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Original Article

Tumor-stroma ratio is an independent predictor for survival in NSCLC

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Abstract: Tumor-stroma ratio (TSR) has been identified as a new and practicable prognostic factor in some solid tumors. The aim of the study is to evaluate the prognostic value of TSR in non-small cell lung cancer (NSCLC). A total of 404 patients who underwent surgery resection for NSCLC were included in this study. TSR was assessed visually on the hematoxylin-stained tissue sections of surgical specimens. Patients with more than 50% intratumor stroma were quantified as the stroma-rich group and those with less than 50% as the stroma-poor group. In 404 cases of tissue samples, 302 cases were included in the stroma-poor group, while 102 cases in stroma-rich group. The different expression of TSR in NSCLC tissue was not correlated with gender, age, smoking history, tumor diameter, histology, differentiation grade and pTNM staging. In the Cox univariate and multivariate analyses of the 5-year OS, the HRs of the TSR were 1.818 (95% CI; 1.323-2.497; $P<0.001$) and 1.748 (95% CI; 1.262-2.422; $P<0.05$), respectively. As for DFS, the HRs were 1.715 (95% CI; 1.249-2.354; $P<0.001$) and 1.570 (95% CI; 1.135-2.172; $P<0.05$). Stroma-rich tumors were associated with poor prognosis and an increased risk of relapse, which may serve as a new prognostic histological characteristic in NSCLC.

Keywords: TSR, non-small cell lung cancer, prognosis, targeted therapy

Introduction

Lung cancer is the most lethal cancer worldwide due to its high incidence and mortality [1], of which the most common variant is non-small cell lung cancer (NSCLC). To date, the 5-year overall survival rate for patients with lung cancer is about 15%, highlighting the need for novel treatment strategies. Based on the latest global cancer statistics, lung cancer accounted for about 13% of total cancer diagnoses and was the leading cause of cancer death among males [2]. During the last decades, researchers have spared no effort to find prognostic factors, which could be used to predict disease control or survival. Nevertheless, we cannot predict the relative risk for patients more precisely and define those at high risk of recurrence to receive individual treatment. Thus, identification of new prognostic factors is desirable for adequate determination of prognosis and therapeutic strategies.

The prognostic factors of NSCLC include smoking history [3], tumor size [4], lymph node status [5], histology [6], and pTNM stage [7]. Although there are many well-established prognostic factors, it is still hard to predict the prognosis of individual patients accurately. Clinicians and researchers urgently need to identify economical and convenient predictors.

Tumors are complex tissue composed of carcinoma cells and surrounding stroma. It is increasingly acknowledged that tumor stroma is indispensable for cancer initiation, progression, and metastasis, and the stromal elements of tumor hold prognostic potential [8, 9]. Recently, the ratio of tumor to stroma (TSR) has been identified as a new and practical prognostic histologic characteristic of several solid tumors, such as esophageal cancer [10], breast cancer [11], colon cancer [12], and hepatocellular carcinoma [13]. However, the prognostic

