



Published in final edited form as:

Nat Rev Cancer. 2014 February ; 14(2): 92–107. doi:10.1038/nrc3655.

Paediatric and adult glioblastoma: multiform (epi)genomic culprits emerge

Dominik Sturm^{1,2,*}, Sebastian Bender^{1,2,*}, David T.W. Jones¹, Peter Lichter³, Jacques Grill⁴, Oren Becher⁵, Cynthia Hawkins⁶, Jacek Majewski⁷, Chris Jones⁸, Joseph F. Costello⁹, Antonio Iavarone¹⁰, Kenneth Aldape¹¹, Cameron W. Brennan¹², Nada Jabado^{7,#}, and Stefan M. Pfister^{1,2,#}

¹Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ) Heidelberg, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany

²Department of Pediatric Oncology, Hematology, and Immunology, Heidelberg University Hospital, Im Neuenheimer Feld 430, D-69120 Heidelberg, Germany

³Division of Molecular Genetics, German Cancer Research Center (DKFZ) Heidelberg, Im Neuenheimer Feld 580, D-69120 Heidelberg, Germany

⁴Brain Tumor Program, Department of Pediatric and Adolescent Oncology, Gustave Roussy Cancer Institute, Université Paris Sud, 114 Rue Edouard Vaillant, 94805 Villejuif, France

⁵Division of Pediatric Hematology/Oncology, Duke University Medical Center, DUMC 91001, Durham, NC 27710, USA

⁶The Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, 555 University Avenue, Toronto, ON, M5G 1X8, Canada

⁷Division of Experimental Medicine and Department of Human Genetics, McGill University and McGill University Health Centre, 2155 Guy Street, Montreal, QC, H3H 2R9, Canada

⁸Divisions of Molecular Pathology and Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK

⁹Brain Tumor Research Center, Department of Neurosurgery, University of California, 2340 Sutter St., San Francisco, CA 94143, USA

¹⁰Institute for Cancer Genetics and Departments of Pathology and Neurology, Columbia University Medical Center, 1130 St. Nicholas Avenue, New York, NY 10032, USA

¹¹Department of Neuro-Oncology, University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd. Unit 0085, Houston, TX 77030, USA

@To whom correspondence should be addressed: Nada Jabado, Division of Experimental Medicine and Department of Human Genetics, McGill University and McGill University Health Centre, 2155 Guy Street, Montreal, QC, H3H 2R9, Canada, nada.jabado@mcgill.ca, Phone: +1 514 412 4400, Fax: +1 514 412 4331 and/or Stefan M. Pfister, Department of Pediatric Neurooncology, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany, s.pfister@dkfz.de, Phone: +49 6221 42 4618, Fax: +49 6221 42 4639.

*These authors contributed equally.

#Joint senior authors

Copyright information

None of the figures or other display items have been modified or reproduced from any other publication (either in print or online).

¹²Human Oncology & Pathogenesis Program and Department of Neurosurgery, Brain Tumor Center, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA

Preface

We have extended our understanding of the molecular biology underlying adult glioblastoma over many years. In contrast, high-grade gliomas in children and adolescents have remained a relatively under-investigated disease. The latest large-scale genomic and epigenomic profiling studies have yielded an unprecedented abundance of novel data and revealed deeper insights into gliomagenesis across all age groups, highlighting key distinctions, but also some commonalities. As we are on the verge of dissecting glioblastomas into meaningful biological subgroups, this Review summarizes the hallmark genetic alterations associated with distinct epigenetic features and patient characteristics in both paediatric and adult disease, and examines the complex interplay between the glioblastoma genome and epigenome.

Introduction

Glioblastoma (GBM; synonymous with the formerly used term ‘glioblastoma multiforme’) is the most frequent and most aggressive malignant primary brain tumour^{1, 2}, classified as grade IV in the World Health Organization (WHO) classification of tumours of the central nervous system (CNS)¹. GBM can develop from lower-grade diffuse astrocytoma (WHO grade II) and anaplastic astrocytoma (WHO grade III), and is then termed ‘secondary’ GBM. ‘Primary’ (*de novo*) GBMs are more common, and typically manifest rapidly after a short clinical history and without recognizable signs of a preceding precursor lesion³. Diagnostic criteria for GBM, including high mitotic activity, microvascular proliferation and/or necrotic areas, are fulfilled by both primary and secondary GBM, making them indistinguishable by histology alone¹.

