

The prognostic value and immune landscapes of m¹A/m⁵C/m⁶A-associated lncRNA signature in osteosarcoma

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Abstract. – OBJECTIVE: RNA methylation modifications, mainly including N1-methyladenosine (m¹A), 5-methylcytosine (m⁵C), and N6-methyladenosine (m⁶A), are widely existed in osteosarcoma and involved in the biological processes of cancers. However, there is still no study regarding the relationship between osteosarcoma and m¹A/m⁵C/m⁶A-associated long non-coding RNAs (lncRNAs).

PATIENTS AND METHODS: Here, expression data of osteosarcoma from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) database were retrieved to identify ER-related lncRNAs associated with the overall survival (OS) of osteosarcoma patients. Then, Lasso penalized Cox regression analysis was applied to construct a lncRNAs risk signature. Meanwhile, patients were stratified into two clusters based on the identified m¹A/m⁵C/m⁶A-associated lncRNAs. The prognostic value and immune landscape of the identified signature and clusters were further evaluated.

RESULTS: Two m¹A/m⁵C/m⁶A-associated lncRNAs were incorporated into our risk signature. The functional analyses indicated that the prognostic model was correlated with patient survival, and cancer metastasis and growth. Meanwhile, the signature model was significantly associated with the infiltration of immune cells, immune microenvironment, as well as several immune checkpoint genes. Similar results were detected for the lncRNAs clusters, which were significantly correlated with immune infiltration, cancer microenvironment, and immune-associated genes, and contributed to predicting the prognosis of patients. Moreover, our risk signature and clusters might help guide the application of immunotherapeutic drugs for osteosarcoma patients. Finally, a nomogram based on the risk score was established.

CONCLUSIONS: Overall, a risk signature based on two m¹A/m⁵C/m⁶A-associated lncRNAs was generated and presented predictive value for the prognosis and immune landscapes of osteosarcoma patients. This signature can be further used in the development of novel therapeutic strategies for osteosarcoma.

Key Words:

Osteosarcoma, m¹A/m⁵C/m⁶A, lncRNAs, Risk signature, Prognostic value, Immune landscapes.

Introduction

Osteosarcoma is an aggressive malignant tumor that threatens human health^{1,2}. Due to its predisposition to metastases, especially in the lungs, the 5-year survival of these osteosarcoma patients is < 20%³. On the other hand, the 5-year survival rate of osteosarcoma patients without lung metastases is 70%⁴, suggesting that the early diagnosis and treatment of osteosarcoma affect its outcome. Recently, many investigators have attempted to identify novel biomarkers that can be used for prognostic prediction and personalized therapy of osteosarcoma patients. However, due to its genomic complexity and instability, only a few biomarkers of clinical significance were identified⁵. Therefore, the identification of new biomarkers that can accurately predict the prognosis of osteosarcoma patients is urgently needed.

Recently, RNA modification was identified to be connected with various cancers and human physiologies, especially tumor immunity^{6,7}. Among the identified epigenetic modifications, methylation is the most abundant in human cells, including N1-methyladenosine (m¹A), 5-methylcytosine (m⁵C), and N6-methyladenosine (m⁶A)⁸. The most abundant methylation in eukaryotic RNAs is m⁶A, which mainly occurs on the adenine of the RRACH sequence. The function of m⁶A methylation is jointly regulated by writers: METTL16, WTAP⁹; erasers: FTO and ALKBH5; and readers: YTHDC family, IGF2BP family^{9,10}. Another prevalent methylation modification is m⁵C. This modification is enriched around 3'UTR and 5'UTR and conserved in rRNAs and tRNAs.

Meanwhile, m⁵C is also regulated by several enzymes, including writers: NSUN family, DNMT3 family¹¹; erasers: TET2 and YBX1; and readers: ALYREF. The m¹A methylation is mainly located within the 5' UTR and can also be regulated by writers: TRMT family, BMT2¹²; erasers: ALKBH family; and readers: YTHDF family and YTH-DC1. Besides modulating RNA metabolism, m¹A, m⁵C, and m⁶A methylations are also involved in various biological processes, such as mitochondrial dysfunction, stem cell differentiation, and gametogenesis¹³. Nevertheless, few studies have reported the prognostic value of m¹A/m⁵C/m⁶A in osteosarcoma progression, and the role of m¹A/m⁵C/m⁶A methylation in cancer immunity remains unknown.

Long non-coding RNAs (lncRNAs) are a subset of non-coding RNAs longer than 200 base pairs. In addition to various cellular biological processes, lncRNAs can contribute to tumor progression, including tumorigenesis, cell proliferation, and tumor metastasis^{14,15}. However, there are no systematic analyses aiming at the identification of hub m¹A/m⁵C/m⁶A-associated lncRNAs in osteosarcoma that can be associated with prognosis or progression.

Therefore, in the present study, we conducted univariate and Lasso penalized Cox regression analyses to characterize the hub m¹A/m⁵C/m⁶A-associated lncRNAs and construct a risk signature. The prognostic value and immune landscapes of this model were further validated in osteosarcoma patients. The risk signature generated according to the expression of m¹A/m⁵C/m⁶A-associated lncRNAs in osteosarcoma has not been previously performed. Thus, to the best of our knowledge, this is the first study demonstrating the use of m¹A/m⁵C/m⁶A-associated lncRNAs for the prediction of osteosarcoma prognosis.

Patients and Methods

Raw Data Acquisition

The transcriptomic data of 88 osteosarcoma tissues were collected from the Therapeutically Applicable Research to Generate Effective Treatments (TARGET; <https://ocg.cancer.gov/programs/target>) database. The Log₂-transformation was performed using the “sva” R package to remove batch effects^{16,17}. According to previous publications, 52 specific m¹A/m⁵C/m⁶A methylation complexes, including writers, erasers, and readers (Table I), were applied for further analysis.

Construction of the lncRNAs Signature and Clustering of Osteosarcoma Samples Based on m¹A/m⁵C/m⁶A-Associated lncRNAs

After assessing the association between m¹A/m⁵C/m⁶A-associated lncRNAs and osteosarcoma using the Pearson correlation analysis ($|R_2| > 0.4$, $p < 0.001$). Prognostic m¹A/m⁵C/m⁶A-associated lncRNAs were identified by the univariate Cox regression analysis using the “survival” R package with a cutoff of $p < 0.001$. Then, prognostic lncRNAs were integrated into a Lasso penalized Cox regression analysis to identify hub lncRNAs and to generate lncRNA risk signature and cluster osteosarcoma samples. Based on the expression of identified lncRNAs, osteosarcoma patients were classified into different subgroups using the “ConsensusClusterPlus” R package. Next, osteosarcoma patients were categorized into low- and high-risk subgroups using the median risk score as the threshold. The risk score was calculated as follows:

$$\text{risk score} = \sum \exp(\text{lncRNA}_i) * \beta_i$$

Where $\exp(\text{lncRNA}_i)$ represents the relative expression of the i th hub m¹A/m⁵C/m⁶A-associated lncRNA, and β the regression coefficient¹⁸.

Predictive Value of the lncRNA Signature and Clusters

To explore the distribution of the risk signature and cluster subgroups, t-Distributed Stochastic Neighbor Embedding (t-SNE) and Principal Component Analysis (PCA) were performed using the “Rtsne” and “ggplot2” R packages. The “survival” R package was further applied to compare the overall survival (OS) between the two risk subgroups and clusters of osteosarcoma patients. To verify the predictive accuracy of the risk signature, the “timeROC” R package was applied for both the lncRNA signature and traditional clinical features. Univariate and multivariate Cox regression analyses were performed to evaluate the relationship between the risk score and clinical characteristics. Finally, a nomogram based on the levels of calculated risk scores was constructed to predict the outcomes of osteosarcoma patients at 1, 3, and 5 years using the “rms” R package. The calibration curves constructed by the Hosmer–Lemeshow test were applied to illustrate the consistency of our nomogram.

