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Sex-stratified evaluation of immune and cell cycle transcriptional programs in lower grade glioma

Arylic Singh¹ · Katrina Shute² · Cameron Hawk³ · Muhammed Sulman⁴ · Allan R. Martin¹

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Abstract

Purpose Sex influences the incidence, molecular characteristics and clinical outcomes of gliomas, but the biological pathways contributing to these differences are not fully understood. In lower grade glioma (LGG), immune activity and cell cycle dysregulation are key components of tumor behavior. Because sex heavily influences baseline survival, it is critical to determine if the prognostic relevance of these transcriptional programs differs between males and females, or if they offer universal utility. This study evaluated the robustness of immune and cell cycle gene expression signatures as prognostic markers across sex-stratified cohorts in LGG using data from The Cancer Genome Atlas (TCGA).

Methods Clinical and Ribonucleic acid (RNA) sequencing data from 510 patients with lower grade glioma in The Cancer Genome Atlas were analyzed. Only primary tumors with complete information on survival, age, sex, grade and primary diagnosis were included. Raw RNA counts were normalized to log-counts per million using the trimmed mean of M values (TMM) method in edgeR. Two predefined transcriptional signatures were examined: an immune activation signature (CD3D, CD3E, CD8A, CXCL9, CXCL10, GZMB, PDCD1) and a cell cycle proliferation signature (MKI67, PCNA, TOP2A, CCNB1, CDK1, BIRC5). Signature scores were calculated as the mean expression of component genes, standardized to z-scores, and categorized into within-sex tertiles. Associations with overall survival were evaluated using Kaplan–Meier curves and multivariable Cox proportional hazards models. Models adjusted for age and grade included interaction terms to evaluate whether prognostic utility varied by sex.

Results Higher immune and cell cycle signature scores were each associated with shorter overall survival (immune: log-rank $p=0.0028$; cell cycle: $p<0.0001$). In multivariable Cox models adjusted for age and grade, both signatures remained independently associated with mortality (immune HR per SD = 1.58, 95% CI 1.25–2.00; cell-cycle HR = 1.47, 95% CI 1.15–1.89). Crucially, interaction analyses indicated that the prognostic impact of these signatures did not significantly differ between sexes, demonstrating consistent adverse associations across both male and female cohorts.

Conclusion Immune and cell cycle transcriptional programs show robust, independent associations with survival in lower grade glioma that are consistent across sexes. Elevated activity in either pathway is universally linked to poorer outcomes. These findings validate the broad prognostic utility of these transcriptional signatures in LGG, highlighting their potential clinical relevance regardless of patient sex.

Keywords Lower grade glioma · Sex differences · Transcriptomics · Tumor microenvironment · Cell cycle · Prognostic biomarker

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Introduction

Diffuse lower grade gliomas (LGGs), defined by the World Health Organization (WHO) as adult-type diffuse gliomas of grade II and grade III, represent a biologically and clinically heterogeneous group of primary brain tumors. Although outcomes are generally more favorable than for glioblastoma, survival varies widely, with median overall survival ranging from approximately 6 to 15 years depending on molecular subtype and clinical context [1, 2]. Epidemiologic studies consistently demonstrate a male predominance, with men accounting for nearly 60% of glioma cases [3, 4]. Beyond this higher incidence, males often experience poorer clinical outcomes, establishing sex as an important demographic and biological variable in glioma behavior [5].

Historically, these sex-related disparities have been most extensively described in glioblastoma. Several transcriptomic and clinical analyses have reported a survival advantage in females, independent of age, extent of resection, or adjuvant therapy [6]. A landmark study by Yang et al. applied sex-stratified transcriptomic profiling and found that male survival correlated with lower expression of cell cycle and proliferation genes, while female survival was associated with integrin and extracellular matrix pathways [7]. These results demonstrate that the molecular processes driving glioma progression can differ heavily between males and females. However, comparable analyses in LGG remain limited. Because patient sex dictates different baseline risks, it is critical to determine whether emerging molecular biomarkers are universally applicable or if their prognostic utility is restricted by sex.

Two major molecular axes drive LGG prognosis: proliferative signaling and immune regulation. Increased cell cycle activity is a known marker of aggressive glioma biology [8, 9]. This activity involves Early 2 factor (E2F), cyclin-dependent kinase, and mitotic checkpoint pathways. Concurrently, the immune microenvironment is a central factor in glioma progression, actively impacting tumor invasion, angiogenesis, and resistance to therapy [10]. Significant sex differences in systemic immunity are also well documented. Females typically demonstrate stronger adaptive immune responses and distinct microglial activation patterns [11, 12]. Furthermore, the clinical impact of proliferative activity can vary by sex in other types of gliomas [7, 13]. Because of these known biological differences, there is a strong rationale to question whether these molecular pathways predict survival equally well for both men and women.

To our knowledge, while sex biased gene expression has been described in glioma, previous TCGA-LGG analyses have not systematically evaluated the formal statistical sex-by-pathway interactions of these specific immune and cell cycle transcriptional programs in relation to survival. This

study analyzed ribonucleic acid (RNA) sequencing and clinical data from The Cancer Genome Atlas (TCGA) to quantify immune and cell cycle related transcriptional activity. Rather than treating sex as a simple adjustment variable, the goal of this work was to evaluate whether the prognostic relevance of immune and proliferative signatures remains robust and independent across both male and female cohorts. Validating the universal applicability of these signatures is essential for the future development of reliable, broadly effective prognostic frameworks in neuro-oncology.

Methods

Data source and cohort preparation

Clinical and RNA sequencing data for patients with LGG were obtained from TCGA through the Genomic Data Commons portal (<https://portal.gdc.cancer.gov/>). Only primary tumor samples were included. Extracted clinical variables were patient identifier, sex, age at diagnosis, histologic grade (II or III), primary diagnosis, vital status, days to death and days to last follow up. Overall survival was defined as the time from diagnosis to death or last known follow up and was expressed in months for descriptive and visualization purposes. An event indicator was coded as 1 for death and 0 for censoring. Sex was standardized as Male or Female and cases with incomplete data for age, sex, grade, or survival were excluded. After filtering, each patient had a unique matched clinical and molecular record for analysis.

RNA-sequencing processing

RNA-sequencing count data were downloaded from the TCGA-LGG *Transcriptome Profiling* dataset (workflow type=STAR-Counts). Each patient was represented by one primary tumor sample. Gene identifiers were mapped to HUGO Gene Nomenclature Committee (HGNC) symbols and duplicate entries were collapsed by summation. Library size normalization was performed using the trimmed mean of M values (TMM) method in *edgeR* and normalized expression values were converted to log-counts per million (logCPM) with a prior count of 1. No additional batch correction was applied.

Molecular signature scoring

Two highly targeted molecular signatures were utilized to represent complementary biological pathways. Rather than relying on broad, whole-transcriptome algorithms that are difficult to translate to routine clinical assays, we intentionally selected restricted panels of canonical driver genes

to maximize potential future utility via multiplex Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) or targeted spatial profiling. The specific genes were curated based on established literature detailing the transcriptomic hallmarks of tumor biology. For the immune panel, we utilized canonical gene expression markers of tumor-infiltrating leukocytes [14]. The panel was selected to capture a comprehensive, yet concise, cross-section of the T-cell response, encompassing infiltration (CD3D, CD3E, CD8A), recruitment (CXCL9, CXCL10) [15, 16], and effector/exhaustion states (GZMB, PDCD1) consistent with established glioma microenvironment landscapes [10, 17]. Similarly, the cell cycle panel was anchored by MKI67 (the transcriptomic equivalent of the standard clinical Ki-67 index) [18] and supplemented with essential, universal regulators of mitotic progression (PCNA, TOP2A, CCNB1, CDK1, BIRC5) that comprise the core cancer proliferation metagene [19, 20]. For each patient, a signature score was calculated as the mean logCPM value across the component genes and then standardized to a z score. To assess sex-specific associations, each signature was divided into tertiles within sex (low, middle and high), which provided balanced subgroup sizes and reduced potential distributional differences between males and females.

Survival analysis and multivariable modeling

Kaplan-Meier survival curves were generated separately for males and females, and differences among signature tertiles were compared using the log-rank test. Median survival with 95% confidence intervals was reported. Multivariable Cox proportional hazards models were fit for each signature and included age (continuous), histologic grade (III versus II), sex, the standardized signature score and a sex by signature interaction term. Hazard ratios with 95% confidence intervals were estimated, and statistical significance was defined as a two sided p value less than 0.05. Because only two prespecified pathways were evaluated, no adjustment for multiple comparisons was applied. The proportional hazards assumption was assessed using Schoenfeld residuals on both raw time and rank transformed event times, and visual inspection indicated no meaningful violations.