Overall, primary GBM accounts for 16% of primary brain tumours². The incidence increases with age, affecting an average of 7.2 per 100,000 adults (> 19 years of age) every year, with a peak age at diagnosis between 75 and 84 years (annual incidence rate 14.6 per 100,000)². Due to the lower incidence in children, studies on paediatric high-grade gliomas (HGG) have traditionally combined GBM, anaplastic astrocytomas and diffuse intrinsic pontine gliomas (DIPG). Although most DIPG histologically present as high-grade tumours and share a universally fatal outcome, they comprise tumours with varying histological grade and divergent differentiation⁴⁻⁷. Together, up to 0.8 per 100,000 children (age < 19) are estimated to develop high-grade gliomas each year, making them the most common group of malignant CNS neoplasms in this age group alongside embryonal tumours². The current treatment strategy for GBM patients consists of surgery, radiotherapy and chemotherapy. Complete surgical resection of these infiltrative tumours is virtually impossible and surgery is typically not attempted for DIPG patients due to their location. Concurrent adjuvant radiotherapy in combination with temozolomide (TMZ) represents the standard of care for newly diagnosed GBM, but still less than 5% of patients survive longer than five years post diagnosis^{2, 8-10}. Since no chemotherapeutic drugs have proven to be effective in the treatment of DIPG, radiation therapy alone represents the current standard

regimen^{11, 12}. Prognosis here is even worse, with less than 10% of DIPG patients surviving more than two years after diagnosis^{2, 13}.

Over recent years, large-scale research efforts spearheaded by The Cancer Genome Atlas (TCGA) network and the paediatric neurooncology community have been directed at studying the molecular biology underlying GBM, and have generated detailed catalogues of genomic and epigenomic alterations. The emerging insights into gliomagenesis have triggered an avalanche of novel perceptions on the epigenomic and genomic landscape, biological subgroups and putative cells of origin of GBM, fuelling hopes for more effective treatment strategies in the near future. In this Review, we discuss the major recent advances in GBM molecular research, and we aim to convey important details, as well as a general overview of GBM biology across all ages.

Molecular subtypes of glioblastoma

Gene expression profiling of glioblastoma—Genome-wide profiling studies using gene expression microarrays were successfully used to compare gene expression patterns in primary *vs.* secondary GBM^{14–17} or adult *vs.* paediatric GBM¹⁸, and to identify differentially expressed genes that could be used to distinguish between different groups. In parallel, early reports showed the utility of expression arrays in diagnostic assignment and prognostication within larger glioma cohorts of different grades^{19–21} and within GBM cohorts only²². Phillips and colleagues²³ described three subclasses of high-grade glioma (termed proneural, mesenchymal and proliferative, based on the functional annotation of signature genes) as being associated with different outcomes, namely prolonged survival of the proneural subclass. Similar subclasses of GBM were also detected in a large cohort of mixed gliomas and data from this cohort also revealed a distinct gene expression cluster enriched for secondary GBM²⁴. Partially in line with these previous findings, unsupervised clustering of gene expression data from 200 adult GBM samples from TCGA network^{25, 26} identified four different molecular subtypes: proneural, neural, classical and mesenchymal. The proneural subtype was largely characterized by abnormalities in platelet derived growth factor receptor α (*PDGFRA*) or isocitrate dehydrogenase 1 (*IDH1*), whereas mutation of the epidermal growth factor receptor (*EGFR*) was found in the classical subgroup and mutations in neurofibromin (*NF1*) were common in mesenchymal tumours. This classification system has been refined by subdividing proneural GBM into glioma-CpG island methylator phenotype (G-CIMP)-positive and G-CIMP-negative GBM subsets based on characteristic DNA methylation patterns that strongly correspond with *IDH1* mutation status²⁷ (Figure 1; discussed below). Comparing the transcriptional classification schemes of Phillips *et al.*²³ with those of Verhaak *et al.*²⁵ suggests that the proneural and mesenchymal signatures are the most robust and account for the greatest transcriptional variance, while the exact number and composition of other signatures may depend on the sample cohort compositions and experimental designs^{28, 29}. The transcription factors signal transducer and activator of transcription 3 (*STAT3*), *C/EBP β* and *TAZ* have been identified as epigenetic master regulators of the mesenchymal signature and might predict poor clinical outcome^{30, 31}. Moreover, *CTNND2* (encoding cyclin D2) and *RHPN2* have recently emerged as negative and positive genetic drivers of mesenchymal transformation, respectively^{32, 33}. There is also evidence of plasticity between the proneural and mesenchymal subtypes in GBM²³ that

