



¹⁸F-RGD PET/CT and Systemic Inflammatory Biomarkers Predict Outcomes of Patients With Advanced NSCLC Receiving Combined Antiangiogenic Treatment

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Background: The aim of this study was to evaluate ¹⁸F-AIF-NOTA-PRGD2 positron emission tomography/computed tomography (¹⁸F-RGD PET/CT) and serum inflammation biomarkers for predicting outcomes of patients receiving combined antiangiogenic treatment for advanced non-small cell lung cancer (NSCLC).

Methods: Patients with advanced NSCLC underwent ¹⁸F-RGD PET/CT examination and provided blood samples before treatments commenced. PET/CT parameters included maximum standard uptake value (SUVmax) and mean standard uptake value (SUVmean), peak standard uptake value (SUVpeak) and metabolic tumor volume (MTV) for all contoured lesions. Biomarkers for inflammation included pretreatment neutrophil-to-lymphocyte ratio (PreNLR), pretreatment platelet-to-lymphocyte ratio (PrePLR), and pretreatment lymphocyte-to-monocyte ratio (PreLMR). Receiver operating characteristic (ROC) curve analysis was used to describe response prediction accuracy. Logistic regression and Cox's regression analysis was implemented to identify independent factors for short-term responses and progression-free survival (PFS).

Results: This study included 23 patients. According to ROC curve analysis, there were significant correlations between the SUVmax, SUVmean, and ¹⁸F-RGD PET/CT MTV and short-term responses ($p < 0.05$). SUVmax was identified using logistic regression analysis as a significant predictor of treatment sensitivity ($p = 0.008$). Cox's multivariate regression analysis suggested that high SUVpeak ($p = 0.021$) and high PreLMR ($p = 0.03$) were independent PFS predictors. Combining SUVpeak and PreLMR may also increase the prognostic value for PFS, enabling us to identify a subgroup of patients with intermediate PFS.

Conclusion: ^{18}F -RGD uptake on PET/CT and serum inflammation biomarker pretreatment may predict outcomes for combined antiangiogenic treatments for advanced NSCLC patients. Higher ^{18}F -RGD uptake and higher PreLMR also appear to predict improved short-term responses and PFS. Combining biomarkers may therefore provide a basis for risk stratification, although further research is required.

Keywords: ^{18}F -RGD PET/CT, inflammatory biomarkers, outcome prediction, combined antiangiogenic therapy, NSCLC

INTRODUCTION

Lung cancer remains the leading cause of cancer death, with 80–85% of the total number of lung cancer cases being non-small cell lung cancer (NSCLC) (1). Unfortunately, the vast majority of NSCLC patients are diagnosed late with local progression or evidence of metastasis (2). Bevacizumab combined with chemotherapy was initially approved by the United States Food and Drug Administration (US FDA) in 2006 as a first-line treatment for locally unresectable advanced, recurrent, metastatic, nonsquamous NSCLC due to evidence of a significant survival benefit and acceptable safety (3). However, not every patient benefits from this combination and so predictive markers are increasingly being used to identify NSCLC subpopulations who may benefit from receiving this combined intervention. Unfortunately, to date, research has been largely unsuccessful in identifying even a single response predictor for combined antiangiogenic treatments (4). Therefore, it remains necessary to identify predictive factors to ensure that this treatment is provided to those who will benefit most.

Integrin $\alpha\text{v}\beta_3$, which forms complexes with vascular endothelial growth factor (VEGF) signaling pathways, is highly expressed on newly formed vessels (5, 6). The tripeptide sequence arginine-glycine-aspartate (RGD) also has a high affinity with and can therefore bind to integrin $\alpha\text{v}\beta_3$. As such, ^{18}F -AIF-NOTA-PRGD2 positron emission tomography/computed tomography (^{18}F -RGD PET/CT) has been recommended for noninvasive angiogenesis imaging (7). ^{18}F -RGD is a novel tracer that one-step labels integrin $\alpha\text{v}\beta_3$, which targets the PET probe, and has proven relatively safe (8). In a pilot clinical study, patients with lesions broader than ^{18}F -RGD PET/CT parameters appeared to respond better to antiangiogenic drugs alone (9). These initial studies validated some of the underlying theories that are emerging within this field and further suggest the need to explore the predictive capacity of ^{18}F -RGD PET/CT for combining antiangiogenic and chemotherapeutic interventions.

Serum inflammatory cells have a complex relationship with angiogenesis and can be used to reflect the immune system status by providing insight into the tumor microenvironment (10). In particular, neutrophil and monocyte subpopulations have been found to contribute to angiogenesis (11, 12). However, activated T cells appear to inhibit neoangiogenesis by releasing the immune-related cytokine IFN- γ (4). Antiangiogenic therapies with bevacizumab may therefore increase the infiltration of immune

effector cells and convert an intrinsically immunosuppressive tumor microenvironment into an immunosupportive microenvironment (13). Here, we propose that systemic immune factors, including the pretreatment neutrophil-to-lymphocyte ratio (PreNLR), pretreatment platelet-to-lymphocyte ratio (PrePLR), and pretreatment lymphocyte-to-monocyte ratio (PreLMR), may be useful predictors for combined antiangiogenic treatments. As such, we investigated whether these ^{18}F -RGD PET/CT parameters with systemic immune factors could be used as predictive markers for combined antiangiogenic treatments.

