Am J Transl Res 2026;18(10):9564-9578
www.ajtr.org /ISSN:1943-8141/AJTR0175380

Original Article

WHO CNS5-based diagnostic reclassification and prognostic stratification of adult gliomas: a retrospective cohort study

Kan Cao[1,2*], Cheng Luo[1*], Bo Chen[2], Wei Chen[2], Xiaotian Zhang[3], Lianshu Ding[1,2]

[1]Department of Neurosurgery, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Northern Jiangsu Institute of Clinical Medicine, Nanjing Medical University, Huai'an 223300, Jiangsu, China; [2]Department of Neurosurgery, The First People's Hospital of Zhenjiang, Zhenjiang 212000, Jiangsu, China; [3]Department of Neurosurgery, Hongze District People's Hospital of Huai'an, Huai'an 223100, Jiangsu, China. [*]Co-first authors.

Received July 15, 2026; Accepted September 11, 2026; Epub October 15, 2026; Published October 30, 2026

Abstract: Aim: To evaluate the impact of the fifth edition of the *World Health Organization Classification of Tumors of the Central Nervous System* (WHO CNS5) on diagnostic categorization, grade allocation, and prognostic stratification in adult gliomas. Methods: We retrospectively analyzed 160 adult glioma patients diagnosed via surgical resection or biopsy between January 2021 and December 2025. Tumors were reassessed using the WHO CNS5 integrated diagnostic framework, which combines histological review with molecular markers, including isocitrate dehydrogenase 1/2 (*IDH1/2*) status, 1p/19q codeletion, telomerase reverse transcriptase (*TERT*) promoter mutation, epidermal growth factor receptor (*EGFR*) amplification, chromosome +7/-10 signature, cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*) homozygous deletion, O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation, alpha thalassemia/mental retardation syndrome X-linked (ATRX) expression, and tumor protein p53 (TP53) overexpression. Diagnostic reassignment and grade migration were analyzed separately. Overall survival (OS) and progression-free survival (PFS) were assessed using Kaplan-Meier analysis, Cox regression, and comparative model performance evaluations. Results: After WHO CNS5 reassessment, glioblastoma, *IDH*-wildtype was the most frequent diagnosis (76/160, 47.5%), followed by astrocytoma, *IDH*-mutant (51/160, 31.9%), oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted (27/160, 16.9%), and diffuse astrocytic glioma, *IDH*-wildtype, not elsewhere classified (6/160, 3.8%). Entity-level reassignment occurred in 86 cases (53.8%), while grade migration occurred in 37 cases (23.1%). Overall, 92 cases (57.5%) showed either diagnostic reassignment or grade migration. WHO CNS5 grading provided clearer stratification of both OS and PFS than traditional grading. For OS, the concordance index increased from 0.726 in the traditional grade model to 0.806 in the WHO CNS5 model and to 0.873 in the molecular model; the corresponding values for PFS were 0.784, 0.858, and 0.875, respectively. Pairwise comparisons confirmed improved discrimination of the WHO CNS5 model over the traditional model for both endpoints, whereas the additional improvement of the molecular model over WHO CNS5 was significant for OS but not for PFS. Conclusion: WHO CNS5-based reassessment substantially changed diagnostic assignment and offered superior prognostic discrimination for both OS and PFS compared with traditional histological grading.

Keywords: Glioma, WHO CNS5, *IDH* mutation, 1p/19q codeletion, molecular subtyping, prognostic stratification

Introduction

Glioma is one of the most common primary malignant tumors of the central nervous system that occurs in adults, exhibiting highly heterogeneous clinical courses and biological behaviors. Patients with similar low- or high-grade morphological diagnoses often show markedly different survival outcomes, recurrence risks, and therapeutic responses. The conventional grading system, based solely on histology, has been used clinically for many years, but its limitations in inter-observer reproducibility, prognostic subclassification, and treatment guidance are increasingly recognized. Molecular alterations are now used to define tumor lineage and grade, and they correlate with clinically relevant prognostic differences. Accordingly, the fifth edition of the World Health Organization Classification of

https://doi.org/10.62347/PUAE9217

Tumors of the Central Nervous System (WHO CNS5), adopted in 2021, uses an "integrated diagnosis" as its main approach. This strategy emphasizes the use of pivotal molecular markers for tumor typing and incorporates specific molecular features as part of grading criteria to ensure more reproducible and generalizable risk stratification [1, 2].

The most important diagnostic branch point for adult-type diffuse gliomas is isocitrate dehydrogenase 1/2 (*IDH1/2*) mutation status. Along with alpha thalassemia/mental retardation syndrome X-linked (*ATRX*) loss and tumor protein p53 (*TP53*) mutation, it is a marker of tumor lineage: *IDH*-mutant tumors with 1p/19q codeletion are classified as oligodendroglioma; *IDH*-mutant tumors without 1p/19q codeletion, often harboring *ATRX/TP53* alterations, are classified as astrocytoma; and *IDH*-wildtype tumors typically belong to the more aggressive glioblastoma lineage [3-5]. A key innovation of WHO CNS5 is the incorporation of molecular evidence to refine tumor upgrading. For example, some *IDH*-wildtype diffuse astrocytic tumors with specific molecular markers, despite being morphologically low-grade, should be treated as glioblastoma-equivalent, avoiding potential underestimation of risk based on morphology alone [6, 7]. Systematic evaluation of the magnitude and direction of WHO CNS5-related reclassification, together with the molecular criteria underlying diagnostic reassignment and grade migration, may clarify the diagnostic and prognostic implications of the integrated framework in real-world cohorts [8-11].

Against this background, we systematically reassessed adult gliomas according to the WHO CNS5 integrated diagnostic framework. The primary objective was to quantify entity-level diagnostic reassignment and grade migration after incorporation of WHO CNS5-defining histological and molecular criteria. The secondary objective was to compare the prognostic stratification provided by traditional histological grading and WHO CNS5 grading. This study was intended to provide real-world evidence on the diagnostic and prognostic implications of implementing WHO CNS5 in routine clinical practice.

Materials and methods

Study design

This retrospective single-center cohort study evaluated changes in diagnostic assignment and grade after application of the WHO CNS5 integrated histological-molecular criteria and assessed their associations with survival outcomes. The study included 160 consecutive patients with pathologically confirmed glioma who underwent neurosurgical resection or biopsy at our hospital from January 2021 to December 2025. Clinical, pathological, molecular, treatment, and follow-up data were available for all cases. The study adhered to the diagnosis and grading of adult-type diffuse gliomas as outlined in the WHO CNS5. A pathological review was performed by two independent pathologists; any discrepancies were resolved by consensus with a third senior neuropathologist in a discussion meeting to achieve a final consensus diagnosis. The study protocol was reviewed and approved by the Ethics Committee of the First People's Hospital of Zhenjiang (Approval No. K-20260168-Y). All clinical and molecular data were analyzed anonymously.

Inclusion criteria were: 1) age ≥ 18 years; 2) histologically proven diagnosis of glioma (surgical resection or biopsy); 3) availability of baseline clinicopathological data; 4) availability of key molecular data necessary for WHO CNS5 integrated reassessment; and 5) availability of follow-up data for survival analysis. Exclusion criteria were: 1) insufficient tumor tissue for integrated diagnosis; 2) unclear primary tumor diagnosis; 3) lack of reliable information for first diagnosis of recurrent tumors; 4) prior treatment before pathological assessment that could significantly influence the diagnosis; and 5) lack of key clinical or molecular data.

Data collection

Clinical, pathological, molecular, treatment, and follow-up data were extracted from medical records, pathology reports, molecular diagnostics, and follow-up databases. The collected variables included age, sex, tumor location, tumor size, preoperative Karnofsky Performance Status (KPS), surgical procedure, traditional histological diagnosis and grade, Ki-67

index, molecular alterations, adjuvant radiotherapy, temozolomide treatment, concurrent chemoradiotherapy, procarbazine, lomustine, and vincristine (PCV) chemotherapy, Stupp regimen, progression status, death status, and follow-up time. Surgical procedure was categorized as gross total resection, subtotal resection, or biopsy.

Traditional diagnosis and grade were compared with the final WHO CNS5 integrated diagnosis and grade. Entity-level reassignment was defined as any change from the traditional histological diagnostic category to a WHO CNS5 integrated entity. Grade migration was defined as any change between traditional WHO grade and the final WHO CNS5 grade. Upgrading and downgrading were defined as a higher or lower WHO CNS5 grade, respectively, compared with the traditional grade. Any reclassification was defined as either entity-level reassignment or grade migration.

The basis of reclassification was grouped into: *IDH*-wildtype tumors with glioblastoma-defining molecular features, *IDH*-mutant astrocytoma lineage reassignment, *IDH*-mutant and 1p/19q-codeleted oligodendroglioma reassignment, cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B*)-related grade 4 upgrade, consensus histological grade reassessment, and reassignment to the not elsewhere classified (NEC) category.

