Immune Cell Infiltration Score and Predicting Model Among Lower-grade Gliomas Based on Immunogenomic Clusters

Zhile Wang  
PUMCH: Peking Union Medical College Hospital  
https://orcid.org/0000-0001-6820-8383

Fucun Xie  
PUMCH: Peking Union Medical College Hospital

Yijun Wu  
PUMCH: Peking Union Medical College Hospital

Li Wang  
PUMCH: Peking Union Medical College Hospital

Yi Bai  
Tianjin First Central Hospital

Junyu Long  
PUMCH: Peking Union Medical College Hospital

Xiang Wang  
Department of Medical Oncology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China.  
https://orcid.org/0000-0002-2202-2871

Primary research

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Abstract

**Background and objectives:** Recurrent malignancies had become a significant problem for the treatment of gliomas. Though immunotherapy was regarded as a possible solution, verification of immune-checkpoint inhibitors in multiple clinical cases failed. We aimed to explore target genes for immunotherapy and evaluate the genes through new scoring criteria.

**Methods:** We firstly classified the patients through k-means clustering in immune cell level and gene level. Differential prognostic genes were weighted through principal component analysis (PCA) and Boruta algorithm. The comprehensive scoring of each component was defined as the ICI score. We further analyzed the relationship between ICI score and various clinical factors and prognosis. Moreover, a nomogram based on the ICI scores was built and validated through both internal and external validation.

**Results:** The study cohort finally enrolled 495 patients. We identified a list of differential genes of which the expression level was closely related to prognosis. ICI scores were calculated for each case according to the expression level of prognostic genes. The cases in high ICI score group showed significantly lower survival probabilities (log-rank test; p<0.001). We further built a nomogram model based on the ICI scores. The area under the receiver operating characteristic (ROC) curve (AUC) values of the nomogram were 0.851, 0.86, and 0.768 in internal validation, and 0.725, 0.744, and 0.735 for 1-year, 3-year and 5-year truncation time in external validation.

**Conclusions:** The differential genes were listed for further studies. ICI score had been confirmed to be closely related to prognosis and even genome instability. And the nomogram model based on ICI scores showed the feasibility in clinical practice in both internal and external validation.

Introduction

Gliomas comprised the majority of brain malignancies for the last few decades[1]. In clinical practice, glioma was divided into lower-grade glioma (LGG, grade II/III) and glioblastoma (GBM, grade IV) based on the grades[2]. Lower-grade gliomas were mainly observed among childhood brain primary malignancy cases[3]. 2016 WHO classification divided adulthood gliomas into three categories: IDH-mutant with 1p/19q-co-deleted tumors, IDH-mutant with non-1p/19q-co-deleted tumors, and IDH-wild-type tumors[4]. The differences in classification influenced both the treatment and prognosis of glioma patients. Surgical resection with postoperative radiation was still recommended for LGG patients, especially for those with superficial lesions[5-7]. And relevant studies recommended chemotherapy agents as additional strategies for unresectable cases[8]. Despite all the strategies, recurrence or progression of original lesions was still a challenge. Novel strategies for the treatment of recurrent gliomas were urgently needed.

Though the brain had been recognized as an immune-privileged organ for over half a century, recent studies showed that tumor microenvironment (TME) of brain malignancies were filled with enough immune cells to generate immune responses[9, 10]. Naturally, researchers focused on immune-checkpoint inhibitors as possible solutions for recurrent glioma. An earlier study proved that Inhibitors of PD-1/PD-L1
or CTLA-4 were effective to gliomas in vitro[11]. Unfortunately, the failure among in vivo clinical trials[12, 13] stalled further exploration for immunotherapies.

The study was initially designed to explore possible genes as immunotherapy targets and evaluate immune status by scores. We collected the study cohort from The Cancer Genome Atlas (TCGA) database and enrolled a total of 495 patients. Extra figures of the study were shown in additional files in details [see Additional file 1].

Data And Materials

Datasets and Samples

The original dataset of LGG samples (including transcriptome sequencing data, clinical data, and mutation information) was derived from the TCGA-LGG database, and accessed through the TCGA dataset website (https://portal.gdc.cancer.gov/). mRNAseq_693[14, 15] and mRNAseq_325[16, 17] [from the Chinese Glioma Genome Atlas (CGGA) database, http://www.cgga.org.cn/] were included in the study as external validation datasets for the nomogram model.