Gene Set Enrichment Analysis (GSEA)

For the hub m¹A/m⁵C/m⁶A-associated lncRNAs, a Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed using GSEA 4.2.2 with the two risk subgroups. The statistical significance was defined as FDR < 0.05.

Table I. RNA methylation-related genes of m¹A/m⁵C/m⁶A.

RNA methylation	Writer	Reader	Eraser
m ¹ A	TRMT6, TRMT61A, TRMT61B, TRMT61C, TRMT10C, BMT2, RRP8	YTHDF1, YTHDF2, YTHDF3, YTHDC1	ALKBH1, ALKBH3
m ⁵ C	NOP2, NSUN1, NSUN2, NSUN3, NSUN4, NSUN5, NSUN7, DNMT1, TRDMT1, DNMT3A, DNMT3B	ALYREF	TET2, YBX1
m ⁶ A	METTL3, METTL14, METTL16, WTAP, KIAA1499, RBM15, RBM15B, RBM1, ZC3H13	YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3, IGF2BP1, IGF2BP2, IGF2BP3, HNRNPA2B1, HNRNPC, HNRNPG, RBMX, FMR1, LRPPRC	FTO, ALKBH5

Immune Landscapes Assessment

A Spearman correlation analysis was performed to test the relationship between the risk score, osteosarcoma clusters, and ESTIMATE, stromal, and immune scores. Single-sample gene set enrichment analysis (ssGSEA) was used to compare immune cell infiltration in the two risk subgroups and osteosarcoma clusters and to test immune functions. Meanwhile, potential immune checkpoint molecules retrieved from a previous study were used to explore the connection between immune-related checkpoints, risk signatures, and osteosarcoma clusters¹⁹. Next, the correlation between the risk signature and the key immune regulator PD-L2 was evaluated.

Drug Sensitivity Exploration

The “Prophetic” R package was applied to evaluate the drug sensitivity of osteosarcoma samples from two risk subgroups and clusters. In this case, the sensitivity was determined by the half-maximal inhibitory concentration (IC₅₀)²⁰.

Results

Screening of Candidate Prognostic lncRNAs

The workflow of this study is illustrated in Figure 1. First, we identified 669 lncRNAs related to the expression of m¹A/m⁵C/m⁶A-associated genes expression in osteosarcoma patients. In the univariate Cox regression, 7 lncRNAs were identified as prognostic m¹A/m⁵C/m⁶A-associated lncRNAs (Figure 2A).

Identification of Hub m¹A/m⁵C/m⁶A-Associated lncRNAs

Prognostic m¹A/m⁵C/m⁶A-associated lncRNAs were further analyzed by a Lasso penalized Cox

regression. Two hub lncRNAs, LINC01517 and GAS5, were finally used to construct the risk signature (Table II). The connection between hub lncRNAs and m¹A/m⁵C/m⁶A-associated genes is shown in Figure 2B. The prognostic value of the m¹A/m⁵C/m⁶A-associated lncRNAs LINC01517 and GAS5 is shown in Figure 2C.

Consensus Clustering of m¹A/m⁵C/m⁶A-Associated lncRNAs for Osteosarcoma Patients

Furthermore, we used cluster analysis to classify osteosarcoma patients into two clusters (optimal k = 2) (Figure 3A). The PCA (Figure 3B) and t-SNE (Figure 3C) indicated that the osteosarcoma samples in the two clusters were distinguished. The KM survival curves showed that the survival probability of osteosarcoma patients in Cluster 1 was significantly lower than in Cluster 2 ($p < 0.05$; Figure 3D). Additionally, the Sankey diagram demonstrated that all osteosarcoma patients in Cluster 1 had higher risk scores, while patients in Cluster 2 mainly belonged to the low-risk subgroup (Figure 3E). These results partly explained the survival status results of the above clusters.

Immune Landscapes of Identified Clusters

The immune microenvironment of osteosarcoma patients in Cluster 2 had significantly higher ESTIMATE, stromal, and immune scores compared to those in Cluster 1 (Figures 4A-C). The immune infiltration analyses of the two clusters demonstrated that osteosarcoma patients in Cluster 2 had significantly higher infiltration of immune cells, and levels of immune-related pathways and functions than in Cluster 1 (Figures 4D and E). Only the scores for DCs, CD8+ T cells, DCs, iDCs, NK cells, and T helper cells

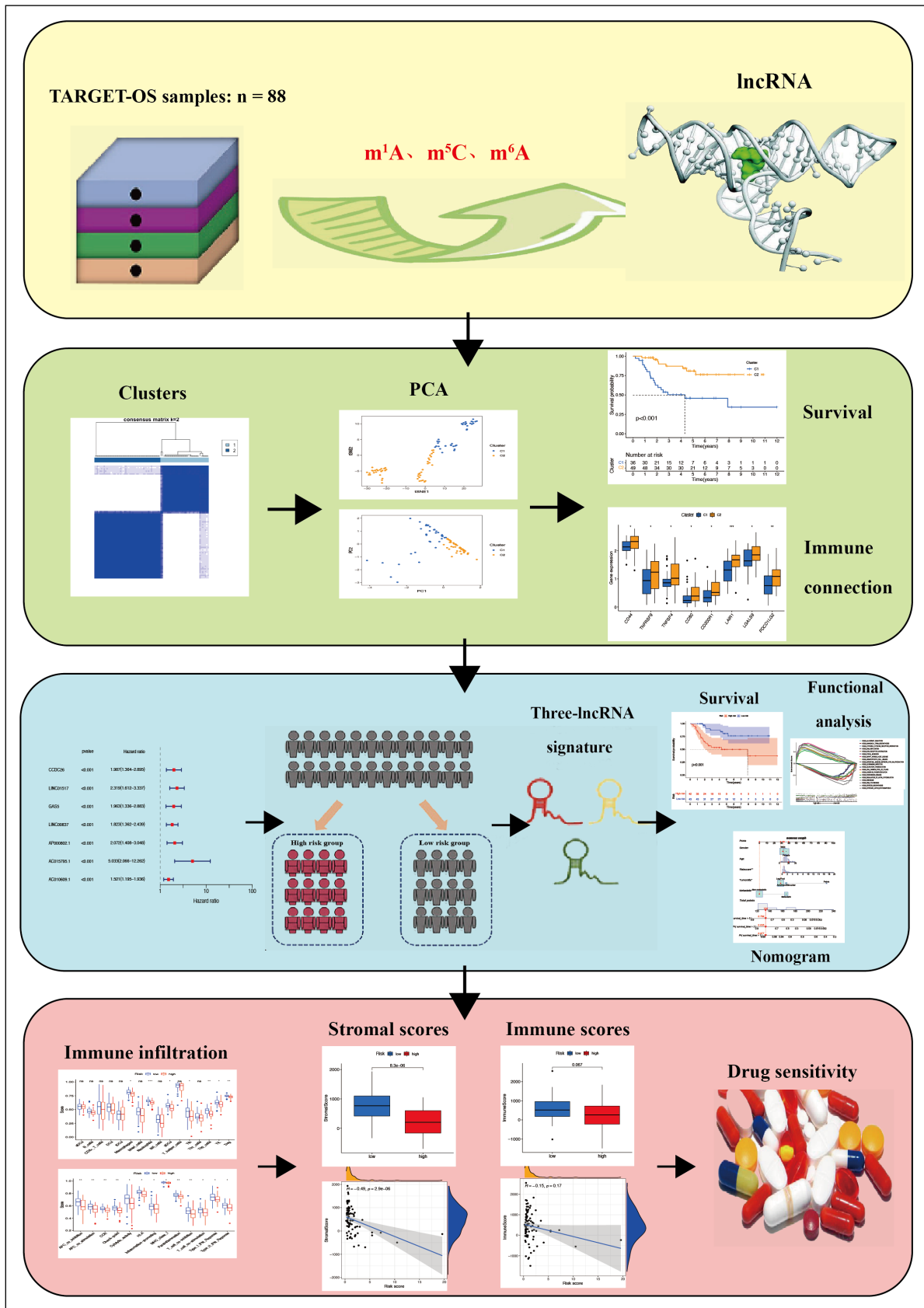


Figure 1. Schema of the study.