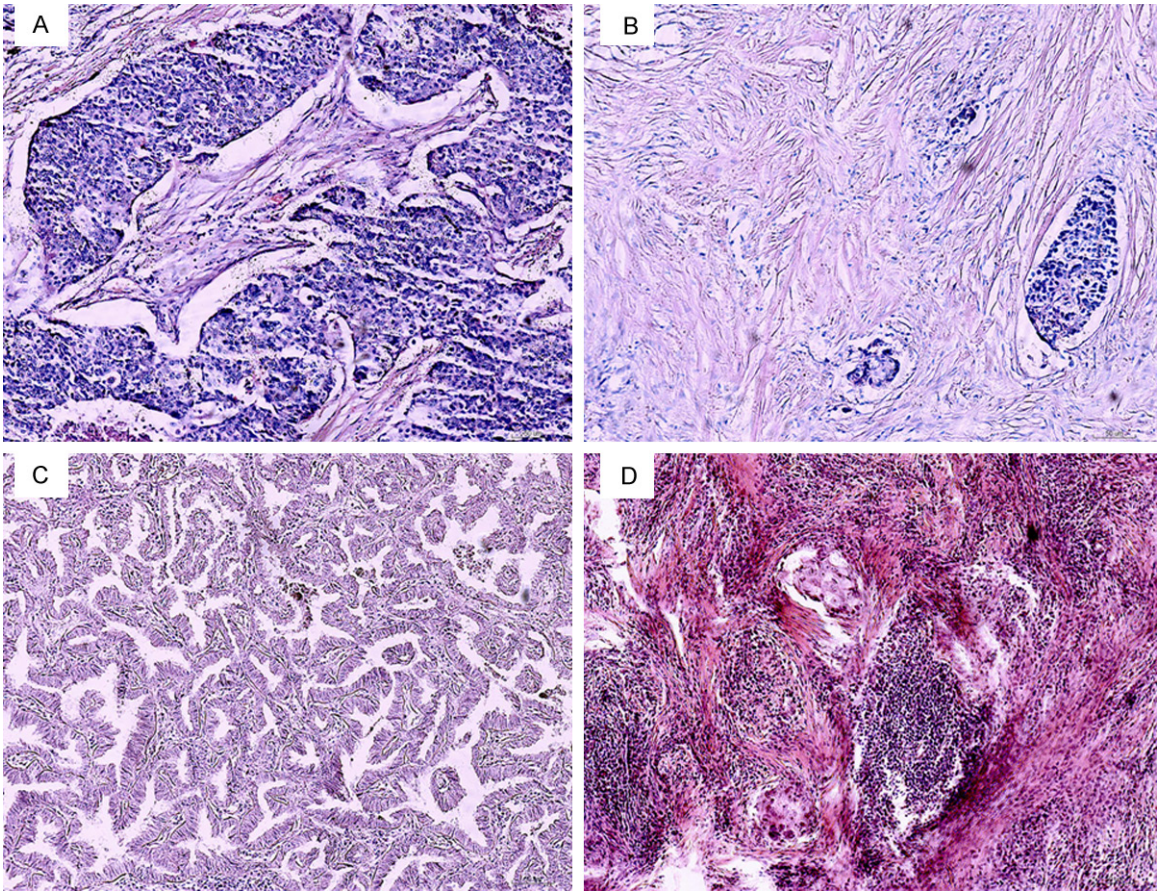

Figure 1. The expression of TSR in lung tissue of different kinds of histology. A: The stroma-poor group of squamous cell lung carcinoma. B: The stroma-rich group of squamous cell lung carcinoma. C: The stroma-poor group of lung adenocarcinoma. D: The stroma-rich group of lung adenocarcinoma.

value of TSR has not been explored in detail or systematically for NSCLC.

Therefore, we conducted the present study to evaluate the prognostic value of TSR in 404 patients with NSCLC and the relationship between TSR and other known prognostic parameters.

Materials and methods

Study population

404 patients with NSCLC who underwent complete surgery in Shandong Provincial Hospital affiliated to Shandong University, Shandong, China, from January 2007 to December 2009 were included in our study. Our this research was approved by Ethical Committee of Provincial Hospital affiliated to Shandong University, and the informed written consent for the use of their clinical study was obtained from the investigated patients. The clinical information was obtained from retrieving the medical records including gender, age, smoking history, tumor diameter, histology, differentiation grade, pTNM stage. Patients treated with neoadjuvant therapy, which could interfere with the evaluation of TSR were excluded, as were patients who died within 30 days after surgery.

Staining and evaluation

The 5-μm HE-stained sections from the primary tumor were routinely analyzed on microscopic examination. In case of tumor heterogeneity, areas with the lowest TSR value were considered decisive as is performed in routine pathology to determine tumor differentiation. In general, areas rich in stroma were found near the site of deepest microscopic infiltration. The surrounding stromal tissue not containing tumor cells was considered not to be connected with