Sensitivity analysis with molecular covariates

To assess whether the pathway associations were independent of established molecular markers, additional Cox models were adjusted for isocitrate dehydrogenase (IDH) mutation and 1p/19q codeletion status. Molecular annotations were obtained from the TCGA Pan Cancer Atlas and merged with the clinical and transcriptomic dataset using patient barcodes. Analyses were restricted to complete cases

with available IDH and 1p/19q information, representing approximately 81% of the cohort. Covariates in these models included age, sex, histologic grade, IDH status, 1p/19q status and the standardized pathway score, along with the corresponding sex by signature interaction term. Hazard ratios with 95% confidence intervals were reported.

Software and reproducibility

All analyses were performed in R (version 4.5.1). Data retrieval, preprocessing, and statistical modeling were conducted using the TCGAAbiolinks, SummarizedExperiment, edgeR, limma, tidyverse, survival, survminer, broom, patchwork, and ggrepel packages. Graphs and graphics were generated using R. To support full transparency and computational reproducibility, the complete R analytical workflow, alongside the exported session information detailing all specific package versions, is provided in the Supplementary Material.

Results

Patient characteristics

After excluding recurrent tumors and cases with incomplete survival, age, sex or grade information, a total of 510 primary lower grade glioma cases from TCGA were included in the final cohort, representing more than 95% of the available TCGA LGG dataset. The median age was 41 years (interquartile range 32.2 to 53.0). Males comprised 55.3% of the cohort ($n=282$) and females comprised 44.7% ($n=228$). Tumor grade was distributed as 48.2% grade II ($n=246$) and 51.8% grade III ($n=264$). The median overall survival for the full cohort was 87.4 months (95% confidence interval 67.4 to 114.0). The median follow up time, estimated using the reverse Kaplan-Meier method, was 27.8 months (95% confidence interval 24.4 to 31.1). Histopathologic subtypes included astrocytoma, oligodendroglioma and mixed glioma in proportions consistent with prior TCGA LGG reports.

The immune signature had a median score of -1.61 (interquartile range -2.58 to -0.53), and the cell cycle signature had a median score of 3.50 (interquartile range 2.60 to 4.43). Both signatures were divided into tertiles within each sex to create balanced low, middle and high groups, each representing approximately one third of male and female patients. These distributions are shown in Table 1.

Prior to survival modeling, we evaluated the baseline distribution of both transcriptional signatures across clinical and demographic variables. As expected, both immune and cell cycle activities were significantly elevated in Grade III tumors compared to Grade II (Fig. 1A-B; $p=1.3 \times 10^{-8}$

Table 1 Baseline clinical and molecular characteristics (TCGA-LGG). Summary of 510 patients with LGG from TCGA included after filtering for complete clinical and RNA-sequencing data. Continuous variables are presented as median (interquartile range, IQR) and categorical variables as n (%). Primary tumor diagnoses comprised astrocytoma, oligodendroglioma and mixed glioma subtypes, reflecting the histopathologic diversity of the TCGA-LGG cohort. Immune and cell cycle signature scores were derived from predefined gene sets and expressed as the mean logCPM per sample. Both signatures were stratified into within-sex tertiles (Low, Mid, High) to ensure balanced subgroup sizes and accurate assessment of sex-specific survival associations

Primary tumors with matched clinical and RNA-seq data (N=510)	
All Patients	
Clinical characteristics	
Age, years — median (IQR)	41 (IQR 32.2–53.0)
Sex — Male, n (%)	282 (55.3%)
Sex — Female, n (%)	228 (44.7%)
Grade — G2, n (%)	246 (48.2%)
Grade — G3, n (%)	264 (51.8%)
Primary diagnosis — n (%)	Astrocytoma — 193 (37.8%) Oligodendroglioma — 187 (36.7%) Mixed glioma — 130 (25.5%)
Molecular signatures	
Immune signature — median (IQR)	-1.61 (IQR -2.58– -0.53)
Cell cycle signature — median (IQR)	3.50 (IQR 2.60–4.43)
Immune tertiles — Low/Mid/High, n (%)	Low: 170 (33.3%) Mid: 170 (33.3%) High: 170 (33.3%)
Cell-cycle tertiles — Low/Mid/High, n (%)	Low: 170 (33.3%) Mid: 170 (33.3%) High: 170 (33.3%)

Immune and cell cycle tertiles were defined within sex to ensure balanced subgroup sizes ($\approx 33\%$ each); counts are equal by design

and $p < 2 \times 10^{-16}$, respectively). Notably, when stratified by patient sex, the baseline distributions of both the immune and cell cycle signatures were largely comparable between males and females, with no statistically significant differences observed (Fig. 1C–D; $p=0.2$ and $p=0.55$, respectively). This confirms that subsequent sex-stratified survival analyses are not fundamentally confounded by massive baseline expression skew between genders.

Immune signature and survival

When stratified by sex and immune activity tertiles, clear survival gradients were observed. Among males, median overall survival decreased across the low, middle, and high immune tertiles, with estimates of 98.2, 79.9, and 62.9 months respectively (log-rank $p=0.0028$). Among females, a similar pattern was present, with median survival estimates of 93.1, 114.0, and 62.0 months for the low, middle, and high tertiles (log-rank $p=0.032$) Fig. 2. Kaplan-Meier

curves showed that higher levels of immune-related gene expression were consistently associated with shorter survival in both sexes.

In multivariable Cox regression adjusted for age, grade, and sex, the immune signature remained independently associated with poorer survival (hazard ratio 1.58 per one standard deviation increase, 95% confidence interval 1.25 to 2.00, $p=1.35 \times 10^{-4}$). The sex-by-immune interaction term yielded a hazard ratio of 0.78 (95% confidence interval 0.56 to 1.09, $p=0.143$). Importantly, the lack of statistical significance for this interaction demonstrates that the adverse prognostic impact of the immune signature does not significantly differ between males and females. Age and histologic grade were also independently associated with survival (age hazard ratio 1.05 per year, $p=1.37 \times 10^{-12}$; grade III versus grade II hazard ratio 2.42, $p=2.97 \times 10^{-5}$). Sex alone did not show a significant main effect (hazard ratio 1.00, $p=0.983$) Fig. 3.

These results indicate that higher immune signature activity is an independent predictor of shorter survival in lower grade glioma. Furthermore, formal interaction testing confirmed that this prognostic utility is robust and comparable across both sexes.

Cell cycle signature and survival

Sex stratified tertiles of cell cycle activity showed clear and consistent survival gradients. Among males, median overall survival declined across the low, middle, and high tertiles with estimates of 144.9, 105.1, and 41.1 months respectively (log-rank $p=1.28 \times 10^{-5}$). Among females, a parallel pattern was observed, with median survival estimates of 95.5, 93.1, and 51.8 months for the low, middle, and high tertiles (log-rank $p=0.0026$) Fig. 2. In both cohorts, higher cell cycle related gene expression was consistently associated with significantly shorter survival.

In multivariable Cox proportional hazards models adjusted for age, sex, and grade, the cell cycle signature remained independently associated with poorer survival (hazard ratio 1.47 per one standard deviation increase, 95% confidence interval 1.15 to 1.89, $p=2.39 \times 10^{-3}$). The sex by cell cycle interaction term yielded a hazard ratio of 0.95 (95% confidence interval 0.65 to 1.38, $p=0.778$). This lack of statistical significance confirms that the adverse prognostic impact of the cell cycle signature is robust and comparable across both males and females. Age and histologic grade again showed expected adverse effects (age hazard ratio 1.05 per year, $p=3.20 \times 10^{-11}$; grade III versus grade II hazard ratio 2.22, $p=2.78 \times 10^{-4}$). Sex alone did not demonstrate a significant main effect (hazard ratio 0.94, $p=0.781$) Fig. 3.

