Molecular pathology and clinical implications of diffuse glioma

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Abstract

The prognosis for diffusely infiltrating gliomas at World Health Organization (WHO) grade 2–4 remains dismal due to their heterogeneity. The rapid development of genome-wide molecular-profiling-associated studies has greatly promoted the accuracy of glioma classification. Thus, the latest version of the WHO classification of the central nervous system tumors published in 2021 has incorporated more molecular biomarkers together with histological features for the diagnosis of gliomas. Advanced usage of molecular pathology in clinical diagnostic practice provides also new opportunities for the therapy of patients with glioma, including surgery, radiotherapy and chemotherapy, targeted therapy, immunotherapy, and more precision clinical trials. Herein, we highlight the updates in the classification of gliomas according to the latest WHO guidelines and summarize the clinically relevant molecular markers by focusing on their applications in clinical practice. We also review the advances in molecular features of gliomas, which can facilitate the development of glioma therapies, thereby discussing the challenges and future directions of molecular pathology toward precision medicine for patients with glioma.

Keywords: Glioma; Molecular pathology; tumor classification; O^6-methylguanine-DNA methyltransferase; Therapy

Introduction

Diffusely infiltrating gliomas or diffuse gliomas are the most common primary tumors of the central nervous system (CNS), accounting for 30% and 80% of all primary and malignant primary CNS tumors, respectively.[1] Currently, the prognosis of diffuse gliomas remains dismal, even after comprehensive treatments, including surgery, radiotherapy (RT) and/or chemotherapy, and tumor treating fields. More than 60% of cases of diffuse glioma are glioblastoma, the most aggressive type of CNS tumors, with a median overall survival of approximately 14 to 16 months.[1,2] There has been limited progress in improving glioma outcomes over the past 15 years. This is largely attributed to the unique anatomic location, biological characteristics, developmental, genetic, epigenetic, and microenvironmental features of gliomas that render them resistant to conventional and novel treatments.[3-6] Additionally, the traditional classification of diffuse gliomas only by histological features cannot provide enough information for clinicians to have a better understanding of the prognosis and optimal therapy for patients with specific subgroups of gliomas.[7-10] However, the rapid development of molecular pathology brings new hope for improving prognosis and consequently the outcome of gliomas.

Since the 2016 World Health Organization (WHO) classification of the CNS tumors (WHO CNS2016), the diagnosis of diffuse gliomas is determined by both molecular and pathological features, implying that glioma diagnoses should be structured in the molecular era.[8] Both mutations in isocitrate dehydrogenase gene (*IDH*) and the chromosomal 1p/19q codeletion have been integrated with morphologic observations to determine the final diagnosis of diffuse gliomas. Additional molecular variations and their clinical relevancies are being continuously discovered, accompanied with an expansion of knowledge on the genetic basis of tumorigenesis.[11-15] Accumulating evidence has shown that more molecular features can contribute to a more accurate diagnosis and risk stratification of gliomas.[11,13] Based on these findings, especially the recommendations of the Consortium to Inform Molecular and Practical Approaches to CNS tumor classification,[11,13,16] the summary of the fifth edition of the WHO Classification of CNS Tumors, published in 2021 (WHO CNS2021), advanced the role of molecular pathology in CNS tumor classification.[2]

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In particular, WHO CNS2021 classifies gliomas into more biologically and molecularly defined types/subtypes, which thus provide new opportunities to improve the management of gliomas, clinical trial design, and evaluation of new therapies.

In this review, we highlight the major advances of molecular pathology in WHO CNS2021, with a particular focus on their applications in clinical practices rather than providing an exhaustive review of each molecular marker. In addition, we summarize the potential implications of molecular pathology advances for the therapy of gliomas and discuss current challenges and future development directions.

Molecular Pathology Plays an Advancing Role in the Classification of Gliomas

The substantial changes incorporated in WHO CNS2021 are advancing the role of molecular diagnostics in CNS tumor classification, which remain rooted in histological and immunohistochemistry analyses. The key molecular features, which are important for the integrated classifications of gliomas, are summarized in Table 1. Among these molecular features, some are readily and consistently used for the classification or grading of tumors, whereas others are not required but support tumor classification.[1,2,17] In WHO CNS2021, the term "type" is used instead of "entity," and "subtype" is used instead of "variant." Grading is also considered within tumor types, while modifier terms, such as "anaplastic" are excluded for the diagnosis of gliomas. Although glioma grades were traditionally written in Roman numerals, WHO CNS2021 changed them to Arabic numerals.

WHO CNS2021 reclassified diffuse gliomas in WHO CNS2016 according to their similarities in molecular/genetic features and divided them into three different families: (1) adult-type diffuse gliomas, which are the majority of primary brain tumors in adults; (2) pediatric-type diffuse low-grade gliomas, which are expected to have good prognoses; and (3) pediatric-type diffuse high-grade gliomas, which are expected to be aggressive. A number of molecular markers, such as *CDKN2A/2B* homozygous deletion, *EGFR* amplification, *TERT* promoter mutation, and the combined whole chromosome 7 gain and whole chromosome 10 loss (+7/−10), have contributed to the classification and grading of gliomas in WHO CNS2021.[8,11,13] Currently, all *IDH*-mutant diffuse astrocytic tumors are considered a single type (astrocytoma, *IDH*-mutant), which could be further classified into WHO grade 2, 3, or 4 according to both their histological and *CDKN2A/B* homozygous deletion status, as recommended in the the Consortium to Inform Molecular and Practical Approaches to CNS tumor classification -not official WHO (cIMPACT-NOW) update.[13]*IDH*-wildtype diffuse astrocytic gliomas in adults are diagnosed as glioblastomas with *IDH*-wildtype if there is microvascular proliferation or necrosis, or the presence of one or more of three genetic parameters (*TERT* promoter mutation, *EGFR* gene amplification, +7/−10) according to the cIMPACT-NOW updates 3 and 6.[11,18] Here, we provide a comprehensive overview on the major changes in the diagnosis of gliomas according to the latest edition of WHO classification compared with WHO CNS 2016 [Table 2].

