nature communications

Article

https://doi.org/10.1038/s41467-025-58186-7

Serum VEGF-A as a biomarker for the addition of bevacizumab to chemo-immunotherapy in metastatic NSCLC

Received: 6 April 2024

Accepted: 7 March 2025

Published online: 22 March 2025

Check for updates

Kentaro Tanaka[1,2,22] ✉, Jun Sugisaka[3], Yoshimasa Shiraishi[1], Yasutaka Watanabe[4], Haruko Daga[5], Koichi Azuma[6], Kazumi Nishino[7], Masahide Mori[8], Takayo Ota[9], Haruhiro Saito[10], Akito Hata[11], Tadashi Sakaguchi[12], Toshiyuki Kozuki[13], Hiroaki Akamatsu[14], Hirotaka Matsumoto[15], Motoko Tachihara[16], Kazushige Wakuda[17], Yuki Sato[18], Tomohiro Ozaki[19], Yuko Tsuchiya-Kawano[20], Nobuyuki Yamamoto[13], Kazuhiko Nakagawa[21] & Isamu Okamoto[1]

Anti-vascular endothelial growth factor (VEGF) agents in combination with immunotherapies have improved outcomes for cancer patients, but predictive biomarkers have not been elucidated. We report here a preplanned analysis in the previously reported APPLE study, a phase 3 trial evaluating the efficacy of the bevacizumab in combination with atezolizumab, plus platinum chemotherapy in metastatic, nonsquamous non-small cell lung cancer (NSCLC). We investigated the correlation of serum VEGF-A and its isoforms at baseline with treatment response by using an enzyme-linked immunosorbent assay. We reveal that the addition of bevacizumab significantly improves the progression-free survival in patients with the low VEGF-A level. Our results demonstrate that measuring serum VEGF-A or its isoforms may identify NSCLC patients who are likely to benefit from the addition of bevacizumab to immunotherapy. These assays are easy to measure and have significant potential for further clinical development.

Immune checkpoint inhibitors (ICIs) have greatly changed the treatment landscape for various types of cancer including non-small cell lung cancer (NSCLC)[1–3]. Antibodies to programmed cell death−1 (PD-1) or to its ligand PD-L1 (hereafter, PD-1 pathway inhibitors) are the most widely administered ICIs, with the combination of a PD-1 pathway inhibitor and platinum-doublet chemotherapy having been established as a standard treatment option for NSCLC, not only for individuals with metastatic disease[4–6] but also in the perioperative setting[7,8].

Attempts to increase the efficacy of such combined immunotherapies have involved the examination of new combinations of agents, including other types of ICI such as antibodies to cytotoxic T lymphocyte antigen−4 (CTLA-4)[9,10] as well as immunomodulators such as antibodies to interleukin-1β[11]. Bevacizumab, an antibody to vascular endothelial growth factor−A (VEGF-A), is also a promising candidate for such combination therapies. VEGF-A is a cytokine produced by tumor cells as well as by immune cells[12]. It impairs the maturation of and antigen presentation by dendritic cells and thereby promotes the differentiation of regulatory T cells and attenuates $CD8^+$ T cell−mediated cytotoxic killing[13,14]. In addition, VEGF-A contributes to the upregulation of PD-L1 expression on tumor cells and to that of various immune checkpoint molecules including PD-1 that are associated with $CD8^+$ T cell exhaustion[15]. VEGF-A also recruits myeloid-derived suppressor cells from bone marrow to tumor sites, and interaction of VEGF-A with its receptors on these cells serves to maintain their function through an autocrine loop[16]. VEGF-A thus plays a major role in promoting and maintaining an immunosuppressive tumor microenvironment, and its inhibition in cancer patients might be expected to increase the efficacy of

A full list of affiliations appears at the end of the paper. ✉e-mail: k9288090@kadai.jp

immunotherapy. Indeed, bevacizumab is administered in combination with ICIs as a standard treatment option for several cancer types including NSCLC and hepatocellular carcinoma[17,18]. However, there is currently no available biomarker to identify patients likely to experience a survival benefit from the addition of bevacizumab to immunotherapy.

The APPLE study was a randomized phase 3 trial that recently evaluated the benefit of adding bevacizumab to the combination of platinum-doublet chemotherapy plus the PD-L1 inhibitor atezolizumab for individuals with metastatic or recurrent nonsquamous NSCLC[19]. The trial did not meet its primary endpoint of demonstrating superiority of the bevacizumab-containing regimen. In the present study, we performed a preplanned exploratory measurement of VEGF-A and its isoforms $VEGF_{121}$ and $VEGF_{165}$ in serum of peripheral blood collected from patients of the APPLE trial before treatment. Measurement of $VEGF_{121}$ and $VEGF_{165}$ was performed with an enzyme-linked immunosorbent assay (ELISA) system designed specifically to detect these isoforms[20], and the relation of the serum levels of total VEGF-A (tVEGF-A) and the two isoforms to treatment efficacy was examined to determine their utility as a predictive biomarker for the identification of patients likely to benefit from the addition of bevacizumab to the combination of a PD-1 pathway inhibitor and platinum-based chemotherapy.

Results

Patient characteristics

The APPLE study was an open-label phase 3 trial that was conducted between January 2019 and August 2020 with 412 patients enrolled and randomized, 206 (50%) to the carboplatin-pemetrexed-atezolizumab (Chemo/Atezo) arm and 206 (50%) to the carboplatin-pemetrexed-atezolizumab plus bevacizumab (Chemo/Atezo/Bev) arm. Of these patients, 152 individuals joined the present study, a prospective biomarker study associated with the APPLE trial, between August 2019 and August 2020. The CONSORT diagram for the present study is presented in Fig. 1. Two patient samples were not adequately preserved for analysis, and one patient was found to harbor an *ALK* fusion gene and therefore excluded. A total of 149 samples was therefore analyzed in this study, including 114 from patients wild type (WT) for *EGFR* and 35 from those with activating mutations (MT) of *EGFR*. Among the study participants, 76 individuals were treated with Chemo/Atezo and 73 with Chemo/Atezo/Bev. The clinical characteristics of the patients analyzed are shown in Table 1 and were well balanced between the two groups, with the exception that the proportion of patients of unknown PD-L1 status was higher in the Chemo/Atezo/Bev group and that of those with a PD-L1 TPS of ≥50% was higher in the Chemo/Atezo group.

VEGF-A quantification in peripheral blood serum

Serum concentrations of tVEGF-A, $VEGF_{121}$, or $VEGF_{165}$, at baseline did not differ significantly between the two treatment arms (Fig. 2). The median value (range) for tVEGF-A was 405 pg/mL (51–1619 pg/mL) and 353 pg/mL (54–2237 pg/mL) for the Chemo/Atezo and Chemo/Atezo/Bev groups, respectively. $VEGF_{121}$ was detected in all patients, with the median value (range) being 212.5 pg/mL (34–803 pg/mL) and 221 pg/mL (52–1629 pg/mL) for the Chemo/Atezo group and the Chemo/Atezo/Bev group, respectively. In contrast, $VEGF_{165}$ was not detected in 17 patients of the Chemo/Atezo group and 12 patients of the Chemo/Atezo/Bev group, with the median value (range) being 178 pg/mL (0–845 pg/mL) and 127 pg/mL (0–914 pg/mL), respectively.

We then analyzed the correlation between the expression level of VEGF and the percentage of PD-L1 expression on tumor cells (Tumor Proportion Score; TPS), which is a predictor of the efficacy of PD-1 pathway inhibitors in *EGFR* wild-type NSCLC[3]. No significant correlation was found between VEGF expression levels in the three groups: >50% (high), 1-49% (low), and 0% (Supplementary Fig. 1A). In addition, we examined whether VEGF expression by TPS correlated with response to immunotherapy. We classified patients in the Chemo/Atezo and Chemo/Atezo/Bev groups as responders or non-responders based on their respective median PFS of 7.7 and 9.6 months in the Apple study. We found that among responders, the Chemo/Atezo group had higher VEGF expression than the Chemo/Atezo/Bev group in the TPS-high patient population (Supplementary Fig. 1B–D).