might be driven by the tumour microenvironment, perhaps through microglia and the NF- κ B pathway³⁴. The role that the proneural and mesenchymal signatures and other signatures might have as predictors of clinical outcome remains to be clarified. The initially reported better prognosis of the proneural subclass^{23, 25} was recently shown to only prove true for the G-CIMP subset, while non-G-CIMP proneural and mesenchymal GBM tend to show less favourable outcomes in the first twelve months post diagnosis compared with other GBM subtypes²⁶.

Initial gene expression studies of paediatric HGG highlighted significant differences compared to tumours from adult patients, and suggested the existence of molecularly diverse subsets within paediatric cohorts^{18, 35–37}. Activation of a *PDGFRA*-driven gene expression signature has been described in a large proportion of paediatric HGG^{4, 36}, correlating with the higher frequency of *PDGFRA* amplifications observed in this age group^{36–40}. Interestingly, one of these studies further revealed shared gene expression patterns between DIPG and a proportion of midline/thalamic GBM, pointing towards the closely related pathogenesis of these tumours, which was later confirmed by genome sequencing studies^{40–42}. Indeed, comparing midline GBM and DIPG that harboured a K27M amino acid change owing to a mutation in *H3F3A*, one of the histone H3.3 genes, with hemispheric GBM with *H3F3A* mutations that result in amino acid changes at G34 revealed specific gene expression profiles that are distinct from each other and from tumours with wild-type *H3F3A* status^{40, 41, 43, 44}.

DNA methylation profiling of glioblastoma—A number of studies have reported promoter-associated hypermethylation of specific loci in GBM, frequently affecting the expression of genes with known tumour suppressor function such as cyclin dependent kinase inhibitor 2A (*CDKN2A*), *RBI*, *PTEN* and *TP53*^{45–48}, as well as previously unrecognised regulatory genes involved in cell proliferation (epithelial membrane protein 3 (*EMP3*) and *PDGFB*)^{49, 50}, invasion (protocadherin γ subfamily A, member 11 (*PCDHGA11*))⁵¹, or radiation sensitivity (suppressor of cytokine signalling 1 (*SOC1*))^{52, 53}. Most prominently, promoter methylation of *MGMT* (encoding O⁶-methylguanine methyltransferase) occurs in ~ 45% of GBM in adult patients^{26, 54–57}, leading to gene silencing and consequently to a reduced proficiency for repairing DNA damage induced by alkylating agent chemotherapy^{58–61}. Thus, methylation at this locus has been established as a biomarker for predicting the benefit of TMZ chemotherapy, particularly in elderly GBM patients^{56, 57, 61, 62}, while both the frequency (16–50%)^{63–66} and prognostic significance of *MGMT* silencing in childhood HGG remain controversial^{63, 66}.

DNA microarray techniques have successfully been applied to study the GBM methylome in a genome-wide manner, and have led to the discovery of clearly defined GBM subgroups based on their global DNA methylation patterns (Figure 1). The first large study, with a purely adult patient cohort, reported three DNA methylation subgroups. One group was tightly associated with *IDH1* mutations and displayed concerted hypermethylation at a large number of loci, and was therefore termed G-CIMP-positive²⁷. A later study comparing DNA methylation patterns across both paediatric and adult GBM patients found a similar clustering in tumours from adult patients, and further identified three more distinct clusters composed predominantly of children and adolescents⁴⁰. Two of these corresponded strictly