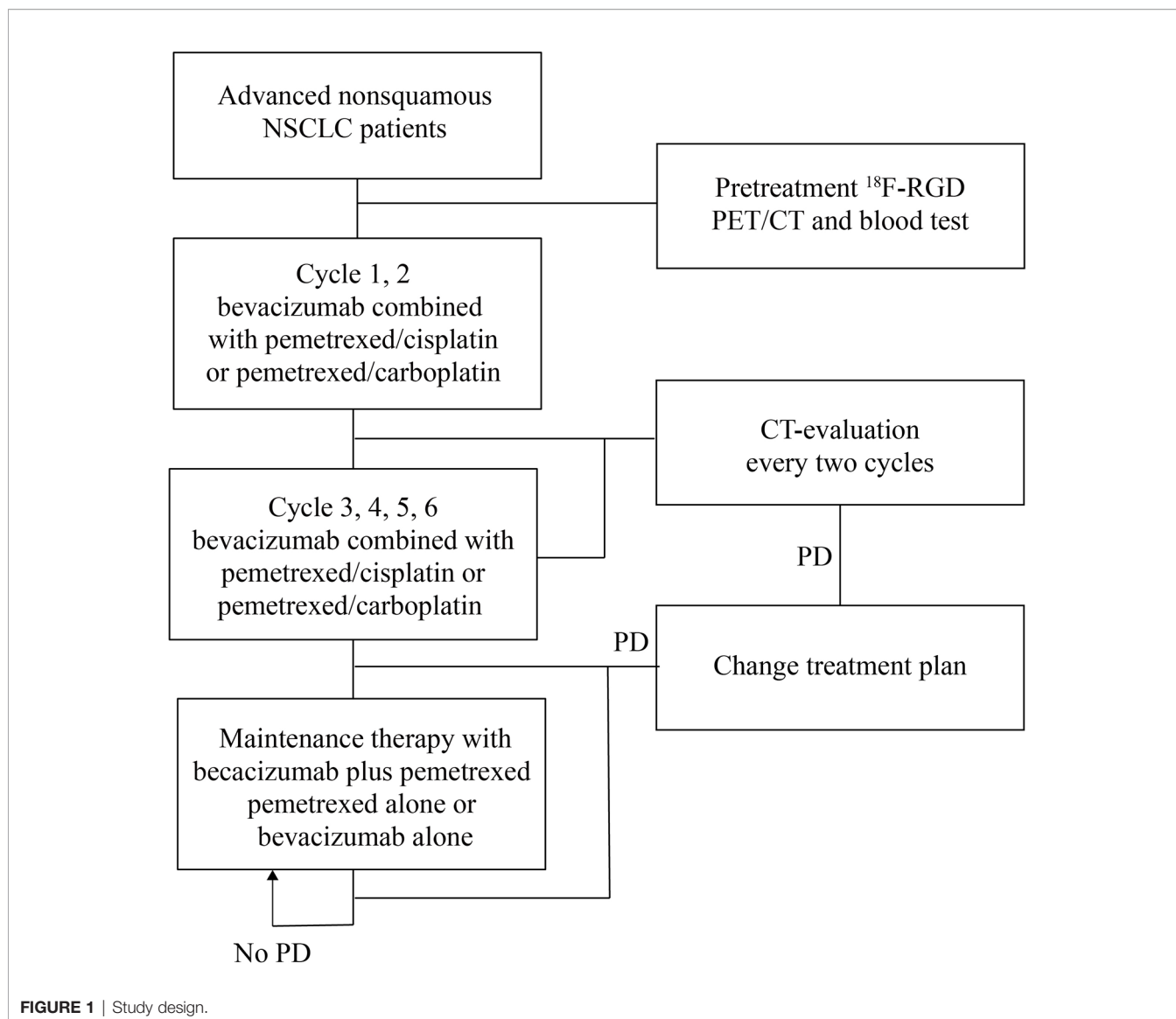
MATERIALS AND METHODS

Patients and Eligibility

In this prospective study, 30 patients with advanced nonsquamous NSCLC were initially enrolled. Each patient had been diagnosed through histologic examination and had pretreatment ^{18}F -RGD PET/CT scans. This study involved patients from Shandong Cancer Hospital over a one-year period, from December 2018 to December 2019. To be included, patients had to be at least 18 years of age with a Karnofsky performance status (KPS) of ≥ 70 . Patients who did not have objectively measurable lesions or those with autoimmune diseases or active comorbid infections were excluded. A total of 23 patients were included in the final analysis. Formal consent was requested and received from all prospective candidates prior to participation. This study was approved by the Institutional Review Board and the ethics committee within Shandong Cancer Hospital (reference no. SDTHEC 20180311). A flow chart of the study design is provided as **Figure 1**.

Treatment Regimen

Patients received bevacizumab combined with pemetrexed/cisplatin or pemetrexed/carboplatin every three weeks. Those who did not progress during four or six therapeutic cycles were assigned to continuous maintenance therapy with bevacizumab plus pemetrexed, pemetrexed alone, or bevacizumab alone, according to treatment plans devised by a patient's physician. Treatments continued until there was evidence of progressive disease (PD) according to RECIST criteria (v.1.1) or intolerable toxicity or patients requested to withdraw from further participation. Only one patient with advanced squamous cell carcinoma was treated with endostar plus docetaxel/carboplatin. This patient returned to hospital regularly for follow-ups after six treatment cycles.



¹⁸F-RGD PET/CT Scanning

Baseline ¹⁸F-RGD PET/CTs were performed within five days prior to the commencement of treatment. Lyophilized kits for labeling PRGD2 peptides were purchased from Jiangsu Institute of Nuclear Medicine, and synthesis progress was performed according to the standards described in a previous related study (14).

Radiochemical ¹⁸F-RGD purity exceeded 95%, and specific radioactivity exceeded 37 GBq (1,000 mCi)/ μ mol. Patients were required to rest for approximately one hour after receiving intravenous ¹⁸F-RGD injections at 219.24 ± 25.7 MBq. Patients were then asked to remain calm and sustain slow breathing during image acquisition. PET images were acquired from head to thigh (with patients in a supine position) for five minutes for each perspective, with an axial sampling thickness of 4.25 mm per slice after intravenous administration of ¹⁸F-RGD.

Images were attenuated with transmission data from the CT scans. This device was utilized because it is capable of multi-slice helical CT for anatomic imaging and attenuation correction. Attenuation-corrected PET images, CT images, and fused PET/CT images are presented as coronal, sagittal and transaxial slices and were viewed through a Xeleris workstation (GE Healthcare).

Image Analysis

Two qualified, experienced physicians analyzed the PET/CT images using MIM software (MIM, 6.1.0, Ohio, USA) without knowledge of the patients' respective histories. Significant differences between the physicians' opinions in terms of regions of interest (ROIs) and tumor uptake values, were discussed until consensus was reached. ROIs were drawn around lesions, referencing anatomical structures according to CT images and PET/CT fused images for ROI accuracy. ROIs were defined as a

closed area in the image, which usually has similar characteristics. In this study, ROIs were the anatomical structures of tumors from CT images, which help to acquire the tumor uptake value of ^{18}F -RGD.

The maximum standard uptake value (SUVmax) and mean standard uptake value (SUVmean) of all tumors were generated using a vendor-provided automated contouring program based on a 2.5 threshold. The peak standard uptake value (SUVpeak) was acquired as the average SUV within a one cubic centimeter sphere surrounding the voxel with the SUVmax. Metabolic tumor volume (MTV) was measured through attenuation-corrected ^{18}F -RGD PET images using an SUV-based automated contouring program with an iso-counter threshold method based on 41% of the SUVmax, defined as the total volume of all tumors in the body in milliliters.

