



Negative impact of ratio of the microvascular area to tumor area on the response to EGFR-TKI in non-small cell lung cancer with an *EGFR* mutation

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Background: The clinical impact of tumor microvessels on the efficacy of epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs) in *EGFR* mutation-positive non-small cell lung cancer (NSCLC) is unclear. Thus, the aim of this study was to investigate whether a tumor microenvironment, abundant in microvessels, affects EGFR-TKI efficacy in patients with NSCLC and *EGFR* mutations.

Methods: We retrospectively studied the data of 40 post-operative patients with recurrent NSCLC and *EGFR* mutations who received EGFR-TKIs as a first-line treatment at Kumamoto University Hospital from January 2010 to February 2021. Tumor sections were retrieved from the tissue registry and analyzed for CD34-positive microvessels using immunohistochemical techniques. The ratio of microvascular area to tumor area (RMV), which is the CD34-positive microvascular area compared to the total tumor area, was measured using StrataQuest. The predictive value of RMV on treatment outcome, assessed via progression-free survival (PFS), was evaluated using a multivariate Cox proportional hazard model.

Results: The median PFS in the high RMV group (≥ 0.058) was significantly shorter than that in the low RMV group [< 0.058 ; 296 days, 95% confidence interval (CI): 217–374 *vs.* 918 days, 95% CI: 279–1,556, $P=0.002$]. Multivariate analysis revealed that high RMV was an independent negative predictor of PFS (hazard ratio, 3.21; 95% CI: 1.18–8.76, $P=0.022$).

Conclusions: High RMV may critically affect EGFR-TKI resistance in patients with NSCLC and *EGFR* mutations.

Keywords: Non-small cell lung cancer (NSCLC); epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI); microvascular area; microvascular density (MVD)

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Introduction

Background

Tumor angiogenesis plays a crucial role in cancer progression and distant metastasis. Compared to normal vasculature, tumor vasculature resulting from angiogenesis has abnormal structural and functional features, such as tortuosity, leakage, ramification, and poor pericyte support (1). These blood vessels characterized by high permeability lead to interstitial hypertension, which decreases drug delivery into the tumor and is likely to lead to resistance to treatment (2).

Rationale and knowledge gap

Microvascular density (MVD) based on pathological methods is generally recognized as an indicator of tumor angiogenesis in various cancers, including lung cancer. MVD is calculated as the ratio of microvessels number per area of cancer tissue and has been reported to be a prognostic factor in lung and other types of cancers (3-7). However, assessment of MVD, in which observers count the number of microvessels via immunohistochemistry staining of vascular endothelial cells, involves the subjectivity of observers, such as in determining the size and morphology of microvessels (8). Development of quantitative evaluation methods based on image analysis software has enabled automatic analysis of the microvascular area. These analyses

are expected to allow for more objective and reproducible measurements for microvascular evaluations.

Epidermal growth factor receptor (*EGFR*) mutations are the most common driver mutations in non-small cell lung cancer (NSCLC) and are detected in approximately 40% of lung adenocarcinomas in Asian populations (9). *EGFR*-tyrosine kinase inhibitors (*EGFR*-TKIs) for patients with NSCLC with *EGFR* mutations provided therapeutic benefits compared to cytotoxic anticancer agents in several randomized phase III studies and are used as the standard treatment in the first-line setting (10-13). In *EGFR* mutation-positive adenocarcinoma, a tumor microenvironment with abundant microvessels was reported to be associated with aggressive behavior (14); however, the clinical impact of tumor microvessels on the efficacy of *EGFR*-TKIs in *EGFR* mutation-positive NSCLC is unclear.

Objective

In this study, we measured tumor microvessel areas (MVAs) using image analysis software and calculated the ratio of microvascular area to tumor area (RMV), which is the microvessel areas divided by the field view area. Additionally, we aimed to clarify the impact of RMV on the efficacy of *EGFR*-TKIs in patients with NSCLC with *EGFR* mutations. We present this article in accordance with the REMARK reporting checklist (available at <https://jtd.amegroups.com/article/view/10.21037/jtd-23-1723/rc>).

