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ORIGINAL RESEARCH

Prognostic value of *SOX2*, Cyclin D1, P53, and ki-67 in patients with esophageal squamous cell carcinoma

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Abstract: In this study, we evaluated *SOX2*, Cyclin D1, p53, and ki-67 expression immunohistochemically in 117 samples of surgically resected esophageal squamous cell carcinoma (ESCC) and matched normal tumor adjacent tissues and correlated the expression with clinicopathological finding and patient survival. Lymph node metastasis was observed in 36.8% of patients, and organ metastasis was observed in 17.9%. We detected high expression of *SOX2*, Cyclin D1, p53, and ki-67 in 46.1%, 70.1%, 54.7%, and 32.5% of ESCC tissues, respectively. *SOX2* is localized in the tumor cell nuclei, and its expression was significantly associated with N stage (p=0.034) and differentiation (p=0.003) and ki-67 expression (p=0.001), whereas increased Cyclin D1 expression was correlated with high p53 (p=0.015). With regard to survival, we found that ESCC patients with high *SOX2* expression had significantly better survival time than those with low *SOX2* expression (p=0.021). A multivariate Cox analysis revealed that therapy and high p53 expression and venous invasion were independent predictors of unfavorable prognosis in overall survival (p=0.039, p=0.004, and p=0.023, respectively). Furthermore, higher T stage, clinical stage (pTNM), venous invasion, and high p53 expression were independent predictors of a worse progression-free survival. Notably, co-overexpression of p53 and Cyclin D1 was significantly correlated with poor overall survival and progression-free survival (p=0.029 and p=0.0227, respectively). Therefore, *SOX2* might be considered as a potential prognostic indicator and a potential target for therapeutic targets in ESCC. p53 staining and combined p53 and Cyclin D1 expression had significantly unfavorable prognostic value for patients with ESCC. These findings provide more insight into ESCC; thus, further investigations into molecular mechanisms of drug resistance are essential.

Keywords: esophageal squamous cell carcinoma, *SOX2*, Cyclin D1, p53, ki-67, prognosis

Introduction

Esophageal carcinoma is one of the most common malignancies and also remains a leading cause of cancer-related death worldwide.[1] More than 90% cases present as esophageal squamous cell carcinoma (ESCC) in Asian countries.[2] China is a high incidence area of esophageal cancer, and mortality rate due to this disease is as high as 15.2/100 thousand.[3] Esophageal cancer is prone to lymph node metastasis in the early stages of cancer because of its extensive lymphatic drainage network.[4] The overall survival (OS) of esophageal carcinoma patients remains poor, although some improvements have been achieved in treatment.[5] Unlike lung carcinoma, ESCC was not a special molecular routine practice for therapy. Therefore, identification of genes responsible for prognosis of ESCC and understanding their clinical significance are critical in the diagnosis and treatment of this cancer.

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More and more genes involved in the development and tumorigenesis of ESCC are being identified. *SOX2* was the first identified one, as a 317 amino acid transcription factor containing an HMG domain.[6] *SOX2* gene is located at chromosome 3q26.33 and is involved in cell proliferation, apoptosis, and differentiation and affects prognosis.[7,8] *SOX2* was recently shown to be found in several malignancies including lung, pancreatic, colon, cervical, and prostate cancers.[9–12] Furthermore, *SOX2* was reported to be associated with lymph node metastasis in oral squamous cell carcinomas.[13] Intriguingly, *SOX2* was frequently found in squamous cell carcinomas of various organs.[14] However, its expression in ESCC was controversial for prognosis.

Cyclin D1, a well-known cell cycle regulator gene, is located at chromosome 11q13.[15] Cyclin D1 was recognized as a nuclear protein by inactivating the cell cycle suppressive retinoblastoma protein. Ki-67 is a common cell proliferation marker and codes for a 359 KD non-histone nuclear protein.[16] Ki-67 labeling index was found to be significantly associated with poor prognosis in many malignancies, such as lymphoma,[17] neuroendocrine tumors,[18] and bladder cancer.[19] P53 protein was identified as the classic tumor suppressor gene TP53.[20] Yao et al[21] reported that high expression of p53 was associated with poor prognosis in patients with early-stage ESCC. However, the prognostic value of coexpression p53 and Cyclin D1 in ESCC remains unknown.

The present investigation was designed to examine the value of using the combination of p53 and ki-67 or Cyclin D1 in ESCC as prognostic markers. Although some investigations have studied the prognostic value of *SOX2*, no consistent conclusion has been drawn. We aimed to examine the prognostic significance of expression of *SOX2*, Cyclin D1, p53, and ki-67 in ESCC.

Materials and methods

Patients

This study encompassed a retrospective series of 117 patients identified as having ESCC diagnosed between 2008 and 2014 and treated at the First Affiliated Hospital of Xinjiang Medical University. Ethical approval was obtained from the ethics committee of the First Affiliated Hospital of Xinjiang Medical University. Written informed consent for the scientific use of the tissue samples and medical records were obtained from each patient.

Lymph node biopsy was performed in 43 (36.8%) patients, each of whom had at least 1 positive lymph node. According to lymph node ratios (LNR) previously reported,[4] the patients were classified into two groups (LNR: <0.2, LNR: ≥ 0.2). LNR ≥ 0.2 was seen in 21 (17.9%) cases. Vascular invasion was present in 28 (23.9%) cases, and perineural invasion was reported in 30 (25.6%) cases.

According to 2010 the American Joint Committee on Cancer TNM classification, 35 (29.9%), 34 (29.1%), 28 (23.9%), and 20 (17.1%) of the ESCC patients had pTNM stage 1, 2, 3, and 4 disease, respectively. 21 (17.9%) had metastasis at the time of last follow-up. The metastatic sites included lung (n=8), liver (n=3), bone (n=2), peritoneum (n=2), mediastinum (n=1), and multiple organ (n=5).