Overall survival (OS) was defined as the interval from surgery or biopsy to death from any cause or to the last follow-up. Progression-free survival (PFS) was defined as the interval from surgery or biopsy to radiological progression, recurrence, death, or last follow-up. Patients without events were censored at the last follow-up.

Histological review and molecular testing

All available hematoxylin- and eosin-stained sections were independently reviewed by two pathologists. The histological features assessed included cellularity, nuclear atypia, mitotic activity, necrosis, microvascular proliferation, and high-grade components. Grade 2 versus grade 3 was determined according to morphological criteria, particularly mitotic activity and anaplastic features. Histological grade 4 features were defined as necrosis and/or microvascular proliferation. Discrepancies were resolved by consensus review or adjudicated by a senior pathologist.

Molecular markers included *IDH1/2* mutation, 1p/19q codeletion, ATRX expression, TP53 overexpression, O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation, telomerase reverse transcriptase (*TERT*) promoter mutation, epidermal growth factor receptor (*EGFR*) amplification, chromosome +7/-10 signature, *CDKN2A/B* homozygous deletion, B-Raf proto-oncogene serine/threonine kinase (*BRAF*) V600E, and H3 K27/G34 alterations. Core WHO CNS5-defining markers, including *IDH1/2*, 1p/19q, TERT, *EGFR*, chromosome +7/-10, and *CDKN2A/B*, were available for all cases. ATRX and TP53 immunohistochemistry (IHC) was evaluable in 118 cases and was used as supportive lineage evidence when available. In cases without ATRX/TP53 results, astrocytic lineage assignment was based on the presence of an *IDH* mutation and the absence of 1p/19q codeletion.

IDH1 status was primarily assessed by IHC using an *IDH1* R132H mutation-specific antibody, with sequencing performed for negative or inconclusive cases when clinically indicated. *IDH2* mutation testing was performed in selected cases using polymerase chain reaction (PCR)- or sequencing-based methods. 1p/19q codeletion was determined by fluorescence in situ hybridization (FISH). ATRX expression and TP53 overexpression were evaluated by IHC, and *MGMT* promoter methylation was assessed by methylation-specific PCR (MSP). *TERT* promoter hotspot mutations, including C228T and C250T, were detected by Sanger sequencing. *EGFR* amplification and *CDKN2A/B* homozygous deletion were assessed by FISH. The chromosome +7/-10 signature was assessed by copy-number profiling. *BRAF* V600E and H3 K27/G34 alterations were evaluated by IHC and/or sequencing when required for differential diagnosis. Ki-67 was recorded as a proliferative marker but was not used as an independent WHO CNS5 grading criterion.

WHO CNS5 integrated diagnostic reassessment

All cases were reassessed according to the WHO CNS5 integrated diagnostic framework for adult-type diffuse gliomas. *IDH1/2* status

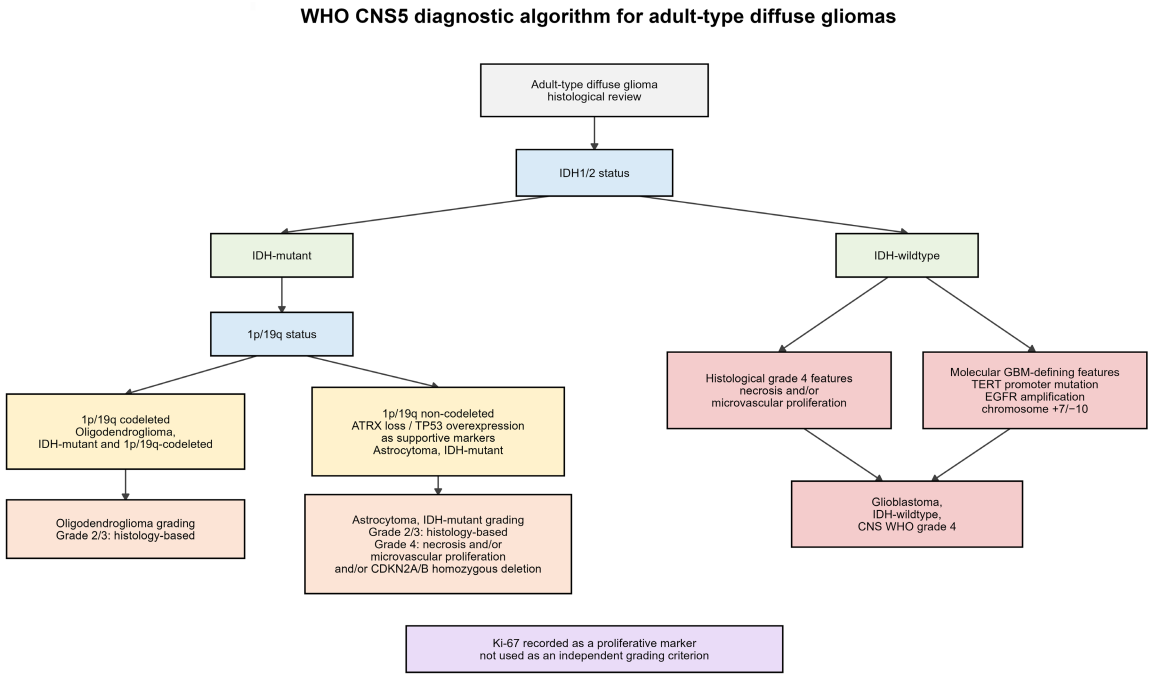


Figure 1. Diagnostic algorithm for WHO CNS5 reassessment of adult-type diffuse gliomas. Notes: WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System.

was used as the primary diagnostic branch point. *IDH*-mutant tumors with 1p/19q codeletion were classified as oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted, and were graded as CNS WHO grade 2 or 3 based on histology. *IDH*-mutant tumors without 1p/19q codeletion were classified as astrocytoma, *IDH*-mutant, with ATRX loss and/or TP53 overexpression used as supportive markers when available. Astrocytoma, *IDH*-mutant was graded as CNS WHO grade 2 or 3 based on histology, and was assigned CNS WHO grade 4 if necrosis, microvascular proliferation, and/or *CDKN2A/B* homozygous deletion was present.

IDH-wildtype diffuse astrocytic tumors were classified as glioblastoma, *IDH*-wildtype, CNS WHO grade 4 if histological grade 4 features or glioblastoma-defining molecular alterations were identified. Glioblastoma-defining molecular alterations included *TERT* promoter mutation, *EGFR* amplification, and the chromosome +7/-10 signature. *IDH*-wildtype diffuse astrocytic tumors without histological grade 4 features or glioblastoma-defining molecular alterations were categorized as diffuse astrocytic glioma, *IDH*-wildtype, NEC. The diagnostic algorithm is shown in **Figure 1**.

Statistical analysis

Statistical analyses were performed using R version 4.5.2. Continuous variables were summarized as medians and interquartile ranges (IQR), and categorical variables as counts and percentages. Molecular alteration frequencies were calculated among evaluable cases. Entity-level reassignment, grade migration, and the basis of reclassification were summarized descriptively, and a migration matrix was constructed to compare traditional histological grade with final WHO CNS5 grade.

Kaplan-Meier analysis was used to estimate OS and PFS according to traditional histological grade and WHO CNS5 integrated grade, with differences assessed using the log-rank test. Multivariable Cox proportional hazards regression was performed separately for OS and PFS, and results were reported as hazard ratios (HR) with 95% confidence intervals (CI). The multivariable Cox models included preoperative KPS, surgical procedure, the listed molecular markers, and treatment variables as adjustment covariates. Treatment effects were not interpreted causally because of potential confounding by indication. The proportional

Table 1. Patient characteristics and treatment profiles

Characteristics	Overall cohort, n = 160
Age, years	49.0 (39.0-61.0)
Sex, n (%)	
Female	66 (41.2%)
Male	94 (58.8%)
Preoperative KPS	80.0 (70.0-90.0)
Tumor size, cm	4.5 (3.2-5.5)
Tumor location, n (%)	
Frontal lobe	62 (38.8%)
Temporal lobe	41 (25.6%)
Parietal lobe	29 (18.1%)
Deep structures	28 (17.5%)
Surgical procedure, n (%)	
Gross total resection	71 (44.4%)
Subtotal resection	54 (33.8%)
Biopsy	35 (21.9%)
Traditional WHO grade, n (%)	
Grade 2	54 (33.8%)
Grade 3	46 (28.8%)
Grade 4	60 (37.5%)
Ki-67 index, %	17.0 (10.8-35.2)
Treatment profiles, n (%)	
Radiotherapy	129 (80.6%)
Temozolomide	124 (77.5%)
Concurrent chemoradiotherapy	108 (67.5%)
PCV chemotherapy	6 (3.8%)
Stupp regimen	92 (57.5%)
Follow-up and outcomes	
OS, months	23.5 (14.3-32.9)
PFS, months	13.5 (9.4-21.3)
Death events, n (%)	67 (41.9%)
Progression events, n (%)	98 (61.2%)

Notes: KPS, Karnofsky Performance Status; WHO, World Health Organization; PCV, procarbazine, lomustine, and vincristine; OS, overall survival; PFS, progression-free survival.

hazards assumption was assessed using Schoenfeld residuals.

