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Impact of ramucirumab pharmacokinetics in combination with docetaxel on the efficacy and survival in patients with advanced non-small cell lung cancer

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ABSTRACT

Objectives: Ramucirumab, an anti-vascular endothelial growth factor receptor-2 antibody, has been approved for the treatment of non-small cell lung cancer (NSCLC); however, its pharmacokinetic properties in clinical practice are unknown. We aimed to measure ramucirumab concentrations and conduct a retrospective pharmacokinetic analysis using real-world data.

Materials and Methods: Patients with stage III–IV and recurrent NSCLC who received ramucirumab plus docetaxel were evaluated in this study. After the first administration, the ramucirumab trough concentration (C_{trough}) was measured using liquid chromatography-mass spectrometry. Patient characteristics, adverse events, tumor response, and survival time were retrospectively extracted from medical records from August 2, 2016 to July 16, 2021.

Results: A total of 131 patients were examined to assess serum ramucirumab concentrations. C_{trough} ranged from below the lower limit of quantification (BLQ) to 48.8 μg/mL (BLQ ≤ 1st quartile (Q1) ≤ 7.34, 7.34 < 2nd quartile (Q2) ≤ 14.7, 14.7 < 3rd quartile (Q3) ≤ 21.9 and 21.9 < 4th quartile (Q4) ≤ 48.8 μg/mL). The overall response rate was significantly higher in Q2–4 than that in Q1 ($p = 0.011$). The median progression-free survival was marginally longer, and overall survival was significantly longer in Q2–4 ($p = 0.009$). The Glasgow prognostic score (GPS) in Q1 was significantly higher than in Q2–4 ($p = 0.034$) and associated with C_{trough} ($p = 0.002$).

Conclusion: Patients with higher ramucirumab exposure had a high ORR and prolonged survival time, whereas patients with lower ramucirumab exposure were characterized by a high GPS and poor prognosis. Cachexia may reduce the exposure level of ramucirumab in certain patients, reducing the clinical benefits of ramucirumab treatment.

1. Introduction

Ramucirumab is a human IgG1 monoclonal antibody that targets human vascular endothelial growth factor receptor-2 (VEGFR-2). Ramucirumab binds to VEGFR-2, preventing binding to human vascular endothelial growth factor (VEGF). Angiogenesis is inhibited through the VEGFR-2 signaling pathway, leading to the suppression of tumor growth [1]. In the phase III REVEL study, second-line ramucirumab plus

Abbreviations: ADA, Anti-drug antibodies; BLQ, Below the lower limit of quantification; CRP, C-reactive protein; DCR, Disease control rate; ECOG, Eastern Cooperative Oncology Group; EGFR, Epidermal growth factor receptor; FN, Febrile neutropenia; GPS, Glasgow prognostic score; ICI, Immune checkpoint inhibitors; NLR, Neutrophil-to-lymphocyte ratios; NSCLC, Non-small cell lung cancer; ORR, Overall response rate; OS, Overall survival; PFS, Progression-free survival; PS, Performance status; VEGF, Vascular endothelial growth factor.

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docetaxel (DTX) improved progression-free survival (PFS) and overall survival (OS) of patients with stage IV non-small cell lung cancer (NSCLC) after disease progression despite platinum-based therapy [2]. In the phase II JVCG study, ramucirumab plus DTX also improved PFS with acceptable toxicities in Japanese patients [3]. Ramucirumab plus DTX has therefore been approved as a standard regimen for stage IV NSCLC after disease progression. Ramucirumab combined with erlotinib has subsequently been approved for untreated epidermal growth factor receptor (*EGFR*)-mutated NSCLC [4], and various clinical trials evaluating ramucirumab combined with immune checkpoint inhibitors (ICI) are currently underway, making ramucirumab an important option in NSCLC treatment.

Exposure to bevacizumab, an anti-VEGF antibody, is positively correlated with survival in metastatic colorectal cancer [5]. Regarding ramucirumab, the population pharmacokinetic (PPK) analysis from the REVEL study indicated that the predicted minimum ramucirumab concentration after the first infusion was significantly associated with OS, and the predicted average ramucirumab concentration at a steady state was significantly associated with toxicity [6]. However, the pharmacokinetics (PK) of antibodies are susceptible to hypercatabolism in cancer cachexia [7], which is reflected by the Glasgow prognostic score (GPS, 0: albumin [Alb] $\geq$ 3.5 g/dL and C-reactive protein [CRP] $\leq$ 1.0 mg/dL, 1: Alb $<$ 3.5 g/dL and CRP $\leq$ 1.0 mg/dL or Alb $\geq$ 3.5 g/dL and CRP $>$ 1.0 mg/dL, 2: Alb $<$ 3.5 g/dL and CRP $>$ 1.0 mg/dL) [8]. Since patients with poor Eastern Cooperative Oncology Group (ECOG) performance status (PS) and advanced age were excluded and only patients with more than three infusions of ramucirumab and one line of prior treatment were included in the PPK analysis of the REVEL study, the response and survival may have been overestimated due to the lack of a population that did not benefit from ramucirumab. Herein, we selected a cohort that included patients with multiple treatments or an early discontinuation history and conducted a PK analysis of ramucirumab. We aimed to evaluate the relationship between ramucirumab exposure and response, toxicity, survival, and GPS in a real-world Japanese population.

2. Materials and Methods

2.1. Study design

Data of patients with stage III, IV, and recurrent NSCLC who received ramucirumab plus DTX at the National Cancer Center Hospital between August 2, 2016, and July 16, 2021, were extracted, and 166 patients provided written consent for specimen use. First, 32 patients who received more than three infusions of ramucirumab and one line of prior treatment were evaluated as the primary cohort in reference to the PPK analysis of the REVEL study [6]. The PK data were confirmed to be similar to those previously reported [9], and 105 patients who were excluded from the primary cohort and had available study samples were evaluated in the additional cohort as in the real-world cohort. The serum ramucirumab concentration after the first infusion (C_{trough}) was measured using liquid chromatography-mass spectrometry (LC-MS/MS), and 131 patients were eligible for PK analysis (Fig. S1). Patient serum samples were collected for at least 18 days within the maximum infusion interval from the first infusion date. Patient characteristics, adverse events, tumor response, and survival time were retrospectively collected from the medical records. Efficacy was assessed in accordance with the Response Evaluation Criteria in Solid Tumors version 1.1 [10], and adverse events were assessed using the criteria of the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 [11]. PFS was defined as the time from the start date of ramucirumab treatment to disease progression or death from any cause, whereas OS was defined as the time from the start date of ramucirumab treatment to the last day confirmed to be alive or dead from any cause. Preserved serum samples were obtained from the National Cancer Center (Biobank, Tokyo, Japan). This study was approved by the Institutional Review Board of our institute (approval no. 2019–123) and was performed in accordance with the Declaration of Helsinki. The participants provided comprehensive consents for specimen use.

2.2. Reagents

Ramucirumab was purchased from Toho Pharmaceutical Co. Ltd. (Tokyo, Japan), and rituximab was purchased from Zenyaku Kogyo (Tokyo, Japan). The nano-surface and molecular-orientation-limited kits were purchased from Shimadzu (Kyoto, Japan). Pooled human serum was purchased from Cosmo Bio (Tokyo, Japan). All other reagents were purchased from commercial sources.

2.3. Analysis of ramucirumab

ASQGIDNWLGWYQQKPGK (m/z 1038.50 $>$ 1091.55 [y9]) peptide was selected as the signature ramucirumab peptide using the Skyline software (MacCoss Laboratory, Washington DC, USA, version 20.2). The median C_{trough} after three infusions was approximately 30.1 μg/mL [9], and the quantification range was set as 3–200 μg/mL. Linearity (Fig. S2), selectivity (Fig. S3), matrix effect, carryover, precision and accuracy within-run or between runs (Table S1), dilution effect, and stability (when stored at −20 ℃, −80 ℃, or room temperature for 6 h, during freeze and thaw, post-prepared for 24 h, and when stored at −80 °C or −20 °C for 6 months) were evaluated and satisfied the criteria of the guidelines [12].

2.4. Statistical analysis

Continuous variables are presented as means and medians for parametric and nonparametric variables, respectively. Fisher's exact test was used for group comparisons of categorical variables. Student's *t*-test was used to compare parametric continuous variables between groups, whereas the Mann–Whitney *U* test was used to compare nonparametric continuous variables between groups. Multiple regression and logistic regression analyses were used to assess continuous and categorical variables, respectively. Cox multivariate regression analysis was used to assess survival, and the log-rank test was used for inter-group comparisons. The covariates were selected based on previous reports and the results of univariate analysis. Statistical significance was set at $p < 0.05$. Stata SE version 16.1 (Stata Corp, College Station, TX, USA) and GraphPad Prism version 9.3.1 software (GraphPad Software, San Diego, CA, USA) were used for analysis.