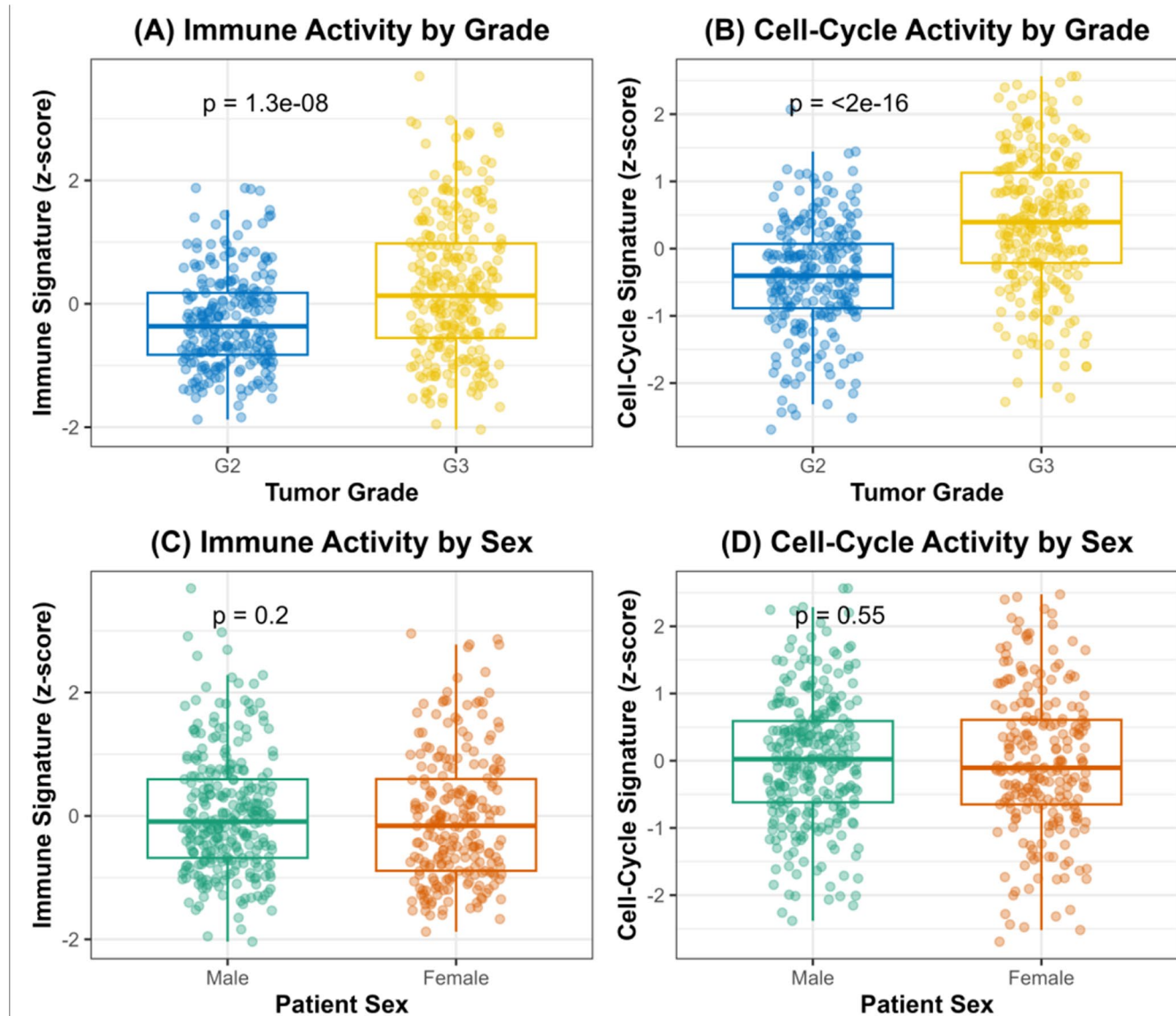


Fig. 1 Baseline distribution of molecular signatures by tumor grade and patient sex. Boxplots depicting (A) immune signature and (B) cell cycle signature z-scores stratified by WHO Grade (G2 vs. G3), demonstrating significantly elevated transcriptional activity in higher-grade tumors. Panels (C) and (D) display the distribution of the immune and

cell cycle signatures, respectively, stratified by patient sex (Male vs. Female), illustrating comparable baseline activity between cohorts. Boxes represent interquartile ranges, horizontal lines indicate medians, and individual points denote patient-level values. Statistical comparisons were performed using the Wilcoxon rank-sum test

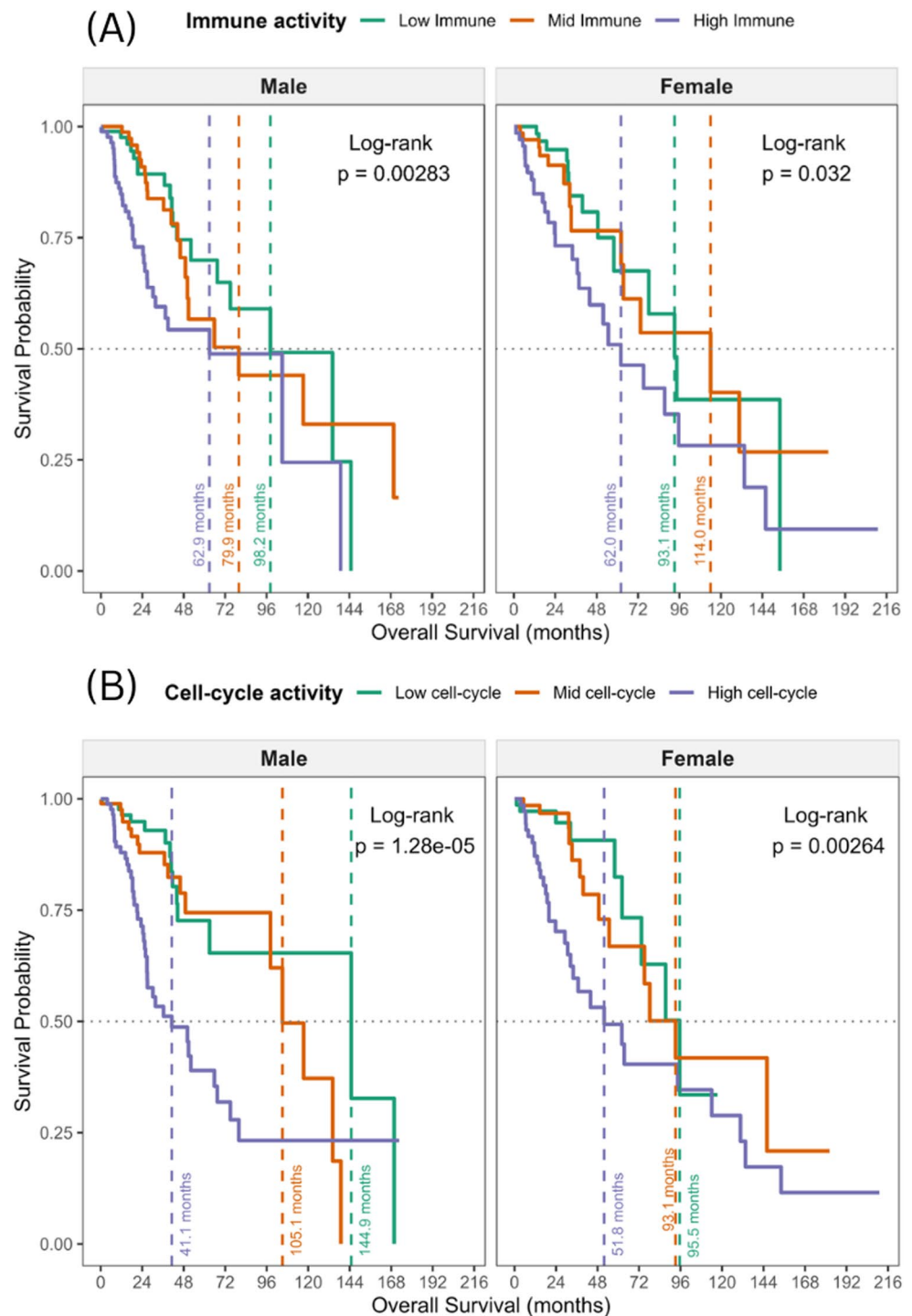
Taken together, these results show that higher cell cycle signature activity is a strong independent predictor of shorter survival in lower grade glioma, with comparable effect sizes validating its utility in both males and females.