Inflammatory Factors

Complete blood counts were obtained three days prior to the start of treatment and were all performed in the Department of Clinical Laboratory within Shandong Cancer Hospital. Inflammatory factors, including baseline NLR, PLR and LMR. These baseline data were abstracted from patients' records and then anonymized. The NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The PLR was calculated by dividing the absolute platelet count by the absolute lymphocyte count. Likewise, the LMR was calculated by dividing the absolute lymphocyte count by the absolute monocyte count.

Study Endpoint

Short-term treatment responses were assessed after the 2nd cycle using the treatment response evaluation according to RECIST criteria (v.1.1) using CT. Patients with a complete response (CR) or partial response (PR) were classified as 'responders'. Those who appeared with stable disease (SD) or PD were defined as 'nonresponders'. Progression-free survival (PFS) was measured from the start of treatment until the date when disease progression was ascertained or in the occurrence of death due to any cause. Patients who died without documented PD were considered to have had PD at the time of death.

Statistical Analyses

All statistical tests were performed with SPSS 23.0 (SPSS Inc., Chicago, IL) and R software (version 3.6.2). Quantitative data are expressed as the means with corresponding standard deviations (SD). Two-sample *t*-tests and Wilcoxon rank-sum tests were used to compare PET/CT parameters and inflammatory factors between responders and nonresponders. ROC curves and area under the ROC curve (AUC) analyses were used to describe response prediction accuracy. Logistic regression analysis was applied to identify correlations between variables and short-term outcomes. Cox's proportional hazard regression model was utilized to investigate the independence of survival and other related factors. Variables with $p < 0.1$ under univariate analysis were entered into Cox's multivariate regression to build prognostic models.

The results are presented as hazard ratios (HRs) with corresponding 95% confidence intervals (95% CIs). Continuous prognostic variables were dichotomized for PFS before Kaplan-Meier analysis using optimal cutoff values determined using the "surv_cutpoint" function in the "survminer" package in R. Correlations between PET parameters and inflammatory factors were calculated using Spearman's rank correlation. All variables with $p < 0.05$ were considered statistically significant.

RESULTS

Patients and Short-Term Outcomes

23 patients were included for complete short-term response and survival analyses. Seven patients were excluded from the original 30 eligible participants, four of whom refused combined antiangiogenic therapy with chemotherapy, and three discontinued treatment after the first cycle. One patient died from bleeding caused by tumor-infiltrating blood vessels before response assessment. Therefore, 22 patients were included in this short-term response analysis. Among these patients, nine were assessed as PR, and the overall response rate was 40.91%. By contrast, 11 patients achieved SD, and two patients attained PD. Patient characteristics are summarized in **Table 1** and listed in **Table 2** and **Supplementary Table 1**. ^{18}F -RGD PET/CT and CT scans for a typical responder and nonresponder are presented in **Figure 2**.

Survival Outcome

Median follow-up time was 12 months (range 7.08-16.92). The median PFS (mPFS) was 5.83 months (range 3.17-8.49). The 6-month actuarial PFS rate was 43.48%. During follow-up, four patients were still alive without known recurrent disease. Seven

TABLE 1 | Patient characteristics.

Characteristics	Participants (N=23)	Percentage (%)
Age		
<60	7	30.43
≥60	16	69.57
Gender		
Male	15	65.22
Female	8	34.78
Tumor stage		
IIIb/C	4	17.39
IVa/B	19	82.61
Histopathologic subtype		
Adenocarcinoma	22	95.65
Squamous cell carcinoma	1	4.35
Smoking history		
Yes	12	52.17
No	11	47.83
KPS		
≤80	14	60.87
>80	9	39.13
Number of treatment regimens		
1	17	73.91
2	6	26.09

*N denotes whole sample.

TABLE 2 | Patients characteristics, outcomes, tumor pre-treatment ¹⁸F-RGD PET uptake and inflammatory biomarkers.

Patient no.	Gender	Age (y)	Tumor stage	Smoking history	No. of treatment regimens	Histology	EGFR	Pretreatment ¹⁸ F-RGD PET uptake				PreNLR	PrePLR	PreLMR	2 Cycles Response	Time to progression (mo)
								SUVmax	SUVmean	SUVpeak	MVT					
1	M	59	IIIB	Former	1	Adenocarcinoma	Negative	2.61	1.32	2.05	45.95	1.73	162.57	3.73	NA	0.37
2	F	42	IIIC	None	1	Adenocarcinoma	Not available	5.29	3.34	4.24	17.50	2.44	204.98	5.58	SD	8.43
3	M	68	IVB	Former	1	Adenocarcinoma	Negative	2.80	2.58	2.36	0.44	20.53	776.36	0.38	PD	1.77
4	F	73	IVB	None	1	Adenocarcinoma	Not available	7.52	3.42	5.58	12.67	1.92	167.62	2.14	SD	6.87
5	M	68	IVB	Former	2	Adenocarcinoma	Negative	3.66	2.70	3.23	19.31	2.68	127.63	2.62	SD	3.23
6	M	69	IIIC	Former	1	Squamous cell carcinoma	Not available	7.01	3.57	5.61	43.97	2.34	129.07	3.74	SD	14.57+
7	M	69	IVB	None	2	Adenocarcinoma	Not available	12.92	8.59	3.39	394.97	2.40	120.10	6.69	PR	10.00
8	M	35	IVA	None	1	Adenocarcinoma	Sensitive mutations	4.59	3.02	3.84	21.45	2.78	214.11	4.29	PR	14.13+
9	F	52	IVB	None	1	Adenocarcinoma	Negative	2.86	2.60	2.36	7.52	3.02	185.08	1.35	SD	5.93
10	M	52	IVB	Former	1	Adenocarcinoma	Negative	3.90	2.93	3.00	12.30	5.47	337.50	1.75	SD	2.80
11	F	66	IVB	None	1	Adenocarcinoma	Negative	4.23	2.86	3.65	23.25	2.88	163.53	2.66	PR	5.27
12	M	61	IVA	Former	2	Adenocarcinoma	Sensitive mutations	4.21	2.98	3.64	30.29	5.04	157.55	1.28	PR	6.20
13	M	64	IVB	Former	1	Adenocarcinoma	Negative	5.34	3.06	3.99	25.37	3.86	255.08	1.76	PR	5.83
14	F	56	IVA	None	2	Adenocarcinoma	Sensitive mutations	4.21	2.91	3.17	2.82	2.69	219.66	4.50	SD	7.70
15	F	62	IVB	None	1	Adenocarcinoma	Not available	4.18	3.03	3.71	22.63	3.66	269.23	2.10	SD	7.80
16	M	62	IIIC	Former	2	Adenocarcinoma	Sensitive mutations	3.84	2.86	3.00	8.20	7.00	433.33	1.46	SD	3.60
17	M	60	IVB	Former	2	Adenocarcinoma	Negative	3.93	2.86	2.98	2.32	1.73	66.90	3.09	SD	3.50
18	M	69	IVB	Former	1	Adenocarcinoma	Negative	6.28	3.10	5.38	75.49	3.22	146.91	2.98	PR	5.53
19	F	66	IVB	None	1	Adenocarcinoma	Negative	3.38	2.76	2.71	2.07	4.17	396.15	2.23	PD	2.07
20	M	68	IVB	Former	1	Adenocarcinoma	Negative	4.59	3.00	3.55	2.40	5.31	125.96	2.30	SD	4.23
21	M	31	IVB	None	1	Adenocarcinoma	Negative	6.83	3.08	5.40	23.29	12.47	424.29	5.00	PR	6.27+
22	M	66	IVB	Former	1	Adenocarcinoma	Negative	11.03	3.98	8.83	166.50	7.38	262.50	0.67	PR	6.10+
23	F	65	IVB	None	1	Adenocarcinoma	Negative	7.99	3.39	5.02	45.75	2.89	284.58	2.46	PR	2.87

