Received: 23 January 2024 | Accepted: 5 August 2024

DOI: 10.1111/bpa.13300

RESEARCH ARTICLE

Brain Pathology

The molecular history of *IDH*-mutant astrocytomas without adjuvant treatment

Zhi-Feng Shi[1,2] | **Kay Ka-Wai Li**[3] | **Johnny Sheung-Him Kwan**[3,4] | **Nellie Yuk-Fei Chung**[3] | **Sze-Ching Wong**[3] | **Abby Wai-Yan Chu**[3] | **Hong Chen**[5] | **Danny Tat-Ming Chan**[6] | **Ying Mao**[1,2] | **Ho-Keung Ng**[2,3]

[1]Department of Neurosurgery, Huashan Hospital, Fudan University, Shanghai, China

[2]Hong Kong and Shanghai Brain Consortium (HSBC), Hong Kong, China

[3]Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, China

[4]Hong Kong Genome Institute, Hong Kong Science Park, Shatin, Hong Kong, China

[5]Department of Pathology, Huashan Hospital, Fudan University, Shanghai, China

[6]Division of Neurosurgery, Department of Surgery, The Chinese University of Hong Kong, Shatin, Hong Kong, China

Correspondence
Ho-Keung Ng and Ying Mao, Department of Anatomical and Cellular Pathology, The Chinese University of Hong Kong, Hong Kong, China.
Email: hkng@cuhk.edu.hk and maoying@fudan.edu.cn

Funding information
National Natural Science Foundation of China, Grant/Award Number: 82072020

Abstract

Hypermutation and malignant transformation are potential complications arising from temozolomide treatment of *IDH*-mutant gliomas. However, the natural history of *IDH*-mutant low-grade gliomas without temozolomide treatment is actually under-studied. We retrieved retrospectively from our hospitals paired tumors from 19 patients with *IDH*-mutant, 1p19q non-codeleted Grade 2 astrocytomas where no interim adjuvant treatment with either temozolomide or radiotherapy was given between primary resections and first recurrences. Tissues from multiple recurrences were available from two patients and radiotherapy but not temozolomide was given before the last specimens were resected. We studied the natural molecular history of these low-grade *IDH*-mutant astrocytomas without pressure of temozolomide with DNA methylation profiling and copy number variation (CNV) analyses, targeted DNA sequencing, *TERT*p sequencing, FISH for ALT and selected biomarkers. Recurrences were mostly higher grades (15/19 patients) and characterized by new CNVs not present in the primary tumors (17/19 cases). Few novel mutations were identified in recurrences. Tumors from 17/19 (89.5%) patients showed either *CDKN2A* homozygous deletion, *MYC* or *PDGFRA* focal and non-focal gains at recurrences. There was no case of hypermutation. Phylogenetic trees constructed for tumors for the two patients with multiple recurrences suggested a lack of subclone development in their evolution when under no pressure from temozolomide. In summary, our studies demonstrated, in contrast to the phenomenon of temozolomide-induced hypermutation, *IDH*-mutant, 1p19q non-codeleted Grade 2 astrocytomas which had not been treated by temozolomide, acquired new CNVs at tumor recurrences. These findings improve our understanding of the molecular life history of *IDH*-mutant astrocytomas.

KEYWORDS
CDKN2A, *IDH*-mutant astrocytomas without adjuvant treatment, *MYC*

Zhi-Feng Shi, Kay Ka-Wai Li contributed equally to this work and share first authorship.

1 | INTRODUCTION

Although the majority of *IDH*-mutant gliomas are generally slow growing, most of the low-grade cases eventually undergo malignant transformation [1]. Alkylating agents such as temozolomide have been shown to prolong the survival of adult patients with lower-grade gliomas [2, 3]. It is also known that temozolomide treatment potentially induces hypermutation in gliomas as first shown by Johnson et al [4] and this is associated with malignant transformation of the tumors [5]. Hypermutation can occur in different subtypes of gliomas [6] but is more common in *IDH*-mutant gliomas [5]. The majority of hypermutated *IDH*-mutant gliomas are high-grade [5]. A "wait-and-see" approach or a non-temozolomide chemotherapy regimen after resection is therefore acceptable post-operative management for Grade 2 *IDH*-mutant, 1p19 non-codeleted astrocytomas with both the SNO and EANO recommendations; this is especially with regard for the low-risk younger patients [7–9]. Adjuvant temozolomide therapy has been shown to be of benefit to patients with Grade 3 *IDH*-mutant, 1p19q non-codeleted astrocytomas by the CATNON trial [10]. With the availability of vorasidenib in the future for the treatment of Grade 2 *IDH*-mutant astrocytoma, it is possible that standard recommendations for this group of tumors may change in the future [11].

In fact, the natural history of low-grade *IDH*-mutant astrocytomas not associated with adjuvant treatment is not well known. Although a small number of paired *IDH*-mutant low-grade astrocytomas without interim adjuvant treatment have been embedded in well-known series of longitudinal gliomas, their detailed molecular pathology has not been clearly separated from that of tumors which have been treated by adjuvant treatment like temozolomide, and only few paired tumor samples unassociated with adjuvant treatment in the interim were included [12]. Touat et al [12] described two pathways of hypermutation in *IDH*-mutant gliomas, one de novo and associated with constitutional defects in DNA polymerase and mismatch repair (MMR) gene mutations and a more common one associated with post-temozolomide treatment. However, of the *IDH*-mutant gliomas examined in that study, there were few cases without adjuvant treatment. Bai et al. studied 41 pairs of *IDH*-mutant gliomas and found that activation of *MYC*, *RTK-RAS-PI3K* pathways, upregulation of *FOXM1*- and *E2F2*-mediated cell cycle transitions and epigenetic silencing of developmental transcription factor genes were implicated in tumor progression and 21 pairs were adjuvant treatment-naïve [13]. However, these results were derived from both temozolomide-treated and non-treated cases. Moreover, 1p19q codeleted tumors were not clearly separated from 1p19q non-codeleted tumors in the analyses presented. This is important as hypermutation after alkylator treatment may be more prevalent with 1p19q codeleted tumors [14] and 1p19q codeleted Grade 2 oligodendrogliomas generally have longer survival than the *IDH*-mutant astrocytomas of similar grades so the pressure for giving upfront post-operative adjuvant treatment is less and a balance of the various clinical factors is recommended for clinical management [8, 9].

Many of the longitudinally paired gliomas studied in the literature have been treated by alkylator therapy in the interim between the resections [4, 6, 13, 15, 16]. A significant proportion of paired *IDH*-mutant gliomas treated by temozolomide in the interim showed hypermutation in their recurrences: 31% in Mu et al. study [16], 18% in Bai et al. study [13], 29% in Seifert et al. study [17], and 55% in Johnson et al. study [4]. However, little specific information is available concerning the natural evolution of *IDH*-mutant astrocytomas without the selection pressures of temozolomide and irradiation and it is not always possible to separate out the detailed data of those pairs which received and those which did not receive temozolomide as interim treatment in these papers in our literature review [4, 6, 13, 15–17].

