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Vascular endothelial growth factor receptor 2 expression and immunotherapy efficacy in non–small cell lung cancer

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Abstract

It is unclear whether tumor vascular endothelial growth factor receptor 2 expression affects the therapeutic efficacy of immune-checkpoint inhibitors and antiangiogenic agents. This retrospective, multicenter study included patients with advanced non–small cell lung cancer who were treated with immune-checkpoint inhibitors. We constructed tissue microarrays and performed immunohistochemistry with an anti-vascular endothelial growth factor receptor 2 antibody. We analyzed immune and tumor cell staining separately in order to determine their correlation with the objective response rate, progression-free survival, and overall survival in patients receiving immune-checkpoint inhibitors. Of 364 patients, 37 (10%) expressed vascular endothelial growth factor receptor 2 in immune cells and 165 (45%) in tumor cells. The objective response rate, progression-free survival, and overall survival were significantly worse in patients treated with immune checkpoint inhibitor monotherapy who expressed vascular endothelial growth factor receptor 2 in immune cells than those who did not (10% vs 30%, $p = 0.028$; median = 2.2 vs 3.6 months, $p = 0.012$; median = 7.9 vs 17.0 months, $p = 0.049$, respectively), while there was no significant difference based on tumor cell expression (24% vs 30%, $p = 0.33$; median = 3.1 vs 3.5 months, $p = 0.55$; median = 13.6 vs 16.8 months, $p = 0.31$). There was no significant difference in overall survival between patients treated with and without antiangiogenic agents in any treatment period based on vascular endothelial growth factor receptor 2 expression. Immune checkpoint inhibitor efficacy was limited in patients expressing vascular endothelial growth factor receptor 2 in immune cells.

KEYWORDS
immune checkpoint inhibitor, lung cancer, programmed death-ligand 1, tissue microarray, vascular endothelial growth factor receptor

Abbreviations: EGFR, epidermal growth factor receptor; IC, immune cells; ICI, immune checkpoint inhibitor; NSCLC, non–small cell lung cancer; ORR, objective response rate; OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TC, tumor cells; TME, tumor microenvironment; TPS, tumor proportion score; VEGFR2, vascular endothelial growth factor receptor 2.

1 | INTRODUCTION

Lung cancer remains the leading cause of cancer-related death worldwide.[1] Although the prognosis of lung cancer patients is gradually improving with the emergence of various therapeutic agent classes, such as molecular-targeted drugs and immune checkpoint inhibitors (ICIs),[2–6] achieving long-term survival remains a challenge. Thus, improved therapeutic strategies need to be developed.

Increased vascularization and the expression of proangiogenic factors in tumors are associated with poor prognosis in various cancer types.[7–9] In particular, the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) signaling pathway plays a key role in angiogenesis, which promotes cancer proliferation and metastasis.[10,11] The resultant aberrant vessels exhibit vascular permeability and induce hypoxia, leading to an immunosuppressive tumor microenvironment (TME).[12,13]

Therapy based on the VEGF/VEGFR axis blockade has been effective in various types of cancer.[14–17] For example, bevacizumab, a humanized monoclonal antibody against VEGF-A, in combination with platinum doublet chemotherapy, was effective against advanced non-squamous non–small cell lung cancer (NSCLC).[18,19] In a phase III trial of ramucirumab, a recombinant human IgG1 monoclonal antibody against VEGFR2, combination with docetaxel achieved longer overall survival (OS) than docetaxel alone as second-line treatment for stage IV NSCLC patients experiencing disease progression following platinum-based chemotherapy.[20] Ramucirumab together with erlotinib also exhibited significant efficacy in non-squamous NSCLC patients harboring epidermal growth factor receptor (*EGFR*) mutations.[21]

The VEGF/VEGFR pathway negatively regulates the immune system through various mechanisms, such as the recruitment of regulatory T cells,[22,23] inhibition of dendritic cell maturation,[24] conversion of tumor-associated macrophages from the M1 to the M2 phenotype,[25] and the induction of T cell exhaustion.[26] Therefore, blocking the VEGF/VEGFR pathway represents a potential strategy for reprogramming the immunosuppressive TME and improving the efficacy of ICIs. In fact, combination treatment of anti–programmed death-ligand 1 (PD-L1) monoclonal antibody atezolizumab together with carboplatin, paclitaxel, and bevacizumab resulted in longer progression-free survival (PFS) as well as OS than without atezolizumab in advanced non-squamous NSCLC patients.[3]

Thus, drugs targeting the VEGF/VEGFR pathway are expected to have a synergistic effect with ICIs. However, it is unclear which patients are most likely to experience these synergistic effects and whether the expression of VEGF/VEGFR in tumor tissues is associated with the efficacy of ICIs and antiangiogenic agents. Hence, we conducted a multicenter, retrospective study to investigate the relationship between expression of VEGFR2, the main signaling tyrosine kinase receptor of the VEGF/VEGFR pathway, and the therapeutic effects of ICIs as well as antiangiogenic agents in patients with advanced NSCLC.

2 | MATERIALS AND METHODS

2.1 | Study design and patients

We conducted a retrospective, multicenter study of advanced NSCLC patients treated with ICIs at Osaka Metropolitan University Hospital, Ishikiriseiki Hospital, and Bell Land General Hospital between December 2015 and July 2020. The protocol was approved by the institutional review boards and ethics committees of all participating institutions. We obtained written informed consent from most patients. Because the application of the opt-out method in this research is permitted under Japan's most preferential law governing clinical research, additional informed consent was obtained in the form of an opt-out on the website.

Patients who had previously received anticytotoxic T lymphocyte–associated antigen 4 therapy or durvalumab as consolidation therapy after chemoradiation, as well as those with insufficient residual tissue for creating tissue microarrays or evaluating immunostaining results due to too few tumor cells were excluded.

2.2 | Data collection

We collected patient data, including age, sex, Eastern Cooperative Oncology Group Performance Status (ECOG PS) at the time of receiving ICI therapy, TNM stage according to the eighth edition, smoking history, histological type, *EGFR* mutation status, ICI administration line, use of antiangiogenic agents in any treatment period, tumor proportion score (TPS) of PD-L1, objective response rate (ORR) to ICIs, PFS, and OS. Tumor responses were assessed using the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.[27] PFS was estimated from the date of the first ICI administration until disease progression or death from any cause. OS was calculated from the date of the first ICI administration until death from any cause. The data cutoff date was May 31, 2021. The TPS of PD-L1 was assessed from clinical pathology reports based on detection in formalin-fixed tumor samples using the commercially available PD-L1 IHC 22C3 pharmDx assay (Dako North America, RRID: AB_2889976) at each institution.