PreNLR, pre-treatment neutrophil-to-lymphocyte ratio; PrePLR, pre-treatment platelet-to-lymphocyte ratio; PreLMR, pre-treatment lymphocyte-to-monocyte ratio; PD, progressive disease; PR, partial response; SD, stable disease; NA, not applicable.

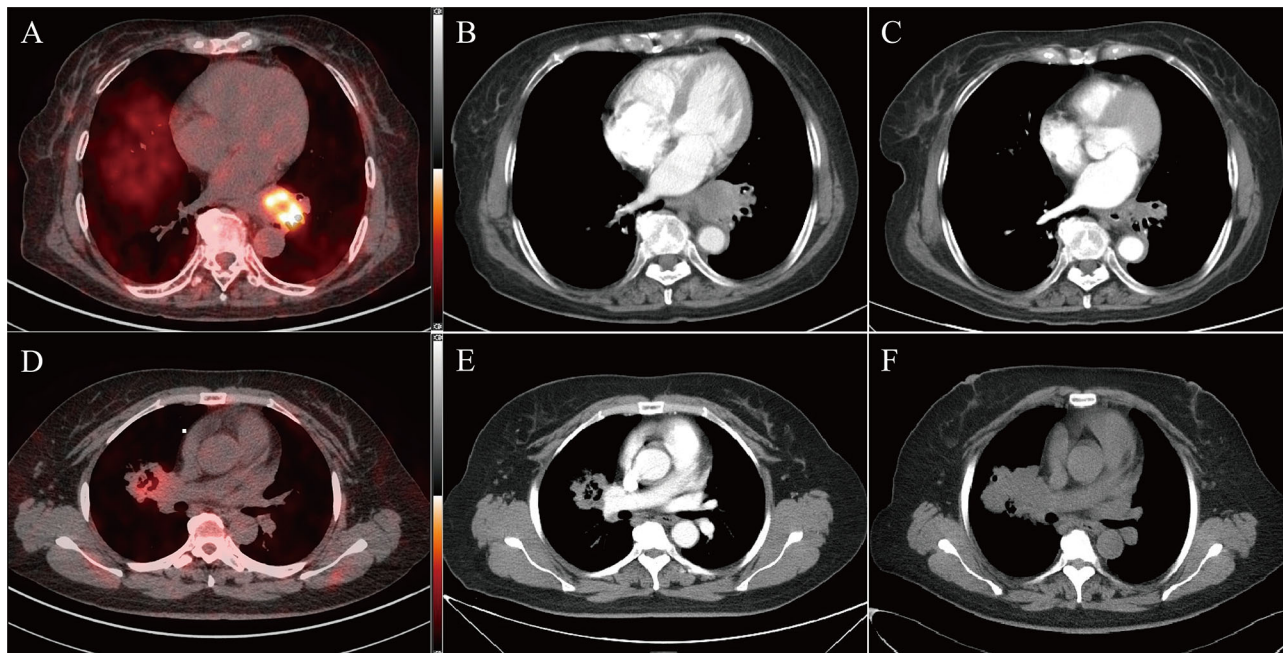


FIGURE 2 | Two typical examples of ^{18}F -RGD PET/CT scans in responder (top panel, SUVmax = 7.99, SUVmean = 3.39, MTV = 5.02, Response evaluated as PR) and non-responder (bottom panel, SUVmax = 3.38, SUVmean = 2.76, MTV = 2.07, Response evaluated as PD). Baseline PET/CT images (**A, D**) and corresponding CT slices from before treatment (**B, E**) and 2 cycles after treatment (**C, F**).

patients had locoregional recurrence or metastasis but were still alive after salvage or palliative treatment. Four patients died of tumor recurrence, six patients died of distant organ metastasis, including metastasis to the brain and lung, and one patient died of both tumor recurrence and distant organ metastasis. Another patient died of bleeding caused by tumor-infiltrating blood vessels.

Predictors of Short-Term Responses Among ^{18}F -RGD PET/CT, Inflammatory Biomarkers, and Clinical Parameters

^{18}F -RGD PET/CT and inflammation parameters are provided in Table 3. The SUVmax, SUVmean, and MTV were significantly higher in responders compared to nonresponders ($p < 0.05$). ROC curve analysis suggested that there were significant correlations among SUVmax, SUVmean, and MTV and ^{18}F -RGD PET/CT

and short-term responses ($p < 0.05$). Additional details can be found in Figure 3.

^{18}F -RGD PET/CT parameters, including the SUVmax, SUVmean, and MTV, and multiple clinical variables combined with the biomarkers of inflammation were tested through binary logistic regression analysis. According to univariate analysis, SUVmax and MTV could predict short-term outcomes ($p < 0.05$), although SUVmean could not ($p = 0.396$). Multivariate analysis revealed that SUVmax was a predictor of sensitivity to the combined antiangiogenic treatment ($p = 0.008$).

