Comprehensive analyses of $N^6$-methyladenosine-related long noncoding RNA profiles with prognosis, chemotherapy response, and immune landscape in small cell lung cancer

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Abstract

Small cell lung cancer (SCLC) is the most devastating subtype of lung cancer with no clinically available prognostic biomarkers. $N^6$-methyladenosine ($m^6A$) and noncoding RNAs play critical roles in cancer development and treatment response. However, little is known about $m^6A$-related long noncoding RNAs (lncRNAs) in SCLC. We used 206 limited-stage SCLC (LS-SCLC) samples from two cohorts to undertake the first and most comprehensive exploration of the $m^6A$-related lncRNA profile in SCLC and constructed a relevant prognostic signature. In total, 289 $m^6A$-related lncRNAs were screened out. We then built a seven-lncRNA-based signature in the training cohort with 48 RNA sequencing data using univariate and multivariate Cox regression models. The signature was well validated in an independent cohort containing 158 cases with quantitative PCR data. In both cohorts, the signature divided patients into high- and low-risk groups with significantly different survival rates (both $p<0.001$). Our signature predicted chemotherapy survival benefit in patients with LS-SCLC. Receiver operating characteristic and C-index analyses indicated that the signature was better at predicting prognosis and chemotherapy benefit than other clinicopathologic features. Moreover, the signature was identified as an independent predictor of prognosis and chemotherapy response in different cohorts. Furthermore, functional analysis...
showed that multiple activated immune-related pathways were enriched in the low-risk group. Additionally, the signature was also closely related to various immune checkpoints and inflammatory responses. We generated the first available m<sup>6</sup>A-related lncRNA signature to predict prognosis and chemotherapy benefit in patients with LS-SCLC. Our findings could help optimize the clinical management of patients with LS-SCLC and inform future therapeutic targets for SCLC.

**KEYWORDS**

immune response, individualized medicine, lncRNA, m<sup>6</sup>A-methyladenosine, small cell lung cancer

1 | INTRODUCTION

Small cell lung cancer is a highly invasive, malignant, high-grade neuroendocrine carcinoma with unparalleled growth and early metastases." Currently, SCLC accounts for approximately 15% of all lung cancer cases and is the leading cause of cancer-related deaths worldwide." Due to its elusive pathophysiology, prognosis for patients with SCLC is generally bleak." The US NCI considers SCLC to be a recalcitrant cancer." Given the dismal prognosis of patients with SCLC, more effective therapeutic targets are urgently needed.

N<sub>6</sub>-methyladenosine modification is the most prevalent and significant type of epigenetic methylated modification in mRNA and ncRNA. This modification regulates RNA export, splicing, stability, and translation." N<sub>6</sub>-methyladenosine modification is a reversible and dynamic process controlled by specialized regulators, including methyltransferases (writers), demethylases (erasers), and binding proteins (readers)." N<sub>6</sub>-methyladenosine regulators are involved in the genesis and progression of multiple cancers." Long noncoding RNAs—a subgroup of ncRNAs more than 200 nt in length—actively participate in the biological process regulated by m<sup>6</sup>A methylation tumors." The m<sup>6</sup>A reader YTHDF3 inhibits oncogenesis and tumor progression by negatively regulating the link between IncRNA GAS5 and YAP signaling in colorectal cancer;" the m<sup>6</sup>A eraser ALKBH5 can sustain the expression of the IncRNA FOXM1, resulting in tumorigenesis and proliferation of glioblastoma cells." The m<sup>6</sup>A writer METTL3 also mediates IncRNA MALAT1 stability to enhance and promote the invasion and spread of lung cancer cells." Both m<sup>6</sup>A regulators and some IncRNAs have therapeutic potential and could serve as prognostic biomarkers across various malignancies." There is a close interaction between m<sup>6</sup>A regulators and IncRNAs; they cooperate to modulate essential biological processes in tumorigenesis and development.

