

ALK-Positive Renal Cell Carcinoma in a Large Series of Consecutively Resected Korean Renal Cell Carcinoma Patients

Cheol Lee¹ · Jeong Whan Park¹
Ja Hee Suh¹ · Kyung Han Nam¹
Kyung Chul Moon^{1,2}

¹Department of Pathology, Seoul National University College of Medicine, ²Kidney Research Institute, Medical Research Center, Seoul National University College of Medicine, Seoul, Korea

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Corresponding Author

Kyung Chul Moon, M.D.
Department of Pathology, Kidney Research Institute,
Medical Research Center, Seoul National University
College of Medicine, 103 Daehak-ro, Jongno-gu,
Seoul 110-799, Korea
Tel: +82-2-2072-1767
Fax: +82-2-743-5530
E-mail: blue7270@snu.ac.kr

Background: Recently, there have been a few reports of renal cell carcinoma (RCC) cases with anaplastic lymphoma kinase (*ALK*) gene fusion. In this study, we screened consecutively resected RCCs from a single institution for *ALK* protein expression by immunohistochemistry, and then we performed fluorescence *in situ* hybridization to confirm the *ALK* gene alteration in *ALK* immunohistochemistry-positive cases. **Methods:** We screened 829 RCCs by *ALK* immunohistochemistry, and performed fluorescence *in situ* hybridization analysis using *ALK* dual-color break-apart rearrangement probe. Histological review and additional immunohistochemistry analyses were done in positive cases. **Results:** One *ALK*-positive case was found. Initial diagnosis of this case was papillary RCC type 2. This comprises 0.12% of all RCCs (1/829) and 1.9% of papillary RCCs (1/53). This patient was a 44-year-old male with RCC found during routine health check-up. He was alive without evidence of disease 12 years after surgery. The tumor showed a papillary and tubular pattern, and showed positivity for CD10 (focal), epithelial membrane antigen, cytokeratin 7, pan-cytokeratin, PAX-2, and vimentin. **Conclusions:** We found the first RCC case with *ALK* gene rearrangement in Korean patients by *ALK* immunohistochemistry among 829 RCCs. This case showed similar histological and immunohistochemical features to those of previous adult cases with *ALK* rearrangement, and showed relatively good prognosis.

Key Words: *ALK*; Carcinoma, renal cell; Immunohistochemistry; *In situ* hybridization, fluorescence

Renal cancer is a common cancer, with an incidence of about 10.1% in Korean males in 2010.¹ Renal cell carcinoma (RCC) of adults represents 90% of all renal cancers.² The 5-year survival rate of patients with localized RCC is more than 70%, which is relatively excellent, although there are some differences according to the subtype. However, the 5-year survival rate of patients with advanced RCC is poor.³ Recently, many targeted therapies have been tried on advanced RCC patients.⁴

After the anaplastic lymphoma kinase (*ALK*) gene rearrangement was first reported in anaplastic large-cell lymphoma in 1994, it was reported in several human cancers including diffuse large B-cell lymphoma, plasmacytoma, non-small cell lung cancer, and neuroblastoma and its oncogenic role is still under research.⁵⁻⁹ Even though the physiologic role of the *ALK* gene is still being uncovered, the clinical therapeutic trial for a kinase inhibitor against *ALK* in non-small lung cancer patients is ongoing, and early reports indicate excellent interim result.¹⁰ The recent case report showed an excellent treatment outcome with

the *ALK* inhibitor on an *ALK*-rearranged inflammatory myofibroblastic tumor patient,¹¹ and some *in vitro* studies showed the effectiveness of the *ALK* inhibitor on *ALK*-positive anaplastic large cell lymphoma, diffuse large B-cell lymphoma, and neuroblastoma.¹²⁻¹⁴ Therefore, we anticipate increased use of *ALK* inhibitors in the treatment of human cancers in the future.

The detection methods of *ALK* gene rearrangement are divided into two major categories. One is the method detecting gene rearrangement indirectly via *ALK* immunohistochemistry, which detects protein overexpression, and the other is the direct method of *in situ* hybridization.¹⁵⁻¹⁷

Several studies have reported RCC cases with *ALK* gene fusion and the fusion partners of these genes have been diverse. The subtypes of these RCCs were non-clear cell type such as papillary type, medullary type, and unclassified type.¹⁸⁻²¹ But there has been no study on *ALK* gene rearrangement in Korean RCC patients.