We believe that knowledge of the natural evolution of *IDH*-mutant astrocytomas will contribute to the identification of the best management of patients with Grade 2 *IDH*-mutant gliomas. In this study, we evaluated 19 patients for tissues from primary and recurrent *IDH*-mutant 1p19q non-codeleted Grade 2 astrocytomas and where no alkylating therapy or radiation was given between primary tumors and recurrences (with 2 patients with multiple recurrences) in this article.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

We went through the files of the Prince of Wales Hospital, Chinese University of Hong Kong and Huashan Hospital, Fudan University, Shanghai and retrieved patients where consecutive or multiple operations on *IDH*-mutant gliomas were performed. We included only cases which were known to be *IDH*-mutant and 1p19q non-codeleted by our usual diagnostic methods [18] and were diagnosed as Grade 2 in the initial tumors. We found cases from 19 patients in which no adjuvant treatment, either alkylator therapy or radiotherapy, was given between the primary and first recurrences. Histology was reviewed by both HKN and HC. Initial tumors were all confirmed as Grade 2 *IDH*-mutant 1p19q non-codeleted astrocytomas and there was progression in grades in the recurrences as per WHO Classification [19]. Histologic criteria for Grade 3 *IDH*-mutant astrocytomas were mitotic figures, or increased cellularity and nuclear atypia. Histologic criteria for Grade 4 were presence of necrosis and/or microvascular proliferation. Data on patient demographics and treatments were retrieved from clinic visits or hospital records or obtained from close relatives by phone.

2.2 | Illumina Infinium methylation EPIC BeadChip array

This array was performed as previously by us [20]. Briefly, formalin-fixed, paraffin-embedded (FFPE) sections were sent to Sinotech Genomics Co., Ltd, Shanghai. DNA was extracted, bisulfite modified and restored by the company. DNA was hybridized to EPIC Infinium Human (850k) array and then washed and scanned. Signal intensities in the IDAT files were subjected to background correction and dye-bias normalization as per Capper et al. [21]. Probes known to have common single nucleotide polymorphisms at the CpG site were filtered out. Sex chromosomes and cross-reactive probes were excluded from the analysis. Tumors were then classified into either G-CIMP-high or G-CIMP-low subtypes by a machine learning algorithm described in previous studies [22]. IDAT files were also uploaded to the Heidelberg Classifier (www.molecularneuropathology.org, Heidelberg Brain Tumor Classifier v12.8).

2.3 | Identification of *MGMT* methylation with DNA methylation array

The DNA methylation status of the promoter of the gene *MGMT* and the *MGMT* score were determined using the *MGMT*-STP27 algorithm Bady et al. [23]. A cut-off of 0.358 was used to classify tumors into methylated *MGMT* and unmethylated *MGMT*.

2.4 | Identification of copy number variations with DNA methylation array

Copy number variations (CNVs) were determined as previously used by Capper et al. and by the authors [21, 24]. To determine copy number variations, probe-level signal intensities from the IDAT files were subjected to background correction and dye-bias normalization according to Capper et al [21]. Probes targeting the sex chromosomes, containing single-nucleotide polymorphisms, or mapping to multiple locations in the human genome were excluded to avoid inaccurate assessment. Two sets of 50 control samples derived from male and female donors were used for normalization [21]. CNV analysis was performed via the R package conumee in Bioconductor (http://www.bioconductor.org/packages/release/bioc/html/conumee.html). Log_2 ratios ±0.35 and −0.415 were used as cutoffs for gain/loss and homozygous loss of genes, respectively, as per methodology by DKFZ and other authors [25–28] and also as used by us previously [29, 30].

2.5 | Targeted sequencing

A custom-designed, capture-based targeted panel consisting 416 cancer relevant genes was designed for this study. This gene panel examined approximately 1.3 Mb of the human genome and a gene list is shown in Table S1. Targeted sequencing was performed on FFPE tissues. In brief, DNA was purified with truXTRAC FFPE Kits (Covaris) according to the manufacturer's protocol. Libraries were prepared using KAPA HyperPrep kit (Roche), enriched with the hybrid capture panel (IDT) sequenced on Illumina HiSeq platform (Illumina, San Diego, CA, USA) at 150 bp paired-end to achieve a mean coverage of ≥200×. Data analysis was performed as described [31, 32].

2.6 | Fluorescence in-situ hybridization (FISH) analysis for *MYC, CDKN2A, PDGFRA* and *EGFR*

Fluorescence in-situ hybridization was performed on FFPE tissues on the tumors from patients 7 and 18 as previously used by us to confirm CNV findings concerning *MYC, CDKN2A, PDGFRA* and *EGFR* obtained from DNA methylation profiling [20]. The details of FISH probes for *MYC*, *EGFR*, *PDGFRA* and *CDKN2A* utilized are summarized in Table S2. Briefly, tumor-specific region was identified on matched FFPE unstained section and H&E-stained section. After deparaffinization and dehydration in xylene and ethanol, sections were soaked in 1 M sodium thiocyanate for 10 min at 80°C. Sections were subsequently digested in pepsin solution, rinsed in Millipore water and dehydrated. Gain (*MYC, EGFR*, and *PDGFRA*) or deletion (*CDKN2A*) probes were designed to span target genes with bacterial artificial chromosome (BAC) clones, which were prepared by nick translation with Spectrum Orange deoxyuridine triphosphate (target probe) or Spectrum Green deoxyuridine triphosphate (reference probe). FISH probes and sections were denatured and hybridized overnight in a humified incubator at 37°C. Sections were washed in 1.5 M Urea/2X saline sodium citrate (SSC) at 46°C for 10 min, stained with Vectasheid mounting medium (Vector Laboratories), mounted with coverslips, and visualized under a Zeiss Axioplan fluorescence microscope. At least 100 non-overlapping nuclei and at least 5% of nuclei population were scored and analyzed in this study. Gene gain was assigned when >5% of the tumor cells showed target-to-reference ratio >2 [33]. *CDKN2A* homozygous deletion was considered when >20% of tumor cells showed loss of two target signals and presence of two reference signals [34].

2.7 | Telomere specific FISH analysis

ALT (Alternative Lengthening of Telomeres) phenotype was examined on FFPE tissues with the use of Telomere PNA FISH kit (K532511, Dako) as previously used by us [20, 35]. First, sections were treated with xylene and ethanol, followed by 1 M sodium thiocyanate and pepsin

solution. FISH probes and tissues were denatured and hybridized to sections. Afterwards, they were washed in Urea/SSC, stained with Vectasheid mounting medium (Vector Laboratories), mounted with coverslips, and visualized under a Zeiss Axioplan fluorescence microscope. Samples were considered ALT positive when ≥5% of tumor cells exhibited large and bright intranuclear foci of telomere FISH signals [36]. Normal brain tissues were used as negative controls. Previous positive cases from our other studies were used as positive controls [20, 35].

2.8 | *TERT* promoter mutation analysis

The hotspot mutations in the *TERT* promoter, C228T and C250T, were being examined in this study. DNA extracted from FFPE sections using QIAamp® DNA FFPE Advanced kit (Qiagen) was subjected to DNA amplification using KAPA HiFi HotStart ReadyMix (Sigma), forward primer 5′- GTCCTGCCCCTTCACCTT-3′ and reverse primer 5′-CAGCGCTGCCTGAAACTC-3′. The PCR products were purified and sequenced using BigDye Terminator Cycle Sequencing kit (Life Technologies).

2.9 | Construction of phylogenetic trees

Phylogenetic trees as constructed in Bi et al's study [37] and also previously used by us [35] were made for tumors from two patients where there was more than one recurrence (patients 7 and 18). The analysis was carried out with PHYLIP software and molecular data of CNVs, gene mutations, *MGMT* methylation and ALT were included in the analysis [38].

2.10 | Statistical analysis

Statistical analysis was conducted using IBM SPSS software (version 26). PFS and OS were defined as the time between operations of the primary tumor and the first recurrence and between the first operation and death, respectively. Frequency distribution was calculated for all clinical and molecular variables. Chi-square or Fisher's test was used to define relationship between clinical parameters and molecular alterations. Log–rank test was used to compare the survival differences between groups. All statistical tests were 2-sided. $p = 0.05$ was considered as the threshold for statistical significance.