For each endpoint, three Cox models were compared: a traditional histological grade model, a WHO CNS5 integrated grade model, and a molecular model. Model performance was evaluated using Harrell's concordance index (C-index), the Akaike information criterion (AIC), and the Bayesian information criterion (BIC). Pairwise differences in C-index were assessed using patient-level paired bootstrap resampling with 2,000 replicates. At 12 months, time-dependent AUCs were estimated using inverse probability of censoring weighting, calibration was assessed using bootstrap resampling with 1,000 replicates, and clinical net benefit was evaluated using survival decision curve analysis. A sensitivity analysis excluding the eight IDH-mutant astrocytomas with unavailable ATRX and TP53 assessments was performed by recalculating diagnostic reclassification rates and refitting the OS and PFS models using the same specifications as in the primary analysis. All tests were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics and treatment profiles

This retrospective cohort included 160 adult glioma patients. The median age was 49.0 years (IQR, 39.0-61.0), and the median preoperative KPS score was 80.0 (IQR, 70.0-90.0). Ninety-four patients were male (58.8%) and 66 were female (41.2%). The most common tumor location was the frontal lobe (62/160, 38.8%), followed by the temporal lobe (41/160, 25.6%), parietal lobe (29/160, 18.1%), and deep structures (28/160, 17.5%). Gross total resection was performed in 71 patients (44.4%), subtotal resection in 54 patients (33.8%) and biopsy in 35 patients (21.9%). According to traditional histological grading, 54 tumors were grade 2, 46 were grade 3, and 60 were grade 4. During follow-up, 67 deaths (41.9%) and 98 progression events (61.2%) were recorded. Radiotherapy, temozolomide, and concurrent chemoradiotherapy were administered to 129 (80.6%), 124 (77.5%), and 108 (67.5%) patients, respectively (**Table 1**).

Molecular testing coverage and molecular alteration profiles

Molecular testing coverage is summarized in **Table 2**. All cases had available results for *IDH1/2* status, 1p/19q codeletion, *MGMT* promoter methylation, *TERT* promoter mutation, *EGFR* amplification, chromosome +7/-10 signature, and *CDKN2A/B* homozygous deletion. *IDH1/2* mutation was identified in 78 cases (48.8%), and 1p/19q codeletion was detected in 27 cases (16.9%). *TERT* promoter mutation,

Table 2. Molecular testing coverage and molecular alteration profiles

Molecular marker	Tested cases, n	Positive/abnormal cases, n (%)
IDH1/2 mutation	160	78 (48.8%)
1p/19q codeletion	160	27 (16.9%)
ATRX loss	118	46 (39.0%)
TP53 overexpression	118	52 (44.1%)
MGMT promoter methylation	160	85 (53.1%)
TERT promoter mutation	160	71 (44.4%)
EGFR amplification	160	36 (22.5%)
Chromosome +7/-10 signature	160	39 (24.4%)
CDKN2A/B homozygous deletion	160	15 (9.4%)
BRAF V600E	160	2 (1.2%)
H3 K27 alteration	160	0 (0.0%)

Notes: *IDH1/2*, isocitrate dehydrogenase 1/2; *ATRX*, alpha thalassemia/mental retardation syndrome X-linked; *TP53*, tumor protein p53; *MGMT*, O^6-methylguanine-DNA methyltransferase; *TERT*, telomerase reverse transcriptase; *EGFR*, epidermal growth factor receptor; *CDKN2A/B*, cyclin-dependent kinase inhibitor 2A/B; *BRAF*, B-Raf proto-oncogene serine/threonine kinase.

Table 3. Distribution of WHO CNS5 integrated diagnoses and final CNS WHO grades

WHO CNS5 classification	n (%)
Integrated diagnosis	
Glioblastoma, *IDH*-wildtype	76 (47.5%)
Astrocytoma, *IDH*-mutant	51 (31.9%)
Oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted	27 (16.9%)
Diffuse astrocytic glioma, *IDH*-wildtype, NEC	6 (3.8%)
Final CNS WHO grade	
CNS WHO grade 2	38 (23.8%)
CNS WHO grade 3	30 (18.8%)
CNS WHO grade 4	92 (57.5%)

Notes: WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System; *IDH*, isocitrate dehydrogenase; NEC, not elsewhere classified.

EGFR amplification, chromosome +7/-10 signature, and *CDKN2A/B* homozygous deletion were observed in 71 (44.4%), 36 (22.5%), 39 (24.4%), and 15 (9.4%) cases, respectively. *MGMT* promoter methylation was present in 85 cases (53.1%). Among the 51 astrocytomas, *IDH*-mutant, ATRX and TP53 status was available in 37 (72.5%) and 32 (62.7%) cases, respectively; markers both were available in 26 cases, whereas both were unavailable in eight cases. The clinical and molecular characteristics of these eight cases are provided in Supplementary Table 1. All 51 cases had complete *IDH* and 1p/19q results, including those lacking ATRX/TP53 IHC. *BRAF* V600E was detected in two cases, whereas H3 K27 and H3 G34 alterations were not identified (**Table 2**).

Distribution of WHO CNS5 integrated diagnoses and final CNS WHO grades

After reassessment according to the WHO CNS5 integrated diagnostic framework, the most frequent diagnosis was glioblastoma, *IDH*-wildtype (76/160, 47.5%), followed by astrocytoma, *IDH*-mutant (51/160, 31.9%), oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted (27/160, 16.9%), and diffuse astrocytic glioma, *IDH*-wildtype, NEC (6/160, 3.8%). Among the six NEC cases, all were 1p/19q non-codeleted and lacked *TERT* promoter mutation, *EGFR* amplification, chromosome +7/-10 signature, *CDKN2A/B* homozygous deletion, *BRAF* V600E, and H3 K27/G34 alterations. *MGMT* promoter methylation was detected in one case. ATRX was evaluable in three cases and TP53 in four cases. During follow-up, one PFS event occurred and no deaths were recorded. The final CNS WHO grade distribution was grade 2 in 38 patients (23.8%), grade 3 in 30 patients (18.8%), and grade 4 in 92 patients (57.5%) (**Table 3**).

Grade migration from traditional WHO grading to WHO CNS5 grading

Grade migration from traditional histological grading to WHO CNS5 grading is summarized in **Table 4**. Among 54 tumors initially classified as traditional WHO grade 2, 38 cases (70.4%) remained grade 2, whereas 3 cases (5.6%) were upgraded to WHO CNS5 grade 3 and 13 cases (24.1%) were upgraded to WHO CNS5 grade 4. Among 46 traditional WHO grade 3 tumors, 26 cases (56.5%) remained grade 3

Table 4. Grade migration from traditional WHO grading to WHO CNS5 grading

Traditional WHO grade	WHO CNS5 grade 2	WHO CNS5 grade 3	WHO CNS5 grade 4	Total
Grade 2	38 (70.4%)	3 (5.6%)	13 (24.1%)	54
Grade 3	0 (0.0%)	26 (56.5%)	20 (43.5%)	46
Grade 4	0 (0.0%)	1 (1.7%)	59 (98.3%)	60
Total	38	30	92	160

Notes: Percentages were calculated within each traditional WHO grade group. Grade migration was defined as any change between traditional histological grade and final WHO CNS5 grade. WHO, World Health Organization; WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System.

