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Rapid diagnosis of adult-type diffuse glioma using a layered scheme

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Abstract

Background Molecular biomarkers have become an essential part of the diagnosis of adult-type diffuse glioma. Still, complex detection methods and long-term turnaround for these biomarkers hinder integrated diagnosis in clinical practice. We hypothesized that *IDH* and *TERT* promoter (*TERTp*) mutations play similar roles in accurately classifying adult-type diffuse glioma compared to the complicated WHO CNS5-recommended biomarkers, and the detection of *IDH* and *TERTp* mutations should be the first layer in clinical practice.

Methods We propose a novel layered diagnostic scheme for adult-type diffuse gliomas, with *IDH* and *TERTp* mutation detection as the initial layer. We also developed a rapid intraoperative testing technology capable of detecting *TERTp* and *IDH* mutations within 35 min. This study involved both a retrospective cohort and a prospective multicenter diagnostic test. The diagnostic accuracy of the layered approach was evaluated using sensitivity, specificity, and the area under the receiver operating characteristic curve (AUC), with a 95% confidence interval.

Results In retrospective cohort, the *TERTp* mutation demonstrated comparable statistical power to 1p/19q codeletion in distinguishing oligodendrogliomas from astrocytomas ($\kappa = 0.96$, $P < 0.001$). Additionally, 91.8% of glioblastomas with either *EGFR* amplification or +7/-10 exhibited *TERTp* mutations. In the prospective application of the layered diagnostic scheme and rapid testing, 223 gliomas and 2 non-gliomas (76.5%) were accurately classified intraoperatively. With the addition of postoperative permanent section analysis, 249 gliomas and 24 non-gliomas (92.9%) were correctly classified following the detection of the first-layer biomarkers.

Conclusions The proposed layered diagnostic scheme offers a rapid and accurate means of classifying adult-type diffuse gliomas, facilitating the broader use of molecular classification. It expands its applicability from postoperative to intraoperative settings for the majority of patients, enhancing diagnostic efficiency and accuracy.

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Keywords Layered diagnostic scheme, Adult-type diffuse gliomas, *IDH* mutation, *TERT* promoter mutation, Intraoperative integrated diagnosis

Background

Diffuse gliomas are devastating tumors with poor prognoses [1]. Traditionally, the diagnosis of gliomas has been dependent on their histological features [2]; however, the unequivocal histopathological diagnosis of diffuse glioma subtypes can be challenging and may not accurately predict clinical outcomes [3]. The identification of subtype-specific molecular markers has led to the development of a robust and objective classification system, which can assist in glioma diagnosis [4–7]. This system has been incorporated into the recently revised 2021 WHO Classification of Tumors of the Central Nervous System, fifth edition (WHO CNS5) [8–10], which defines three major subtypes of adult-type diffuse gliomas: astrocytoma, *IDH*-mutant; oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted; and glioblastoma, *IDH*-wildtype. The 1p/19q codeletion of *IDH*-mutant gliomas can differentiate oligodendrogliomas from astrocytomas. Glioblastomas, *IDH*-wildtype, are characterized by either microvascular proliferation or necrosis, the *TERT* promoter (*TERTp*) mutation, *EGFR* amplification (*EGFR*amp), or combined whole chromosome 7 gain and whole chromosome 10 loss (+7/-10) [11, 12]. Notwithstanding, a small number of diffuse gliomas do not meet the above criteria and are classified by the WHO as not elsewhere classified (NEC) [11]. The molecular markers have revolutionized the diagnosis and classification of gliomas and hold great promise for the development of personalized patient treatments.

However, molecular testing presents challenges related to technical requirements, cost, and assay-specific design limitations. Although detecting single-nucleotide variants is relatively straightforward and less time-consuming [13, 14], complex genetic changes pose challenges in execution and interpretation. Fluorescence in situ hybridization-based testing for 1p/19q codeletion, for example, can be complicated by probe selection, data interpretation, and indeterminate features, which risk misdiagnoses due to false positives or negatives [15–18]. Similar technical challenges are apparent with other routine markers used in glioma diagnoses, such as *EGFR*amp, +7/-10, and *CDKN2A/B* deletion testing [6, 19–21]. These challenges have hindered the implementation of a consensus platform for glioma molecular diagnosis across institutions, especially in areas without access to comprehensive molecular diagnostic facilities or experienced staff. The layered detection of these molecular biomarkers provides a wise option for the integrated diagnosis of adult-type diffuse glioma. The priority detection of *IDH* and *TERTp* mutations would be possible since they are the most common and simple mutations in adult-type diffuse gliomas. Furthermore, intraoperative frozen section pathologic confirmation is standard practice during resection, and the extent of resection impacts patient prognosis [22, 23]. However, frozen section diagnosis can be challenging in low cellularity infiltrative gliomas and/or those with minimal nuclear atypia where determining the presence of gliomas can be difficult [24, 25]. Several molecular approaches for intraoperative mutation detection have been proposed [13, 26–28], but their clinical viability has not been validated in real-world settings following the publication of the WHO CNS5.

Addressing the aforementioned challenges in molecular testing is critical for advancing personalized treatments. Previous studies have suggested that gliomas can be classified into molecular subgroups with distinct survival outcomes based on only a few frequently altered recurrent biomarkers, such as *IDH* and *TERTp* mutations [29, 30]. We therefore hypothesized that *IDH* and *TERTp* mutations play similar roles in accurately classifying adult-type diffuse glioma compared to the complicated WHO CNS5-recommended biomarkers, and the detection of *IDH* and *TERTp* mutations should be the first layer in clinical practice. Moreover, the combination of histology and the detection of *IDH* and *TERTp* mutations could be used to rapidly diagnose patients with suspected gliomas intra- and postoperatively.

In this study, we aimed to address the various diagnostic challenges by establishing a practical and feasible layered diagnostic scheme. A multicenter prospective diagnostic test was conducted during surgery, offering a prototype for a streamlined, integrated diagnostic approach that delivers results within tens of minutes. This technique shows significant potential for advancing personalized treatment strategies for adult-type diffuse gliomas.