In this study, we screened consecutively resected RCCs from

a single institution for ALK protein expression by immunohistochemistry, and then we performed fluorescence *in situ* hybridization (FISH) analysis to confirm the *ALK* gene alteration in ALK immunohistochemistry-positive cases. Here, we report a RCC case with *ALK* gene rearrangement among 829 RCCs.

MATERIALS AND METHODS

Patients and tissue microarray

We examined 829 RCCs from patients who underwent radical or partial nephrectomy between 1995 and 2005 at the Seoul National University Hospital. RCCs included 689 (83.1%) clear cell RCC, 73 (8.8%) chromophobe RCC, 53 (6.4%) papillary RCC, and 14 (1.7%) other rare subtypes. Tissue microarray (TMA) blocks consisted of a representative tumor core section (2 mm in diameter) from each formalin-fixed paraffin block (SuperBioChips Laboratories, Seoul, Korea). Clinical and pathological information were collected from electronic medical records and pathologic reports. This study was approved by the Institutional Review Board (IRB) of Seoul National University Hospital.

Immunohistochemistry

Immunohistochemical staining for ALK expression was performed on 4 μ m-thick sections taken from the TMA blocks. Monoclonal mouse anti-human ALK antibody (Novocastra, Newcastle upon Tyne, UK) was diluted 1:100 and immunohistochemistry was performed using the Ventana Benchmark XT automated staining system (Ventana Medical Systems, Tucson, AZ, USA).¹⁵⁻¹⁷ Additional immunohistochemistry for CD10 (ready-to-use, Novocastra), cytokeratin 7 (1:300, Dako, Glos-

trup, Denmark), cytokeratin 20 (1:50, Dako), pan-cytokeratin (1:300, Dako), PAX-2 (1:100, Epitomics, Burlingame, CA, USA), epithelial membrane antigen (EMA; 1:300, Dako), vimentin (1:500, Dako), alpha-methylacyl-CoA racemase (AM-ACR; 1:300, Dako), c-kit (1:300, Dako), TFE3 (1:1,500, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), and human melanoma black 45 (HMB45; 1:200, Dako) were also performed for ALK immunohistochemistry-positive cases.

Fluorescence *in situ* hybridization analysis

For analysis of the *ALK* gene alteration, we performed FISH analysis for ALK immunohistochemistry-positive cases. Two-micrometer-thick sections were deparaffinized, dehydrated, immersed in 0.2 N HCl, boiled in a microwave in citrate buffer (pH 6.0), and incubated in 1 M NaSCN for 35 minutes at 80°C. Sections were then immersed in pepsin solution and the tissues were fixed in 10% neutral-buffered formalin. Dual-probe hybridization was performed as described previously²² using Vysis *ALK* dual-color break-apart rearrangement probe (Abbott Molecular, Abbott Park, IL, USA). FISH signals for each locus-specific FISH probe were assessed under an Olympus BX51TRF microscope (Olympus, Tokyo, Japan) equipped with a triple-pass filter (DAPI/Green/Orange).

RESULTS

Identification of ALK immunohistochemical positivity and *ALK* gene alteration

ALK immunohistochemical staining showed only one positive case among 829 RCCs (Fig. 1A), and there were no cases