Immunohistochemistry (IHC)

Tissues were fixed in 10% formalin, sectioned at 5 μm, subsequently deparaffinized in xylene, and rehydrated in 100%, 95%, 80%, and 70% ethanol. All tissues were blocked with hydrogen peroxide for 10 min and heated in a microwave for antigen retrieval. After blocking with 1% goat serum, the sections were incubated with primary antibodies *SOX2* (Abcam, Stoke-on-Trent, UK, dilution 1:1,000), ki-67 (Dako, Santa Clara, CA, USA, dilution 1:100), p53 (Dako, dilution 1:100), and Cyclin D1 (Dako, dilution 1:500) for 60 min at 37°C. After washing in PBS, the sections were incubated with secondary antibody for 30 min at 37°C. After using DAB for staining, the section were dehydrated, and tread with xylene.

The cutoff values for positivity were as follows: *SOX2* staining of 50% or more was considered as positive, having $<50\%$ positivity in the tumor samples was considered as negative. Immunoreactivity to Cyclin D1 was "low" if nuclear staining of tumor cells was $<20\%$ (low Cyclin D1) and "high" if $\geq 20\%$ (high Cyclin D1). p53 was scored depending on the staining intensity (0–3+), as no staining (0), weak (1+), moderate (2+), and strong (3+). Cases with scores of 0–1+ were considerate negative, whereas those with score of 2+–3+ were considered positive. For Ki-67, tumors with nuclear immunoreactivity of 50% or more were considered positive, whereas those with $<50\%$ staining were considered as negative.[22]

Statistical analysis

All statistical analyses were performed using SPSS 14.0 (SPSS Inc, Chicago, IL, USA). The characteristics of the ESCC patients were compared using the χ^2 test. OS and progression-free survival (PFS) were assessed using the Kaplan–Meier method and the log-rank test. Multivariate analysis was carried out using the Cox proportional hazard regression model. $p<0.05$ was considered to be statistically significant.

Results

Clinicopathologic characteristics

The demographic data of the 117 patients with ESCC included in the study and the pathological characteristics are summarized in Table 1. The median age of patients at the time of diagnosis was 62 years (35–83 years). The patients were followed up for a mean of 30 months (range 1–72). 54 (46.1%) patients died during the follow-up period. Of 117 patients, 30 (25.6%) patients received radical surgery and chemotherapy. Nine (7.7%) patients received radical surgery, chemotherapy, and radiotherapy.

Table 1 General characteristics of ESCC patients

Characteristics and finding	n=117
Age (years)	
Range	35–83
Median	62.00
Tumor size (cm)	
Range	0.2–8.0
Median	3.8
Gender	
Male	87 (74.4%)
Female	30 (25.6%)
Differentiation	
Well	14 (12.0%)
Moderate	65 (55.5%)
Poor	38 (32.5%)
Pathological stage	
T1	5 (4.3%)
T2	55 (47.0%)
T3	57 (48.7%)
Lymph metastasis	
Negative	74 (63.2%)
Positive	43 (36.8%)
N stage	
N0	75 (64.1%)
N1	22 (18.8%)
N2	13 (11.1%)
N3	7 (6.0%)
LNR	
<0.2	96 (82.1%)
≥ 0.2	21 (17.9%)
Venous invasion	
Negative	89 (76.1%)
Positive	28 (23.9%)
Perineuronal invasion	
Negative	87 (74.4%)
Positive	30 (25.6%)
pTNM	
I	35 (29.9%)
II	34 (29.1%)
III	28 (23.9%)
IV	20 (17.1%)

Abbreviations: ESCC, esophageal squamous cell carcinoma; LNR, lymph node ratio.

Association of *SOX2* and Cyclin D1 expression with clinicopathological parameters in ESCC

We detected *SOX2*, Cyclin D1, p53, and ki-67 expression in both adjacent normal esophageal mucosa epithelium and ESCC tissues by IHC. We found that those factors were localized in cell nuclei, and representative IHC images of *SOX2*, Cyclin D1, p53, and ki-67 are presented in Figure 1. The blank controls for *SOX2*, Cyclin D1, p53, and ki-67 are presented in Figure 2.

SOX2 expression was detected in both adjacent normal esophageal mucosa epithelium and ESCC tissues. *SOX2* was localized in cell nuclei and was mainly expressed in the basal layer of the normal esophageal squamous epithelial. Of the cancer specimens, *SOX2* strong positivity was observed in 46.1% (54/117) of samples, *SOX2* status of the samples is shown in Figure 1. To evaluate the role of *SOX2* protein in ESCC progression, we analyzed the association between those protein expression and clinicopathological characteristic in ESCC using Pearson's χ^2 test (Table 2). We observed that upregulation of *SOX2* was associated with N stage (p=0.034), differentiation (p=0.003), and ki-67 expression (p=0.001).

Cyclin D1 was localized in cell nuclei and was also expressed in basal layer of esophagus. Positive Cyclin D1 expression was seen in 70.1% (82/117) of samples. Notably, high Cyclin D1 was correlated with increased p53 expression (p=0.015). There was no significant relationship between Cyclin D1 and other clinicopathological parameters.

p53 and ki-67 were both localized in cell nuclei and expressed in basal layer of the normal esophageal squamous epithelial, respectively. We also analyzed p53 and ki-67 expression and observed these factors to be positive in 54.7% (64/117) and 32.5% (38/117) of samples, respectively. p53 expression was significantly associated with Cyclin D1 (p=0.015) and ki-67 (p=0.048). However, p53 expression was not correlated with *SOX2* (p=0.582).