Prognostic consistency of signatures across sexes

Kaplan-Meier survival curves stratified by tertiles of molecular activity showed significant and parallel separation across groups in both males and females (Fig. 2). For the immune signature, higher pathway activity was consistently associated with shorter overall survival in both

cohorts. Among males, median survival declined from 98.2 months in the low tertile to 62.9 months in the high tertile (log-rank $p=0.0028$). Among females, median survival similarly declined from 93.1 to 62.0 months across the same tertiles (log-rank $p=0.032$). For the cell cycle signature, an even steeper adverse gradient was observed. Among males, median survival decreased from 144.9 to 41.1 months across the low and high tertiles (log-rank $p=1.3 \times 10^{-5}$). Among females, median survival declined from 95.5 to 51.8 months across these tertiles (log-rank $p=0.0026$). Crucially, although absolute baseline survival times naturally differed between males and females, both signatures demonstrated

Fig. 2 Overall survival by immune and cell cycle transcriptional activity. Kaplan–Meier survival curves stratified by molecular activity tertiles within each sex. Top panels (A) display overall survival by immune signature activity for males and females; bottom panels (B) display survival by cell cycle activity. In all cohorts, higher transcriptional activity (High) was significantly associated with progressively shorter overall survival compared to baseline (Low). Dashed vertical lines denote median survival estimates



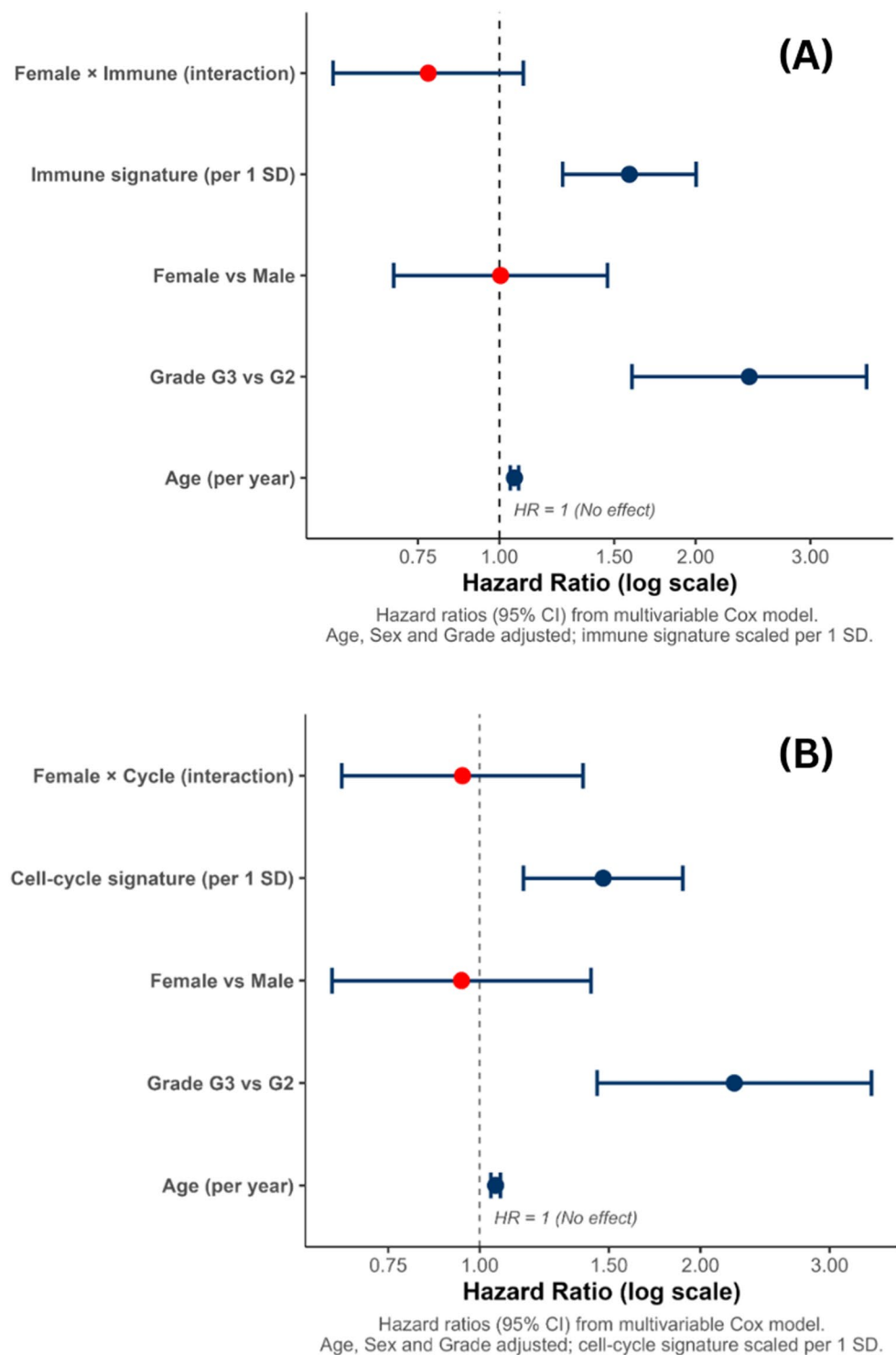
highly consistent trajectories. This confirms that elevated immune or cell cycle transcriptional activity universally predicts shorter survival, independent of patient sex.

Sensitivity analysis with molecular covariates

After merging molecular annotations, 414 cases had available IDH and 1p/19q status for analysis. Age and histologic

grade remained strong predictors of outcome (age hazard ratio 1.03 per year, 95% confidence interval 1.01 to 1.06, $p=0.001$; grade III versus grade II hazard ratio 2.7, 95% confidence interval 1.6 to 4.5, $p<0.001$). After adjustment for molecular subtype, the immune signature (hazard ratio 0.88, 95% confidence interval 0.58 to 1.33, $p=0.55$) and the cell cycle signature (hazard ratio 1.29, 95% confidence interval 0.91 to 1.84, $p=0.15$) did not maintain independent

Fig. 3 Multivariable Cox models for immune and cell cycle signatures. Forest plots depicting hazard ratios (HR, log-scale) with 95% confidence intervals from sex-interaction Cox regression models for the (A) immune and (B) cell cycle signatures. Both signatures (per 1 SD increase) were independently associated with shorter overall survival after adjustment for age, grade, and sex. The non-significant interaction terms confirm the universal prognostic utility of the signatures across sexes. The dashed vertical lines represent the null effect (HR = 1)



statistical significance. This attenuation highlights a strong biological collinearity; the immune and proliferative transcriptional states identified here are heavily intertwined with IDH mutation and 1p/19q codeletion status, which act as primary drivers of these tumor microenvironments. IDH and 1p/19q covariates were not estimable in some models because of the high prevalence of IDH mutation and 1p/19q

codeletion among complete cases. Even within these molecularly adjusted models, the sex by signature interaction terms remained non-significant (immune interaction $p=0.49$, with no evidence of a sex interaction for the cell cycle), further confirming that these biological relationships do not diverge by sex. In combined models, the estimated directions of association for both signatures were completely consistent

Table 2 Multivariable Cox models adjusted for age, histologic grade, sex and molecular markers (complete-case analysis $N=414$). Results of multivariable Cox proportional hazards models adjusted for age, histologic grade, sex and molecular markers (IDH mutation and 1p/19q codeletion status) in the complete case cohort. Age and histologic grade remained strong independent predictors of survival. As detailed in the text, adjustment for molecular subtype attenuated the independent statistical significance of both transcriptional signatures, highlighting their strong biological collinearity with aggressive IDH-wildtype tumor biology. The sex by signature interaction terms remained non-significant across all adjusted models, further confirming that these prognostic relationships are robust and do not diverge by patient sex. Dashes (—) indicate molecular covariates that were unestimable in the models due to the high prevalence of IDH mutations and 1p/19q codeletions among complete cases

Covariate	Cell-cycle model			Immune model			Joint model		
	HR (95% CI)	<i>P</i>	Sig	HR (95% CI)	<i>P</i>	Sig	HR (95% CI)	<i>P</i>	Sig
Age (per year)	1.03 (1.01–1.05)	0.00137	**	1.03 (1.01–1.06)	0.001313	**	1.03 (1.01–1.05)	0.00175	**
G3 vs. G2	2.27 (1.34–3.84)	0.00237	**	2.70 (1.61–4.54)	0.000177	**	2.32 (1.35–3.98)	0.00241	**
Female (vs. Male)	1.18 (0.72–1.94)	0.50369		1.29 (0.81–2.08)	0.286650		1.21 (0.73–2.00)	0.46818	
IDHwt (vs. IDHmut)	—	—		—	—		—	—	
1p/19q non-codel (vs. codel)	—	—		—	—		—	—	
Immune signature (per 1 SD)	—	—	—	0.88 (0.58–1.33)	0.549346		0.90 (0.60–1.36)	0.61400	
Female x Immune	—	—	—	1.22 (0.69–2.14)	0.489005		1.14 (0.64–2.01)	0.65813	
Cell-cycle Signature (per 1 SD)	1.29 (0.91–1.84)	0.15098		—	—	—	1.29 (0.91–1.84)	0.15385	
Female x Cycle	1.17 (0.69–2.00)	0.55578		—	—	—	1.17 (0.68–2.00)	0.57710	

HR Hazard Ratio. Sig: ** $P < 0.05$ | Estimates from multivariable Cox models adjusted for age, grade, sex and molecular markers (complete-case analysis $N=414$). | Grade 3 (G3), Grade 2 (G2), IDHwt (IDH-wild type), IDHmut (IDH-mutant)

with the primary analyses. Results from these fully adjusted molecular models are summarized in Table 2 (and visually represented in Supplementary Fig. S1).