3 | RESULTS

For this cohort of 19 patients, there were 10 males and 9 females. The age was 23–56 years (mean 40.3 years ±6.7). The initial tumors of all cases were *IDH*-mutant, 1p19q non-codeleted Grade 2 astrocytomas. No chemotherapy or radiotherapy was given between primary tumors and the initial recurrences. For two patients (patients 7 and 18), there were specimens from multiple recurrences. Radiotherapy was given before the last specimens were taken but the patients did not receive any temozolomide before any specimen was taken. Patient 7 received temozolomide treatment after the last specimen was resected and later the patient passed away. *IDH* mutation and 1p19q status were first obtained as per our previous work [18] and they were subsequently confirmed by target sequencing and DNA methylation profiling as described above. Mean and median follow-ups were 118.8 and 103.8 months, respectively. The mean and median overall survivals (OS) were 111.4 months and 120.7 months, respectively (range 34.7–171.3 months). For 15 of the 19 patients, tumors progressed from Grade 2 to either Grade 3 or Grade 4 *IDH*-mutant astrocytomas upon recurrences. Progression free survival (PFS) for these fifteen patients was not different from that of the other four patients, whose tumors recurred later as Grade 2 ($p = 0.351$). However, the number for the latter was small.

Figure 1 summarizes the DNA methylation data and prevalent CNVs and mutations found in this study. Recurrences were characterized by copy number changes (Figure 1). We evaluated the frequencies of CNVs in the genomes of the primary and recurrent samples as used by Mirchia et al. [39, 40]. The average percentage of the genome with CNVs was significantly lower in the primary tumors compared with that of the recurrent samples ($p = 0.005$; 1.64% ± 2.92 vs 7.35% ± 7.85). The percentages of the genomes with CNVs in each matched primary and recurrent tumors are shown in Figure 2. Furthermore, the average percentage of the genome with CNVs was 2.92% ± 3.27 in Grade 2 tumors, a significantly lower level than that of Grade 3 tumors (6.67% ± 8.58; $p = 0.030$) or Grade 4 tumors (15.17% ± 6.84; $p < 0.01$). No significant difference was found between *MGMT* methylated cases and unmethylated cases ($p = 0.267$). There was also no difference in percentage of the genome with CNVs between ALT-positive and ALT-negative cases ($p = 0.990$).

At the gene level, of the 26 CNS tumor-related genes analyzed by the Heidelberg Classifier [41], new CNVs as determined by the $\log_2$ status were found in the recurrences when compared with the initial tumors in 16/19 patients (Figure 1; Table S3), These new CNVs at the gene level were only found in the recurrences but not the primary samples. Segmental plots as per the Classifier reports and FISH confirmed CNV changes of *CDKN2A, EGFR, PDGFRA*, and *MYC* (Figures 3, 4 and S1–S4). Both genetic and segmental gains can be seen. The results showed that recurrences in *IDH*-mutant astrocytomas exhibited increases in CNVs.

Classifier diagnosis was consistent with the diagnosis of *IDH*-mutant astrocytoma in 79% of cases with confidence score >0.9. G-CIMP status was determined as per

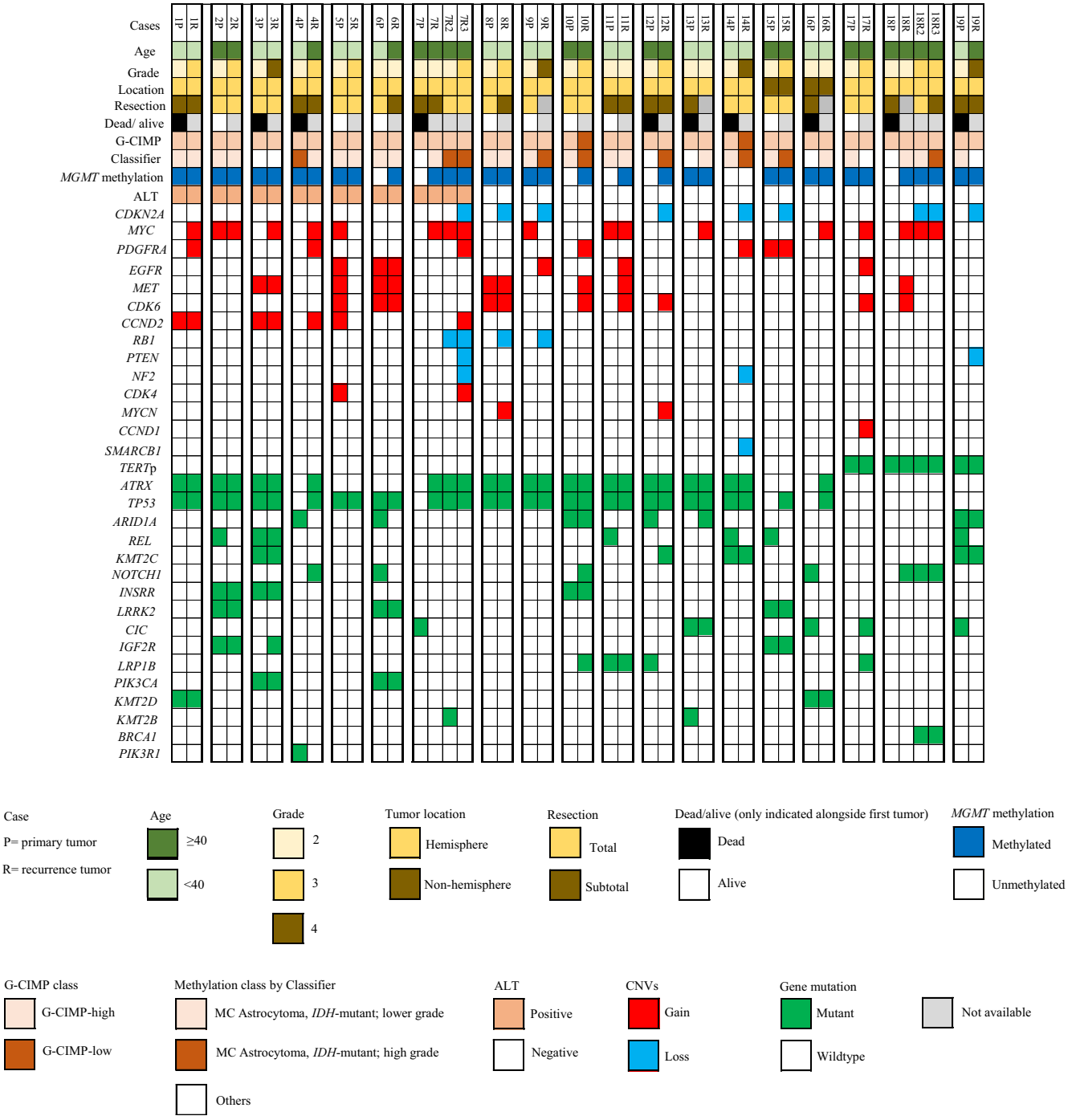

FIGURE 1 Oncoprint summary of clinical and molecular profiles of 19 pairs of *IDH*-mutant 1p19q non-codeleted astrocytomas.

Ceccarelli et al. [22]. The bulk of the cases, both primary tumors and recurrences, in this cohort were assigned to the G-CIMP-high subtype (95%) as can be expected for *IDH*-mutant gliomas [22]. In two patients, G-CIMP status changed from high to low upon recurrences. The recurrent samples of these two patients harbored *PDGFRA* gain that has been shown to be enriched in G-CIMP-low tumors [22]. Overall, 81% of the samples displayed *MGMT* methylation and similar frequency was found in *IDH*-mutant astrocytomas [42]. *MGMT* methylation status was unchanged in most of the patients (13/19) upon tumor recurrences. *MGMT* methylation at primary tumors was unassociated with OS ($p = 0.827$) and PFS ($p = 0.843$). *MGMT* methylation was also reported to have no prognostication in *IDH*-mutant astrocytomas in another study [42]. *MGMT* should be unassociated with hypermutation at recurrences in this series, since there was no case with hypermutation (see below).