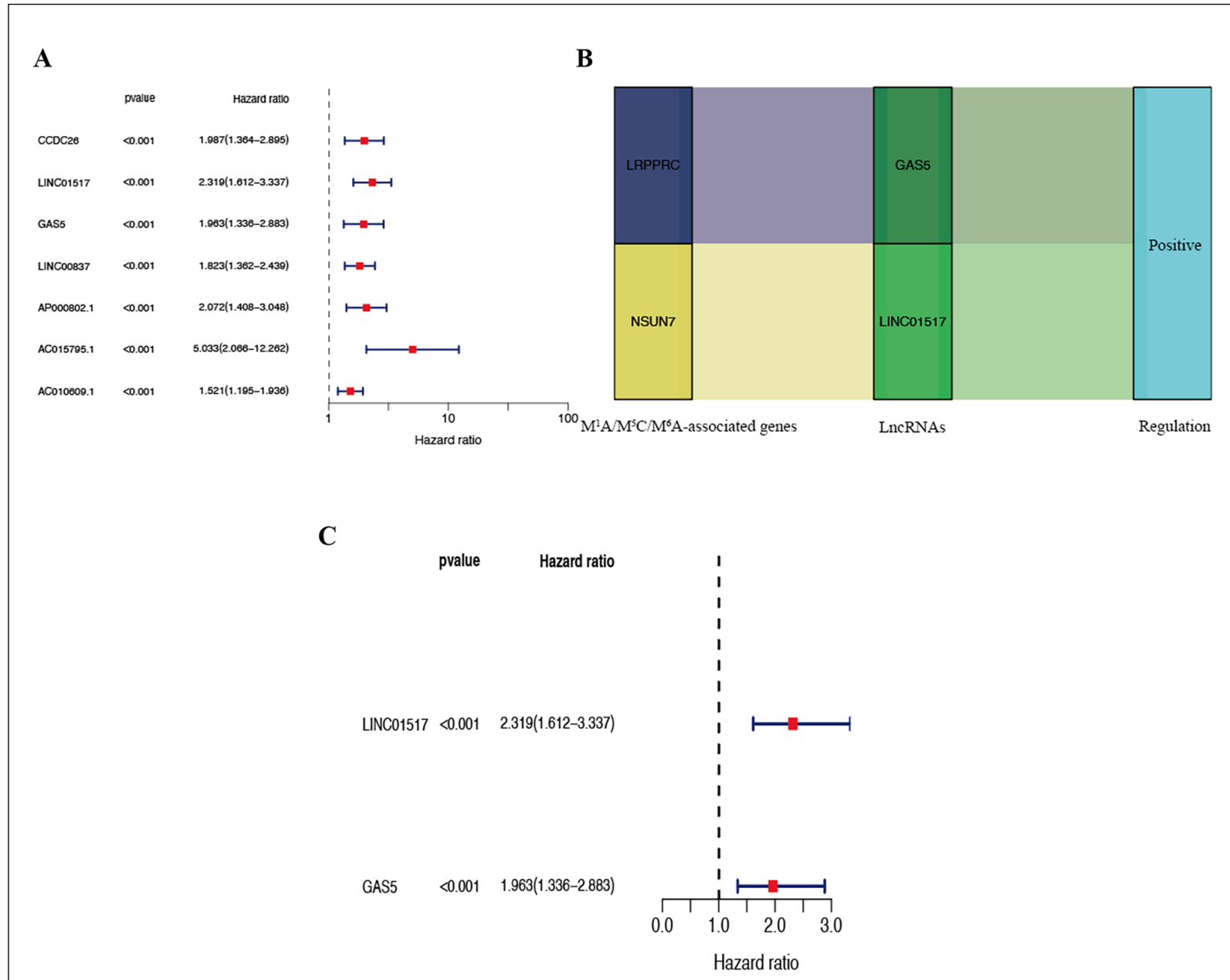


Figure 2. Identification of prognostic m¹A/m⁵C/m⁶A-associated lncRNAs. **A**, Univariate Cox analyses of prognostic m¹A/m⁵C/m⁶A-associated lncRNAs. **B**, Correlation network of hub lncRNAs and their associated mRNAs. **C**, Forest plots of correlations between hub lncRNAs and overall survival of osteosarcoma patients.

Table II. The correlation coefficient of m¹A/m⁵C/m⁶A-associated lncRNAs.

Identified lncRNAs	Coef
LINC01517	0.673727215581825
GAS5	0.552563451962601

did not significantly differ between the two clusters ($p > 0.05$). Additionally, the levels of the immune checkpoints CD44, TNFRSF9, TNFRSF4, CD80, CD200R1, LAIR1, LGALS9, and PDCD1LG2 were significantly higher in Cluster 2 compared to patients in Cluster 1 ($p < 0.05$; Figure 4F). Overall, patients in Cluster 2 had significantly higher OS than those in Cluster 1, partly due to their higher immunotherapeutic responses to osteosarcoma. Additionally, patients in Cluster 2 might be more susceptible to immunotherapies.

Construction of the lncRNA Risk Signature for Osteosarcoma

According to the expression levels of identified hub lncRNAs, the risk score of each osteosarcoma patient was calculated. Then, patients were separated into low- and high-risk subgroups based on the median risk scores (Figures 5A and B). Similar to the clusters of osteosarcoma patients, the PCA and t-SNE showed that the two risk subgroups were clearly separated (Figures 5C and D). The analyses of associations between the risk signature and clinical characteristics indicated that the OS of osteosarcoma patients in the high-risk subgroup was significantly lower than in the low-risk subgroup ($p < 0.05$; Figure 5E). Meanwhile, the results regarding the relationship between the risk score and expression of lncRNAs showed that both lncRNAs (LINC01517 and GAS5) were significantly higher expressed in the high-risk subgroup (Figure 5F). Finally, the receiver operating characteristic (ROC) curve analysis indicated that the lncRNA signature had strong predictive accuracy at 1 (ROC = 0.784), 2 (ROC = 0.844), and 3 (ROC = 0.785) years (Figure 5G). Further, we demonstrated that m¹A/m⁵C/m⁶A-associated lncRNAs signature has greater accuracy compared to all other clinicopathological features in osteosarcoma, even cancer metastatic status (Figure 5H). These results indicated that our m¹A/m⁵C/m⁶A-associated lncRNA signature was a sensitive and specific predictor of the OS of osteosarcoma patients.