Model diagnostics

Proportional hazards assumptions were evaluated using Schoenfeld residuals on both raw time and rank-transformed time scales. Global tests indicated mild time-dependent effects in both models (immune model $p=0.0007$, cell cycle model $p=0.0004$), primarily driven by age and the molecular signature terms. However, visual inspection of residual plots confirmed minimal deviation from proportionality, with the observed trends being small in magnitude (Supplementary Fig. S2a and S2b). These minor time-dependent patterns do not compromise the overall stability or interpretation of the model coefficients.

Correlation between immune and proliferative pathways

Pearson correlation analysis demonstrated a modest but statistically significant positive association between the immune and cell cycle signature scores ($r=0.23$, 95% confidence interval 0.14 to 0.31, $p=1.8 \times 10^{-7}$) Supplementary Fig. S3. This biological relationship was highly consistent across sexes (males: $r=0.22$; females: $r=0.24$) and remained significant after adjustment for histologic grade. Additionally, immune signature scores were significantly higher in grade III tumors compared with grade II tumors (median z score 0.41 versus minus 0.37, $p=3.2 \times 10^{-4}$) Fig. 1A, reflecting greater inflammatory signaling in more advanced disease. These findings indicate that immune-related and

proliferative transcriptional activities share a partial positive correlation that is fundamentally conserved across both male and female patients.

Summary of key findings

Higher immune and cell cycle transcriptional activities were consistently associated with poorer overall survival in lower grade glioma. Formal interaction testing confirmed that these adverse prognostic gradients are robust and parallel across both male and female patients. Moreover, sensitivity analyses revealed a strong biological collinearity between these signatures and established molecular subtypes, indicating that these transcriptional states successfully capture the aggressive microenvironments driven by IDH-wildtype biology. Ultimately, these results validate immune and proliferative gene expression as reliable, universal indicators of tumor severity in LGG that operate independently of patient sex.

Discussion

Overview of principal findings

In this targeted gene expression analysis of 510 primary lower grade gliomas, elevated immune and proliferative gene expression consistently predicted shorter overall survival across both male and female cohorts. These findings align with established molecular paradigms identifying inflammatory and proliferative hyperactivation as core drivers of aggressive glioma biology, which also exhibited partial biological overlap in our cohort [21, 22]. Formal

interaction testing confirmed the absence of statistically significant sex-specific modification, demonstrating that the adverse prognostic impact of these transcriptional states operates independently of patient sex. Furthermore, the attenuation of their independent prognostic significance upon adjustment for IDH mutation and 1p/19q codeletion highlights strong biological collinearity. Rather than a statistical limitation, this indicates these signatures effectively capture the inherently aggressive microenvironmental states driven by advanced IDH-wildtype biology. Ultimately, these results validate immune and cell cycle transcriptional activities as robust, universal prognostic indicators of tumor severity in lower grade glioma.

Biological mechanisms linking immune activation to poor prognosis

Although increased immune-related transcriptional activity is sometimes interpreted as evidence of an antitumor response, recent literature confirms that immune activation in LGG frequently reflects a dysfunctional, exhausted state. Gliomas establish heavily immunosuppressive microenvironments characterized by severe T-cell exhaustion and myeloid-driven immunoregulation [10, 23]. This aligns with our sensitivity analysis, where the immune signature's independent prognostic value was attenuated by molecular subtype adjustment, indicating it successfully captures the dysfunctional inflammatory state driven by advanced tumor biology. Given known sex-related differences, such as females typically exhibiting stronger baseline adaptive immune responses [12, 24], there is a strong biological rationale to question whether one sex might better resist these tumor-induced subversions. However, our findings demonstrate that once this exhausted immune transcriptional program is established within the tumor microenvironment, it is equally detrimental to survival in both sexes. The lack of a significant sex-by-immune interaction suggests that local, tumor-driven immunosuppression ultimately overrides baseline systemic sex advantages, validating the signature as a universally applicable marker of poor prognosis.

Biological mechanisms linking cell cycle activity to glioma progression

The association between higher cell cycle transcriptional activity and shorter survival is consistent with prior evidence identifying proliferative signaling as a core hallmark of malignant progression in glioma [18, 20]. The attenuation of the signature's independent prognostic value when adjusted for IDH status confirms that this transcriptional program successfully captures the proliferative burst characteristic of advanced, high-risk molecular evolution. Our

findings add a critical layer to this paradigm by formally evaluating its prognostic impact across patient sex. While epidemiologic data consistently shows worse overall baseline survival for males with glioma, our interaction analyses revealed that elevated cell cycle activity is equally detrimental in both sexes. This suggests that once the fundamental machinery of mitotic proliferation is unleashed, it drives tumor aggressiveness and mortality entirely independently of a patient's baseline demographic risk. This pattern confirms that proliferative activity is not just a key component of the molecular landscape, but a universally reliable prognostic marker across all lower grade glioma patients.

Sex-based transcriptomic and clinical differences in glioma

Epidemiologic and molecular studies consistently demonstrate sex-based disparities in glioma, with males exhibiting higher incidence, shorter survival [25], and distinct baseline transcriptomic landscapes [5]. However, our findings add a critical nuance to this paradigm: while baseline risk and overall molecular profiles differ by sex, the prognostic penalties of these targeted T-cell inflammatory signatures and core mitotic proliferation metrics do not. The lack of a statistically significant sex by signature interaction confirms that these targeted inflammatory and mitotic programs represent shared, convergent mechanisms of mortality that operate independently of baseline demographic risk.

Known disparities in systemic immune biology, such as stronger adaptive immune responses in females [26] and distinct pro-inflammatory microglial phenotypes in males [27], actually underscore the profound immunosuppressive power of the developing glioma. Rather than confounding our results, these inherent systemic differences highlight that the local, tumor driven microenvironment ultimately subverts and overrides baseline sex advantages, driving equally poor outcomes once these malignant programs are established. Overall, these findings support the continued evaluation of sex as a biological variable in neuro-oncology, while simultaneously validating these specific transcriptional pathways as reliable, sex-independent biomarkers for clinical risk stratification.

Comparison to prior studies

Our findings strongly align with prior studies linking immune and proliferative hyperactivation to aggressive LGG behavior. Consistent with our survival gradients, Lin et al. [23] and Martinez et al. [28] demonstrated that immune-inflamed LGGs paradoxically exhibit T-cell exhaustion, checkpoint upregulation, and poorer prognoses. Similarly, our cell cycle results corroborate Ceccarelli et al. [20], who

identified proliferative activation as a hallmark of malignant progression. These alignments confirm that our signatures successfully capture fundamentally aggressive tumor states.

Building on prior work, this study specifically tests the statistical interaction between sex and the identified transcriptional programs in LGG. While previous studies by Tewari et al. [5] and Khan et al. [29] have extensively detailed sex-specific transcriptomic and metabolic baseline differences in glioma, our interaction analysis adds an important biological nuance. It demonstrates that once these hyperactive immune and proliferative programs are established in the tumor microenvironment, their detrimental impact on overall survival is remarkably consistent across both males and females. Therefore, while foundational sex disparities exist in glioma incidence and baseline biology, the clinical consequences of these advanced transcriptional states represent a convergent, shared driver of poor prognosis.

Clinical implications

The central question of this study was whether the established prognostic impact of immune and proliferative pathways diverges between males and females. By critically evaluating these transcriptional signatures across sex-stratified cohorts, we demonstrated that their adverse impact represents a convergent mechanism of mortality that operates independently of baseline demographic risk. Therefore, the most immediate clinical implication of our findings is the validation of immune and proliferative transcriptomic signatures as universally applicable prognostic tools. Because the adverse impact of these transcriptional states does not diverge by sex, future clinical risk-stratification models can safely utilize unified scoring thresholds for all patients, simplifying pathway derived risk assessment [30]. Their strong collinearity with high-risk molecular subtypes suggests these signatures successfully capture the aggressive microenvironments characteristic of advanced glioma, providing robust prognostic context that complements standard histopathologic grading.