Methods

Study design and patients

This study included 40 consecutive post-operative recurrent NSCLC patients with *EGFR* mutations who were treated with *EGFR*-TKIs as first-line treatment at Kumamoto University Hospital from 1 January 2010 to 28 February 2021. We retrospectively collected the following data from the medical records: age, sex, smoking history, Eastern Cooperative Oncology Group (ECOG) performance status (PS), histology, pathological stage, *EGFR* mutation status, and the type of *EGFR*-TKI (gefitinib, erlotinib, afatinib, or osimertinib). *EGFR* mutations were examined using the Cobas® *EGFR* mutation test v2 (Roche Molecular Systems Inc., Pleasanton, CA, USA) or Oncomine Dx Target Test (Thermo Fisher, Waltham, MA, USA).

The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study

Highlight box

Key findings

- High ratio of microvascular area to tumor area (RMV) in non-small cell lung cancer (NSCLC) harboring epidermal growth factor receptor (*EGFR*) mutations is a negative predictive factor for *EGFR*-tyrosine kinase inhibitor (*EGFR*-TKI), suggesting that the tumor microenvironment with abundant microvessels in *EGFR* mutation-positive NSCLC is associated with resistance to *EGFR*-TKI.

What is known and what is new?

- Tumor angiogenesis plays a crucial role in cancer progression and distant metastasis. In *EGFR* mutation-positive adenocarcinoma, a tumor microenvironment with abundant microvessels was associated with aggressive behavior.
- This study clarifies the impact of RMV on the efficacy of *EGFR*-TKIs in patients with NSCLCs with *EGFR* mutations.

What is the implication, and what should change now?

- The combination of *EGFR*-TKIs and angiogenesis inhibitors may be an appropriate treatment strategy.

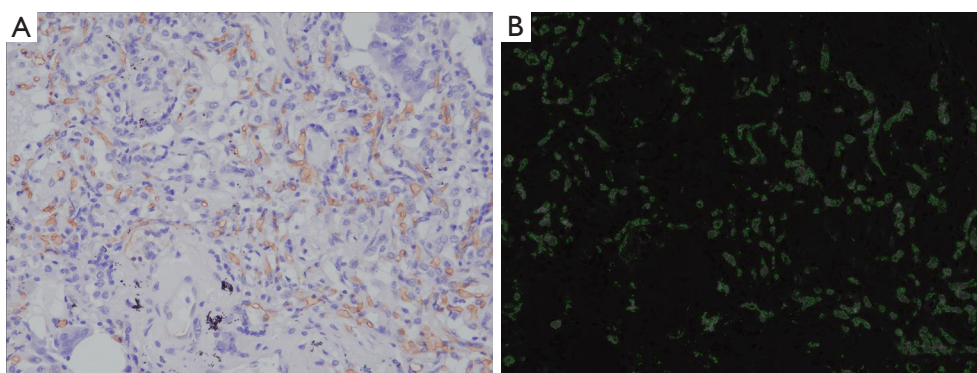


Figure 1 Representative measurement of RMV. (A) Microvascular vessels were observed using CD34 immunohistochemically stained specimens (original magnification $\times 200$). (B) CD34-positive microvascular endothelial cells, which were identified using StrataQuest, are represented in green. In this case, the total area of the green zone was 0.012 mm^2 in this field of view (0.175 mm^2), and RMV was recorded as 0.069. RMV, ratio of microvascular area to tumor area.

was approved by the institutional ethics review board of Kumamoto University Hospital (IRB No. 2364) and individual consent for this retrospective analysis was waived.