Low serum VEGF-A levels as a potential biomarker for the addition of bevacizumab to platinum-based chemotherapy and immunotherapy

For the patients of the present study, PFS did not differ significantly between the Chemo/Atezo group and the Chemo/Atezo/Bev group (median of 6.4 vs. 7.7 months, respectively; hazard ratio [HR] of 0.93, with a 95% confidence interval [CI] of 0.66–1.31) (Supplementary Fig. 2). This finding was consistent with the results for the overall population of the APPLE study[19].

The relation of pretreatment serum levels of tVEGF-A, $VEGF_{121}$, or $VEGF_{165}$ to PFS was then examined in both treatment groups (Fig. 3). For patients with low concentrations of these analytes, individuals in the Chemo/Atezo group had a less favorable PFS than did those in the Chemo/Atezo/Bev group (median of 5.8 vs. 10.4 months [HR of 0.62] for tVEGF-A, median of 5.8 vs. 10.9 months [HR of 0.58] for $VEGF_{121}$, and median of 5.7 vs. 10.4 months [HR of 0.63] for $VEGF_{165}$), with the difference in PFS between the two treatment arms being significant for individuals with low $VEGF_{121}$ levels. In contrast, for patients with high concentrations of tVEGF-A (median of 7.4 vs. 7.5 months, HR of 1.26), $VEGF_{121}$ (median of 7.6 vs. 6.6 months, HR of 1.32), or $VEGF_{165}$ (median

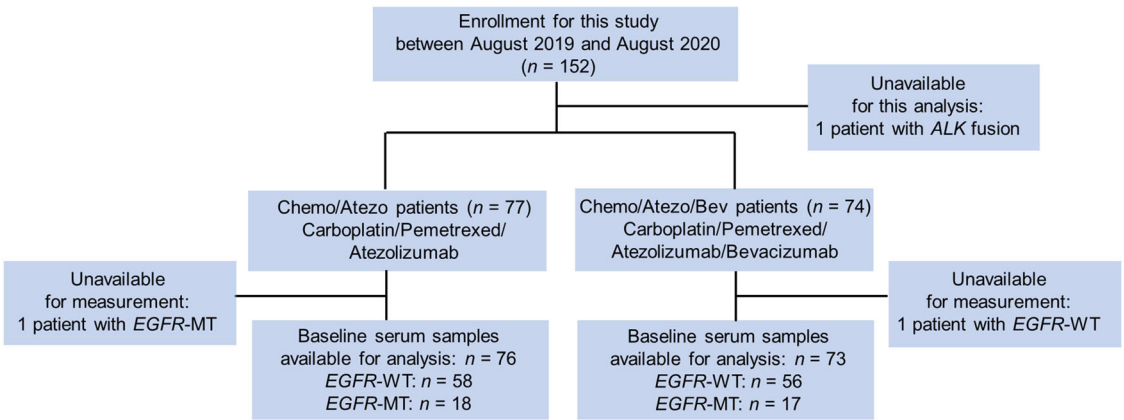

Fig. 1 | CONSORT diagram. *EGFR* epidermal growth factor receptor gene, WT wild type, MT mutation.

of 7.6 vs. 7.4 months, HR of 1.29), PFS did not differ substantially between the Chemo/Atezo and Chemo/Atezo/Bev arms, respectively. Analysis of OS revealed that patients in the two treatment arms showed similar survival curves regardless of tVEGF-A, $VEGF_{121}$, or $VEGF_{165}$ levels (Supplementary Fig. 3), consistent with the results for the overall population of the APPLE study[19].

In the analysis for the APPLE study, PFS was similar in the Chemo/Atezo and Chemo/Atezo/Bev groups for *EGFR*-WT patients (median of 9.5 vs. 9.3 months, respectively; HR of 0.97, with a 95% CI of 0.75–1.25)[19]. To examine whether serum tVEGF-A, $VEGF_{121}$, or $VEGF_{165}$ levels at baseline might be a predictive biomarker for the response to bevacizumab in *EGFR*-WT patients, we analyzed PFS for such patients according to treatment arm and high or low analyte levels (Fig. 4). Patients with low levels of tVEGF-A, $VEGF_{121}$, or $VEGF_{165}$ showed a more favorable PFS in the Chemo/Atezo/Bev group than in the Chemo/Atezo group, whereas those with high tVEGF-A, $VEGF_{121}$, or $VEGF_{165}$ levels showed no such benefit from the addition of bevacizumab. In addition, when we analyzed the interaction term of VEGF levels and chemotherapy arms in *EGFR*-WT patients, the p-value was low enough (Table 2), revealing the nature of VEGF values as a predictive biomarker. Furthermore, we performed multivariable analyses using Cox models. Our analyses, using type of chemotherapy, gender, age, and smoking history, showed that Chemo/Atezo/Bev treatment significantly prolonged PFS compared with Chemo/Atezo only in the population with low tVEGF-A (Supplementary Table 1). Taken together, we concluded that as a predictive biomarker, tVEGF-A should be measured preferentially among three VEGFs in NSCLC patients with *EGFR*-WT.

Table 1 | Characteristics of the study patients according to treatment arm

Characteristics	Carboplatin-pemetrexed-atezolizumab (*n* = 76)	Carboplatin-pemetrexed-atezolizumab-bevacizumab (*n* = 73)
Median age (range), years	66.5 (38–82)	67 (38–83)
Sex, *n* (%)		
Male	53 (69.7%)	50 (68.5%)
Female	23 (30.3%)	23 (31.5%)
Smoking status, *n* (%)		
Never	18 (23.7%)	17 (23.3%)
Former or current	58 (76.3%)	56 (76.7%)
***EGFR* mutation status, *n* (%)**		
Negative	58 (76.3%)	56 (76.7%)
Exon 19 deletion	9 (11.9%)	8 (11.0%)
L858R	7 (9.2%)	8 (11.0%)
Other mutations	2 (2.6%)	1 (1.3%)
PD-L1 TPS, *n* (%)		
<1%	27 (35.5%)	27 (37.0%)
1–49%	22 (29.0%)	19 (26.0%)
≥50%	15 (19.7%)	10 (13.7%)
Unknown	12 (15.8%)	17 (23.3%)
Metastases, *n* (%)		
Liver	5 (6.6%)	5 (6.8%)
Brain	11 (14.5%)	11 (15.1%)
Pleural effusion	20 (26.3%)	18 (24.7%)

PD-L1 programmed cell death–ligand 1, *TPS* tumor proportion score.

Potential of $VEGF_{165}$ measurement in combination with tVEGF-A for optimizing selections of patients who should avoid Bev-containing therapy

Based on the above results, we hypothesized that additional VEGF isoform measurements in addition to tVEGF-A would allow us to more accurately select patients who should receive or avoid Chemo/Atezo/Bev regimen. First, the high and low distributions of each of the three measured VEGFs were analyzed (Table 3). 105 (70.5%) of the total population ($n = 149$) and 86 (75.4%) of the *EGFR*-WT population ($n = 114$) were matched for all three isoforms, suggesting that the three isoforms show similar dynamics in many patients. On the other hand, a certain number of patients (41 (27.5%) of all population, 26 (22.8%) of *EGFR*-WT) had either $VEGF_{121}$ or $VEGF_{165}$ levels different from total VEGF-A. There were no clear differences in the distribution between all and WT population.