Pediatric-type diffuse low-grade gliomas are often characterized by the presence of genetic alterations, such as *BRAF* V600E mutation, *FGFR1* alteration, *MYB* or *MYBL1* rearrangement, or other MAPK pathway alterations.[16,18] Their classification on the following types: "diffuse astrocytoma, *MYB*- or *MYBL1*-altered; angiocentric glioma; polymorphous low-grade neuroepithelial tumor of the young; and diffuse low-grade glioma MAPK pathway-altered," is based on both morphological characteristics and genetic features of these tumors.

Pediatric-type diffuse high-grade gliomas also include the following four types: "diffuse midline glioma with H3 K27-altered; diffuse hemispheric glioma with H3G34-mutant; diffuse pediatric-type high-grade glioma with *H3*-wildtype and *IDH*-wildtype; and infant-type hemispheric glioma." Diffuse midline gliomas with H3 K27-altered involve thalamic, spinal, and diffuse brainstem gliomas, which usually occur in children but rarely in adults, in which H3 K27-altered is characterized by K27M mutations in either *H3F3A* or *HIST1H3B/C*, and other changes, such as overexpression of the EZHIP protein or *EGFR* mutations.[19-21] Recently, it has been reported that adult midline gliomas with H3 K27-altered tumors have distinct molecular features with that of child patients, including a higher proportion of localization in the tumulus or spinal cord, with longer survival.[14,22] Diffuse pediatric-type high-grade gliomas with *H3*-wildtype and *IDH*-wildtype are wildtype for both *H3* and *IDH* gene families and require the integration of histopathological and molecular data, such as mutational and methylome data, for final diagnosis.[23] Infant type hemispheric gliomas are novel high-grade gliomas that occur in newborns (commonly <4 years old) and are characterized by the fusion of *ALK*, *ROS1*, *NTRK1/2/3*, or *MET* genes.[24,25]

Advances and Challenges of Molecular Testing in Clinical Practice

WHO CNS2021 does not recommend specific methods for the molecular diagnostic assessment of individual genetic alterations. With the increasing use of molecular markers in the diagnosis of gliomas, challenges have arisen regarding the methodology of molecular testing for gliomas; and the same is true for performing integrated diagnostics.[26] The traditional technologies used in pathological diagnosis, including light microscopy, histochemical stains, electron microscopy, immunohistochemistry, and DNA fluorescence *in situ* hybridization (FISH) cannot fulfill the requirements for the diagnosis of gliomas.[6,17] A variety of nucleic acid detection methods, such as DNA/RNA sequencing and RNA expression profiles, have clearly shown their contribution to the diagnosis and classification of gliomas.[1] However, the means by which to properly incorporate these novel methodologies into routine molecular testing of formalin-fixed paraffin-embedded samples remains a challenge. These challenges include: (1) the availability and choice of

Table 1: Molecular markers and their clinical relevance in gliomas.

Markers	Genetic alterations	Clinical importance	Detection methods	Therapeutic potential/ target/guidance
IDH1	Mutations (R132H/C/L/S/G)	Diagnostic value, an essential marker for diagnosis for astrocytoma, *IDH*-mutant; Differential diagnosis between diffuse and non-diffuse gliomas (WHO grade 1) or gliosis; Prognostic value, associated with relatively favorable prognosis	IHC, Sequencing	Target therapy; Tumor vaccine
IDH2	Mutations (R172K/M/G/W)	Diagnostic value, essential marker for diagnosis for astrocytoma, *IDH*-mutant; Molecular parameters for glioma classification; Differential diagnosis between diffuse and non-diffuse gliomas (WHO grade 1) or gliosis; Prognostic value, associated with relatively favorable prognosis	Sequencing	Target therapy; Tumor vaccine
Chromosome 1p/19q	Whole arm codeletion	Diagnostic value, essential marker for diagnosis of oligodendroglioma; Prognostic value, associated with relatively favorable prognosis; Predictive of response to alkylating chemotherapy and combination of radiation and alkylating chemotherapy.	FISH, PCR, array-based methods, Sequencing	Chemotherapy; Surgery
ATRX	Loss of function mutations	Diagnostic value, *IDH*-mutant astrocytomas, with loss of ATRX nuclear expression and/or strong, diffuse p53 immunopositivity, could be diagnosed without 1p/19q testing.	IHC, Sequencing	NA
TP53	Mutations	Diagnostic value, *IDH*-mutant astrocytomas, with loss of ATRX nuclear expression and/or strong, diffuse p53 immunopositivity, could be diagnosed without 1p/19q testing, and differential diagnosis between diffuse and no-diffuse gliomas (WHO grade 1) or gliosis.	IHC, Sequencing	NA
CDKN2A/B	Homozygous deletion	Diagnostic value, diagnostic parameters for astrocytoma, *IDH*-mutant, grade 4, in the absence of necrosis and/or microvascular proliferation	FISH, qPCR, MLPA, array- or sequencing-based methods	NA
TERT	Promoter mutations (C228T/C250T)	Diagnostic value, diagnostic parameters for GBM, *IDH*-wildtype, in the absence of necrosis and/or microvascular proliferation; Frequent in oligodendroglioma and glioblastoma	Sequencing	NA
EGFR	Amplification	Diagnostic value, diagnostic parameters for GBM, *IDH*-wildtype, in the absence of necrosis and/or microvascular proliferation	FISH, digital PCR, array- or sequencing-based methods	Target therapy
Chromosome 7/10	7 gain/10 loss	Diagnostic value, diagnostic parameters for GBM, *IDH*-wildtype, in the absence of necrosis and/or microvascular proliferation	FISH, array- or sequencing-based methods	NA
H3 K27	Mutation (K27M/I)	Diagnostic value, diagnostic parameters for Diffuse midline glioma, H3 K27 altered	IHC, Sequencing	NA
EZHIP	Overexpression	Diagnostic value, diagnostic parameters for Diffuse midline glioma, H3 K27 altered	IHC	NA
H3 G34	Mutations (G34R/V)	Diagnostic value, diagnostic parameters for diffuse hemispheric glioma, H3 G34-mutant.	IHC, Sequencing	NA

(Continued)

Table 1
(Continued)