2.3 | Microarray construction

To prepare the tissue microarrays, formalin-fixed paraffin-embedded tumor blocks from surgically resected and biopsy specimens were collected in a routine clinical setting. The most representative tumor areas were carefully selected based on the matched H&E-stained slides and marked directly on the donor block. We then removed a tissue sample 2.0 mm in diameter from the selected region in each donor block using a manual tissue microarrayer and embedded it directly into the premade recipient block. The manual microarrayer and premade recipient block were purchased from Funakoshi Co., Ltd. A total of ten tissue microarray blocks were constructed.

2.4 | Immunohistochemistry

From each tissue microarray block, 4-μm sections were deparaffinized and rehydrated. The sections were then incubated with citric acid (pH 6.0) for 20 minutes at 100°C. The sections were stained with an anti-VEGFR2 antibody (polyclonal, ab2349, 1:200; Abcam, RRID: AB_302998) at 4°C overnight. Mouse skin tissue was used as the positive control. The negative controls were treated with Tris-buffered saline instead of primary antibodies. Following incubation with the secondary antibodies for 30 min at room temperature (approximately 25°C), the specimens were stained using the VECTASTAIN Elite ABC Universal PLUS Kit (Vector Laboratories, Inc.). All sections were counterstained with 100% hematoxylin for 30 seconds at room temperature (approximately 25°C). All immunohistochemistry results were assessed by an experienced pathologist. Immune cells staining (IC-VEGFR2), including lymphocytes, macrophages, granulocytes, dendritic cells, plasma cells, and tumor cells staining (TC-VEGFR2), was separately evaluated. IC-VEGFR2 was defined as positive when any immune cells were stained with an anti-VEGFR2 antibody in the tumor stroma or intratumor area. TC-VEGFR2 was defined as positive when any tumor cells were stained with an anti-VEGFR2 antibody in the specimen (Figure 1).

2.5 | Statistical analyses

We analyzed the association of IC-VEGFR2 and TC-VEGFR2 positivity with ORR, PFS, and OS. ORR was assessed in patients who received ICI monotherapy, while PFS and OS were assessed in patients who received ICI monotherapy as well as in those who received ICI concurrently with chemotherapy. We also analyzed OS in all patients based on the administration of antiangiogenic agents in any treatment line. We compared the categorical variables using Fisher's exact test. Survival curves were estimated via the Kaplan-Meier method, and the differences between groups were compared using the log-rank test. Univariate and multivariate analyses were performed using the Cox proportional hazards model with the following variables: IC-VEGFR2 (positive vs negative), TC-VEGFR2 (positive vs negative), age (<75 vs ≥75), sex (male vs female), smoking status (never smoker vs current or former smoker), ECOG PS (0 or 1 vs ≥2), TNM stage (stage III vs stage IV or recurrent), histological type (squamous cell carcinoma vs non-squamous cell carcinoma), EGFR mutation status (mutant vs wild type), ICI administration line (first line vs second or later line), TPS of PD-L1 (≥1% vs <1%), ICI treatment (monotherapy vs combination therapy), and the use of antiangiogenic agents (administered in any treatment period vs not administered in any treatment period). The effect of missing data was explored via multiple imputation, and a total of 10 imputed datasets were created for multivariate analysis. Statistical significance was set at $p < 0.05$. Fisher's exact test, Kaplan-Meier analysis, and log-rank tests were performed using EZR on R commander version 1.55 (Saitama Medical Center, Jichi Medical University). Multivariate analyses for the Cox proportional hazards model using multiple imputation were performed using IBM SPSS statistics software, version 25 (IBM Corp.).