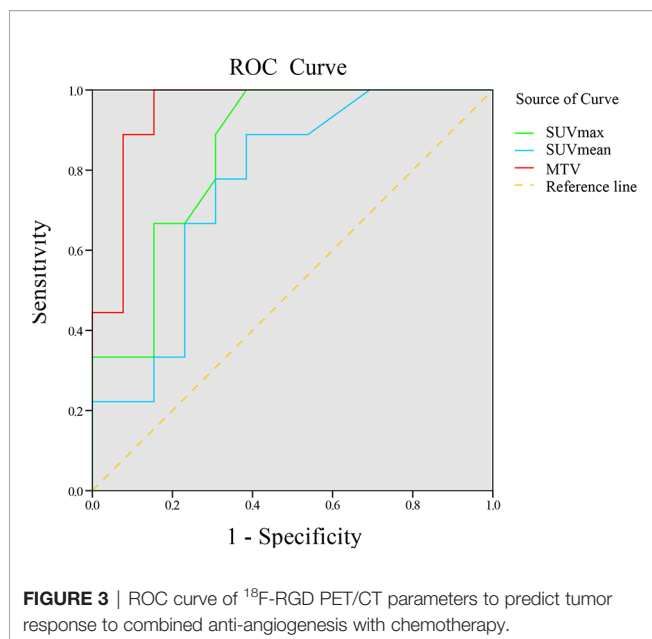
Independent Predictors of PFS Among ^{18}F -RGD PET/CT, Inflammatory Biomarkers, and Clinical Parameters

^{18}F -RGD PET/CT parameters, including SUVmax, SUVmean, SUVpeak and MTV, as well as multiple clinical variables and

TABLE 3 | Pretreatment ^{18}F -RGD PET/CT and inflammatory biomarkers for patients, and AUC curve analysis for predicting tumor responses.

Parameters	All patients	Responders	Non-responders	P	ROC Curve Analysis					
					Area	P	Threshold	Sensitivity	Specificity	Accuracy
SUVmax	4.41 ± 2.57	6.28 ± 3.10	3.93 ± 1.44	0.030	0.846 ± 0.083	0.007	4.195	100	61.5	77.3
SUVmean	3.01 ± 1.23	3.08 ± 1.83	2.91 ± 0.31	0.036	0.761 ± 0.103	0.042	2.955	88.9	61.5	72.7
SUVpeak	3.65 ± 1.47	3.99 ± 1.70	3.17 ± 1.06	0.287	–	–	–	–	–	–
MTV	20.38 ± 86.23	30.29 ± 123.68	8.20 ± 12.10	0.004	0.949 ± 0.047	0.000	20.38	100	84.6	90.9
PreNLR	3.12 ± 4.27	3.22 ± 3.28	3.02 ± 4.97	0.969	–	–	–	–	–	–
PrePLR	209.54 ± 155.69	214.11 ± 94.29	204.98 ± 189.24	0.547	–	–	–	–	–	–
PreLMR	2.38 ± 1.61	2.66 ± 1.92	2.23 ± 1.39	0.461	–	–	–	–	–	–

PreNLR, pre-treatment neutrophil-to-lymphocyte ratio; PrePLR, pre-treatment platelet-to-lymphocyte ratio; PreLMR, pre-treatment lymphocyte-to-monocyte ratio.



biomarkers for inflammation in relation to PFS, were further investigated using univariate Cox regression analysis. As shown in **Table 4**, SUVpeak ($p=0.015$) and PreLMR ($p=0.030$) were significantly associated with PFS.

Variables considered highly significant with a $p<0.1$ were then intercalated into a multivariate regression model. SUVpeak ($p=0.021$) and PreLMR ($p=0.030$) were verified as independent prognostic factors for PFS. Patients with SUVpeak >3.23 and PreLMR >3.09 experienced prolonged PFS (HR=0.204, 95% CI: 0.071-0.556, $p<0.001$; HR=0.118, 95% CI: 0.025-0.556, $p=0.002$). Further details are provided in **Figure 4**.

Combining the SUVpeak and PreLMR Parameters

Baseline SUVpeak appears not to correlate significantly with PreLMR (Spearman rank correlation 0.057, $p=0.341$). When applying the respective cutoff values, we combined SUVpeak with PreLMR to stratify patients into three risk groups: high-SUVpeak patients with a high PreLMR (low-risk group; mPFS=10 months, $n=5$); high-SUVpeak patients with a low PreLMR and low-SUVpeak patients with a high PreLMR (intermediate-risk group; mPFS=5.53 months, $n=13$; low-risk group vs. intermediate-risk group $p=0.001$); and low-SUVpeak patients with a low PreLMR (high-risk group; mPFS=2.80 months, $n=5$; low-risk group vs. high-risk group $p=0.02$; intermediate-risk group vs. high-risk group, $p=0.049$). See **Figure 5** for further details.

DISCUSSION

This prospective study provided the first evidence that tumor ¹⁸F-RGD uptake parameters and systemic biomarkers for inflammation at baseline can be used to predict outcomes in

TABLE 4 | Univariate and multivariate progression-free survival analyses for patients with advanced NSCLC.