with recurrent age-specific (K27 or G34) mutations in *H3F3A*, and another group of tumours was enriched for *PDGFRA* alterations (and termed receptor tyrosine kinase 1 (RTK I) and consisted of patients from a more widespread age range⁴⁰. Aberrations of *PDGFRA* are present in approximately half of G-CIMP-negative GBM of the proneural subtype in children and adults^{25, 40, 67}. Epigenetic silencing by promoter hypermethylation of the neural lineage markers *OLIG1* and *OLIG2* was observed as a novel and exclusive characteristic in GBM from the G34 cluster and points towards a different pathogenesis of this subgroup. These tumours also showed a considerable decrease in overall DNA methylation on a global scale⁴⁰. This has been previously reported as a common epigenetic feature in primary GBM and was linked to genomic instability of affected regions, deficiency of the methyl group metabolism gene *MTHFR* and increased proliferative activity⁶⁸. A distinct G-CIMP-positive IDH cluster was also identified and, in line with previous reports, tumours from this group were found among younger adult patients, showed proneural gene expression patterns, lacked typical copy-number aberrations (gain of chromosome 7, loss of chromosome 10, *EGFR* amplification, *CDKN2A* and *CDKN2B* deletion), and were associated with a favourable prognosis compared to all other GBM subgroups^{27, 40}, explaining the increased survival observed in the proneural GBM subtype^{23, 25}. The remaining G-CIMP-negative DNA methylation clusters comprised GBM from elderly patients, displaying predominantly classical gene expression, combined gain of chromosome 7 and loss of chromosome 10, and *EGFR*, *CDKN2A* and *CDKN2B* alterations (termed cluster #2 and RTK II)^{27, 40}, and GBM enriched for mesenchymal gene expression patterns and mutations in *NF1* and *PTEN* (termed cluster #3 and mesenchymal subtype)^{27, 40}.

Although mutations in *H3F3A* and *IDH1* seem to map one-to-one with three distinct DNA methylation subclasses, recent in-depth analysis of genome-wide DNA methylation and sequencing data by TCGA has found no similarly striking pathognomonic mutations for the additional non-G-CIMP subclasses of adult GBM²⁶. This study further confirmed the notion that, despite considerable overlap, established gene expression subclasses do not directly correspond to those defined by DNA methylation profiling²⁶, unlike DNA methylation profiling in medulloblastoma⁶⁹. Whether DNA methylation profiling provides a more robust and clinically useful platform for GBM subgrouping remains to be tested, but the logistics involved are probably less error-prone than for any RNA-based analyses.

Structural variations in glioblastoma

DNA copy-number aberrations (CNAs) are commonly observed in GBM, and can affect a significant fraction of the tumour genome^{67, 70}. Assessing DNA copy-number using high-resolution techniques in large collections of tumour samples has allowed the precise characterization of focal CNA target regions, and genome-wide sequencing studies are beginning to unravel more complex structural rearrangements and hitherto unknown fusion events.

Chromosomal aberrations and genomic rearrangements—Genomic gains of chromosome 7 and losses of chromosome 10 (most often occurring concomitantly; 7⁺/10⁻) represent by far the most common gross chromosomal abnormalities in GBM, being

detected in 83–85% of GBM in adults^{26, 67} (Table 1). Consistently throughout reported studies, 7⁺/10⁻ is more frequent in older (age > 70 years) compared to younger (age < 40 years) patients⁷¹, and is therefore highly enriched (> 95 %) in GBM from the classical (or RTK II) subclass, but less common in GBM of the proneural gene expression subtype^{23, 25}, and virtually absent in *H3F3A*- or *IDH*-mutant G-CIMP-positive tumours^{27, 40} (Figure 1). Further broad genomic copy-number changes seen at high frequency (> 20%) in adult GBM patients include gains of chromosomes 19 and 20 (35–40%; enriched in the classical (or RTK II) subtype), and losses affecting chromosomes 9p (38%), 22q (33%), 13q (33%), 14q (27%) and 6q (22%)^{26, 67} (Table 1). The number of chromosomal imbalances is generally lower in childhood HGG and a proportion of these tumours (~ 15%) lack any detectable copy-number abnormalities^{36, 38, 40, 72}. Paediatric tumours also differ from their adult counterparts in displaying frequent gain of chromosome 1q (20%; enriched in *H3F3A* G34-mutated GBM), while only rarely harbouring aberrations of chromosomes 7 and 10^{36, 38}.