Association Between the lncRNA Signature and Clinical Features of Osteosarcoma Patients

Multivariate and univariate Cox regression analyses revealed that our newly identified lncRNA signature was an independent prognostic factor for osteosarcoma patients (Figures 6A and B). Interestingly, osteosarcoma patients diagnosed with metastatic cancer also had significantly higher risk scores than patients with primary osteosarcoma ($p < 0.05$; Figure 6C). The heatmap of clinical characteristics and m¹A/m⁵C/m⁶A-associated lncRNAs signature showed that our risk signature was associated with the metastatic status of osteosarcoma patients (Figure 6D). These results demonstrated the protective value of the risk signature for osteosarcoma metastatic patients.

The GSEA showed that the lncRNA signature was significantly enriched in several pathways (FDR < 0.05), including focal adhesion, Leishmania infection, cytokine-cytokine receptor interaction, ribosome, steroid biosynthesis, and oxidative phosphorylation (Figure 7F). Moreover, several immune-associated pathways, such as the intestinal immune network for IgA production, were also enriched in the risk signature.

Associations with Immune Landscapes

In the analysis of associations between the m¹A/m⁵C/m⁶A-associated lncRNAs signature and cancer immunity, all components of immune-related pathways and functions were significantly reduced in the high-risk subgroup compared to the low-risk subgroup ($p < 0.05$; Figure 7A). Meanwhile, the proportion of several immune cell subpopulations, including macrophages, neutrophils, pDCs, Tfh, Th2 cells, TILs, and Tregs, were significantly inhibited in the high-risk subgroup ($p < 0.05$; Figure 7B). The immune microenvironment, including immune and stromal scores, are both key modulators of cancer progression. Herein, the stromal scores significantly declined in the high-risk subgroup compared to the low-risk subgroup ($p < 0.05$; Figure 7C). Meanwhile, they were significantly negatively correlated with the m¹A/m⁵C/m⁶A-associated lncRNA signature (Figure 7D). However, the immune scores did not differ between the two risk subgroups and were not significantly associated with the risk scores ($p > 0.05$; Figures 7E and F).

Regarding the immune checkpoints, the levels of CD44, TNFRSF9, CD200R1, LAIR1, and PDCD1LG2 were lower in the high-risk subgroup (Figure 7G). Moreover, considering the roles of the

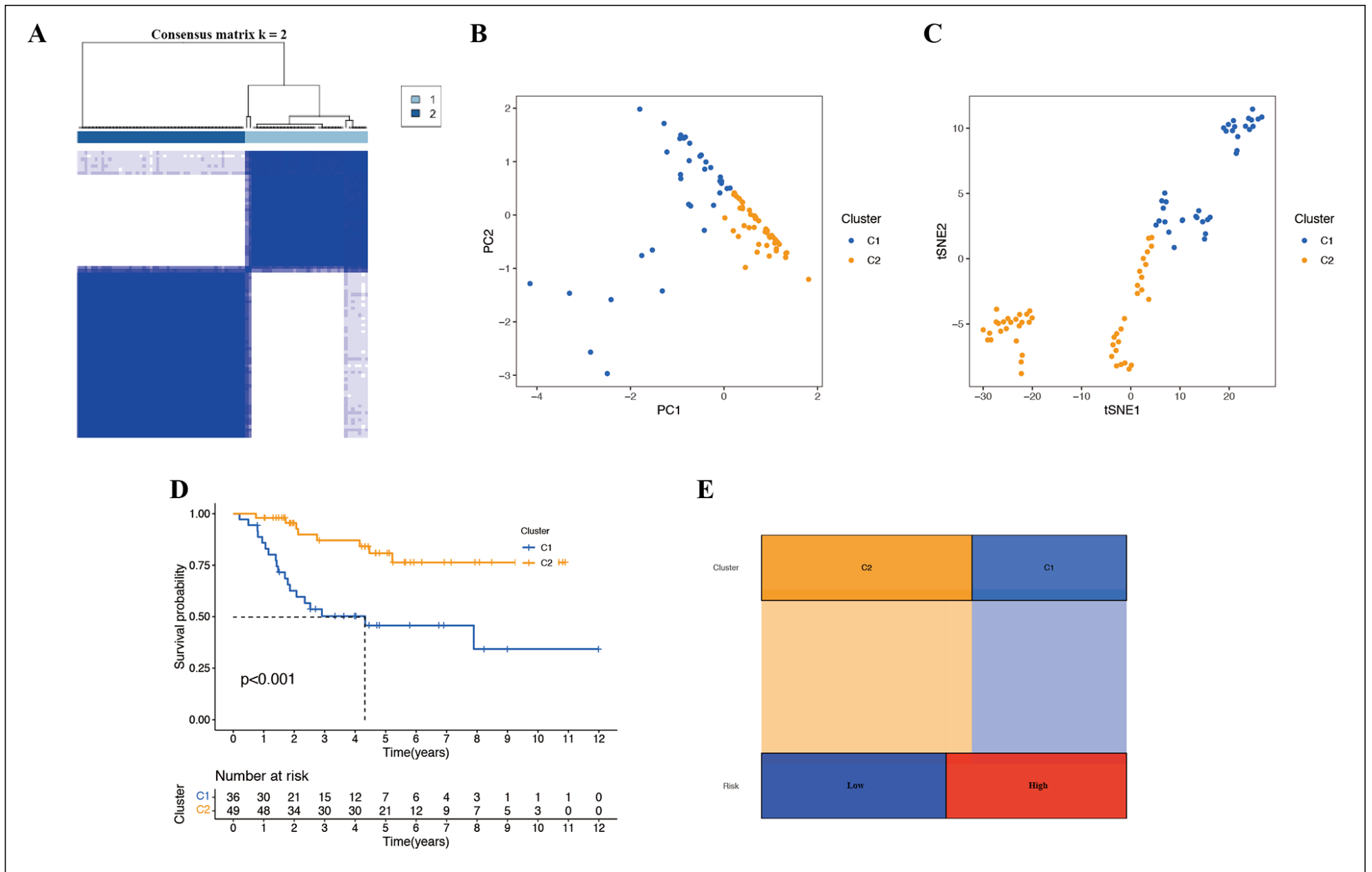


Figure 3. Clinical characteristics and overall survival among different osteosarcoma clusters. **A**, Consensus matrix for optimal $k = 2$. PCA plot (**B**), and t-SNE (**C**) analysis of clusters. **D**, KM curve of overall survival time in two clusters. **E**, Forest plots of correlations between osteosarcoma clusters and risk score.