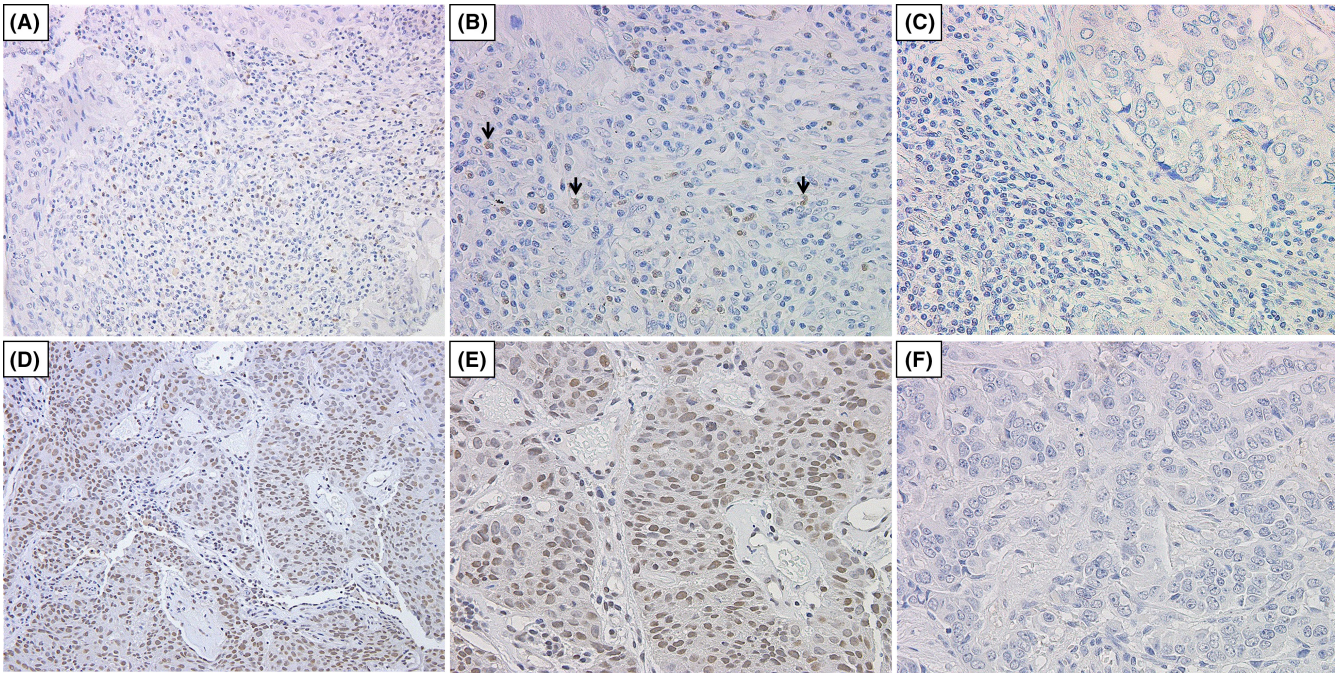

FIGURE 1 Representative immunohistochemical staining for non–small cell lung cancer. (A,B) Vascular endothelial growth factor receptor 2 (VEGFR2) expression was observed in immune cells; arrows indicate the VEGFR2 positive immune cells. (C) VEGFR2 was negative for immune cells. (D,E) VEGFR2 expression was observed in tumor cells. F, VEGFR2 was negative for tumor cells. Original magnification: ×100 (A,D); ×200 (B,C,E,F)

3 | RESULTS

3.1 | Patient characteristics

We reviewed the medical records of 496 NSCLC patients who received ICI therapy at participating institutions during the study period. Eventually, immunohistochemically evaluable specimens on microarray blocks were available for 364 patients (Table 1). Of the 364 patients, 37 (10%) were IC-VEGFR2 (+), 327 (90%) were IC-VEGFR2 (−), 165 (45%) were TC-VEGFR2 (+), and 199 (55%) were TC-VEGFR2 (−). The rate of TC-VEGFR2 positivity was significantly higher in IC-VEGFR2 (+) patients than in IC-VEGFR2 (−) patients (84% vs 41%, $p < 0.001$). The median age was 71 (33-90) years, the majority were male (76%), most patients had a smoking habit (88%), and 35% of the patients had squamous cell carcinoma. Five percent of patients had tumors harboring an *EGFR* mutation. There was no significant association between IC-VEGFR2 or TC-VEGFR2 positivity and the TPS of PD-L1 ≥ 1% ($p = 0.35$, $p = 0.39$, respectively). The TC-VEGFR2 (+) group had a lower rate of EGFR mutant, had more frequently received ICI concurrent with chemotherapy, were less frequently treated with antiangiogenic agents in any treatment period, and were more frequently treated with ICI as first-line treatment than the TC-VEGFR2 (−) group (2% vs 8%, $p = 0.020$, 35% vs 22%, $p = 0.007$, 18% vs 32%, $p = 0.004$, 67% vs 49%, $p = 0.001$, respectively) (Table 1).

3.2 | ORR to ICI based on VEGFR2 expression status

3.2.1 | ORR to ICI monotherapy

Among those who received ICI monotherapy, the ORR was significantly lower in IC-VEGFR2 (+) than in IC-VEGFR2 (−) patients (10% vs 30%, $p = 0.028$, respectively), whereas there was no significant difference in the ORR between TC-VEGFR2 (+) and TC-VEGFR2 (−) patients (24% vs 30%, $p = 0.33$, respectively) (Figure 2).

3.2.2 | ORR to ICI concurrent with chemotherapy

Among the patients who received ICI with concurrent chemotherapy, there was no significant difference in the ORR between IC-VEGFR2 (+) and IC-VEGFR2 (−) patients (43% vs 55%, $p = 0.70$, respectively), whereas the ORR of TC-VEGFR2 (+) patients was significantly higher than that of TC-VEGFR2 (−) patients (63% vs 42%, $p = 0.044$, respectively) (Figure 2). The proportion of patients treated with antiangiogenic agents as part of a combination regimen was 7% (4/57) for TC-VEGFR2 (+) and 21% (9/43) for TC-VEGFR2 (−) patients ($p = 0.069$). The proportion of patients with a TPS ≥1% of PD-L1 expression was similar between TC-VEGFR2 (+) and TC-VEGFR2 (−) patients who received concurrent chemotherapy with ICI (76% vs 74%, $p = 1.00$, respectively).

3.2.3 | ORR to ICI monotherapy when groups are divided by the PD-L1 expression status

Among patients with PD-L1 TPS ≥ 1%, the ORR of IC-VEGFR2 (+) patients was significantly lower than that of IC-VEGFR2 (−) patients (13% vs 39%, $p = 0.011$, respectively) (Figure 3). The ORR of TC-VEGFR2 (+) patients was also significantly lower than that of TC-VEGFR2 (−) patients (27% vs 43%, $p = 0.038$, respectively). In contrast, among patients with PD-L1 TPS <1%, there was no significant difference in ORR between IC-VEGFR2 (+) and IC-VEGFR2 (−) patients (0% vs 6%, $p = 1.00$, respectively) nor between TC-VEGFR2 (+) and TC-VEGFR2 (−) patients (0% vs 9%, $p = 0.53$, respectively) (Figure 3).

3.3 | PFS and OS based on VEGFR2 expression status

3.3.1 | PFS and OS for ICI monotherapy

For patients treated with ICI monotherapy, the PFS was significantly poorer for IC-VEGFR2 (+) than for IC-VEGFR2 (−) patients (median = 2.2 vs 3.7 months, $p = 0.012$, respectively) (Figure 4A). The OS was also significantly poorer for IC-VEGFR2 (+) than for IC-VEGFR2 (−) patients (median = 7.9 vs 17.0 months, $p = 0.049$, respectively) (Figure 4C). There was no significant difference in the PFS and OS between TC-VEGFR2 (+) and TC-VEGFR2 (−) patients (median = 3.1 vs 3.5 months, $p = 0.55$ and median = 13.6 vs 16.8 months, $p = 0.31$, respectively) (Figure 4B,D).

3.3.2 | PFS and OS for ICI concurrent with chemotherapy

Of the patients treated with ICI concurrent with cytotoxic chemotherapy, there was no significant difference between the IC-VEGFR2 (+) and IC-VEGFR2 (−) groups in terms of PFS (median = 13.5 vs 10.3 months, $p = 0.95$, respectively) and OS (median = 14.2 vs not estimable, $p = 0.25$) (Figure 5A,C). The TC-VEGFR2 (+) and TC-VEGFR2 (−) groups also exhibited no significant difference in PFS (median = 12.8 vs 9.1 months, $p = 0.96$, respectively) nor OS (median = 20.6 vs not estimable, $p = 0.14$, respectively) (Figure 5B,D).