Recent data indicate that the incidence of complex chromosomal rearrangements in the context of a single catastrophic event (*chromothripsis*) is significantly higher in GBM (> 30%) relative to most other tumour types (9%), including breast, colon and lung cancer⁷⁰. Interchromosomal, intrachromosomal (intergenic) and intragenic rearrangements can be detected in the majority (69%) of GBM samples, and these frequently (56%) co-occur with intragenic copy-number differences at the breakpoints⁷³. The most prominent intragenic deletions in GBM target parts of the gene encoding either the extracellular domain of *EGFR* (exons 2–7 to form *EGFRvIII*) or the carboxy terminus^{73–76}, and are almost always associated with amplification and co-expression of the wild-type *EGFR* allele^{26, 76, 77} (Figure 2 and Table 2). Similarly, a fraction of *PDGFRA*-amplified GBM from both adults and children harbour age-specific intragenic deletion rearrangements of this kinase with constitutively increased activity^{78, 79}. Further intragenic deletions disrupt the function of the tumour suppressor candidate gene Fas-associated factor 1 (*FAF1*) as a result of focal deletion of the adjacent gene, *CDKN2C*⁷³.

A breakpoint-enriched region on chromosome 12q (12q14-15), identified in ~ 5% of GBM, was recently shown to harbour copy-number equal co-amplification of the two known GBM oncogenes *CDK4* and *MDM2*, assembled into double minute chromosomes^{73, 80}. Additional fusion transcripts between genes included in the two amplicon segments were also observed⁷³. This phenomenon was found to be mutually exclusive with *CDKN2A* and *CDKN2B* deletions and *TP53* mutations, and associated with adverse patient survival⁷³. Similarly, a number of gliomas were previously found to harbour double minute chromosomes bearing amplified copies of the *EGFR* gene⁸¹ (Figure 2). More recently, *EGFR* was shown to be further activated by recurrent translocations in 7% of GBM samples, most frequently being fused in-frame to septin 14 (*SEPT14*) or phosphoserine phosphatase (*PSPH*) as the 3' gene segment, and almost always occurring within amplified regions of the fusion partner genes^{26, 32}. In a smaller fraction of GBM (~ 3%), local inversion and in-frame fusion of the tyrosine kinase coding region of fibroblast growth factor receptor 1 (*FGFR1*) to the transforming acidic coiled-coil (TACC) coding domain of *TAC1* (or alternatively fusion of *FGFR3* to *TACC3*) results in constitutive kinase activity^{82, 83}. Therefore, recurrent fusion events involving RTK-encoding genes may represent a

promising therapeutic target and provide a strong rationale for the inclusion of these patients in future clinical trials using RTK inhibitors.

Focal copy-number aberrations—The spectrum of high-amplitude focal copy-number alterations in adult GBM highlights a key role of *EGFR* amplifications (43% of cases) and *CDKN2A* and *CDKN2B* deletions (53% of cases)²⁶, although these events are rarely detected in paediatric HGG (< 5% and ~ 20% of cases, respectively)^{38, 40, 72} (Figure 1 and Table 2). Both events are enriched in the classical (or RTK II) and neural molecular subtypes²⁵ and co-occur with *EGFR* intragenic deletions and/or point mutations^{25, 26, 76}. Known genes less frequently targeted by homozygous deletion in adult GBM include *PTEN* (10%), *RBI* (3%), *CDKN2C* and *FAF1* (3%), *QKI* (1.6%), *NF1* (1.3%), *NPAS3* (1.3%) and *TP53* (1%)²⁶. High-level amplifications of *CDK4* (13%), *PDGFRA* (11%), *MDM2* (8%), *MDM4* (7%), *MET* (2%) and *CDK6* (1.5%) often co-occur in a variety of combinations²⁶, with common co-amplification of RTK-encoding genes in a mosaic-like pattern (Box 1). Amplifications of *CCND2*, *CCNE1*, *SOX2*, *MYC* and *MYCN* represent recurrent but less frequent events in adult GBM (< 3%)²⁶. Although to some extent most of these gains are also observed in paediatric HGG, focal amplifications of *PDGFRA* (14%) and *MYC* or *MYCN* (8%) are more frequent in the paediatric population^{38, 40, 72, 84}. Recurrent focal amplification of *PDGFRA* has been suggested as a key oncogenic event in DIPG in a number of studies^{4, 37, 85}. With increasing molecular data on treatment-naïve DIPG, however, the occurrence of this feature seems to be slightly lower than previously reported (< 40%), and enriched in DIPG harbouring histone H3 mutations⁴⁰ (C.J., J.G., O.B., C.H. J.M. and N.J., unpublished observations). As *PDGFRA* amplification is somewhat more commonly observed in radiation-induced gliomas³⁶, one might hypothesize a possible link between *PDGFRA* amplifications and prior radiotherapy, a caveat when studying *post mortem* DIPG (and GBM) tissue obtained from autopsy cases.