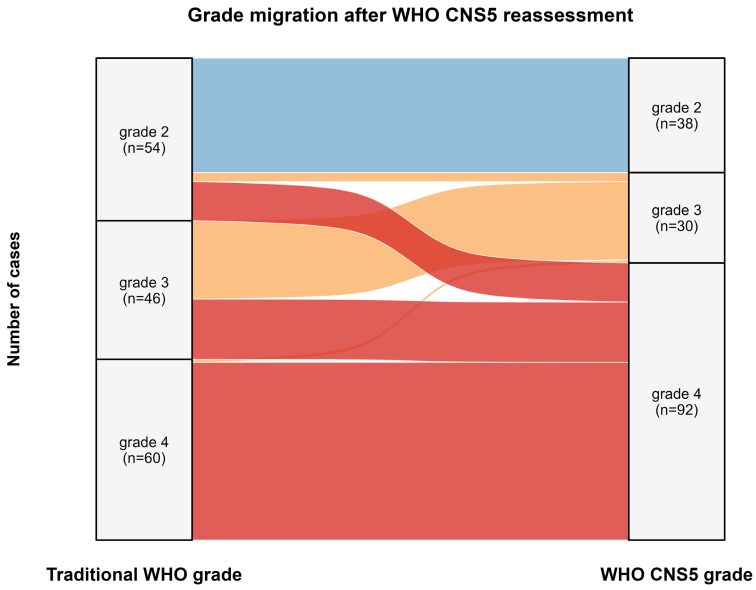


Figure 2. Grade migration from traditional histological grading to WHO CNS5 grading. Notes: The alluvial plot shows the redistribution of tumors from traditional WHO grade 2, grade 3, and grade 4 categories to final WHO CNS5 grades. Upward grade migration occurred mainly among tumors initially diagnosed as traditional grade 2 or grade 3. WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System.

and 20 cases (43.5%) were upgraded to grade 4. Among 60 traditional WHO grade 4 tumors, 59 cases (98.3%) remained grade 4, whereas one case (1.7%) was reassigned to WHO CNS5 grade 3. Overall, grade migration occurred in 37 of 160 cases (23.1%), including 36 upgrades and one downgrade (**Table 4**; **Figure 2**).

Type and basis of reclassification after WHO CNS5 reassessment

The type and basis of reclassification are summarized in **Table 5**. Entity-level reassignment occurred in 86 cases (53.8%), whereas grade migration occurred in 37 cases (23.1%). Overall, 92 cases (57.5%) showed either diagnostic entity reassignment or grade migration after WHO CNS5 reassessment. Among cases with grade migration, 36 were upgraded and one was downgraded.

Among the 92 reclassified cases, the most frequent reclassification category was *IDH*-wildtype diffuse astrocytic tumors with WHO CNS5 glioblastoma-defining molecular features, including *TERT* promoter mutation, *EGFR* amplification, and/or chromosome +7/-10 signature (40/92, 43.5%). *IDH*-mutant tumors were reassigned according to 1p/19q status and astrocytic lineage markers, including 21 cases reassigned to the *IDH*-mutant astrocytoma lineage and 23 cases reassigned to oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted. Among the eight *IDH*-mutant astrocytomas with *CDKN2A/B* homozygous deletion, five were upgraded from a lower traditional histological grade to CNS WHO grade 4, whereas three had already been classified as traditional grade 4 and therefore did not undergo grade migration. Only one case underwent grade reassessment based on a consensus histological review.

Among 92 tumors assigned CNS WHO grade 4, 9 cases (9.8%) were classified as grade 4 based on histological grade 4 features alone, whereas 27 cases (29.3%) were assigned grade 4 based solely on molecular glioblastoma-defining features. Forty-eight cases (52.2%) showed both histological grade 4 features and molecular glioblastoma-defining alterations. *CDKN2A/B* homozygous deletion was present in eight *IDH*-mutant astrocytomas assigned to

Table 5. Type and basis of reclassification after WHO CNS5 reassessment

Category	n (%)
Type of reclassification	
Entity-level reassignment	86 (53.8%)
Grade migration	37 (23.1%)
Any diagnostic or grade reclassification	92 (57.5%)
Direction of grade migration	
Upgrade	36 (22.5%)
Downgrade	1 (0.6%)
Unchanged grade	123 (76.9%)
Basis of diagnostic or grade reclassification among reclassified cases, n = 92	
IDH-wildtype with WHO CNS5 glioblastoma-defining feature(s)	40 (43.5%)
IDH-mutant astrocytoma lineage reassignment	21 (22.8%)
IDH-mutant and 1p/19q-codeleted oligodendroglioma reassignment	23 (25.0%)
Grade migration to CNS WHO grade 4 in cases with *CDKN2A/B* homozygous deletion	5 (5.4%)
Consensus histological grade reassessment	1 (1.1%)
Reassignment to NEC category	2 (2.2%)
Basis for CNS WHO grade 4 classification among grade 4 tumors, n = 92	
Histological grade 4 features only	9 (9.8%)
Molecular glioblastoma-defining feature(s) only	27 (29.3%)
Both histological and molecular glioblastoma-defining features	48 (52.2%)
CDKN2A/B homozygous deletion only	4 (4.3%)
Histological feature(s) plus *CDKN2A/B* homozygous deletion	4 (4.3%)

Notes: Percentages for type and direction of reclassification were calculated using the total cohort as denominator, n = 160. Percentages for basis of reclassification were calculated among reclassified cases, n = 92. Percentages for basis of CNS WHO grade 4 classification were calculated among WHO CNS5 grade 4 tumors, n = 92. Molecular glioblastoma-defining features included *TERT* promoter mutation, *EGFR* amplification, and/or chromosome +7/-10 signature. Histological grade 4 features included necrosis and/or microvascular proliferation. WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System; *IDH*, isocitrate dehydrogenase; *CDKN2A/B*, cyclin-dependent kinase inhibitor 2A/B; NEC, not elsewhere classified.

CNS WHO grade 4. It was the sole grade 4 criterion in four cases and co-occurred with histological grade 4 features in the remaining four cases (**Table 5**). Kaplan-Meier analysis showed significant differences in OS across traditional histological grades and WHO CNS5 integrated grades (both log-rank $P < 0.001$; **Figure 3A**, **3B**), with similar findings for PFS (both log-rank $P < 0.001$; **Figure 3C**, **3D**).

Multivariable Cox regression analysis for OS and PFS

Multivariate Cox regression analysis for OS is summarized in **Table 6**. A lower preoperative KPS was independently associated with poorer OS, whereas a higher KPS was protective (HR = 0.955, 95% CI: 0.928-0.983, $P = 0.002$). Compared with gross total resection, a biopsy-only procedure was associated with significantly increased mortality risk (HR = 2.523, 95% CI: 1.288-4.945, $P = 0.007$), while subtotal resection showed a borderline association with poorer OS (HR = 1.847, 95% CI: 0.997-3.422, $P = 0.052$).

Among molecular markers, *MGMT* promoter methylation was associated with improved OS (HR = 0.193, 95% CI: 0.077-0.484, $P < 0.001$), whereas *EGFR* amplification and chromosome +7/-10 signature were associated with increased mortality risk (*EGFR* amplification: HR = 3.072, 95% CI: 1.514-6.234, $P = 0.002$; chromosome +7/-10: HR = 2.115, 95% CI: 1.064-4.204, $P = 0.033$). *IDH*-wildtype status, *TERT* promoter mutation, 1p/19q codeletion, and *CDKN2A/B* homozygous deletion were not independently significant in this multivariate model. Treatment variables were retained as adjustment covariates but were not interpreted causally because of potential confounding by indication.