Table 1. Clinicopathological characteristics of 404 patients with NSCLC

Characteristics	Total		Stroma-poor group		Stroma-rich group		P
	n	%	n	%	n	%	
Gender							0.154
Women	109	27.0%	87	28.8%	22	21.6%	
Men	295	73.0%	215	71.2%	80	78.4%	
Age							0.998
<60	206	51.0%	154	51.0%	52	52.9%	
≥60	198	49.0%	148	49.0%	50	47.1%	
Smoking history							0.15
<20 P.Y	211	52.2%	164	54.3%	47	46.1%	
≥20 P.Y	193	47.8%	138	45.7%	55	53.9%	
Tumor diameter							0.417
<3.5	216	53.5%	165	54.6%	51	50.0%	
≥3.5	188	46.5%	137	45.4%	51	50.0%	
Histology							0.287
ADC	236	58.4%	181	59.9%	55	53.9%	
SCC	168	41.6%	121	40.1%	47	46.1%	
Differentiation grade							0.311
Well	39	9.7%	33	10.9%	6	5.9%	
Moderate	252	62.3%	187	61.9%	65	63.7%	
Poor	113	28.0%	82	20.2%	31	30.4%	
pTNM stage							0.104
I	171	42.3%	137	33.9%	34	33.3%	
II	102	25.2%	72	17.8%	30	29.4%	
III	131	32.5%	93	23.0%	38	37.3%	

the tumor. Using a 5× microscope objective (50× total magnification), the most invasive tumor area of the whole tissue slide was selected. Subsequently, microscopical fields, where both stroma and tumor were present, and tumor cells were visualized on all sides and were scored with a 10 × objective (100 × total magnification). The assessment was done on the basis of the analysis of at least one microscopic field. The estimate was then recorded as the TSR. With this protocol, the tumor-stroma ratio was visually estimated in a blinded manner by two investigators and scored per tenfold percentage (10, 20, 30% etc.). A third observer was consulted when the two observers disagreed. The cut-off of the TSR was taken as 50%, as described before. TSR was defined as stroma poor (the proportion of stroma <50%) or stroma rich (the proportion of stroma ≥50%). Representative examples of microscopical fields selected for tumor-stroma ratio quantification from stroma-rich and stroma-poor tumors are shown in **Figure 1**.

Statistical analysis

Statistical analysis was performed using SPSS software version 17.0. The associations with other clinicopathological parameters were assessed with the Chi-squared test. Overall survival [9] was defined as the time period between the randomization date and the last period of follow-up or date of death. Disease-free survival (DFS) was defined as the time between the randomization date and the date of death or the date of first locoregional or distant recurrence. If no recurrence occurred, DFS was calculated as the time period until the date of last follow-up [14]. Analysis of the survival curves was performed using Kaplan-Meier survival analysis and differences in survival distributions were tested using log-rank statistics. The Cox proportional hazard model was used to estimate the hazard ratio (HR) and 95% confidence intervals (95% CI) of explanatory variables for OS and DFS. $P<0.05$ was considered statistically significant.

Results

Clinicopathological features

404 patients (295 men and 109 women) were included in this study. The median age of the patients was 60 (range, 30-81) years at the date of surgery. The median follow-up time was 51 (range, 1-60) months. Clinicopathological and treatment characteristics of patients are shown in **Table 1**.

Tumor-stroma ratio in NSCLC

Tumor-stroma ratio in NSCLC with 5× and 10× objectives, routine HE-stained sections from the primary tumors were analyzed for the presence of stromal involvement. TSR was assessed on one section derived from the most invasive