Association clinical feathers in ESCC with patient survival

OS and PFS were analyzed through Kaplan–Meier plots. Univariate analysis showed that N stage (p=0.014), higher pTNM (p=0.006), adjuvant therapy (p=0.044), presence of venous invasion (p=0.015), lymph node metastasis (p=0.007), and high expression of P53 (p=0.018) were associated with OS (Table 3). Additionally, increased p53 expression (p=0.006) and higher pTNM (p=0.0001) were significantly correlated with PFS (Figure 3). Lymph node

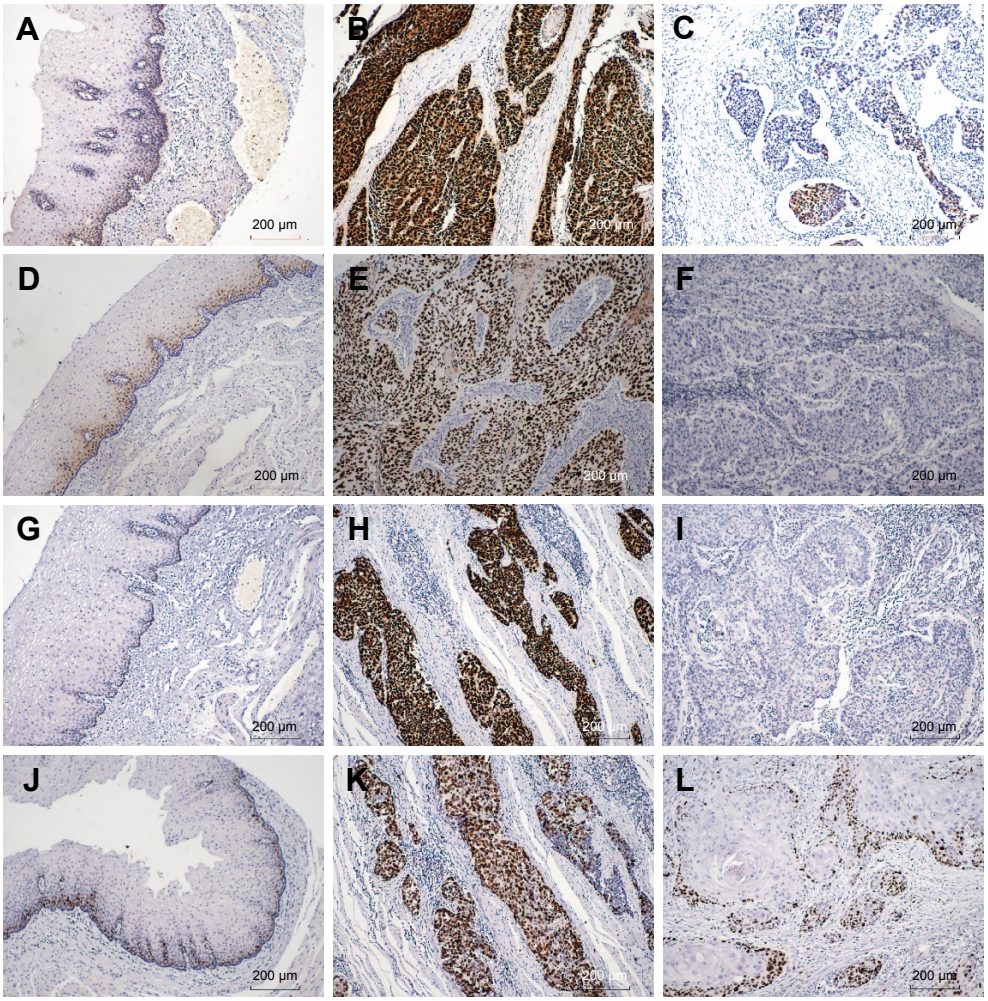

Figure 1 IHC staining of *SOX2*, Cyclin D1, P53, and KI-67 in ESCC tissues.
Notes: (**A**) In normal esophagus mucosa, *SOX2* is localized to the basal layer (100×). (**B**) Strong positive expression of *SOX2* in ESCC (100×). (**C**) *SOX2* was expressed at a low level in ESCC (100×). (**D**) Cyclin D1 expression in normal esophagus epithelium (100×). (**E**) High expression of Cyclin D1 in ESCC (100×). (**F**) Low expression of Cyclin D1 in ESCC (100×). (**G**) P53 was expressed in adjacent normal esophagus mucosa (100×). (**H**) P53 was strongly positive in ESCC (100×). (**I**) P53 was negative in ESCC (100×). (**J**) In normal esophagus mucosa, ki-67 is localized to the basal layer (100×). (**K**) Ki-67 upregulation in ESCC (100×). (**L**) Low expression of ki-67 in ESCC (100×).
Abbreviations: ESCC, esophageal squamous cell carcinoma; IHC, immunohistochemistry.

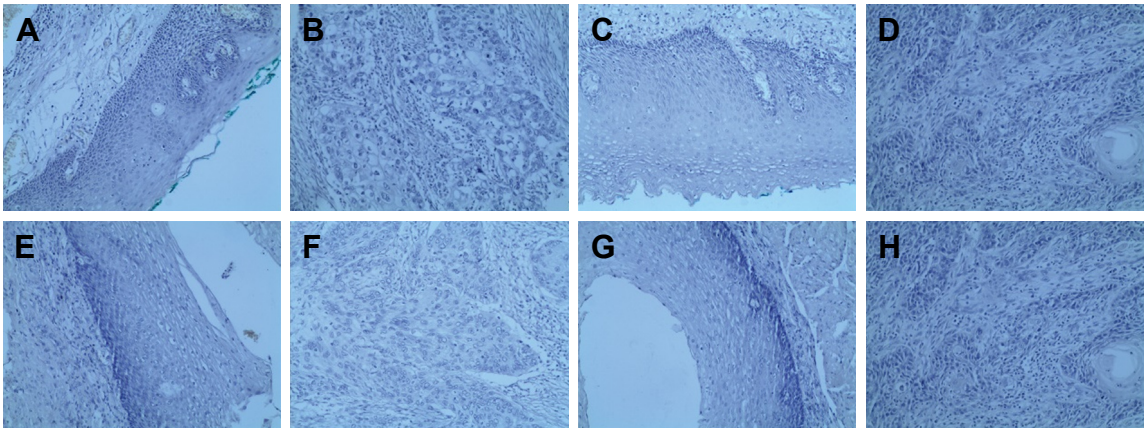

Figure 2 Blank staining of *SOX2*, Cyclin D1, p53, and Ki-67 in ESCC tissues (100×).
Notes: (**A**) Negative control of *SOX2* in esophagus mucosa; (**B**) Negative control of *SOX2* in ESCC; (**C**) Negative control of Cyclin D1 in esophagus mucosa; (**D**) Negative control of Cyclin D1 in ESCC; (**E**) Negative control of p53 in esophagus mucosa; (**F**) Negative control of p53 in ESCC; (**G**) Negative control of Ki-67 in esophagus mucosa; and (**H**) Negative control of Ki-67 in ESCC.
Abbreviation: ESCC, esophageal squamous cell carcinoma.