Despite these observations, the relationship between m<sup>6</sup>A regulators and aberrant lncRNA expression in SCLC remains largely unclear. Few have explored the underlying mechanisms of IncRNA-dependent biological processes modulated by m<sup>6</sup>A modification in SCLC. Thus, determining how m<sup>6</sup>A regulatory elements and IncRNAs are linked in SCLC could help us to identify biomarkers as therapeutic targets and aid prognostication. We first identified the m<sup>6</sup>A-related IncRNAs with prognostic value in patients with LS-SCLC. Then we constructed a seven-m<sup>6</sup>A-related IncRNA signature in LS-SCLC using bioinformatic methods. This signature precisely stratified ACT benefit and prognosis in patients with LS-SCLC and was well-validated in multiple clinical subgroups and with an independent qRT-PCR dataset. We also explored the relationship between the signature and immune landscape in SCLC samples. This signature is the first molecular model associated with m<sup>6</sup>A modification and IncRNAs in SCLC to demonstrate precise and robust prognostic and predictive ability in patients with LS-SCLC. These findings could help optimize precision medicine approaches for patients with LS-SCLC and elucidate promising therapeutic targets in SCLC.

2 | MATERIALS AND METHODS

2.1 | Samples and m<sup>6</sup>A-related regulators

For the training cohort, we collected 79 patients diagnosed with SCLC and the corresponding microarray data from GSE65002. We analyzed the m<sup>6</sup>A regulator expression profiles between normal lung and LS-SCLC tissues from GSE40275. Clinicopathological data were also downloaded from Gene Expression Omnibus datasets (http://www.ncbi.nlm.nih.gov/geo). The validation set consisted of 158 patients with SCLC. All independent cohort cases underwent surgery for SCLC at the Chinese Academy of Medical Sciences Cancer Hospital, 2009-2018; their corresponding FFPE tissues were used in this research. All patients provided informed consent for tissues collection. We determined the OS from the day of surgery to death or the last follow-up and the RFS from the day of surgery to relapse, metastasis, or last follow-up. We identified an additional 30 m<sup>6</sup>A regulators from recently published reports," including 11 writers (METTL3, METTL14, METTL16, METTL5, WTAP, VIRMA, RBM15, RBM15B, ZC3H13, CBLL1, and ZCCHC4), 2 erasers (FTO and ALKBH5), and 17 readers (YTHDF1, YTHDF2, YTHDF3, YTHDC1, YTHDC2, HNRNPA2B1, HNRNPC, FMR1, EIF3A, IGF2BP1, IGF2BP2, IGF2BP3, ELAVL1, G3BP1, G3BP2, PRRC2A, and RBMX).

Patients’ clinical information is presented in Table 1. The Ethics Committee Board of the Chinese Academy of Medical Sciences Cancer Hospital approved this research.
**TABLE 1** Clinical characteristics of small cell lung cancer (SCLC) patients from different cohorts

| Characteristic       | Training cohort (N = 48) | Validation cohort (N = 158) |
|----------------------|--------------------------|----------------------------|
| Age, years           |                          |                            |
| <60                  | 27 (56.25%)              | 84 (53.16%)                |
| ≥60                  | 21 (43.75%)              | 74 (46.84%)                |
| Sex                  |                          |                            |
| Male                 | 43 (89.58%)              | 124 (78.48%)               |
| Female               | 5 (10.42%)               | 34 (21.52%)                |
| Smoking history      |                          |                            |
| Yes                  | 33 (68.75%)              | 99 (62.66%)                |
| No                   | 15 (31.25%)              | 59 (37.34%)                |
| SCLC staging         |                          |                            |
| I                    | 8 (16.67%)               | 51 (32.28%)                |
| II                   | 8 (16.67%)               | 52 (32.91%)                |
| III                  | 31 (62.50%)              | 55 (34.81%)                |
| IV                   | 1 (2.08%)                | 0 (0.00%)                  |
| OS state             |                          |                            |
| Alive                | 25 (52.08)               | 70 (44.30)                 |
| Death                | 23 (47.92)               | 88 (55.70)                 |

Note: Data are shown as n (%). Abbreviation: OS, overall survival.

2.2 | Identification of m^6^A-related lncRNAs

We identified the lncRNA profile based on previously published studies. We used the log2 transformation method to normalize the raw GSE60052 microarray data. We mapped the annotation file (GPL11154) with gene code v36 IDs; this screened out 2942 lncRNA transcripts with corresponding probes. We only included high-expression lncRNAs in the subsequent analysis. Then we undertook Pearson’s correlation analysis to identify the m^6^A-related lncRNAs in the training cohort (with |r| > 0.5 and p < 0.0001). We ultimately identified 289 m^6^A-related lncRNAs.