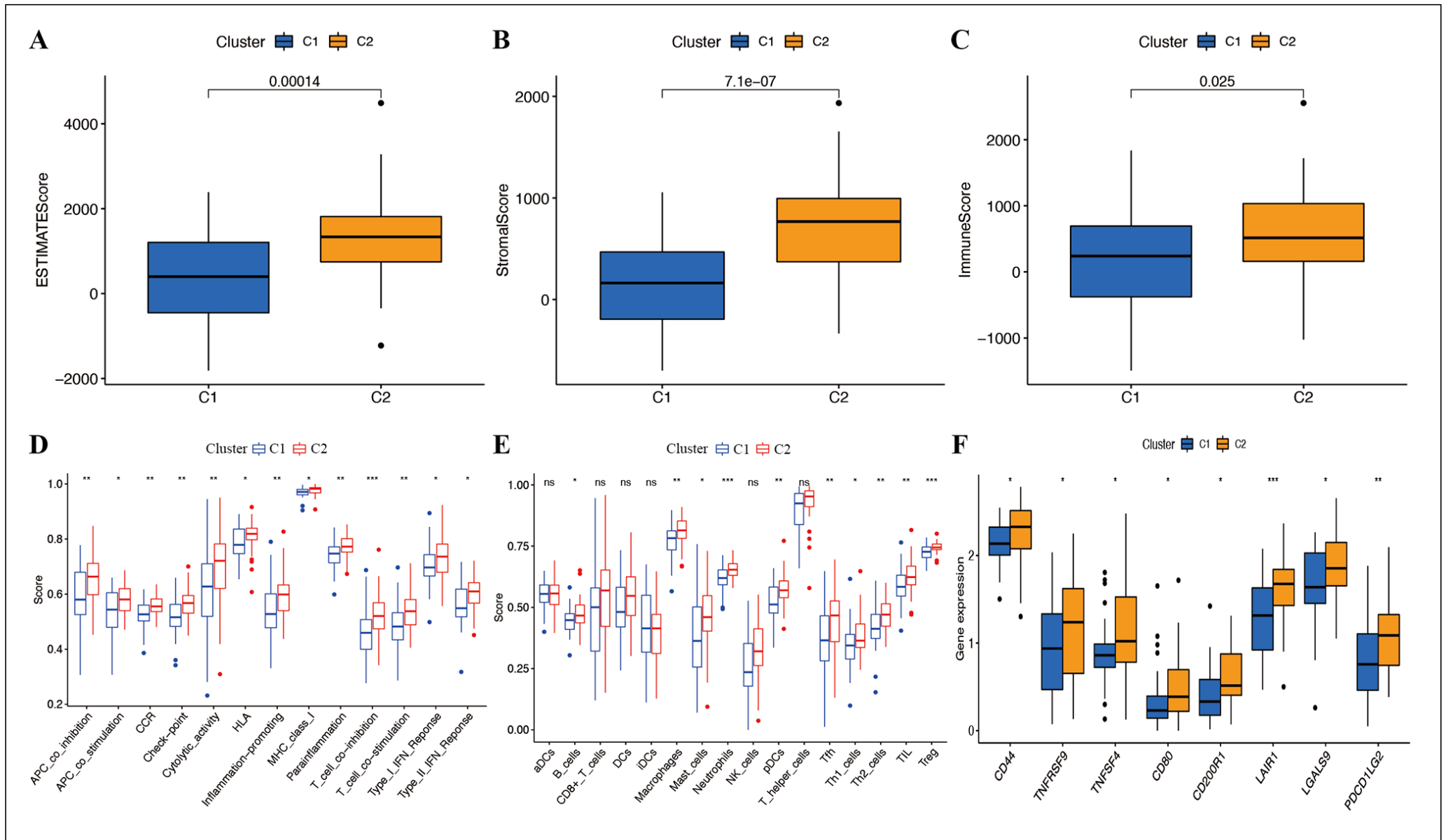


Figure 4. Potential role of osteosarcoma clusters in immune landscapes. Associations between osteosarcoma clusters and ESTIMATE scores (A), stromal scores (B), immune scores (C). Boxplots of scores of immune-associated functions (D) and immune cells (E) among two clusters. F, Expression of immune checkpoints among two clusters in osteosarcoma patients.

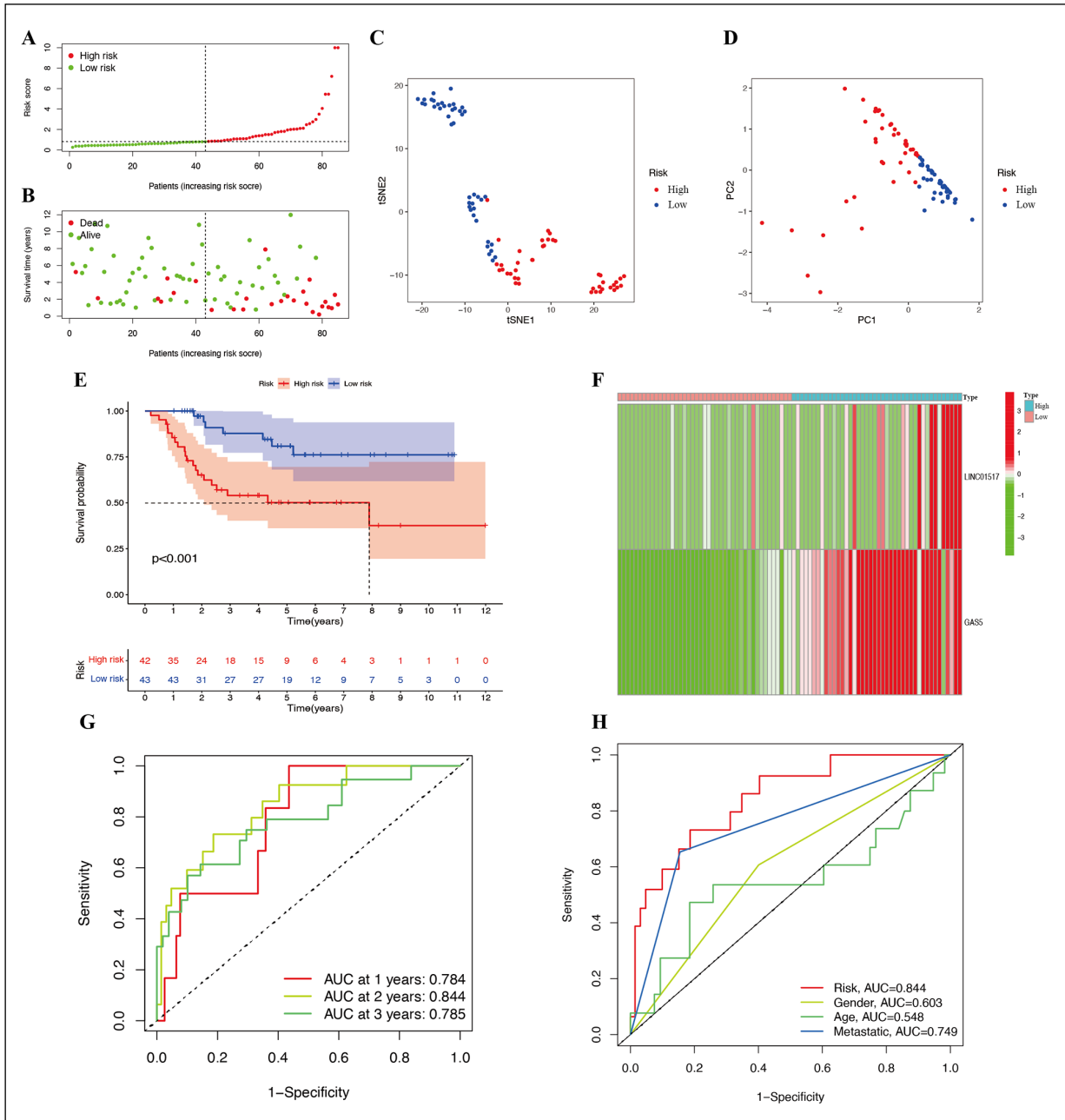


Figure 5. Construction of $m^1A/m^5C/m^6A$ -associated lncRNAs risk signature. Risk score distribution (A), survival status (B), t-SNE (C), and PCA plot (D) analysis of TARGET-osteosarcoma cohort. E, Survival curve of osteosarcoma patients. F, Heatmap of lncRNAs LINC01517 and GAS5 distribution in two risk subgroups. TimeROC (G) and ClinicalROC (H) curves to forecast overall survival of patients.

immune checkpoint protein PD-L2 in immune evasion, we analyzed the relationship between these loci and the $m^1A/m^5C/m^6A$ -associated lncRNA signature. The gene expression levels of PD-L2 were significantly negatively correlated with the risk score (Figure 7H). Additionally, the expression of PD-L2 was significantly higher in the low-risk subgroup than in the high-risk subgroup (Figure 7I).