Table 2 Correlation of Cyclin D1, *SOX2*, and p53 expression with clinicopathological features in 117 ESCC patients

Characteristic	Cyclin D1		*p*-value	*SOX2*		*p*-value	P53		*p*-value
	Low	High		Low	High		Low	High	
Gender									
Male	30	57		48	39		41	46	
Female	5	25	0.104	15	15	0.674	12	18	0.531
Age (years)									
<65	21	47		39	29		33	35	
≥65	14	35	0.840	24	25	0.453	20	29	0.455
Tumor size									
<4 cm	25	60		47	38		37	48	
≥4 cm	10	22	0.825	16	16	0.679	16	16	0.540
Differentiation									
Well	4	10		13	1		7	7	
Moderate	18	47		28	37		30	35	
Poor	13	25	0.779	22	16	0.003	16	22	0.861
Lymph node									
Absent	19	55		41	33		35	39	
Present	16	27	0.213	22	21	0.703	18	25	0.569
N stage									
N0	19	55		42	32		35	39	
N1	7	18		9	16		12	13	
N2	6	6		6	6		4	8	
N3	3	3	0.247	6	0	0.034	2	4	0.745
Pathological stage									
T1	2	3		2	3		4	1	
T2	15	40		27	28		23	32	
T3	18	39	0.778	34	23	0.436	26	31	0.259
Venous invasion									
Absent	26	63		46	43		39	50	
Present	9	19	0.815	17	11	0.515	14	14	0.665
Perineuronal invasion									
Absent	26	61		46	41		38	49	
Present	9	21	1.000	17	13	0.833	15	15	0.671
LNR									
<0.2	26	70		50	46		44	52	
≥0.2	9	12	0.190	13	8	0.475	9	12	1.000
P53									
Low	22	31		27	26				
High	13	51	0.015	36	28	0.582			
Ki-67									
Low	25	54		51	28		41	38	
High	10	28	0.668	12	26	0.001	12	26	0.048
pTNM									
I	11	24		18	17		18	17	
II	8	26		16	18		18	16	
III	13	15		19	9		8	20	
IV	3	17	0.091	10	10	0.385	9	11	0.213
Therapy									
Surgery	24	52		40	36		34	42	
Chemotherapy	7	23		17	13		15	15	
Radiotherapy	3	6	0.681	4	5	0.806	2	7	0.337

Abbreviations: ESCC, esophageal squamous cell carcinoma; LNR, lymph node ratio.

metastasis was not associated with PFS (p=0.052). Moreover, ki-67 was not associated with prognosis in both OS and PFS. This was similar to previous reports[23] that patients receiving both surgery and chemotherapy or radiotherapy showed significantly better prognosis than those only receiving surgery radical (p=0.044).

Cox proportional multivariate analysis of relationships between all the significant variables and patient survival

Table 3 Univariate analysis of factors associated with OS and PFS in ESCC patients

Characteristic	OS		PFS	
	χ^2	*p*-value	χ^2	*p*-value
Gender				
Male/female	0.381	0.537	0.314	0.575
Age, years				
≥65/<65	2.453	0.117	0.291	0.590
Size, cm				
≥4/<4	2.694	1.101	0.465	0.495
Differentiation				
Poor/moderate/well	0.278	0.870	0.080	0.961
Pathological stage				
T1/T2/T3	0.897	0.639	0.611	0.737
Venous invasion				
Present/absent	5.953	0.015	1.751	0.186
Perineuronal invasion				
Present/absent	3.080	0.079	0.805	0.370
Lymph metastasis				
Present/absent	7.172	0.007	3.264	0.052
N stage				
N0/N1/N2/N3	10.623	0.014	1.863	0.601
Involved LNR				
≥0.2/<0.2	3.441	0.064	0.107	0.743
Therapy				
Surgery/chemotherapy/ radiotherapy	6.233	0.044	0.265	0.876
pTNM				
I/II/III/IV	12.316	0.006	28.801	0.0001
Ki-67				
Low/high	0.779	0.378	0.139	0.709
P53				
Low/high	5.623	0.018	7.621	0.006
Cyclin D1				
Low/high	1.412	0.235	0.933	0.334
SOX2				
Low/high	5.291	0.021	1.477	0.224
P53 + Cyclin D1				
Low/high	3.949	0.047	11.470	0.001
P53 + ki-67				
Low/high	0.040	0.841	2.080	0.149

Abbreviations: ESCC, esophageal squamous cell carcinoma; OS, overall survival; PFS, progression-free survival; LNR, lymph node ratio.

are shown in Table 4. We found that therapy (hazard ratio [HR]: 0.490, 95% confidence interval [CI]: 0.249–0.964, *p*=0.039), high expression of p53 (HR: 2.697, 95% CI: 1.373–5.299, *p*=0.004), and venous invasion (HR: 2.373, 95% CI: 1.129–4.987, *p*=0.023) were significant independent predictors of OS. However, *SOX2* overexpression was not a significant independent prognostic factor in prognosis of ESCC (*p*=0.103). In the multivariate analysis, higher T stage, pTNM stage, venous invasion, and high p53 expression were also found to be independent factors affecting PFS (Table 4).