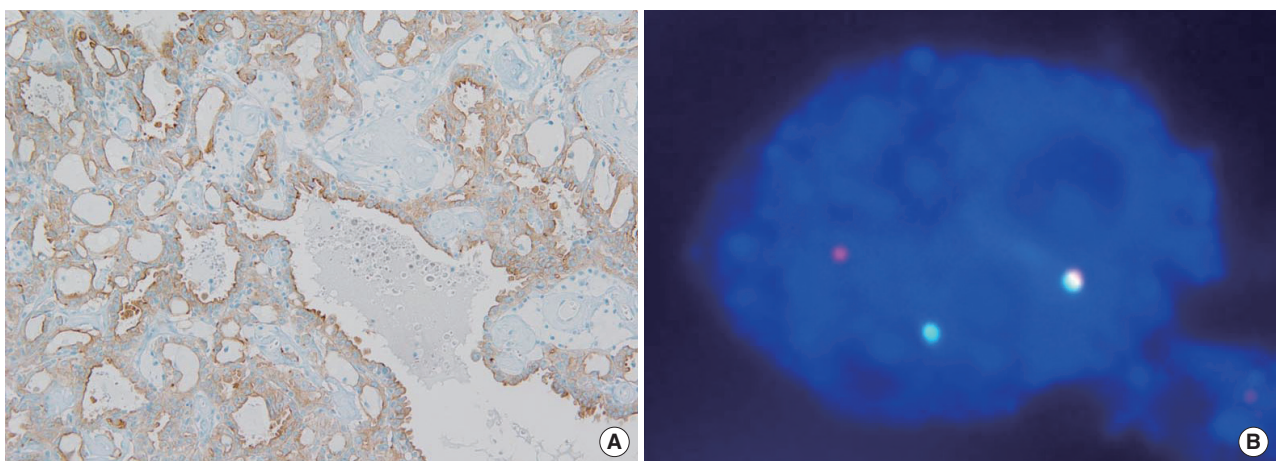


Fig. 1. Anaplastic lymphoma kinase (ALK) immunohistochemistry and fluorescence *in situ* hybridization. (A) Tumor cells show diffuse strong positivity for *ALK* in immunohistochemistry. (B) FISH analysis using dual color *ALK* break-apart probe shows one red, one green, and one fused signal indicating one rearranged *ALK* gene and one intact *ALK* gene.