Then we conducted survival analyses to determine whether additional measurements of $VEGF_{121}$ and $VEGF_{165}$ levels could be used to more accurately determine the indication for treatment with Chemo/Atezo/Bev in patients with *EGFR*-WT. In the low VEGF-A

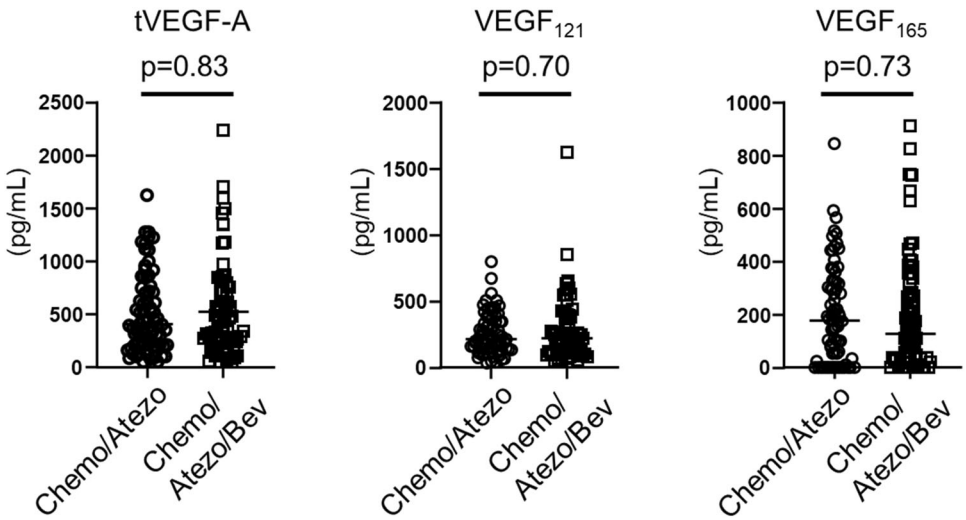

Fig. 2 | Serum concentrations of total VEGF-A (tVEGF-A), $VEGF_{121}$, and $VEGF_{165}$ at baseline for all study patients according to treatment arm. Horizontal bars indicate median values for Chemo/Atezo ($n = 76$) and Chemo/Atezo/Bev ($n = 73$) groups, and the statistical analysis was performed with two-sided Mann–Whitney *U* test. tVEGF-A total vascular endothelial growth factor–A, VEGF vascular endothelial growth factor, Chemo carboplatin-pemetrexed chemotherapy, Atezo atezolizumab, Bev bevacizumab.

https://doi.org/10.1038/s41467-025-58186-7

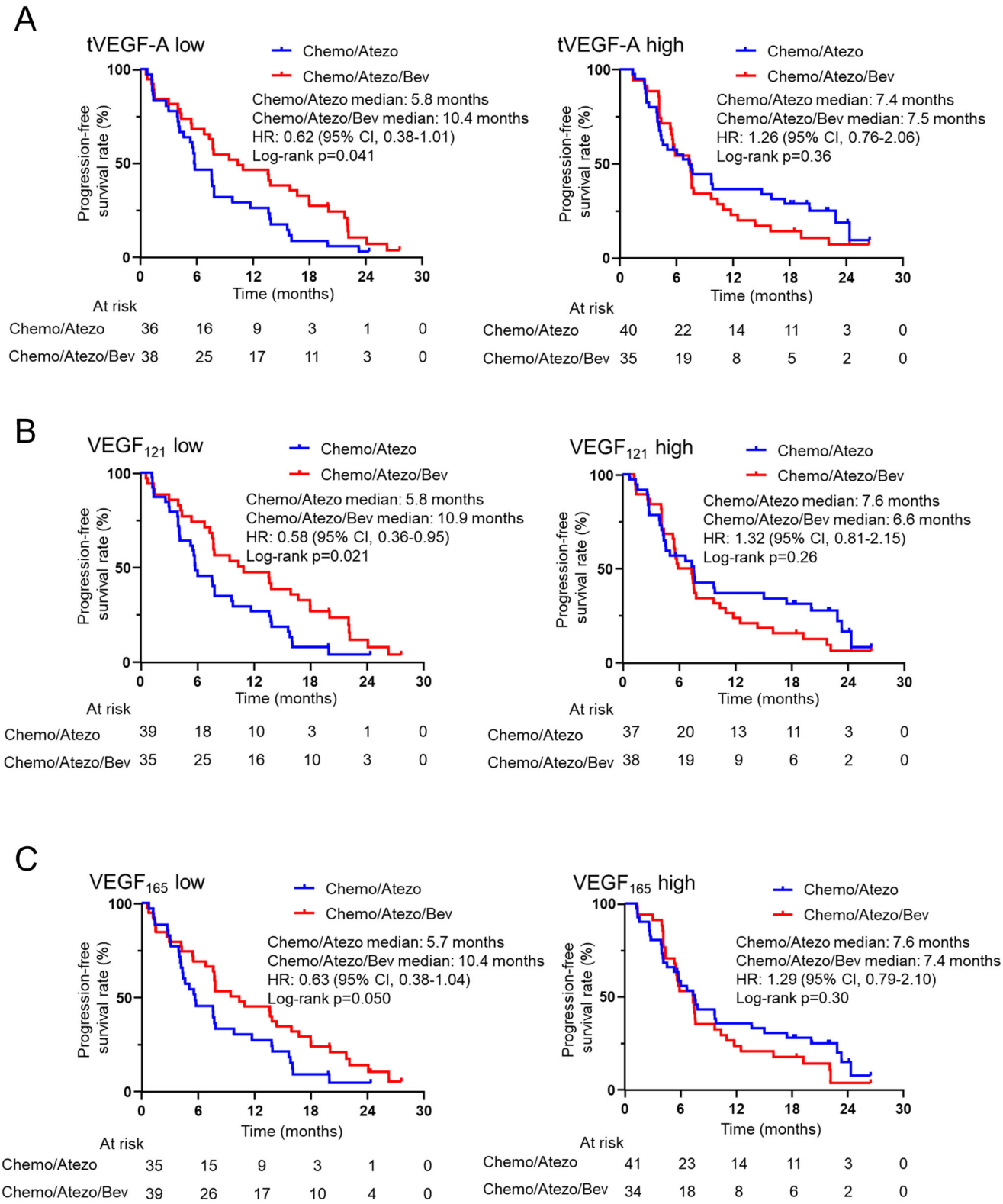

Fig. 3 | Kaplan–Meier estimates of PFS according to treatment arm for patients with low or high serum concentrations of VEGF-A at baseline. All study patients with low (left) or high (right) concentrations of tVEGF-A (**A**), $VEGF_{121}$ (**B**), or $VEGF_{165}$ (**C**) defined according to the corresponding median value were examined. The survival curve for Chemo/Atezo is shown in blue, and the survival curve for Chemo/Atezo/Bev is shown in red. PFS progression-free survival, tVEGF-A total vascular endothelial growth factor–A, VEGF vascular endothelial growth factor, Chemo carboplatin-pemetrexed chemotherapy, Atezo atezolizumab, Bev bevacizumab, HR hazard ratio, CI confidence interval.