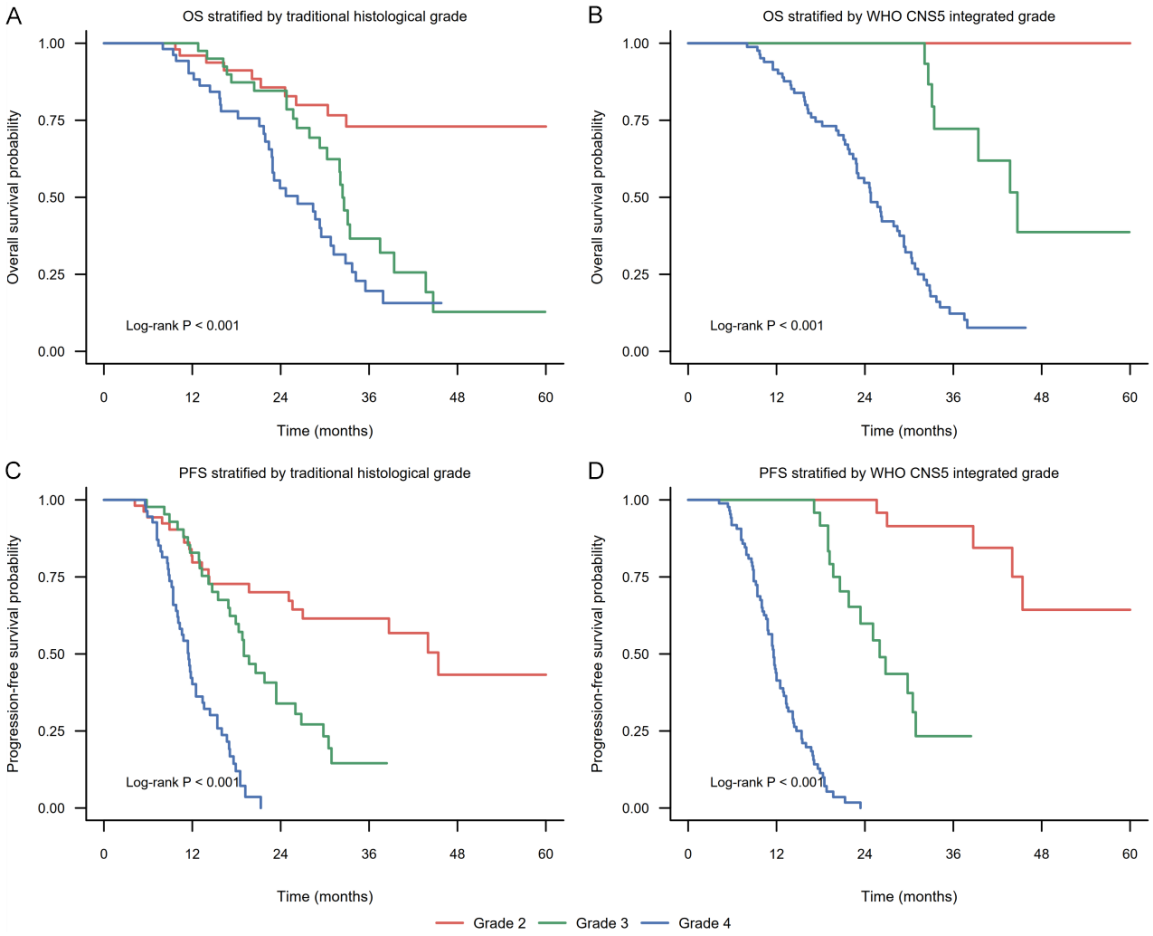


Figure 3. OS and PFS stratification before and after WHO CNS5 reassessment. Notes: Kaplan-Meier curves show OS stratified by traditional histological grade (A) and WHO CNS5 integrated grade (B), and PFS stratified by traditional histological grade (C) and WHO CNS5 integrated grade (D). Differences among grade groups were assessed using the log-rank test. OS, overall survival; PFS, progression-free survival; WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System.

Table 6. Multivariable Cox regression analysis for OS and PFS

Variable	OS adjusted HR (95% CI)	OS *P* value	PFS adjusted HR (95% CI)	PFS *P* value
Preoperative KPS	0.955 (0.928-0.983)	0.002	0.946 (0.924-0.968)	< 0.001
Surgical procedure				
Gross total resection	Reference	-	Reference	-
Subtotal resection	1.847 (0.997-3.422)	0.052	3.058 (1.826-5.121)	< 0.001
Biopsy	2.523 (1.288-4.945)	0.007	2.938 (1.682-5.129)	< 0.001
IDH-wildtype	2.589 (0.773-8.670)	0.124	6.447 (2.589-16.051)	< 0.001
1p/19q codeletion	0.275 (0.051-1.492)	0.134	0.509 (0.194-1.334)	0.170
MGMT promoter methylation	0.193 (0.077-0.484)	< 0.001	0.326 (0.204-0.523)	< 0.001
TERT promoter mutation	1.766 (0.870-3.584)	0.116	2.961 (1.673-5.240)	< 0.001
EGFR amplification	3.072 (1.514-6.234)	0.002	2.889 (1.643-5.080)	< 0.001
Chromosome +7/-10 signature	2.115 (1.064-4.204)	0.033	1.878 (1.059-3.330)	0.031
CDKN2A/B homozygous deletion	1.101 (0.325-3.733)	0.878	5.555 (2.106-14.654)	< 0.001

Notes: OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; KPS, Karnofsky Performance Status; *IDH*, isocitrate dehydrogenase; *MGMT*, O^6-methylguanine-DNA methyltransferase; *TERT*, telomerase reverse transcriptase; *EGFR*, epidermal growth factor receptor; *CDKN2A/B*, cyclin-dependent kinase inhibitor 2A/B. The multivariable models included preoperative KPS, surgical procedure, and the listed molecular markers. Treatment variables were included as adjustment covariates and were not interpreted causally because of potential confounding by indication.

Table 7. Prognostic performance of traditional, WHO CNS5, and molecular models for OS and PFS

Endpoint	Model	N	Events	C-index	AIC	BIC
OS	Traditional histological grade model	160	67	0.726	525.21	542.85
	WHO CNS5 integrated grade model	160	67	0.806	488.72	506.35
	Molecular model	160	67	0.873	463.65	492.31
PFS	Traditional histological grade model	160	98	0.784	753.26	773.94
	WHO CNS5 integrated grade model	160	98	0.858	675.87	696.55
	Molecular model	160	98	0.875	671.7	705.3

Notes: The traditional histological grade model included preoperative KPS, surgical procedure, and traditional WHO grade. The WHO CNS5 integrated grade model included preoperative KPS, surgical procedure, and WHO CNS5 grade. The molecular model included preoperative KPS, surgical procedure, *IDH* status, 1p/19q codeletion, *MGMT* promoter methylation, *TERT* promoter mutation, *EGFR* amplification, chromosome +7/-10 signature, and *CDKN2A/B* homozygous deletion. WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System; OS, overall survival; PFS, progression-free survival; C-index, Harrell's concordance index; AIC, Akaike information criterion; BIC, Bayesian information criterion.

Table 8. Pairwise bootstrap comparisons of C-indices among prognostic models

Endpoint	Comparison	ΔC-index	95% CI	*P* value
OS	WHO CNS5 vs Traditional	0.080	0.020-0.107	0.001
	Molecular vs Traditional	0.147	0.075-0.183	< 0.001
	Molecular vs WHO CNS5	0.067	0.025-0.107	< 0.001
PFS	WHO CNS5 vs Traditional	0.073	0.023-0.107	0.002
	Molecular vs Traditional	0.091	0.050-0.138	< 0.001
	Molecular vs WHO CNS5	0.018	-0.001-0.059	0.069

Notes: ΔC-index was calculated as the C-index of the first model minus that of the second model in each comparison; positive values therefore indicate higher discrimination for the first model. OS, overall survival; PFS, progression-free survival; WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System; C-index, Harrell's concordance index; CI, confidence interval.

For PFS, higher preoperative KPS and *MGMT* promoter methylation were independently associated with favorable outcomes, whereas subtotal resection, biopsy, *IDH*-wildtype status, *TERT* promoter mutation, *EGFR* amplification, chromosome +7/-10 signature, and *CDKN2A/B* homozygous deletion were associated with poorer PFS (all $P < 0.05$). 1p/19q codeletion was not independently significant (**Table 6**). Treatment variables were retained as adjustment covariates and were not interpreted causally.

Prognostic performance of traditional, WHO CNS5, and molecular models for OS and PFS

Compared with the traditional histological grade model, the WHO CNS5 integrated grade model showed improved prognostic discrimination for OS, as reflected by a higher C-index and lower AIC and BIC. The molecular model showed a further increase in the C-index for OS. Similar trends were observed for PFS: the C-index increased from 0.784 in the traditional histological grade model to 0.858 in the WHO CNS5 integrated grade model and 0.875 in the molecular model. The WHO CNS5 model also showed lower AIC and BIC than the traditional model, whereas the molecular model had the lowest AIC but a slightly higher BIC than the WHO CNS5 model for PFS (**Table 7**). Pairwise bootstrap comparisons showed that the WHO CNS5 model had a significantly higher C-index than the traditional model for both OS (ΔC-index = 0.080, 95% CI: 0.020-0.107, P = 0.001) and PFS (ΔC-index = 0.073, 95% CI: 0.023-0.107, P = 0.002). The molecular model also had a significantly higher C-index than the WHO CNS5 model for OS (ΔC-index = 0.067, 95% CI: 0.025-0.107, P < 0.001), whereas the difference for PFS was not statistically significant (ΔC-index = 0.018, 95% CI: -0.001-0.059, P = 0.069) (**Table 8**). Sensitivity analyses excluding the eight cases with unavailable ATRX and TP53 assessments yielded similar diagnostic reclassification rates and prognostic model performance, with no material change in the main findings (Supplementary Table 2).