Beyond prognostication, defining these hyperactive pathways provides critical context for therapeutic development. The association between elevated immune activity and shortened survival reinforces that LGG immune responses are often profoundly exhausted or myeloid suppressed [31, 32]. Consequently, future immunotherapeutic strategies must focus on reversing local microenvironmental immunosuppression rather than merely stimulating peripheral T-cell proliferation. The robust prognostic penalty of cell cycle activation highlights the potential utility of targeted cell cycle regulators, such as Cyclin-dependent kinase inhibitors (CDK-inhibitors), particularly for tumors exhibiting rapid molecular evolution.

Ultimately, while patient sex remains a critical baseline biological variable in neuro-oncology, these specific transcriptional signatures offer robust, sex-independent clinical utility. Integrating these universally applicable pathway measurements into standard workflows could refine patient risk-stratification and better guide the selection of microenvironment targeted therapies.

Future directions

Several critical opportunities for future research emerge from this work. First, integrating single-cell and spatial transcriptomic platforms [33, 34] is essential to resolve the specific cellular architectures driving these bulk tissue signatures. Identifying the precise microenvironmental niches, such as exhausted T-cell clusters or localized proliferative hubs, will clarify how these aggressive states physically manifest. Second, future multi-omic studies must investigate the mechanistic basis of this prognostic convergence. Understanding the specific epigenetic or metabolic mechanisms the tumor microenvironment uses to override baseline systemic sex differences could reveal novel therapeutic vulnerabilities. Finally, prospective longitudinal profiling, via serial tissue sampling or liquid biopsies, is necessary to map the spatiotemporal evolution of these immune and proliferative pathways. Tracking these signatures across the disease course will determine their dynamic response to standard therapies and their potential utility for real-time clinical monitoring.

Limitations

This study presents several limitations. First, the analysis relies on retrospective TCGA data lacking detailed clinical variables, such as extent of resection and specific therapeutic regimens, which are known survival modifiers. Second, our use of bulk RNA sequencing and predefined gene signatures provides a macro-level transcriptomic view but cannot resolve the underlying spatial and cellular heterogeneity of the tumor microenvironment. Third, while we successfully adjusted our multivariable models for IDH mutation and 1p/19q codeletion status, missing molecular annotations in the database restricted these specific sensitivity models to a complete-case subset (81% of the cohort). Fourth, without an independent external validation cohort, the broader generalizability of these findings remains to be prospectively confirmed. Finally, while our analytical framework relied on traditional Kaplan-Meier and Cox regression models rather than complex machine learning algorithms, this was an intentional choice to preserve clinical interpretability and allow for formal statistical interaction testing, which is the gold standard for evaluating specific biological hypotheses.

such as sex-based pathway divergence. However, despite these constraints, our cohort was sufficiently powered to demonstrate the robust, sex-independent prognostic value of these immune and proliferative signatures, providing a strong and reliable foundation for future clinical validation.

Conclusion

In this targeted gene expression analysis of primary lower grade glioma, elevated immune and proliferative transcriptional activities emerged as strong predictors of shorter overall survival. Formal interaction testing confirmed that the prognostic penalties of these aggressive biological states are robust and universally applicable across both male and female patients. Rather than diverging by demographic factors, these hyperactive transcriptional programs represent convergent pathways of mortality that are deeply intertwined with advanced, high-risk molecular subtypes. Ultimately, these findings validate immune and cell cycle signatures as reliable, sex-independent biomarkers. Integrating these universal pathway measurements into future prognostic frameworks could significantly refine clinical risk stratification and help guide therapeutic development for all patients with lower grade glioma.

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Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Arylic Singh and Katrina Shute. The first draft of the manuscript was written by Arylic Singh and all authors commented on previous versions of the manuscript. Overall supervision was provided by Allan Martin. All authors read and approved the final manuscript.

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Data availability The datasets analyzed during the current study are available in The Cancer Genome Atlas repository, Link : <https://portal.gdc.cancer.gov/projects/TCGA-LGG>.

Declarations

Ethics approval and consent to participate All methods were carried out in accordance with relevant guidelines and regulations. The data analyzed in this study were obtained from the publicly available The Cancer Genome Atlas (TCGA) database. The original TCGA data collection protocols were approved by their respective institutional review boards, and informed consent was obtained from all subjects prior to data collection. Because this study strictly involves the secondary analysis of fully de-identified, open-access genomic data, it does not constitute human subjects research and is exempt from requiring ethical approval by the University of California, Davis Institutional Review Board (IRB). Consequently, additional informed consent to participate was not required.

Clinical trial number not applicable.

Competing interests The authors declare no competing interests.

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References

1. D.N. Louis, A. Perry, P. Wesseling, D.J. Brat, I.A. Cree, D. Figarella-Branger, C. Hawkins, H.K. Ng, S.M. Pfister, G. Reifenberger, R. Soffietti, von A. Deimling, D.W. Ellison, The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol.* **23**(8), 1231–1251 (2021). <https://doi.org/10.1093/neuonc/noab106>
2. J.E. Eckel-Passow, D.H. Lachance, A.M. Molinaro, K.M. Walsh, P.A. Decker, H. Sicotte, M. Pekmezci, T. Rice, M.L. Kosel, I.V. Smirnov, G. Sarkar, A.A. Caron, T.M. Kollmeyer, C.E. Praska, A.R. Chada, C. Halder, H.M. Hansen, L.S. McCoy, P.M. Bracci, R. Marshall, S. Zheng, G.F. Reis, A.R. Pico, B.P. O'Neill, J.C. Buckner, C. Giannini, J.T. Huse, A. Perry, T. Tihan, M.S. Berger, S.M. Chang, M.D. Prados, J. Wiemels, J.K. Wiencke, M.R. Wrensch, R.B. Jenkins, Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. *N Engl J Med.* **372**(26):2499–508 (2015). <https://doi.org/10.1056/NEJMoa1407279>
3. Q.T. Ostrom, M. Price, C. Neff, G. Cioffi, K.A. Waite, C. Kruchko, J.S. Barnholtz-Sloan, CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2015–2019. *Neuro Oncol.* **24**(Suppl 5), v1–v95 (2022). <https://doi.org/10.1093/neuonc/noac202>
4. R. Hoogendijk, van der J. Lugt, J. Baugh, C. Kline, M. Kranendonk, E. Hoving, L. Kremer, P. Wesseling, H. Karim-Kos, van D. Vuurden, Sex-related incidence and survival differences in pediatric high-grade glioma subtypes: A population-based cohort study. *iScience.* **26**(10), 107957 (2023)
5. S. Tewari, M.C. Tom, D.Y.J. Park, W. Wei, S.T. Chao, J.S. Yu, J.H. Suh, S. Kilic, D.M. Peereboom, G.H.J. Stevens, J.D. Lathia, R. Prayson, G.H. Barnett, L. Angelov, A.M. Mohammadi, M.S. Ahluwalia, E.S. Murphy, Sex-specific differences in low-grade glioma presentation and outcome. *Int J Radiat Oncol Biol Phys.* **114**(2):283–292 (2022). <https://doi.org/10.1016/j.ijrobp.2022.05.036>
6. N. Jovanovich, A. Habib, A. Chilukuri, N.U. Hameed, Farrukh, Deng, Hansen, R. Shanahan, Head, Jeffrey, Zinn, Pascal, Sex-specific molecular differences in glioblastoma: assessing the clinical significance of genetic variants. *Front. Oncol.* **13**, 1340386 (2024). <https://doi.org/10.3389/fonc.2023.1340386>
7. W. Yang, N.M. Warrington, S.J. Taylor, P. Whitmire, E. Carrasco, K.W. Singleton, N. Wu, J.D. Lathia, M.E. Berens, A.H. Kim, J.S. Barnholtz-Sloan, K.R. Swanson, J. Luo, J.B. Rubin, Sex differences in GBM revealed by analysis of patient