Clinical Features

The clinicopathological features of all enrolled patients included overall survival status, gender, age, histology, WHO grade, and IDH/1p19q subtype. Age was recorded when the patients were diagnosed. The latest WHO classification was based on IDH mutation and 1p/19q codeletion. The molecular biological features in the study included immune cell types, transcriptome expression level, and mutation status of genes. tumor mutation burden (TMB) values were further calculated based on the mutation data. And the ICI scores were further calculated based on the different genes among ICI clusters.

Immune Cell Infiltration

The ICI levels of tumor cells were quantified through the 'CIBERSORT' R source (R version 4.0.1)[18]. The 22 immune cell types included: naïve B cells, memory B cells, Plasma cells, CD8 T cells, CD4 naïve T cells, CD4 memory resting T cells, CD4 memory activated T cells, follicular helper T cells, regulatory Treg T cells, gamma delta T cells, resting NK cells, activated NK cells, Monocytes, M0 Macrophages, M1 Macrophages, M2 Macrophages, resting Dendritic cells, activated Dendritic cells, resting Mast cells, activated Mast cells, Eosinophils, and Neutrophils (employing leukocyte signature matrix and 1,000 permutations during calculation). The stromal, immune, and ESTIMATE scores were collected from single sample gene set enrichment analysis (ssGSEA) algorithm by 'estimate' R package.

Consensus Clustering

The k-means clustering algorithm was conducted based on the immune cell types using Euclidean distance. The consensus matrix plot (shown in Figure S1) and Probably Approximately Correct algorithm were set as the cluster number selection criteria. The clustering process for genes was similarly
conducted. A final of four ICI clusters and two gene clusters were selected, and were named "A", "B", "C", "D" ICI clusters and "A", "B" gene clusters individually.

ICI Score Calculation

ICI score was defined to describe the overall status of prognostic genes in the study. And the calculation of the ICI scores was accomplished in the following process: The Boruta algorithm trained a dataset based on the random forest classification[19], and adopted a feature importance ranking measure to evaluate each immune cell type's importance. After continuous deletion of unimportant features, the Boruta would score the importance of each immune cell type. The values of ICI score were calculated through the following equation.

\[
\text{ICI score} = \sum PC_1^A - \sum PC_1^B
\]

Gene Set Enrichment Analysis

To quantify the enrichment level of immune cells and immune-related pathways among samples, we conducted enrichment analysis by employing single-sample gene set enrichment analysis (ssGSEA) based on the Kyoto Encyclopedia of Genes and Genomes database. The pathways were scored according to the expression level of genes among samples. And the highly enriched pathways were selected and plotted based on both the enrichment score and the ICI score.

Tumor Mutation Burden

TMB was defined as the total count number of somatic mutations in samples. TMB values were calculated based on the mutation data from TCGA-LGG. The clinical value of the TMB score had been verified in earlier studies. And the relationship among TMB, ICI score, and overall survival were further analyzed through the Kaplan-Meier method.

Statistical Analysis

All statistical analyses were conducted using R software version 4.0.1 (http://www.r-project.org/) and GSEA (version 4.1.0) (http://software.broadinstitute.org/gsea/). A two-tailed P value < 0.05 was considered statistically significant in the study.

The levels of immune cell types were calculated through the 'CIBERSORT' R source based on the transcriptome expression levels. The scoring process of infiltrated immune environment was conducted by 'estimate' R package. Both ICI and gene expression were clustered ('ConsensusClusterPlus' R package) for further analysis. The correlation picture of immune cells was plotted ('corrplot' R package). And the heatmap of ICI and gene clusters were drawn ('pheatmap' R package). Kaplan-Meier analysis and log-rank tests ('survival' R package) further plotted survival curves among clusters. The study's box plots were plotted using 'ggpubr' R package, with dot plots using 'enrichplot' R package. Additional enrichment
analysis was conducted through GSEA software (version 4.1.0). TMB values were calculated through R software, with oncoplots plotted using 'maftools' R package.