https://doi.org/10.1038/s41467-025-58186-7

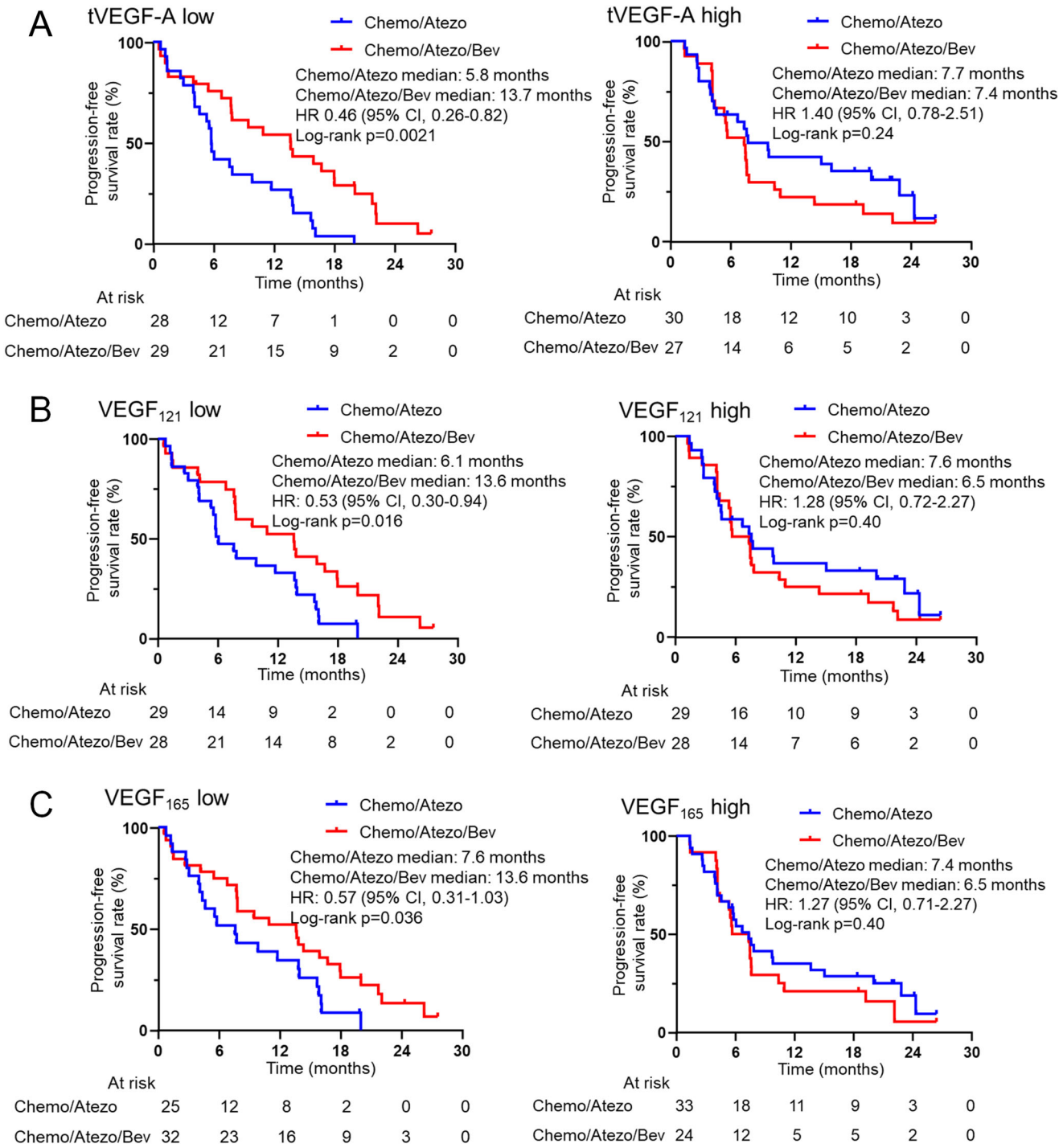

Fig. 4 | Kaplan–Meier estimates of PFS according to treatment arm for patients with *EGFR*-WT tumors and either low or high concentrations of VEGF-A at baseline. Patients with *EGFR*-WT tumors and either low (left) or high (right) concentrations of tVEGF-A (**A**), $VEGF_{121}$ (**B**), or $VEGF_{165}$ (**C**) defined according to the corresponding median value were examined. The survival curve for Chemo/Atezo is shown in blue, and the survival curve for Chemo/Atezo/Bev is shown in red. *EGFR* epidermal growth factor receptor gene, PFS progression-free survival, WT wild type, tVEGF-A total vascular endothelial growth factor–A, VEGF vascular endothelial growth factor, Chemo carboplatin-pemetrexed chemotherapy, Atezo atezolizumab, Bev bevacizumab, HR hazard ratio, CI confidence interval.

population, it did not appear meaningful to measure other VEGF isoforms to increase the likelihood of response to Bev-containing therapy (Fig. 5A–C). On the other hand, in the total VEGF-A high population (Fig. 5D–F), the tendency to be less likely to benefit from Chemo/Atezo/Bev was more evident when $VEGF_{165}$ was evaluated to be high (median of 9.7 vs. 6.5 months [HR of 1.54] for tVEGF-A/$VEGF_{165}$ high (Fig. 5E)) (median of 9.7 vs. 6.5 months [HR of 1.58] for tVEGF-A/$VEGF_{121}$/$VEGF_{165}$ high (Fig. 5F)). These findings suggest that measuring $VEGF_{165}$ in addition to total VEGF-A may be useful in optimizing such selection.

Discussion

Our study identified a potential biomarker for prediction of which patients are likely to benefit from the addition of bevacizumab to platinum-based chemotherapy and a PD-1 pathway inhibitor for the treatment of individuals with metastatic nonsquamous NSCLC. We

Table 2 | The interaction term of VEGF levels and chemotherapy arms in *EGFR*-WT patients

	P value for interaction
Chemotherapy and tVEGF-A	0.0177
Chemotherapy and $VEGF_{121}$	0.0384
Chemotherapy and $VEGF_{165}$	0.0763

Table 3 | High and low distributions of each of the three measured VEGFs (8 groups)

t/VEGF-A/$VEGF_{121}$/$VEGF_{165}$	Overall (*n* = 149)	*EGFR*-WT (*n* = 114)
High/high/high	54	44
High/high/low	11	8
High/low/high	7	3
High/low/low	3	2
Low/high/high	0	0
Low/high/low	8	5
Low/low/high	15	10
Low/low/low	51	42

found that lower levels of tVEGF-A, $VEGF_{121}$, and $VEGF_{165}$ in serum of peripheral blood before treatment indicate that patients with *EGFR*-WT tumors may benefit from the addition of bevacizumab. Given that VEGF-A is easily measured, its assay can be readily applied in daily clinical practice to support treatment with VEGF-A axis inhibitors as part of the standard of care for advanced cancer patients with low baseline VEGF-A levels. In addition, by multivariable analyses, Chemo/Bev/Atezo treatment was found to be a significant factor for predicting better PFS in low tVEGF-A population. Furthermore, we demonstrated the in many patients, expression levels of isoforms are consistent with that of tVEGF-A and additional evaluation of $VEGF_{165}$ with tVEGF-A may find patients who tend to be less likely to benefit from Chemo/Atezo/Bev therapy.

The APPLE study did not demonstrate a benefit of adding bevacizumab as an immunostimulant to the combination of platinum-based chemotherapy plus atezolizumab in patients with metastatic nonsquamous NSCLC. PFS was thus similar in the Chemo/Atezo and Chemo/Atezo/Bev groups for both the overall population as well as the subgroup of patients with *EGFR*-WT tumors[19]. Our present results indicate that low serum levels of VEGF-A at baseline are able to predict response to bevacizumab in this treatment combination for *EGFR*-WT patients. Previous studies of bevacizumab as an agent to enhance the effect of cytotoxic agents in patients with various tumor types have found that the concentration of VEGF-A in peripheral blood can serve as a prognostic but not predictive biomarker[21–23]. We here show that VEGF-A is a potential predictive biomarker for the efficacy of the combination of bevacizumab with platinum-based chemotherapy and an ICI in advanced nonsquamous NSCLC. In contrast to patients with low VEGF-A levels, the addition of bevacizumab actually tended to shorten PFS in patients with high concentrations of VEGF-A (Figs. 3 and 4). On the basis of these findings as well as the known properties of VEGF-A in tumor immunity, we speculate that higher levels of VEGF-A may not only prevent immune activation by bevacizumab but increase the likelihood that ICI treatment will be ineffective as a result of bevacizumab-specific adverse events, such as hypertension, bleeding, and hematologic toxicity[24]. We also found that an OS benefit for the addition of bevacizumab was not apparent in patients with lower VEGF-A levels, consistent with the notion that low VEGF-A concentrations are a predictor of treatment response to bevacizumab combined with platinum-based chemotherapy and an ICI.