At 12 months, time-dependent ROC analysis yielded AUCs of 0.691, 0.757, and 0.919 for OS and 0.844, 0.912, and 0.953 for PFS in the traditional, WHO CNS5, and molecular models, respectively. Calibration plots showed generally

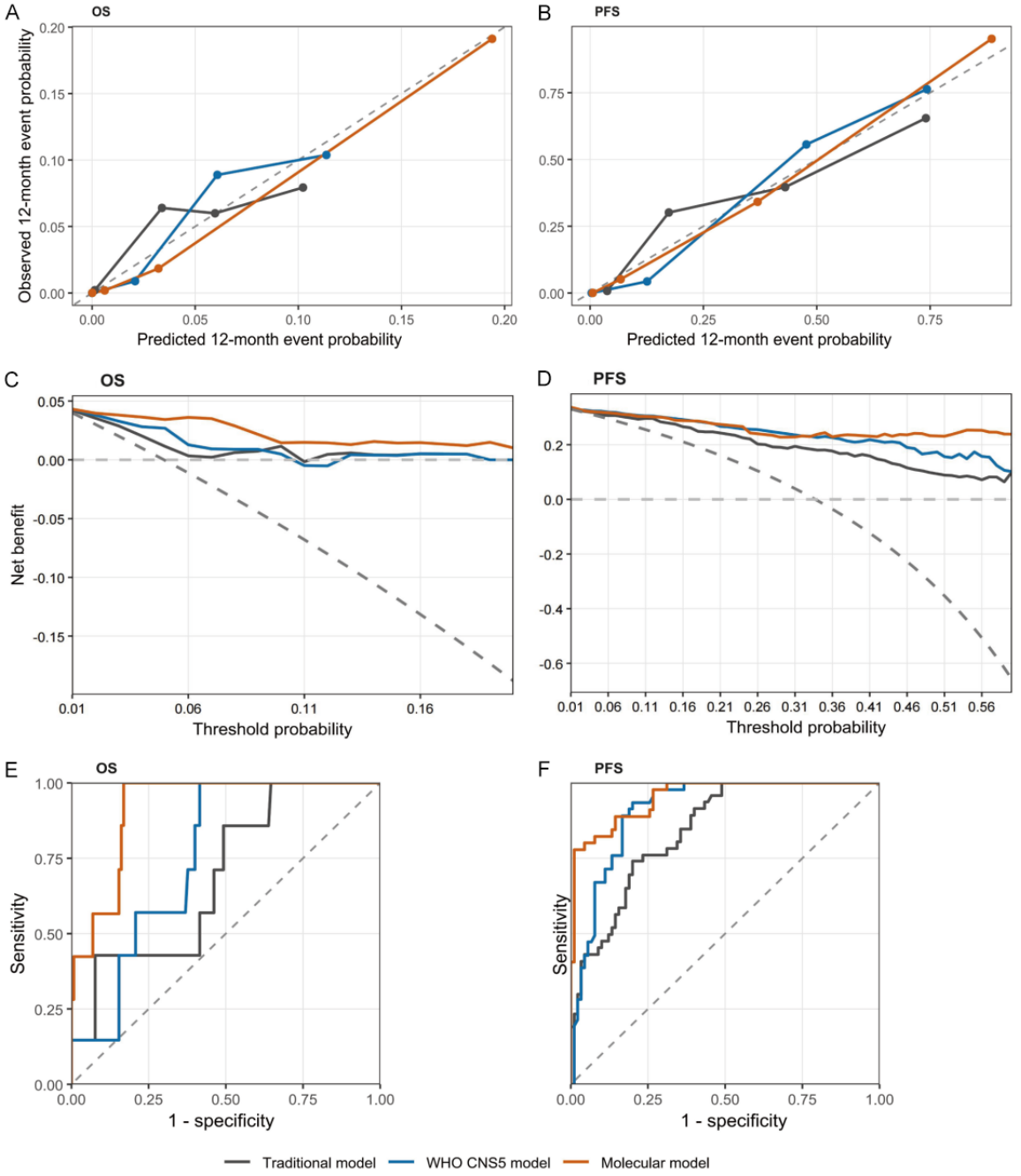


Figure 4. Twelve-month performance evaluation of the traditional, WHO CNS5, and molecular prognostic models. Notes: Calibration plots for OS (A) and PFS (B), decision curve analyses for OS (C) and PFS (D), and time-dependent ROC curves for OS (E) and PFS (F) at 12 months. In the calibration plots, the diagonal dashed line represents perfect agreement between predicted and observed event probabilities. In the decision curve analyses, the dashed gray and light-gray lines represent the treat-all and treat-none strategies, respectively. In the ROC plots, the diagonal dashed line represents no discrimination. OS, overall survival; PFS, progression-free survival; WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System; ROC, receiver operating characteristic.

acceptable agreement between predicted and observed 12-month risks across the three models. Decision curve analysis was further used to compare the net benefit of the models across the evaluated threshold probabilities (**Figure 4**).

Discussion

Based on a single-center retrospective cohort of 160 adult gliomas, this study systematically evaluated the diagnostic, grading, and prognostic impact of WHO CNS5 integrated molecular classification. Compared with the original histology-centered framework, our revised analysis included core WHO CNS5-defining molecular markers, including *IDH1/2* status, 1p/19q codeletion, *TERT* promoter mutation, *EGFR* amplification, the chromosome +7/-10 signature, and *CDKN2A/B* homozygous deletion. The WHO CNS5 emphasizes an integrated histological-molecular diagnosis and classifies adult-type diffuse gliomas into three major molecularly defined entities: astrocytoma, *IDH*-mutant; oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted; and glioblastoma, *IDH*-wildtype [1, 2, 8]. After WHO CNS5 reassessment, entity-level reassignment occurred in 86 cases (53.8%), while grade migration occurred in 37 cases (23.1%). More importantly, WHO CNS5 integrated grading showed improved OS stratification compared with traditional histological grading, with the C-index increasing from 0.726 in the traditional grade model to 0.806 in the WHO CNS5 grade model. These findings indicate that WHO CNS5 reassessment is not merely a change in diagnostic terminology, but also provides additional prognostic discrimination in adult gliomas.

Grade migration was concentrated primarily among tumors originally assigned to traditional WHO grades 2 and 3. Among traditional grade 2 tumors, 13 cases were upgraded to CNS WHO grade 4, and among traditional grade 3 tumors, 20 cases were upgraded to grade 4. The predominance of upward grade migration was consistent with the WHO CNS5 criteria. *IDH*-wildtype diffuse astrocytic tumors harboring a *TERT* promoter mutation, *EGFR* amplification, or a chromosome +7/-10 signature may be assigned to glioblastoma, *IDH*-wildtype, CNS WHO grade 4 even in the absence of histological grade 4 features [5], whereas *CDKN2A/B* homozygous deletion is a grade 4 criterion for astrocytoma, *IDH*-mutant [6, 12]. Accordingly, the observed upward migration illustrates how WHO CNS5 molecular criteria can reclassify tumors that would otherwise have been assigned to lower-risk categories on the basis of morphology alone, particularly in biopsy specimens and morphologically borderline cases. Morphological interpretation of atypia, mitotic activity, microvascular proliferation, and necrosis are heavily relied upon in the conventional grading system, which can be influenced by inter-observer variability and sampling bias [13]. The WHO CNS5 introduces lineage-defining and grade-defining molecular events to the schema, which will facilitate risk stratification and cross-center diagnostic comparability by adding a more reproducible framework.

The distribution of integrated diagnoses in our cohort was consistent with the central role of *IDH* status and 1p/19q codeletion in lineage assignment under WHO CNS5. Following integrated reassessment, glioblastoma, *IDH*-wildtype was the most common WHO CNS5 diagnosis followed by astrocytoma, *IDH*-mutant, and oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted. The main branch points for the classification of adult-type diffuse gliomas within WHO CNS5 are *IDH* status and 1p/19q codeletion [13]. An integrated diagnostic process, involving both pathological and molecular tests, is supported by the fact that the same number of patients were found with the 1p/19q codeletion as with the diagnosis of oligodendroglioma. ATRX loss and TP53 overexpression, however, were considered supportive lineage markers but not required for grading. This distinction is important because ATRX and TP53 are helpful for supporting astrocytic lineage in *IDH*-mutant, 1p/19q-non-codeleted tumors, but they are not independent WHO CNS5 grade-defining events. The six diffuse astrocytic gliomas, *IDH*-wildtype, NEC represented a small diagnostically unresolved subgroup lacking glioblastoma-defining molecular alterations. Given the limited number of cases and outcome events, their prognosis could not be reliably evaluated, and additional molecular testing beyond the routine panel may help refine classification in such cases.

The prognostic relevance was further assessed by correlating molecular subtypes with survival outcomes. Kaplan-Meier analysis showed that WHO CNS5 integrated grading demonstrated clearer separation of both OS and PFS than traditional histological grading. Consistently, replacing traditional grade with WHO CNS5 grade improved prognostic discrimination and model fit for both endpoints. These findings support the prognostic relevance of integrated histological-molecular classification

in adult gliomas [14]. The molecular model provided additional discrimination over the WHO CNS5 model for OS, whereas its incremental improvement for PFS was not statistically significant. However, its clinical utility requires validation in independent cohorts before it can be used to guide management decisions.

In the multivariate molecular Cox model, *MGMT* promoter methylation was associated with improved OS. This finding is consistent with previous evidence that *MGMT* promoter methylation is associated with improved outcomes in patients with glioblastoma treated with temozolomide [15, 16]. Conversely, *EGFR* amplification and the chromosome +7/-10 signature were associated with poorer OS. These alterations are established molecular features of glioblastoma, *IDH*-wildtype, and were associated with poorer OS in this cohort [5]. *IDH*-wildtype status and *TERT* promoter mutation were not independently associated with OS in the fully adjusted model; however, this finding does not preclude a prognostic relevance. Their effects may have been partly absorbed by closely related glioblastoma-defining alterations such as *EGFR* amplification and the +7/-10 signature, reflecting collinearity within the molecular glioblastoma signature.