Markers	Genetic alterations	Clinical importance	Detection methods	Therapeutic potential/ target/guidance
MYB	Rearrangement/Fusion	Diagnostic value, essential marker for diagnosis of diffuse astrocytoma, *MYB*- or *MYBL1*-altered, MYB-QKI fusion is a characteristic of angiocentric glioma	IHC, PCR, Sequencing	NA
MYBL1	Rearrangement/ Fusion	Diagnostic value, essential marker for diagnosis of diffuse astrocytoma, *MYB*- or *MYBL1*-altered	IHC, PCR, Sequencing	NA
BRAF	Activating mutation (BRAF V600E) or fusion	Diagnostic value, one of molecular marker for diagnosis of diffuse low-grade glioma, MAPK pathway-altered; presented in a variety of gliomas, including epithelioid glioblastoma; Potential parameter for target therapy (e.g., vemurafenib).	IHC, PCR, Sequencing	Target therapy
FGFR1-4	Mutations, TKD-duplicated, Rearrangement/ Fusion (FGFR-TACC)	Diagnostic value, one of molecular marker for diagnosis of diffuse low-grade glioma, MAPK pathway-altered. Potential parameter for target therapy (e.g., FGFR inhibitors).	Sequencing	Target therapy
MET	Fusion	Diagnostic value, one of molecular markers of infant-type hemispheric glioma, also occur in GBM and *IDH*-mutant astrocytoma. Potential parameter for target therapy (e.g., MET inhibitors).	PCR, Sequencing	Target therapy
ALK	Fusion	Diagnostic value, one of molecular markers of infant-type hemispheric glioma. Potential parameter for target therapy.	PCR, Sequencing	Target therapy
NTRK	Fusion	Diagnostic value, one of molecular markers of infant-type hemispheric glioma. Potential parameter for target therapy.	PCR, Sequencing	Target therapy
ROS1	Fusion	Diagnostic value, one of molecular markers of infant-type hemispheric glioma. Potential parameter for target therapy.	PCR, Sequencing	Target therapy

FISH: Fluorescence *in situ* hybridization; IHC: immunohistochemistry; MAPK: Mitogen-activated protein kinase; MLPA: Multiplex ligation-dependent probe amplification; PCR: Polymerase chain reaction; qPCR: Quantitate polymerase chain reaction; *IDH*: Isocitrate dehydrogenase gene.

high-throughput DNA/RNA sequencing methods; (2) a cost- and time-effective workflow; (3) intensive communication and collaboration among people with different academic backgrounds (e.g., pathologists, molecular biologists, and bioinformaticians); (4) comparability of test results between different testing centers; and (5) security of human genetic data. The implementation of combined phenotypic-genotypic diagnostics in some large canters has suggested that most of these challenges can be readily overcome in the near future.[27] Here, we also provide a roadmap for the diagnosis of gliomas according to the experience in our institute [Figure 1].

Clinical Implications of Molecular Pathology in the Therapy of Glioma

Although WHO CNS2021 is likely only an intermediate stage to an even more precise classification in the future, it has the potential to enable clinicians to have a better understanding of the prognosis and optimal therapy for patients with specific gliomas. Several recent studies have revealed differences in the benefits of total resection of different glioma subtypes,[28] suggesting that glioma surgery should be planned according to its classification. Classification of gliomas into types according to their molecular features is also useful for both reasonable treatment design as it might explain the variability in patient response to same therapeutic approaches.[4] Predictive molecular markers can be used to identify gliomas that are sensitive to distinct postoperative therapeutic approaches.[29] Gliomas with different molecular features have their own unique immune microenvironment.[15] The increasing use of molecular markers has also brought the implementation of targeted therapeutic approaches for some subtypes of gliomas, such as pediatric gliomas with *BRAF* mutations.[16,30] Additionally, genomically defined patient subgroups allow for the study of more homogenous populations in clinical trials.[10] Moreover, longitudinal molecular testing can facilitate precision medicine and an even better design of clinical trials.[9] Altogether, the advances in molecular pathological detection of gliomas not only promote the precision diagnosis of tumors but also facilitate the progress of glioma therapy from surgery to clinical trials.

Table 2: Changes in the classification of diffuse gliomas in WHO CNS2021 compared with WHO CNS2016.

WHO CNS2021[*]	WHO CNS2016
Adult-type diffuse gliomas	Diffuse astrocytic and oligodendroglial tumors
Astrocytoma, *IDH*-mutant	
Astrocytoma, *IDH*-mutant, grade 2	Diffuse astrocytoma, *IDH*-mutant, grade II
Astrocytoma, *IDH*-mutant, grade 3	Anaplastic astrocytoma, *IDH*-mutant, grade II
Astrocytoma, *IDH*-mutant, grade 4	Glioblastoma, *IDH*-mutant, grade IV
	Diffuse astrocytoma, *IDH*-mutant, grade II[†]
	Anaplastic astrocytoma, *IDH*-mutant, grade III[†]
Oligodendrogliomas, *IDH*-mutant, and 1p/19q-coledeted	
Oligodendroglioma, *IDH*-mutant, and 1p/19q-codeleted, grade 2	Oligodendroglioma, *IDH*-mutant, and 1p/19q-codeleted, grade II
Oligodendroglioma, *IDH*-mutant, and 1p/19q-codeleted, grade 3	Anaplastic oligodendroglioma, *IDH*-mutant, and 1p/19q-codeleted, grade III
Glioblastoma, *IDH*-wildtype, grade 4	Glioblastoma, *IDH*-wildtype, grade IV
	Diffuse astrocytoma, *IDH*-wildtype, grade II[‡]
	Anaplastic astrocytoma, *IDH*-wildtype, grade III[‡]
Pediatric-type diffuse low-grade gliomas	
Diffuse astrocytoma, MYB- or MYBL1-altered	*Diffuse astrocytoma, IDH-wildtype*, grade II[§]
Angiocentric glioma	Diffuse gliomas with specific histological features
Polymorphous low-grade neuroepithelial tumor of the young	Diffuse gliomas with specific histological features
Diffuse low-grade glioma, MAPK pathway-altered	*Diffuse astrocytoma, IDH-wildtype*, grade II[‖]
Pediatric-type diffuse high-grade gliomas	
Diffuse midline glioma, *H3* K27-altered	Diffuse midline glioma, H3K27M-mutant, grade IV
	Other diffuse gliomas in midline[¶]
Diffuse hemispheric glioma, *H3 G34*-mutant	Glioblastoma, *IDH*-wildtype, grade IV[**]
	Anaplastic astrocytoma, *IDH*-wildtype, grade III[**]
Diffuse pediatric-type high-grade glioma, *H3*-wildtype, and *IDH*-wildtype	Glioblastoma, *IDH*-wildtype, grade IV[††]
	Anaplastic astrocytoma, *IDH*-wildtype, grade III[††]
	Others
Infant-type hemispheric glioma	Glioblastoma, *IDH*-wildtype grade IV[††]
	Anaplastic astrocytoma, *IDH*-wildtype, grade III[‡‡]
	Diffuse astrocytoma, IDH-wildtype, grade II[‡‡]