Methods

Study participants

The institutional review boards of all participating hospitals approved this study. For the respective cohort, frozen tissue and paired peripheral blood samples were obtained from the Huashan Glioma Biobank between October 2010 and August 2018. Seven hundred and

fifty-three patients aged 18–80 years who had been diagnosed with supratentorial diffuse glioma (WHO grades 2–4) were selected for the study. Seven cases with *H3F3A* mutations were excluded from the study, so 746 patients under long-term follow-up were chosen to formulate the layered diagnostic scheme (Fig. 1). For the prospective cohort, a total of 296 patients, admitted to four clinical centers (129 patients from the Central Campus of Huashan Hospital of Fudan University, 132 patients from the West Campus of Huashan Hospital of Fudan University, 15 patients from General Hospital of Ningxia Medical University, and 20 patients from The First Affiliated Hospital of Fujian Medical University) between May 2021 and January 2022, were included in the study (Fig. 1). Detailed descriptions of these two cohorts are presented in Additional file 1: Method S1 [9].

Molecular pathology and rapid genotyping

A targeted NGS panel and a qPCR test were used to detect hotspot mutations in *IDH*, *TERTp*, *BRAF*, *H3F3A*, and *HIST1H3B* (Additional file 1: Methods S2–S4) [31–33]. Additionally, shallow whole-genome sequencing was used to determine the copy number variation (CNV) and arm-level alterations such as *EGFR*amp, *CDKN2A/B* homozygous deletion, 1p/19q codeletion, and +7/-10 (Additional file 1: Methods S6–S7) [34–48]. Status of 1p/19q was analyzed by fluorescence in situ hybridization (FISH) (Additional file 1: Method S8) [49]. Two senior neuropathologists established the permanently integrated diagnosis by combining the histology and molecular alterations and following the WHO CNS5 standard (Additional file 1: Methods S9, S13) [50]. Methylation profile of three IDH-mutated cases without concurrence of *TERTp* mutation and 1p/19q codeletion was analysis by methylation sequencing (Additional file 1: Method S14) [51, 52].

We developed a fast qPCR-based test using the amplification refractory mutation system and locked nucleic acid oligonucleotides to specifically detect all the hotspot mutations in *IDH1*, *IDH2*, and *TERTp* against wildtype alleles (Additional file 1: Method S10, Additional file 2: Fig. S1) [26, 53]. The technology was validated using Sanger sequencing and targeted NGS sequencing.

Statistical analysis

The primary outcome was the diagnostic accuracy of the layered diagnostic scheme. The accuracy of the layered diagnostic scheme was evaluated via its sensitivity, specificity, and area under receiver operating characteristic curve (AUC) with a 95% CI. The results of the patients

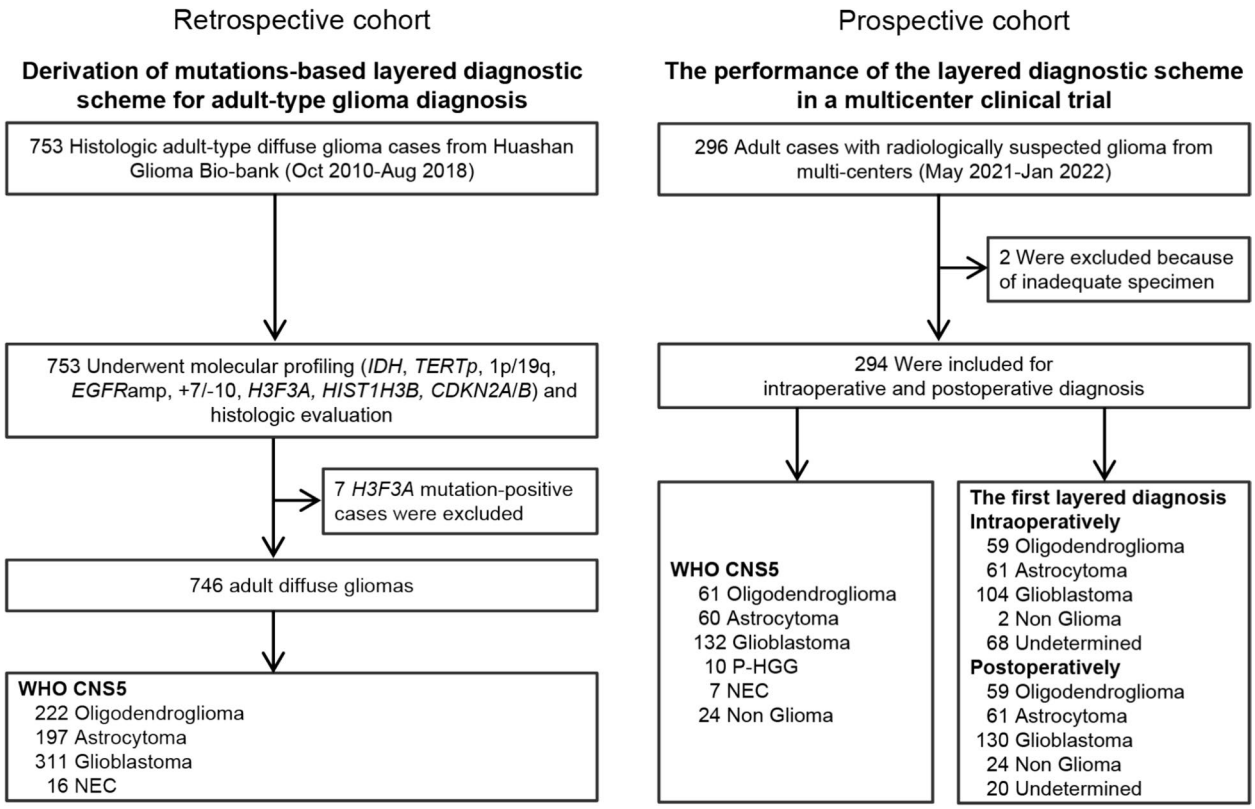

Fig. 1 Overview of the study design. This figure illustrates the study design used to derive the mutation-based layered diagnostic scheme

who received a definitive integrated diagnosis during or after surgery were used to calculate the diagnostic accuracy through comparisons with the standard WHO CNS5 criteria. All the statistical analyses were performed using R (version 4.0.2) (https://cran.r-project.org/). Intraoperative integrated diagnoses were achieved for the glioma patients with *IDH* or *TERTp* mutations and the non-glioma patients with apparent histologic features. Postoperatively, glioblastomas without *IDH* or *TERTp* mutations could be diagnosed based on their typical histologic features.