Box 1

Tumour heterogeneity in glioblastoma

Intratumoural heterogeneity, referring to the presence of multiple, epigenetically and genetically different cell sub-populations within a single tumour, might contribute to tumour growth, progression and treatment failure¹⁸⁰. In glioblastoma (GBM), the previously used term ‘multiforme’ describes the histopathologically observed co-existence of morphologically heterogeneous areas¹. On a molecular level, this phenomenon is well reflected by different area-specific chromosome aberrations^{181–183}, mutations¹⁸⁴ and gene expression patterns^{22, 185, 186}. Multiple receptor tyrosine kinase (RTK) genes (epidermal growth factor receptor (*EGFR*), platelet derived growth factor receptor α (*PDGFRA*) and *MET*) can be amplified and activated in a mutually exclusive manner in adjacent intermingled tumour cells^{178, 179, 187}, and even multiple *EGFR* mutant alleles can commonly be expressed in a single tumour²⁶. Arising from a single precursor and following an early driver event (such as loss of cyclin dependent kinase inhibitor 2A (*CDKN2A*) and *CDKN2B*)^{186, 187}, activation of different RTKs in equally proliferating sub-populations may have distinct effects on downstream signalling pathways not only within but also between and among heterogeneous sub-populations *via*

cell-cell interactions¹⁸⁷. Indeed, a paracrine mechanism has been identified by which cells expressing EGFRvIII recruit wild-type EGFR-expressing cells into accelerated proliferation through upregulation of interleukin 6 (IL-6), leukaemia inhibitor factor (LIF) and GP13, thereby potentially driving growth of the entire tumour mass and maintaining tumour cell heterogeneity¹⁸⁸. These observations have important implications regarding the effects of RTK-targeted therapies, as tumours that have mosaic driver RTK amplifications will probably require the simultaneous use of more than one targeted agent^{178, 189}. Furthermore, the impact of sampling bias over space and time is an important diagnostic consideration, particularly in the era of targeted therapy¹⁸⁶. Tumour heterogeneity in GBM may also arise from clonal evolution, i.e. through the expansion and acquisition of mutations during tumour progression, promoting genetic variability¹⁹⁰. This concept may be linked with the hierarchical cancer stem cell (CSC) model, which postulates a small sub-population of stem-like cells that give rise to a tumour and are maintained through their capability to self-renew and to produce diverse daughter cells that populate the tumour bulk¹⁹¹.

Mutational spectrum of glioblastoma

Advances in sequencing technology have led to the identification of increasing numbers of recurrent somatic mutations in the GBM genome of adult patients (Table 2). The number of coding mutations per tumour sample is highly variable (median: ~ 53; range: 3–179)²⁶. Frequent mutations in genes such as *PTEN* (29%), *TP53* (29%), *EGFR* (20%), *NF1* (9%), *RBI* (8%), phosphatidylinositol 3 catalytic α (*PIK3CA*; 7%), *PIK3R1* (6%) and *IDH1* (5%) have been reported as significant GBM signature events in earlier studies^{67, 86}. The increasing number of samples with genome-wide sequencing data has now facilitated the detection of lower-frequency events in both cancer-related as well as previously un-associated genes such as spectrin α (*SPTA1*; 9%), *ATRX* (6%), *KEL* (5%), gaba-aminobutyric acid α 6 (*GABRA6*; 4%), leucine zipper-like transcriptional regulator 1 (*LZTR1*; 3%), *BCOR* (2%), *BRAF* (2%), catenin δ 2 (*CTNND2* (2%)) and *QKI* (2%)²⁶. Some of those genes (such as *LZTR1*, *CTNND2*, *QKI*, *BRAF* and *BCOR*) are recurrently targeted by both copy-number changes and point mutations, thus underscoring their role as new driver genes³². Of note, there is a non-random pattern of mutual exclusivities (such as *PIK3R1* and *PIK3CA*)^{26, 67} (C.J., J.G., J.M. and N.J., unpublished observations) and co-occurrences (such as *IDH1*, *TP53* and *ATRX*)^{26, 87, 88}, and multiple but overall mutually exclusive mutations in genes implicated in regulation of chromatin modification²⁶, a phenomenon recently observed in various other cancer types^{89, 90}. Based on genome-wide sequencing data from 284 GBM samples generated by TCGA²⁶, 46% of cases were found to have at least one somatic mutation in a gene associated with DNA methylation (*IDH1*), histone modifications (SET domain containing protein 2 (*SETD2*) and lysine-specific demethylase 6A (*KDM6A*), mixed lineage leukaemia 2 (*MLL2*), *MLL3*, *MLL4*, *EZH2* and histone deacetylase 2 (*HDAC2*)) or chromatin remodelling (*ATRX*, CREB binding protein (*CREBBP*), chromodomain helicase DNA binding protein 5 (*CHD5*), *CHD6*, *CHD7*, *CHD8*, *CHD9* and SWI/SNF-related matrix-associated, actin-dependent regulator of chromatin A2 (*SMARCA2*)) (Figure 2).