- imaging, transcriptome, and survival data. *Sci. Transl. Med.* **11**(473), eaao5253 (2019). <https://doi.org/10.1126/scitranslmed.aao5253>
8. H. Suzuki, K. Aoki, K. Chiba, Y. Sato, Y. Shiozawa, Y. Shiraishi, T. Shimamura, A. Niida, K. Motomura, F. Ohka, T. Yamamoto, K. Tanahashi, M. Ranjit, T. Wakabayashi, T. Yoshizato, K. Kataoka, K. Yoshida, Y. Nagata, A. Sato-Osubo, H. Tanaka, M. Sanada, Y. Kondo, H. Nakamura, M. Mizoguchi, T. Abe, Y. Muragaki, R. Watanabe, I. Ito, S. Miyano, A. Natsume, S. Ogawa, Mutational landscape and clonal architecture in grade II and III gliomas. *Nat. Genet.* **47**(5), 458–468 (2015). <https://doi.org/10.1038/ng.3273>
 9. Q. Wang, B. Hu, X. Hu, H. Kim, M. Squatrito, L. Scarpacci, A.C. deCarvalho, S. Lyu, P. Li, Y. Li, F. Barthel, H.J. Cho, Y.H. Lin, N. Satani, E. Martinez-Ledesma, S. Zheng, E. Chang, C.G. Sauv  , A. Olar, Z.D. Lan, G. Finocchiaro, J.J. Phillips, M.S. Berger, K.R. Gabrusiewicz, G. Wang, E. Eskilsson, J. Hu, T. Mikkelsen, R.A. DePinho, F. Muller, A.B. Heimberger, E.P. Sulman, D.H. Nam, R.G.W. Verhaak, Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. *Cancer Cell.* **2017**;32(1):42–56.e6. [10.1016/j.ccell.2017.06.003](https://doi.org/10.1016/j.ccell.2017.06.003). Erratum in: *Cancer Cell.* **2018**;33(1):152. <https://doi.org/10.1016/j.ccell.2017.12.012>
 10. F. Klemm, R.R. Maas, R.L. Bowman, M. Kornete, K. Soukup, S. Nassiri, J.P. Brouland, C.A. Iacobuzio-Donahue, C. Brennan, V. Tabar, P.H. Gutin, R.T. Daniel, M.E. Hegi, J.A. Joyce, Interrogation of the Microenvironmental Landscape in Brain Tumors Reveals Disease-Specific Alterations of Immune Cells. *Cell.* **181**(7), 1643–1660.e17 (2020)
 11. K.M. Lenz, M.M. McCarthy, A starring role for microglia in brain sex differences. *Neuroscientist.* **21**(3):306–21 (2015). <https://doi.org/10.1177/1073858414536468>
 12. S.L. Klein, K.L. Flanagan, Sex differences in immune responses. *Nat. Rev. Immunol.* **16**(10), 626–638 (2016). <https://doi.org/10.1038/nri.2016.90>
 13. M. Tian, W. Ma, Y. Chen, Y. Yu, D. Zhu, J. Shi, Y. Zhang, Impact of gender on the survival of patients with glioblastoma. *Biosci. Rep.* **38**(6), BSR20180752 (2018). <https://doi.org/10.1042/BSR20180752>
 14. P. Danaher, S. Warren, L. Dennis, L. D’Amico, A. White, M.L. Disis, M.A. Geller, K. Odunsi, J. Beechem, S.P. Fling, Gene expression markers of Tumor Infiltrating Leukocytes. *J. Immunother. Cancer.* **5**, 18 (2017). <https://doi.org/10.1186/s40425-017-0215-8>
 15. D.S. Chen, I. Mellman, Elements of cancer immunity and the cancer-immune set point. *Nature.* **541**(7637):321–330 (2017). <https://doi.org/10.1038/nature21349>
 16. M.S. Rooney, S.A. Shukla, C.J. Wu, G. Getz, N. Hacohen, Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell.* **160**(1–2), 48–61 (2015)
 17. A.S. Berghoff, B. Kiesel, G. Widhalm, O. Rajky, G. Ricken, A. W  hr, K. Dieckmann, M. Filipits, A. Brandstetter, M. Weller, S. Kurscheid, M.E. Hegi, C.C. Zielinski, C. Marosi, J.A. Hainfellner, M. Preusser, W. Wick, Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro Oncol.* **17**(8), 1064–1075 (2015). <https://doi.org/10.1093/neuonc/nou307>
 18. K. Aldape, G. Zadeh, S. Mansouri, G. Reifenberger, von A. Deimling, Glioblastoma: pathology, molecular mechanisms and markers. *Acta Neuropathol.* **129**(6), 829–848 (2015). <https://doi.org/10.1007/s00401-015-1432-1>
 19. M.L. Whitfield, G. Sherlock, A.J. Saldanha, J.I. Murray, C.A. Ball, K.E. Alexander, J.C. Matese, C.M. Perou, M.M. Hurt, P.O. Brown, D. Botstein, Identification of genes periodically expressed in the human cell cycle and their expression in tumors. *Mol. Biol. Cell.* **13**(6), 1977–2000 (2002). <https://doi.org/10.1091/mbc.02-02-0030>
 20. M. Ceccarelli, F.P. Barthel, T.M. Malta, T.S. Sabedot, S.R. Salama, B.A. Murray, O. Morozova, Y. Newton, A. Radenbaugh, S.M. Pagnotta, S. Anjum, J. Wang, G. Manyam, P. Zoppi, S. Ling, A.A. Rao, M. Grifford, A.D. Cherniack, H. Zhang, L. Poisson, C.G. Jr Carlotti, D.P. Tirapelli, A. Rao, T. Mikkelsen, C.C. Lau, W.K. Yung, R. Rabadan, J. Huse, D.J. Brat, N.L. Lehman, J.S. Barnholtz-Sloan, S. Zheng, K. Hess, G. Rao, M. Meyerson, R. Beroukhim, L. Cooper, R. Akbani, M. Wrensch, D. Haussler, K.D. Aldape, P.W. Laird, D.H. Gutmann, TCGA Research Network, H. Nounmehr, A. Iavarone, R.G. Verhaak, Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. *Cell.* **164**(3), 550–563 (2016). <https://doi.org/10.1016/j.cell.2015.12.028>
 21. Cancer Genome Atlas Research Network; Brat DJ, Verhaak RG, Aldape KD, Yung WK, Salama SR, Cooper LA, Rheinbay E, Miller CR, Vitucci M, Morozova O, Robertson AG, Nounmehr H, Laird PW, Cherniack AD, Akbani R, Huse JT, Ciriello G, Poisson LM, Barnholtz-Sloan JS, Berger MS, Brennan C, Colen RR, Colman H, Flanders AE, Giannini C, Grifford M, Iavarone A, Jain R, Joseph I, Kim J, Kasaiian K, Mikkelsen T, Murray BA, O’Neill BP, Pachter L, Parsons DW, Sougnez C, Sulman EP, Vandenberg SR, Van Meir EG, von Deimling A, Zhang H, Crain D, Lau K, Mallery D, Morris S, Paulauskis J, Penny R, Shelton T, Sherman M, Yena P, Black A, Bowen J, Dicostanzo K, Gastier-Foster J, Leraas KM, Lichtenberg TM, Pierson CR, Ramirez NC, Taylor C, Weaver S, Wise L, Zmuda E, Davidsen T, Demchok JA, Eley G, Ferguson ML, Hutter CM, Mills Shaw KR, Ozenberger BA, Sheth M, Sofia HJ, Tarnuzzer R, Wang Z, Yang L, Zenklusen JC, Ayala B, Baboud J, Chudamani S, Jensen MA, Liu J, Pihl T, Raman R, Wan Y, Wu Y, Ally A, Auman JT, Balasundaram M, Balu S, Baylin SB, Beroukhim R, Bootwalla MS, Bowlby R, Bristow CA, Brooks D, Butterfield Y, Carlsen R, Carter S, Chin L, Chu A, Chuah E, Cibulskis K, Clarke A, Coetzee SG, Dhalla N, Fennell T, Fisher S, Gabriel S, Getz G, Gibbs R, Guin R, Hadjipanayis A, Hayes DN, Hinoue T, Hoadley K, Holt RA, Hoyle AP, Jefferys SR, Jones S, Jones CD, Kucherlapati R, Lai PH, Lander E, Lee S, Lichtenstein L, Ma Y, Maglinte DT, Mahadeshwar HS, Marra MA, Mayo M, Meng S, Meyerson ML, Mieczkowski PA, Moore RA, Mose LE, Mungall AJ, Pantazi A, Parfenov M, Park PJ, Parker JS, Perou CM, Protopopov A, Ren X, Roach J, Sabedot TS, Schein J, Schumacher SE, Seidman JG, Seth S, Shen H, Simons JV, Sipahimalani P, Soloway MG, Song X, Sun H, Tabak B, Tam A, Tan D, Tang J, Thiessen N, Triche T Jr, Van Den Berg DJ, Veluvolu U, Waring S, Weisenberger DJ, Wilkerson MD, Wong T, Wu J, Xi L, Xu AW, Yang L, Zack TJ, Zhang J, Aksoy BA, Arachchi H, Benz C, Bernard B, Carlin D, Cho J, DiCara D, Frazer S, Fuller GN, Gao J, Gehlenborg N, Haussler D, Heiman DI, Iype L, Jacobsen A, Ju Z, Katzman S, Kim H, Knijnenburg T, Kreisberg RB, Lawrence MS, Lee W, Leinonen K, Lin P, Ling S, Liu Y, Liu Y, Liu Y, Lu Y, Mills G, Ng S, Noble MS, Paull E, Rao A, Reynolds S, Saksena G, Sanborn Z, Sander C, Schultz N, Senbabaoglu Y, Shen R, Shmulevich I, Sinha R, Stuart J, Sumer SO, Sun Y, Tasman N, Taylor BS, Voet D, Weinhold N, Weinstein JN, Yang D, Yoshihara K, Zheng S, Zhang W, Zou L, Abel T, Sadeghi S, Cohen ML, Eschbacher J, Hattab EM, Raghunathan A, Schniederjan MJ, Aziz D, Barnett G, Barrett W, Bigner DD, Boice L, Brewer C, Calatuzzolo C, Campos B, Carlotti CG Jr, Chan TA, Cuppini L, Curley E, Cuzzubbo S, Devine K, DiMeco F, Duell R, Elder JB, Fehrenbach A, Finocchiaro G, Friedman W, Fulop J, Gardner J, Hermes B, Herold-Mende C, Jungk C, Kendler A, Lehman NL, Lipp E, Liu O, Mandt R, McGraw M, Mclendon R, McPherson C, Neder L, Nguyen P, Noss A, Nunziata R, Ostrom QT, Palmer C, Perin A, Pollo B, Potapov A, Potapova O, Rathmell WK, Rotin D, Scarpacci L, Schilero C, Senecal K, Shimmel K, Shurkhay V, Sifri S, Singh R, Sloan AE, Smolenski K, Staugaitis SM, Steele R, Thorne L, Tirapelli DP, Unterberg