According to the results of the retrospective study, the diagnostic specificity of the layered diagnostic scheme was about 99.0% for each subtype of adult-type diffuse glioma. We therefore used this value to set the expected specificity for the control and case arms. The type 1 error was set to 5.0% and the marginal error to 10.0%. About 85% of the preoperative patients with radiologically suspected glioma were confirmed as having adult-type diffuse glioma postoperatively. A sample size of 25 patients was thus established for each subtype of adult-type diffuse glioma. Additional details of the statistical analysis are provided in Additional file 1: Method S11.

Results

Concurrent IDH and TERTp mutations as prominent biomarkers in adult-type diffuse glioma subgroups

The assessment of molecular alterations is critical for developing a layered, reliable, and clinically practical patient stratification process. Recently, several molecular biomarkers, including the well-recognized *IDH* mutations and 1p/19q codeletion, along with *TERTp* mutations, *CDKN2A/B* homozygous deletions, *EGFR*amp, and +7/-10 alterations, have been incorporated into the WHO CNS5 classification of adult-type diffuse gliomas. To evaluate the relative contributions of these biomarkers in glioma classification, we analyzed their presence in a retrospective cohort of 746 adult-type diffuse gliomas (Figs. 1 and 2A, Additional file 2: Tables S1–S2).

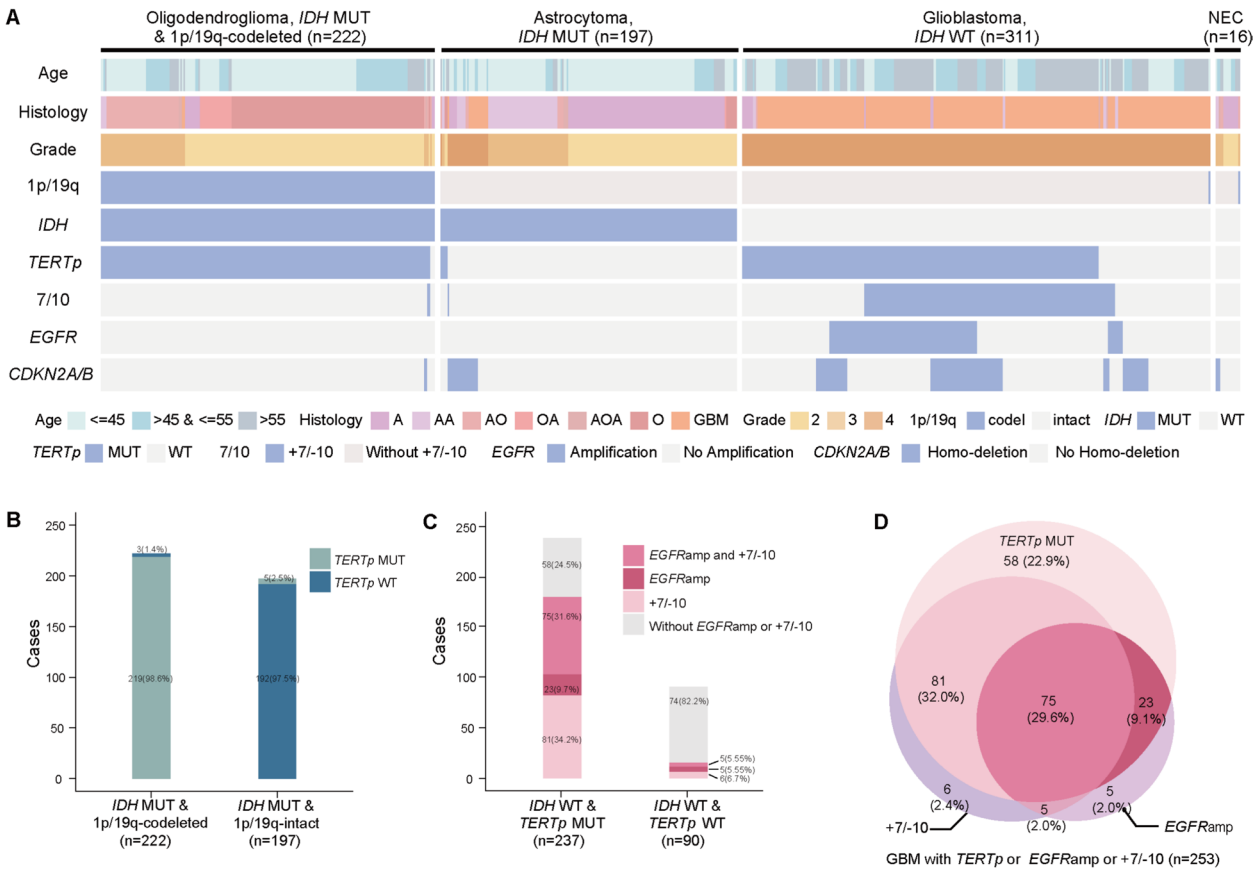

Fig. 2 Genetic signatures for adult-type diffuse gliomas. **A** Clinical and molecular profiles of adult-type diffuse gliomas in a retrospective cohort. The WHO CNS5 criteria were used for the subgroup diagnoses. **B** Prevalence of the *TERTp* mutation in *IDH*-mutated astrocytomas and oligodendrogliomas. **C** Prevalence of the *EGFR*amp and +7/-10 signatures in *IDH*-wildtype gliomas based on their *TERTp* mutation status. **D** Venn diagram showing the numbers of patients with a *TERTp* mutation, *EGFR*amp, and a +7/-10 signature in *IDH*-wildtype glioblastoma

Among the 222 oligodendrogliomas with *IDH* mutations and 1p/19q codeletion, 98.6% also harbored concurrent *TERTp* mutations. In contrast, only 2.5% of the 197 IDH-mutated astrocytomas without 1p/19q codeletion carried *TERTp* mutations (Fig. 2B). Notably, *TERTp* mutation demonstrated statistical power comparable to 1p/19q codeletion in distinguishing oligodendrogliomas from astrocytomas ($\kappa = 0.96$, $P < 0.001$).