Immunohistochemical analysis

Sections of formalin-fixed, paraffin-embedded tissues were depleted of paraffin and hydrated. They were incubated with 3% H_2O_2 for 5 min, washed with 0.05% Tween in Tris-buffered saline (TBST), exposed to blocking buffer [5% goat serum and 0.5% bovine serum albumin in phosphate-buffered saline (PBS)] in PBS for 10 min at 24 degrees Celsius, and incubated with mouse anti-CD34 antibodies (ab8536; clone QBEND-10; Abcam, Cambridge, UK) for 60 min. They were then washed with TBST, incubated with biotinylated secondary antibodies (Vector Laboratories, CA, USA) for 10 min at room temperature, and washed again, after which immune complexes were detected using an avidin-peroxidase complex (Vector Laboratories) and 3,3'-diaminobenzidine (Vector Laboratories). The sections were counterstained with Mayer's hematoxylin, dehydrated using a graded series of ethanol, and mounted in Marinol (Muto Pure Chemicals, Tokyo, Japan).

Assessment of microvessels

Three images with the highest density of CD34-positive microvessels as the hotspot field of the tumor section were captured using a BX51 microscope (Olympus, Tokyo, Japan) at $\times 200$ magnification. The number of CD34-positive microvessels was counted by two independent investigators

(M.A. and K.S.). MVD was recorded as the average count of CD34-positive microvessels in three images. The MVAs in each image were automatically measured using StrataQuest (TissueGnostics, Vienna, Austria). The RMV in each image was calculated as the area occupied by CD34-positive microvessels divided by the total area of the field of view (area of 0.175 mm^2 per field of view). The RMV in each case was recorded as the average RMV of three images. Representative images of the RMV measurements are shown in *Figure 1*.

Clinical outcome parameter

Progression-free survival (PFS) was calculated from the initial date of EGFR-TKI therapy to the date of progressive disease or last follow-up. Overall survival (OS) was calculated from the initial date of EGFR-TKI therapy to the date of death or last follow-up. The best tumor response was evaluated based on the Response Evaluation Criteria in Solid Tumours guidelines version 1.1.

Statistical analysis

Primary endpoint of this study was to assess the correlation between MVD and RMV, to compare PFS and OS according to RMV, and to analyze whether RMV was independent predictive factors of PFS and OS. Chi-square test, Fisher's exact test, or Mann-Whitney *U* test was used to investigate the association of clinical factors. The Spearman's rank correlation coefficient was used to assess the correlation between MVD and RMV. Survival curves were generated

Table 1 Baseline patient characteristics

Characteristics	Subtypes	Value (n=40)
Age, years, median [range]		71.5 [43–89]
Sex	Male	22 (55.0)
	Female	18 (45.0)
Smoking	Yes	17 (42.5)
	No	23 (57.5)
ECOG PS	0	20 (50.0)
	1	16 (40.0)
	2	3 (7.5)
	3	0
	4	1 (2.5)
Histology	Ad	38 (95.0)
	Non-Ad	2 (5.0)
EGFR mutation type	Exon 19 deletions	24 (60.0)
	Exon 21 mutations	
	L858R	15 (37.5)
	L861Q	1 (2.5)
Vascular invasion	Absence	17 (42.5)
	Presence	23 (57.5)
Pleural invasion	Absence	21 (52.5)
	Presence	19 (47.5)
Pathological stage	I	19 (47.5)
	II	12 (30.0)
	III	9 (22.5)
EGFR-TKI	Gefitinib	11 (27.5)
	Erlotinib	7 (17.5)
	Afatinib	11 (27.5)
	Osimertinib	11 (27.5)

Data are presented as n (%) unless otherwise specified. ECOG PS, Eastern Cooperative Oncology Group performance status; Ad, adenocarcinoma; EGFR, epidermal growth factor receptor mutation; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

using the Kaplan-Meier method and compared using the log-rank test. Univariate and multivariate analyses were performed using a Cox proportional hazard model to identify independent predictive factors of PFS and OS. Age, sex, smoking, PS, pathological stage, *EGFR* mutation type, and RMV were included in the multivariable model as covariates. All statistical analyses were performed using SPSS (version 23.0; IBM, Armonk, NY, USA). Statistical significance was set at $P < 0.05$.