Drug Sensitivity

In the drug sensitivity analysis, several immunotherapeutic drugs, such as AZD8055, AP.24534, Bexarotene, and Camptothecin, were significantly sensitive to the risk subgroups and patient clusters ($p < 0.05$; Figure 8). This indicated that the risk signature and clusters can be applied in further immunotherapy responses

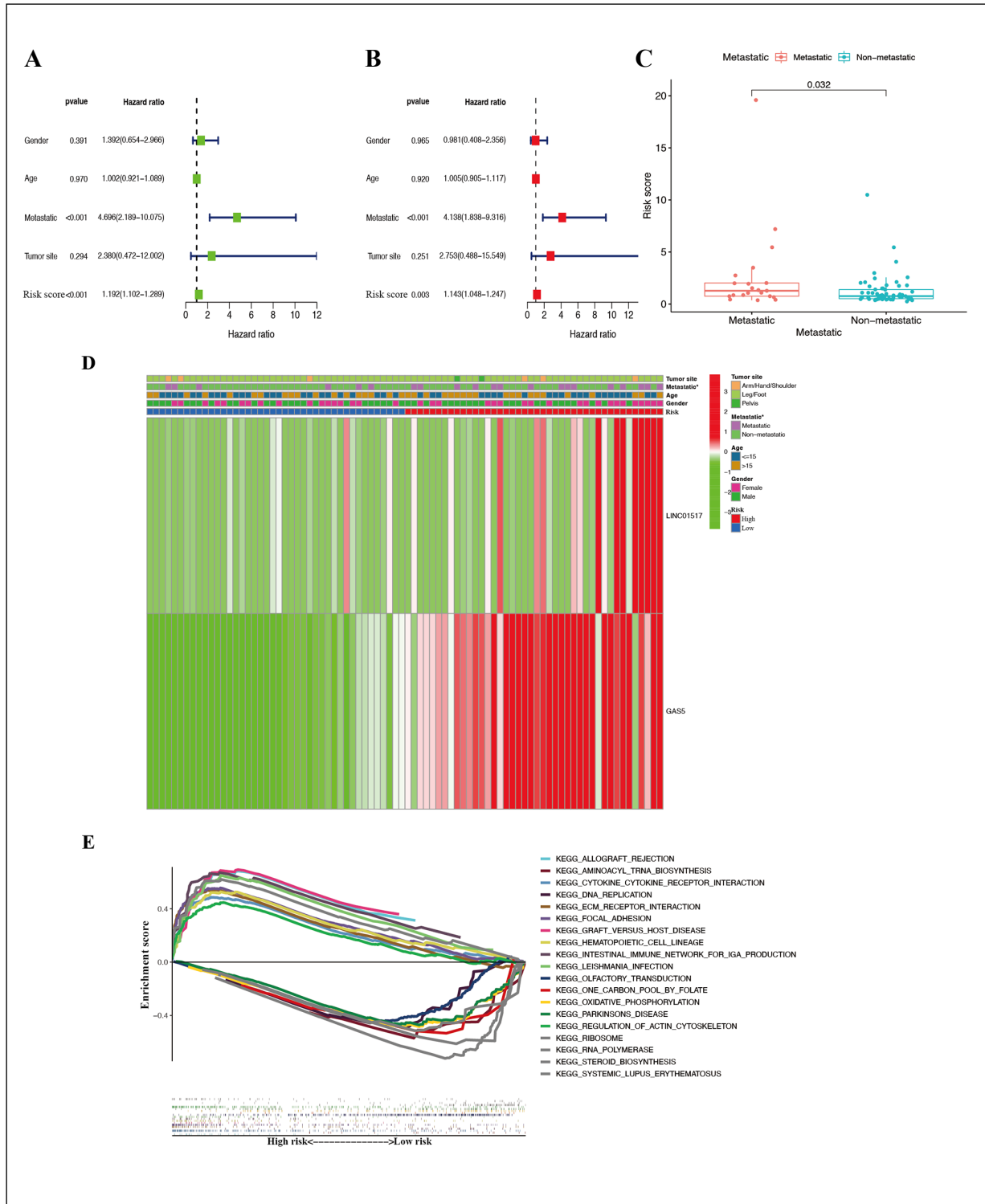


Figure 6. Associations between risk signature and clinicopathological factors. Univariate (A) and multivariate Cox (B) regression of clinicopathological features in TARGET-osteosarcoma cohort. C, Correlations between risk scores and metastatic capacity. D, The heatmap of clinicopathological features and hub lncRNAs expression in two risk subgroups. E, GSEA of top 13 enriched pathways in risk signature.