3.3.3 | Univariate and multivariate analysis for PFS and OS

In the univariate analysis for PFS of patients treated with ICI monotherapy, the factors of IC-VEGFR2 (+), no history of smoking, ECOG PS ≥ 2, stage IV or recurrent, EGFR mutant, ICI administered in second or later line, and PD-L1 TPS < 1% were significantly associated with poor PFS. Furthermore, multivariate analysis revealed that the factors of IC-VEGFR2 (+), no history of smoking, ECOG PS ≥ 2, stage

TABLE 1 Patient characteristics

	VEGFR2-positive in immune cells ($n = 37$)	VEGFR2-negative in immune cells ($n = 327$)	p value	VEGFR2-positive in tumor cells ($n = 165$)	VEGFR2-negative in tumor cells ($n = 199$)	p value
Age						
Median (range)	72 (33–84)	71 (38–90)	0.13	72 (33–87)	71 (38–90)	0.81
Sex						
Male	27 (73)	251 (77)	0.68	123 (75)	155 (78)	0.46
Female	10 (27)	76 (23)		42 (25)	44 (22)	
Smoking status						
Current or former smoker	34 (92)	277 (85)	0.45	141 (85)	170 (85)	0.88
Never smoker	3 (8)	46 (14)		23 (14)	26 (13)	
Unknown	0 (0)	4 (1)		1 (1)	3 (2)	
ECOG PS						
0–1	30 (81)	274 (84)	0.64	137 (83)	167 (84)	0.89
≥2	7 (19)	53 (16)		28 (17)	32 (16)	
TNM stage						
Stage III	10 (27)	70 (21)	0.41	35 (21)	45 (23)	0.80
Stage IV or recurrent	27 (73)	257 (79)		130 (79)	154 (77)	
Histological type						
Adenocarcinoma	18 (49)	187 (57)	0.24	88 (53)	117 (59)	0.55
Squamous cell carcinoma	13 (35)	113 (35)		62 (38)	64 (32)	
Other	6 (16)	27 (8)		15 (9)	18 (9)	
EGFR mutation status						
Wild type	27 (73)	198 (61)	0.14	106 (64)	119 (60)	0.020
Mutant	0 (0)	20 (6)		4 (2)	16 (8)	
Unknown	10 (27)	109 (33)		55 (33)	64 (32)	
Treatment						
ICI monotherapy	30 (81)	234 (72)	0.25	108 (65)	156 (78)	0.007
ICI concurrent with chemotherapy	7 (19)	93 (28)		57 (35)	43 (22)	
Antiangiogenic agents						
Administered in any treatment period	7 (19)	86 (26)	0.43	30 (18)	63 (32)	0.004
No use in any treatment period	30 (81)	241 (74)		135 (82)	136 (68)	
ICI administration line						

TABLE 1 (Continued)

	VEGFR2-positive in immune cells (*n* = 37)	VEGFR2-negative in immune cells (*n* = 327)	*p* value	VEGFR2-positive in tumor cells (*n* = 165)	VEGFR2-negative in tumor cells (*n* = 199)	*p* value
1	19 (51)	188 (57)	0.49	110 (67)	97 (49)	0.001
≥2	18 (49)	139 (43)		55 (33)	102 (51)	
PD-L1 TPS						
<1%	4 (11)	56 (17)	0.35	26 (16)	34 (17)	0.39
≥1%	29 (78)	216 (66)		122 (74)	123 (62)	
Unknown	4 (11)	55 (17)		17 (10)	42 (21)	
VEGFR2 in immune cells						
Positive	37 (100)	0 (0)	-	31 (19)	6 (3)	<0.001
Negative	0 (0)	327 (100)		134 (81)	193 (97)	
VEGFR2 in tumor cells						
Positive	31 (84)	134 (41)	<0.001	165 (100)	0 (0)	-
Negative	6 (16)	193 (59)		0 (0)	199 (100)	

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; ICI, immune checkpoint inhibitor; PD-L1, programmed death-ligand 1; TNM, tumor, node, metastasis; TPS, tumor proportion score; VEGFR2, vascular endothelial growth factor receptor 2.

IV or recurrent, and ICI administered in the second or later line were independent negative prognostic factors for PFS of patients treated with ICI monotherapy (Table 2). In the univariate analysis for OS of all patients, IC-VEGFR2 status, ECOG PS, stage, histological type, EGFR mutation status, ICI treatment line, and the method of ICI administration were significantly associated with OS, and among these factors, IC-VEGFR2 (+), ECOG PS ≥ 2, and stage IV or recurrent were independent unfavorable prognostic factors for OS of all patients in the multivariate analysis (Table 3).

3.4 | Overall survival based on the use of antiangiogenic agents

We analyzed whether there was a significant difference in the median OS with or without the use of antiangiogenic agents in any treatment period based on TC-VEGFR2 and IC-VEGFR2 status. Among the IC-VEGFR2 (−), IC-VEGFR2 (+), TC-VEGFR2 (−), and TC-VEGFR2 (+) patients, there was no significant difference in the OS between those treated with and without antiangiogenic agents in any treatment period (median = 21.5 vs 18.2 months, $p = 0.15$, median = 5.9 vs 11.8 months, $p = 0.81$, median = 18.4 vs 17.9 months, $p = 0.41$, 21.5 vs 14.8 months, $p = 0.27$, respectively) (Figure S1).

4 | DISCUSSION

In this study, IC-VEGFR2 (+) patients exhibited a significantly poorer ORR, PFS, and OS than those of IC-VEGFR2 (−) patients with NSCLC receiving ICI monotherapy. Among the patients with TPS of PD-L1 ≥ 1%, those with TC-VEGFR2 (+) who received ICI monotherapy also had a significantly lower ORR than TC-VEGFR2 (−) patients. As per multivariate analysis, IC-VEGFR (+) was a negative prognostic factor for PFS in patients treated with ICI monotherapy and OS in all patients, whereas TC-VEGFR2 (+) was not.