Results

Patient characteristics

The median age at diagnosis was 71.5 years. Of the patients, 22 (55%) were male, 36 (90%) had ECOG PS 0–1, 38 (95%) had adenocarcinoma, 19 (47.5%) had pathological stage I, 23 (57.5%) had vascular invasion, and 19 (47.5%) had pleural invasion (*Table 1*). Regarding the type of *EGFR* mutation, 24 patients (60%) had exon 19 deletions, and 15 patients (37.5%) had exon 21 L858R mutation. The EGFR-TKI drugs administered as first-line treatment for post-operative recurrence were as follows: gefitinib in 11 (27.5%), erlotinib in 7 (17.5%), afatinib in 11 (27.5%), and osimertinib in 11 (27.5%) patients.

Assessment of correlation between MVD and RMV

The median MVD and RMV were $123/\text{mm}^2$ (range, $70\text{--}470/\text{mm}^2$) and 0.058 (range, 0.013–0.094), respectively. Scatter plots of the MVD and RMV data are shown in *Figure 2*. A significant moderate positive correlation was observed between MVD and RMV ($r=0.47$, $P=0.020$).

Comparison of clinicopathological features according to RMV

Using the median RMV as the cut-off value, we divided the patients into high (≥ 0.058) and low (< 0.058) RMV groups. No significant difference in clinicopathological factors was observed between the two groups (*Table 2*).

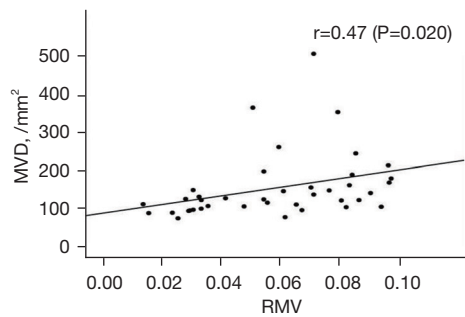


Figure 2 Correlation between MVD and RMV. RMV, ratio of microvascular area to tumor area; MVD, microvascular density.

Tumor response, PFS, and OS analyses with EGFR-TKI according to RMV

Among the 30 patients with measurable lesions, four had complete response, seventeen had partial response, five had stable disease, one had progressive disease, and three were not evaluable (Table S1). The best objective response rate was 70% [95% confidence interval (95% CI): 52.0–83.5%], and no significant difference was observed between the high and low RMV groups.

The median follow-up time from the initiation of

Table 2 Patient characteristics and clinicopathological features according to RMV status

Variables	Subtypes	RMV		P
		High (N=20)	Low (N=20)	
Age, years, median [range]		72.5 [46–89]	71 [43–85]	0.659
Sex	Male	13 [65]	9 [45]	0.341
	Female	7 [35]	11 [55]	
Smoking	Yes	11 [55]	6 [30]	0.2
	No	9 [45]	14 [70]	
ECOG PS	0–1	17 [85]	20 [100]	0.231
	2–4	3 [15]	0 [0]	
Histology	Ad	19 [95]	19 [95]	>0.99
	Non-Ad	1 [5]	1 [5]	
Vascular invasion	Absence	8 [40]	15 [75]	0.054
	Presence	12 [60]	5 [25]	
Pleural invasion	Absence	14 [70]	7 [35]	0.056
	Presence	6 [30]	13 [65]	
Pathological stage	I–II	16 [80]	15 [75]	>0.99
	III	4 [20]	5 [25]	
EGFR mutation type	Exon 19 deletions	9 [45]	15 [75]	0.105
	Exon 21 mutations	11 [55]	5 [25]	
EGFR-TKI	Gefitinib	5 [25]	6 [30]	0.6
	Erlotinib	4 [20]	3 [15]	
	Afatinib	4 [20]	7 [35]	
	Osimertinib	7 [35]	4 [20]	

Data are presented as n [%] unless otherwise specified. RMV, ratio of microvascular area to tumor area; ECOG PS, Eastern Cooperative Oncology Group performance status; Ad, adenocarcinoma; EGFR, epidermal growth factor receptor mutation; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