Among the 327 IDH-wildtype gliomas, *TERTp* mutations, +7/-10 alterations, and *EGFR*amp were detected in 72.5%, 51.1%, and 33.0% of cases, respectively (Fig. 2A). Of the 237 gliomas with *TERTp* mutations, 65.8% also exhibited +7/-10 alterations, and 41.4% had *EGFR*amp. Notably, 91.8% of glioblastomas with either *EGFR*amp or +7/-10 alterations also carried *TERTp* mutations (Fig. 2C and D). Similar results were confirmed in the prospective cohorts (Additional file 2: Fig. S2). Given its high prevalence, *TERTp* mutation may serve as a reliable substitute for diagnosing +7/-10 alterations and *EGFR*amp in IDH-wildtype diffuse gliomas.

Histologic and molecular characteristics of eight discordant cases in the retrospective cohort

Eight IDH-mutated cases were identified that did not exhibit concurrent 1p/19q codeletion or *TERTp* mutation. These cases were further investigated through histologic assessment, FISH, and WGS.

Among the five gliomas with both *IDH* and *TERTp* mutations but without 1p/19q codeletion, the median overall survival was 127.9 months, with three of these cases displaying classic features of oligodendroglioma. Similar findings have been reported in previous studies, suggesting that *IDH* and *TERTp* mutations could serve as reliable surrogate biomarkers for oligodendroglioma classification. FISH analysis revealed the following genetic alterations: RP652TH1 exhibited a 1p/19q codeletion, RP258TH1 showed intact 1p and 19q deletions, RP890TH1 had a 1p deletion and intact 19q, and RP917TH1 and RP1381TH1 both displayed intact 1p and 19q. However, shallow WGS analysis indicated more complex genetic alterations: RP652TH1 had a partial 1p deletion and 19q deletion, RP890TH1 exhibited a partial 1p deletion and intact 19q, RP258TH1 had a 1p/19q partial deletion, and RP917TH1 and RP1381TH1 had intact 1p and 19q. B-allele frequency analysis of polymorphic sites using WGS revealed an initial deletion of the entire 1p and a copy-neutral loss of heterozygosity (LOH) at p35.2-p21.1 in RP652TH1. Additionally, RP890TH1 exhibited subclonal deletion of chromosome 19. Furthermore, mutations in *FUBP1* were detected in RP652TH1, while mutations in *ATRX* and *TP53* were identified in RP917TH1 (Additional file 2: Table S3 and Fig. S3).

The remaining three IDH-mutated, 1p/19q codeleted tumors without *TERTp* mutations also had favorable prognoses, with all patients remaining alive at the last follow-up, showing overall survival times of 64.2, 102.7, and 85.2 months for RP1084TH1, RP742TH1, and RP723TH1, respectively. FISH and WGS confirmed the 1p/19q codeletion status in all three cases. Additionally, mutations in *FUBP1* and *NOTCH1* were found in RP742TH1 through WGS (Additional file 2: Table S3 and Fig. S3).

Clinical performance of the layered diagnostic scheme intra- and postoperatively

IDH and *TERTp* mutations are highly specific biomarkers, with their distinct frequency and specificity within each glioma subgroup making them valuable for patient stratification. Thus, the detection of *IDH* and *TERTp* mutations should be prioritized in cases with 1p/19q codeletion, *EGFR* amplification, and +7/-10 alterations to enhance the stratified classification of adult-type diffuse gliomas. Based on this principle, a layered diagnostic scheme was developed (Fig. 3). To further evaluate the performance of this diagnostic scheme in real clinical settings, we created a rapid test for *IDH* and *TERTp* mutation detection (Additional file 1: Methods S2 and S10) and conducted a multicenter prospective diagnostic test during surgery involving 296 adults with radiologically suspected gliomas (Fig. 1, Additional file 2: Tables S1–S2). The objective was to assess whether the layered diagnostic scheme, using *IDH* and *TERTp* mutations alongside histology, could accurately classify gliomas.

Intraoperatively, 224 gliomas and 2 non-gliomas were classified after detecting the first-layer biomarkers. These included 61 astrocytomas, 59 oligodendrogliomas, and 104 glioblastomas (Fig. 4, Table 1, Additional file 2: Fig. S4A). However, a definitive diagnosis could not be made for the remaining 68 *IDH* and *TERTp* wild-type cases that lacked clear histological features and additional biomarkers. Postoperatively, review of the permanent sections by two pathologists led to the classification of 26 cases as glioblastomas and 22 as non-gliomas, in alignment with the WHO CNS5 criteria (Fig. 4A and C). Accordingly, after detecting the first-layer biomarkers, 249 gliomas (comprising 60 astrocytomas, 59 oligodendrogliomas, and 130 glioblastomas) and 24 non-gliomas were accurately classified (Fig. 4, Table 1, Additional file 2: Fig. S4B).

Further classification was achieved by detecting second-layer biomarkers in the remaining 20 cases. Ten cases were found to have an *H3K27M* mutation and were diagnosed as pediatric-type high-grade gliomas. One case exhibited both *EGFR*amp and +7/-10 alterations but lacked typical glioblastoma histology. Another