Clinical features with ICI score groups were screened to find prognostic factors through the least absolute shrinkage and selection operator (LASSO) regression ('glmnet' R package). Univariate and multivariate Cox regression ('survival' R package) was then conducted to select independent prognostic factors for model-building. The nomogram model was built using the 'rms' R package based on samples randomly selected from the TCGA-LGG dataset. The internal validation was based on the testing group (the rest samples of TCGA-LGG). And external validation was conducted using data from the CGGA dataset (combination of mRNAseq_693 and mRNAseq_325 with batch effect confounding removed through 'sva' R package). ROC curves of the nomogram in different datasets were plotted using 'survivalROC' R package. And calibration curves were plotted through 'rms' R package. Time-dependent decision curve analysis (DCA) was conducted using the 'stdca' R source.

Results

Data Preparation

We collected the information of 511 patients (529 samples) was from the TCGA-LGG dataset. Due to the limited mutation data, 495 patients were finally enrolled. Moreover, we recorded another 1,018 patients for external validation from the CGGA database (mRNAseq_693 and mRNAseq_325). All transcriptome expression data among datasets accepted the following transition before data analysis: .

We acquired the immune infiltration status of 529 samples through 'CIBERSORT' analysis. 314 out of 529 samples (305 out of 511 patients) passed the verification process (p<0.05). Furthermore, we calculated the stromal, immune, and ESTIMATE scores. The Pearson correlation plot of immune cell types among all patients was shown in Figure 1C.

Immune Cell Infiltration Cluster

Four ICI clusters were built based on the types of infiltrated immune cells among all 305 patients. The consensus matrix plot was shown in additional files [see Additional file 1]. Heatmap of four immune cell infiltration (ICI) clusters were plotted in Figure 1A. Survival curves of each ICI cluster were plotted and shown in Figure 1D. Significant differences were observed between ICI cluster A-B (log-rank test; p=0.003; Figure 1D), B-C (log-rank test; p<0.001), and C-D (log-rank test; p=0.034).

The differences in prognosis among different ICI clusters might result from the differences in specific genes. Moreover, we compared these clusters in immune-checkpoint gene expression and immune cell types. Cluster B showed significantly higher gene expression levels in PD-1, PD-L1, CTLA-4, IDO1, and CD161 expression than the rest three clusters (Kruskal-Wallis test; p<0.01; Figure 1E-I). Cluster C had a better prognosis than cluster D. However, the differences between the two clusters were not significant enough in PD-1, CTLA-4, and IDO1 expression (Kruskal-Wallis test; p>0.05). We further compared the ICI
clusters in immune cell types. ICI cluster B had significantly more infiltrated immune cells in the following types: naïve B cells (Kruskal-Wallis test; p<0.01; **Figure 1B**), CD8 T cells (Kruskal-Wallis test; p<0.001), resting memory CD4 T cells (Kruskal-Wallis test; p<0.001), M0 macrophage (Kruskal-Wallis test; p<0.001), M1 macrophage (Kruskal-Wallis test; p<0.001), and neutrophile (Kruskal-Wallis test; p<0.001). And cluster B also got the highest stromal and immune score (Kruskal-Wallis test; p<0.001). Cluster C had more activated mast cells and eosinophils (Kruskal-Wallis test; p<0.001) with a better prognosis than other clusters. For cluster D, which received a relatively worse prognosis, had the most M2 macrophage than other clusters (Kruskal-Wallis test; p<0.001).

**Gene Expression Cluster**

To identify the critical prognostic factor among the ICI clusters, we selected the genes that differed in expression levels for further analysis. We reclassified all 511 patients into two consensus clusters according to listed genes, including gene cluster A (397 patients) and B (114 patients). The consensus matrix plots for gene clusters were also shown in additional files [see Additional file 1]. The heatmap of gene clusters was shown in **Figure 2A**. We further classified genotypes according to the correlation coefficients between expression level and gene cluster. Genotype A included data of positive coefficients, and genotype B consisted of negative-coefficient data. We conducted intersection (495 patients) of the gene cluster dataset (511 patients) and clinical dataset (495 patients) for Kaplan-Meier analysis. And gene cluster A (383 patients) acquired significantly higher survival probabilities than gene cluster B (112 patients) (log-rank test; p<0.001; **Figure 2B**). Gene ontology enrichment analysis showed the high-level expression pathways concerning biological process, cellular component, and molecular function in both gene cluster A and B groups (**Figure 2C**). All pathways listed in the bubble plot were significantly highly expressed in tumor tissues.