The VEGF/VEGFR axis plays a key role in aberrant angiogenesis in tumor tissues, including those of lung cancer,[10,11] with VEGF/VEGFR expression being associated with NSCLC patient survival. VEGFR2 expression was observed in 43%–58% of patients with NSCLC and associated with worse survival outcomes.[28–31] Moreover, gene polymorphisms of *VEGFR2* were associated with NSCLC prognosis.[32] Thus, several studies have reported the association between VEGFR2 expression in tumor cells and worse survival in patients with NSCLC. However, no study has previously investigated the association between tumor cell VEGFR2 expression and the outcome of patients with advanced NSCLC receiving ICIs. In our study, VEGFR2 positivity in tumor cells was negatively correlated with the ORR in ICI monotherapy–treated NSCLC patients with a TPS of PD-L1 ≥ 1%. The significance of VEGFR2 positivity in tumor cells in the context of ICI treatment might differ based on PD-L1 expression status. As for the association between VEGFR2 and immune cells, a few reports described that VEGFR2 was also expressed in several immune cell types, including regulatory T cells,[33] M2

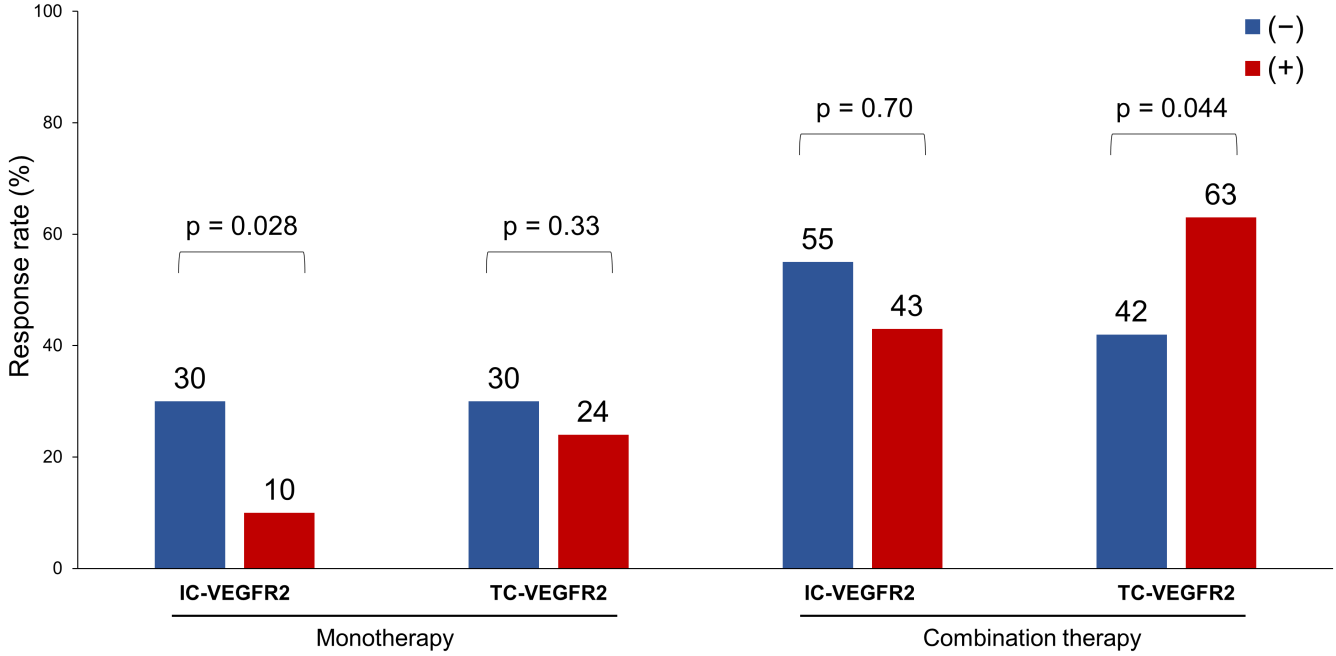

FIGURE 2 Objective response rate based on VEGFR2 expression status. IC, immune cells, TC, tumor cells; VEGFR2, vascular endothelial growth factor receptor 2

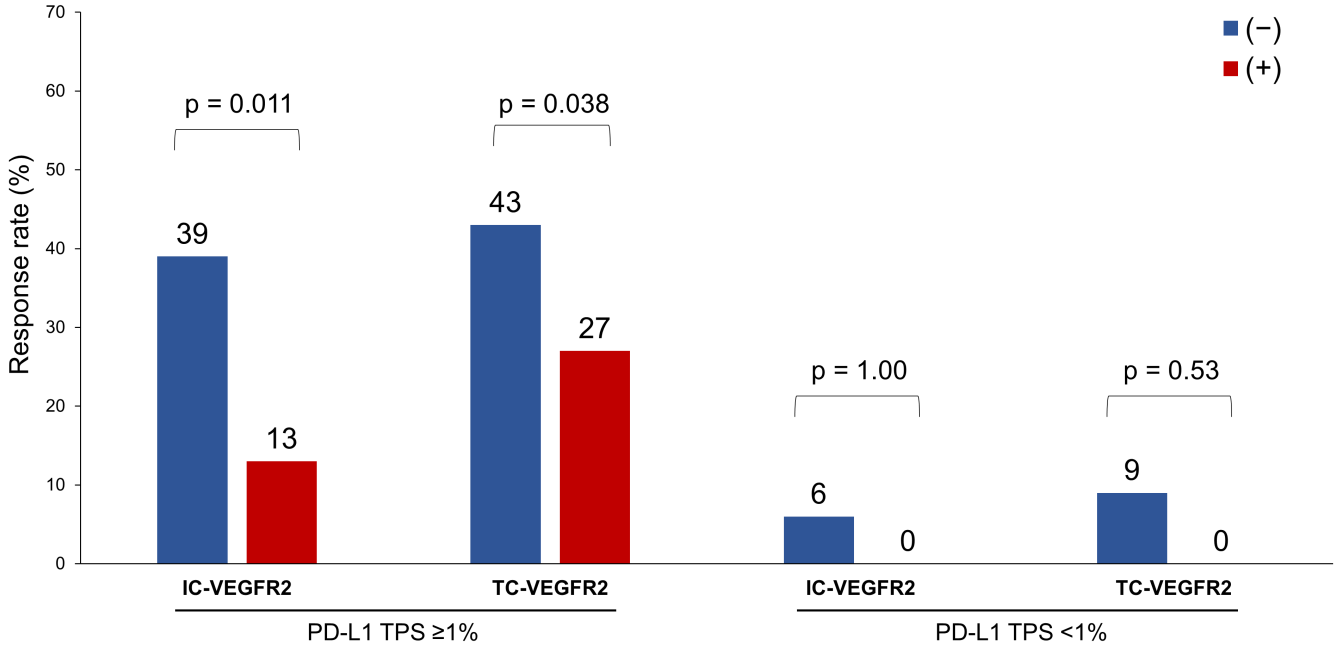

FIGURE 3 Objective response rate based on VEGFR2 and PD-L1 expression status. IC, immune cells; PD-L1 TPS, programmed death-ligand 1 tumor proportion score; TC, tumor cells; VEGFR2, vascular endothelial growth factor receptor 2