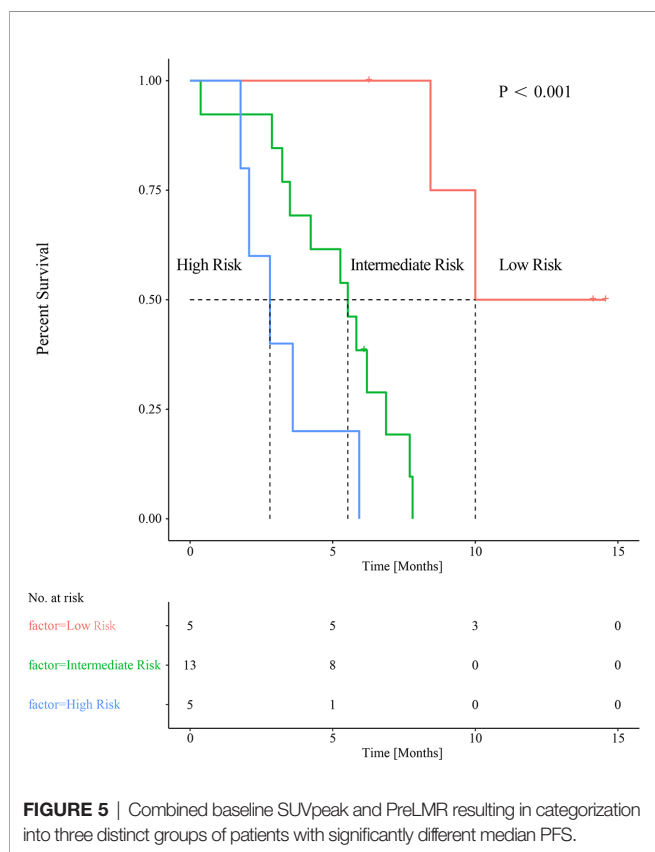
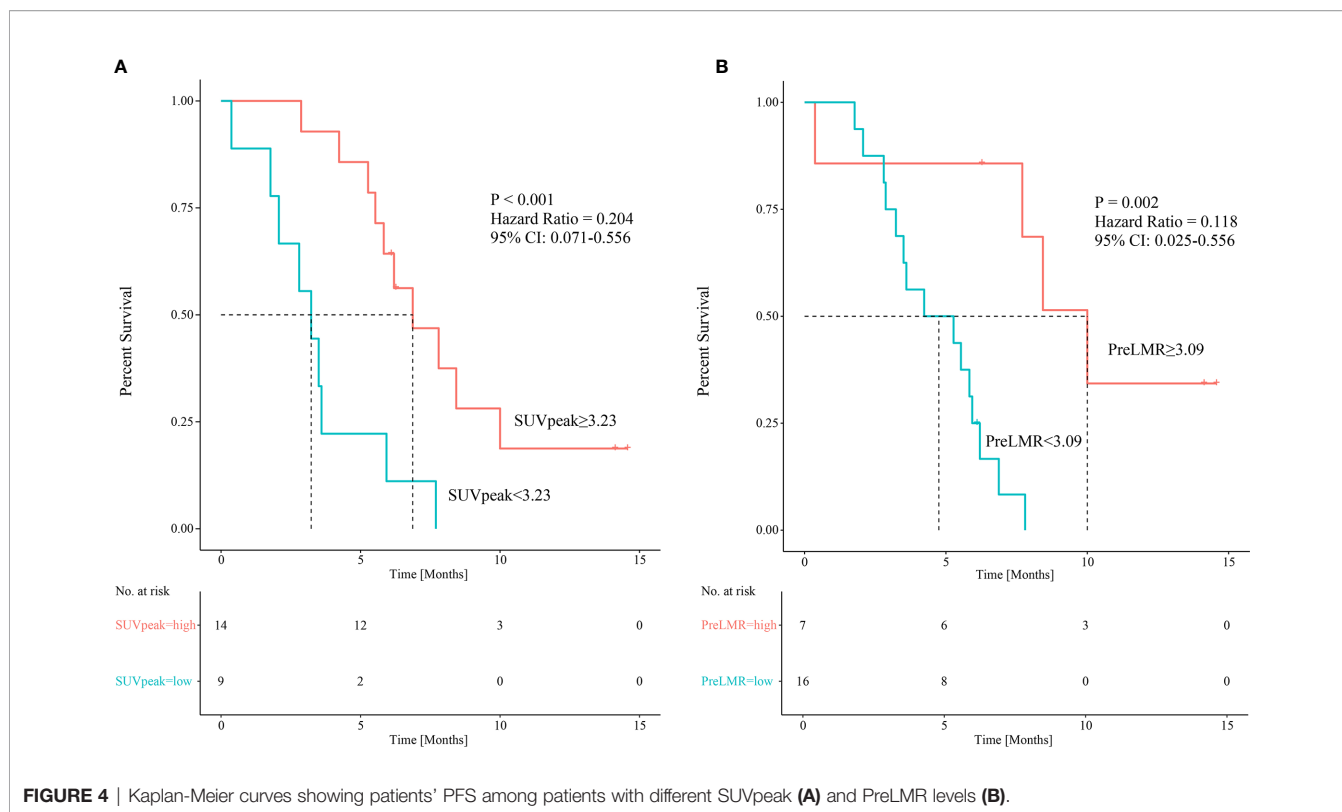
Variables	PFS					
	Univariate analysis			Multivariate analysis		
	HR	95%CI	P value	HR	95%CI	P value
Age	1.031	0.988-1.077	0.157			
Gender	1.227	0.480-3.137	0.670			
Smoking History	0.493	0.193-1.258	0.139			
Anatomical location	1.883	0.620-5.713	0.264			
EGFR mutation	0.881	0.291-2.670	0.823			
KPS	0.933	0.850-1.024	0.144			
No. of treatment lines	1.531	0.599-3.908	0.373			
Treatments procedures	1.593	0.367-6.921	0.535			
Tumor size	1.048	0.923-1.191	0.462			
Tumor stage	1.447	0.166-12.658	0.736			
SUVmax	0.766	0.583-1.006	0.055			
SUVmean	0.651	0.332-1.274	0.210			
SUVpeak	0.438	0.268-0.870	0.015	0.535	0.315-0.910	0.021
MTV	0.997	0.991-1.003	0.292			
Pre-NLR	1.072	0.933-1.232	0.327			
Pre-PLR	1.003	0.999-1.007	0.089			
Pre-LMR	0.684	0.486-0.965	0.030	0.675	0.474-0.963	0.030

No., number; PreNLR, pre-treatment neutrophil-to-lymphocyte ratio; PrePLR, pre-treatment platelet-to-lymphocyte ratio; PreLMR, pre-treatment lymphocyte-to-monocyte ratio.

patients receiving combined antiangiogenic therapy for advanced NSCLC. The tumor SUVmax obtained from ¹⁸F-RGD PET/CT was significantly higher in responders than nonresponders. Patients with tumors with higher SUVpeak or higher PreLMR had comparatively longer PFS. Although without a linear correlation, the combination of SUVpeak and PreLMR may increase the prognostic value and enable the identification of a subgroup of patients with an intermediate PFS. The exclusion of the seven patients did not change the prognostic value of these parameters since the included patients received a standard treatment.

A large proportion of the included patients had received two prior combined antiangiogenic treatments (26.09%). This may be the reason why we observed a median PFS slightly lower than 6.2 months in the bevacizumab combined treatment arm in the ECOG4599 trial. For tumors with high microvessel density (MVD), anti-angiogenic drugs are more likely to induce blood vessel normalization, thereby increasing the effect of anti-tumor treatment, which in turn makes the tumor insensitive to treatment. Therefore, the baseline MVD level has the potential to predict the efficacy of anti-angiogenic therapy. However, immunohistochemistry of tumor is an invasive test, and the heterogeneity of the tumor is likely to cause unstable results. Therefore, molecular imaging and hematology markers are expected to become potential indicators.

In a previous preclinical study, we found that the degree of tumor responses to bevacizumab alone, apatinib alone, or bevacizumab combined with chemotherapy or radiotherapy



was positively associated with ¹⁸F-RGD tumor uptake prior to treatment (15). ¹⁸F-RGD uptake decreased more in high-uptake tumors than in low-uptake tumors. Several other preclinical studies have found that RGD uptake from pre- to post-antiangiogenic monotherapy decreased more than that in the control groups, and this occurs much earlier than the fluorodeoxyglucose (FDG) metabolic response (16). From a preclinical perspective, tumor RGD uptake at baseline or RGD uptake changes in the early stage seems to be a strong predictor for antiangiogenic therapies.