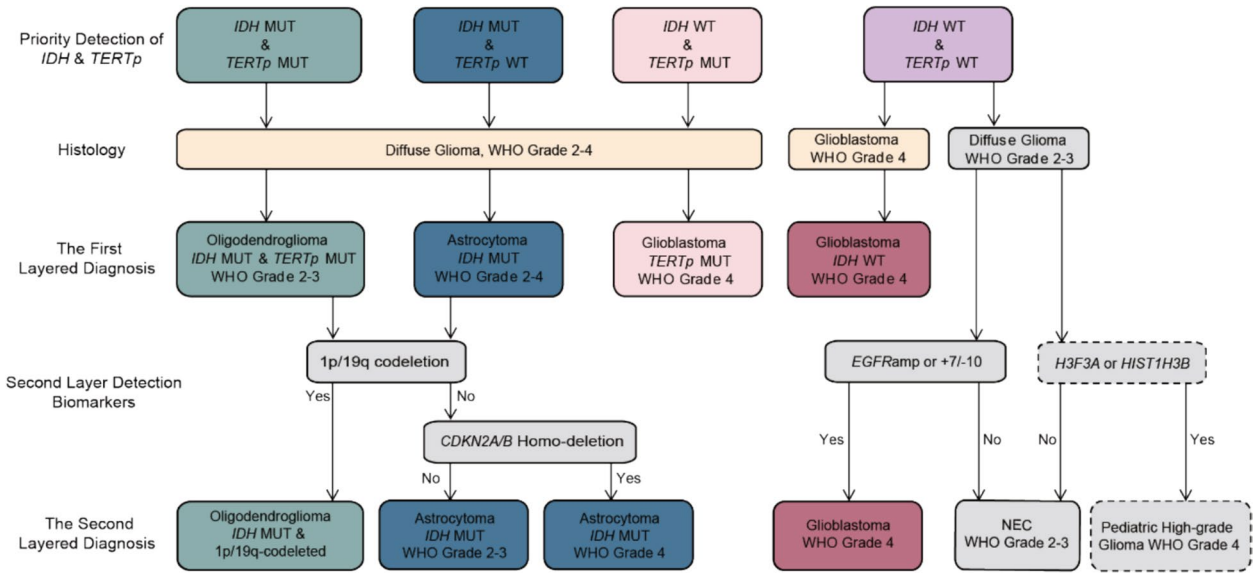

Fig. 3 Layered diagnostic scheme for adult-type diffuse gliomas. Layered diagnostic scheme based on the mutation status of *IDH* and *TERTp*, and histology

case was diagnosed as glioblastoma after re-testing for *TERTp* mutations postoperatively. Additionally, one case was diagnosed as oligodendroglioma due to a rare *TERTp* mutation (c.-57A > C), while the remaining seven cases were classified as NEC based on WHO CNS5 criteria (Fig. 4C).

In summary, intraoperative detection of *IDH* and *TERTp* mutations as the first-layer biomarkers accurately classified 84.0% of radiologically suspected gliomas, achieving a sensitivity of 0.829 (0.777 to 0.871), specificity of 0.960 (0.777 to 0.998), and an area under the curve (AUC) of 0.915 (0.892 to 0.937) (Fig. 5, Additional file 2: Fig. S4A). Postoperatively, with the assistance of permanent section analysis, 92.9% of cases were classified accurately, with a sensitivity of 0.926 (0.886 to 0.953), specificity of 0.960 (0.777 to 0.998), and an AUC of 0.963 (0.947 to 0.979) (Fig. 5, Additional file 2: Fig. S4B). These results demonstrate the high clinical utility and accuracy of the layered diagnostic scheme for classifying adult-type diffuse gliomas both intra- and postoperatively.

Discussion

We determined that the detection of *IDH* and *TERTp* mutations should be prioritized in cases involving 1p/19q codeletion, *EGFR*amp, and + 7/-10 for the stratified classification of adult-type diffuse glioma. To address this, we developed a novel layered diagnostic scheme prioritizing the detection of *IDH* and *TERTp* mutations. Additionally, we designed a rapid test capable of identifying all hotspot mutations in *IDH* and TERTp within 35 min. Using this novel diagnostic approach, 92.9% of adult-type diffuse gliomas were accurately classified postoperatively by detecting the first-layer biomarkers. Furthermore, with the rapid test applied intraoperatively, we successfully achieved integrated diagnoses for adult-type diffuse gliomas harboring *IDH* and/or *TERTp* mutations during surgery.

To the best of our knowledge, this study represents the largest evaluation of stratified diagnosis for adult-type diffuse gliomas with a focus on the prioritized detection of *IDH* and *TERTp* mutations. A total of 1049 cases from two cohorts were included in this analysis. The inclusion of comprehensive and uniformly detected molecular biomarkers across this extensive study population enabled a robust assessment of the diagnostic performance of the novel layered diagnostic scheme. Furthermore, the development of the rapid test significantly enhances the accessibility of molecular diagnostics, extending their application from postoperative settings to intraoperative use for most adult-type diffuse gliomas.

The WHO CNS5 recommends a stratified diagnostic approach for adult-type diffuse gliomas. However, the complexity of the associated biomarkers significantly limits the clinical feasibility of implementing a layered diagnostic scheme. Furthermore, the challenges of detecting copy number variations (CNVs), particularly at the chromosomal arm level, prevent many centers from completing the necessary biomarker testing. Additionally, intraoperative frozen section analysis is limited by the lack of molecular pathology, leading to low diagnostic accuracy during surgery and the inability to achieve an integrated diagnosis intraoperatively.