In the present study, we used a recently developed ELISA system[20] that detects the VEGF-A isoforms $VEGF_{121}$ and $VEGF_{165}$ at higher concentrations in serum than in plasma. Four different isoforms, $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$ are generated from the human eight-exon VEGFA gene by alternative exon splicing[25]. $VEGF_{121}$, and $VEGF_{165}$, a most major isoform, have been shown in several studies to promote and inhibit tumor growth, respectively[26,27], but the precise functions of these isoforms, including the regulatory mechanisms of their production and their involvement in tumor immunity, remain unclear. In our study, the combination of tVEGF-A and $VEGF_{165}$ levels showed the potential to optimize the selection of patients who should avoid the addition of bevacizumab to immunotherapy (Fig. 5). Further confirmative clinical studies are needed to determine the appropriate combination of VEGF isoforms with tVEGF-A for predicting treatment outcome in this setting.

The APPLE study also examined PFS among patients with *EGFR*-MT tumors as a preplanned subgroup analysis and found that median PFS was 9.6 months in the Chemo/Atezo/Bev group and 5.7 months in the Chemo/Atezo group (HR of 0.70, with a 95% CI of 0.46–1.06), suggesting that the addition of bevacizumab improves PFS for such patients[19]. *EGFR* mutation has been found to increase VEGF-A expression in NSCLC cell lines[28]. We previously hypothesized that higher circulating levels of VEGF-A in patients with *EGFR*-MT tumors than in those with *EGFR*-WT tumors might contribute to the poorly immunogenic microenvironment—characterized by a low tumor mutation burden, abundant immunosuppressive cytokines and chemokines, and greater infiltration of regulatory T cells than of $CD8^+$ T cells—of the former tumors[29–31]. However, in the present study, we found that serum levels of tVEGF-A, $VEGF_{121}$, or $VEGF_{165}$ at baseline did not differ significantly between patients with *EGFR*-WT tumors and those with *EGFR*-MT tumors (Supplementary Fig. 4). Our results thus indicate that VEGF-A production was similar in patients of both treatment groups and that its regulation was independent of the presence or absence of *EGFR* activating mutations. In addition, low VEGF-A concentrations did not appear to be a predictive biomarker for PFS prolongation by bevacizumab in patients with *EGFR*-MT tumors, although the number of patients for this analysis was relatively small (Supplementary Fig. 5). On the basis of these results, we conclude that the increased efficacy of the bevacizumab combination in the *EGFR*-MT subgroup of the APPLE study was due to a mechanism independent of VEGF-A. Other cytokines and chemokines—such as interleukin-8[32], transforming growth factor-β[33,34], and CCL22[31]—are candidates for factors that contribute to this effect of bevacizumab.

There are several limitations to the present study. First, the number of cases in the association analysis for VEGF-A levels and treatment efficacy was not statistically determined. Second, the genes that influence the levels of VEGF-A in serum of NSCLC patients remain largely unknown. Third, the relation between VEGF-A levels in serum of peripheral blood and those in tumor tissue was not examined, with the cell source and amount of VEGF-A isoforms produced in tumors remaining to be determined. Fourth, the biological and clinical significance of the discrepancy between VEGF levels and those of isoforms observed in a certain number of patients are not clear in this study.

In conclusion, our results identify low serum levels of total or individual isoforms of VEGF-A at baseline as a potential predictive biomarker for the selection of patients with advanced nonsquamous NSCLC likely to benefit from the addition of bevacizumab to platinum-based chemotherapy plus a PD-1 pathway inhibitor. Further studies are warranted to confirm this finding as well as to determine its biological basis.

Methods

Study design

The design details of the APPLE study have been described previously[19]. In brief, patients with histologically or cytologically confirmed unresectable locally advanced, metastatic, or recurrent nonsquamous NSCLC were randomized (1:1) to receive either

https://doi.org/10.1038/s41467-025-58186-7

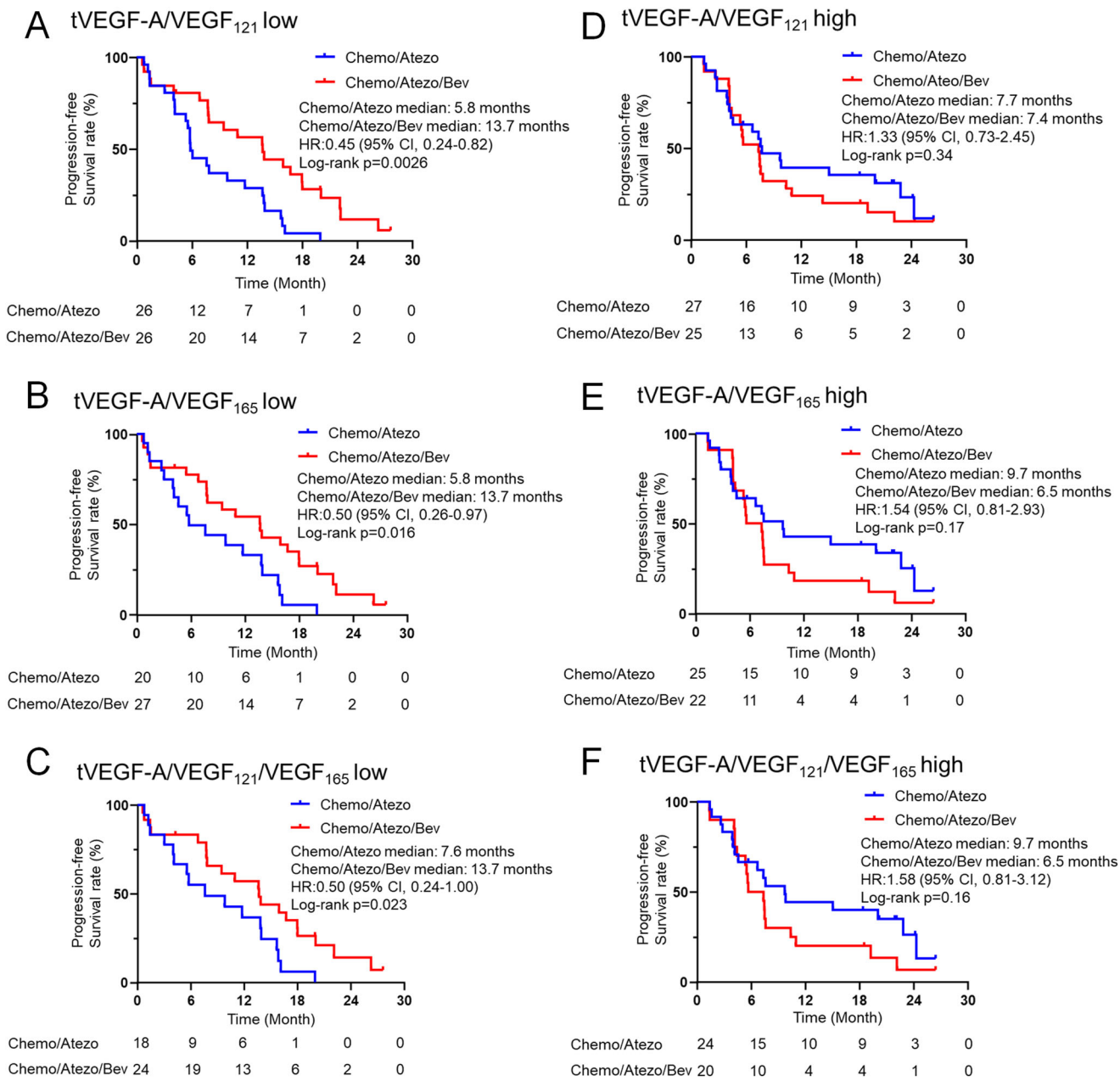

Fig. 5 | Kaplan–Meier estimates of PFS according to treatment arm for patients with *EGFR*-WT tumors and $VEGF_{121}$ and $VEGF_{165}$ measurement added to either low or high concentrations of VEGF-A at baseline. Patients with *EGFR*-WT tumors and $VEGF_{121}$ and $VEGF_{165}$ measurement added to either low (left) or high (right) concentrations of tVEGF-A (**A**, **D**), $VEGF_{121}$ (**B**, **E**), or $VEGF_{165}$ (**C**, **F**) defined according to the corresponding median value were examined. The survival curve for Chemo/Atezo is shown in blue, and the survival curve for Chemo/Atezo/Bev is shown in red. *EGFR* epidermal growth factor receptor gene, PFS progression-free survival, WT wild type, tVEGF-A total vascular endothelial growth factor–A, VEGF vascular endothelial growth factor, Chemo carboplatin-pemetrexed chemotherapy, Atezo atezolizumab, Bev bevacizumab, HR hazard ratio, CI confidence interval.