Comparison results of immune cell types and expression of immune-checkpoint genes were shown in **Figure 2D-H**. Gene cluster B showed worse survival outcome with a higher level of immune cells in the following types: naïve B cells (Kruskal-Wallis test; p<0.01; **Figure 2D**), CD8 T cells (Kruskal-Wallis test; p<0.001), resting memory CD4 T cells (Kruskal-Wallis test; p<0.001), activated memory CD4 T cells (Kruskal-Wallis test; p<0.05), regulatory T cells (Kruskal-Wallis test; p<0.05), M0 macrophage (Kruskal-Wallis test; p<0.001), M1 macrophage (Kruskal-Wallis test; p<0.001), resting mast cells (Kruskal-Wallis test; p<0.01), eosinophils (Kruskal-Wallis test; p<0.05), and neutrophils (Kruskal-Wallis test; p<0.001). Stromal and immune scores of gene cluster B were significantly higher than cluster A (Kruskal-Wallis test; p<0.001; **Figure 2D**). As for immune-checkpoint genes, gene cluster B also had relatively higher expression levels. The relevant genes included PD-L1 (Kruskal-Wallis test; p<0.001; **Figure 2E**), CTLA-4 (Kruskal-Wallis test; p<0.001; **Figure 2F**), IDO1 (Kruskal-Wallis test; p<0.001; **Figure 2G**), and CD161 (Kruskal-Wallis test; p<0.001; **Figure 2H**).

**Immune Cell Infiltration Score**

To better describe the correlations between gene clusters and expression levels, we divided the patients by the correlation coefficients into two genotypes through Boruta analysis (Boruta analysis showed
significantly better performance in large number gene weighting than traditional regression models[20]). Further reduction of dimensions was conducted through PCA. The ICI scores (based on results of Boruta and PCA) contained the information of prognostic genes among LGG patients. The relationship among ICI score, gene cluster, and the prognosis was shown in the alluvial plot (Figure 3A). The high ICI score group had significantly lower survival probabilities than the low ICI score group (log-rank test; p<0.001; Figure 3B). Moreover, the high ICI score group showed higher gene expression in CXCL10, PD1, IDO1, LAG3, GZMB, GZMA, CD8A, HAVCR2, CXCL9, CTLA4, PRF1, and PD-L1 (Kruskal-Wallis test; all p values<0.001; Figure 3C). Enrichment analysis was conducted at the pathway level, and the immune-related highly-enriched pathways for the high ICI score group were shown in Figure 3D.

Tumor Mutation Burden

Mutation information for both the high ICI score group and low ICI score group was firstly analyzed through the ‘maftool’ R package, and the oncoplots for each group were plotted in Figure 4A and 4B. Mutations in IDH1, TP53, ATRX, and CIC frequently happened in both groups, and non-missense mutation occurred most frequently in ATRX. Further, we calculated the tumor mutation burden value, and the high ICI score group got significantly higher TMB values (Kruskal-Wallis test; p<0.001; Figure 4C). The correlation coefficient between TMB values and ICI scores was 0.17 (Spearman test; p<0.001; Figure 4D). Patients with high TMB values acquired significantly lower survival probabilities (log-rank test; p<0.001; Figure 4E). To better describe the prognostic relationship of both ICI scores and TMB values, patients were divided into four groups. High TMB values with high ICI score subgroup acquired the minuscule survival profits (log-rank test; p<0.001; Figure 4F), and low values for both TMB and ICI scores received the most survival benefits (log-rank test; p<0.001; Figure 4F).

Clinical Features and ICI Score Group

The differences in prognosis between high and low ICI score groups were significant. Nevertheless, there still might be selection bias during the enrolling process. The limited number of patients prevented us from conducting propensity score matching (PSM) to minimize the effect of selection bias. Therefore, we further compared patients’ survival outcomes with different ICI scores in multiple clinical feature subgroups. Significant differences were observed between high and low ICI score subgroups in different groups divided by age (log-rank test; p≤0.001; Figure 5B and C), gender (log-rank test; p<0.001; Figure 5E and F), and histology (log-rank test; p≤0.01; Figure 5H-J). And we also observed that the ICI score distribution was not relatively even among patients with different clinical features. Patients in the following groups had significantly lower ICI scores: age < 56 years (vs. ≥ 56 years; Wilcox test; p<0.01; Figure 5A) and oligodendroglioma (vs. astrocytoma; Wilcox test; p<0.01; Figure 5G).