2.3 | RNA extraction and qRT-PCR

We used the Ambion RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Thermo Fisher Scientific) to isolate the total RNA from FFPE SCLC tissues based on the manufacturer’s protocols. We applied the NanoDrop 2000C spectrophotometer (Thermo Fisher Scientific) to evaluate the quality and quantity of extracted RNA. We used a 10-μl system which included 1 μl of each PCR primer, 1 μl cDNA, 3 μl nuclease-free water, and 5-μl SYBR in the 7900HT Fast Real-Time PCR system (Applied Biosystems). All validation and independent cohort samples were subjected to qRT-PCR analysis. The expression of target m^6^A-related lncRNAs was evaluated using the 2−ΔΔCt method. The primer sequences applied to this research are displayed in Table S1.

2.4 | Biological pathway enrichment analysis and immune cell infiltration estimation

The GO and KEGG analyses were implemented in DAVID 6.8 (http://david.abcc.ncifcrf.gov/home.jsp). In addition, GSEA was carried out to explore the underlying and relevant molecular mechanisms (http://www.broadinstitute.org/gsea/index.jsp).

2.5 | Signature establishment and statistical analysis

We used univariate and multivariate Cox regression models to determine the m^6^A-related lncRNAs most closely linked to prognosis and established a seven-m^6^A-related lncRNA signature.

R software (version 3.5.1; https://www.r-project.org) and SPSS Statistics 25.0 software were used for statistical analysis and figure generation. Student’s t-test was utilized to evaluate the signature or clinicalopathologic information-based subgroups and prognostic outcomes in various cohorts. Kaplan-Meier curves and log-rank tests were applied to compare prognosis between low- and high-risk groups. Gene Set Variation Analysis was carried out using the GSVA package in R software (version 3.5.1). Univariate and multivariate Cox regression analyses were undertaken to identify the independent predictor value of the seven-m^6^A-related lncRNA signature regarding OS. The R RMS package was used for these analyses. The ROC curves (the timeROC package) and AUC curves were used to assess the prognostic prediction capacity of the nomogram and other variables (TNM stage and risk score) for 1-, 3-, and 5-year OS. A significant difference was defined as p < 0.05.

3 | RESULTS

3.1 | Screening prognostic m^6^A-related lncRNAs in LS-SCLC

Considering the indispensable role of m^6^A regulators in tumorigenesis and progression, we investigated the m^6^A regulator expression profiles in normal lung and SCLC tissues. Based on the principal component analysis, we found that the expression patterns of these 30 regulators in normal lung and SCLC specimens were remarkably different (Figure 1A). The heatmap of different expression profiles between normal lung and SCLC cases is shown in Figure 1B; the corresponding expression pattern details are displayed in Figure S1. As m^6^A regulators actively participate in the development and progression of SCLC, we identified prognostic m^6^A-related lncRNAs in LS-SCLC.

The study flow is depicted in Figure S2. We reannotated 2942 lncRNAs from 79 patients with LS-SCLC from the GSE60052 dataset after mapping the gene code v36 IDs to the annotation document. The GSE60052 with 79 samples was designated as the training cohort, and the corresponding clinical features are shown in Table 1.
We also extracted 30 m₆A regulator expression levels from 79 patients. To confirm the clinical significance of our analysis, we excluded lncRNAs with low expression levels (≥50% expression values were zero). Next, Pearson's correlation analysis was applied between the remaining 1202 lncRNAs and 30 m₆A regulators in GSE65002. The lncRNA with Pearson |r| > 0.5 and p < 0.0001 was defined as the m₆A-related lncRNA. During this process, 289 m₆A-related lncRNAs were identified. To screen the prognostic m₆A-related lncRNAs, we implemented the univariate Cox regression analysis in 48 cases with survival information from GSE65002. Afterward, 19 m₆A-related lncRNAs were potentially associated with OS in patients with SCLC included from the GSE60052 dataset (Figure 1C, p < 0.2). Multivariate stepwise regression analysis was used to identify seven predictive m₆A-related lncRNAs in patients with LS-SCLC. The m₆A regulators associated with the seven m₆A-related lncRNAs are illustrated in Figure 1D.