atezolizumab plus carboplatin-pemetrexed or atezolizumab, carboplatin-pemetrexed, and bevacizumab. Participants were stratified according to clinical stage (III or IV versus recurrence), driver genetic alterations (EGFR, ALK, ROS1, or BRAF alteration positive versus negative or unknown), and PD-L1 tumor proportion score (TPS, ≥50% versus <50% or unknown). Patient eligibility criteria included an age of ≥20 years; no prior treatment with cytotoxic chemotherapy and PD-1/PD-L1 antibodies; an Eastern Cooperative Oncology Group performance status of 0 or 1; no risk factors for bevacizumab-induced hemoptysis; and adequate hematologic, hepatic, and renal function. Prior treatment with EGFR, ALK, ROS1, or BRAF kinase inhibitors was required for patients with activating alterations of these genes. For this analysis, driver genetic mutations other than EGFR was excluded.

The protocol for this biomarker study affiliated with the APPLE trial was approved by Kyushu university institutional review board as well as an independent ethics committee or institutional review board at each participating site, and the study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent prior to study entry.

Sample collection

Baseline blood samples (7 mL) for serum isolation were collected from the study participants between random assignment and treatment onset. The blood was centrifuged at 1300×*g* for 10 min at room temperature, and the serum supernatant was immediately placed in cryovials and frozen at or below −20 °C until analysis.

Measurement of VEGF-A

VEGF-A isoforms were measured with a newly developed ELISA at Shino-test (Kanagawa, Japan).20 Polystyrene 96-well microtiter plates were incubated overnight at 4 °C with 100 μL per well of rabbit polyclonal antibodies to human VEGF-A (#AB-293-NA; R&D Biosystems, Minneapolis, MN, USA) in phosphate-buffered saline (PBS). The plates were washed three times with PBS containing 0.05% Tween 20, and any remaining binding sites in the wells were blocked by incubation of the plates for 2 h at room temperature with 400 μL per well of PBS containing 1% bovine serum albumin. The plates were washed again with PBS containing 0.05% Tween 20 and then incubated for 15 h at 25 °C with 100 μL per well of dilutions of the calibrator and samples (1:1 dilution in a solution containing 0.2 M Tris-HCl [pH 8.5], 0.15 M NaCl, and 1% casein). The plates were washed with PBS containing 0.05% Tween 20 and then incubated for 2 h at 25 °C with 100 μL per well of horseradish peroxidase-conjugated mouse monoclonal antibodies to human $VEGF_{121}$ or $VEGF_{165}$. After an additional washing step, 100 μL of the chromogenic substrate 3,3′,5,5′-tetramethylbenzidine (Dojindo, Kumamoto, Japan) were added to each well. The reaction was terminated and the absorbance of each well at 450 nm was measured with a microplate reader (model 680; Bio-Rad, Irvine, CA, USA). Standard curves were constructed with the use of recombinant human forms of $VEGF_{121}$ or $VEGF_{165}$, with the linear range of the assay being 10 to 2000 pg/mL for each isoform. Total VEGF-A levels were measured with a separate ELISA (Human VEGF Quantikine ELISA Kit, R&D Biosystems).

Statistical analysis

The relation between serum levels of $VEGF_{121}$, $VEGF_{165}$, or tVEGF-A at baseline and APPLE study endpoints including progression-free survival (PFS) and overall survival (OS) was analyzed with the Kaplan–Meier method and log-rank test. Differences in analyte levels between two groups were evaluated with the Mann–Whitney *U* test, and those among three groups were with the Kruskal–Wallis test. The cutoff for high versus low levels of each analyte was the median of all study participants in analyses of all populations. In analyses specific to patients with *EGFR*-WT or *EGFR*-MT, this cutoff was the median of each population. Given that the study was designed as an exploratory analysis, the number of patients was not statistically prespecified. Calculation of the interaction term and multivariable analyses were performed using JMP version 18.0.1.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All patients' data generated in this study are provided in the Supplementary Information/Source Data file. Source data are provided with this paper.

References

1. Gettinger, S. et al. Five-year follow-up of nivolumab in previously treated advanced non-small-cell lung cancer: results from the CA209-003 study. *J. Clin. Oncol.* **36**, 1675–1684 (2018).
2. Chamoto, K., Hatae, R. & Honjo, T. Current issues and perspectives in PD-1 blockade cancer immunotherapy. *Int. J. Clin. Oncol.* **25**, 790–800 (2020).
3. Reck, M. et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *New Engl. J. Med.* **375**, 1823–1833 (2016).
4. Gandhi, L. et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. *New Engl. J. Med.* **378**, 2078–2092 (2018).
5. Paz-Ares, L. et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *New Engl. J. Med.* **379**, 2040–2051 (2018).
6. West, H. et al. Atezolizumab in combination with carboplatin plus nab-paclitaxel chemotherapy compared with chemotherapy alone as first-line treatment for metastatic non-squamous non-small-cell lung cancer (IMpower130): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* **20**, 924–937 (2019).
7. Forde, P. M. et al. Neoadjuvant nivolumab plus chemotherapy in resectable lung cancer. *New Engl. J. Med.* **386**, 1973–1985 (2022).
8. Heymach, J. V. et al. Perioperative durvalumab for resectable non-small-cell lung cancer. *New Engl. J. Med.* **389**, 1672–1684 (2023).
9. Paz-Ares, L. et al. First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomised, open-label, phase 3 trial. *Lancet Oncol.* **22**, 198–211 (2021).
10. Johnson, M. L. et al. Durvalumab with or without tremelimumab in combination with chemotherapy as first-line therapy for metastatic non-small-cell lung cancer: the phase III POSEIDON study. *J. Clin. Oncol.* **41**, 1213–1227 (2023).
11. Tan, D. S. W. et al. Canakinumab versus placebo in combination with first-line pembrolizumab plus chemotherapy for advanced non-small-cell lung cancer: results from the CANOPY-1 trial. *J. Clin. Oncol.* **42**, 192–204 (2024).
12. Patel, S. A. et al. Molecular mechanisms and future implications of VEGF/VEGFR in cancer therapy. *Clin. Cancer Res.* **29**, 30–39 (2023).
13. Gabrilovich, D. I. et al. Production of vascular endothelial growth factor by human tumors inhibits the functional maturation of dendritic cells. *Nat. Med.* **2**, 1096–1103 (1996).
14. Dikov, M. M. et al. Differential roles of vascular endothelial growth factor receptors 1 and 2 in dendritic cell differentiation. *J. Immunol.* **174**, 215–222 (2005).
15. Voron, T. et al. VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. *J. Exp. Med.* **212**, 139–148 (2015).
16. Parker, K. H., Beury, D. W. & Ostrand-Rosenberg, S. Myeloid-derived suppressor cells: critical cells driving immune suppression in the tumor microenvironment. *Adv. Cancer Res.* **128**, 95–139 (2015).
17. Socinski, M. A. et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. *New Engl. J. Med.* **378**, 2288–2301 (2018).
18. Finn, R. S. et al. Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *New Engl. J. Med.* **382**, 1894–1905 (2020).
19. Shiraishi, Y. et al. Atezolizumab and platinum plus pemetrexed with or without bevacizumab for metastatic nonsquamous non-small cell lung cancer: a phase 3 randomized clinical trial. *JAMA Oncol.* **10**, 315–324 (2023).
20. Yamakuchi, M. et al. VEGF-A165 is the predominant VEGF-A isoform in platelets, while VEGF-A121 is abundant in serum and plasma from healthy individuals. *PLoS ONE* **18**, e0284131 (2023).
21. Hegde, P. S. et al. Predictive impact of circulating vascular endothelial growth factor in four phase III trials evaluating bevacizumab. *Clin. Cancer Res.* **19**, 929–937 (2013).
22. Van Cutsem, E. et al. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a biomarker evaluation from the AVAGAST randomized phase III trial. *J. Clin. Oncol.* **30**, 2119–2127 (2012).
23. Miles, D. et al. Bevacizumab plus paclitaxel versus placebo plus paclitaxel as first-line therapy for HER2-negative metastatic breast cancer (MERiDiAN): a double-blind placebo-controlled randomised phase III trial with prospective biomarker evaluation. *Eur. J. Cancer* **70**, 146–155 (2017).
24. Sandler, A. et al. Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *New Engl. J. Med.* **355**, 2542–2550 (2006).
25. Ferrara, N., Gerber, H. P. & LeCouter, J. The biology of VEGF and its receptors. *Nat. Med.* **9**, 669–676 (2003).
26. Carrer, A. et al. Neuropilin-1 identifies a subset of bone marrow Gr1- monocytes that can induce tumor vessel normalization and inhibit tumor growth. *Cancer Res.* **72**, 6371–6381 (2012).
27. Kazemi, M. et al. VEGF121 and VEGF165 differentially promote vessel maturation and tumor growth in mice and humans. *Cancer Gene Ther.* **23**, 125–132 (2016).