tumor-associated macrophages,[34] and dendritic cells.[35] However, no study has focused on the influence of immune cell VEGFR2 expression on the efficacy of ICIs against NSCLC to date. In our study, the presence of VEGFR2-positive immune cells was strongly associated with a low ORR as well as poor PFS and OS among ICI-treated NSCLC patients. To our knowledge, this is the first report to show the relationship of VEGFR2 expression in tumor cells and immune cells with the efficacy of ICI treatment in patients with NSCLC. Furthermore, among those treated with ICI combination therapy, TC-VEGFR2 (+) patients showed a higher response rate than TC-VEGFR2 (−) patients. Although the reason remains unclear, due to the lower rate of antiangiogenic agents used in combination therapy for the TC-VEGFR2 (+) group, regimens without an antiangiogenic agent might be more effective for TC-VEGFR2 (+) NSCLC patients.

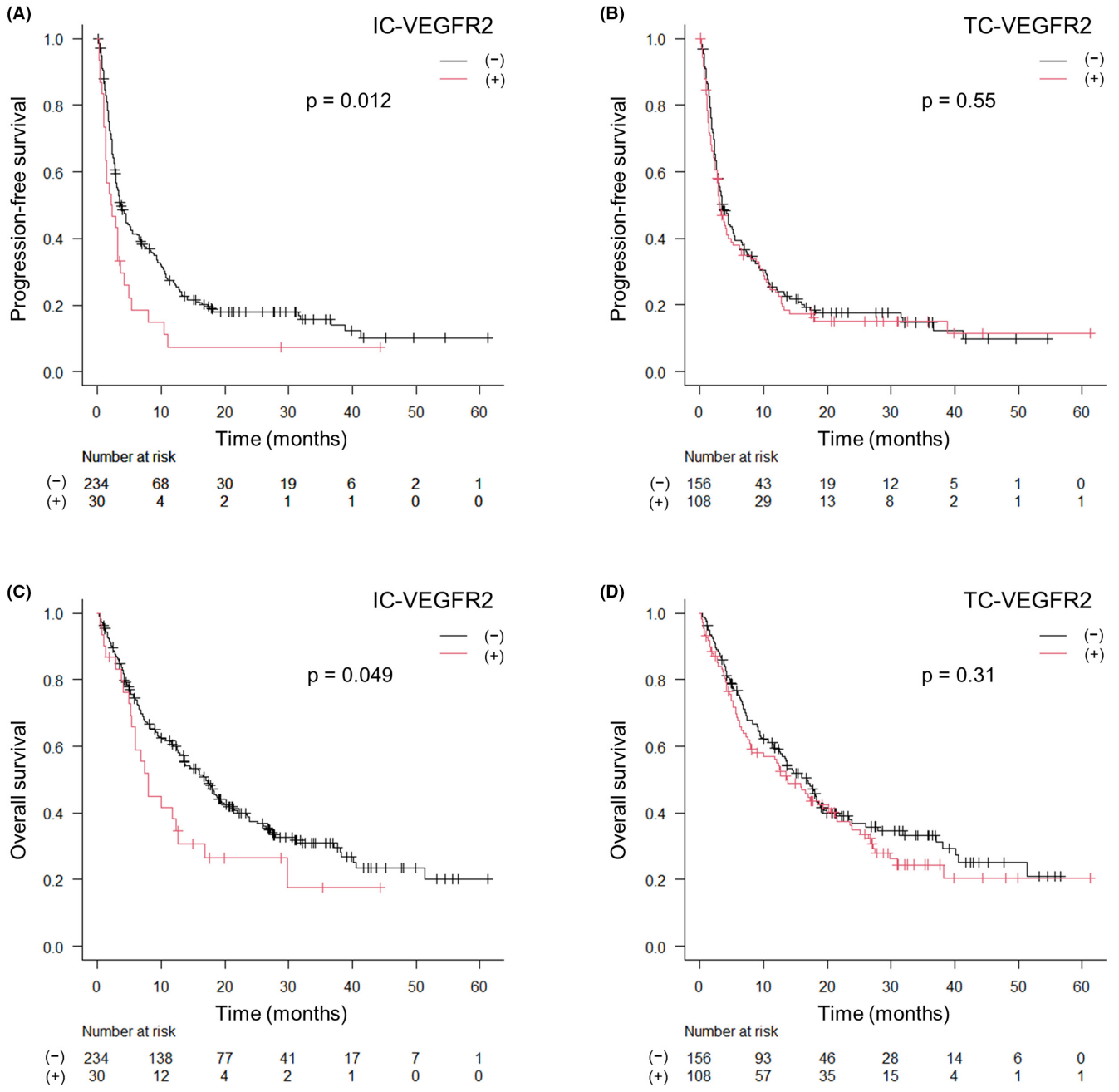

FIGURE 4 Progression-free survival (PFS) and overall survival (OS) based on VEGFR2 expression status in patients treated with immune checkpoint inhibitor (ICI) monotherapy therapy. PFS and OS were analyzed according to IC-VEGFR2 (A,C) and TC-VEGFR2 (B,D) positivity in patients treated with ICI monotherapy. IC, immune cells; TC, tumor cells; VEGFR2, vascular endothelial growth factor receptor 2