3. Results

3.1. Clinical data of All patients

The median age of the patients was 62 years (range 32–78 years), and 85 men and 52 women were included. Of these patients, 8 (5.8%) had an ECOG PS of 2, and 96 (70.1%) received multiple lines of treatment (Table 1). Neutropenia occurred in 23 (16.8%) patients and was grade 3 or higher in each case, and febrile neutropenia (FN) occurred in 8 (6.1%). The primary prophylaxis rate by granulocyte-colony stimulating factor was 66.4%, and the incidence rates of FN with and without primary prophylaxis were 4.4% and 8.7%, respectively, and did not differ significantly ($p = 0.446$). Adverse events of ramucirumab included bleeding (27.7%), proteinuria (11.7%), hypertension (8.0%), and thromboembolic events (1.5%; Table S2). The overall response rate (ORR) was 26.3%, the disease control rate (DCR) was 77.4%, the median PFS was 5.1 months, and the median OS was 14.7 months. The additional cohort was more likely to be female than the primary cohort, have no smoking history, harbor *EGFR* mutations, have elevated neutrophil-to-lymphocyte ratios (NLRs) and pleural effusions, and receive no subsequent treatment, indicating a marginally shorter OS (Fig. S4). GPS was significantly associated with PFS (HR = 2.28, 95% confidence interval [CI]: 1.53–3.40, $p < 0.001$) and OS (HR = 1.98, 95% CI: 2.26–3.12, $p =$

Table 1
Patient characteristics.

	Overall (n = 137)		Primary cohort (n = 32)		Additional cohort (n = 105)		
	n	%	n	%	n	%	*p*-value
Age (median, range) (≥65 vs. < 65 years)	62 (32–78)		62 (35–74)		62 (32–78)		
≥ 65/< 65 years	58/79	42.3/57.6	12/20	37.5/62.5	46/59	43.8/56.2	0.548
Sex (male vs. female)							
male/female	85/52	62.0/38.0	25/7	78.1/21.9	60/45	57.1/42.9	0.038
Body weight (kg, [a]mean ± SD)	60.9 ± 11.7		64.6 ± 9.7		59.8 ± 12.0		0.045
[b]ECOG-performance status (2 vs. 0/1)							
0/1/2	41/88/8	29.9/64.2/5.8	15/16/1	46.9/50.0/3.1	26/72/7	24.8/68.6/6.7	0.681
Clinical stage (III vs. IV/recurrent)							
III/IV/recurrent	27/70/40	19.7/51.1/29.2	7/20/5	21.9/62.5/15.6	20/50/35	19.0/47.6/33.3	0.800
Tumor histology (Sq[c] vs. non-Sq)							
Ad[d]/Sq/NSCLC[e]	107/24/6	78.1/17.5/4.4	22/9/1	68.8/28.1/3.1	85/15/5	81.0/14.3/4.8	0.108
Smoking history (yes vs. no or unknown)							
yes/no or unknown	88/49	64.2/35.8	26/6	81.3/18.8	62/43	59.1/10.9	0.022
Driver gene (positive vs. negative)							
EGFR[f] mutation/ALK[g] fusion/ROS1[h] fusion/unknown	53/2/2/17	38.7/1.5/1.5/12.4	1/0/0/3	3.1/0.0/0.0/9.4	52/2/2/14	49.5/1.9/1.9/13.3	<0.001/1.000/1.000/0.762
PD-L1[i] TPS[j] (%) (positive vs. negative)							
< 1/1–49/≥ 50/unknown	38/34/20/45	27.7/24.8/14.6/32.9	16/9/3/4	50.0/28.1/9.4/12.5	22/25/17/41	20.1/23.8/16.2/39.1	0.839
C-reactive protein (mg/dL, median, IQR[k])	0.8 (0.2–2.6)		0.5 (0.1–1.4)		1.1 (0.2–2.9)		0.175
Albumin (g/dL, median, IQR)	3.7 (3.3–4.1)		3.8 (3.4–4.2)		3.7 (3.3–4.1)		0.658
Glasgow prognostic score (2 vs. 0/1)							
0/1/2	65/25/47	47.5/18.3/34.3	19/4/9	59.4/12.5/28.1	46/21/38	43.8/20.0/36.2	0.524
Neutrophil-to-lymphocyte ratio (median, IQR)	3.7 (2.6–6.4)		2.5 (1.7–3.6)		4.1 (3.0–6.8)		0.001
eGFR[l] (mL/min, mean ± SD)	69.0 ± 16.7		71.6 ± 11.3		68.3 ± 17.8		0.324
Pleural effusion (positive vs. negative)							
positive/negative	67/70	48.9/51.1	9/23	28.1/71.9	58/47	55.2/44.8	0.009
Proteinuria (positive vs. negative)							
positive/negative	16/121	11.7/88.3	2/30	6.3/93.8	14/91	13.3/86.7	0.359
Ramucirumab infusions (≤2 vs. ≥ 3)							
≤ 2/≥ 3	39/98	28.5/71.5	0/32	0.0/100	39/66	37.1/62.9	<0.001
Treatment line (≥3rd vs. ≤ 2nd)							
1st/2nd/3rd/4th/≥ 5th	1/40/41/34/21	0.7/29.2/29.9/24.8/15.3	1/31/0/0/0	3.1/96.9/0.0/0.0/0.0	0/9/41/34/21	0.0/8.6/39.1/32.4/20.0	<0.001
Best response to previous therapy (SD[m]/PD[n]/NE[o] vs. CR[p]/PR[q])							
CR/PR/SD/PD/NE	0/87/41/4/5	0.0/63.5/29.9/1.9/3.6	0/21/8/2/1	0.0/65.6/25.0/6.3/3.1	0/66/33/2/4	0.0/62.9/31.4/1.9/3.8	0.836
Previous treatment (yes vs. no)							
taxan/VEGF[r]/VEGFR[s] inhibitor/ICI[t]/EGFR-TKI[u]	28/27/77/49	20.4/19.7/56.2/35.8	11/1/20/0	34.4/3.1/64.5/0.0	17/26/57/49	16.2/24.8/54.3/46.7	0.043/0.005/0.542/<0.001
Following treatment (yes vs. no)							
best supportive care/ICI	42/17	30.7/12.4	3/8	9.4/25.0	39/9	37.1/8.6	0.003/0.025

Abbreviations: [a]mean ± SD, mean ± standard deviation; [b]ECOG, Eastern Cooperative Oncology group; [c]Sq, squamous cell carcinoma; [d]Ad, adenocarcinoma; [e]NSCLC, non-small cell lung cancer; [f]EGFR, epidermal growth factor receptor; [g]ALK, anaplastic lymphoma kinase; [h]ROS1, ROS proto-oncogene 1; [i]PD-L1, programmed death ligand 1; [j]TPS, tumor proportion score; [k]IQR, interquartile range; [l]eGFR, estimated glomerular filtration rate; [m]SD, stable disease; [n]PD, progressive disease; [o]NE, not evaluable; [p]CR, complete response; [q]PR, partial response; [r]VEGF, vascular endothelial growth factor; [s]VEGFR, VEGF receptor; [t]ICI, immune checkpoint inhibitor; [u]TKI, tyrosine kinase inhibitor.

0.003; Fig. S5).

3.2. Profile of study samples

The serum ramucirumab trough concentrations in the primary cohort ranged from below the lower limit of quantification (BLQ) to 95.7 μg/mL (Fig. 1, Table S3), which was consistent with the steady state after the third infusion reported previously [9]. C_{trough} ranged from BLQ–48.8 μg/mL, and the median was 14.7 μg/mL. C_{trough} was sorted from Q1–Q4 by interquartile range (BLQ ≤ Q1 ≤ 7.34, 7.34 < Q2 ≤ 14.7, 14.7 < Q3 ≤ 21.9 and 21.9 < Q4 ≤ 48.8 μg/mL; Fig. 2a, Table S4a). BLQ was observed in 17 (13.0%) patients, accounting for

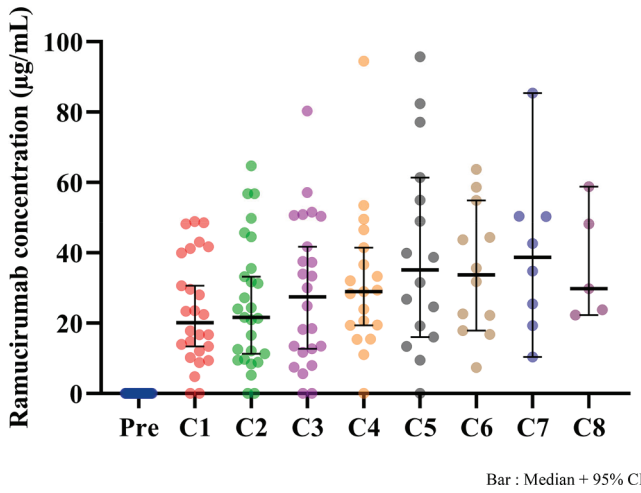

Fig. 1. Profile of ramucirumab trough concentration in the primary cohort. Ramucirumab trough concentration in the primary cohort range from below the lower limit of quantification (3.0 μg/mL) to 95.7 μg/mL. The bar shows the median and 95% CI. Abbreviation:CI, confidence interval;C, cycle.

most patients in the Q1 group. The C_{trough} in the additional cohort was significantly lower than that in the primary cohort (Fig. 2b, Table S4b).