CDKN2A/B homozygous deletion contributed to CNS WHO grade 4 classification in a subset of *IDH*-mutant astrocytomas. *CDKN2A/B* homozygous deletion was not independently associated with OS, which may be attributable to the limited sample size, as only eight *IDH*-mutant astrocytomas harbored this alteration, limiting reliable subgroup-specific prognostic assessment. *CDKN2A/B* homozygous deletion has been incorporated as a grade 4 criterion for *IDH*-mutant astrocytoma in the WHO CNS5. Recent studies also support its adverse prognostic value in *IDH*-mutant astrocytoma [17]. WHO CNS5 classifies adult-type diffuse gliomas into three molecularly defined entities: glioblastoma, *IDH*-wildtype; astrocytoma, *IDH*-mutant; and oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted, highlighting the central role of these markers in lineage assignment and prognostic stratification [18, 19]. *IDH* mutation status has been established as an important prognostic factor, and 1p/19q-codeleted tumors are generally associated with more favorable survival outcomes [20]. Furthermore, it has been demonstrated that *CDKN2A/B* homozygous deletion correlates with poor prognosis in *IDH* mutant gliomas [21], so it is recommended to include a more comprehensive integrated diagnostic panel in future studies. Accordingly, the lack of independent significance for OS should be interpreted cautiously and does not conflict with its status as a WHO CNS5 grade-defining alteration. Larger cohorts of *IDH*-mutant astrocytoma are needed to more precisely evaluate its prognostic impact.

Ki-67 is still a valuable auxiliary marker of proliferative activity, but should not be used as a single factor to determine WHO CNS5 grade. Previous research has suggested that the Ki-67 labelling index is frequently raised with the grade of the glioma and may be associated with the risk of recurrence and survival [22, 23]. Ki-67 was specifically analyzed as a proliferation marker in our updated analysis and not used for grading. This is crucial because WHO CNS5 grading is based on specific histological and molecular parameters, whereas Ki-67 is a downstream expression of proliferative behavior and therefore dependent on the tumor type and molecular context. Accordingly, Ki-67 should be regarded as a supportive proliferative marker rather than an independent determinant of integrated grade.

Clinically, these findings support the value of molecular testing within the WHO CNS5 framework, although the availability of some assays, particularly copy-number assessment for the chromosome +7/-10 signature, may remain limited in resource-constrained settings. For lineage assignment, *IDH1/2* and 1p/19q are mandatory. Among diffuse astrocytic tumors with *IDH*-wildtype status, mutation of the *TERT* promoter, amplification of the *EGFR* gene, and the chromosome +7/-10 signature are key for the identification of molecular glioblastoma. Since it has a direct grading implication, *CDKN2A/B* homozygous deletion should be evaluated in *IDH*-mutant astrocytomas [12]. *MGMT* promoter methylation, although not a WHO grading criterion, remains clinically relevant for prognostic assessment [15, 24]. In this context, integrated diagnosis represents more than a change in pathological terminology; it provides a standardized framework for diagnostic assignment and prognostic stratification.

There are some limitations to be noted in this research. First, this was a single-center retrospective study with a moderate sample size, and selection bias cannot be excluded. Second, ATRX and TP53 assessments were not available in all cases, and both markers were unavailable in eight IDH-mutant astrocytomas. Although astrocytic lineage in these cases was assigned based on IDH mutation and absence of 1p/19q codeletion, the lack of these supportive lineage markers remains an important limitation because uncommon molecular profiles may not have been fully characterized. Sensitivity analyses excluding these eight cases yielded similar diagnostic reclassification rates and prognostic model performance. Nevertheless, more complete assessment of ATRX and TP53 should be pursued whenever feasible in future studies. Third, treatment allocation was not randomized, and patients with higher-risk tumors were more likely to receive radiotherapy, temozolomide, or the Stupp regimen. Although treatment variables were included as adjustment covariates, residual confounding by indication remained possible, and no treatment-stratified or treatment-by-molecular interaction analyses were performed. Therefore, treatment-specific prognostic or predictive effects could not be established, and the present findings do not support individualized treatment recommendations. Fourth, model performance was evaluated within a single-center cohort and requires external validation. Although 12-month time-dependent discrimination, calibration, and decision curve analyses were additionally performed, these assessments represent internal evaluation and should be interpreted cautiously. In addition, some molecular subgroups were small, particularly *IDH*-mutant astrocytomas with *CDKN2A/B* homozygous deletion, limiting subgroup-specific prognostic analyses. Finally, this study was designed as a clinicopathological reassessment rather than a mechanistic investigation. Accordingly, the observed associations do not establish causality, and the molecular pathways underlying differences in tumor behavior require separate functional studies using cellular or *in vivo* models.

Conclusion

This study demonstrates that WHO CNS5 integrated molecular reassessment substantially changes diagnostic assignment and grade distribution in adult gliomas. Grade migration occurred in nearly one-quarter of patients, mainly among traditional grade 2 and grade 3 tumors. More importantly, WHO CNS5 integrated grading provided improved prognostic stratification for both OS and PFS compared with traditional histological grading, and integrated molecular information provided additional prognostic discrimination. The results support the value of comprehensive molecular testing within the WHO CNS5 diagnostic framework and underscores the importance of combined histological-molecular classification for diagnostic assignment and prognostic stratification.

Acknowledgements

This work was supported by the Key Projects of Jiangsu Provincial Health Commission (Grant No. ZD2021051, awarded to Lianshu Ding) and the Key Research Training Program of Northern Jiangsu Institute of Clinical Medicine, Nanjing Medical University (Grant No. HAKY202400406, awarded to Lianshu Ding).

Disclosure of conflict of interest

None.

Address correspondence to: Lianshu Ding, Department of Neurosurgery, The Affiliated Huai'an No. 1 People's Hospital of Nanjing Medical University, Northern Jiangsu Institute of Clinical Medicine, Nanjing Medical University, No. 1 Huanghe West Road, Huaiyin District, Huai'an 223300, Jiangsu, China. E-mail: Dls1391511@163.com; Xiaotian Zhang, Department of Neurosurgery, Hongze District People's Hospital of Huai'an, No. 102 Dongfeng Road, Hongze District, Huai'an 223100, Jiangsu, China. E-mail: 15195307883@163.com

References

[1] Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, Hawkins C, Ng HK, Pfister SM, Reifenberger G, Soffietti R, von Deimling A and Ellison DW. The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol 2021; 23: 1231-1251.

[2] Smith HL, Wadhwani N and Horbinski C. Major features of the 2021 WHO classification of CNS tumors. Neurotherapeutics 2022; 19: 1691-1704.

[3] Antonelli M and Poliani PL. Adult type diffuse gliomas in the new 2021 WHO Classification. Pathologica 2022; 114: 397-409.