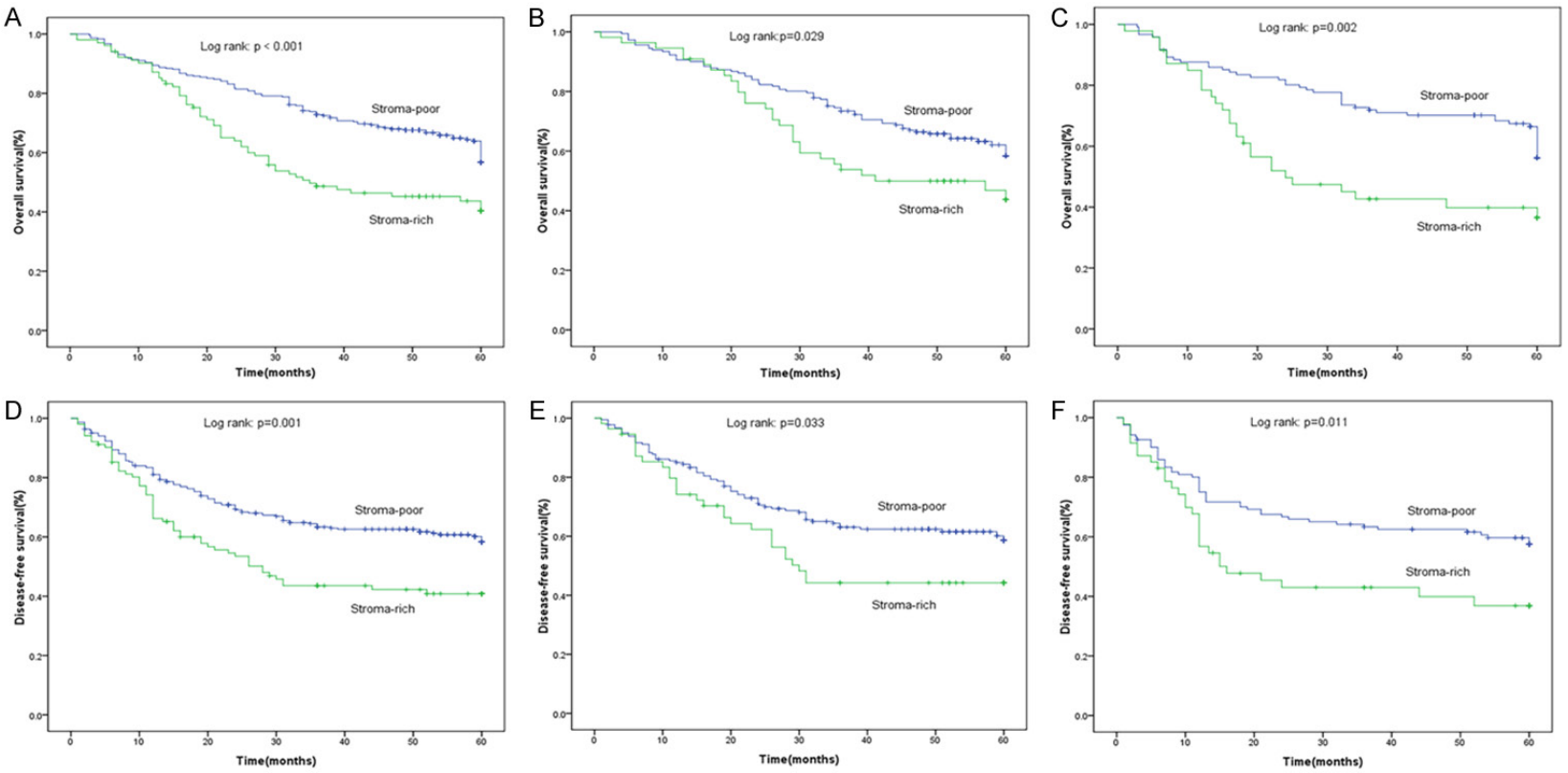

Figure 2. Difference in survival between stroma-poor and stroma-rich groups. A: Kaplan-Meier curve of OS for NSCLC; B: Kaplan-Meier curve of OS for ADC; C: Kaplan-Meier curve of OS for SCC; D: Kaplan-Meier curve of DFS for NSCLC; E: Kaplan-Meier curve of DFS for ADC; F: Kaplan-Meier curve of DFS for SCC.

Table 2. Cox univariate analysis for survival in 404 patients of NSCLC

Variables	Overall survival			Disease-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
TSR						
Stroma-poor	1.000	Ref.	-	1.000	Ref.	-
Stroma-rich	1.818	1.323-2.497	<0.001	1.715	1.249-2.354	<0.001
Gender						
Women	1.000	Ref.	-	1.000	Ref.	-
Men	1.339	0.944-1.900	0.102	1.399	0.986-1.985	0.06
Age						
<60	1.000	Ref.	-	1.000	Ref.	-
≥60	1.484	1.101-2.002	0.01	1.471	1.091-1.984	0.011
Smoking history						
<20 P.Y	1.000	Ref.	-	1.000	Ref.	-
≥20 P.Y	1.163	0.864-1.564	0.319	1.241	0.923-1.670	0.153
Tumor diameter						
<3.5	1.000	Ref.	-	1.000	Ref.	-
≥3.5	2.498	1.838-3.394	<0.001	2.490	1.832-3.390	<0.001
Histology						
ADC	1.000	Ref.	-	1.000	Ref.	-
SCC	1.143	0.847-1.542	0.382	1.189	0.882-1.603	0.256
Differentiation grade						
Well	1.000	<0.001	0.045	1.000	Ref.	<0.001
Moderate	2.654	0.012	0.021	2.799	1.302-6.018	0.008
Poor	4.779	<0.001	0.125	4.852	2.222-10.598	<0.001
pTNM stage						
I	1.000	Ref.	<0.001	1.000	Ref.	<0.001
II	3.133	2.054-4.780	<0.001	3.23	2.117-4.930	<0.001
III	5.033	3.407-7.437	<0.001	4.724	3.200-6.973	<0.001