- A, Vallurupalli M, Wang Y, Warnick R, Williams F, Wolinsky Y, Bell S, Rosenberg M, Stewart C, Huang F, Grimsby JL, Radenbaugh AJ, Zhang J. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. *N Engl J Med*. **372**(26):2481-98 (2015). <https://doi.org/10.1056/NEJMoa1402121>
22. R.G. Verhaak, K.A. Hoadley, E. Purdom, V. Wang, Y. Qi, M.D. Wilkerson, C.R. Miller, L. Ding, T. Golub, J.P. Mesirov, G. Alexe, M. Lawrence, M. O'Kelly, P. Tamayo, B.A. Weir, S. Gabriel, W. Winckler, S. Gupta, L. Jakkula, H.S. Feiler, J.G. Hodgson, C.D. James, J.N. Sarkaria, C. Brennan, A. Kahn, P.T. Spellman, R.K. Wilson, T.P. Speed, J.W. Gray, M. Meyerson, G. Getz, C.M. Perou, D.N. Hayes, Cancer Genome Atlas Research Network. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell*. **17**(1), 98–110 (2010). <https://doi.org/10.1016/j.ccr.2009.12.020>
 23. H. Lin, C. Liu, A. Hu, D. Zhang, H. Yang, Y. Mao, Understanding the immunosuppressive microenvironment of glioma: mechanistic insights and clinical perspectives. *J. Hematol. Oncol.* **17**(1), 31 (2024). <https://doi.org/10.1186/s13045-024-01544-7>
 24. L.M. Pennell, C.L. Galligan, E.N. Fish, Sex affects immunity. *J Autoimmun.* **38**(2–3):J282-91 (2012). <https://doi.org/10.1016/j.jaut.2011.11.013>
 25. T. Sun, N.M. Warrington, J. Luo, M.D. Brooks, S. Dahiya, S.C. Snyder, R. Sengupta, J.B. Rubin, Sexually dimorphic RB inactivation underlies mesenchymal glioblastoma prevalence in males. *J. Clin. Invest.* **124**(9), 4123–4133 (2014). <https://doi.org/10.1172/JCI71048>
 26. I. Ben-Batalla, M.E. Vargas-Delgado, von G. Amsberg, M. Janning, S. Loges, Influence of Androgens on Immunity to Self and Foreign: Effects on Immunity and Cancer. *Front. Immunol.* **11**, 1184 (2020). <https://doi.org/10.3389/fimmu.2020.01184>
 27. D. Guneykaya, A. Ivanov, D.P. Hernandez, V. Haage, B. Wojtas, N. Meyer, M. Maricos, P. Jordan, A. Buonfiglioli, B. Gielniewski, N. Ochocka, C. Cömert, C. Friedrich, L.S. Artiles, B. Kaminska, P. Mertins, D. Beule, H. Kettenmann, S.A. Wolf, Transcriptional and translational differences of microglia from male and female brains. *Cell Rep.* **24**(10):2773–2783.e6 (2018). <https://doi.org/10.1016/j.celrep.2018.08.001>
 28. M. Martinez-Lage, T.M. Lynch, Y. Bi, C. Cocito, G.P. Way, S. Pal, J. Haller, R.E. Yan, A. Ziober, A. Nguyen, M. Kandpal, D.M. O'Rourke, J.P. Greenfield, C.S. Greene, R.V. Davuluri, N. Dahmane, Immune landscapes associated with different glioblastoma molecular subtypes. *Acta Neuropathol. Commun.* **7**(1), 203 (2019). <https://doi.org/10.1186/s40478-019-0803-6>
 29. M.T. Khan, B. Prajapati, S. Lakhina, M. Sharma, S. Prajapati, K. Chosdol, S. Sinha, Identification of Gender-Specific Molecular Differences in Glioblastoma (GBM) and Low-Grade Glioma (LGG) by the Analysis of Large Transcriptomic and Epigenomic Datasets. *Front. Oncol.* **11**, 699594 (2021)
 30. X. Zheng, C.I. Amos, H.R. Frost, Comparison of pathway and gene-level models for cancer prognosis prediction. *BMC Bioinform.* **21**, 76 (2020). <https://doi.org/10.1186/s12859-020-3423-z>
 31. C. Neftel, J. Laffy, M.G. Filbin, T. Hara, M.E. Shore, G.J. Rahme, A.R. Richman, D. Silverbush, M.L. Shaw, C.M. Hebert, J. Dewitt, S. Gritsch, E.M. Perez, L.N. Gonzalez Castro, X. Lan, N. Druck, C. Rodman, D. Dionne, A. Kaplan, M.S. Bertalan, J. Small, K. Pelton, S. Becker, D. Bonal, Q.D. Nguyen, R.L. Servis, J.M. Fung, R. Mylvaganam, L. Mayr, J. Gojo, C. Haberler, R. Geyeregger, T. Czech, I. Slave, B.V. Nahed, W.T. Curry, B.S. Carter, H. Wakimoto, P.K. Brastianos, T.T. Batchelor, A. Stemmer-Rachamimov, M. Martinez-Lage, M.P. Frosch, I. Stamenkovic, N. Riggi, E. Rheinbay, M. Monje, O. Rozenblatt-Rosen, D.P. Cahill, A.P. Patel, T. Hunter, I.M. Verma, K.L. Ligon, D.N. Louis, A. Regev, B.E. Bernstein, I. Tirosh, M.L. Suvà, An Integrative Model of Cellular States, Plasticity, and Genetics for Glioblastoma. *Cell*. **178**(4), 835–849.e21 (2019). <https://doi.org/10.1016/j.cell.2019.06.024>
 32. D.A. Reardon, K. Wucherpfennig, E.A. Chiocca, Immunotherapy for glioblastoma: on the sidelines or in the game? *Discov Med.* **24**(133), 201–208 (2017)
 33. I. Tirosh, A.S. Venteicher, C. Hebert, L.E. Escalante, A.P. Patel, K. Yizhak, J.M. Fisher, C. Rodman, C. Mount, M.G. Filbin, C. Neftel, N. Desai, J. Nyman, B. Izar, C.C. Luo, J.M. Francis, A.A. Patel, M.L. Onozato, N. Riggi, K.J. Livak, D. Gennert, R. Satija, B.V. Nahed, W.T. Curry, R.L. Martuza, R. Mylvaganam, A.J. Iafrate, M.P. Frosch, T.R. Golub, M.N. Rivera, G. Getz, O. Rozenblatt-Rosen, D.P. Cahill, M. Monje, B.E. Bernstein, D.N. Louis, A. Regev, M.L. Suvà, Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. *Nature*. **539**(7628), 309–313 (2016). <https://doi.org/10.1038/nature20123>
 34. Y. Liu, M. Yang, Y. Deng, G. Su, A. Enninfu, C.C. Guo, T. Tebaldi, D. Zhang, D. Kim, Z. Bai, E. Norris, A. Pan, J. Li, Y. Xiao, S. Halene, R. Fan, High-Spatial-Resolution Multi-Omics Sequencing via Deterministic Barcoding in Tissue. *Cell*. **183**(6), 1665–1681.e18 (2020)

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