### 3.2 Establishment of m₆A-related lncRNAs in LS-SCLC

We created an equation that consisted of the seven m₆A-related lncRNAs and their corresponding coefficients for risk stratification of patients with LS-SCLC. Here, the risk score = (0.5622 × WARS2-IT1 expression) + (1.0842 × AC005162 expression) + (1.1170 × AC130352 expression) + (1.5938 × AC015971 expression) − (0.6460 × FOXP1-IT1 expression) − (0.0665 × AC040963 expression) − (0.5835 × LINC00355 expression) (Figure 2A). The relationship between 7 m₆A-related
IncRNAs and the risk score is illustrated in Figure 2B. All patients in the training group were assigned a risk value and assigned to the high- or low-risk groups based on the optimal cut-off point (Figure 2C). Importantly, high-risk patients had worse OS than low-risk patients (Figure 2D). The ROC analysis produced AUC values of 0.775, 0.826, and 0.86 for forecasting OS in the GSE60052 cohort at 1-, 3-, and 5-years, respectively (Figure 2E).

3.3 Validation of m^6A-related IncRNAs in LS-SCLC

To further validate the prognostic performance of this signature, we included 158 FFPE samples from an independent validation set. We tested the expression levels of the seven m^6A-related IncRNAs in these 158 patients using a qRT-PCR analysis. The cases were similarly assigned to low- or high-risk groups based on individual risk scores. High-risk patients showed a remarkably worse OS than low-risk candidates (Figure 3A). The ROC curves analysis revealed that AUCs of this risk score in predicting 5-year OS was 0.645 (Figure 3B). This m^6A-related IncRNAs signature could predict OS better than other critical clinical features (Figure 3C). Furthermore, we explored whether this m^6A-related IncRNA classifier effectively predicted the RFS of patients with LS-SCLC. Intriguingly, high-risk cases presented with significantly shorter RFS versus low-risk patients (Figure 3D). We found the AUCs of this classifier predicting RFS at 5-year was up to 0.666 (Figure 3E). This classifier also showed superior predictive performance in RFS than other clinical characteristics (Figure 3F).

Considering the pivotal role of ACT in SCLC treatment, we tested the predictive ability of this m^6A-related IncRNA signature to ACT response in patients with LS-SCLC. In the independent cohort, 138 of 154 cases received ACT, and the risk score categorized them into high- and low-risk groups based on the optimal cut-off point. Low-risk cases benefited more from ACT treatment than their high-risk counterparts, achieving longer OS and RFS (Figure 3G,J). The signature AUCs for predicting 1-, 3-, and 5-year OS and RFS are displayed in Figure 3H,K. Importantly, the signature was a better predictor of 5-year OS and RFS than other clinical features (Figure 3I,L). We also validated the predictive capacity of the risk score in clinical features.
(sex, age, smoking status, and disease stage) subgroups from the independent cohort. Once again, low-risk patients achieved better OS and RFS within the clinical characteristic subgroups (Figures S3 and S4).

### 3.4 $N^6$-methyladenosine-related lncRNA signature is an independent prognostic factor for patients with LS-SCLC

Univariate and multivariate Cox regression models were used to determine whether our $m^6$A-related lncRNA signature could independently predict prognosis in patients with SCLC. Our signature was superior to other clinical features (sex, age, smoking status, and SCLC staging) for predicting OS and RFS among patients with LS-SCLC (Figure 4A). After including these clinical features, the multivariate Cox analysis confirmed the $m^6$A-related lncRNAs independently predicted OS and RFS in patients with LS-SCLC in the training and independent cohorts (Figure 4B). Additionally, we sought to determine whether this classifier could serve as an independent predictor of ACT efficacy in LS-SCLC. Consistent with the previous results, the model independently forecasted OS and RFS in patients receiving ACT from the independent cohort (Figure 4C,D). Our signature independently predicted ACT efficacy and prognosis among patients with LS-SCLC; thus, we believe our signature could assist with clinical management of these patients.
3.5 | Functional analysis of m6A-related lncRNA signature