Tumors from 17 of the 19 (89.5%) patients showed either *CDKN2A* homozygous deletion, *MYC* or *PDGFRA* focal and non-focal gains at recurrences. For

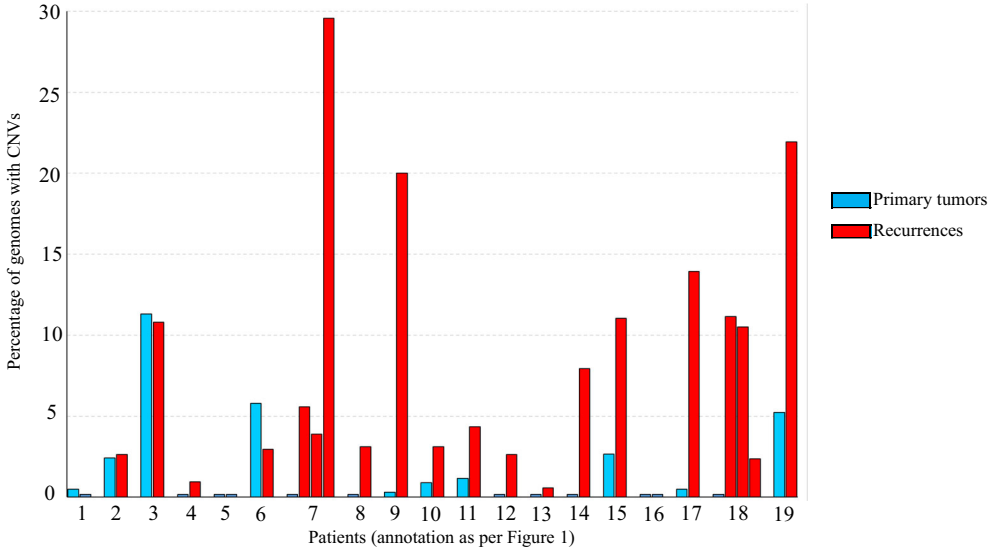

FIGURE 2 Percentage of copy number alterations in the genomes of matched primary tumors and recurrences.

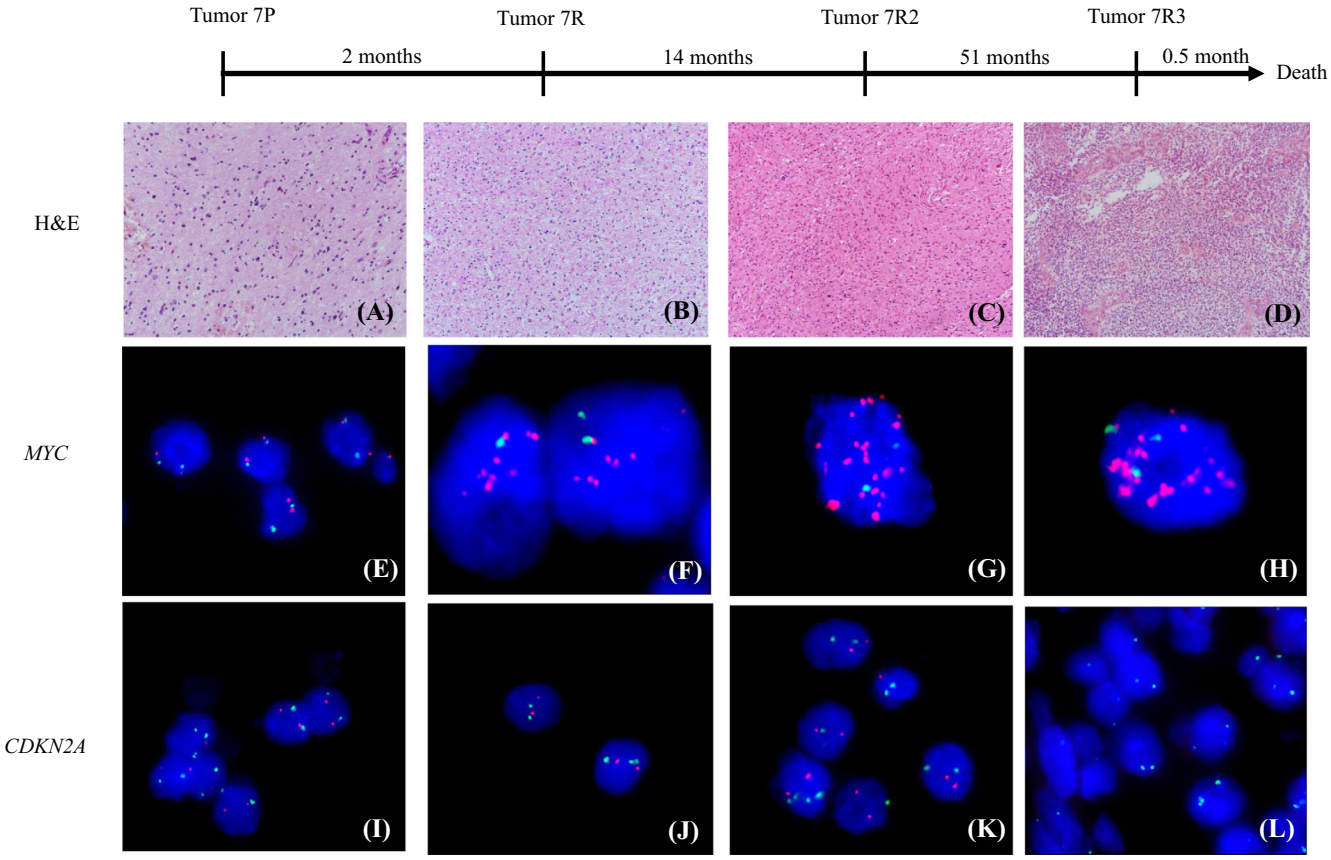

FIGURE 3 Histological and FISH findings of primary and recurrent tumors of Patient 7, a 42-year-old female with an initial diagnosis of Grade 2 astrocytoma located at the frontal lobe. The patient received no temozolomide before all samples were collected. Top to bottom: Patient history timeline. H&E shows the histology at each tumor occurrence (all ×200). FISH confirmed the results of DNA methylation profiling. *MYC* was not amplified in the initial tumor. Upon the first, second and third recurrences, tumors acquired *MYC* gain. FISH also confirmed *CDKN2A* homozygous deletion at the third recurrence. *CDKN2A* was retained in the initial tumor and the first and second recurrences.

CDKN2A homozygous deletion, tumors from 8 of the 19 patients showed deletion, and in all eight patients, the deletion was found in the recurrent tumors but not the primary tumors. *MYC* gain was found in tumors from 12 of the 19 patients, with 10 of the 12 being found in recurrences. For 8 patients, *MYC* gain occurred in the recurrent tumors but not the primary tumors. *PDGFRA* gain was seen in tumors from 6 of the 19 patients, all