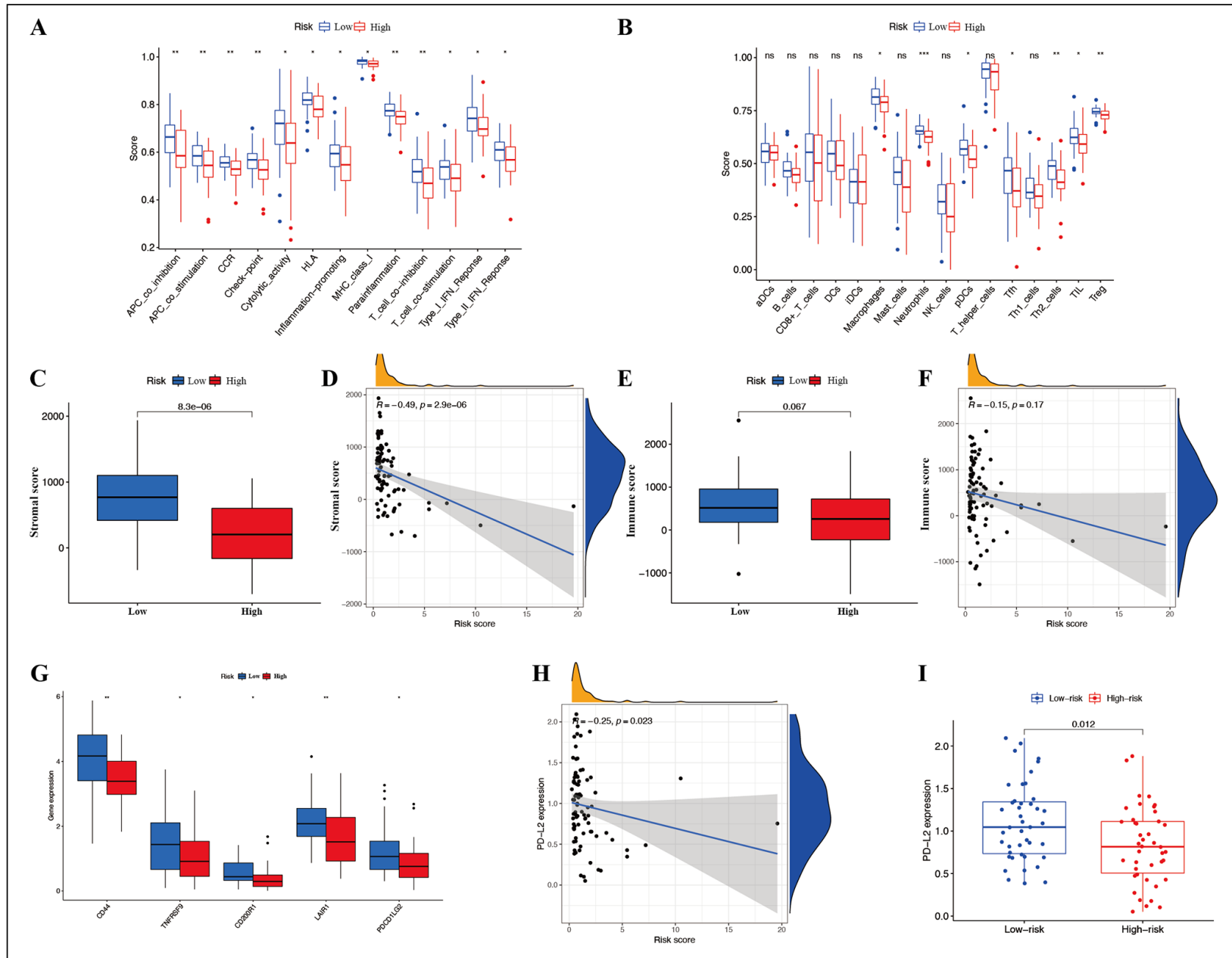


Figure 7. Potential role of risk signature in osteosarcoma immune status. Boxplots of scores of immune-associated functions (A) and immune cells (B) in risk subgroups. The scores of stromal (C) and immune (E) in two risk subgroups. Associations between risk signature, stromal scores (D), and immune scores (F). G, Expression of immune checkpoints among two risk subgroups in osteosarcoma patients. Correlation analysis between risk score and PD-L2 (H). Expression levels of genes PD-L2 (I) in risk subgroups.

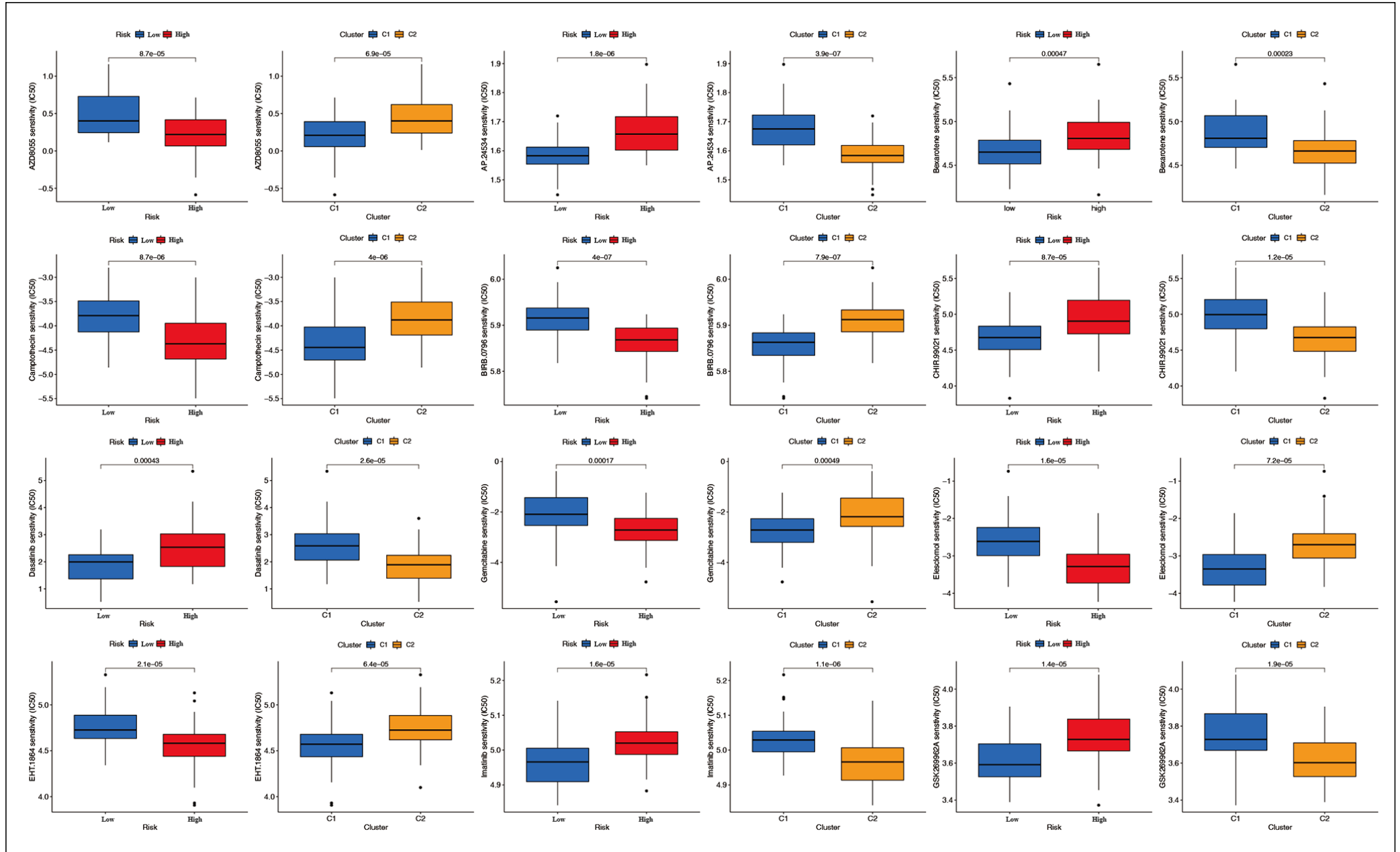


Figure 8. Drug sensitivity analysis of top 9 immunotherapeutic drugs solely showed significant IC50 difference among osteosarcoma clusters and two risk subgroups.