3.3. *Characteristics of patients according to C_{trough}*

Q1 exhibited significantly higher CRP and NLR levels and lower Alb levels compared with Q2–4 (Table 2). GPS was significantly higher in Q1 compared with Q2–4, and C_{trough} was significantly lower in the GPS 2 group than in the GPS 0 or 1 groups, suggesting the presence of a hypercatabolic state in the Q1 group (Fig. 2c, Table S4c). Multivariate regression analysis revealed that estimated glomerular filtration rate (eGFR), treatment line, infusion interval, and GPS were significantly associated with C_{trough} (Table 3).

3.4. *C_{trough} and adverse events*

The incidences of bleeding, hypertension, and proteinuria did not differ significantly between Q1 and Q2–4. FN was more frequently observed in Q1 than in Q2–4 (Table S5).

3.5. *C_{trough}, Efficacy, and survival time*

The ORR was significantly higher in Q2–4 than in Q1 (31.6 vs. 9.1%, $p = 0.011$), and the DCR did not significantly differ between Q1 and Q2–4 (69.7 vs. 78.4%, $p = 0.346$). The median PFS was 4.0 months in Q1 and 5.3 months in Q2–4, which was not significantly different. The Kaplan–Meier curve indicated a trend toward longer PFS in the Q2–4 group (Fig. 3a). Cox multivariate regression analysis revealed that C_{trough} had the most significant effect on PFS among the examined covariates (Table S6). The median OS was 7.1 and 15.5 months in Q1 and Q2–4, respectively, and was significantly longer in Q2–4 (HR = 0.51, 95% CI: 0.30–0.85, $p = 0.009$; Fig. 3b). The Cox multivariate regression analysis revealed that C_{trough} had the most significant effect on OS among the examined covariates ($p = 0.020$; Table S7).

4. Discussion

To the best of our knowledge, this is the first retrospective PK analysis of ramucirumab in real-world patients with NSCLC in Japan. A previous PPK analysis from the REVEL study [6] suggested that the predicted ramucirumab C_{trough} was associated with OS in a limited number of patients who received more than three infusions of ramucirumab and one line of prior treatment. The present study obtained real-world data, including cases of late line ramucirumab administration and cases in which ramucirumab was discontinued early, by setting up an additional cohort without restrictions on the number of ramucirumab infusions or previous treatments, revealing that C_{trough} was associated with efficacy and survival and may be reduced due to hypercatabolism in patients with cachexia.

Although the PPK analysis of the REVEL study [6] indicated that the average ramucirumab concentration at the steady state was associated with ≥ grade 3 FN and hypertension, C_{trough} was not correlated with degrees of proteinuria, hypertension, or bleeding in the present study.

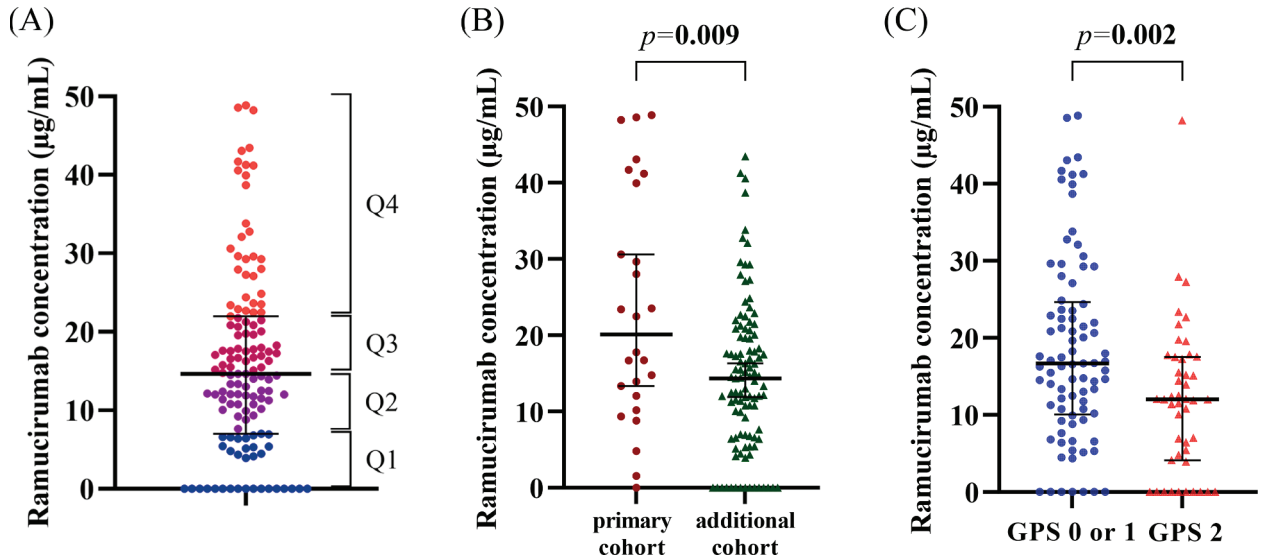

Fig. 2. Profile of ramucirumab study samples. A. C_{trough} is sorted by IQR as below the lower limit of quantification (BLQ, below 3.0 μg/mL) ≤ Q1 ≤ 7.34, 7.34 < Q2 ≤ 14.7, 14.7 < Q3 ≤ 21.9, and 21.9 < Q4 ≤ 48.8 μg/mL. The BLQ concentration was regarded as 0 μg/mL. B. The C_{trough} levels of the study cohorts were compared. The C_{trough} in the additional cohort was significantly lower than that in the primary cohort ($p = 0.009$). C. The C_{trough} was compared between the Glasgow prognostic score (GPS) groups. The C_{trough} was significantly lower in the GPS 2 group than that in the GPS 0 or 1 groups ($p = 0.002$). All bars in panels A–C show the median concentration and 95% CI. Abbreviation: CI, confidence interval; GPS, Glasgow prognostic score; IQR, interquartile range.

Table 2
Characteristics of patients by concentration group (Q1 vs. Q2–4).

	Q1 (n = 33)		Q2–4 (n = 98)		
	n	%	n	%	*p*-value
Age (median, range) (≥65 vs. < 65 years)	63 (32–78)		62 (35–74)		
≥ 65/< 65 years	18/ 15	54.5/ 45.5	55/ 43	56.1/ 43.9	1.000
Sex (male vs. female)					
male/female	21/ 12	63.6/ 36.4	58/ 40	59.2/ 40.8	0.686
Body weight (kg, [a]mean ± SD)	57.9 ± 11.9		61.8 ± 11.8		0.108
[b]ECOG-performance status (2 vs. 0/1)					
0/1/2	6/ 23/4	18.2/ 69.7/ 12.1	32/ 62/4	32.7/ 63.3/4.1	0.110
Clinical stage (III vs. IV/ recurrent)					
III/IV/recurrent	6/ 15/ 12	18.2/ 45.5/ 36.4	20/ 50/ 28	20.4/ 51.0/ 28.6	1.000
Tumor histology (Sq[c] vs. non-Sq)					
Ad[d]/Sq/NSCLC[e]	26/ 6/1	78.8/ 18.2/3.0	78/ 15/5	79.6/ 15.3/5.1	0.785
Smoking history (yes vs. no or unknown)					
yes/no or unknown	24/9	72.7/ 27.3	59/ 39	60.2 /39.8	0.217
Driver gene (positive vs. negative)					
EGFR[f] mutation/ALK[g] fusion/ROS1[h] fusion/ unknown	14/ 1/0/ 6	42.4/ 3.0/0.0/ 18.2	38/1/ 2/9	38.8/ 1.0/2.0/ 9.2	0.837/ 0.442/ NA/0.205
PD-L1[i] TPS[j] (%) (positive vs. negative)					
< 1/1–49/≥ 50/ unknown	7/ 10/ 6/10	21.2/ 30.3/ 18.2/ 30.3	30/ 22/ 13/ 33	30.6/ 22.4/ 13.3/ 33.7	0.219
C-reactive protein (mg/dL, median, IQR[k])	2.3 (0.7–3.8)		0.7 (0.1–2.1)		0.003
Albumin (g/dL, median, IQR)	3.3 (3.0–3.8)		3.8 (3.4–4.2)		< 0.001
Glasgow prognostic score (2 vs. 0/1)					
0/1/2	10/ 6/17	30.3/ 18.2/ 51.5	51/ 18/ 29	52.0/ 18.4/ 29.6	0.034
Neutrophil-to-lymphocyte ratio (median, IQR)	5.1 (3.3–8.9)		3.6 (2.5–5.8)		0.016
eGFR[l] (mL/min, mean ± SD)	73.4 ± 17.2		67.6 ± 16.8		0.090
Pleural effusion (positive vs. negative)					
positive/negative	21/ 12	63.6/ 36.4	44/ 54	44.9/ 55.1	0.072
Proteinuria (positive vs. negative)					
positive/negative	6/27	18.2/ 81.8	10/ 88	10.2/ 89.8	0.234
Ramucirumab infusions (≤2 vs. ≥ 3)					
≤ 2/≥ 3	14/ 19	42.4/ 57.6	24/ 73	25.5/ 74.5	0.080
Treatment line (≥3rd vs. ≤ 2nd)					
1st/2nd/3rd/4th/≥ 5th	0/7/ 9/ 10/7	0.0/ 21.2/ 27.3/ 30.3/ 21.2	1/ 27/ 32/ 24/ 14	1.0/ 27.6/ 32.7/ 24.5/ 14.3	0.499
Best response to previous therapy (SD[m]/PD[n]/NE[o] vs. CR[p]/PR[q])					
CR/PR/SD/PD/NE	0/ 20/ 11/ 1/1	0.0/ 60.6/ 33.3/ 3.0/3.0	0/62/ 29/3/ 4	0.0/ 63.3/ 29.6/ 3.1/4.1	0.837
Previous treatment (yes vs. no)					
taxan/VEGF[r]/VEGFR[s] inhibitor/ICI[t]/EGFR-TKI[u]	6/ 11/ 12/ 14	18.2/ 33.3/ 66.7/ 42.4	19/ 16/ 50/ 35	19.4/ 16.3/ 51.0/ 35.7	1.000/ 0.047/ 0.157/ 0.536
Following treatment (yes vs. no)					
best supportive care/ICI	16/0	48.5/0.0	26/ 13	28.3/ 13.7	0.028/ 0.037