To further confirm the role of *SOX2* expression in ESCC, we analyzed OS and PFS using the Kaplan–Meier method (Figure 4). We found that that ESCC patients with negative *SOX2* expression had a shorter OS (*p*=0.021). However, *SOX2* expression was not associated with PFS (*p*=0.224). Next, we used Kaplan–Meier and a log-rank test to analyze OS rate in ESCC after stratification by *SOX2* expression and clinical feathers. *SOX2* expression was significantly correlated with favorable prognosis in the group with age <65 years (*p*=0.020), tumor size <4 cm (*p*=0.031), moderate differentiation (*p*=0.018), T2 pathological stage (*p*=0.032), no lymph node metastasis (*p*=0.017), the combination of radical surgery and chemotherapy (*p*=0.017), LNR >0.2 (*p*=0.026), lower p53 expression (*p*=0.029), and higher Cyclin D1 expression (*p*=0.031). Moreover, *SOX2* expression correlated significant with OS among female patients (*p*=0.027).

The Kaplan–Meier survival analysis with log-rank test showed that high p53 expression was associated with shorter OS and PFS (*p*=0.018 and *p*=0.006, respectively) in ESCC. Notably, the combination of increased p53 and Cyclin D1 was associated with OS and PFS (*p*=0.047 and *p*=0.001, respectively). However, the combination of positive p53 and ki-67 was not associated with OS and PFS (*p*=0.047 and *p*=0.001, respectively, in Figure 5). In multivariate analysis, the combination of increased p53 and Cyclin D1 was an independent predictor of poor prognosis of ESCC patients (Table 4). Next, we found that the combination of p53 and Cyclin D1 was significantly correlated with poor prognosis in radical surgery (*p*=0.020) and in the chemotherapy (*p*=0.020) after stratification.

Discussion

ESCC remains an aggressively and lethal malignant carcinoma. Previous studies have reported that the survival rate at 5 years was 40%.[24] Thus, it is important to investigate the probable molecular markers to improve the outcome of patients with ESCC.

SOX2 is an important transcription factor and an embryonic stem cell factor. Several studies found that *SOX2* was expressed in a wide variety of squamous cell carcinomas by promoting tumor growth, invasion, and metastasis.[25] *SOX2* could promote breast cancer cell proliferation and tumorigenesis in in vivo and vitro studies.[26] As previously described, high level of *SOX2* is associated with poor prognosis in human oral squamous cell carcinomas.[27] On the other hand, Maehara et al[28] demonstrated that reduced expression of *SOX2* was correlated with advanced clinical T stage (*p*=0.003) and poor differentiation (*p*=0.002) of tumors in ESCC patients. Wilbertz et al[29] also found that overexpression of *SOX2* was correlated with favorable prognosis in lung

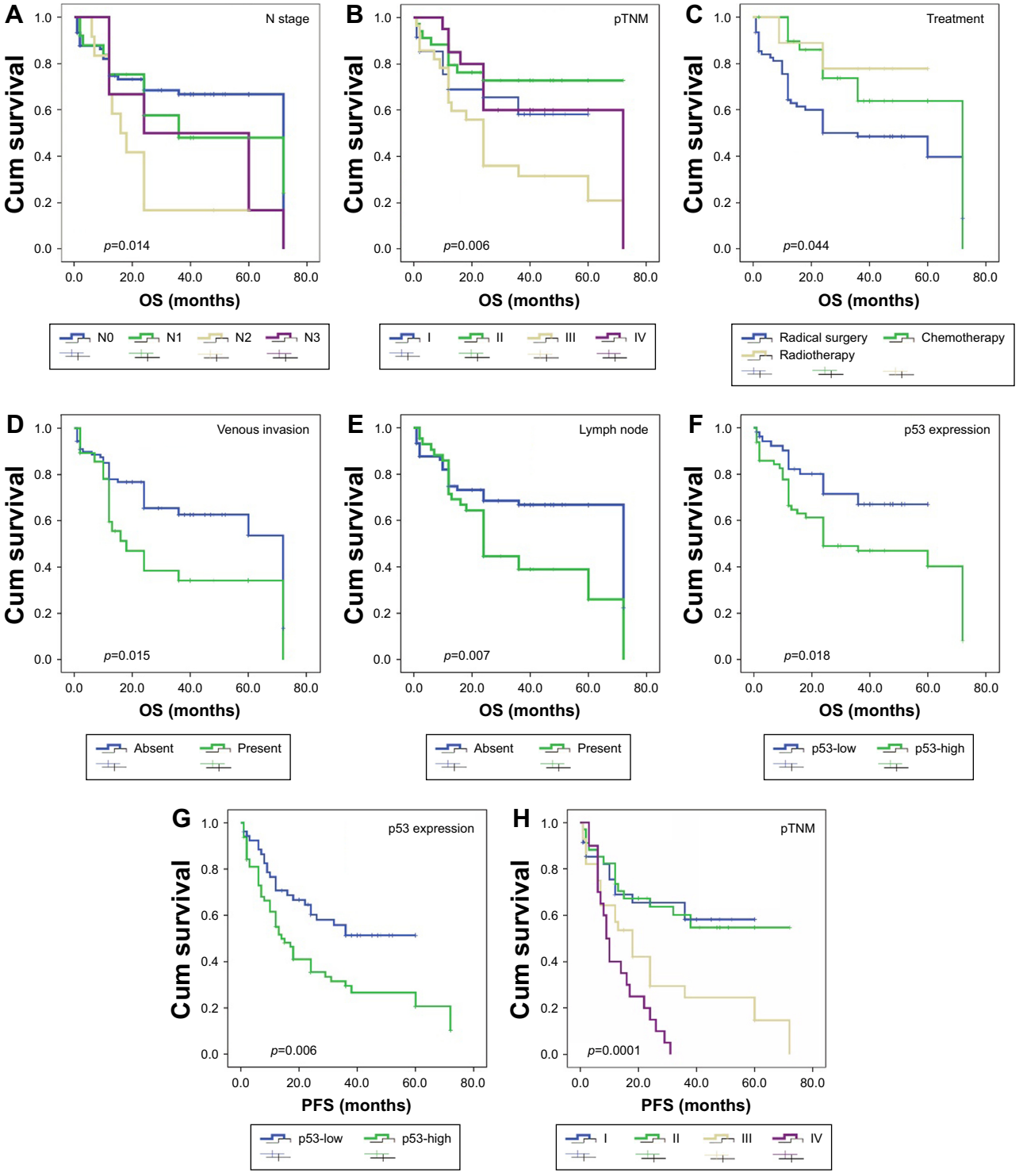

Figure 3 OS and PFS analysis of patients with ESCC using the Kaplan–Meier method.
Notes: OS according to (**A**) N stage; (**B**) pTNM; (**C**) treatment; (**D**) venous invasion; (**E**) lymph node; (**F**) p53 expression (low vs high). PFS according to (**G**) p53 expression (low vs high); (**H**) pTNM.
Abbreviations: OS, overall survival; PFS, progression-free survival; ESCC, esophageal squamous cell carcinoma.

squamous cell carcinoma. There is still controversy regarding the significance of *SOX2* expression for prognosis.