CNS: Central nervous system; WHO: World Health Organization; WHO CNS2016: The 2016 World Health Organization classification of the central nervous system tumors; WHO CNS2021: The 2021 World Health Organization classification of the central nervous system tumors. [*] The DNA methylation profile could also be used to determine the classification of each type in the WHO CNS2021. [†] With *CDKN2A* homozygous deletion. [‡] With anyone of *TERT* promoter mutation, *EGFR* amplification, or +7/−10. [§] With *MYB*- or *MYBL1* re-arrangement. [‖] With alterations in MAPK pathway, but without *CDKN2A* homozygous deletion. [¶] With p.K28I (K27I) mutation, *H3*-wildtype with EZHIP overexpression, or *H3*-wildtype EGFR-mutant. [**] With *H3* G34R/V mutation. [††] Absence of *H3* mutation, with methylation profile aligned with "pHGG RTK1, pHGG RTK2, or pHGG *MYCN*" or with anyone of molecular features of *PDGFRA* alteration, *EGFR* alteration, or *MYCN* amplification. [‡‡] Presentation in early childhood, and with cerebral hemispheric location, and with anyone of fusions in an NTRK family gene or in *ROS1, MET*, or *ALK*.

Surgery

Tumor resection is the most important step in the therapy of gliomas. The main aims of surgery are: (1) to perform histopathological and molecular pathology assessment that will guide postoperative adjuvant therapy, such as chemotherapy, radiation therapy, and immunotherapy; (2) to relieve the effect of tumor occupation; (3) to delay the malignant progression of tumor and improve prognosis; and (4) to alleviate glioma-related neurological deficits, including headache, postoperative glioma-related epilepsy, and other side effects.[31] An earlier surgical resection is important for an improved prognosis of patients. Accumulated evidence have suggested that early surgical resection can prolong the overall survival of patients with glioma, delay malignant progression, and avoid neurological deficits.[32]

Total surgical resection is currently the safest resection approach for all different subtypes of gliomas.[33] This means removing as much as possible of the tumor without causing permanent neurological dysfunction. Hence, if gliomas are not involved in eloquent areas, their total resection or supratotal resection is recommended for improving survival outcomes. In addition, compared with total resection, supratotal resection has been suggested to be more beneficial for prolonging the survival period and controlling glioma-related epilepsy.[34] The definition of supratotal resection for glioblastoma is that the surgical region is larger than the enhancement region on T1-enhancement images compared with the region with high-intensity signals on T2-flair images. However, these conclusions have been mainly derived from studies of gliomas in the anterior temporal lobe, which is responsible for less neurological functions than other areas, implying that they might not be applicable to gliomas in other brain regions.

To improve the accuracy of surgical resection and preserve fundamental neurological functions, such as motor,