In a previous clinical study with bevacizumab-containing therapy in ovarian and cervical cancers, larger decreases in the SUVmean related to RGD uptake were found in two patients with an early objective response compared to one patient with disease progression (17). However, this initial study provided only preliminary findings, which need to be confirmed in larger validation studies. In another clinical trial, researchers found that a higher ¹⁸F-RGD uptake in pretreated tumors predicted better responses to apatinib alone (9). The results of this study were, however, not entirely consistent with previous research. For example, higher RGD uptake values at baseline have been shown to predict poorer outcomes after concurrent chemoradiotherapy in both NSCLC and glioblastoma (18, 19). The primary role of antiangiogenic therapy alone, for example, apatinib, endostar or bevacizumab, may be to prevent new vessels from forming and maintaining tumor vessels in an inactive state, although with different mechanisms of action (20). Integrin αvβ3, which is highly expressed on newly

formed vessels, should be reduced by these drugs. It seems therefore that higher RGD uptake can represent a high density of effective target receptors, which increases the sensitivity to antiangiogenic treatments alone and therefore also increases the predictive power.

However, dilated, tortuous, and hyperpermeable angiogenesis represented by RGD uptake values increased tumor interstitial fluid pressure and led to poor blood flow and severe hypoxia, which increased tumor resistance to chemoradiotherapy (19, 21). In this trial, the predominant role of the antiangiogenic drug was to prune tumor vessels and improve the function of the remaining vessels to induce chemopotentiality by enhanced delivery of chemotherapeutic agents according to the vascular normalization theory. In this instance, high RGD uptake not only predicted improved outcomes with antiangiogenic agents but also suggests that the penetrative capacity of chemotherapy is enhanced and therefore can kill tumors with a higher sensitivity.

Previous studies have provided evidence that serum inflammatory factors are associated with immunological status within tumor microenvironments (10). In a retrospective study of patients receiving bevacizumab combined with chemotherapy, researchers found that NLR and PLR in sera decreased in patients with CR/PR but increased in patients with PD, while LMR was elevated in patients with CR/PR and reduced in those with evidence of PD (22). Additionally, low levels of baseline LMR, an increased NLR and a decreased LMR were all independent risk factors for death. Our findings further validate the predictive value of inflammatory factors, confirming that LMR pretreatment is an independent prognostic factor for PFS. This phenomenon may be explained simply because more lymphocytes (and fewer monocytes) migrate into the tumor microenvironment and therefore improve immunological responses, which help to prevent tumor growth. Lymphocytes are thought to play a critical role in immunology by inhibiting tumor cell proliferation and migration (23). Additionally, neutrophils and macrophages, derived from monocytic precursors within tumors, promote angiogenesis. By releasing proangiogenic growth factors such as VEGF and triggering VEGF-independent angiogenesis, this process ultimately leads to antiangiogenic agent resistance (24, 25). Therefore, inflammatory factors certainly appear to be useful predictors of combined antiangiogenic responses.

Although there was no significant correlation between the SUV_{peak} and PreLMR, the combination of the two parameters may provide a more accurate prognostic value and enable the identification of a subgroup of patients with a high SUV_{peak} with a low PreLMR and patients with a low SUV_{peak} with a high PreLMR who encountered intermediate survival. While this study may advance shared decision-making processes allowing patients to plan their futures, it also suggests that intercalating parameters may further increase predictive power.

To the best of our knowledge, this is the first clinical study to apply ¹⁸F-RGD uptake on PET/CT and the combined biomarker

with serum inflammatory factors for selecting optimal advanced NSCLC patients to receive combined antiangiogenic treatment. As such, we ought to reflect on the limitations of this study design and our testing. First and foremost, the sample size was relatively small, and the credibility of the results need to be further verified. Therefore, larger studies are needed, not only to verify the results but also to further develop and validate biomarkers. Additionally, predictors of ¹⁸F-RGD PET/CT for short-term efficacy and survival were inconsistent. The earlier the effective treatment is determined, the greater the benefit for patients (26); however, the objective response rate did not appear to have a predictive function for PFS in this study. This may have led to inconsistent predictions, and studies with a larger sample size are needed. Second, inflammatory factors may be influenced by other causes, such as comorbid infections and the use of steroids. Even though patients with autoimmune diseases and active infections were excluded from this study, some of our participants may have been in the early stages of developing other conditions.

In conclusion, pretreatment ¹⁸F-RGD uptake on PET/CT imaging and systemic inflammatory biomarker may predict outcomes of combined antiangiogenic treatment and chemotherapy, and a higher ¹⁸F-RGD uptake or higher PreLMR may better predict short-term responses and PFS. Combined biomarkers based on molecular imaging with ¹⁸F-RGD PET/CT and serum inflammatory biomarkers may increase the prognostic value and provide a basis for risk stratification.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shandong cancer hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

JL, LW, and ZL collected the data. JL wrote the manuscript. JNL and ZG helped to collect literature and participated in discussions. JY and XM designed and verified the study. SS helped to write and then edited this report for publication. All authors contributed to the article and approved the submitted version.