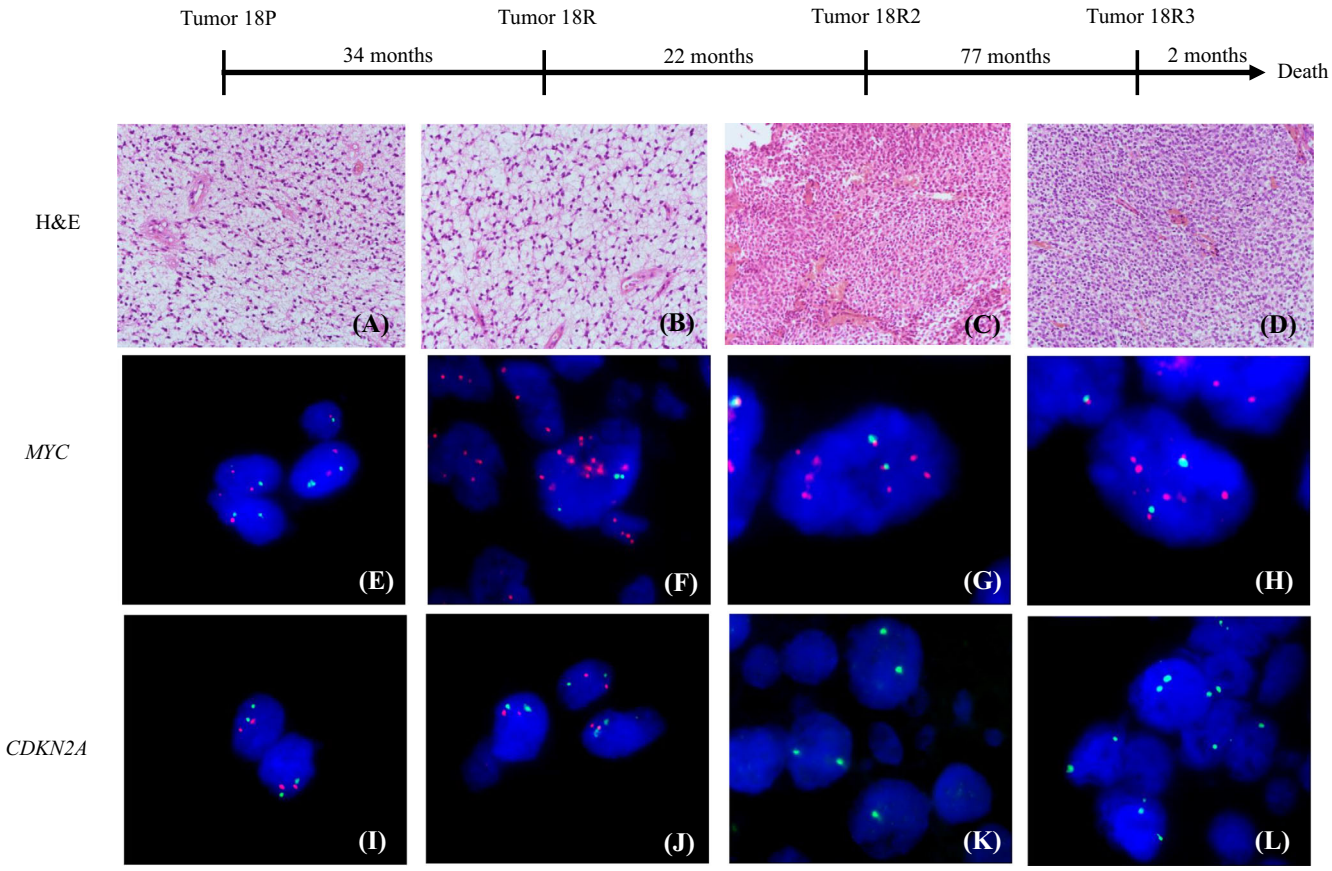

FIGURE 4 Histological and molecular features of primary and recurrent tumors of Patient 18, a 45-year-old male with an initial diagnosis of Grade 2 astrocytoma at the frontal lobe. This patient received no temozolomide treatment before all specimens were taken. Top to bottom: Patient history timeline. H&E staining illustrated the histology at each tumor occurrence (all ×200). FISH confirmed the results of DNA methylation profiling. The initial tumor showed no *MYC* gain, but at the first, second and third recurrences, the tumors acquired *MYC* gain. FISH also detected no *CDKN2A* homozygous deletion in the initial and first recurrent tumors. Upon the second and third recurrences, the tumors acquired *CDKN2A* homozygous deletion.

being found in the recurrences. In five of these patients, gain was found in the recurrent tumors but not primary tumors. *EGFR* gain was found in tumors from five patients with 4 of the 5 of the recurrent tumors showing copy number gain. For three patients, *EGFR* gain was found in the recurrent but not the primary tumors. *MET* gain was found in tumors from 7 of the 19 patients, with 6 of the 7 of the recurrent tumors showing gain. For three patients, *MET* gain was found in the recurrent tumors but not the primary tumors. *CDK6* gain was found in the recurrent tumors but not primary tumors in six patients. *PDGFRA* and *CCND2* gains at recurrences were associated with shorter OS ($p = 0.008$ and $p = 0.013$, respectively) but the numbers were small. These results showed that CNV changes characterized the evolution of *IDH*-mutant astrocytomas unassociated with adjuvant treatment of *IDH*-mutant astrocytomas. The raw DNA methylation data is available online at https://www.surgery.cuhk.edu.hk/BTC/HSBC/.

In contrast, gene mutation signatures were not very different between the primary tumors and recurrences of *IDH*-mutant astrocytomas in this cohort. We detected no difference in the tumor mutation burden between primary and recurrent tumors ($p = 0.836$; 5.04 mutations/Mb ± 1.39 vs 5.16 mutations/Mb ± 1.55). Overall, tumor mutation burdens were low and no case of hypermutation, either in the initial or recurrent tumors, were found. We concede that we have not examined the whole genome for mutations and whole exome sequencing (WES) should allow a more comprehensive tumor mutational burden (TMB) estimation. However, a number of clinical studies have shown that targeted sequencing of a few hundred genes of interest can be used to estimate TMB, and the results were comparable with those of WES [43–45]. With the targeted panel we used, and when recurrences were compared with the primary tumors, there were on average only 3 new mutations in the recurrent samples across the 19 patients. This showed that *IDH*-mutant, 1p19q non-codeleted tumors without selection pressures from temozolomide and radiotherapy acquired few new mutations at recurrences. Three *MSH6* mutations were found in two patients. However, no mutations of the other mismatch repair (MMR) genes, namely *MLH1*, *MSH2*, and *PMS2*, were found. *IDH*

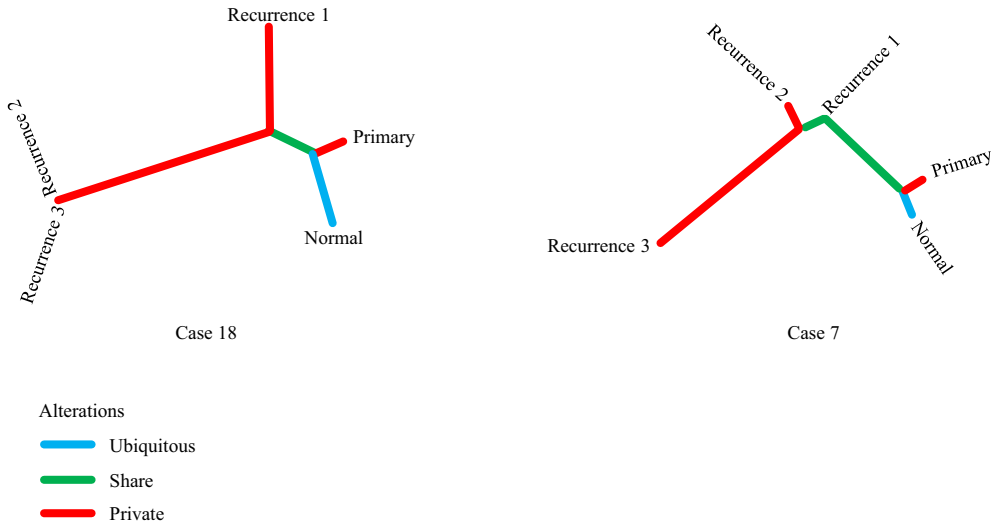

FIGURE 5 Phylogenetic trees of two *IDH*-mutant, 1p19q non-codeleted astrocytomas with multiple recurrences from Patients 7 and 18.

and *TERT*p mutations co-occurred in both primary and recurrent tumors for the same patients in all cases. *TERT*p mutations had no prognostication for PFS ($p = 0.275$) and OS ($p = 0.988$). This was in contrast to a previous study of a favorable prognostic significance of *TERT*p in *IDH*-mutant astrocytomas but our numbers and the numbers in that series ($n = 31$) were both small [46]. Tumors from 13/19 patients showed *ATRX* mutations and only in three patients were the mutations absent in their primary tumors. Similarly, tumors from 16 of the 19 patients showed *TP53* mutations but only in four patients were the mutations absent in their primary tumors. Significantly, *NOTCH1* mutations were found in tumors from five patients and they were present in the recurrent tumors in three patients. NOTCH pathway mutations have been identified as drivers for unfavorable prognosis in *IDH*-mutant, 1p19q codeleted gliomas [47]. ALT was found in 7 of the 19 tumors and was conserved between primary and recurrent tumors for all seven patients.