28. Hung, M. S. et al. Epidermal growth factor receptor mutation enhances expression of vascular endothelial growth factor in lung cancer. *Oncol. Lett.* **12**, 4598–4604 (2016).
29. Rooney, M. S., Shukla, S. A., Wu, C. J., Getz, G. & Hacohen, N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell* **160**, 48–61 (2015).
30. Saito, M. et al. Development of lung adenocarcinomas with exclusive dependence on oncogene fusions. *Cancer Res.* **75**, 2264–2271 (2015).
31. Sugiyama, E. et al. Blockade of EGFR improves responsiveness to PD-1 blockade in EGFR-mutated non-small cell lung cancer. *Sci. Immunol.* **5**, eaav3937 (2020).
32. Schalper, K. A. et al. Elevated serum interleukin-8 is associated with enhanced intratumor neutrophils and reduced clinical benefit of immune-checkpoint inhibitors. *Nat. Med.* **26**, 688–692 (2020).
33. Mariathasan, S. et al. TGFbeta attenuates tumour response to PD-L1 blockade by contributing to exclusion of T cells. *Nature* **554**, 544–548 (2018).
34. Pinter, M., Jain, R. K. & Duda, D. G. The current landscape of immune checkpoint blockade in hepatocellular carcinoma: a review. *JAMA Oncol.* **7**, 113–123 (2021).

Acknowledgements

No specific funds, grants, or other financial support were received for the conduct of this study. We thank all patients, their families, and the investigators who participated in this study as well as N. Takayanagi, K. Funakoshi, and other staff at the Center for Clinical and Translational Research at Kyushu University Hospital and members of the West Japan Oncology Group Data Center for their support.

Author contributions

K.T. contributed conceptualization, data curation, investigation, methodology, resources, formal analysis, validation, visualization, writing—original draft, writing—review and editing, and approval. J.S., Y Shiraishi, T.W., H.D., K.A., K.N., M.M., T Ota., H.S., A.H., T.S., T.K., H.A., H.M. M.T., K.W., Y Sato, T Ozaki and Y.T.K. contributed investigation, writing—review and editing, and approval. N.Y. and K.N. contributed supervision, writing—review and editing, and approval. I.O. contributed conceptualization, investigation, methodology, resources, supervision, visualization, writing—original draft, writing—review and editing, and approval.