Nomogram Model

Clinical features and ICI score group were analyzed through the LASSO regression, univariate Cox regression, and multivariate Cox regression. Age, grade, and ICI score were identified as independent prognostic factors (Wald test; all p values<0.05; Table 1). We randomly divided all 495 available patients
into the training group (248 patients) and test group (247 patients) based on the three risk factors. The nomogram model was built based on the training group (Figure 6A). Further internal validation was conducted among 247 patients in the test group. The 1-year, 3-year, and 5-year ROC curves in the training and test groups were shown in Figure 6B and C. The AUC values were 0.895, 0.754, and 0.73 for the training set. And the AUC values of internal validation were 0.851, 0.86, and 0.768 for 1-year, 3-year, and 5-year truncation time. We further draw the calibration curve for internal validation to correct the model's possible bias (Figure 6E). Figure 6G showed the 3-year time-dependent DCA curve in internal validation, and the rest DCA curves were shown in additional files [see Additional file 1]. External validation was conducted based on the 591 patients from the CGGA database. The ROC was plotted in Figure 6D, and the AUC value was 0.725, 0.744, and 0.735 for 1-year, 3-year, and 5-year truncation time. The calibration curve and time-dependent DCA of external validation were shown in Figure 6F and H.

**Discussion**

Currently, surgery was recommended as first-line therapy for regional resectable glioma[3]. Relapse of glioma, especially among glioblastoma patients, was quite common after surgery. Therapeutic options for relapse glioma did exist, but the patients' prognosis was not satisfying[5]. New treatment strategies for glioma were in urgent demand.

Ingression of immune cells in the microenvironment of glioma was widely reported, and the differences in immune cell types were closely related to prognosis of glioma patients. Myeloid cells were the key mediators of immune suppression[3]. Microglia, dendritic cells (DCs), and macrophages, especially M2 macrophages, were significantly highly expressed in the TME[21-23]. The distribution of immune cells among different clusters were shown in Figure 1B and 2D. All cluster which had worse prognosis showed higher level of the following immune cells in common: naïve B cells, CD8 T cells, resting memory CD4 T cells, M0 macrophage, M1 macrophage, and neutrophiles. Though these immune cell types had not been directly proved as risk factors for glioma, these cells were possible targets for immunotherapy in the future. Due to the lack of information, we decided to list the immune cell types for reference of further studies.

Immune-checkpoint had been a significant focus among all immunotherapies. Indoleamine 2,3-deoxygenase enzymes and PD-1/PD-L1[24] were highly expressed to affect the immune tolerance of T cells[25]. Besides, CTLA-4 and LAG-3 were also highly expressed in glioma cells and were related to immune suppression among glioma patients[26]. Despite all the immune-checkpoint studies' efforts, anti-PD-1 therapies failed to prove the survival benefits in phase III clinical trials[12, 13]. Highly immunosuppressive TME might have influenced the efficacy of the therapy. Furthermore, new immune targets were also a possible solution for the reduced efficacy. Recently, CD161 was considered as another possible molecular target for immunotherapy. Infiltrated T cells highly expressed CD161 to inhibit the anti-tumor effect of killer T cells. The process might share some features with the PD-1 pathway, since the CD161 was also expressed in immunosuppressive myeloid cells and tumor cells[27]. In the study, ICI cluster B showed higher immune-checkpoint gene expression levels in PD-1, PD-L1, CTLA-4, IDO1, and
CD161 with significantly worse prognosis (Figure 1D-I). Similar results were also observed in gene clusters and ICI score clusters (Figure 2B, E-H and Figure 3B-C). Based on the results of ICI score groups, the following immune-checkpoint genes were negatively related to prognosis: CXCL-10, PD-1, IFN-gamma, IDO1, LAG-3, CD161, GZMB, GZMA, CD8A, TIM3, CXCL9, CTLA-4, PRF1, and PD-L1. And part of the conclusion just supported previous studies[28-33].