Importantly, 58–84% of primary GBM in adults have hotspot mutations in the *TERT* gene promoter. These mutations are tightly coupled with *EGFR* amplifications and inversely correlated with *IDH1*, *IDH2*, *TP53* and *ATRX* mutations^{26, 91–95}, and are associated with increased telomerase expression and activity^{26, 92, 94, 96} (Figure 3). *TERT* promoter mutations are found at a much lower rate (3–11%) in childhood GBM^{91, 95}, which instead frequently display loss of *ATRX* and an alternative lengthening of telomeres (ALT) phenotype to maintain or increase telomere length^{41, 97}.

Moreover, recent work has identified novel oncogenic mutations in *PDGFRA* in a proportion of paediatric HGG, often in combination with amplification of the *PDGFRA* locus^{4, 79}. Of further note and clinical relevance, inactivating mutations in DNA mismatch repair (MMR) genes (such as *MSH6*) can be induced during TMZ treatment and evoke a GBM hypermutator phenotype, which is causally related to TMZ resistance^{67, 98–100}.

Histone mutations in childhood high-grade gliomas—The first recurrent histone mutations in human cancer were uncovered by sequencing studies on a large number of paediatric GBM and DIPG patient samples^{41, 101}. Heterozygous mutations in *H3F3A* result in amino acid substitutions at positions K27 or G34, and K27 was also found to be mutated in the H3.1 histone genes *HIST1H3B* and *HIST1H3C*^{41, 101} (C.J., J.G., O.B., C.H., J.M. and N.J., unpublished observations). These mutations directly or indirectly target important sites on the histone tail for posttranslational modifications (Figure 2). Occurring exclusively in ~38% of childhood and young adult (< 30 years) HGG^{41, 102} (Table 2) and in a mutually exclusive fashion with each other and with mutations in *IDH1*, each histone mutation shows a strong association with distinct molecular features as well as clinical patient characteristics^{5, 40, 42} (Figure 1). Mutations in *H3F3A* that result in amino acid changes at G34 occur in adolescents around the age of 20 years that have disease exclusively located in hemispheric regions. A significant number of these tumours have mutations in *TP53*, *ATRX* and (less frequently) *DAXX*^{40, 41}, and commonly display a global pattern of DNA hypomethylation and an ALT phenotype⁴⁰. Mutations in *H3F3A* that result in amino acid changes at K27 occur in 70–80% of midline GBM and DIPG in younger children^{5, 40, 101}. The same substitutions in *HIST1H3B* or *HIST1H3C* seem to be less common and specific to DIPG (11–31%) and to coincide with novel activating mutations in *ACVR1* (also known as *ALK2*) identified in ~20% of DIPG cases (C.J., J.G., O.B., C.H., J.M. and N.J., unpublished observations; Suzanne J. Baker, personal communication), providing a potentially druggable target in this subset. Interestingly, recent studies also detected *H3F3A* K27M mutations in a small fraction (< 2%) of midline pilocytic astrocytomas (WHO grade I)^{103, 104}, conceivably indicating some overlap between the genetics of paediatric low-grade and high-grade gliomas or, alternatively, a remaining degree of diagnostic uncertainty. GBM harbouring mutations in histone H3 genes are mostly devoid of hallmark cytogenetic aberrations, such as *EGFR* alterations and *CDKN2A* and *CDKN2B* deletions observed in adult GBM patients⁴⁰ (Figure 1). Interestingly, mutations at K27 seem to confer a dismal prognosis^{5, 40}, while G34 mutations might be associated with slightly prolonged overall survival⁴⁰. However, these initial observations will have to be tested in larger, prospective patient cohorts.