The GSEA was carried out to discover the potential mechanisms of the signature. Several immune-related pathways were enriched in the low-risk group, including those involved in cytokine production for the inflammatory response (ES = 0.5748, NES = 1.6625, \( p < 0.001 \)), regulation of type I interferon-mediated signaling pathway (ES = 0.4695, NES = 1.6175, \( p = 0.0205 \)), and response to interferon \( \beta \) (ES = 0.5852, NES = 1.6699, \( p = 0.0212 \)) and \( \alpha \) (ES = 0.6612, NES = 1.7345, \( p < 0.001 \)) (Figure 5A–D). Thus, the signature was correlated with tumor immunity. Therefore, we closely examined the relationship between this signature and immune genes. The immune genes with Pearson \( |r| > 0.3 \) were considered to be correlated with this signature, and finally 277 immune genes were identified. The resultant heatmap of these immune genes and various clinical features of cases from the GSE60052 dataset is presented as Figure 5E. The GO and KEGG analyses were used to identify the underlying biological functions. Our risk score was closely associated with various immune response processes, such as the T-cell receptor signaling pathway, antigen processing and presentation of exogenous peptide antigens through MHC class II, tumor necrosis factor-mediated signaling pathway, the nuclear factor-kappa B signaling pathway, and the Toll-like receptor signaling pathway (Figure 5F,G). This m6A-related lncRNA signature is linked to immune responses and biological processes and provides insights into tumor immunity in SCLC.

3.6 | Immune landscape of m6A-related lncRNA signature

We applied the seven clusters metagenes (HCK, interferon, LCK, MHC-I, MHC-II, IgG, and STATA) to further validate the relationship between this signature and tumor immunity relative to various inflammatory and immune responses (Figure 6A). The risk score was positively correlated with the IgG cluster, and negatively correlated with the HCK, LCK, MHC-I, MHC-II, and STAT1 clusters (Figure 6B). The GSVA results agreed with our prior findings. Low-risk patients were more likely to be associated with T cell signaling transduction and macrophage activation.

Immune checkpoints are an important component of the tumor immune microenvironment and directly influence the antitumor response. Thus, we also tested the relationship between risk score and the expressions of various immune checkpoints. The risk score was positively related to various immune checkpoint expression levels in the training cohort, including TNFRSF4, TNFRSF9, CMTM4,
CMTM6, and CD226 (Figure 6C,D). Most of these immune checkpoints are promising therapeutic targets for immunotherapies. High-risk patients could benefit from emerging therapies that target these immune checkpoints.

4 | DISCUSSION

Past studies have indicated that m<sup>6</sup>A modification actively participates in tumor pathogenesis and progression. Several m<sup>6</sup>A regulators modulate the progression of multiple malignancies by affecting the degradation, stability, expression, and translation of different lncRNAs.

For example, the m<sup>6</sup>A writer METTL3 accelerates tumor invasion and metastasis in lung cancer by modifying the stability of the lncRNA MALAT1. Similarly, METTL14 restrains the development and progression of colon cancer by decreasing the lncRNA XIST. The m<sup>6</sup>A eraser ALKBH5 enhances the expression of the lncRNA FOXM1, maintaining the stemness of glioblastoma cells. N<sup>6</sup>-methyladenosine modification of lncRNAs can regulate various tumor-related biological processes and affect tumor development; substantial lncRNAs might act as competing endogenous RNAs. Therefore, m<sup>6</sup>A
regulators could emerge as targets for tumor elimination. While lncRNAs are the essential targets of (and closely associated with) m^6^A modification, few studies have examined m^6^A modification of lncRNAs in SCLC. The links between m^6^A regulators and lncRNAs in SCLC could help us identify potential prognostic biomarkers and therapeutic targets.

In this study, we aimed to explore the m^6^A-related lncRNA expression profile and its clinical significance in LS-SCLC. We also constructed a seven m^6^A-related lncRNA signature that could precisely predict ACT efficacy and prognostic risk for patients with LS-SCLC. Our signature also showed better predictive ability than other clinical characteristics. This m^6^A-related lncRNA classifier was well validated in the various clinical subgroups and the independent cohort, and served as an independent prognostic predictor in LS-SCLC. We believe that our findings could help optimize the clinical precision management for patients with SCLC and shed some light on the therapeutic targets in SCLC.