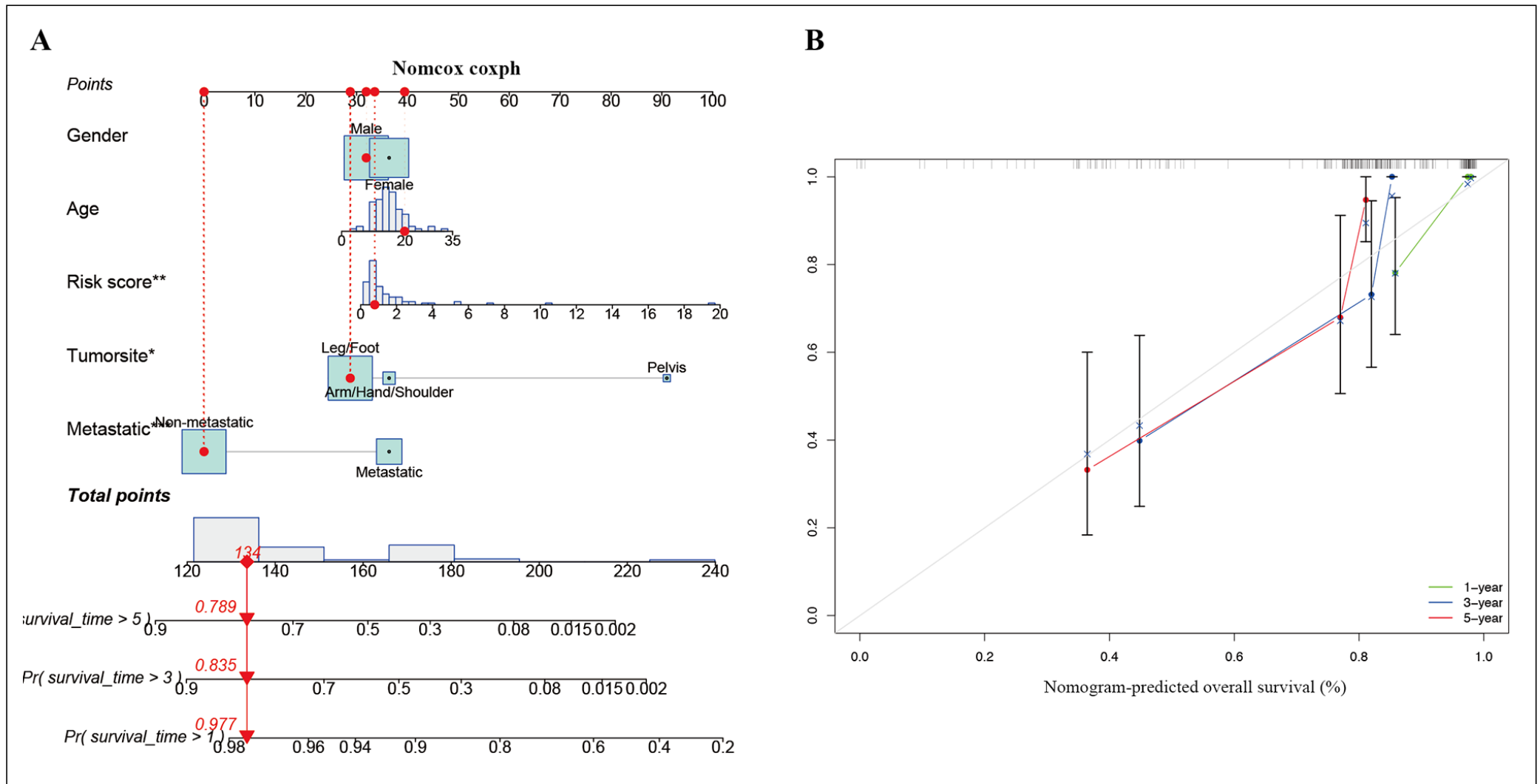


Figure 9. Construction of nomogram. **A**, Decision curve analysis of risk signature and other clinicopathological features. **B**, Nomogram for predicting osteosarcoma 1-, 3-, and 5-year overall survival.

studies and precise medication of osteosarcoma patients.

Nomogram Construction

Furthermore, the risk signature was used to construct a nomogram to predict the outcomes of osteosarcoma patients (Figure 9A). The calibration plots indicated that the predictive model had good conformity between observed and predicted outcomes at 1, 3, and 5 years (Figure 9B). Overall, the risk signature was associated with the development of osteosarcoma and might be a valuable tool for the clinical management of patients.

Discussion

Although next-generation sequencing technology has resulted in the discovery of various biomarkers for osteosarcoma, there is still a need for novel markers that are more closely associated with the early detection and prognosis of osteosarcoma patients. m¹A/m⁵C/m⁶A methylation is widely present in human cells and participates in various biological processes, such as mitochondrial dysfunction, stem cell differentiation, and gametogenesis. However, its role in osteosarcoma remains unclear. Additionally, a m¹A/m⁵C/m⁶A-associated lncRNA signature has not been reported for osteosarcoma yet.

Therefore, in the present study, m¹A/m⁵C/m⁶A methylation complexes (including 52 genes) were systematically analyzed to identify lncRNAs associated with the OS of osteosarcoma patients. Two hub m¹A/m⁵C/m⁶A-associated lncRNAs, LINC01517 and GAS5, were applied to construct a novel prognostic signature for osteosarcoma. Its prognostic value for osteosarcoma was verified by various approaches. The identified signature was significantly correlated with cancer metastasis, which is considered the main factor affecting the survival rate of osteosarcoma patients⁴. Compared to single clinical characteristics, regardless of age, gender, or metastatic status, the constructed risk signature not only showed higher accuracy for prognostic prediction but could also be used to predict the metastatic potential of osteosarcoma. Finally, the nomogram analysis revealed the effectiveness of our risk signature for predicting the outcomes of osteosarcoma patients.

Based on the GSEA, the risk signature was associated with immune-related pathways, such as the intestinal immune network for IgA production. Then, we evaluated the predictive

value of the m¹A/m⁵C/m⁶A-associated lncRNA signature in immune landscapes. Interestingly, all immune functions were significantly inhibited in the high-risk subgroup. Several immune cells, including macrophages, neutrophils, pDCs, Tfh, Th2 cells, TILs, and Tregs, also showed reduced infiltration in osteosarcoma. Given the critical roles of these immune cells in stimulating anti-tumor immunity²¹, it is reasonable to conclude that the degree of anti-tumor immunity of osteosarcoma patients in the high-risk subgroup was substantially reduced. Additionally, the ESTIMATE algorithm demonstrated that the stromal cell scores were negatively correlated with the risk score. This confirmed that the stromal cell infiltration was poor in the high-risk subgroup. To explore the value of the newly constructed gene signature in guiding chemotherapy, we conducted a drug sensitivity analysis. The results indicated that several immunotherapeutic drugs were significantly associated with the risk signature and osteosarcoma clusters. However, the specific mechanisms underlying these relationships require further exploration.

Cancer immunotherapies targeting immune checkpoints have improved the outcomes for various cancers²². PD-L2 is one of the key regulators of immune responses²³. Herein, we detected significantly differential expression of PD-L2 in the two risk subgroups. PD-L2 levels were also negatively correlated with the risk score. Moreover, the levels of several other immune checkpoints, including CD44, TNFRSF9, CD200R1, and LAIR1, were also significantly higher expressed in the low-risk subgroup and Cluster 2 compared to the other groups. These results indicated that immune responses were dramatically altered in the high-risk subgroup and Cluster 1. In conclusion, the m¹A/m⁵C/m⁶A-associated lncRNA signature could be used to predict the expression of immune checkpoints in osteosarcoma and might be used to guide the implementation of immunotherapy.

Despite the prognostic value of the current risk signature, this study also has some limitations. First, the results from our present retrospective study need further confirmation by prospective studies. Second, more experimental assays are needed to verify and validate the conclusions. In the future, functional studies should be performed to gain mechanistic insights into m¹A/m⁵C/m⁶A-associated lncRNAs and their role in osteosarcoma metastasis.

Conclusions

In the present study, a novel m¹A/m⁵C/m⁶A-associated risk signature consisting of two m¹A/m⁵C/m⁶A-associated lncRNAs was constructed and presented high predictive accuracy. This risk signature was valuable to predict parameters related to immune functions, immune cell infiltration, and the cancer microenvironment of osteosarcoma patients. To the best of our knowledge, this is the first m¹A/m⁵C/m⁶A-associated lncRNA signature for osteosarcoma. These results also provided a novel basis for understanding the specific effects of m¹A/m⁵C/m⁶A-related lncRNAs in osteosarcoma. Therefore, this study comprehends a significant contribution to the literature and can contribute to improvements in the outcomes and individualized treatments of osteosarcoma patients.

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Conflict of Interests

The authors declare that there is no conflict of interests.

Authors' Contributions

Conceived and designed the experiments: Zhaoyang Shi. Performed the experiments: Zhengyuan Wu. Analyzed the data: Zhengyuan Wu. Contributed reagents/materials/analysis tools: Zhengyuan Wu. Wrote the paper: Zhengyuan Wu.

Data Availability Statement

The datasets analyzed during the current study are available sourced from the publicly available TARGET database (https://ocg.cancer.gov/progr_ams/target).

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Ethics Approval and Consent to Participate

Not applicable.

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