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REFERENCES

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* (2018) 68:394–424. doi: 10.3322/caac.21492
- Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer Treatment and Survivorship Statistics, 2016. *CA Cancer J Clin* (2016) 66:271–89. doi: 10.3322/caac.21349
- Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-Carboplatin Alone or With Bevacizumab for Non-Small-Cell Lung Cancer. *New Engl J Med* (2006) 355:2542–50. doi: 10.1056/NEJMoa061884
- Hegde PS, Wallin JJ, Mancao C. Predictive Markers of anti-VEGF and Emerging Role of Angiogenesis Inhibitors as Immunotherapeutics. *Semin Cancer Biol* (2018) 52:117–24. doi: 10.1016/j.semcancer.2017.12.002
- Hood JD, Cheresch DA. Role of Integrins in Cell Invasion and Migration, Nature Reviews. *Cancer* (2002) 2:91–100. doi: 10.1038/nrc727
- Xiong JP, Stehle T, Zhang R, Joachimiak A, Frech M, Goodman SL, et al. Crystal Structure of the Extracellular Segment of Integrin Alpha Vbeta3 in Complex With an Arg-Gly-Asp Ligand. *Science (New York, N. Y.)* (2002) 296:151–5. doi: 10.1126/science.1069040
- Beer AJ, Haubner R, Wolf I, Goebel M, Luderschmidt S, Niemeyer M, et al. Positron Emission Tomography using [18F]Galacto-RGD Identifies the Level of Integrin Alpha(v)beta3 Expression in Man. *Clin Cancer Res* (2006) 12 (13):3942–9. doi: 10.1158/1078-0432.CCR-06-0266
- Gao S, Wu H, Li W, Zhao S, Teng X, Lu H, et al. A Pilot Study Imaging Integrin Alphavbeta3 With RGD PET/CT in Suspected Lung Cancer Patients. *Eur J Nucl Med Mol Imaging* (2015) 42:2029–37. doi: 10.1007/s00259-015-3119-1
- Li L, Ma L, Shang D, Liu Z, Yu Q, Wang S, et al. Pretreatment PET/CT Imaging of Angiogenesis Based on (18F)-RGD Tracer Uptake May Predict Antiangiogenic Response. *Eur J Nucl Med Mol Imaging* (2019) 46:940–7. doi: 10.1007/s00259-018-4143-8
- Hiramatsu S, Tanaka H, Nishimura J, Sakimura C, Tamura T, Toyokawa T, et al. Neutrophils in Primary Gastric Tumors Are Correlated With Neutrophil Infiltration in Tumor-Draining Lymph Nodes and the Systemic Inflammatory Response. *BMC Immunol* (2018) 19:13. doi: 10.1186/s12865-018-0251-2
- Tazzyman S, Niaz H, Murdoch C. Neutrophil-Mediated Tumour Angiogenesis: Subversion of Immune Responses to Promote Tumour Growth. *Semin Cancer Biol* (2013) 23:149–58. doi: 10.1016/j.semcancer.2013.02.003
- Dalton HJ, Armaiz-Pena GN, Gonzalez-Villasana V, Lopez-Berestein G, Bar-Eli M, Sood AK. Monocyte Subpopulations in Angiogenesis. *Cancer Res* (2014) 74:1287–93. doi: 10.1158/0008-5472.CAN-13-2825
- Chen DS, Hurwitz H. Combinations of Bevacizumab With Cancer Immunotherapy. *Cancer J* (2018) 24:193–204. doi: 10.1097/PPO.0000000000000327
- Wan W, Guo N, Pan D, Yu C, Weng Y, Luo S, et al. First Experience of 18F-Alfatide in Lung Cancer Patients Using a New Lyophilized Kit for Rapid Radiofluorination. *J Nucl Med* (2013) 54:691–8. doi: 10.2967/jnumed.112.113563
- Liu J, Wang D, Meng X, Sun X, Yuan S, Yu J. 18F-alfatide Positron Emission Tomography May Predict Antiangiogenic Responses. *Oncol Rep* (2018) 40:2896–905. doi: 10.3892/or.2018.6692
- Bao X, Wang MW, Luo JM, Wang SY, Zhang YP, Zhang YJ. Optimization of Early Response Monitoring and Prediction of Cancer Antiangiogenesis

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fonc.2021.671912/full#supplementary-material>

- Therapy Via Noninvasive Pet Molecular Imaging Strategies of Multifactorial Bioparameters. *Theranostics* (2016) 6:2084–98. doi: 10.7150/tno.13917
- Minamimoto R, Karam A, Jamali M, Barkhodari A, Gambhir SS, Dorigo O, et al. Pilot Prospective Evaluation of (18)F-FPPRGD2 PET/CT in Patients With Cervical and Ovarian Cancer. *Eur J Nucl Med Mol Imaging* (2016) 43:1047–55. doi: 10.1007/s00259-015-3263-7
 - Zhang H, Liu N, Gao S, Hu X, Zhao W, Tao R, et al. Can an (1)(8)F-ALF-NOTA-PRGD2 PET/CT Scan Predict Treatment Sensitivity to Concurrent Chemoradiotherapy in Patients With Newly Diagnosed Glioblastoma? *J Nucl Med* (2016) 57:524–9. doi: 10.2967/jnumed.115.165514
 - Luan X, Huang Y, Gao S, Sun X, Wang S, Ma L, et al. (18)F-Alfatide PET/CT may Predict Short-Term Outcome of Concurrent Chemoradiotherapy in Patients With Advanced Non-Small Cell Lung Cancer. *Eur J Nucl Med Mol Imaging* (2016) 43:2336–42. doi: 10.1007/s00259-016-3505-3
 - Burger RA, Brady MF, Bookman MA, Fleming GF, Monk BJ, Huang H, et al. Incorporation of Bevacizumab in the Primary Treatment of Ovarian Cancer. *New Engl J Med* (2011) 365:2473–83. doi: 10.1056/NEJMoa1104390
 - Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, et al. Normalization of the Vasculature for Treatment of Cancer and Other Diseases. *Physiol Rev* (2011) 91:1071–121. doi: 10.1152/physrev.00038.2010
 - Li B, Wang S, Li C, Guo M, Xu Y, Sun X, et al. The Kinetic Changes of Systemic Inflammatory Factors During Bevacizumab Treatment and Its Prognostic Role in Advanced Non-Small Cell Lung Cancer Patients. *J Cancer* (2019) 10:5082–9. doi: 10.7150/jca.30478
 - Al-Shibli KI, Donnem T, Al-Saad S, Persson M, Bremnes RM, Busund LT. Prognostic Effect of Epithelial and Stromal Lymphocyte Infiltration in Non-Small Cell Lung Cancer. *Clin Cancer Res* (2008) 14:5220–7. doi: 10.1158/1078-0432.CCR-08-0133
 - Nozawa H, Chiu C, Hanahan D. Infiltrating Neutrophils Mediate the Initial Angiogenic Switch in a Mouse Model of Multistage Carcinogenesis. *Proc Natl Acad Sci USA* (2006) 103:12493–8. doi: 10.1073/pnas.0601807103
 - Coffelt SB, Chen YY, Muthana M, Welford AF, Tal AO, Scholz A, et al. Angiopoietin 2 Stimulates TIE2-Expressing Monocytes to Suppress T Cell Activation and to Promote Regulatory T Cell Expansion. *J Immunol (Baltimore Md.: 1950)* (2011) 186:4183–90. doi: 10.4049/jimmunol.1002802
 - Huang W, Zhou T, Ma L, Sun H, Gong H, Wang J, et al. Standard Uptake Value and Metabolic Tumor Volume of ¹⁸F-FDG PET/CT Predict Short-Term Outcome Early in the Course of Chemoradiotherapy in Advanced Non-Small Cell Lung Cancer. *Eur J Nucl Med Mol Imaging* (2011) 38:1628–35. doi: 10.1007/s00259-011-1838-5

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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