HR: hazard ratio; CI: confidence interval; TSR: tumor-stroma ratio; P.Y: package year; ADC: adenocarcinoma; SCC: squamous carcinoma. P<0.05 was considered significant.

part of the tumor (**Figure 1**). Estimation of the TSR was performed successfully in all tumors. Assessed by two independent researchers (Zhang T. and Xu J.), 302 tumors were stroma poor and 102 were stroma rich, and controversial results were adjudicated by a third reviewer (Du J.).

Correlation of TSR with other prognostic factors

Table 1 lists patients and tumor characteristics for the stroma-rich and the stroma-poor groups. There were no significant differences between the two groups. Follow up was complete. The 5-year overall survival rate and disease-free survival rate were 63.9% and 55.7%, respectively, in the stroma-poor group and 40.4% and 37.5%, respectively, in the stroma-rich group. Survival curves are shown in **Figure 2**.

The difference of survival curves between the stroma-poor and stroma-rich groups remained statistically significant. In the Cox univariate and multivariate analyses of the 5-year OS, the HRs of the TSR were 1.818 (95% CI; 1.323-2.497; *P*<0.001) and 1.748 (95% CI; 1.262-2.422; *P*<0.05), respectively. As for DFS, the HRs were 1.715 (95% CI 1.249-2.354; *P*<0.001) and 1.570 (95% CI 1.135-2.172; *P*<0.05). In the Cox univariate model, TSR, age, tumor diameter, differentiation grade and pTNM stage were significantly related to the 5-year OS and DFS. As show in **Tables 2** and **3**, the TSR was an independent prognostic variable for the 5-year OS and DFS. In the multivariate analysis, the TSR was an independent prognostic variable for the 5-year OS with an HR of 1.748 (95% CI; 1.262-2.422; *P*<0.05) and DFS with an HR of 1.570 (95% CI 1.135-2.172; *P*<0.05), independent of other clinicopathological parameters.

Table 3. Multivariate Cox analysis for survival in 404 patients of NSCLC

Variables	Overall survival			Disease-free survival		
	HR	95% CI	*P*	HR	95% CI	*P*
TSR						
Stroma-poor	1.000	Ref.	-	1.000	Ref.	-
Stroma-rich	1.748	1.262-2.422	0.001	1.570	1.135-2.172	0.006
Gender						
Women	1.000	Ref.	-	1.000	Ref.	-
Men	1.061	0.691-1.630	0.786	1.099	0.715-1.689	0.667
Age						
<60	1.000	Ref.	-	1.000	Ref.	-
≥60	1.577	1.157-2.150	0.004	1.611	1.180-2.199	0.003
Smoking history						
<20 P.Y	1.000	Ref.	-	1.000	Ref.	-
≥20 P.Y	0.792	0.549-1.143	0.212	0.837	0.579-1.210	0.343
Tumor diameter						
<3.5	1.000	Ref.	-	1.000	Ref.	-
≥3.5	1.582	1.120-2.535	0.009	1.54	1.086-2.183	0.015
Histology						
ADC	1.000	Ref.	-	1.000	Ref.	-
SCC	1.110	0.779-1.581	0.565	1.095	0.766-1.566	0.613
Differentiation grade						
Well	1.000	Ref.	0.045	1.000	Ref.	0.047
Moderate	1.977	0.908-4.304	0.086	2.116	0.972-4.603	0.059
Poor	2.564	1.151-5.713	0.021	2.645	1.187-5.894	0.017
pTNM stage						
I	1.000	Ref.	<0.001	1.000	Ref.	<0.001
II	2.147	1.361-3.388	0.001	2.192	1.348-3.472	0.001
III	4.011	2.596-6.196	<0.001	3.796	2.453-5.874	<0.001

HR: hazard ratio; CI: confidence interval; TSR: tumor-stroma ratio; P.Y: package year; ADC: adenocarcinoma; SCC: squamous carcinoma. *P*<0.05 was considered significant.