Phylogenetic trees of molecular features of multiple tumors from two patients with multiple recurrences were constructed as used by Bi et al. [37] and us [35] (Figure 5). When samples that did not immediately precede one another were the most closely related, this would be reflecting development of subclones rather than tumor evolving from a single dominant clone. The results suggested samples from both patients showed lack of subclone development in their evolution when under no pressure from temozolomide. The limitation was that we had only tumors from two patients available for such analyses.

4 | DISCUSSION

Alkylating agents such as temozolomide prolong the survival of adult patients with lower grade gliomas [2, 3]. In spite of the known association in some tumors between *IDH*-mutant gliomas and hypermutation and malignant transformation after temozolomide treatment, the molecular evolution of these tumors without pressure of temozolomide is relatively unknown. Partly as a result, there is also uncertainty as to standard-of-care for *IDH*-mutant Grade 2 astrocytomas, especially when the patients are young or regarded as low-risk for recurrence. Although there have been many high profile series of molecular studies of longitudinal gliomas, paired *IDH*-mutant astrocytomas without interim adjuvant treatment were often embedded with tumors which were treated with temozolomide and/or radiotherapy in the interim. Although hypermutation at recurrences has been proposed to be a potential complication of temozolomide treatment for *IDH*-mutant gliomas, it is not always possible to separate out in the papers molecular features of paired *IDH*-mutant astrocytomas which have not been treated by temozolomide in our literature review. Conclusions about *IDH*-mutant gliomas progression was often made from findings from both temozolomide-treated and untreated longitudinal pairs [4, 6, 12, 13, 15–17, 48, 49].

In this study, we recruited a cohort of *IDH*-mutant Grade 2 astrocytomas which progressed as part of their natural history without pressures of temozolomide or radiotherapy, where recurrences were resected and paired tissues obtained. It should therefore be noted that their molecular features may not necessarily be representative of *IDH*-mutant gliomas in general. We showed that recurrences were associated with increased CNVs and most of the recurrences were associated with tumor progression to high-grade lesions. It has been shown in non-paired *IDH*-mutant astrocytomas, CNVs were associated with higher grade tumors [50]. Similarly chromosomal instability is associated with a poor outcome in *IDH*-mutant astrocytomas [51, 52]. *CDKN2A* homozygous deletion and *CDK4* amplification are also associated with a poor prognosis in *IDH*-mutant astrocytomas [34, 53].

However, paired primary and recurrent tumors have not been studied in these studies. *CDKN2A* homozygous deletion is now listed as a molecular criteria for Grade 4 designation in *IDH*-mutant astrocytomas [54]. Experimentally, *PDGFRA* is activated in *IDH*-mutant gliomas [55] and *MYC* is associated in their malignant transformation [13, 56]. Barthel et al. also showed glioma recurrences were typified by acquired alterations in the cell cycle [6]. Bai et al. identified *MYC* as the most frequently amplified locus during progression of *IDH*-mutant gliomas [13]. However, their cases contained both temozolomide-treated and untreated patients for this result.

Gliomas typically have a relatively low mutation profile but are associated hypermutation with temozolomide treatment by Choi et al. [14]. These authors also showed hypermutation was more prevalent with *IDH*-mutated gliomas with *MGMT* hypermethylation [5]. Temozlomide-induced hypermutation is associated with distant recurrences and reduced survival after high-grade transformation of low-grade *IDH*-mutant gliomas [57]. Malignant transformation is also associated with increased mutation burden in *IDH*-mutant gliomas [58]. Mutations in mismatch repair (MMR) genes are also often observed in temozolomide-treated hypermutated recurrences [59]. Intriguingly, there seems to be no difference in OS between hypermutators and non-hypermutators of *IDH*-mutant gliomas treated by alkylating agents [6]. Bai et al. identified *NOTCH1* and *NOTCH2* mutations as major drivers for malignant progression, and *NOTCH1* and other NOTCH pathway gene mutations are noted to be hallmarks of less favorable prognosis in 1p19q codeleted gliomas [13, 47]. Bai et al. found NOTCH and FAT pathway mutations as significant drivers for tumor progression [13]. However, in their study, results are not clearly separable between tumors with and without adjuvant treatment, and between 1p19q codeleted and 1p19q non-codeleted cases. Interestingly, the GLASS studies showed an increase of *KMT2C, KMT2D, NOTCH1* mutations in recurrences of paired *IDH*-mutant gliomas treated by temozolomide [6, 48, 49]. In our cohort of paired *IDH*-mutant astrocytomas without interim adjuvant treatment, we found *NOTCH1* mutations in tumors from five patients and they were present in the recurrent tumors in 3 of the 5 patients. *KMT2C* and *KMT2D* mutations were not particularly enriched in our cohort.

Tumor mutation burdens in this cohort were generally low and relatively few new mutations were identified. It seems that no case of hypermutation could be found. Evolutionary trees drawn from two patients with multiple recurrences showed absence of subclone development. It is appreciated that the number of patients was too small for a definitive conclusion to be drawn.

De Souza et al. reported that 9.5% of their *IDH*-mutant gliomas upon recurrence changed their epigenetic status from G-CIMP-high to G-CIMP-low and we found that 2 of the 19 (10.5%) of our series showed this change and the results are consistent with their study [15]. A low level of methylation has been shown to be a poor prognosticator in *IDH*-mutant gliomas [22, 60]. We also showed *MGMT* methylation was not a prognosticator in *IDH*-mutant gliomas and similar finding was reported by the European group [42].

In summary, we showed that in a series of paired *IDH*-mutant 1p19q non-codeleted astrocytomas, where the initial tumor was Grade 2 and where there had been no interim treatment by temozolomide or radiotherapy, the natural molecular evolution of these tumors was recurrence with CNVs and higher grade progression. For the former, the more common associations were *CDKN2A* homozygous deletion and gain of *MYC* or *PDGFRA*. Hypermutation did not occur and relatively few new mutations were identified. *TERT*, *ATRX*, and ALT status were conserved in spite of histological progression.

AUTHOR CONTRIBUTIONS

Zhi-Feng Shi and Kay Ka-Wai Li contributed equally and are co-first authors. Ying Mao and Ho-Keung Ng are the co-corresponding authors. HKN and ZFS designed the study. HKN and HC performed the histological examination. KKL, NC, SCW and AWC performed the experimental work and molecular analyses. JSK did the bioinformatic analyses. ZFS, DTC and YM provided clinical data and medical history. ZFS and DTC provided imaging data and reviewed them. KKL and HKN wrote the manuscript. All authors approved the submitted version.

FUNDING INFORMATION

This study was supported by Shanghai Science and Technology Committee Rising-Star Program (20QA1401900), National Natural Science Foundation of China (82072020), Science and Technology Commission of Shanghai Municipality (20Z1900100), and Shanghai Municipal Science and Technology Major Project (2018SHZDZX01).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The IDAT files generated during the current study are available at https://www.surgery.cuhk.edu.hk/BTC/HSBC/.