Interestingly, this m^6^A-related lncRNA signature consisted of three protective factors (FOXP1-IT1, AC040963, and LINC00355) and four risk-enhancing factors (WARS2-IT1, AC005162, AC130352, and AC015971). FOXP1-IT1 is significantly downregulated in ovarian cancer and is associated with a better prognosis for colon adenocarcinoma. LINC00355 is a pro-oncogene factor and upregulated in various cancers. It promotes tumor proliferation, migration, and invasion by regulating multiple microRNA axes or epigenetic modification in head and neck squamous cell carcinoma, hepatocellular carcinoma, lung cancer, and gastric cancer. Importantly, the exosomal LINC00355 enhances the resistance of bladder cancer cells to chemotherapy by its interaction with the microRNA axis. WARS2-IT1 promotes tumor growth and is associated with
an unfavorable prognosis in hepatocellular carcinoma.\textsuperscript{29} AC005162 could accelerate tumor growth and worsens prognosis for patients with breast cancer.\textsuperscript{30} However, little is known about the roles of AC040963, AC130352, or AC015971; further studies are needed determine their function. The specific functions of the seven m\textsuperscript{3}A-related IncRNAs relative to SCLC development warrants further exploration.

The m\textsuperscript{3}A-related molecular model appears closely linked to various immune-related pathways, including cytokine production involved in the inflammatory response, regulation of type I interferon-mediated signaling pathway, and the responses to interferon $\beta$ and $\alpha$. Low-risk patients showed different forms of immune phenotype activation than high-risk patients. Some essential immune response processes were linked to this molecular model—including the T-cell receptor signaling pathway, antigen processing and presentation of exogenous peptide antigen through MHC class II, the tumor necrosis factor-mediated signaling pathway, the nuclear factor-kappa B signaling pathway, and the Toll-like receptor signaling pathway. Collectively, this signature appears to be actively involved in tumor immunity.

Notably, various immune checkpoints (TNFRSF4, TNFRSF9, CD226, CMTM4, and CMTM6) expression emerged as relevant to this risk score. TNFRSF4 and TNFRSF9, TNF receptor superfamily candidates, actively participate in co-stimulatory and co-inhibitory signaling of specific immunity. Harnessing TNFRSF members can increase tumor immunity, and relevant preclinical studies and clinical trials are being carried out.\textsuperscript{31,32} CD226 is a critical activator of natural killer cells and CD8$^+$ T cells to mediate the immune reactivity against cancer; additionally, CD226 can affect the efficacy of PD-1/PD-L1 blockade therapy.\textsuperscript{33} CMTM4 and CMTM6 mediate PD-L1 protein expression and function, enhancing tumor cells with high expression of PD-L1 to weaken T cell-dominated antitumor effects.\textsuperscript{34} These results could guide future clinical application of immunotherapies for SCLC.

Investigations of prognostic biomarkers for SCLC have been impeded due to difficulties obtaining SCLC samples under standard care conditions. Our study was the first large-cohort, IncRNA-related research in LS-SCLC. Before our study, several researchers constructed molecular models to predict the prognostic risk of patients with SCLC, which is mostly limited in mRNAs and microRNAs with small size of samples.\textsuperscript{35,36} This was the first time the IncRNA expression profile for LS-SCLC was identified; we constructed an m\textsuperscript{3}A-related IncRNA-based signature to predict ACT responses and prognosis in patients with LS-SCLC.

Our results should be considered within the context of several study limitations. First, the IncRNA expression profile was primarily decided by GPL11154; this might not include all IncRNAs identified to date. Second, despite our best efforts to collect validation samples, our model requires validation within a larger study cohort. Finally, our retrospective results should be confirmed within the context of future, prospective studies.

In summary, we determined the expression profile of m\textsuperscript{3}A-related IncRNAs in SCLC and established a seven-m\textsuperscript{3}A-related IncRNA-based molecular model to forecast chemotherapy response and prognostic risk for patients with SCLC. Our results could inform future clinical applications of chemotherapy and immunotherapy in patients with SCLC.

**AUTHOR CONTRIBUTIONS**

NS and JH supervised the project, designed, edited and led out the experiments of this study. YJL, ZHZ, and PW conducted the experiments and data analysis. YJL and ZHZ prepared all the figures and tables. YJL, ZHZ, and BZ drafted the manuscript. GCZ, LDW, QPZ, ZYY, LYX, HZ, FWT, QX, and SGG collected clinical samples and provided material support. All the authors reviewed and approved the final manuscript.

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**DISCLOSURE**

The authors declare that they have no conflict of interest.

**DATA AVAILABILITY STATEMENT**

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

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**SUPPORTING INFORMATION**

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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