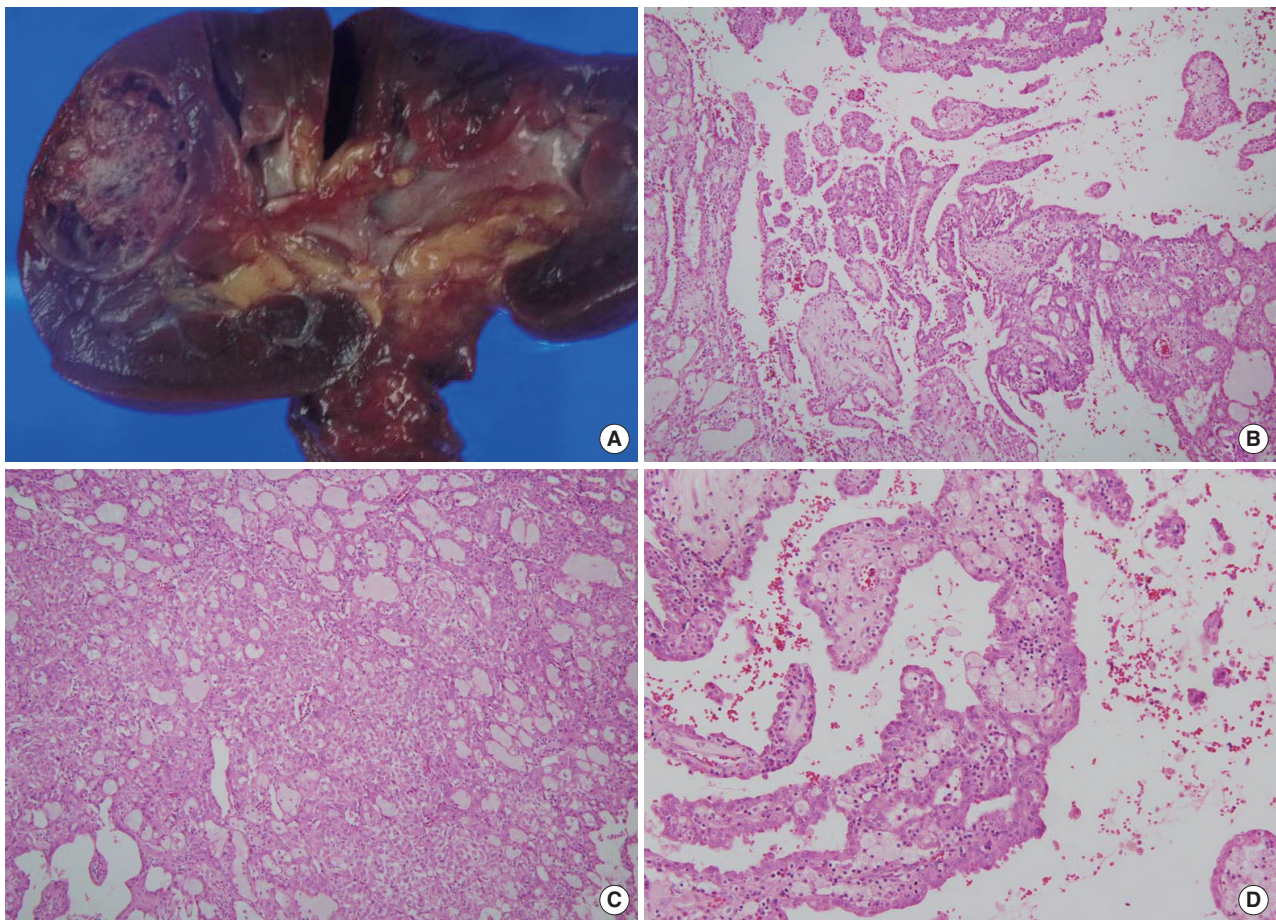


Fig. 2. Pathologic features of renal cell carcinoma showing anaplastic lymphoma kinase (*ALK*) rearrangement. (A) Grossly, the tumor is a well-circumscribed, partially cystic mass. Microscopically, the tumor shows a papillary (B), tubular or solid pattern (C) with occasional foam cell collections (D).

showing borderline positivity. The initial diagnosis of this case was papillary RCC type 2. This case also revealed the break-apart signals in *ALK* FISH analysis (Fig. 1B). This comprises of 0.12% of all RCCs (1/829) and 1.9% of papillary RCCs (1/53).

Patient information

The patient was a 44-year-old male. The RCC was incidentally found by ultrasonography during a routine health check-up and radical nephrectomy was done. The patient had no history of sickle cell trait. He was alive, without evidence of disease, 12 years after surgery.

Pathologic findings

The size of tumor was 3×3×2.5 cm. The tumor was confined to the renal parenchyme and there was no regional lymph node metastases in three regional lymph nodes (pT1aN0 by American Joint Committee on Cancer [AJCC] 7th edition²³).

Grossly, the tumor was a well-circumscribed, partially cystic mass (Fig. 2A). Microscopically, the tumor showed a papillary, tubular or solid pattern with occasional foam cell collections. Tumor cells were columnar or cuboidal cells with abundant eosinophilic cytoplasm (Fig. 2B-D). No psammoma bodies were found. Initial diagnosis of this case was papillary RCC type 2 and the Fuhrman nuclear grade was 3. Immunohistochemical staining showed positivity for CD10 (focal), EMA, cytokeratin 7, pan-cytokeratin, PAX-2, and vimentin and negativity for cytokeratin 20, AMACR, c-kit, TFE3, and HMB45 (Fig. 3).

DISCUSSION

Recently, six RCC cases showing *ALK* gene rearrangement were reported by 4 independent groups.¹⁸⁻²¹ Two of the reported cases were pediatric cases (6- and 16-year-old) with a history of sickle cell trait and the RCC subtypes of these cases were renal