ETHICS STATEMENT

The study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee and the Ethics Committee of Huashan Hospital, Fudan University, Shanghai.

ORCID

Kay Ka-Wai Li https://orcid.org/0000-0001-9588-3042
Ho-Keung Ng https://orcid.org/0000-0002-2614-2213

REFERENCES

1. Sun X, Turcan S. From laboratory studies to clinical trials: temozolomide use in IDH-mutant gliomas. Cells. 2021;10(5):1225.
2. Baumert BG, Hegi ME, van den Bent MJ, von Deimling A, Gorlia T, Hoang-Xuan K, et al. Temozolomide chemotherapy versus radiotherapy in high-risk low-grade glioma (EORTC 22033-26033): a randomised, open-label, phase 3 intergroup study. Lancet Oncol. 2016;17(11):1521–32.
3. Buckner JC, Shaw EG, Pugh SL, Chakravarti A, Gilbert MR, Barger GR, et al. Radiation plus procarbazine, CCNU, and vincristine in low-grade glioma. N Engl J Med. 2016;374(14):1344–55.
4. Johnson BE, Mazor T, Hong C, Barnes M, Aihara K, McLean CY, et al. Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma. Science. 2014;343(6167):189–93.
5. Jonsson P, Lin AL, Young RJ, DiStefano NM, Hyman DM, Li BT, et al. Genomic correlates of disease progression and treatment response in prospectively characterized gliomas. Clin Cancer Res. 2019;25(18):5537–47.
6. Barthel FP, Johnson KC, Varn FS, Moskalik AD, Tanner G, Kocakavuk E, et al. Longitudinal molecular trajectories of diffuse glioma in adults. Nature. 2019;576(7785):112–20.
7. Weller M, van den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat Rev Clin Oncol. 2021;18(3):170–86.
8. Mohile NA, Messersmith H, Gatson NT, Hottinger AF, Lassman A, Morton J, et al. Therapy for diffuse astrocytic and oligodendroglial tumors in adults: ASCO-SNO guideline. J Clin Oncol. 2022;40(4):403–26.
9. Miller JJ, Gonzalez Castro LN, McBrayer S, Weller M, Cloughesy T, Portnow J, et al. Isocitrate dehydrogenase (IDH) mutant gliomas: a Society for Neuro-Oncology (SNO) consensus review on diagnosis, management, and future directions. Neuro Oncol. 2023;25(1):4–25.
10. van den Bent MJ, Tesileanu CMS, Wick W, Sanson M, Brandes AA, Clement PM, et al. Adjuvant and concurrent temozolomide for 1p/19q non-co-deleted anaplastic glioma (CATNON; EORTC study 26053-22054): second interim analysis of a randomised, open-label, phase 3 study. Lancet Oncol. 2021;22(6):813–23.
11. Mellinghoff IK, van den Bent MJ, Blumenthal DT, Touat M, Peters KB, Clarke J, et al. Vorasidenib in IDH1- or IDH2-mutant low-grade glioma. N Engl J Med. 2023;389(7):589–601.
12. Touat M, Li YY, Boynton AN, Spurr LF, Iorgulescu JB, Bohrson CL, et al. Mechanisms and therapeutic implications of hypermutation in gliomas. Nature. 2020;580(7804):517–23.
13. Bai H, Harmanci AS, Erson-Omay EZ, Li J, Coskun S, Simon M, et al. Integrated genomic characterization of IDH1-mutant glioma malignant progression. Nat Genet. 2016;48(1):59–66.
14. Choi S, Yu Y, Grimmer MR, Wahl M, Chang SM, Costello JF. Temozolomide-associated hypermutation in gliomas. Neuro Oncol. 2018;20(10):1300–9.
15. de Souza CF, Sabedot TS, Malta TM, Stetson L, Morozova O, Sokolov A, et al. A distinct DNA methylation shift in a subset of glioma CpG Island Methylator phenotypes during tumor recurrence. Cell Rep. 2018;23(2):637–51.
16. Mu Q, Chai R, Pang B, Yang Y, Liu H, Zhao Z, et al. Identifying predictors of glioma evolution from longitudinal sequencing. Sci Transl Med. 2023;15(716):eadh4181.
17. Seifert M, Schackert G, Temme A, Schröck E, Deutsch A, Klink B. Molecular characterization of astrocytoma progression towards secondary glioblastomas utilizing patient-matched tumor pairs. Cancers (Basel). 2020;12(6):1696.
18. Chan AK, Shi ZF, Li KK, Wang WW, Chen H, Chung NY, et al. Combinations of single-gene biomarkers can precisely stratify 1,028 adult gliomas for prognostication. Front Oncol. 2022;12:839302.
19. Brat DJ, Reuss DE, Huse JT, Kleinschmidt-DeMasters BK, Komori T, Reuss DE, et al. IDH-mutant astrocytoma. Central nervous system. 5th ed. Lyon (France): International Agency for Research on Cancer; 2021. p. 19–27.
20. Liu EM, Shi ZF, Li KK, Malta TM, Chung NY, Chen H, et al. Molecular landscape of IDH-wild type, pTERT-wild type adult glioblastomas. Brain Pathol. 2022;32(6):e13107.
21. Capper D, Jones DTW, Sill M, Hovestadt V, Schrimpf D, Sturm D, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469–74.
22. Ceccarelli M, Barthel FP, Malta TM, Sabedot TS, Salama SR, Murray BA, et al. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell. 2016;164(3):550–63.
23. Bady P, Delorenzi M, Hegi ME. Sensitivity analysis of the MGMT-STP27 model and impact of genetic and epigenetic context to predict the MGMT methylation status in gliomas and other tumors. J Mol Diagn. 2016;18(3):350–61.
24. Shi ZF, Li KK, Huang QJ, Wang WW, Kwan JS, Chen H, et al. Molecular landscape of IDH-wild-type, H3-wild-type glioblastomas of adolescents and young adults. Neuropathol Appl Neurobiol. 2022;48(4):e12802.
25. Shirahata M, Ono T, Stichel D, Schrimpf D, Reuss DE, Sahm F, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. Acta Neuropathol. 2018;136(1):153–66.
26. Ferreyra Vega S, Olsson Bontell T, Corell A, Smits A, Jakola AS, Carén H. DNA methylation profiling for molecular classification of adult diffuse lower-grade gliomas. Clin Epigenetics. 2021;13(1):102.
27. Ferreyra Vega S, Olsson Bontell T, Kling T, Jakola AS, Carén H. Longitudinal DNA methylation analysis of adult-type IDH-mutant gliomas. Acta Neuropathol Commun. 2023;11(1):23.
28. Wenger A, Ferreyra Vega S, Schepke E, Löfgren M, Olsson Bontell T, Tisell M, et al. DNA methylation alterations across time and space in paediatric brain tumours. Acta Neuropathol Commun. 2022;10(1):105. https://doi.org/10.1186/s40478-022-01406-8
29. Li KK, Shi ZF, Malta TM, Chan AK, Cheng S, Kwan JSH, et al. Identification of subsets of IDH-mutant glioblastomas with distinct epigenetic and copy number alterations and stratified clinical risks. Neurooncol Adv. 2019;1(1):vdz015.
30. Wong QH, Li KK, Wang WW, Malta TM, Noushmehr H, Grabovska Y, et al. Molecular landscape of IDH-mutant primary astrocytoma grade IV/glioblastomas. Mod Pathol. 2021;34(7):1245–60.
31. Chau SL, Tong JH, Chow C, Kwan JS, Lung RW, Chung LY, et al. Distinct molecular landscape of Epstein-Barr virus associated pulmonary Lymphoepithelioma-like carcinoma revealed by genomic sequencing. Cancers (Basel). 2020;12(8):2065.
32. Chan AW, Chau SL, Tong JH, Chow C, Kwan JSH, Chung LY, et al. The landscape of actionable molecular alterations in immunomarker-defined large-cell carcinoma of the lung. J Thorac Oncol. 2019;14(7):1213–22.
33. Horbinski C, Miller CR, Perry A. Gone FISHing: clinical lessons learned in brain tumor molecular diagnostics over the last decade. Brain Pathol. 2011;21(1):57–73.
34. Yang RR, Shi ZF, Zhang ZY, Chan AK, Aibaidula A, Wang WW, et al. IDH mutant lower grade (WHO grades II/III) astrocytomas can be stratified for risk by CDKN2A, CDK4 and PDGFRA copy number alterations. Brain Pathol. 2020;30(3):541–53.