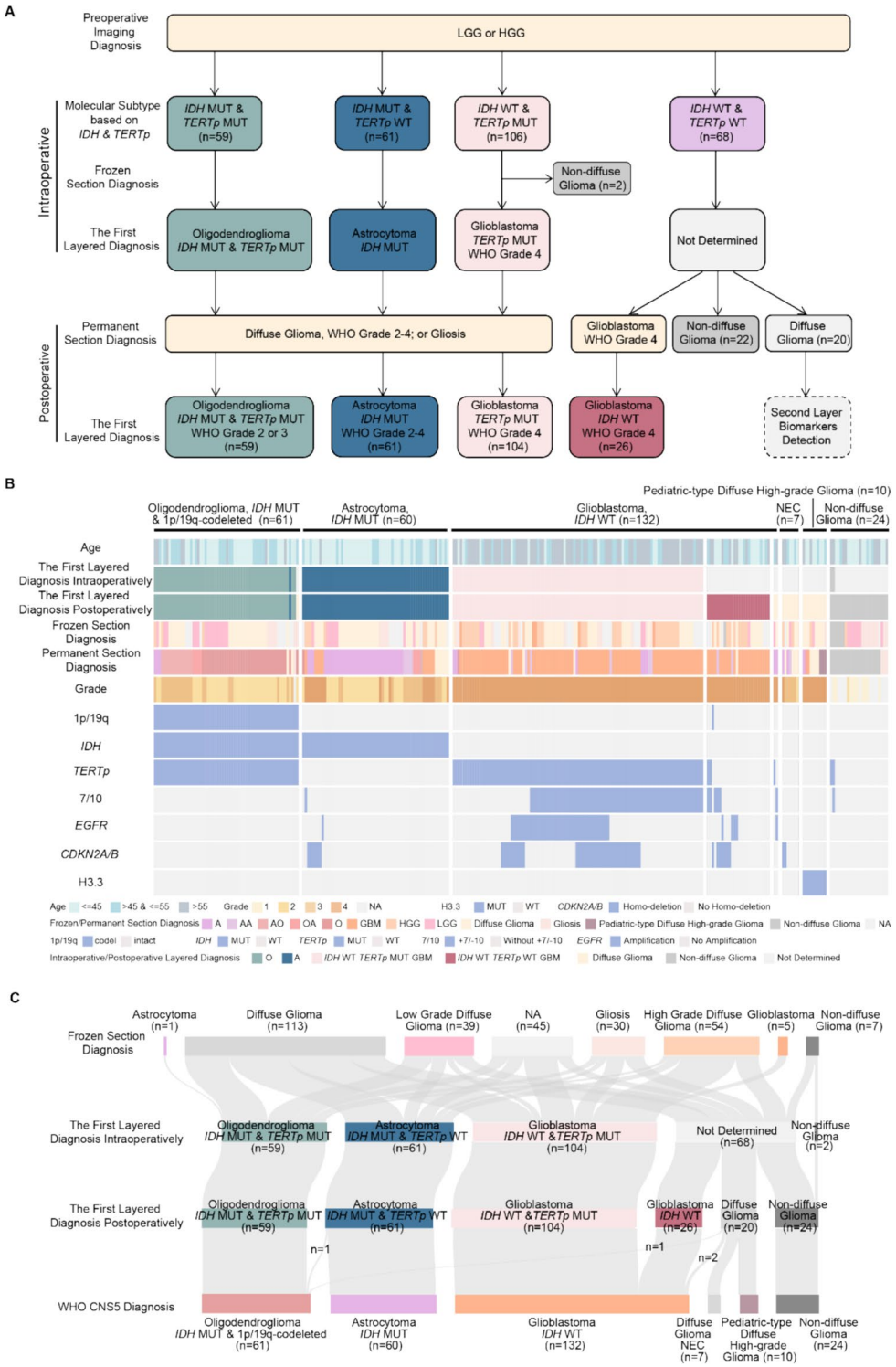

Fig. 4 Evaluation of the clinical performance of the layered diagnostic scheme in a prospective diagnostic test. **A** The clinical workflow and diagnostic results following the application of the layered diagnostic scheme for 294 patients highly suspected of having diffuse glioma in a prospective clinical study. **B** The clinical and molecular profiles of the patients in this cohort, along with their final diagnoses based on the WHO CNS5 criteria. **C** Comparison of the diagnoses made intra- and postoperatively based on frozen sections, the layered diagnostic scheme, and the WHO CNS5 criteria

Table 1 Performance of the layered diagnostic scheme compared to the WHO CNS5

Subtype	Prospective diagnostic test intra-op ($n=294$)				Prospective diagnostic test post-op ($n=294$)			
	Accuracy	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	Accuracy	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)
All	0.840	0.829 (0.777–0.871)	0.960 (0.777–0.998)	0.915 (0.892–0.937)	0.929	0.926 (0.886–0.953)	0.960 (0.777–0.998)	0.963 (0.947–0.979)
A	0.997	1.000 (0.925–1.000)	0.996 (0.973–1.000)	1.000 (1.000–1.000)	0.997	1.000 (0.925–1.000)	0.996 (0.973–1.000)	1.000 (1.000–1.000)
O	0.993	0.967 (0.876–0.994)	1.000 (0.980–1.000)	0.984 (0.961–1.000)	0.993	0.967 (0.876–0.994)	1.000 (0.980–1.000)	0.984 (0.961–1.000)
GBM	0.905	0.788 (0.706–0.852)	1.000 (0.971–1.000)	0.894 (0.859–0.929)	0.993	0.985 (0.941–0.997)	1.000 (0.971–1.000)	0.992 (0.982–1.000)

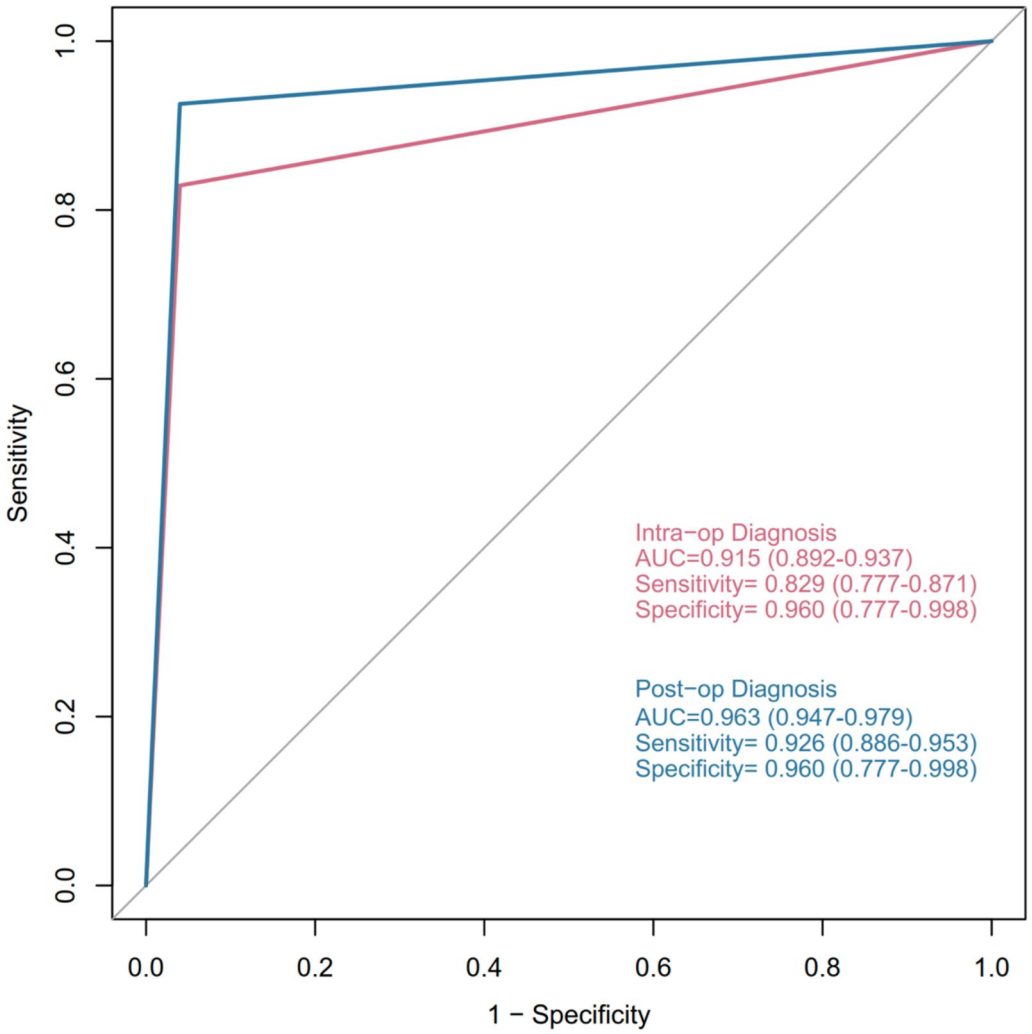

Fig. 5 Diagnostic accuracy of the layered diagnostic scheme compared to the WHO CNS5 criteria. The diagnostic accuracy of the layered diagnostic scheme in a prospective cohort intra- and postoperatively