Abbreviations: [a]mean ± SD, mean ± standard deviation; [b]ECOG, Eastern Cooperative Oncology group; [c]Sq, squamous cell carcinoma; [d]Ad, adenocarcinoma; [e]NSCLC, non-small cell lung cancer; [f]EGFR, epidermal growth factor receptor; [g]ALK, anaplastic lymphoma kinase; [h]ROS1, ROS proto-oncogene 1; [i]PD-L1, programmed death ligand 1; [j]TPS, tumor proportion score; [k]IQR, interquartile range; [l]eGFR, estimated glomerular filtration rate; [m]SD, stable disease; [n]PD, progressive disease; [o]NE, not evaluable; [p]CR, complete response; [q]PR, partial response; [r]VEGF, vascular endothelial growth factor; [s]VEGFR, VEGF receptor; [t]ICI, immune checkpoint inhibitor; [u]TKI, tyrosine kinase inhibitor.

Measuring the steady state or maximum concentration values may reveal additional correlations between treatment and clinical outcomes.

Even in real-world settings, the ORR was significantly higher in Q2–4 than in Q1, confirming a positive exposure-efficacy relationship, which may have contributed to the prolonged survival of patients. The difference in survival between Q1 and Q2–4 was more apparent in OS than in PFS, similar to previously reported data [6]. The differences between the following treatments were examined. Patients in Q1 received significantly more "best supportive care," suggesting that Q1 may be especially vulnerable to aggressive therapy, leading to a shorter survival time.

The characteristics of Q1, including high CRP level, NLR, and low Alb level, were all associated with systemic inflammation and a hypercatabolic state. Low body weight and high NLR were also observed in the additional cohort. Cachexia, which was observed in approximately 50–80% of patients with cancer [13] is defined as a multifactorial syndrome characterized by an ongoing loss of skeletal muscle mass with or without loss of fat mass that cannot be fully reversed by conventional nutritional support, leading to progressive functional impairment [14]. In cachexia, weight loss or sarcopenia is accompanied by a loss of appetite or systemic inflammation, which results in refractory cachexia when accompanied by hypercatabolism. GPS is a simplified index that reflects inflammation and hypercatabolism in cachexia and is a prognostic factor in NSCLC [8]. A significantly higher number of patients in the GPS 2 group converged on Q1, suggesting that a large number of patients in Q1 may be in a cachexic state.

In cachexia, impaired gut wall function, malabsorption, reduced fat tissue, or altered expression of metabolizing enzymes generally results in higher blood concentrations of small-molecule drugs [15]. Antibodies are less susceptible to drug absorption, distribution, liver metabolism, and renal excretion. They are mostly eliminated via lysosomal degradation after uptake via pinocytosis or receptor-mediated endocytosis through target receptors, fragment crystallizable (Fc) gamma receptors, or neonatal Fc receptors [7]. Hypercatabolism of proteins, including IgG, occurs in advanced cancer cachexia, and the whole-body protein turnover was 50–70% greater in patients with cancer than in healthy individuals [16]. The endogenous catabolic rate of Alb is highly correlated with the catabolic degradation of IgG [17], and CRP levels correlate positively with monoclonal antibody clearance [18]. We hypothesized that in a population with high GPS, characterized by low

Table 3
Results of regression analysis for ramucirumab trough concentration after the first infusion.

	Univariate analysis			Multivariate analysis		
	Coefficients	95% CI[a]	*p*-value	Coefficients	95% CI	*p*-value
Body weight (kg)	0.20	0.03–0.37	0.024	0.06	−0.08 to −0.19	0.405
eGFR[b] (mL/min)	−0.14	−0.26 to −0.02	0.018	−0.12	−0.21 to −0.03	0.013
Treatment line (≥3rd vs. ≤ 2nd)	−5.49	−10.0 to −0.96	0.018	−3.98	−7.52 to −0.44	0.028
Ramucirumab infusions (≤2 vs. ≥ 3)	−6.49	−10.8 to −2.16	0.004	−1.96	−5.53–1.61	0.279
Pleural effusion (positive vs. negative)	−5.92	−9.89 to −1.95	0.004	−2.71	−5.82–0.40	0.087
Glasgow prognostic score (2 vs. 0/1)	−6.86	−11.0 to −2.74	0.001	−5.39	−8.81 to −1.97	0.002
Infusion interval (days)	−11.8	−15.4 to −8.20	0.000	−1.43	−1.78 to −1.08	<0.001

Abbreviations: [a]CI, confidence interval; [b]eGFR, estimated glomerular filtration rate.

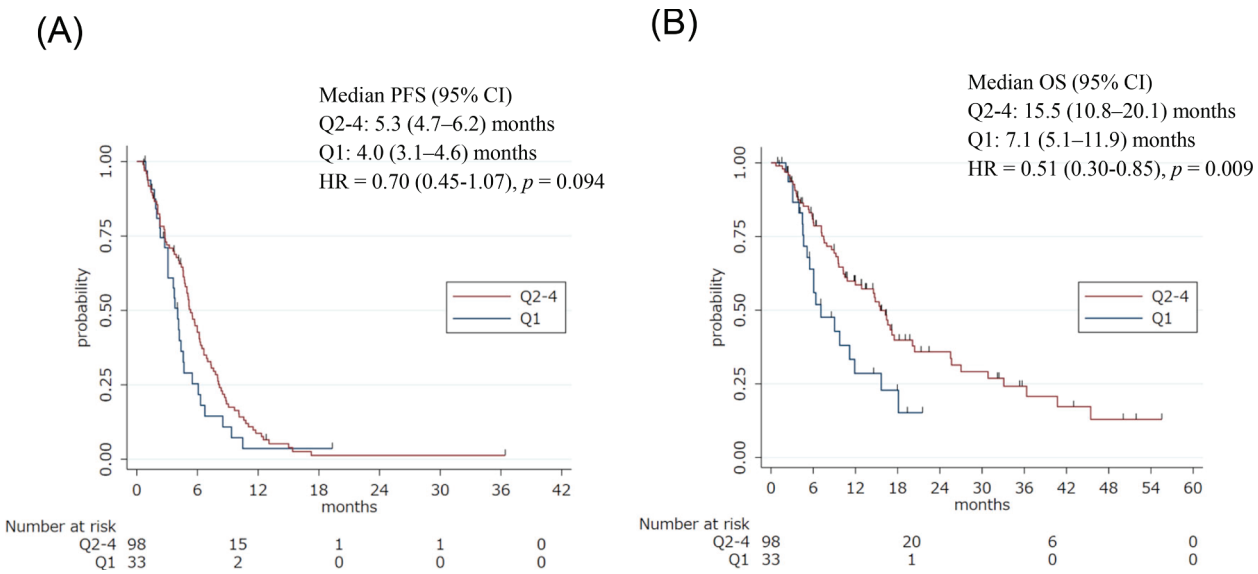

Fig. 3. Kaplan–Meier estimates of survival in the ramucirumab concentration group. A. Kaplan–Meier curves showing progression-free survival (PFS). PFS was marginally longer in Q2–4 than in Q1, but the difference was not statistically significant. B. Kaplan–Meier curves showing the overall survival. OS was significantly longer in Q2–4 than in Q1. Abbreviations: CI, confidence interval; HR, hazard ratio; NR, not reached.

Alb and high CRP levels, ramucirumab catabolism may be enhanced due to increased protein catabolism, resulting in a lower ramucirumab concentration. Additionally, as VEGF production may be elevated in cachexia [19] and VEGFR-2 expression in target cells is expected to increase, VEGFR-2-mediated intracellular uptake and elimination of ramucirumab could be enhanced, resulting in a lower ramucirumab concentration.

The emergence of anti-drug antibodies (ADAs) should also be considered. An enzyme-linked immunosorbent assay did not detect ADAs in the primary cohort (data not shown); however, false negatives should be considered. Since ADAs that form immune complexes after binding to the target drug are more likely to be recognized and eliminated by the reticuloendothelial system [20], it cannot be ruled out that the ADA-drug complex had been eliminated by the time of serum collection. The affinity of ADAs also matures over time, resulting in increased drug clearance by encountering more monoclonal antibodies [21]. This may explain why BLQ was observed more frequently in the additional cohort with a high history of antibody administration.