Discussion

The present investigation shows that TSR is a new independent prognostic factor for NSCLC. Stroma-rich tumors were associated with poor prognosis and an increased risk of relapse.

The use of TSR as a prognostic factor has been introduced by previous studies. After analysis of 122 patients with stage I to III colon carcinoma, Mesker et al. found that patients with TSR less than 50% showed significant worse overall and disease-free survivals, which suggested that [15] TSR could serve as an independent parameter for confident prediction of clinical outcome in early-stage colon cancer [12]. Recently, TSR was also confirmed to be a new and practicable prognostic tumor characteristic in early cervical carcinoma [16]. Similar results were presented in esophageal squamous cell carcinoma [10]. Our hypothesis was that TSR might also be an important prognostic parameter for NSCLC. In the study, we found that 5-year overall survival rate and disease-free survival rate were 63.9% and 55.7%, respectively, in the stroma-poor group and 40.4% and 37.5%, respectively, in the stroma-rich group. The TSR was of prognostic value by both univariate and multivariate analysis.

As we know, many prognostic factors have been found for NSCLC, such as smoking history, tumor size, histology, differentiation grade, and pTNM stage. All these factors have been included in our analysis. In the study, we found that age, tumor diameter, differentiation grade and pTNM stage were significantly related to 5-year OS and DFS in the univariate analysis,

and the TSR was also an independent prognostic factor in the multivariate analysis.

Tumor tissue is composed of both carcinoma cells and stromal cells recruited from normal tissue. In normal tissue, the stroma may actually act as a barrier in tumorigenesis by constraining tumor cell proliferation. In tumor tissue, however, stromal components-the main part of tumor microenvironment-could facilitate the process of tumor progression [15]. The majority studies of neoplastic transformation have focused on events that occur within cancer cells, while other studies have addressed the microenvironment of tumor cells supporting tumor progression [9, 17]. Recent study provides more insight into possible initiation and progression of malignant cells. The mechanism underlying tumor-promoting effect of stroma is still not completely clear, which may be might be attributed to tumor microenvironment (i.e., fibroblasts, myoepithelial cells, macrophages, proteases etc.). Increase in abundance of fibroblasts in a tumor causes deposition of fibrotic extra-cellular matrix (ECM). Changes in ECM structure can be further stimulated by proteases, which degrade stroma. Together, this results in disruption of epithelial tissue and remodeling of the ECM, facilitating invasion of tumors cells. In addition, fibroblasts could produce various growth factors, cytokines, and extracellular matrix proteins to promote angiogenesis, which all contribute to tumor growth and progression [18, 19]. In breast tumors and prostatic tumors, it was shown that fibroblast from tumor environment, compared to fibroblasts derived from areas that were not intimately associated with invasive carcinoma, significantly increased growth of epithelium and provided better support for cancer growth [20]. All these findings support the hypothesis that tumor-associated stroma plays an important role in some aspects of cancer biology including tumor growth, transformation and progression, and large numbers of tumor-associated stroma is often associated with the high-grade malignancies and poor prognosis.

In conclusion, our findings indicate that TSR is an independent factor predicting outcome in NSCLC. Because of its low cost, simplicity and availability, it has potential to facilitate the assessment of prognosis and even to stratify the high-risk patients of NSCLC for individual treatment in the future. In the last decades, tumor cells have drawn the attention of the researchers as the main target for therapeutic interventions. However, evidence is growing that the peritumoral microenvironment plays key roles in tumor progression. Future plans are necessary to analyze the underlying mechanisms of the stroma formation using molecular techniques and model systems. Targeting components of the tumor microenvironment have great potential in the clinical practice, particularly when used in combination with other therapeutic agents.

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Disclosure of conflict of interest

None.

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