Given the high mutation frequency of *IDH* and *TERTp* in adult-type diffuse gliomas and the ease of detecting these point mutations, numerous studies have explored their diagnostic and prognostic significance in this malignancy. However, the priority of these two mutations over other biomarkers, such as the three CNV variations, remains a topic of debate, particularly following the publication of the WHO CNS5 guidelines.

Using a large cohort encompassing all subtypes of adult-type diffuse gliomas and employing uniform detection methods, we systematically analyzed the correlations between *IDH* and *TERTp* mutations and 1p/19q codeletion, +7/-10 alterations, and *EGFR*amp. Our findings confirmed the superior diagnostic utility of *IDH* and *TERTp* mutations compared to the three CNV biomarkers, supporting the establishment of a layered diagnostic scheme prioritizing the detection of these mutations. By applying this layered diagnostic scheme, only a small subset of patients required additional testing—such as fluorescence in situ hybridization (FISH), next-generation sequencing (NGS), or Sanger sequencing—to determine the status of 1p/19q codeletion, *EGFR*amp, +7/-10 alterations, or *H3* mutations. In the prospective cohort, this subset accounted for just 4.8% of cases, with only a few yielding positive results necessary for an integrated diagnosis. These findings suggest that it is unnecessary to simultaneously test all biomarkers required by the CNS5 guidelines for every case. Instead, prioritizing the layered detection of *IDH* and *TERTp* mutations offers a more efficient and practical diagnostic pathway for adult-type diffuse gliomas.

Despite the effectiveness of the layered diagnostic scheme, a challenge remains in the further classification of a small subset of IDH-mutated gliomas based on the presence of *TERTp* mutations or 1p/19q codeletion. In the retrospective cohort, eight IDH-mutated cases lacked concurrence of both 1p/19q codeletion and *TERTp* mutation (Additional file 2: Table S3 and Fig. S3). To address this, methylation sequencing was performed to explore the subtypes of three IDH-mutated cases without concurrence of *TERTp* mutation and 1p/19q codeletion. The methylation profiles revealed that the IDH-mutated case with a 1p/19q codeletion had signatures consistent with typical oligodendrogliomas. Conversely, the methylation profiles of the two IDH-mutated cases with *TERTp* mutations were consistent with, respectively, typical astrocytomas and oligodendrogliomas (Additional file 2: Fig. S5). These findings highlight the complexity of subtyping these rare cases, underscoring the need for further research to refine classification approaches for this unique subset of gliomas.

The integration of molecular features into glioma diagnoses has significantly extended the time required for a final diagnosis from days to weeks, often impacting patient eligibility for clinical trials and delaying treatment initiation. Our layered diagnostic scheme addresses this challenge by enabling precise diagnoses within 35 min, thereby expediting decision-making for most patients and increasing the likelihood of successful treatment outcomes. Furthermore, this method requires only basic instruments and facilities, making it easily implementable in existing molecular pathology laboratories. By facilitating the adoption of WHO CNS5 molecular pathology guidelines, the layered diagnostic scheme promotes accurate and timely glioma diagnoses, supports equitable access to advanced diagnostics and treatments, and advances the goal of medical fairness.

Intraoperative frozen section analysis plays a critical role in guiding surgical decision-making for glioma patients, yet its accuracy and reliability can be limited [24]. To address this, we developed a PCR-based rapid test capable of detecting *IDH* and *TERTp* mutations at an exceptionally low cost (test reagent cost of $20 per reaction). This test offers several advantages: minimal surgical time (single-person operation time of 8 min), low tissue requirement (less than 100 ng, enabling even biopsy samples to be tested), a short turnaround time, and high accuracy. The intraoperative application of our novel layered diagnostic scheme and rapid test enables an integrated diagnosis of adult-type diffuse gliomas with *IDH* or *TERTp* mutations during surgery (Table 1). This approach significantly influences surgical strategies and decision-making. For instance, 40 patients initially diagnosed with lower-grade gliomas by frozen section were later identified as carrying *TERTp* mutations in their IDH-wildtype tumors, supporting a glioblastoma diagnosis, which was subsequently confirmed postoperatively.

Previous studies have demonstrated that molecular diagnostics can aid in differentiating gliomas from reactive gliosis in challenging cases [54, 55]. Consistently, in our prospective study, 20 patients initially diagnosed with gliosis by frozen section histology were identified through molecular analysis as harboring *IDH* or *TERTp* mutations and were accurately diagnosed with diffuse gliomas and their respective subtypes (Fig. 4C). Postoperative sections confirmed these revised molecular diagnoses. The infiltrative growth pattern of adult-type diffuse gliomas complicates the definition of tumor boundaries. However, our rapid test, with its high sensitivity, offers the potential to redefine the molecular margins of these tumors, further improving diagnostic precision and surgical outcomes.