Competing interests

K.T. has received personal fees from Chugai Pharmaceutical, Ono Pharmaceutical, AstraZeneca, Daiichi Sankyo, Eli Lilly, Merck, Takeda Pharmaceutical, Pfizer, MSD, Novartis and Bristol Myers Squibb outside the submitted work. Y Shiraishi has received personal fees from Ono Pharmaceutical, Taiho Pharmaceutical, AstraZeneca, Ono Pharmaceutical and Bristol Myers Squibb outside the submitted work. H.D. has received personal fees from AstraZeneca outside the submitted work. K.A. has received personal fees from Chugai Pharmaceutical, Takeda Pharmaceutical, MSD, AstraZeneca, and Bristol Myers Squibb outside the submitted work. K.N. has received grants from Ono Pharmaceutical, Taiho Pharmaceutical, MSD, AbbVie, Daiichi Sankyo, Amgen, Eisai, Sanofi, Janssen Pharmaceutical, Novartis, Pfizer, Eli Lilly, Merck, Takeda Pharmaceutical, Chugai Pharmaceutical and AstraZeneca; and personal fees from AstraZeneca, Ono Pharmaceutical, Boehringer Ingelheim, Eli Lilly, Novartis, Pfizer, Merck, Janssen Pharmaceutical, Bristol Myers Squibb and Nihon Kayaku outside the submitted work. M M. has received personal fees from AstraZeneca, Chugai Pharmaceutical, Ono Pharmaceutical, Daiichi-Sankyo, Eli Lilly, Kyowa Hakko Kirin, MSD, Nihon Kayaku, Pfizer, Taiho Pharmaceutical, Takeda Pharmaceutical and AbbVie outside the submitted work. H.S. has received grants from AstraZeneca, Chugai Pharmaceutical, Ono Pharmaceutical and Bristol Myers Squibb; and personal fees from AstraZeneca, Chugai Pharmaceutical, Ono Pharmaceutical and Bristol Myers Squibb, Boehringer Ingelheim and Pfizer outside the submitted work. A.H. has received grants from MSD, Eli Lilly, Chugai Pharmaceutical, Taiho Pharmaceutical, Boehringer Ingelheim and AstraZeneca; and personal fees from MSD, Eli Lilly, Chugai Pharmaceutical, Taiho Pharmaceutical, Boehringer Ingelheim, Pfizer and AstraZeneca outside the submitted work. T.S. has received personal fees from MSD, Eli Lilly, Chugai Pharmaceutical, Merck, Ono Pharmaceutical and AstraZeneca outside the submitted work. T.K. has received grants from Chugai Pharmaceutical, AstraZeneca, Eli Lilly, Taiho Pharmaceutical, Bristol Myers Squibb, Ono Pharmaceutical, MSD, Kyowa Hakko Kirin, Merck, Daiichi-Sankyo, Amgen, Abbvie, Sanofi, Labcorp development Japan, IQVIA Services Japan, Gilead Sciences, Pfizer and Bayer; consulting fees from Chugai Pharmaceutical, AstraZeneca, Ono Pharmaceutical, Pfizer, Daiichi-Sankyo, Bayer and Abbvie; and personal fees from Chugai Pharmaceutical, AstraZeneca, Eli Lilly, Taiho Pharmaceutical, Bristol Myers Squibb, Ono Pharmaceutical, MSD, Pfizer, Kyowa Hakko Kirin, Boehringer Ingelheim, Merck, Nihon Kayaku, Novartis, Daiichi-Sankyo, Takeda Pharmaceutical, Bayer, Sawai, Amgen and Eisai outside the submitted work. H.A. has received grants from Amgen, Boehringer Ingelheim; personal fees from Amgen, Chugai Pharmaceutical, AstraZeneca, Eli Lilly, Taiho Pharmaceutical, Bristol Myers Squibb, Ono Pharmaceutical, MSD, Pfizer, Boehringer Ingelheim, Merck, Nihon Kayaku, Novartis, Daiichi-Sankyo, Takeda Pharmaceutical; fees for membership of an advisory board for Amgen, Janssen Pharmaceutical, Sandoz and MSD outside the submitted work. H.M. has personal fees from AstraZeneca, Boehringer Ingelheim, Ono Pharmaceutical, Chugai Pharmaceutical, Taiho Pharmaceutical, Takeda Pharmaceutical and MSD outside the submitted work. M.T. has received grants from AstraZeneca, Chugai Pharmaceutical and Eli Lilly; personal fees from Eli Lilly, Ono Pharmaceutical, Bristol Myers Squibb, Chugai Pharmaceutical, AstraZeneca, MSD, Novartis pharmaceuticals, Takeda Pharmaceutical, Taiho Pharmaceutical, Boehringer Ingelheim, Daiichi Sankyo, Pfizer and Janssen Pharmaceutical outside the submitted work. K.W. has received grants from Chugai Pharmaceutical, AstraZeneca, Novartis, Abbvie, Amgen, MSD and Daiichi Sankyo; personal fees from Chugai Pharmaceutical, Taiho Pharmaceutical, Boehringer Ingelheim, Eli Lilly, Ono Pharmaceutical, MSD, AstraZeneca, Daiichi Sankyo, Janssen Pharmaceutical and Takeda Pharmaceutical outside the submitted work. Y Sato has received personal fees from AstraZeneca, MSD, Novartis, Chugai Pharmaceutical, Ono Pharmaceutical, Pfizer, Taiho Pharmaceutical, Nihon Kayaku, Bristol Myers Squibb, Eli Lilly, Takeda, Kyowa Hakko Kirin and Daiichi Sankyo outside the submitted work. Y. T. K. has received personal fees from Bristol Myers Squibb, Taiho Pharmaceutical, Chugai Pharmaceutical, AstraZeneca, Kyowa Hakko Kirin, MSD, Ono Pharmaceutical and Takeda Pharmaceutical outside the submitted work. N.Y. has received grants from Boehringer Ingelheim, Taiho Pharmaceutical, Chugai Pharmaceutical, Shionogi, Eli Lilly, Daiichi Sankyo, Tumura, Nihon Kayaku, Asahikasei-pharma, AstraZeneca, Janssen, Sanofi, Amgen, Novartis, Astellas, MSD, Esai, Bristol-Myers Squibb, Abbvie and Tosoh; and personal fees from MSD, AstraZeneca, Amgen, Ono Pharmaceutical, Otsuka, Guardant Health Japan, Tumura, Kyowa Hakko Kirin, Kyorin, GlaxoSmithKline, Sanofi, Daiichi Sankyo, Taiho Pharmaceutical, Takeda, Chugai Pharmaceutical, Eli Lilly, Nippon Kayaku, Boehringer Ingelheim, Novartis, Pfizer, Bristol Myers Squibb, Miyarisan, Merck and Janssen; and fees for membership of a data safety monitoring board or an advisory board from AstraZeneca, Eli Lilly, and Takeda outside the submitted work. K.N. has received grants from AstraZeneca, SD, Ono Pharmaceutical, Boehringer Ingelheim, Novartis, Pfizer, Bristol-Myers Squibb, Eli Lilly, Chugai Pharmaceutical, Daiichi Sankyo, Merck, PAREXEL International, PRA HEALTHSCIENCES, EPS Corporation., Kissei Pharmaceutical, EPS International, Taiho Pharmaceutical, PPD-SNBL, SymBio Pharmaceuticals, IQVIA Services JAPAN, SYNEOS HEALTH CLINICAL, Nihon Kayaku, EP-CRSU, Mebix, Janssen, AbbVie, Bayer, Eisai, Mochida Pharmaceutical, Covance Japan, Japan Clinical Research Operations, Takeda, GlaxoSmithKline, Sanofi, Sysmex, Medical Reserch

Support, Otsuka Pharmaceutical, SRL, and Amgen; and consulting fees from Eli Lilly, Kyorin Pharmaceutical, Ono Pharmaceutical and Pfizer; and personal fees from Ono Pharmaceutical, Amgen, Nippon Kayaku, AstraZeneca, Chugai Pharmaceutical, Eli Lilly, MSD, Pfizer, Boehringer Ingelheim, Taiho Pharmaceutical, Bayer, CMIC ShiftZero, Life Technologies Japan, Neo Communication, Roche Diagnostics, AbbVie, Merck, Kyowa hakko Kirin, Takeda, 3H Clinical Trial, Care Net, Medical Review, Medical Mobile Communications, YODOSHA, Nikkei Business Publications, Japan Clinical Research Operations, CMIC, Novartis, TAIYO Pharma, Kyorin Pharmaceutical, and Bristol-Myers Squibb; and patents from Daiichi Sankyo outside submitted work. I.O. has received grants from Chugai Pharmaceutical, AstraZeneca, Taiho Pharmaceutical, Boehringer Ingelheim, Ono Pharmaceutical, MSD, Eli Lilly, Astellas, Bristol Myers Squibb, Novartis, Pfizer, and AbbVie; and consulting fee from AstraZeneca, Bristol Myers Squibb, and AbbVie; and personal fees from AstraZeneca, Taiho Pharmaceutical, Boehringer Ingelheim, Chugai Pharmaceutical, Ono Pharmaceutical, MSD, Eli Lilly, Bristol-Myers Squibb, Novartis, and Pfizer outside the submitted work. J.S., T Ota and T Ozaki declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-025-58186-7.

Correspondence and requests for materials should be addressed to Kentaro Tanaka.

Peer review information *Nature Communications* thanks Liyun Miao and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. A peer review file is available.

[1]Department of Respiratory Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan. [2]Department of Pulmonary Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan. [3]Department of Pulmonary Medicine, Sendai Kousei Hospital, Sendai, Japan. [4]Division of Thoracic Oncology, Saitama Cancer Center, Ina, Japan. [5]Department of Medical Oncology, Osaka City General Hospital, Osaka, Japan. [6]Division of Respirology, Neurology, and Rheumatology, Department of Internal Medicine, Kurume University School of Medicine, Kurume, Japan. [7]Department of Thoracic Oncology, Osaka International Cancer Institute, Osaka, Japan. [8]Department of Thoracic Oncology, NHO Osaka Toneyama Medical Center, Toyonaka, Japan. [9]Department of Breast Medical Oncology, Izumi City General Hospital, Izumi, Japan. [10]Department of Thoracic Oncology, Kanagawa Cancer Center, Yokohama, Japan. [11]Division of Thoracic Oncology, Kobe Minimally Invasive Cancer Center, Kobe, Japan. [12]Respiratory Center, Matsusaka Municipal Hospital, Matsusaka, Japan. [13]Department of Thoracic Oncology and Medicine, NHO Shikoku Cancer Center, Matsuyama, Japan. [14]Internal Medicine III, Wakayama Medical University, Wakayama, Japan. [15]Department of Respiratory Medicine, Hyogo Prefectural Amagasaki General Medical Center, Amagasaki, Japan. [16]Division of Respiratory Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan. [17]Division of Thoracic Oncology, Shizuoka Cancer Center Hospital, Nagaizumi, Japan. [18]Department of Respiratory Medicine, Kobe City Medical Center General Hospital, Kobe, Japan. [19]Department of Medical Oncology, Kishiwada City Hospital, Osaka, Japan. [20]Department of Respiratory Medicine, Kitakyushu Municipal Medical Center, Kitakyushu, Japan. [21]Department of Medical Oncology, Kindai University Faculty of Medicine, Osaka-Sayama, Japan. [22]Present address: Department of Pulmonary Medicine, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan. ✉e-mail: k9288090@kadai.jp