The differences in expression level of immune-checkpoint genes might not completely explain the differences in prognosis. In the study, ICI cluster C and D showed different prognosis with similar expression levels in PD-1, IDO1, and CTLA-4. Between ICI cluster B and D, significant differences in immune-checkpoint genes were observed with similar prognosis. Other prognostic genes might exist besides identified immune-checkpoint genes. Differences in prognosis between gene cluster A and B proved that the differential genes among ICI clusters were related to prognosis (Figure 2B). Further analysis was focused on description of genes based on pathways and weighting the prognostic genes.

Phenomenon of different immune cells influencing prognosis through different pathways had been revealed in many studies. Infiltrated immune cells expressed multiple tumor-intrinsic factors to form the immunosuppression TME of glioma[3]. Signal transducer and activator of transcription 3[34], TGF-β[35], IL-10 signaling pathway had been proved to play an essential role in suppressing the activity of immune cells among glioblastoma patients. Highly expressed pathways between high and low ICI score groups were shown in Figure 3D.

TMB had been regarded as a key prognostic indicator for many tumors including glioma[36, 37]. And we calculated and analyzed the TMB values with many clinical features which had been proved as prognostic factors for reduction of bias[38, 39]. TMB values were positively related to ICI scores (Figure 4D). Furthermore, comparison of prognosis between high and low ICI score groups received same results in prognostic clinical factors (Figure 5). After minimizing the influence of selection bias, the ICI scores were still closely related to prognosis.

Finally, we built a nomogram model to evaluate the ICI score's clinical application's significance. Through both internal and external validation, the model showed impressive predicting ability (Figure 6B-H). The application of both ICI score and the nomogram could be rational in prognosis-prediction and even therapy-selection.

There were still some shortages in the study. 1) Firstly, the dataset was not big enough for us to conduct PSM analysis to minimize the influence of selection bias. We had to compared the prognosis with specific prognostic clinical features, and calculate the TMB values. 2) The dataset of the study was collected from the TCGA database, and the results might not be applicable in some regions. More data were required to ensure the reliability of the results.

**Conclusions**
The ICI score, which was built according to immune-related differential genes, had been proved to be a reliable predictor for prognosis. The nomogram model based on the ICI score showed great guiding significance for clinical practice through multiple validations.

**Abbreviations**

AUC: Area under the ROC curve

CGGA: Chinese Glioma Genome Atlas

DC: Dendritic cells

DCA: Decision curve analysis

GBM: Glioblastoma

ICI: Immune cell infiltration

LASSO: Least absolute shrinkage and selection operator

LGG: Lower-grade glioma

PCA: Principal component analysis

ROC: Receiver operating characteristic

ssGESA: Single sample gene set enrichment analysis

TCGA: The Cancer Genome Atlas

TMB: Tumor mutation burden

TME: Tumor microenvironment

WHO: World Health Organization

**Declarations**

**Ethics approval and consent to participate**

The Ethical Committee of Peking Union Medical College Hospital had reviewed and approved the study. And the study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. The ethics approval and consent to participate were available in the webpages of TCGA and CGGA database.

**Consent for publication**
Not applicable.

**Availability of data and materials**

The datasets supporting the conclusions of this article are available in the TCGA-LGG database, [https://portal.gdc.cancer.gov/](https://portal.gdc.cancer.gov/), and in the mRNAseq_693 and mRNAseq_325 repository, [http://www.cgga.org.cn/](http://www.cgga.org.cn/).

**Competing interests**

All authors have no conflict of interest to declare.

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**Author Contributions**

**Wang Zhile**: Conceptualization, Data Curation, Formal analysis, Visualization, Writing- Original draft preparation, Writing- Reviewing and Editing.

