKI therapy			
First/second generation	1.00		
Osimertinib	0.98	0.65-1.48	0.925
Combination therapy	0.52	0.21-1.31	0.166

CI, confidence interval; EGFR, epidermal growth factor receptor; HR, hazard ratio; OS, overall survival; PD-L1, programmed death ligand-1; PS, performance status.

inhibition of angiogenesis, improvement of hypoxia in the tumor microenvironment, normalization of interstitial fluid pressure, and sensitization of tumors to anticancer therapies (27). Furthermore, the *in vivo* study demonstrated that bevacizumab restored the sensitivity of EGFR-TKI-resistant tumor cells against erlotinib, and increasing concentrations of erlotinib in the tumor tissues were observed (27). This effect was evident in cell lines with increased VEGF expression. The present study cannot confirm whether VEGF was upregulated in PD-L1-positive EGFR-mutant NSCLC. However, it has been reported that VEGF-neuropilin-2 signaling is associated with PD-L1 expression in prostate cancer (28). Thus, increased tumor VEGF expression may underlie the efficacy of combined therapy with EGFR-TKIs plus VEGF inhibitors for PD-L1-positive EGFR-mutant NSCLC.

The phase 3 NEJ009 trial that evaluated the efficacy of combination therapy with gefitinib plus cytotoxic agents showed a longer PFS of 20.9 months (24) and a longer PFS2 (duration between the randomization until the progressive disease of the second-line treatment or death) compared with gefitinib monotherapy (25). In addition, indirect comparisons have revealed that combined therapy with gefitinib plus cytotoxic agents was associated with a significant reduction in the risk of death compared with monotherapy using first-generation EGFR-TKIs and some second-generation EGFR-TKIs (29), suggesting an OS benefit. Although direct

comparison is difficult because of the variations in study designs and patient backgrounds, the patient survival observed in the present study appears to be consistent with these prior clinical trials. The present study suggested that, even in patients with PD-L1-positive EGFR-mutant NSCLC, combined therapy might confer a longer PFS than first- and second-generation EGFR-TKI monotherapy.

Notably, sequential osimertinib therapy may yield a longer OS by prolonging the duration of treatment with EGFR-TKIs after acquiring the secondary T790M mutation because combined therapy regimens also contain first-generation EGFR-TKIs (30). Although the difference was not significant, the Kaplan-Meier curve showed that combined therapy was associated with the longest duration of treatment with EGFR-TKIs and OS, and the point estimate of the hazard ratio for OS was as low as 0.52. However, it is difficult to determine a definitive conclusion about the superiority or equivalence in terms of the OS between combined therapy, osimertinib monotherapy, and first- or second-generation EGFR-TKI monotherapy in patients with PD-L1-positive EGFR-mutant NSCLC because this study was not a predesigned clinical trial and may not have had sufficient statistical power. Additionally, the information on post-TKI treatment is important for this discussion. However, this multicenter study did not collect information on post-TKI therapy, including immune checkpoint inhibitor therapy. This should be mentioned as a limitation in this study.

Moreover, biomarkers that can predict poor response to EGFR-TKIs may help in selecting patients who are likely to benefit from combined therapy (31). PD-L1 expression is upregulated by oncogenes, including *EGFR, ALK, MYC*, hypoxia-inducible factor-1 alpha, phosphatase and tensin homolog loss, mitogen-activated protein kinase, and *KRAS*, and PD-L1 expression in EGFR-mutant NSCLC might result from the expression of multiple oncogenes (14). Additionally, using next-generation sequencing, the expression of several oncogenes, including *ALK, BCL2, KRAS*, and *PIK3CA*, has been observed in EGFR-mutant NSCLCs (32). Even in EGFR-mutant NSCLCs, multiple oncogene signals might contribute to oncogenesis in some cases, making cell proliferation and survival relatively less dependent on EGFR signaling.

AXL is a tyrosine kinase receptor that is associated with resistance and poor outcomes in various cancers. In EGFR-mutant NSCLC, AXL has also been reported to support resistance to osimertinib therapy (33). High expression levels of AXL were detected in 26.1% of tumors in previously untreated cases of EGFR-mutant NSCLC and were associated with a shorter PFS (34). Furthermore, in cell line-based assays, AXL expression was shown to accelerate PD-L1 expression (34), and the expression of AXL and PD-L1 was correlated (34,35). AXL is also a downstream target of Yes-associated protein (YAP) (36), which is one of the mechanisms underlying the development of resistance to EGFR-TKIs (36,37). Based on these reports, overexpression of AXL or YAP might help explain the poor outcomes in PD-L1-positive EGFR-mutant NSCLC patients treated with EGFR-TKIs.

Finally, EGFR exon 21 L858R was associated with the risk of progression and death in the present study.

The presence of compound mutations, defined as multiple mutations in the EGFR tyrosine kinase domain, has been reported in 15.9 and 24.6% of EGFR-mutant NSCLCs, depending on the study (32,38), and is associated with decreased EGFR-TKI sensitivity (38). EGFR compound mutations are more frequently detected in EGFR-mutant NSCLCs harboring the exon 21 L858R mutation than in those harboring the exon 19 deletion (38). Moreover, the RBM10 mutation has been suggested to contribute to decreased EGFR-TKI sensitivity in NSCLC patients harboring the exon 21 L858R mutation of EGFR. RBM10 is associated with mRNA alternative splicing of the Bcl-x gene regulating tumor cell apoptosis, and inactivation of RBM10 diminished apoptosis mediated by EGFR-TKIs. The presence of the RBM10 mutation might be one of the mechanisms underlying poor outcomes in NSCLC patients harboring the exon 21 L858R mutation of EGFR because the RBM10 mutation was more frequently observed in cases with the exon 21 L858R mutation (39).

There were several limitations of the present study. Selection bias or an imbalance of patient characteristics may have affected the results of the analysis, and it is difficult to completely exclude these because of the retrospective nature of the study. In addition, the number of patients who received combined therapy was small, making it difficult to discuss the efficacy of each combined therapy with EGFR-TKIs plus VEGF inhibitors/cytotoxic agents.

In conclusion, the results of the present study showed that combined therapy was associated with a significant reduction in the risk of disease progression compared with first- and second-generation EGFR-TKI monotherapy, suggesting that combined therapy is effective for PD-L1-positive EGFR-mutant NSCLC. The results of the present study not only imply that tumor PD-L1 expression may help predict the efficacy of EGFR-TKI therapy but may also lead to the development of precision medicine for EGFR-mutant NSCLC in the future. However, future studies are needed to validate the findings of the present study.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MI and EM designed the study. YK, RS, DM, HN, MS, YS, SY, TK, DJ, NY and TH contributed to the data collection and investigation. MI wrote the original draft of the manuscript. MI and EM confirm the authenticity of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was conducted following the Declaration of Helsinki and Ethical Guidelines for Medical and Biological Research Involving Human Subjects (Ministry of Health, Labour and Welfare, Japan) and was approved by the Ethics Committee, University of Toyama (approval number: R2022070). The need to obtain informed consent from the study subjects was waived under the approval of the Ethics Committee, University of Toyama, and we disclosed information about the study to the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they do not have any competing interests.

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