GPS was significantly associated with PFS and OS in this study and is considered a more convenient and versatile prognostic factor than C_{trough} in clinical practice. Low C_{trough} may contribute to the hypercatabolism of ramucirumab and resistance to ramucirumab treatment. Similarly, cachexia progression negatively impacts serum nivolumab concentration [22], which may occur universally with other antibodies. One aspect of treatment resistance in cachexia may be explained by a reduced blood concentration of antibodies that fail to show their expected efficacy.

This study had several limitations. First, weight loss, sarcopenia, or loss of appetite should be considered for further cachexia assessment. Since the treatment was administered after second-line and mostly performed in an outpatient setting, there was insufficient data regarding changes in weight and appetite in the medical records; therefore, we were regrettably forced to exclude the data from the present analysis. Second, the serum samples of the additional cohort were obtained only after the first infusion. Third, the effects of concomitant medications were unclear. Fourth, confirmation through a prospective study is desirable.

5. Conclusions

We developed a novel analytical method for measuring serum ramucirumab concentrations using LC-MS/MS. The high ramucirumab concentration group had a high ORR and prolonged survival time, whereas the low ramucirumab concentration group was characterized by a high GPS and a poorer prognosis. Cachexia may reduce the exposure level of ramucirumab in some patients and reduce the clinical benefits of ramucirumab treatment.

6. Classification

Clinical research.

CRediT authorship contribution statement

Kazumasa Akagi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Shigehiro Yagishita:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Mayu Ohuchi:** Validation, Investigation, Writing – review & editing. **Yoshiharu Hayashi:** Validation, Investigation, Writing – review & editing. **Yuki Takeyasu:** Writing – review & editing. **Ken Masuda:** Writing – review & editing. **Yuki Shinno:** Writing – review & editing. **Yusuke Okuma:** Writing – review & editing. **Tatsuya Yoshida:** Writing – review & editing. **Yasushi Goto:** Writing – review & editing. **Hidehito Horinouchi:** Writing – review & editing. **Noboru Yamamoto:** Writing – review & editing. **Hiroshi Mukae:** Writing – review & editing. **Yuichiro Ohe:** Resources, Writing – review & editing. **Akinobu Hamada:** Methodology, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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Data statement

The data generated in this study are available in the article and its supplementary files.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2023.03.001.

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Supplementary methods

Nano-surface and molecular-orientation limited proteolysis

The nano-surface and molecular-orientation limited (nSMOL) proteolysis was conducted in accordance with the manufacturer's protocol. The instructions were as follows: 1. Add immunoglobulin collection resin; 2. Add wash solution 1 and internal standard (rituximab); 3. Add 5 µl of serum and ramucirumab for the calibration curve and quality control sample; 4. Gently agitate the mixture at room temperature for 30 min; 5. Transfer into the filter cup; 6. Centrifuge and add wash solution 1; 7. Centrifuge and add wash solution 2; 8. Repeat steps 6 and 7 twice; 9. Add enhanced reaction solution; 10. Add FG beads trypsin DART; 11. Incubate at 50 ℃ in saturated vapor pressure for 6 h; 12. Add reaction stop solution; 13. Transfer the filter cup and centrifuge; and 14. Place the tube in a magnetic stand and collect the filtrate.

LC-MS/MS settings

As an LC- electrospray ionization (ESI)-MS with triple quadrupole, Nexera X2 and LCMS-8050 (Shimadzu) were utilized for the quantification. Mobile phase A: 0.1% formic acid, mobile phase B: methanol with 0.1% formic acid, column; Aeris 1.7 µm XB-C18 100×2.1 mm (Phenomenex #00D-4506-AN), column temperature: 60 ° C, flow rate: 0.3 mL/min, gradient program: 0.0–1.0 min % B 1, 1.0–8.9 min % B 1 to 55 gradient, 8.9–9.0 min % B 55 to 100, 9.0–10.50 min % B 100, 10.50–10.55 min % B 100 to 1. MS conditions were optimized using the LabSolution software (Shimadzu) as follows: pause time: 1 msec, dwell time: 5 msec, nebulizer gas flow: 3 L/min, heating gas flow: 10 L/min, drying gas flow: 10 L/min, desolvation line temperature: 250 ℃, heat block temperature: 400 ℃. For ramucirumab, the electrode voltage of Q1 pre-bias was -40 V, collision cell Q2 was -42 V, Q3 pre-bias was -30 V, and the transition was 1038.50 > 1091.55 (y9). For the internal standard (rituximab), the electrode voltage of Q1 pre-bias was -30 V, collision cell Q2 was -34 V, Q3 pre-bias was -48 V, and the transition was 1092.10 > 1343.40 (y12).

Ramucirumab signature peptide determination

The amino acid sequence of ramucirumab was obtained from the Kyoto Encyclopedia of Genes and Genomes database. Candidates of ramucirumab signature peptides were provided under the criteria of the variable region, trypsin digestion, the length between 8 and 25 amino acids, excluding cysteine and histidine, and a maximum of three post-translational modifications using Skyline (MacCoss Laboratory, Washington DC, USA, version 20.2). Eleven candidate peptides from the heavy chain and seven from the light chain were obtained using Skyline. Interference with human pooled serum was analyzed for each peptide, and the peptide from the variable region was selected. Finally, the peptide ASQGIDNWLGWYQQKPGK [*m/z* 1038.50 > 1091.55 (y9)] was selected as the signature peptide of

ramucirumab, which, to the best of our knowledge, was inconsistent with previously published signature peptides.

Optimization of nSMOL proteolysis for ramucirumab

We selected the internal standard method and assigned rituximab as an internal standard. The signature peptide of rituximab was GLEWIGAIYPGNGDTSYNQK which had no interference with human pooled serum or the ramucirumab signature peptide (data not shown). The area under the chromatogram increased as the incubation time extended from 4 to 6 h, and we selected 6 h as the incubation time. The enhanced reaction solution increased the area compared to the reaction solution; thus, we selected the enhanced reaction solution.

Calibration standard and quality control samples

The ramucirumab concentration was set from 3 to 200 μg/mL, which was predicted to cover the clinical settings. The lower limit of quantification (LLQC), low-quality control (LQC), middle-quality control (MQC), and high-quality control (HQC) were set as 3, 9, 60, and 160 μg/mL, respectively. Linearity was evaluated by analyzing eight standard calibration samples (3, 7.5, 25, 50, 75, 100, 150, and 200 μg/mL) using the linear regression model. The weighting factor was $1/x^2$ (where x was nominal concentration). The accuracy of every sample was evaluated **(Fig. S2)**.

Selectivity

The interference with human pooled serum obtained from three males and three females was evaluated **(Fig. S3)**.

Precision and accuracy in within-run and between runs

QC samples (n = 5) of LLQC, LQC, MQC, and HQC were processed and evaluated for within-run and between runs assays. Average accuracy and precision were evaluated **(Table S1)**.

Matrix effect

QC samples were processed with human pooled serum obtained from three males and three females as a matrix. The precision between individual QC samples was evaluated.

Carryover

The response peak of a blank sample followed by measurement of the upper limit of quantification (ULOQ; 200 μg/mL) of the calibration curve sample was evaluated.