In the current study, we found that *SOX2* was highly expressed in 46.1% (54/117) of primary ESCC patients. More importantly, we observed that upregulation of *SOX2* was associated with N stage (p=0.034), differentiation (p=0.003), and ki-67 expression (p=0.001). With regard to survival, we found that ESCC patients with high *SOX2* expression had

Table 4 Multivariate analysis of factors associated with OS and PFS for ESCC

Characteristic	OS			PFS		
	95% CI	HR	*p*-value	95% CI	HR	*p*-value
Therapy						
Surgery/chemotherapy/ radiotherapy	0.249–0.964	0.490	0.039	0.336–0.960	0.568	0.035
Venous invasion						
Present/absent	1.129–4.987	2.373	0.023	1.114–4.269	2.181	0.023
P53						
Low/high	1.373–5.299	2.697	0.004	1.222–3.747	2.140	0.008
SOX2						
Low/high	0.272–1.126	0.553	0.103	0.399–1.239	0.703	0.223
pTNM						
I/II/III/IV	0.776–1.603	1.115	0.555	1.592–2.877	2.140	0.0001
P53 + Cyclin D1						
Low/high	1.184–22.649	5.178	0.029	1.17–14.049	4.057	0.027

Abbreviations: CI, confidence interval; ESCC, esophageal squamous cell carcinoma; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

significantly better survival time than those with low *SOX2* expression (p=0.021) by Kaplan–Meier analysis. Subgroup analysis found that in the low *SOX2* expression group, receiving both radical surgery and chemotherapy did not lead to any significant survival benefit. Moreover, subgroup analysis showed that *SOX2* expression was correlated with OS (p=0.017) among ESCC patients without lymph node metastasis. Similar to the present study, a previous study found that increased *SOX2* expression was significantly associated with absence of clinical lymph node metastasis (p=0.011).[30] However, we found that high *SOX2* expression was not associated with PFS (p=0.224). Our findings suggested that *SOX2* might

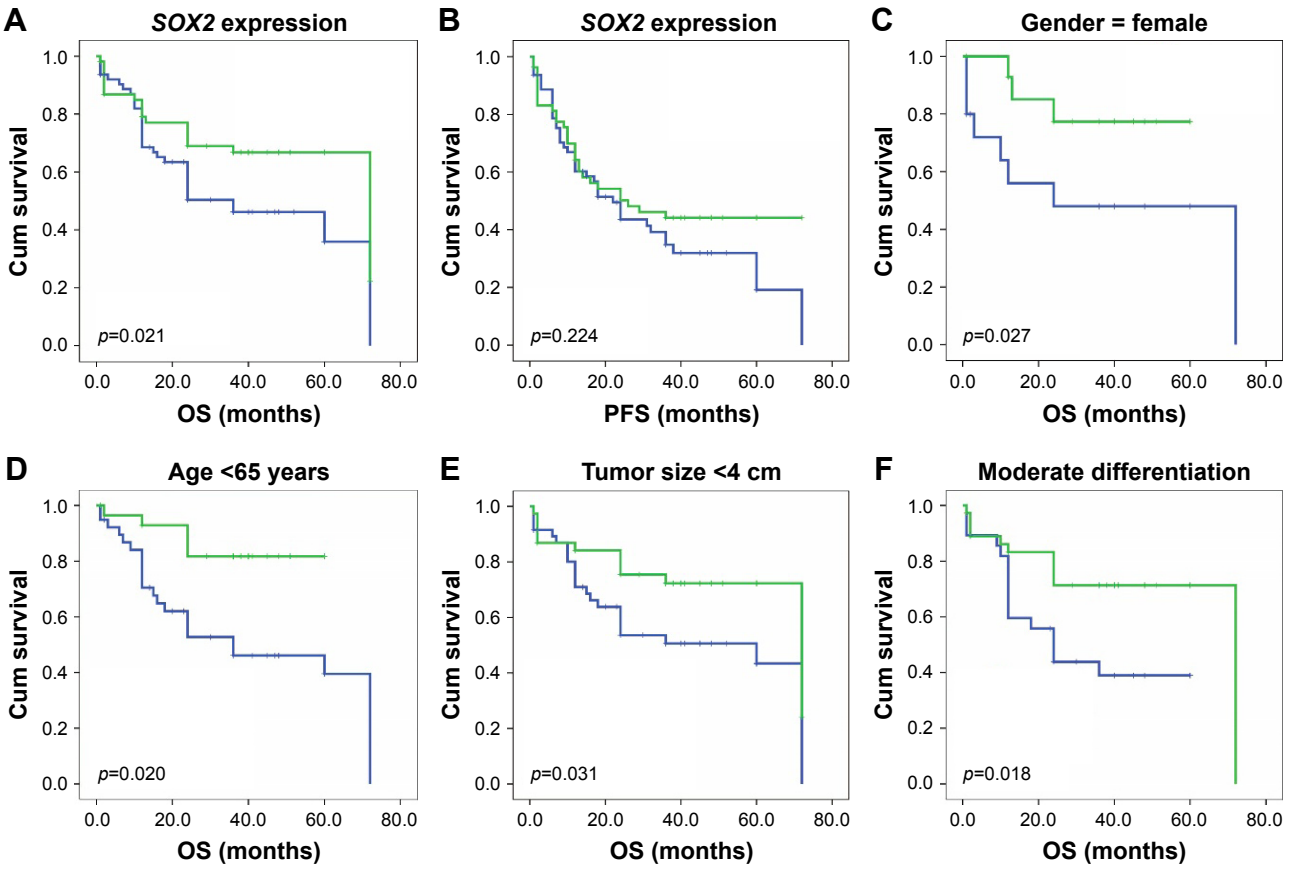

Figure 4 (*Continued*)