Angiogenesis has major influence on immune activity in tumors,[36] and VEGF/VEGFR modulates tumor immunity both directly and indirectly.[22–26] Therefore, the combination of ICIs and angiogenesis inhibitors is expected to have a synergistic effect.[37] In an analysis of 20 patients with advanced gastric cancer, VEGFR2 was highly expressed in regulatory T cells, with PD-L1 expression and $CD8^+$ T cell infiltration increasing after the administration of ramucirumab, which inhibits VEGFR2.[38] Moreover, blocking VEGFR2 in a breast cancer mouse model increased immune cell infiltration and improved the efficacy of anti-PD-1 therapy, especially at low doses of anti-VEGFR2.[39] In a meta-analysis assessing the impact of timing and the sequence of treatment with ICIs and antiangiogenic agents for NSCLC, combination therapy for both improved the ORR, PFS, and OS. However, when ICIs were administered immediately after antiangiogenic agents, there were no benefits compared with ICI monotherapy.[40] Regarding the association between VEGFR2 and PD-L1 expression, a significant positive correlation was reported in the immunohistochemical analysis of 96 patients with renal cell carcinoma[41] and 93 patients with osteosarcoma.[42] In contrast, a significant negative correlation was obtained for 92 patients with large cell neuroendocrine

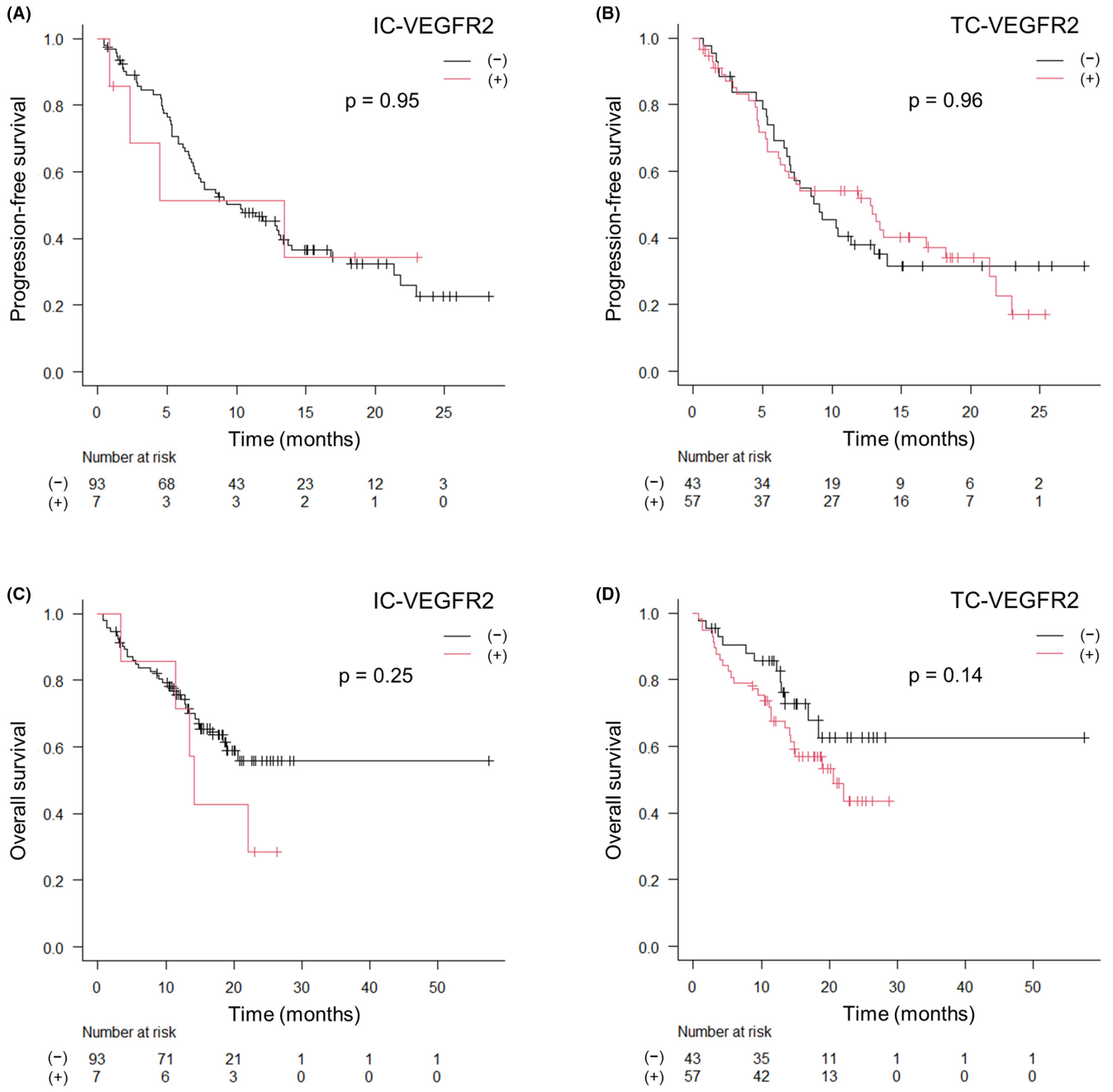

FIGURE 5 Progression-free survival (PFS) and overall survival (OS) based on VEGFR2 expression status in patients treated with immune checkpoint inhibitor (ICI) combination therapy. PFS and OS were analyzed according to IC-VEGFR2 (A,C) and TC-VEGFR2 (B,D) positivity in patients treated with ICI combination therapy. IC, immune cells; TC, tumor cells; VEGFR2, vascular endothelial growth factor receptor 2

carcinoma of the lung via the same analysis.[43] However, no significant correlation between the two was observed in our study of 364 NSCLC patients. An important clinical question was which patients would benefit from angiogenesis inhibitors and whether their therapeutic effect would vary based on VEGFR2 expression in tumor and immune cells. However, there was no significant difference in OS with or without antiangiogenic agents regardless of VEGFR2 expression status in ICI-treated patients with NSCLC.

With regard to the possibility that ICI efficacy might be limited in VEGFR2-positive NSCLC, particularly in tumors with VEGFR2-positive immune cells, a method to improve survival outcomes in such patients is necessary, and ICI in combination with other treatments such as cytotoxic agents or another immunotherapy, rather than monotherapy, might be more effective. Based on the results of our study, antiangiogenic agents may be insufficient to improve survival outcomes in TC-VEGFR2 (+) and IC-VEGFR2 (+) patients. Larger prospective trials evaluating the efficacy of ICI based on VEGFR2 expression in immune and tumor cells are warranted.