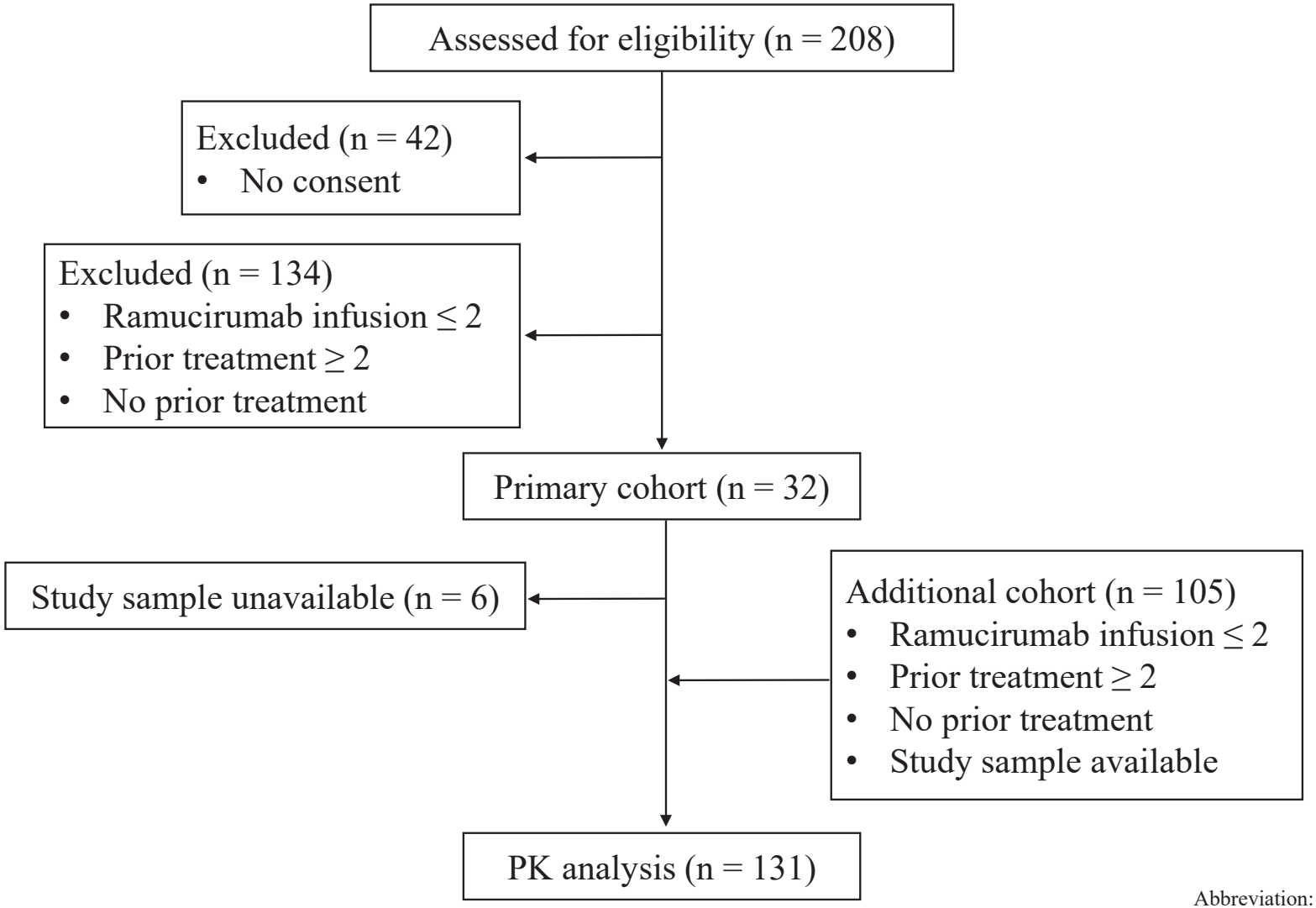
Dilution effect

Dilution was assumed necessary in unpredictable clinical settings. A 200 μg/mL sample (n = 3) was diluted five times and measured, and the average accuracy and precision were evaluated.

Stability

Stability was evaluated in the following situations: stored at -20 ℃, -80 ℃, and room temperature for 6 h, freeze and thaw, post-prepared for 24 h, stored at -20 ℃ and -80 ℃ for 6 months. HQC and LQC samples (n = 3 each) were evaluated in every situation, and the accuracy of every sample was evaluated.

Figure S1. CONSORT diagram



Abbreviation: PK, pharmacokinetics

Figure S2. Typical calibration curve (3–200 μg/mL)

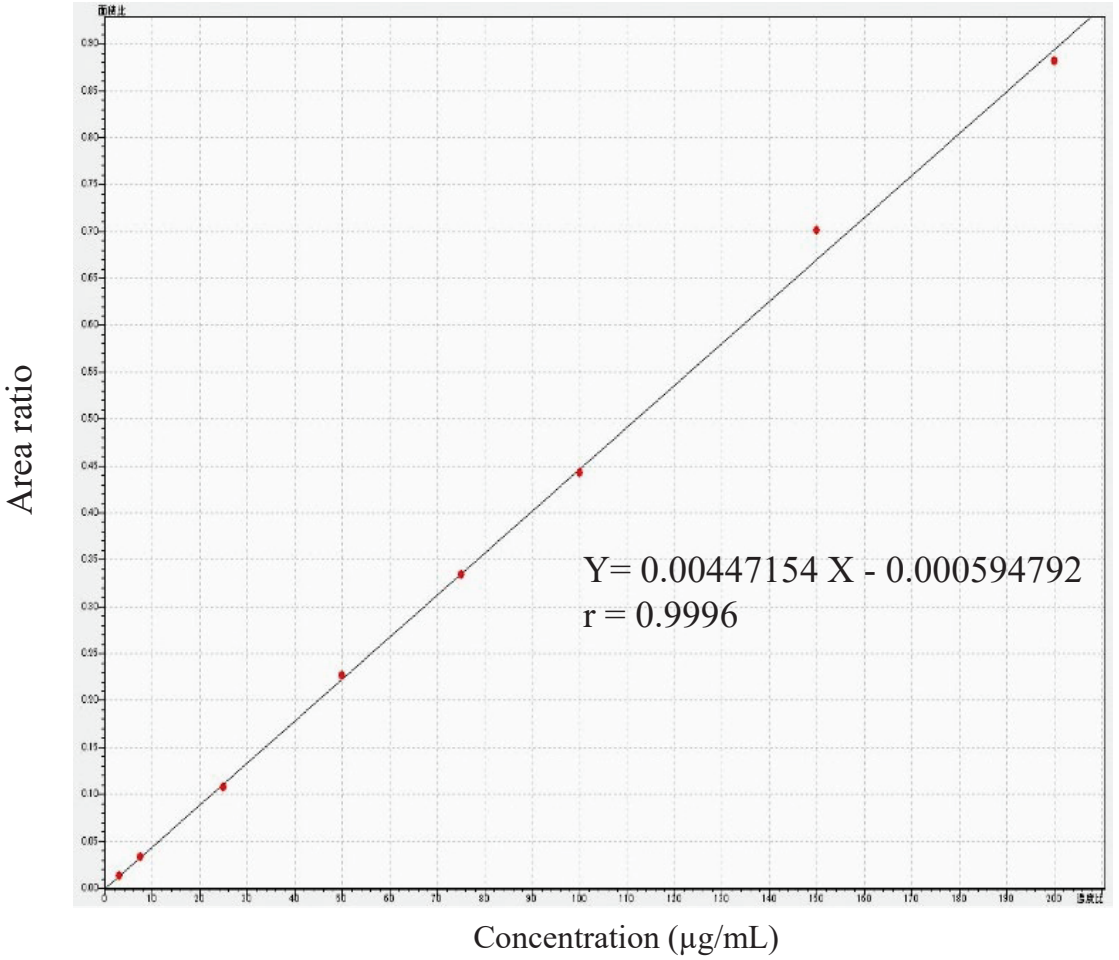
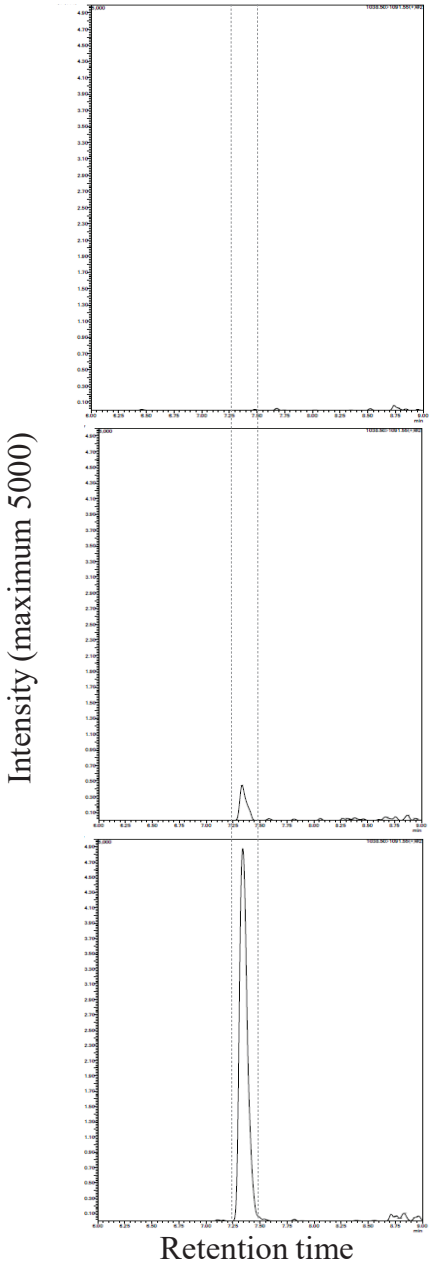


Figure S3.
LC-MS chromatograms of ramucirumab with nSMOL proteolysis in human serum