While the layered diagnostic scheme offers numerous benefits, it is essential to acknowledge its limitations. *IDH* or *TERTp* are not exclusive to diffuse gliomas and can also occur in non-diffuse gliomas, such as meningiomas, xanthoastrocytomas, ependymomas, and

metastatic brain tumors [4, 56, 57]. Thus, histologic evaluation of both frozen and permanent sections remains critical for accurately diagnosing IDH or TERTp-mutated non-gliomas (Additional file 2: Fig. S6). The detection of first-layer biomarkers primarily facilitates the subtyping of adult-type diffuse gliomas but falls short in assisting with tumor grading, particularly in IDH-mutant astrocytomas with *CDKN2A/B* homozygous deletions. Second-layer detection is therefore vital for grading histologically lower-grade IDH-mutant astrocytomas. In this study, among 182 (24.4%) and 44 (15.0%) histologically lower-grade IDH-mutant astrocytomas identified in the two cohorts, only 13 and 1 cases, respectively, exhibited *CDKN2A/B* homozygous deletions (Figs. 2A and 4B, Additional file 2: Table S2). Considering the challenges associated with the intraoperative rapid detection of *CDKN2A/B* homozygous deletions, further research is needed to identify additional biomarkers that correlate with the grading of IDH-mutant gliomas. Additionally, given that *BRAF V600E*, *H3F3A*, and *HIST1H3B* are the most prevalent point mutations in pediatric gliomas and can be detected using PCR, it is crucial to integrate these biomarkers into the first-layer detection in future clinical applications. Expanding the scope of first-layer biomarkers will enhance the clinical utility of the layered diagnostic scheme and improve diagnostic accuracy for various glioma subtypes.

Conclusions

The detection of *IDH* and *TERTp* mutations should serve as the first layer in the classification of adult-type diffuse gliomas due to their low cost, rapid turnaround time, and compatibility with widely available detection platforms. The optional second layer should focus on detecting *CDKN2A/B* homozygous deletion, *EGFR*amp, +7/-10, and *H3* mutations to provide further stratification and grading. The implementation of our layered diagnostic scheme offers a significant advantage by extending its application from postoperative to intraoperative settings for most adult-type diffuse gliomas. This capability facilitates personalized precision surgery, enabling timely and informed decision-making that optimizes surgical outcomes.

Abbreviations

A	Astrocytoma
O	Oligodendroglioma
GBM	Glioblastoma
NEC	Not elsewhere classified
NG	Non-diffuse glioma
P-HGG	Pediatric high-grade glioma
NA	Not available
Intra-op	Intra-operation
Post-op	Post-operation
CC	Corpus callosum
TERTp	*TERT* Promoter
*EGFR*amp	*EGFR* Gene amplification
+7/-10	Whole chromosome 7 gain and whole chromosome 10 loss
WHO CNS5	The 2021 World Health Organization Classification of Tumors of the Central Nervous System

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12916-025-04124-9.

Additional file 1: Supplementary Methods S1–S14. Method S1 Screening and enrollment of patients. Method S2 DNA extraction. Method S3 Detection of IDH and TERTp mutations using Sanger sequencing. Method S4 Detection of IDH1, IDH2, TERTp, H3F3A, and HIST1H3B mutations by targeted sequencing. Method S5 Quantifying the VAFs of IDH and TERTp mutations using droplet digital PCR. Method S6 sWGS and CNV detection. Method S7 Whole-genome sequencing (WGS). Method S8 Fluorescence in situ hybridization (FISH) analysis of chromosomal 1p and19q loss. Method S9 Review of frozen sections and permanent sections. Method S10 A rapid detection test for hotspot mutations of IDH and TERTp. Method S11 Statistical analysis. Method S12 Preoperative imaging diagnosis. Method S13 Intraoperative and postoperative integrated diagnosis of adult-type diffuse glioma using the layered diagnostic scheme. Method S14 Methylation array analysis.

Additional file 2: Supplementary Tables and Figs. S1–S9. Fig. S1 Flow chat of the fast DNA extraction method and rapid qPCR test. Fig. S2 Overlap of TERTp mutations, +7/-10, and EGFRamp in IDH-wildtype gliomas. Fig. S3 Histologic and molecular characteristics of eight cases without concurrence of TERTp and 1p/19q codeletion. Fig. S4 Performance of the layered diagnostic scheme compared to WHO CNS5. Fig. S5 DNA methylation-based classification of three IDH-mutated cases. Fig. S6 Characteristics of two non-glioma cases with TERTp mutations in the prospective cohort. Fig. S7 Sensitivity of detection of different mutant alleles in IDH and TERTp using a qPCR rapid test. Fig. S8 Specificity of detection of different amount of input DNA in IDH and TERTp using a qPCR rapid test. Fig. S9 Accuracy and turnaround time for the rapid test results in prospective cohort. Tables S1–S4. Table S1 Baseline characteristics of the patients. Table S2 Molecular characteristics of all glioma patients in the study. Table S3 Clinical and molecular characteristics of the eight IDH-mutant cases without concurrence of 1p/19q codeletion and TERTp mutation in the retrospective cohort. Table S4 Primer sequences for mutation detection of IDH and TERTp.

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Authors' contributions

J.W. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: J.W., Y.M., Y.J., H.M., D.K., and H.Y.. Study supervision: J.W., Y.M., H.M., D.K., and H.Y.. Collection of samples and clinical information: S.W., Z.X., Y.Z., Z.W., C.L. and H.C.. Acquisition, analysis, or interpretation of data: S.W., D.C., J.Z., Y.Y., Y.S., Z.X., Y.Z., Z.W. and Y.N.. Drafting of the manuscript: S.W., D.C., J.W. and H.Y.. Critical revision of the manuscript for important intellectual content: S.W., D.C., J.W. and H.Y.. Statistical analysis: Y.S., Y.Z.. Administrative, technical, or

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Data availability
All sequence data reported here, including sWGS, WGS and targeted sequencing, have been deposited in the National Genomics Data Center's (NGDC) Genome Sequence Archive for Human (GSA-Human) database (https://ngdc.cncb.ac.cn/gsa-human/s/E00NCsXX) under Accession Number HRA003167. Jinsong Wu had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. These data are available under a controlled access regimen to ensure the protection of personally identifiable data, and access can be obtained by contacting Prof. Jinsong Wu (wujinsong@huashan.org.cn).

Declarations

Ethics approval and consent to participate
This study was registered in the ClinicalTrials.gov (NCT04924127, NCT04904419). All patients and samples used in this study were approved by the institutional review boards of Huashan Hospital of Fudan University (2019–539, 2020–1298), General Hospital of Ningxia Medical University (KYLL-2021–242), and the First Affiliated Hospital of Fujian Medical University (MRCTA, ECFAH of FMU [2021]120).

Consent for publication
Not applicable.

Competing interests
Hai Yan is the Co-founder of Genetron Health, and receives royalties from Agios, Genetron Health and Personal Genome Diagnostics (PGDX). Other authors declare no conflict of interest.

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