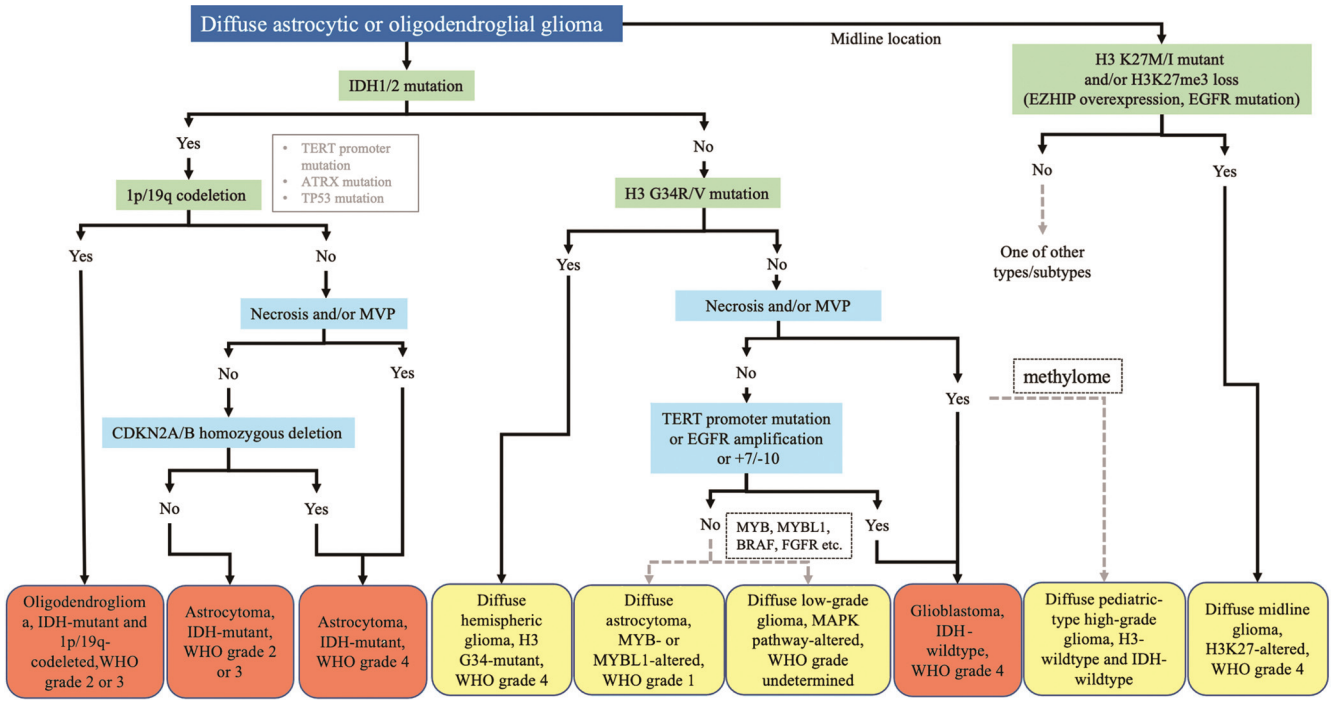

Figure 1: The latest diagnostic algorithm for the integrated classification of diffuse gliomas in adults according to histological and molecular features. The molecular features used for classification were presented in light-green boxes, and the features used for grading, including histological and molecular features, were shown in the light-blue boxes. The adult and pediatric types were shown in orange and yellow boxes, respectively. 1p/1q9: chromosome 1p and chromosome 19q; MVP: microvascular proliferation.

sensory, and language functions, the performance of awaken craniotomy is recommended.[31] Both positive and negative mapping strategies, identifying areas that are associated or not associated with neurological functions, respectively, have been recommended in awaken craniotomy. However, it is still controversial whether positive or negative mapping should be used in craniotomy. Compared with positive mapping, negative mapping results in smaller surgical regions and is accompanied with lower percentages of intraoperative stimulated epilepsy and side effects following resection.[35] However, negative mapping has also been associated with a higher rate of postoperative neurological impairments due to false-negative mapping results caused by the lack of cortical mapping experience or improper mapping measurements. Therefore, there is an urgent need for more advanced strategies or technologies to improve this situation.

The choice of electrical stimulators also affects the outcome of surgery. Recently, we demonstrated that the sensitivity of bipolar electrical stimulators is not enough for the identification of subcortical fibers, possibly due to the limited region of effective stimulation.[36] Given the reduced neuroplasticity, the use of bipolar electrical stimulators might cause surgical-related impairments of neurological functions. Hence, we recommended the use of bipolar electrical stimulators combined with monopolar electrical stimulators and motor-evoked potential technique to identify the cortical-spinal tract before removing gliomas adjacent to the internal capsule.[37] For gliomas that are near to the posterior superior longitude fasciculus/arcuate fasciculus (<5 mm), we suggested a conservative strategy of tumor resection for preserving linguistic functions.[36]

The rapid development of molecular pathology has led to the classification of gliomas into more homogenous types/subtypes.[2,13] However, it seems that their molecular characteristics cannot guide the surgery strategies used for gliomas which are tightly associated with the results of magnetic resonance imaging (MRI).[1,4] Whether different resection approaches should be adopted for gliomas with different molecular characteristics remains unanswered. A multicenter study revealed that the extent of resection cannot stratify the overall survival of patients with oligodendrogliomas with *IDH*-mutation and 1p/19q codeletion.[38] However, gross total resection improved the overall survival of patients with astrocytoma with *IDH*-mutation or glioblastoma with *IDH*-wildtype.[28] Altogether, these findings suggested that different surgery strategies should be adopted for distinct types/subtypes of gliomas. However, obtaining the molecular features of gliomas before/during surgery remains a challenge.

Fortunately, with the development of artificial intelligence (AI) and radiomics, predicting the molecular features of gliomas through models based on radiomic characteristics is a hopeful potential. Accumulating studies have successfully predicted the status of *IDH*, 1p/19q codeletion, and *TERT* mutation by using AI models.[39,40] The increasing accuracy of these predictive models has brought them close to being used in clinical diagnosis, and enlarging the sample size and improving their advanced algorithms have the potential to further improve the accuracy and robustness of these models. We believe that validation of these predictive models through prospective clinical trials will make the use of molecular features for guiding tumor resection a reality in the near future.

Radiotherapy and chemotherapy

The standard postoperative treatment options for adult patients with glioma, including concomitant RT and DNA alkylating agent therapy, have not substantially changed over the last 15 years.[1,17] Although some newly recognized types of glioma have been described in WHO CNS2021, which therapeutic approaches should be adopted for these tumors remains unclear. From the perspective of health economics and reducing overtreatment, it is important to identify which patients might benefit from intensive RT and concurrent or adjuvant chemotherapy. Similarly, it also critical to identify which patients might be cured with less intense RT or chemotherapy, especially in the case of pediatric patients.[4]

Overall, RT is adopted for most adult patients with diffuse gliomas, and different doses are recommended for patients with grade 2 and grade 3/4 tumors, respectively. However, the recommendation of RT doses is based on the results of clinical trials using patients classified by histological features. Thus, the impact of molecular subgroups on the RT dose selection, especially what RT dose should be used for patients with grade 4 tumors defined only by molecular features is still an open question. Recently, both our retrospective and prospective studies indicated that high-dose RT (>54 Gy) should be adopted for patients with histological grade 2/3 *IDH*-mutant astrocytoma and histological grade 2/3 *IDH*-wildtype gliomas.[41,42]

Compared with RT, the association between molecular features and chemotherapy is relatively clearer. Here, we also summarize the molecular markers with predictive significance for guiding postoperative chemotherapy treatment of patients with glioma.