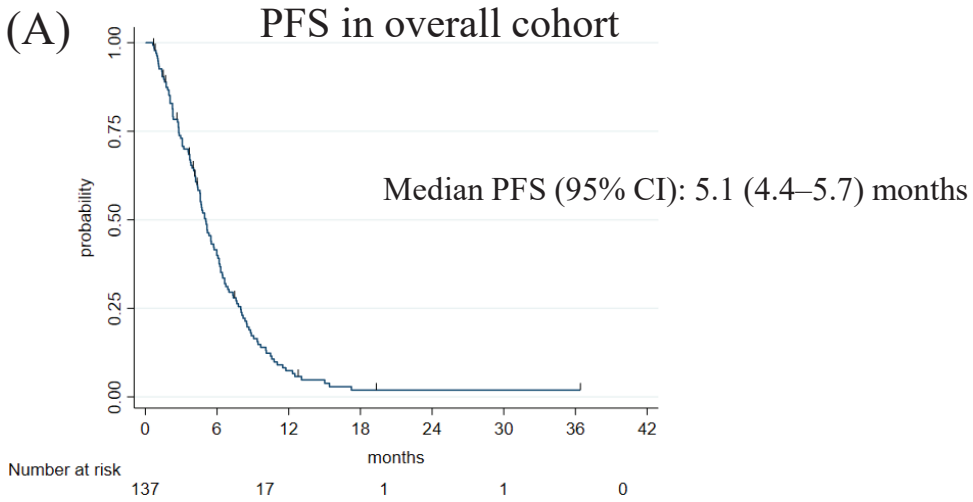
Blank sample

LLOQ

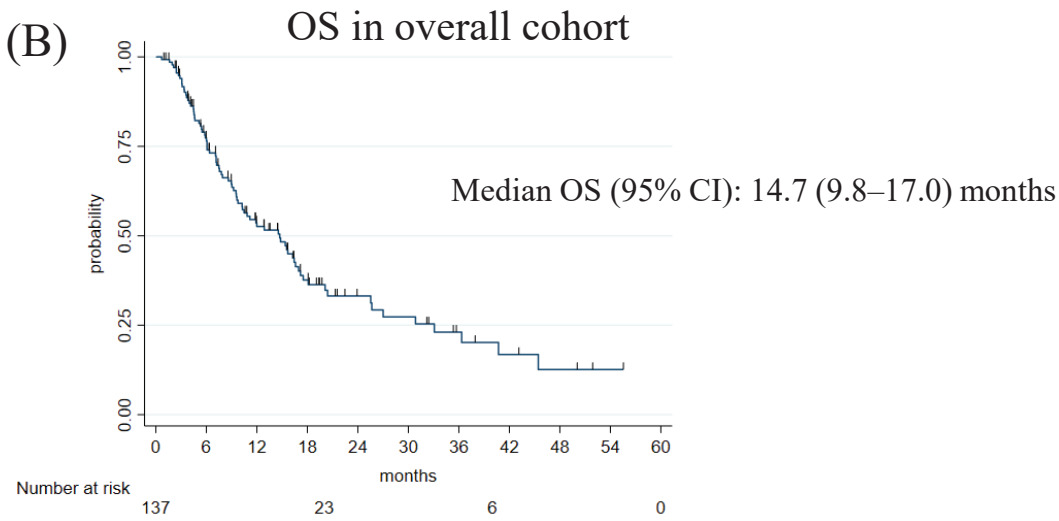
Study sample

Abbreviation: LC-MS, liquid chromatography-mass spectrometry; nSMOL, nano-surface and molecular-orientation limited proteolysis; LLOQ, lower limit of quantitation

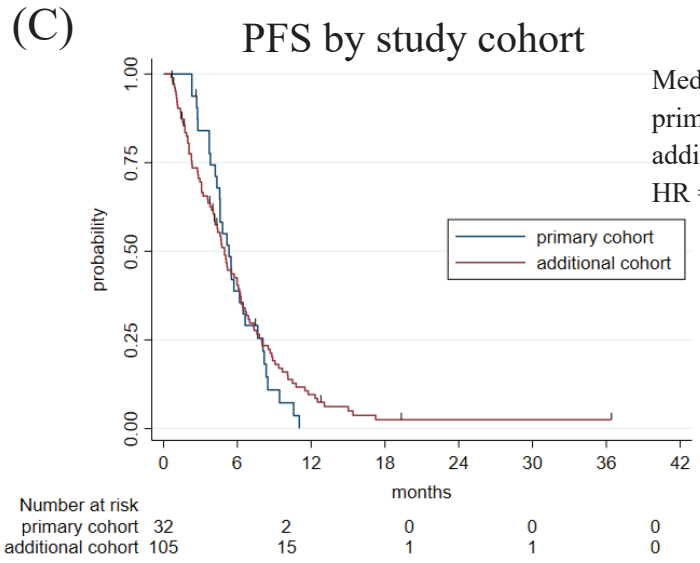
Figure S4. Kaplan–Meier estimates of survival by study cohort



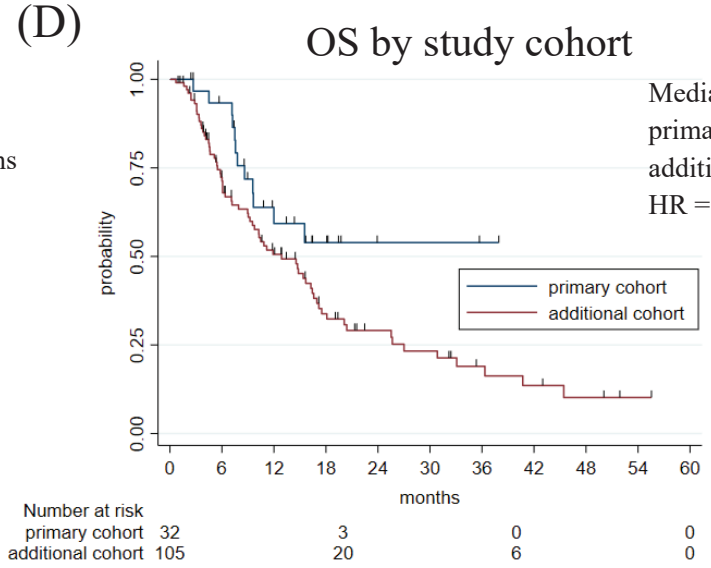
Median PFS (95% CI): 5.1 (4.4–5.7) months



Median OS (95% CI): 14.7 (9.8–17.0) months



Median PFS (95% CI)
primary cohort: 5.3 (4.3–6.5) months
additional cohort: 5.0 (4.1–6.0) months
HR = 0.93 (0.61–1.41), $p = 0.720$

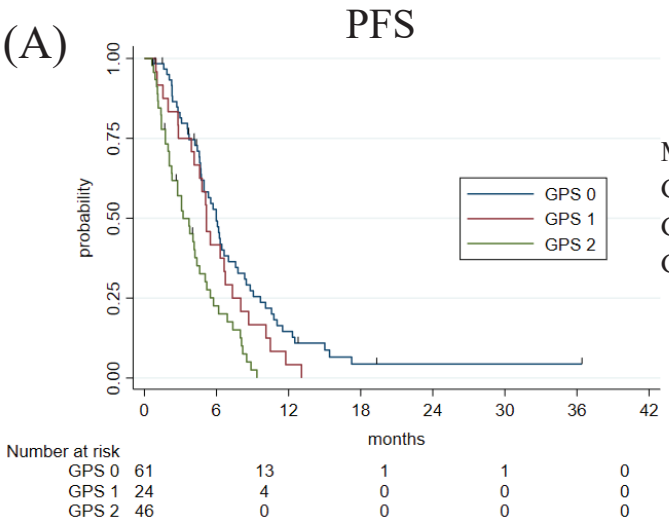


Median OS (95% CI)
primary cohort: NR (9.6–NR) months
additional cohort: 12.9 (9.2–16.4) months
HR = 1.81 (0.98–3.38), $p = 0.005$

Abbreviation: PFS, progression free survival; OS, overall survival; CI, confidence interval; NR, not reached

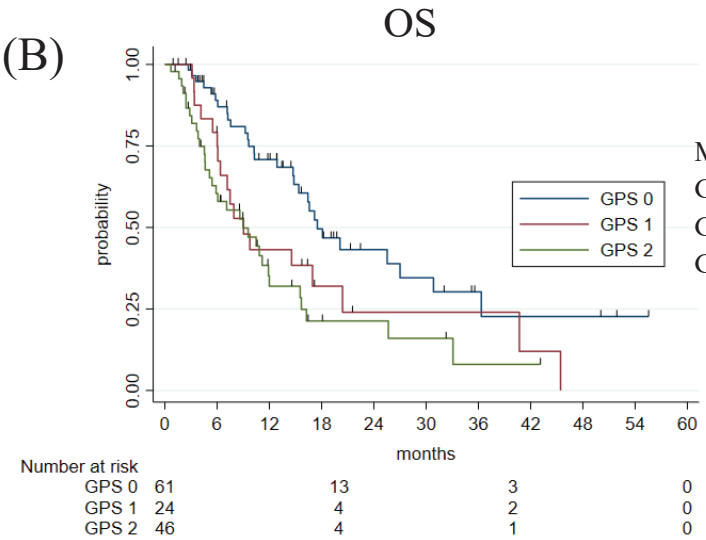
Figure S5. Kaplan–Meier estimates of survival by GPS

(A)

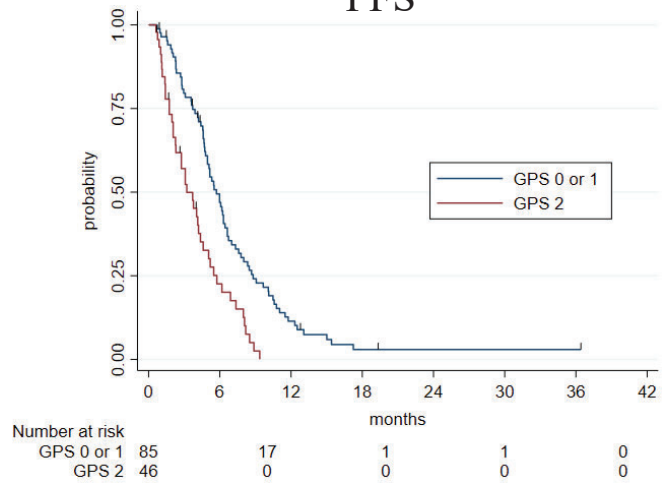


Median PFS (95% CI)
GPS 0: 6.0 (4.7–7.0) months
GPS 1: 5.2 (3.9–6.7) months
GPS 2: 3.2 (2.3–4.4) months