35. Ng HK, Li KK, Chung NY, Chan JY, Poon MF, Wong QH, et al. Molecular landscapes of longitudinal NF2/22q and non-NF2/22q meningiomas show different life histories. Brain Pathol. 2023;33(3):e13120.
36. Heaphy CM, Subhawong AP, Hong SM, Goggins MG, Montgomery EA, Gabrielson E, et al. Prevalence of the alternative lengthening of telomeres telomere maintenance mechanism in human cancer subtypes. Am J Pathol. 2011;179(4):1608–15.
37. Bi WL, Greenwald NF, Abedalthagafi M, Wala J, Gibson WJ, Agarwalla PK, et al. Genomic landscape of high-grade meningiomas. NPJ Genom Med. 2017;2:2.
38. Retief JD. Phylogenetic analysis using PHYLIP. Methods Mol Biol. 2000;132:243–58.
39. Mirchia K, Snuderl M, Galbraith K, Hatanpaa KJ, Walker JM, Richardson TE. Establishing a prognostic threshold for total copy number variation within adult IDH-mutant grade II/III astrocytomas. Acta Neuropathol Commun. 2019;7(1):121.
40. Mirchia K, Sathe AA, Walker JM, Fudym Y, Galbraith K, Viapiano MS, et al. Total copy number variation as a prognostic factor in adult astrocytoma subtypes. Acta Neuropathol Commun. 2019;7(1):92.
41. Capper D, Stichel D, Sahm F, Jones DTW, Schrimpf D, Sill M, et al. Practical implementation of DNA methylation and copy-number-based CNS tumor diagnostics: the Heidelberg experience. Acta Neuropathol. 2018;136(2):181–210.
42. Weller M, Felsberg J, Hentschel B, Gramatzki D, Kubon N, Wolter M, et al. Improved prognostic stratification of patients with isocitrate dehydrogenase-mutant astrocytoma. Acta Neuropathol. 2024;147(1):11.
43. Cheng DT, Mitchell TN, Zehir A, Shah RH, Benayed R, Syed A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn. 2015;17(3):251–64.
44. Rizvi H, Sanchez-Vega F, La K, Chatila W, Jonsson P, Halpenny D, et al. Molecular determinants of response to anti-programmed cell death (PD)-1 and anti-programmed death-ligand 1 (PD-L1) blockade in patients with non-small-cell Lung cancer profiled with targeted next-generation sequencing. J Clin Oncol. 2018;36(7):633–41.
45. Johnson DB, Frampton GM, Rioth MJ, Yusko E, Xu Y, Guo X, et al. Targeted next generation sequencing identifies markers of response to PD-1 blockade. Cancer Immunol Res. 2016;4(11):959–67.
46. Eckel-Passow JE, Lachance DH, Molinaro AM, Walsh KM, Decker PA, Sicotte H, et al. Glioma groups based on 1p/19q, IDH, and TERT promoter mutations in tumors. N Engl J Med. 2015;372(26):2499–508.
47. Aoki K, Nakamura H, Suzuki H, Matsuo K, Kataoka K, Shimamura T, et al. Prognostic relevance of genetic alterations in diffuse lower-grade gliomas. Neuro Oncol. 2018;20(1):66–77.
48. Varn FS, Johnson KC, Martinek J, Huse JT, Nasrallah MP, Wesseling P, et al. Glioma progression is shaped by genetic evolution and microenvironment interactions. Cell. 2022;185(12):2184–2199.e16.
49. Malta TM, Sabedot TS, Morosini NS, Datta I, Garofano L, Vallentgoed W, et al. The epigenetic evolution of glioma is determined by the IDH1 mutation status and treatment regimen. Cancer Res. 2024;84(5):741–56.
50. Richardson TE, Sathe AA, Kanchwala M, Jia G, Habib AA, Xiao G, et al. Genetic and epigenetic features of rapidly progressing IDH-mutant Astrocytomas. J Neuropathol Exp Neurol. 2018;77(7):542–8.
51. Liu Y, Sathe AA, Abdullah KG, McBrayer SK, Adams SH, Brenner AJ, et al. Global DNA methylation profiling reveals chromosomal instability in IDH-mutant astrocytomas. Acta Neuropathol Commun. 2022;10(1):32.
52. Richardson TE, Snuderl M, Serrano J, Karajannis MA, Heguy A, Oliver D, et al. Rapid progression to glioblastoma in a subset of IDH-mutated astrocytomas: a genome-wide analysis. J Neurooncol. 2017;133(1):183–92.
53. Cimino PJ, Holland EC. Targeted copy number analysis outperforms histologic grading in predicting patient survival for WHO grades II/III IDH-mutant astrocytomas. Neuro Oncol. 2019;21(6):819–21.
54. Louis DN, Perry A, Wesseling P, Brat DJ, Cree IA, Figarella-Branger D, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol. 2021;23(8):1231–51.
55. Flavahan WA, Drier Y, Liau BB, Gillespie SM, Venteicher AS, Stemmer-Rachamimov AO, et al. Insulator dysfunction and oncogene activation in IDH mutant gliomas. Nature. 2016;529(7584):110–4.
56. Wakimoto H, Tanaka S, Curry WT, Loebel F, Zhao D, Tateishi K, et al. Targetable signaling pathway mutations are associated with malignant phenotype in IDH-mutant gliomas. Clin Cancer Res. 2014;20(11):2898–909.
57. Yu Y, Villanueva-Meyer J, Grimmer MR, Hilz S, Solomon DA, Choi S, et al. Temozolomide-induced hypermutation is associated with distant recurrence and reduced survival after high-grade transformation of low-grade IDH-mutant gliomas. Neuro Oncol. 2021;23(11):1872–84.
58. Aoki K, Suzuki H, Yamamoto T, Yamamoto KN, Maeda S, Okuno Y, et al. Mathematical modeling and mutational analysis reveal optimal therapy to prevent malignant transformation in grade II IDH-mutant gliomas. Cancer Res. 2021;81(18):4861–73.
59. van Thuijl HF, Mazor T, Johnson BE, Fouse SD, Aihara K, Hong C, et al. Evolution of DNA repair defects during malignant progression of low-grade gliomas after temozolomide treatment. Acta Neuropathol. 2015;129(4):597–607.
60. Mamatjan Y, Voisin MR, Nassiri F, Moraes FY, Bunda S, So J, et al. Integrated molecular analysis reveals hypermethylation and overexpression of HOX genes to be poor prognosticators in isocitrate dehydrogenase mutant glioma. Neuro Oncol. 2023;25(11):2028–41.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Shi Z-F, Li KK-W, Kwan JS-H, Chung NY-F, Wong S-C, Chu AW-Y, et al. The molecular history of *IDH*-mutant astrocytomas without adjuvant treatment. Brain Pathology. 2024. e13300. https://doi.org/10.1111/bpa.13300