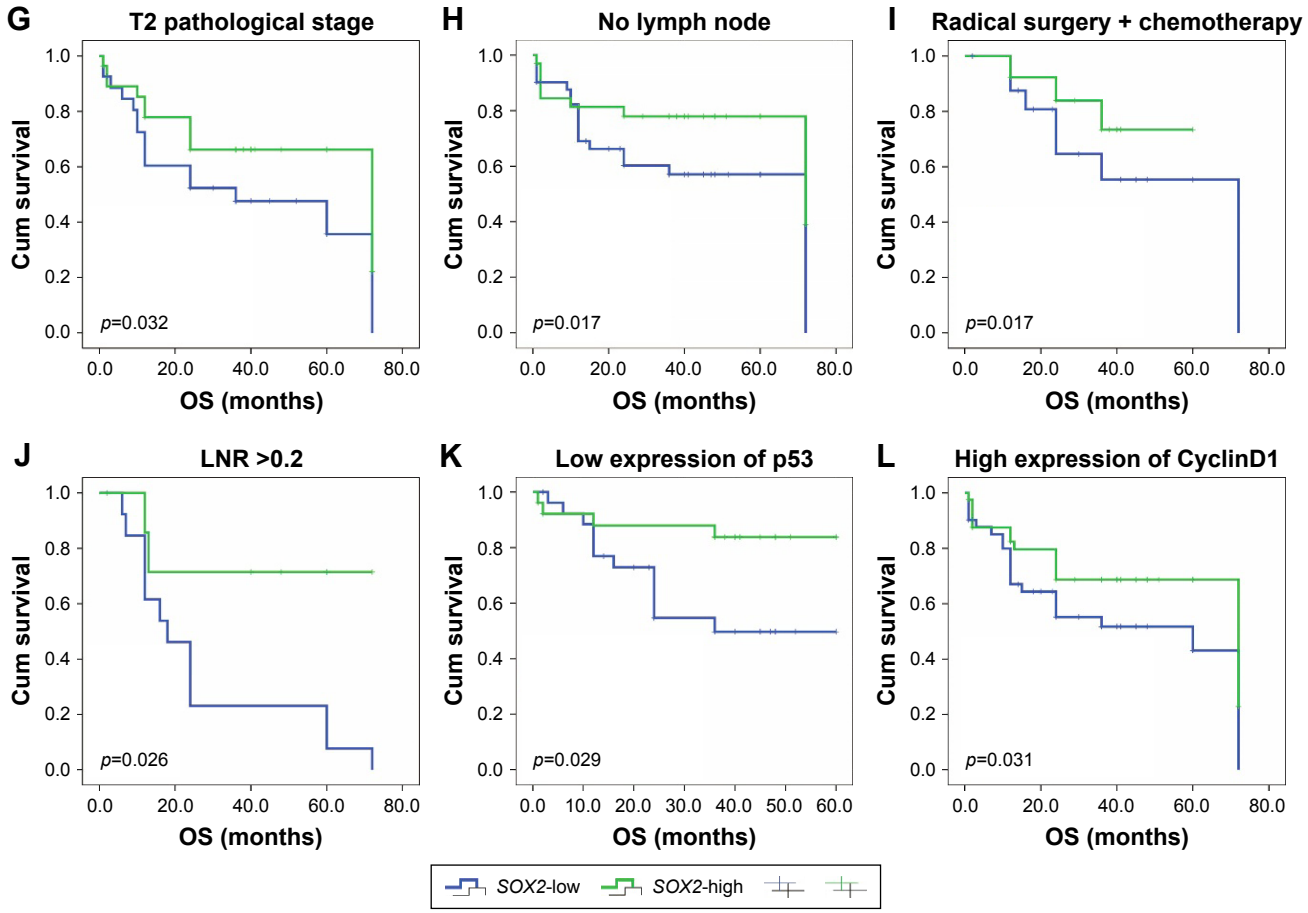

Figure 4 OS and PFS based on *SOX2* expression.
Notes: (**A**) OS; (**B**) PFS. The OS of ESCC patients was analyzed using Kaplan–Meier and stratified by *SOX2* expression level. (**C**) Female; (**D**) age <65 years; (**E**) tumor size <4 cm; (**F**) moderate differentiation; (**G**) pathological stage: T2; (**H**) absent lymph node metastasis; (**I**) receiving both radical surgery and chemotherapy; (**J**) LNRs ≥0.2; (**K**) low expression of p53; and (**L**) high expression of Cyclin D1.
Abbreviations: LNR, lymph node ratio; OS, overall survival; PFS, progression-free survival; ESCC, esophageal squamous cell carcinoma.

potentially act as a prognostic biomarker in ESCC. However, it is still unclear why the absent *SOX2* was correlated with malignant clinical phenotypes.

p53 protein is located at chromosome 17p13.1 and codes for a 393 amino acid protein. Recent findings have shown that p53 expression is upregulated, statistically significantly, in ESCC when compared with normal esophageal squamous epithelial.[31] Xu et al[32] found that higher expression of p53 was correlated with a poorer differentiation level (p=0.044) and associated with a more advanced clinical stage (p=0.015) among 830 operable ESCC patients. In the present study, we also found that p53 was highly expressed in 54.7% (64/117) of primary ESCC patients. Sankalecha et al[33] found that overexpression of p53 was associated with increasing TNM stage in 91 ESCC patients. However, we found that the expression of p53 was not associated with pTNM (p=0.213). With regard to survival, our study revealed that ESCC patients with p53 overexpression had lower OS and PFS rates than patient without p53 expression. Multivariate analysis demonstrated that p53 expression was an independent predicator for poor prognosis of ESCC patients.