O^6-methylguanine-DNA methyltransferase (MGMT) promoter methylation

Among predictive markers, the presence of *MGMT* promoter methylation has been associated with benefit from alkylating-agent chemotherapy in patients with glioblastoma, particularly elderly patients (aged ≥65 years).[29,43,44] The DNA-alkylating agent temozolomide (TMZ) is the first in a class of drugs used in the postoperative treatment of glioblastoma. MGMT, a DNA repair enzyme, can rapidly repair major temozolomide-induced DNA-adducts, 6-O-methylguanine, via self-alkylation.[45] The alkylated MGMT is then degraded through the ubiquitylation pathway. Thus, the levels of expression of MGMT correspond to the repair capacity of cellular 6-O-methylguanine, and deficiency in the expression of MGMT in glioma has been acknowledged as a predictive marker for TMZ sensitivity. The cysteine-phosphate-guanine (CpG) island (CGI) in the 5′ promoter region of *MGMT* is susceptible to DNA methylation, which suppresses *MGMT* transcription.[34] The level of expression of *MGMT* strongly depends on the level of methylation of its promoter region.[43] In particular, *MGMT*-promoter methylation, occurring in approximately 40% of glioblastoma, has been closely associated with the benefit from TMZ therapy and prolonged survival of patients with glioblastoma.[34] In addition, several clinical trials or studies have also revealed that *MGMT*-promoter methylation is a highly relevant biomarker for guiding treatment with temozolomide.[29,46,47] Taken together, *MGMT*-promoter methylation can be used as a predictive marker for TMZ sensitivity.

However, this finding has not been obtained from studies using a homogeneous cohort of patients with glioblastoma with *IDH*-wildtype, as glioblastoma cases included both *IDH*-mutant (<15%) and *IDH*-wildtype (>85%) prior to WHO CNS2021.[14] Several studies have recently shown that the predictive role of *MGMT*-promoter methylation for the response to treatment with temozolomide might be restricted to glioblastoma with *IDH*-wildtype (WHO CNS2016).[29,48] *MGMT* promoter methylation is present in most *IDH*-mutant gliomas and might thus serve as a prognosis but not as a predictive marker.[49] This might be due to the fact that the cutoff value determined in *IDH*-wildtype glioblastoma cases might not be suitable for *IDH*-mutant cases. Our recent study using a homogenous cohort of patients with astrocytoma with *IDH*-mutant grade 4 showed that although *MGMT* promoter methylation has predictive value in this type of glioma, its cutoff value should be higher than that for glioblastoma with *IDH*-wildtype.[29] The levels of methylation of the *MGMT* promoter can also be used for the stratification of the progression-free survival of patients with astrocytoma with *IDH*-mutant grade 2 or 3 under TMZ therapy by using cutoff values significantly higher than those commonly used in *IDH*-wildtype glioblastoma cases.[50] Together, these findings strongly suggested that the predictive cutoff value for *MGMT* promoter methylation in *IDH*-mutant gliomas must be reassessed. The predictive value of *MGMT* promoter methylation should also be reevaluated in cases where there have been changes in tumor classification in WHO CNS2021 (e.g., glioblastoma, *IDH*-wildtype determined by molecular characteristics but without morphological evidence) compared with those in WHO CNS2016. Notably, given that the *MGMT* gene is located on 10q26, whether loss of chromosome 10 or 10q affects the predictive value of *MGMT* promoter methylation remains an important issue.

MGMT promoter methylation status has been used as a stratification factor for patient selection in clinical trials for gliomas, including glioblastoma with *IDH*-wildtype and astrocytoma with *IDH*-mutant.[47] However, the use of *MGMT* promoter methylation has faced challenges in clinical practices due to the fact that there are no alternative treatment choices for cases with unmethylated *MGMT* promoter and due to the perceived uncertainty of test results. Alternative treatment choices rely on the success of clinical trials with unmethylated *MGMT* promoter gliomas. The uncertainty of test results is mainly due to the lack of wide availability of standardized tests, given the absence of an ideal testing method and the lack of a defined accurate cutoff.[29] Currently, there are several methods used for *MGMT* promoter methylation testing, including pyrosequencing (PSQ), gel-based methylation-specific PCR (MSP), methylation-specific quantitative PCR, methylation-specific quantitative PCR plus specific probe, MethyLight quantitative PCR, methylation-sensitive high-resolution melting,

methylation-specific, and multiplex ligation-dependent probe amplification and microarray chips, that is, HM-850K chips. Among these methods, PSQ and MSP appear to be more prognostic for the overall survival of patients with glioma receiving TMZ.[51] However, the best CpG sites and thresholds for these quantitative methods remains ambiguous.

The *MGMT* promoter CGI contains 98 individual CpGs, named CpGs 1–98 depending on their 5′-to 3′-location in the 762 bp sequence of the promoter.[44] Likewise, the CpG sites from 76 bp to 80 bp and 84 bp to 87 bp of *MSP* and those from 72 bp to 95 bp of *PSQ* have also been explored.[43] Compared with *MSP*, high heterogeneity has been reported for CpG methylation in *PSQ*.[44] In spite of this, the number or which CpGs in the *MGMT* promoter CGI should be selected remains a controversial issue for PSQ testing, with various combinations, such as those of CpGs 72–83, 72–80, 72–77, 74–78, 74–89, 76–79, and 80–83 being used in distinct studies.[43,51] We have systematically compared the predictive value of all combinations within CpGs 72–82 on the expression of *MGMT* mRNA through analyzing paired samples using both *MGMT* methylation PSQ testing and mRNA expression data and revealed that the differences in the predictive value among combinations with four or more CpGs within CpGs 72–82 were marginal.[43] This finding might explain the similar results obtained when different CpGs were examined. The cutoff value is another important issue for PSQ testing of *MGMT* promoter methylation, especially for cases in which the levels of methylation are in the "gray zone" between a true methylated and unmethylated status.[52] We have successfully developed a novel analytical model to judge the methylation status of cases in the "gray zone."[44] This novel model evaluates the methylation status of each selected CpG according to its own cutoff value and defines *MGMT* methylation as occurring when the methylation of at least eight CpGs exceeds the respective threshold. We further demonstrated that this novel model is particularly useful in cases with "gray zone" results according to the traditional testing approach. The only drawback was that the optimal cutoff value for each CpG needed to be adjusted as it was limited by the retrospective nature and the relatively small population size of our study.

Taken together, the evaluation of the *MGMT* promoter methylation status should be performed using validated testing methods, and the results should be properly analyzed for the best patient care.