[4] Whitfield BT and Huse JT. Classification of adult-type diffuse gliomas: impact of the World Health Organization 2021 update. Brain Pathol 2022; 32: e13062.
[5] Byun YH and Park CK. Classification and diagnosis of adult glioma: a scoping review. Brain Neurorehabil 2022; 15: e23.
[6] Komori T. Grading of adult diffuse gliomas according to the 2021 WHO Classification of Tumors of the Central Nervous System. Lab Invest 2022; 102: 126-133.
[7] Melhem JM, Detsky J, Lim-Fat MJ and Perry JR. Updates in IDH-wildtype glioblastoma. Neurotherapeutics 2022; 19: 1705-1723.
[8] Martin KC, Ma C and Yip S. From theory to practice: implementing the WHO 2021 classification of adult diffuse gliomas in neuropathology diagnosis. Brain Sci 2023; 13: 817.
[9] Park YW, Vollmuth P, Foltyn-Dumitru M, Sahm F, Ahn SS, Chang JH and Kim SH. The 2021 WHO classification for gliomas and implications on imaging diagnosis: part 1-key points of the fifth edition and summary of imaging findings on adult-type diffuse gliomas. J Magn Reson Imaging 2023; 58: 677-689.
[10] Thomas DL. 2021 updates to the World Health Organization classification of adult-type and pediatric-type diffuse gliomas: a clinical practice review. Chin Clin Oncol 2023; 12: 7.
[11] Torp SH, Solheim O and Skjulsvik AJ. The WHO 2021 Classification of Central Nervous System tumours: a practical update on what neurosurgeons need to know-a minireview. Acta Neurochir (Wien) 2022; 164: 2453-2464.
[12] Yokoda RT, Cobb WS, Yong RL, Crary JF, Viapiano MS, Walker JM, Umphlett M, Tsankova NM and Richardson TE. CDKN2A mutations have equivalent prognostic significance to homozygous deletion in IDH-mutant astrocytoma. J Neuropathol Exp Neurol 2023; 82: 845-852.
[13] Sahm F, Brandner S, Bertero L, Capper D, French PJ, Figarella-Branger D, Giangaspero F, Haberler C, Hegi ME, Kristensen BW, Kurian KM, Preusser M, Tops BBJ, van den Bent M, Wick W, Reifenberger G and Wesseling P. Molecular diagnostic tools for the World Health Organization (WHO) 2021 classification of gliomas, glioneuronal and neuronal tumors; an EANO guideline. Neuro Oncol 2023; 25: 1731-1749.
[14] Sejda A, Grajkowska W, Trubicka J, Szutowicz E, Wojdacz T, Kloc W and Izycka-Swieszewska E. WHO CNS5 2021 classification of gliomas: a practical review and road signs for diagnosing pathologists and proper patho-clinical and neuro-oncological cooperation. Folia Neuropathol 2022; 60: 137-152.
[15] Darlix A, Bady P, Deverdun J, Lefort K, Rigau V, Le Bars E, Meriadec J, Carriere M, Coget A, Santarius T, Matys T, Duffau H and Hegi ME. Clinical value of the MGMT promoter methylation score in IDHmt low-grade glioma for predicting benefit from temozolomide treatment. Neurooncol Adv 2024; 7: vdae224.
[16] Brandner S, McAleenan A, Kelly C, Spiga F, Cheng HY, Dawson S, Schmidt L, Faulkner CL, Wragg C, Jefferies S, Higgins JPT and Kurian KM. MGMT promoter methylation testing to predict overall survival in people with glioblastoma treated with temozolomide: a comprehensive meta-analysis based on a Cochrane Systematic Review. Neuro Oncol 2021; 23: 1457-1469.
[17] Ghosh HS, Patel RV, Claus EB, Gonzalez Castro LN, Wen PY, Ligon KL, Meredith DM and Bi WL. Canonical amplifications and CDKN2A/B loss refine IDH1/2-mutant astrocytoma prognosis. Neuro Oncol 2025; 27: 993-1003.
[18] Santosh V and Rao S. A review of adult-type diffuse gliomas in the WHO CNS5 classification with special reference to Astrocytoma, IDH-mutant and Oligodendroglioma, IDH-mutant and 1p/19q codeleted. Indian J Pathol Microbiol 2022; 65: S14-S23.
[19] Schaff LR and Mellinghoff IK. Glioblastoma and other primary brain malignancies in adults: a review. JAMA 2023; 329: 574-587.
[20] Choate KA, Pratt EPS, Jennings MJ, Winn RJ and Mann PB. IDH mutations in glioma: molecular, cellular, diagnostic, and clinical Implications. Biology (Basel) 2024; 13: 885.
[21] Nakasu S, Deguchi S, Mitsuya K, Notsu A and Nakasu Y. Prognostic implication of CDKN2A/B homozygous deletion on histological grades in isocitrate dehydrogenase-mutant astrocytomas: a systematic review and meta-analysis. Neurooncol Adv 2025; 7: vdaf171.
[22] Yang Z, Ling F, Ruan S, Hu J, Tang M, Sun X and Long W. Clinical and prognostic implications of 1p/19q, IDH, BRAF, MGMT promoter, and TERT promoter alterations, and expression of Ki-67 and p53 in human gliomas. Cancer Manag Res 2021; 13: 8755-8765.
[23] Chen WJ, He DS, Tang RX, Ren FH and Chen G. Ki-67 is a valuable prognostic factor in gliomas: evidence from a systematic review and meta-analysis. Asian Pac J Cancer Prev 2015; 16: 411-420.
[24] Yuile A, Satgunaseelan L, Alexander KL, Thavaneswaran S, Sim HW, Krasovitsky M, Kong BY, Miller S, Buckland ME, Lee M, Wei G, Kastelan M, Wong M, Wilson I, Bayly A, Varikatt W, Lwin Z, Turner C, Back MF, Pavlakis N, Wei JQ, Hudson A, Chan DL, Wheeler HR and Lee A. Clinical outcomes and management of CDKN2A/B homozygously deleted IDH-mutant astrocytomas-a cohort study and patterns of care survey. Neurooncol Pract 2025; 12: 787-796.

Supplementary Table 1. Clinical and molecular characteristics of eight IDH-mutant astrocytomas with unavailable ATRX and TP53 assessments

A. Clinical and diagnostic characteristics

Patient ID	Age, years	Sex	Traditional diagnosis	Traditional grade	WHO CNS5 diagnosis	CNS WHO grade	Surgical procedure	Radiotherapy	Temozolomide
P018	39	Male	Oligoastrocytoma	2	Astrocytoma, IDH-mutant	2	Biopsy	No	No
P021	61	Female	Anaplastic astrocytoma	3	Astrocytoma, IDH-mutant	3	Gross total resection	Yes	Yes
P024	55	Female	Diffuse astrocytoma	2	Astrocytoma, IDH-mutant	2	Gross total resection	No	Yes
P069	74	Female	Anaplastic astrocytoma	3	Astrocytoma, IDH-mutant	4	Subtotal resection	Yes	Yes
P089	61	Male	Diffuse astrocytoma	2	Astrocytoma, IDH-mutant	2	Biopsy	No	No
P121	37	Female	Anaplastic astrocytoma	3	Astrocytoma, IDH-mutant	4	Biopsy	Yes	Yes
P132	32	Male	Anaplastic astrocytoma	3	Astrocytoma, IDH-mutant	3	Subtotal resection	Yes	Yes
P140	37	Male	Diffuse astrocytoma	2	Astrocytoma, IDH-mutant	2	Biopsy	Yes	Yes

B. Molecular characteristics

Patient ID	IDH	1p/19q	ATRX	TP53	MGMT	TERT	EGFR	Chr +7/-10	CDKN2A/B hom. del.	BRAF V600E	H3 K27	H3 G34
P018	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Unmethylated	Wildtype/Negative	Not amplified	Absent	Absent	Negative	Absent	Absent
P021	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Methylated	Wildtype/Negative	Not amplified	Absent	Absent	Negative	Absent	Absent
P024	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Unmethylated	Wildtype/Negative	Not amplified	Absent	Absent	Negative	Absent	Absent
P069	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Methylated	Wildtype/Negative	Not amplified	Absent	Present	Negative	Absent	Absent
P089	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Methylated	Wildtype/Negative	Not amplified	Absent	Absent	Negative	Absent	Absent
P121	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Unmethylated	Wildtype/Negative	Not amplified	Absent	Absent	Negative	Absent	Absent
P132	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Methylated	Wildtype/Negative	Not amplified	Absent	Absent	Negative	Absent	Absent
P140	IDH-mutant	Non-codeleted	Unavailable	Unavailable	Methylated	Wildtype/Negative	Not amplified	Absent	Absent	Negative	Absent	Absent

Note: All eight cases had complete IDH and 1p/19q results but unavailable ATRX and TP53 assessments. IDH, isocitrate dehydrogenase; MGMT, O6-methylguanine-DNA methyltransferase; TERT, telomerase reverse transcriptase; EGFR, epidermal growth factor receptor; CDKN2A/B, cyclin-dependent kinase inhibitor 2A/B; hom. del., homozygous deletion.

Supplementary Table 2. Sensitivity analyses after exclusion of eight IDH-mutant astrocytomas with unavailable ATRX and TP53 assessments

Analysis/Outcome	Full cohort (N = 160)	Sensitivity cohort (N = 152)	Change
Diagnostic reclassification			
Entity-level reassignment	86 (53.8%)	85 (55.9%)	+2.1 pp
Grade migration	37 (23.1%)	35 (23.0%)	-0.1 pp
Any reclassification	92 (57.5%)	89 (58.6%)	+1.1 pp
OS discrimination (C-index)			
Traditional model	0.726	0.722	-0.004
WHO CNS5 model	0.806	0.796	-0.010
Molecular model	0.873	0.873	0.000
PFS discrimination (C-index)			
Traditional model	0.784	0.780	-0.004
WHO CNS5 model	0.858	0.851	-0.007
Molecular model	0.875	0.874	-0.001

Note: The sensitivity cohort excluded eight IDH-mutant astrocytomas with unavailable ATRX and TP53 assessments and included 152 patients, with 64 deaths and 93 progression events; the full cohort included 160 patients, with 67 deaths and 98 progression events. The same model specifications as in the primary analysis were retained without variable reselection, and the directions of the major multivariable Cox associations remained unchanged. For diagnostic reclassification, changes are expressed as percentage points (pp); for prognostic models, changes represent the sensitivity-cohort C-index minus the full-cohort C-index. OS, overall survival; PFS, progression-free survival; WHO CNS5, fifth edition of the World Health Organization Classification of Tumors of the Central Nervous System; C-index, Harrell's concordance index.