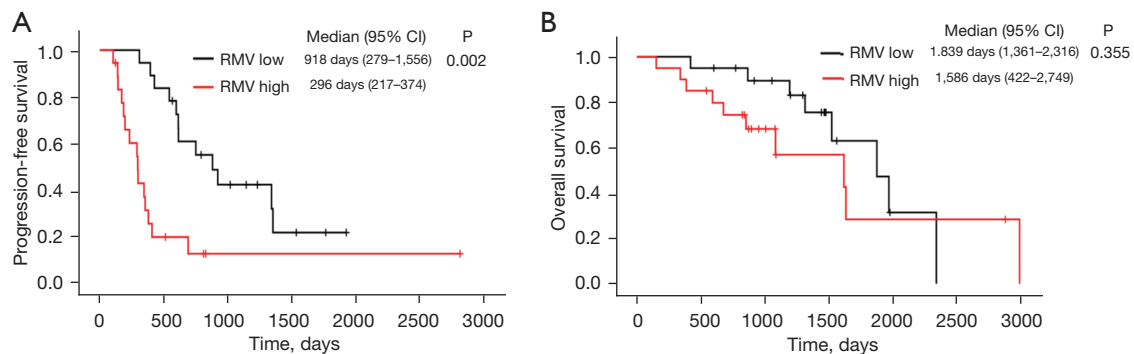


Figure 3 Kaplan-Meier curve of progression-free survival (A) and overall survival (B) according to RMV. RMV, ratio of microvascular area to tumor area; CI, confidence interval.

EGFR-TKI therapy in the first-line setting was 1,059.5 days (range, 120–2,807 days). The median PFS and OS were 610 days (range, 102–2,807 days) and 1,602 days (range, 146–2,933 days), respectively. The median PFS was significantly shorter in the high RMV group than in the low RMV group [296 days (95% CI: 217–374) *vs.* 918 days (95% CI: 279–1,556), $P=0.002$; *Figure 3A*]. However, no significant differences in OS were observed between the high and low RMV groups [1,586 days (95% CI: 422–2,749) *vs.* 1,839 days (95% CI: 1,361–2,316), $P=0.355$; *Figure 3B*].

Univariate and multivariate analysis for PFS and OS

The univariate and multivariate analyses of PFS and OS are summarized in *Tables 3,4*. Univariate analysis showed that PFS was significantly associated with RMV [hazard ratio (HR): 3.25, 95% CI: 1.48–7.16, $P=0.003$]. Multivariate analysis revealed that only high RMV (HR: 3.21, 95% CI: 1.18–8.76, $P=0.022$) were significant independent negative predictive factors for PFS after first-line EGFR-TKI treatment.

Univariate analysis showed that OS was significantly associated with ECOG PS (HR: 2.93, 95% CI: 1.03–6.60, $P=0.009$), but no significant differences were found in RMV (HR: 1.58; 95% CI: 0.60–4.16, $P=0.359$). Multivariate analysis revealed that ECOG PS ≥ 2 (HR: 12.59, 95% CI: 1.34–118.30, $P=0.027$) was a significant independent negative predictive factor for OS.

Discussion

To our knowledge, this is the first study to assess the association between RMV and antitumor efficacy of EGFR-

TKIs in patients with NSCLC harboring *EGFR* mutations. This study showed a significant positive correlation between MVD and RMV, suggesting that RMV and MVD might be indicators of angiogenesis. Moreover, we found that a high RMV in patients with *EGFR* mutation-positive NSCLC was a negative predictive factor for PFS in EGFR-TKI treatment; thus, a novel treatment strategy with EGFR-TKIs, such as a combination of EGFR-TKI and angiogenesis inhibitors, might be necessary for *EGFR* mutation-positive NSCLC with abundant tumor microvessels.