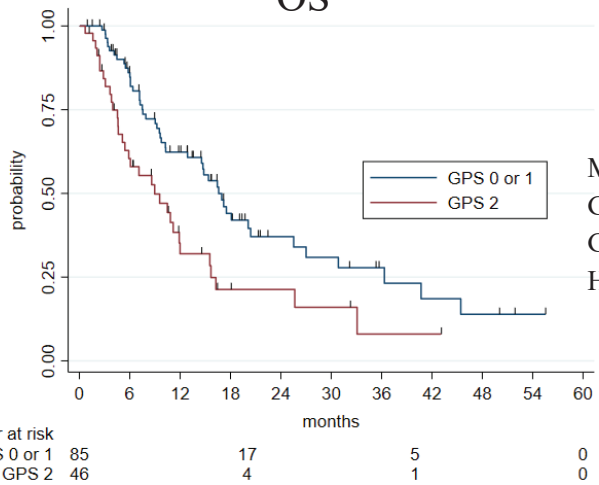
(B)



Median OS (95% CI)
GPS 0: 17.5 (14.8–30.9) months
GPS 1: 9.0 (6.1–20.4) months
GPS 2: 9.0 (5.1–12.0) months



Median PFS (95% CI)
GPS 0 or 1: 5.7 (4.8–6.4) months
GPS 2: 3.2 (2.3–4.4) months
HR = 2.28 (1.53–3.40), $p < 0.001$



Median OS (95% CI)
GPS 0 or 1: 16.6 (12.9–20.4) months
GPS 2: 9.0 (5.1–12.0) months
HR = 1.98 (2.26–3.12), $p = 0.003$

Abbreviation: GPS, Glasgow prognostic score; PFS, progression free survival; OS, overall survival; CI, confidence interval; HR, hazard ratio

Table S1. Within-run and between runs reproducibility

		LLOQ	LQC	MQC	HQC
	Concentration (μg/mL)	3.00	9.00	60.0	160
Assay 1	Accuracy (%)	94.0	104	104	106
(n = 5)	Precision (%)	5.3	2.0	3.5	4.2
Assay 2	Accuracy (%)	99.2	91.3	87.2	91.6
(n = 5)	Precision (%)	14.0	3.7	1.9	11.7
Assay 3	Accuracy (%)	119	102	98.8	96.5
(n = 5)	Precision (%)	16.5	4.5	1.0	2.5
Overall	Accuracy (%)	104.2	99.1	96.6	98.1
	Precision (%)	16.7	6.6	7.8	9.1

Abbreviations: LLOQ, lower limit of quantification; LQC, low quality control; MQC, middle quality control; HQC, high quality control

Table S2. Overall adverse events

Laboratory findings	Any grade	%	Grade ≥ 3	%
Anemia	47	34.3	7	5.1
Aspartate aminotransferase increased	45	32.8	1	0.7
Hypoalbuminemia	39	28.5	1	0.7
White blood cell decreased	32	23.4	20	14.6
Alanine aminotransferase increased	27	19.7	1	0.7
Neutrophil count decreased	23	16.8	23	16.8
Platelet count decreased	22	16.1	2	1.5
Hyperkalemia	20	14.6	0	0.0
Alkaline phosphatase increased	12	8.8	1	0.7
Blood lactate dehydrogenase increased	12	8.8	0	0.0
GGT increased	9	6.6	2	1.5
Hyponatremia	8	5.8	0	0.0
Creatinine increased	2	1.5	0	0.0
Hyperuricemia	1	0.7	0	0.0
Hypernatremia	1	0.7	0	0.0
Hypokalemia	1	0.7	0	0.0

Symptoms	Any grade	%	≥ Grade 3	%
Malaise	77	56.2	1	0.7
Anorexia	77	56.2	1	0.7
Alopecia	70	51.1	NA	NA
Constipation	54	39.4	1	0.7
Nail changes	52	38.0	0	0.0
Dysgeusia	41	29.9	NA	NA
Nausea	39	28.5	0	0.0
Mucositis oral	39	28.5	0	0.0
Peripheral sensory neuropathy	38	27.7	0	0.0
Edema	25	18.2	0	0.0
Fever	18	13.1	0	0.0
Diarrhea	17	12.4	0	0.0
Skin disorders	17	12.4	0	0.0
Vomiting	8	5.8	0	0.0
Pneumonitis	3	2.2	3	2.2
Cough	3	2.2	0	0.0
Skin hyperpigmentation	3	2.2	0	0.0
Urinary tract infection	2	1.5	1	0.7
Sepsis	1	0.7	1	0.7
Lung infection	1	0.7	1	0.7
Peritoneal infection	1	0.7	1	0.7
Stomach pain	1	0.7	0	0.0
Abdominal pain	1	0.7	0	0.0
Arthralgia	1	0.7	0	0.0
Herpes simplex reactivation	1	0.7	0	0.0
Hiccups	1	0.7	0	0.0
Vestibular disorder	1	0.7	0	0.0

Ramucirumab-characteristic events	Any grade	%	Grade ≥ 3	%
Epistaxis	25	18.2	0	0.0
Proteinuria	16	11.7	0	0.0
Hypertension	11	8.0	0	0.0
Febrile neutropenia	8	5.8	8	5.8
Gastrointestinal hemorrhage	6	4.4	3	2.2
Bronchopulmonary hemorrhage	4	2.9	0	0.0
Hematuria	3	2.2	1	0.7
Thromboembolic event	2	1.5	1	0.7
Retinal hemorrhage	1	0.7	0	0.0
Superficial thrombophlebitis	1	0.7	0	0.0
Oral hemorrhage	1	0.7	0	0.0
Intracranial hemorrhage	1	0.7	0	0.0

Abbreviations: GGT, γ-glutamyl transpeptidase ; NA, not applicable

Table S3. Profile of ramucirumab trough concentration in the primary cohort

	Pre	C1	C2	C3	C4	C5	C6	C7	C8
Number of cases	32	26	27	24	19	16	12	8	5
Median trough concentration (μg/mL)	0	20.1	21.6	27.5	28.9	35.1	33.7	38.7	29.8
95% CI	0-0	13.3–30.6	11.3–33.2	12.7–41.7	19.4–41.5	16.1–61.4	17.9–54.9	10.4–85.4	22.3–58.8

Abbreviations: CI, confidence interval; C, cycle

Table S4. Profile of ramucirumab study samples

(A) Profile of ramucirumab study samples

quartile	number of cases	Median C_{trough} (μg/mL)	range
Q1	33	BLQ	BLQ ≤ Q1 ≤ 7.34
Q2	33	12	7.34 < Q2 ≤ 14.7
Q3	32	17.5	14.7 < Q3 ≤ 21.9
Q4	33	29.6	21.9 < Q4 ≤ 48.8

(B) Profile by study cohort

Cohort	Number of cases	Median C_{trough} (μg/mL)	IQR
primary	26	20.1	12.1 – 39.9
additional	105	14.4	6.6 – 20.1

(C) Profile by GPS

Group	Number of cases	Median C_{trough} (μg/mL)	IQR
GPS 0 or 1	85	16.7	10.2 – 24.4
GPS 2	46	12	4.2 – 17.5

Abbreviations: BLQ, below lower limit of quantification; IQR, interquartile range; GPS, Glasgow prognostic score

Table S5. Ramucirumab trough concentration after the first infusion and ramucirumab-characteristic adverse events

	Q1 (n = 33)		Q2-4 (n = 98)		
	n	%	n	%	p-value
Hypertension	1	3.0	10	10.2	0.289
Bleeding	7	21.2	30	30.6	0.374
Proteinuria	6	18.2	10	10.2	0.231
Febrile neutropenia	5	15.2	3	3.1	0.024

Table S6. Cox regression analysis for progression-free survival

	univariate analysis			multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Clinical stage (IV/recurrent vs III)	0.85	0.55–1.33	0.489	0.87	0.55–1.39	0.558
EGFR mutation (positive vs negative or unknown)	0.73	0.50–1.06	0.099	0.7	0.47–1.04	0.077
ECOG-Performance Status (2 vs 0/1)	1.78	0.82–3.86	0.143	1.72	0.79–3.78	0.173
Ramucirumab trough concentration (Q2-4 vs Q1)	0.7	0.45–1.07	0.097	0.65	0.41–1.01	0.054

Abbreviation: ECOG; Eastern cooperative oncology group; CI, confidence interval; HR, hazard ratio; PFS, progression free survival

Table S7. Cox regression analysis for overall survival

	Univariate analysis			Multivariate analysis		
	HR	95% CI	p-value	HR	95% CI	p-value
Clinical stage (IV/recurrent vs III)	0.76	0.45–1.29	0.308	0.75	0.42–1.31	0.306
EGFR mutation (positive vs negative or unknown)	1.1	0.70–1.72	0.675	1.08	0.68–1.73	0.743
ECOG-Performance Status (2 vs 0/1)	1.88	0.75–4.67	0.176	1.61	0.62–4.16	0.328
Ramucirumab trough concentration (Q2-4 vs Q1)	0.51	0.30–0.85	0.011	0.53	0.31–0.90	0.020

Abbreviation: ECOG; Eastern cooperative oncology group; CI, confidence interval; HR, hazard ratio; OS, overall survival