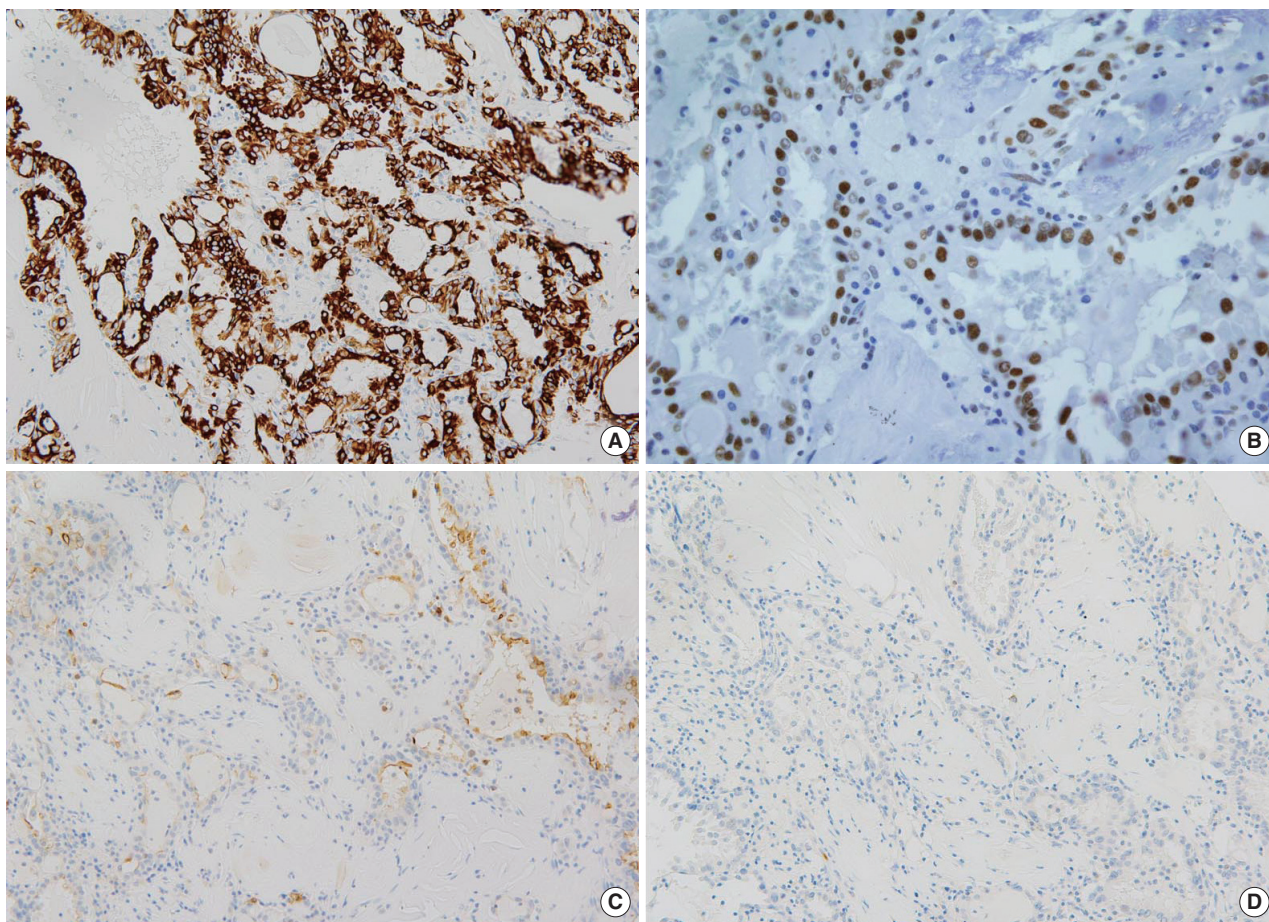


Fig. 3. Immunohistochemical findings of renal cell carcinoma showing anaplastic lymphoma kinase (*ALK*) rearrangement. The tumor cells are positive for cytokeratin 7 (A), PAX-2 (B), and focal positive for CD10 (C) and negative for alpha-methylacyl-CoA racemase (D).

Table 1. Seven *ALK*-positive renal cell carcinomas

Case No.	Age (yr)	Sickle cell trait	Subtype	Size (cm)	Fuhrman grade	TNM stage ²³	Follow-up	Fusion partner	Reference
1	16/M	Yes	Indeterminate	6.2	Not mentioned	T1bN1M0	0.75 yr, alive, no evidence of disease	<i>VCL</i>	Debelenko <i>et al.</i> ¹⁸
2	6/M	Yes	Medullary	4.5	Not mentioned	T1bN0M0	1.75 yr, alive, no evidence of disease	<i>VCL</i>	Marino-Enriquez <i>et al.</i> ¹⁹
3	36/F	No	Unclassified	4	Not mentioned	T1aN0M0	2 yr, alive, no evidence of disease	<i>TPM3</i>	Sugawara <i>et al.</i> ²¹
4	53/F	No	A: papillary, B: clear cell (double cancer)	A: 2.5, B: 0.6	Not mentioned	T1aN0M0	7 yr, alive, no evidence of disease	<i>EML4</i>	Sugawara <i>et al.</i> ²¹
5	61/M	No	Papillary	5	2	T1bNXM0	4 yr, death from disease	Unchecked	Sukov <i>et al.</i> ²⁰
6	59/M	No	Papillary	5.5	3	T1bNXM0	1.4 yr, death from disease	Unchecked	Sukov <i>et al.</i> ²⁰
7	44/M	No	Papillary	3	3	T1aN0M0	12 yr, alive, no evidence of disease	Unchecked	Present case