1p and 19q codeletion

Apart from being a diagnostic markers, two phase III clinical trials have revealed that 1p/19q codeletion is also an independent predictive biomarker of benefit from upfront combined RT and chemotherapy with procarbazine, lomustine (CCNU), and vincristine (PCV).[1] However, the mechanism underlying the favorable treatment responses of patients with *IDH*-mutant and 1p/19q-codeleted gliomas remains poorly understood. A systematic functional investigation of genes located on chromosome 1p and 19q, whose expression levels also have prognostic value for non-1p/19q-codeleted gliomas,[53] might address this question. Importantly, whether codeletion of 1p/19q is also a predictive marker for TMZ treatment remains to be answered in clinical trials, given that PCV has more side effects than TMZ and the relatively long-term survival (median overall survival of >10 years) of patients with oligodendroglioma with *IDH*-mutant and 1p/19q-codeleted. Of note, only whole-arm 1p/19q codeletion but not partial deletions on either chromosome arm are predictive biomarkers. In addition, the frequency of false-positive FISH 1p/19q codeletion in adult diffuse astrocytic gliomas has been found to be relatively high.[54] Thus, special care should be taken in interpreting positive FISH results, especially for *IDH*-wildtype gliomas or tumors with *IDH*-mutant but without *TERT* promoter mutations.

Other molecular features associated with chemotherapy

Although *MGMT* promoter methylation is the only commonly acknowledged predictive biomarker for the response of gliomas to TMZ, the discordance between *MGMT* promoter methylation and the levels of protein expression in a small subset of cases has suggested the existence of other mechanisms contributing to the upregulation of MGMT.[55] Such potential mechanisms include the *MGMT* promoter super-enhancer and *MGMT* rearrangement.[45,55] Likewise, miR-181d has been found to also lead to decreased mRNA stability or reduced protein translation by binding to the 3′ untranslated region of *MGMT* transcripts and thus can be used to predict the TMZ response of glioblastomas with unmethylated *MGMT* promoter.[56] In addition to *MGMT*, DNA mismatch repair (MMR) defects caused by mutations of MMR genes also lead to TMZ resistance in recurrent gliomas. Such MMR defects are more likely to occur in recurrent tumors of astrocytoma with *IDH*-mutant and *MGMT* promoter methylation. We have identified a novel DNA methylation-based signature with 31 CpG sites, which predicts the responses of glioblastomas with unmethylated *MGMT* promoter to TMZ.[57] All of these findings suggested that additional predictive biomarkers should be considered in the precision management of gliomas.

Increasing evidence have shown that RNA regulation also plays important roles in the response of gliomas to TMZ. For instance, the increased expression of c-MET or activation of MET signaling pathway contributes to TMZ resistance, especially in secondary glioblastomas.[58] Upregulation of the expression of long noncoding RNA lnc-TALC also enhances the TMZ resistance of glioblastomas via promoting the expression of c-MET through the competitive binding of miR-20b-3p.[58,59] Circle RNA circASAP1, whose expression is known to be significantly increased in recurrent glioblastoma tissues and TMZ-resistant cells, promotes the TMZ resistance of gliomas via upregulating the expression of NRAS by sponge absorption of miR-502–5p.[60] RNA N6-methyladenosine (m^6A) has also been shown to play an important role in the TMZ-resistance of gliomas.[61] Interestingly, m^6A is dynamically regulated by methyltransferases ("writers"),

binding proteins ("readers"), and demethylases ("erasers"). Increased levels of m^6A modifications have been positively associated with glioma malignancy and chemotherapy resistance, and the elevated levels of expression of METTL3, a writer of m^6A, have also been shown to be required for the malignant progression and TMZ resistance of gliomas.[62,63] These findings suggested that additional stratification based on transcriptome profiles holds promise for further improving the predictive accuracy of the TMZ response of gliomas.

Together, the above findings indicated that a molecular panel consisting of genomic alterations, DNA epigenetic alterations, and RNA profiles has the potential to predict TMZ responses of gliomas with or without *MGMT* promoter methylation.

Targeted therapy

With the increasing understanding of molecular features, the targeted therapy of gliomas has become a reality. In particular, *IDH* mutation, the most prominent genetic feature of adult gliomas, is known to affect cell death, epigenetic status, and metabolism of tumors via the synthesis of 2-hydroxyglutarate.[1] Blocking this impact through the use of IDH1/IDH2 inhibitors has been shown to be promising in preclinical models.[64] In a phase I study, ivosidenib (AG-120), a small-molecule inhibitor of IDH1, was shown to prolong disease control and reduce the growth of advanced gliomas with *IDH* mutations.[65] Several other inhibitors are also currently under evaluation,[64] and further clinical trials are expected to provide pivotal insights about the efficacy and toxicity of these compounds in patients. Regarding *IDH*-wildtype adult gliomas, whole exon sequencing of large samples revealed that the most common mutated oncogenic pathway of adult *IDH*-wildtype gliomas included receptor tyrosine kinase (RTK)–PI3K, TP53, and RB pathways.[66] Both RTK inhibitors targeting EGFR and RTK–PI3K pathway inhibitors have been studied in clinical trials,[66] however, without encouraging results. This might be associated with the high intratumoral heterogeneity and evolution of gliomas. Thus, multitargeted therapeutic approaches have greater potential to improve the survival of patients with gliomas. Regorafenib, a VEGF receptor 2 and multikinase inhibitor, has been found to increase the survival of patients with recurrent glioblastoma compared with CCNU in a randomized phase II trial.[67]

Notably, WHO CNS2021 also explicitly recommends the evaluation of fusion genes in adult gliomas, including FGFR3-TACC3, *MET*, *EGFR*, and *NTRK* fusions.[2] These fusion genes are important therapeutic targets for gliomas. Interestingly, *FGFR–TACC* fusions occur in 3.5% of pediatric gliomas and approximately 2.9% of glioblastomas with *IDH*-wildtype and have been shown to commonly cooccur with *CDK4* amplification. *FGFR3-TACC3*–positive patients benefited from treatment with an FGFR inhibitor in a clinical study with a small sample size.[68] MET fusions, including *TFG–MET*, *CLIP2–MET*, and *PTPRZ1–MET*, have become diagnostic molecular markers for newly defined infant-type gliomas. We have demonstrated that *PTPRZ1–MET* and MET exon 14 skipping exists in about 15% of adult secondary glioblastomas.[59,69] In a phase I clinical trial, a novel small-molecule MET inhibitor, PLB1001, successfully suppressed the growth of tumor harboring a *PTPRZ1–MET* fusion.[59] In addition, *EGFR–SEPT14* (3.7%) and *EGFR–PSPH* (1.9%) fusions have also been reported in glioblastomas, with *EGFR–SEPT14* activating the STAT3 signaling to confer sensitivity to EGFR inhibition in a preclinical study.[70] These findings offer new hope for the treatment of gliomas. However, the mutational evolution of gliomas under therapy cannot be ignored, especially when subclone expansion is influenced by strong selection pressures and is accompanied by adaptation in response to treatment modalities.