**Xie Fucun**: Methodology, Software, Resources.

**Wu Yijun**: Software, Formal analysis, Validation.

**Wang Li**: Software, Validation.

**Bai Yi**: Methodology.

**Junyu Long**: Methodology.

**Wang Xiang**: Supervision.

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### Tables

**Table 1. Univariate and Multivariate Cox regression analysis for selecting prognostic factors.**

| Characteristic   | Classification | Univariate Cox Regression | Multivariate Cox Regression |
|------------------|----------------|---------------------------|-----------------------------|
|                  |                | HR | 95% CI      | P value | HR | 95% CI      | P value |
| Age              | < 56 years     | 3.648 | 1.992-6.678 | <0.001  | 3.419 | 1.833-6.377 | <0.001  |
|                  | ≥ 56 years     | -   | Reference  | -  | -   | Reference  | -  |
| ICI score group  | High           | -   | Reference  | -  | -   | Reference  | -  |
|                  | Low            | 0.325 | 0.188-0.563 | <0.001  | 0.497 | 0.274-0.903 | 0.022  |
| WHO Grade        | II             | -   | Reference  | -  | -   | Reference  | -  |
|                  | III            | 2.742 | 1.544-4.870 | <0.001  | 2.185 | 1.183-4.034 | 0.013  |
| Gender           | Feale          | -   | Reference  | -  | *   | -   | -  |
|                  | Male           | 1.710 | 0.970-3.015 | 0.064  | *   | -   | -  |
| Histology        | Astrocytoma    | 1.641 | 0.964-2.795 | 0.068  | *   | -   | -  |
|                  | Oligodendroglia| 0.624 | 0.348-1.120 | 0.114  | *   | -   | -  |
|                  | Oligoastrocytoma| 0.906 | 0.463-1.773 | 0.773  | *   | -   | -  |

HR: hazard ratio, 95% CI: 95 percent confidence intervals. The bold parts represented a p value < 0.05 among subgroups. * The clinicopathological factors were excluded by univariate Cox regression test as prognostic factors.

### Figures
Figure 1

Plots related to the four ICI clusters (ICI cluster A, B, C, and D) during analysis. (A) Heatmap of four ICI clusters. (B) Boxplots of immune cell types among four ICI clusters. (C) Pearson correlation plot among all enrolled patients. (D) Survival curves among four ICI clusters. (E-I) Violin plots of gene expression among four ICI clusters in PD-L1, PD-1, IDO1, CTLA-4, and CD161.
Figure 2

Plots related to the two gene clusters (gene cluster A and B) during analysis. (A) Heatmap of two gene clusters. (B) Survival curves among two gene clusters. (C) gene ontology enrichment analysis in gene cluster A (left) and gene cluster B (right). (D) Boxplots of immune cell types among two gene clusters. (E-H) Violin plots of gene expression among two gene clusters in PD-L1, CTLA-4, IDO1, and CD161.
Figure 3

ICI score groups relationship and analysis in gene and immune-related pathway level. (A) Alluvial plots of gene clusters, ICI score groups, and overall survival status. (B) Survival curves of high ICI score group (blue) and low ICI score group (yellow). (C) Boxplots of immune-related gene expression between high ICI score group (yellow) and low ICI score group (blue). (D) Enrichment plots of immune-related pathways between high and low ICI score groups.
Figure 4

TMB-related plots during analysis. (A) Oncoprint plot among patients in high ICI score group. (B) Oncoprint plot among patients in low ICI score group. (C) Boxplot of TMB values between high (red) and low (blue) ICI score group. (D) Correlation plot of TMB values in different gene cluster. (E) Survival curves between high TMB group (blue) and low TMB group (yellow). (F) Survival curves among groups with different ICI scores and TMB values.
**Figure 5**

Survival curves and related boxplots of patients with specific clinical features. (A) Boxplot of patients < 56 years (blue) and ≥ 56 years (red). (B, C) Survival curves of patients ≥ 56 years (B) or < 56 years (C) between high (blue) and low (red) ICI score groups. (D) Boxplot of female (blue) and male (red) patients. (E, F) Survival curves of female (E) and male (F) patients between high (blue) and low (red) ICI score groups. (G) Boxplot of patients with astrocytoma (blue), oligoastrocytoma (red), or oligodendroglioma.
(yellow). (H-J) Survival curves of patients with oligoastrocytoma (H), oligodendroglioma (I), or astrocytoma (J) between high (blue) and low (red) ICI score groups.

Figure 6

Nomogram model and validation plots. (A) The nomogram model based on ICI scores. (B) The ROC curve of training set in 1-year, 3-year, and 5-year truncation time. (C) The ROC curve of internal validation in three truncation time. (D) The ROC curve of external validation in three truncation time. (E) Calibration
curve of internal validation. (F) Calibration curve of external validation. (G) 3-year DCA curve of nomogram and multiple prognostic factors through internal validation. (H) 3-year DCA curve of nomogram and multiple prognostic factors through external validation.

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