ALK, anaplastic lymphoma kinase; M, male; *VCL*, vinculin; F, female; *TPM3*, tropomyosin 3; *EML4*, echinoderm microtubule-associated protein like 4.

medullary carcinoma and RCC undetermined.^{18,19} Renal medullary carcinoma was almost exclusively associated with sickle cell trait.² However, the following 4 cases were adult cases without history of sickle cell trait and subtypes were papillary RCC in 3 cases and one unclassified RCC.^{20,21} In this study, we screened 829 RCCs and found one case with *ALK* immunohistochemical

positivity and *ALK* gene rearrangement. Our case is also an adult male case without history of sickle cell trait and the initial diagnosis was papillary RCC type 2. The fusion partners of *ALK* in RCC were variable, with *VCL* in two pediatric cases and *TPM3* and *EML4* in two adult cases.^{18,19,21} In this study, we did not check the *ALK* fusion partner (Table 1).

Two previous adult cases showed poor prognosis,²⁰ and the other two adult cases had a relatively short follow-up period.²¹ Our patient is alive without evidence of RCC for 12 years and the follow-up period for this patient was the longest of all reported cases. This case and the previous cases suggest that RCC with *ALK* gene rearrangement may show variable prognosis.

Histologic features of reported RCC cases with *ALK* rearrangement are variable. Two previous pediatric cases showed typical histologic features of renal medullary carcinoma¹⁹ or mixed features of renal medullary carcinoma, chromophobe RCC and transitional cell carcinoma.¹⁸ Three previous adult cases had the histologic features of papillary RCC and another case showed papillary, tubular or cribriform growth pattern. Psammoma bodies were found in all previous adult cases and foam cell collection was described in 3 out of 4 cases.^{20,21} Our case also showed the features of papillary RCC having foam cell collections but psammoma bodies were not found. Excluding the two pediatric cases with sickle cell trait, all four previous adult cases and our case showed the papillary growth pattern.^{20,21}

Two previous studies included large-scale screenings to detect RCC with *ALK* gene alteration. *ALK* rearrangement was mainly found in papillary RCC in these two studies, and they could not find *ALK* rearrangement in 505 clear cell RCC and 34 chromophobe RCC.^{20,21} We also could not find *ALK* expression in 689 clear cell RCCs, 73 chromophobe RCCs, and 14 rare RCC subtypes by immunohistochemistry. Taken together, the results of previous reports and this study indicate that RCC with *ALK* rearrangement may be a particular subset of papillary RCC.

It is difficult to define the frequency of RCC with *ALK* rearrangement. A previous large-scale study showed the very low incidence of RCC with *ALK* rearrangement (0.56% of all RCCs), but they occupied 3.7% of non-clear cell and non-chromophobe RCC (2/32).²¹

Previous adult RCC cases with *ALK* rearrangement showed similar immunohistochemical staining results, with positivity for cytokeratin, cytokeratin 7, and focal positive or negative for CD10.^{20,21} These immunohistochemical results are compatible with those of typical papillary RCCs and our case also showed similar results of positivity for cytokeratin and cytokeratin 7, and focal positivity for CD10.

Many targeted therapies have been used for advanced RCC. *ALK* rearrangement in RCC may add another option for treatment of advanced RCC. *ALK* inhibition has been tried for treatment of other tumors with *ALK* rearrangement and some successful results has been reported.^{10,11} Therefore, we can try *ALK* inhibition in the treatment of advanced RCC with *ALK* rear-

angement. FISH has been accepted as a standard method for detecting *ALK* gene rearrangement, but also has disadvantages in terms of cost and time. Instead of FISH, *ALK* immunohistochemistry can be a reliable screening tool.²⁴ We would have found our case from screening based on *ALK* immunohistochemistry, which suggests that *ALK* immunohistochemistry is useful for the detection of RCC with *ALK* rearrangement.

In conclusion, as far as we know, we have found the first RCC case with *ALK* gene rearrangement in Korean patients among 829 RCCs. This case showed similar histological and immunohistochemical features to previous adult cases with *ALK* rearrangement, and showed a relatively good prognosis.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

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