Although targeted therapies do not widely improve survival in patients with gliomas, a multimodal treatment approach based on the dynamic changes in molecular characteristics might improve survival outcomes and the quality of life in patients with gliomas.

Immunotherapy

Currently, immunotherapy of glioma remains a profound challenge. Although it has been acknowledged that CNS is not immune privileged, the unique immune microenvironment of gliomas resembles a "cold tumor" phenotype owing to the brain blood barrier.[1,4] The "cold tumor" phenotype of gliomas has been associated with poor responses to immune stimulatory therapies, such as immune checkpoint blockade. Additionally, the relatively few coding mutations and high intratumor heterogeneity also limit the development of immunotherapies for gliomas. Nevertheless, the rapid development of molecular pathology has advanced our understanding on the genetic and immunological features of gliomas, thus offering adequate opportunities for the implementation of immunotherapy as a treatment option for gliomas. For instance, the *IDH* mutation, a genetic driver of about half of adult gliomas, is known to suppress leukocyte chemotaxis via reducing the expression of cytotoxic T lymphocyte-associated genes and interferon-γ (IFNγ)-inducible chemokines, including CXC-chemokine ligand 10 (CXCL10).[71] A study at the single-cell level also demonstrated that lymphocytes, including T-cells and NK cells were enriched in *IDH*-wildtype gliomas.[15] All of these findings indicated the distinct immune microenvironment of gliomas with different genetic characteristics, suggesting that the development of subsequent immunotherapy approaches based on the latest pathological classification of gliomas.

Although clinical trials of immune checkpoint blockade targeting the PD1–PD1 ligand 1 (PDL1) axis failed to improve survival in all enrolled patients,[72] a subsequent study showed that a subgroup of patients with specific molecular features might benefit from the PD1/PDL1 blockade.[73] This controversy suggested the requirement for future studies aiming to identify molecular markers for immunotherapy. In particular, adoptive T-cell therapy holds considerable promise for the treatment of gliomas. Proper target selection is a prerequisite for the success of this therapeutic approach, which requires a full

understanding of the molecular characteristics of gliomas, especially the specific markers expressed on cell plasma membrane. A recent clinical trial of CAR T-cells targeting EGFRvIII, HER2, and IL-13Rα2 failed to achieve benefit in gliomas, and this was attributed to their high intratumoral heterogeneity.[74] Single-targeted CAR T-cells are known to kill only a portion of tumor cells expressing the target molecule, accompanied by the expansion of tumor cells without target expression. Therapeutic vaccination for gliomas is another promising potential therapeutic modality but has not been clinically verified.[75] The vaccination approach is also strongly associated with specific molecular alterations of gliomas, such as *EGFRvIII* and *IDH1*-R132H.[75] A substantial problem with single-peptide vaccination is the immune escape caused by intratumoral heterogeneity and selective pressure that results in antigen loss and glioma recurrence. Thus, prediction and dynamic monitoring of molecular features during treatment has been a major issue for the immunotherapy of gliomas. However, several clinical and ethical barriers for the acquisition of longitudinal biopsy glioma samples still exist. MRI-based monitoring approaches also face difficulties including pseudoprogression, radiation-mediated necrosis, and difficulty in reflecting changes in molecular characteristics during treatment.[4] The emergence of fluid biopsy sequencing based on cerebrospinal fluid has provided an alternative, though it is still in its early stages. Cerebrospinal liquid biopsy has the potential to improve the diagnosis, clinical care, and decision-making for gliomas.[76]

Altogether, current findings have pointed to the need for the continued development of predictive biomarkers and dynamic monitoring methods for immune-based therapies for gliomas.

Conclusion and Future direction

After nearly 20 years of research, gliomas remain universally lethal. However, the rapid development of molecular pathology has enabled the more accurate classification of gliomas. This is expected to gradually impact glioma surgery approaches, RT and chemotherapy regimens, and the development of targeted therapy and immunotherapy of gliomas [Table 1]. Based on the molecular or biologically defined classification of gliomas, the design of clinical review studies and prospective clinical trials of gliomas will be more accurate. However, advances in molecular pathology have also brought challenges to clinical molecular testing, pathological diagnosis, and clinical practice of glioma management. Several challenges regarding accurate diagnosis, balance between testing cost, testing accuracy, and timely diagnosis continue to exist in clinical practice. Importantly, the selection of different therapeutic approaches for different pathological types of gliomas will become a reality in the clinical research of gliomas in the future.

Of note, both the molecular detection and treatment of gliomas have been greatly puzzled by the high intratumor heterogeneity of gliomas, the molecular evolution of tumors under treatment, the switch of subclones, wildly transition of transcription status of tumor, and dynamic alterations of immune infiltration. Tracing the changes in molecular characteristics, immune statues, and transcriptional alterations of gliomas during treatment has the potential to improve the long-term prognosis of gliomas. The emergence of liquid biopsies testing, particularly those based on cerebrospinal fluid, has shed new light on the dynamic monitoring of glioma molecular features.

There have been no clear indications of racial differences regarding the development and type of gliomas. However, it has been shown that glioma risk is associated with the extent of estimated European genetic ancestry in African-Americans and Hispanics.[77] Currently, the classification system of gliomas is mainly based on the molecular features and multiple omics data from Caucasian populations. Thus, whether this molecular classification is suitable for the East Asian populations remains to be addressed. The establishment of an optimized molecular diagnosis and treatment system for the East Asian population should be an important topic for glioma research in China.

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Conflicts of interest

None

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