Our study had certain limitations, including its retrospective nature. The number of patients with IC-VEGFR2 (+) was relatively

TABLE 2 Cox proportional hazards model analysis of factors associated with PFS of patients treated with ICI monotherapy

Factor	N	Median PFS (months)	Univariate analysis			Multivariate analysis		
			HR	95% CI	*p* value	HR	95% CI	*p* value
IC-VEGFR2								
Negative	234	3.7						
Positive	30	2.2	1.67	1.09–2.50	0.020	2.10	1.31–3.35	0.002
TC-VEGFR2								
Negative	156	3.5						
Positive	108	3.1	1.09	0.83–1.42	0.55	1.08	0.80–1.46	0.62
Age								
<75	156	3.2						
≥75	108	4.0	0.96	0.80–1.27	0.80	1.08	0.79–1.46	0.63
Sex								
Female	64	2.4						
Male	200	4.2	0.73	0.54–1.00	0.051	1.22	0.79–1.89	0.37
Smoking status								
Current or former smoker	226	4.2						
Never smoker	35	1.8	2.05	1.39–2.94	<0.001	2.44	1.46–4.09	0.001
ECOG PS								
0–1	210	4.2						
≥2	54	1.9	1.87	1.34–2.56	<0.001	1.83	1.30–2.58	0.001
Stage								
III	63	7.7						
IV or recurrent	201	3.0	1.65	1.20–2.33	0.002	1.51	1.07–2.12	0.018
Histological type								
Squamous cell carcinoma	99	4.2						
Non-squamous cell carcinoma	165	3.3	0.93	0.70–1.22	0.58	0.81	0.59–1.12	0.20
EGFR mutation								
Wild type	154	3.5						
Mutant	18	2.6	2.35	1.37–3.80	0.003	1.36	0.80–2.31	0.26
ICI administration line								
1	112	8.4						
≥2	152	2.8	1.79	1.35–2.40	<0.001	1.68	1.18–2.38	0.004
TPS of PD-L1								
≥1%	172	4.5						
<1%	36	2.3	1.75	1.17–2.55	0.007	1.31	0.83–2.06	0.25

Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; HR, hazard ratio; IC, immune cells; ICI, immune checkpoint inhibitor; PD-L1, programmed death-ligand 1; PFS, progression-free survival; TC, tumor cells; TPS, tumor proportion score; VEGFR, vascular endothelial growth factor receptor.

small, which may in part be due to the use of small tissues for microarray analysis. The number of patients who received antiangiogenic agents was also small, and we could not conduct analysis based on the administration timing of antiangiogenic agents. Finally, although we focused on VEGFR2 in this study, other members of the VEGF/VEGFR pathway, such as VEGF-A and VEGFR1, may affect ICI efficacy and survival outcomes.

Our study suggested that there is a need to develop a novel treatment strategy to improve the outcomes of NSCLC patients expressing VEGFR2 in immune cells, as the therapeutic efficacy of ICIs was limited, especially as monotherapy. The addition of existing angiogenesis inhibitors may be insufficient to significantly improve survival outcomes. Further studies to explore novel treatment strategies for these patients are therefore warranted.

TABLE 3 Cox proportional hazards model analysis of factors associated with OS of all patients

Factor	N	Median OS (months)	Univariate analysis			Multivariate analysis		
			HR	95% CI	p value	HR	95% CI	p value
IC-VEGFR2								
Negative	328	18.4						
Positive	37	11.4	1.65	1.07–2.42	0.023	1.66	1.04–2.66	0.035
TC-VEGFR2								
Negative	200	18.2						
Positive	165	16.5	1.18	0.89–1.54	0.25	1.24	0.92–1.66	0.16
Age								
<75	233	19.3						
≥75	132	16.0	1.30	0.98–1.71	0.067	1.20	0.88–1.63	0.25
Sex								
Female	86	13.8						
Male	279	18.6	0.86	0.63–1.19	0.35	1.06	0.70–1.61	0.78
Smoking status								
Current or former smoker	312	18.2						
Never smoker	49	16.5	1.05	0.69–1.53	0.82	1.20	0.72–1.99	0.48
ECOG PS								
0–1	305	19.9						
≥2	60	5.9	2.95	2.12–4.04	<0.001	2.77	1.95–3.93	<0.001
Stage								
III	80	27.1						
IV or recurrent	285	16.0	1.49	1.06–2.16	0.020	1.65	1.15–2.36	0.007
Histological type								
Squamous cell carcinoma	126	16.9						
Non-squamous cell carcinoma	239	18.4	0.73	0.55–0.96	0.025	0.83	0.59–1.16	0.27
EGFR mutation								
Wild type	226	21.5						
Mutant	20	7.4	2.19	1.25–3.59	0.008	1.49	0.88–2.52	0.14
ICI administration line								
1	208	22.1						
≥2	157	13.3	1.52	1.15–2.00	0.003	1.38	0.93–2.05	0.11
TPS of PD-L1								
≥1%	245	18.7						
<1%	60	19.5	1.05	0.70–1.52	0.80	1.15	0.77–1.72	0.49
ICI treatment								
Concurrent with chemotherapy	100	–						
Monotherapy	265	16.0	1.69	1.20–2.44	0.002	1.22	0.80–1.87	0.36
Antiangiogenic agents								
No use in any treatment period	271	17.0						
Administered in any treatment period	93	19.5	0.77	0.54–1.08	0.13	0.87	0.61–1.24	0.43

Abbreviations: CI, confidence interval; ECOG PS, Eastern Cooperative Oncology Group Performance Status; EGFR, epidermal growth factor receptor; HR, hazard ratio; IC, immune cells; ICI, immune checkpoint inhibitor; OS, overall survival; PD-L1, programmed death-ligand 1; TC, tumor cells; TPS, tumor proportion score; VEGFR, vascular endothelial growth factor receptor.

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DISCLOSURE

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ETHICS STATEMENT

Approval of the research protocol by an institutional reviewer board: The protocol was approved by the institutional review boards and ethics committees of all participating institutions.

Informed consent: We obtained written informed consent from most patients. Additional informed consent was obtained in the form of an opt-out on the website.

Registry and the registration No. of the study: Osaka Metropolitan University Hospital 2020–177, Ishikiriseiki Hospital 20–26, and Bell Land General Hospital 2020–020.

Animal studies: N/A.

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SUPPORTING INFORMATION

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

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