Numerous studies using MVD based on immunohistochemistry have shown that MVD is correlated with lung cancer development and prognosis (3,15,16). In contrast, MVA has been reported to be a prognostic factor in breast cancer and renal cell carcinoma (17–19). Moreover, Sullivan *et al.* described that the evaluation of MVA by automated image analysis was correlated with pathologist-diagnosed MVD (17), indicating that MVA is an alternative angiogenetic marker of MVD. However, MVA is measured as the total area of blood vessels per field of view and is dependent on magnification of the microscope and whole tumor size, which makes it difficult to consistently evaluate the overall assessment of angiogenesis status in whole cancer tissue. In the current study, RMV was defined as the ratio of microvascular area to field of view, likely to be estimated independently of tumor size, and was found to be positively correlated with MVD. Thus, the measurement of RMV using image analysis software could be an alternative indicator of tumor angiogenesis as well as MVD.

We found that the PFS of EGFR-TKI in the higher RMV group was significantly shorter than that in the lower RMV group. Tumor angiogenesis assessed by MVD has

Table 3 Univariate and multivariate analysis for PFS

Variables	Subtypes	Univariate		Multivariate	
		HR (95% CI)	P	HR (95% CI)	P
Age	<75 years	Ref	0.630	Ref	0.830
	≥75 years	0.82 (0.36–1.87)		1.12 (0.40–3.16)	
Sex	Male	Ref	0.371	Ref	0.050
	Female	1.42 (0.66–3.04)		3.77 (1.00–14.23)	
Smoking	No	Ref	0.405	Ref	0.225
	Yes	0.72 (0.33–1.56)		0.39 (0.09–1.78)	
PS	0–1	Ref	0.963	Ref	0.633
	≥2	1.05 (0.14–7.82)		0.58 (0.06–5.337)	
Stage	I–II	Ref	0.367	Ref	0.810
	III	0.61 (0.21–1.78)		0.50 (0.23–1.09)	
EGFR mut	Exon 19 deletions	Ref	0.370	Ref	0.851
	Exon 21 mutations	1.43 (0.66–3.10)		0.913 (0.35–2.36)	
RMV	Low	Ref	0.003	Ref	0.022
	High	3.25 (1.48–7.16)		3.21 (1.18–8.76)	

PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; PS, performance status; EGFR, epidermal growth factor receptor; mut, mutation; RMV, ratio of microvascular area to tumor area.

Table 4 Univariate and multivariate analysis for OS

Variables	Subtypes	Univariate		Multivariate	
		HR (95% CI)	P	HR (95% CI)	P
Age	<75 years	Ref	0.969	Ref	0.926
	≥75 years	0.98 (0.36–2.67)		1.07 (0.27–4.24)	
Sex	Male	Ref	0.271	Ref	0.161
	Female	1.78 (0.63–5.00)		3.11 (0.64–15.12)	
Smoking	No	Ref	0.998	Ref	0.602
	Yes	1.00 (0.38–2.67)		0.65 (0.13–3.29)	
PS	0–1	Ref	0.009	Ref	0.027
	≥2	2.93 (1.03–6.60)		12.59 (1.34–118.30)	
Stage	I–II	Ref	0.801	Ref	0.687
	III	1.16 (0.37–3.60)		0.74 (0.17–3.20)	
EGFR mut	Exon 19 deletions	Ref	0.761	Ref	0.331
	Exon 21 mutations	0.85 (0.30–2.44)		0.53 (0.14–1.92)	
RMV	Low	Ref	0.359	Ref	0.697
	High	1.58 (0.60–4.16)		1.33 (0.32–5.47)	

OS, overall survival; HR, hazard ratio; CI, confidence interval; PS, performance status; EGFR, epidermal growth factor receptor; mut, mutation; RMV, ratio of microvascular area to tumor area.

been reported to be associated with resistance to cytotoxic chemotherapy in several cancers, including breast, cervical, and ovarian cancers (20,21). One cause of drug resistance is the vascular endothelial growth factor (VEGF). VEGF plays an important role in tumor angiogenesis and leads to leakage from microvessels in tumor tissues and elevation of interstitial fluid pressure, which decreases drug delivery and results in drug resistance (22-25). Previous reports have shown MVD to be significantly correlated with plasma and tumor VEGF levels in NSCLC and esophageal and gastric cancers (26,27). Thus, *EGFR* mutation-positive NSCLC with higher RMV is suggested to be a microenvironment with abundant VEGF, which might reduce the efficacy of EGFR-TKIs due to inhibition of drug delivery.