This is the first study reporting that the combination of increased p53 and Cyclin D1 was associated with OS and PFS in ESCC (p=0.047 and p=0.001, respectively). However, coexpression of increased p53 and ki-67 was not associated with OS and PFS. Recent studies showed that p53 expression could predict cisplatin or 5-fluorouracil treatment outcome in ESCC.[23,34] However, our data showed that Cyclin D1, *SOX2*, p53, and ki-67 were not associated with pTNM (p=0.091, p=0.385, p=0.213, and p=0.582, respectively) and treatment (p=0.681, p=0.806, p=0.337, and p=0.668, respectively). Next, we used Kaplan–Meier and a log-rank test to analyze OS rate in ESCC after stratification by coexpression of p53 and Cyclin D1. The combination of p53 and Cyclin D1 was significantly correlated with poor prognosis in the radical surgery (p=0.020) and in the chemotherapy

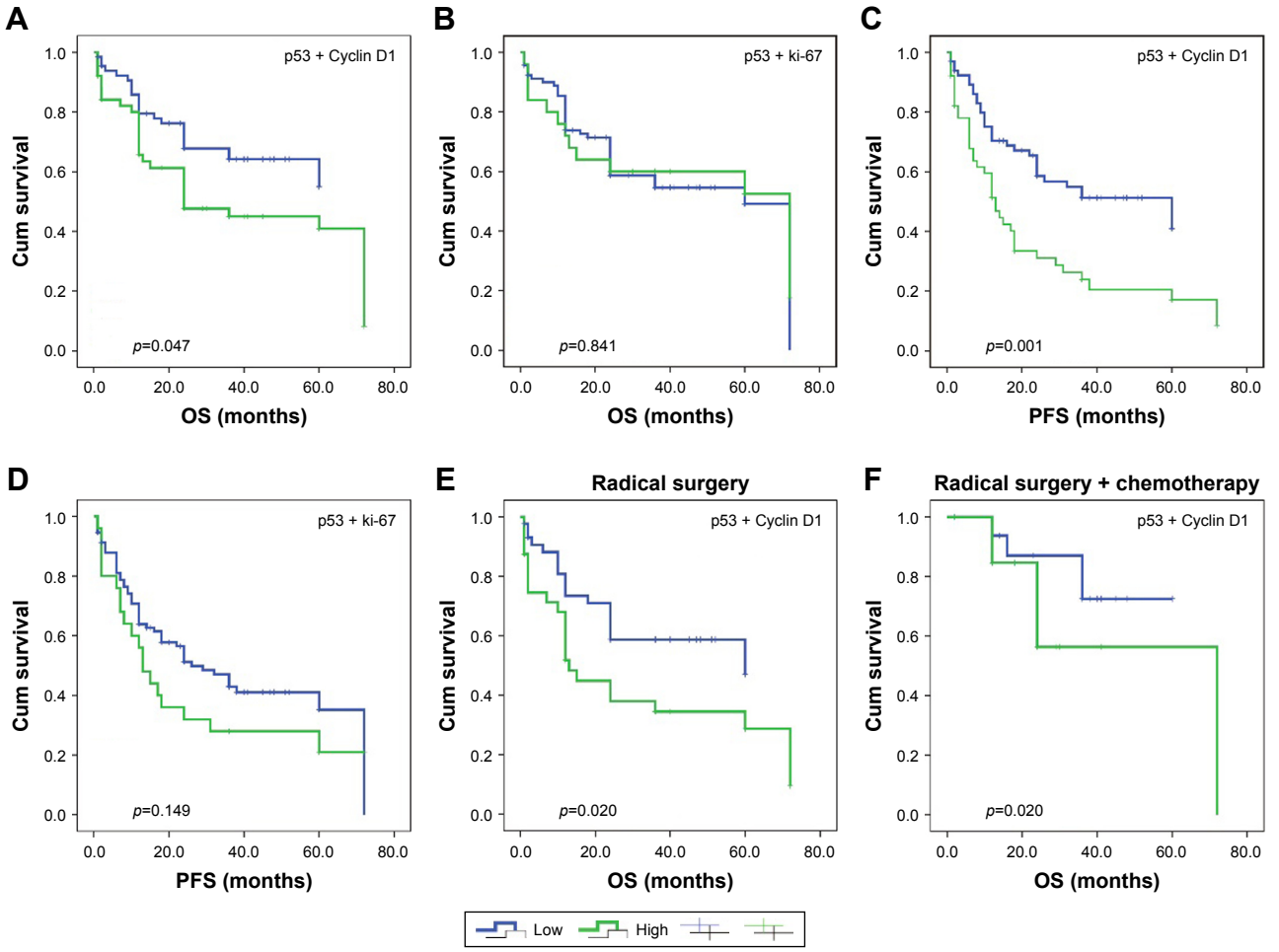

Figure 5 OS according to combined p53-Cyclin D1 expression.
Notes: (**A**) Combined p53-Ki-67 expression; (**B**) PFS based on combined p53-Cyclin D1 expression; (**C**) coexpression of p53 and Ki-67; (**D**) OS and PFS based on coexpression of p53 and Cyclin D1 stratified by treatment; (**E**) receiving radical surgery treatment; and (**F**) receiving both radical surgery and chemotherapy.
Abbreviations: OS, overall survival; PFS, progression-free survival.

(p=0.020) groups. It was suggested that the combination of p53 and Cyclin D1 could be used as a molecular target for ESCC therapy.

In conclusion, our study found that treatment modality, high expression of p53, and venous invasion were significant independent predictors of OS. We also found that higher T stage, pTNM, venous invasion, and high p53 expression were significantly associated with worse PFS. Notably, high expression level of *SOX2* was associated with favorable prognosis in ESCC. The combination of p53 and Cyclin D1 was significantly correlated with poor prognosis in OS and PFS (p=0.047 and p=0.001, respectively). Taken together, *SOX2* and p53 could be used as prognostic factors and as targets for molecular targeted therapy in patients with ESCC.

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Disclosure

The authors report no conflicts of interest in this work.

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