Angiogenesis inhibitors, such as bevacizumab and ramucirumab, have demonstrated clinical efficacy and have been used for patients with NSCLC in routine practice. Recently, randomized phase III studies comparing the combination therapy of EGFR-TKI and angiogenesis inhibitors to EGFR-TKI monotherapy in *EGFR* mutation-positive NSCLC showed that the former resulted in longer PFS (28,29). In ovarian and colorectal cancers, high MVD assessed by immunohistochemistry has been reported to be a potential predictive factor of bevacizumab (30,31). Furthermore, Zhao *et al.* reported a positive correlation between MVD and tumor shrinkage rate in patients with NSCLC treated with platinum doublets and bevacizumab (4). We found that *EGFR* mutation-positive NSCLC with a high RMV was resistant to EGFR-TKI monotherapy compared to that with a low RMV. Based on these findings, *EGFR* mutation-positive NSCLC with abundant tumor microvessels might be preferable in combination with EGFR-TKI and angiogenesis inhibitors.

Despite these findings, our study had several limitations. First, this was a retrospective study performed in a single institution; therefore, this study included small number of Japanese patients and various selection biases might be existed. Second, this study used surgical specimens from patients with post-operative recurrence. The tumor microenvironment, including angiogenesis status, might have changed between the surgical and post-operative recurrent specimens, and these differences in microvasculature might have affected the results of our study. Third, four types of EGFR-TKIs (gefitinib, erlotinib, afatinib and osimertinib) were included in this study. In the LUX-lung 7 and FLAURA trials, afatinib and osimertinib significantly prolonged PFS compared to the first-generation EGFR-TKIs (gefitinib and erlotinib) in *EGFR*-

mutant NSCLC (13,32). Although there was no significant difference in the type of EGFR-TKI between low and high RMV groups, the type of EGFR-TKI might have affected the results of this study. Fourth, image analysis software of RMV evaluation we employed was only StrataQuest in this study although previous studies on RMV have been used various image analysis software to evaluate RMV. It is necessary to verify the reliability and validity for RMV evaluation of each software. Fifth, MVD and RMV were previously reported as a negative prognostic marker for NSCLC (3,33). Although high RMV was significantly associated with shorter PFS of EGFR-TKI for *EGFR*-mutant NSCLC in this study, further study involving large cohort is needed to determine whether RMV is truly predictive factor of EGFR-TKI in patients with *EGFR* mutation-positive NSCLC. Sixth, median value of RMV that we provisionally employed was cut off level in order to analyze the predictive factor of EGFR-TKI. Therefore, a large prospective study using advanced and post-operative recurrent NSCLC specimens is required to clarify the optimal cut-off value of RMV for the predictive value of response to EGFR-TKIs.

Conclusions

High RMV in NSCLC harboring *EGFR* mutations is a negative predictive factor for EGFR-TKI, suggesting that the tumor microenvironment with abundant microvessels in *EGFR* mutation-positive NSCLC is associated with resistance to EGFR-TKI. In these populations, the combination of EGFR-TKIs and angiogenesis inhibitors may be an appropriate treatment strategy.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the institutional ethics review board of Kumamoto University Hospital (IRB No. 2364) and individual consent for this retrospective analysis was waived.

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Table S1 Comparison of response to EGFR-TKI according to RMV

Variables	All	RMV		P
		High (N=14)	Low (N=16)	
CR	4	2	2	
PR	17	9	8	
SD	5	3	2	
PD	1	0	1	
NE	3	0	3	
ORR (95% CI)	70% (52.0-83.5%)	78.6% (51.7-93.2%)	68.8% (44.1-86.1%)	0.427

EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor; RMV, ratio of microvascular area to tumor area; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; NE, not evaluated; ORR, overall response rate; CI, confidence interval.