A gain-of-function K27M mutation in H3.3 or H3.1 leads to global downregulation of the repressive histone mark H3K27me3 through inhibition of the Polycomb repressive complex 2 (PRC2)^{44, 105–107}. K27M-mutant H3.3 was shown to aberrantly bind PRC2 and interfere with the enzymatic activity of EZH2 – the catalytic subunit of PRC2 that establishes the H3K27me3 mark – possibly due to methionine mimicking the structure of monomethyl lysine¹⁰⁶. Consequent loss of repressive PRC2 activity resulting from a combination of reduced H3K27me3 and DNA hypomethylation has therefore been proposed as the main driver of gliomagenesis in K27M-mutant HGG^{44, 106} (Figure 2).

In a similar vein, G34R and G34V-mutant H3.3 can interfere with the regulatory H3K36me3 modification^{43, 106}. Genome-wide mapping of H3K36me3 in a G34V-mutant GBM cell line identified *MYCN* among the genes most strongly enriched for H3K36me3 marks, with increased RNA polymerase II binding and transcriptional upregulation of this locus in G34V-mutant cells⁴³. Therefore, in addition to *MYCN* focal amplification, H3.3 G34 mutations may represent an alternative mechanism to overexpress *MYCN*, which was recently shown to drive glioma formation in neural stem cells (NSCs) *in vivo*¹⁰⁸, and could potentially be targeted by bromodomain inhibition¹⁰⁹. H3K36me3 is further disrupted by mutations in the H3K36 trimethyltransferase SETD2 in children and young adults, which are mutually exclusive with *H3F3A* G34 mutations¹¹⁰ (Figure 2). Besides its role in transcriptional elongation¹⁰⁶, H3K36me3 was found to be important as a recruitment platform for MMR proteins¹¹¹. Depletion of SETD2 and therefore H3K36me3 levels in HeLa cells leads to an increased spontaneous mutation frequency and chromosomal instability¹¹¹. In line with this, altered histone modifications in subtelomeric regions have been suggested to contribute to deregulated telomere length, also resulting in chromosomal instability. This is reflected by a particularly high number of paediatric GBM displaying ALT⁴¹ (Figure 3). Although genetic hits in *ATRX* and *DAXX* (both essential components for H3.3 incorporation at pericentromeric heterochromatin and at telomeres) are known to promote the ALT phenotype, the contribution of mutant p53¹¹², histone modifications and subtelomeric DNA hypomethylation to GBM development needs further investigation.

IDH mutations in glioblastomas of young adults—In the early stages of the next-generation sequencing era, the first genome-wide exon sequencing effort in glioma identified heterozygous hotspot mutations at codon 132 (most commonly R132H) in *IDH1* in 12% of GBM⁸⁶. Today, mutations in *IDH1* (and to a lesser extent in *IDH2*) are commonly established as a hallmark molecular feature of secondary GBM in young adults (age 25–45 years; being present in ~ 70% compared to < 5% in primary GBM)^{113–116} with predominant localisation in the frontal and temporal lobes¹¹⁷. The small number of *IDH*-mutant primary GBM may therefore have rapidly progressed from clinically undiagnosed lower-grade astrocytomas. Mutations in the *IDH* genes are thought to be causative of G-CIMP within the proneural GBM subgroup^{23, 25, 27, 40, 118} (Figure 1). The role of mutations in *IDH* genes in gliomas with respect to molecular pathogenesis as well as clinical implications has recently been reviewed in detail^{119, 120}. *IDH* mutations seem to require cooperating mutations in *TP53* and *ATRX*^{87, 88}, and possibly *SETD2*¹¹⁰, while being less frequently detected in GBM with classical RTK pathway alterations^{40, 67, 121}. Besides being a powerful diagnostic marker¹²², IDH-mutant GBM have a favourable clinical outcome compared to their non-

mutated counterparts^{26, 123}. The main mechanism by which mutant IDH contributes to the pathogenesis of GBM is ascribed to the neomorphic enzymatic activity of mutant IDH proteins, which produce high amounts of the R enantiomer of 2-hydroxyglutarate (2-HG) (a putative oncometabolite) from 2-oxoglutarate (2-OG; also known as α -ketoglutarate)^{124, 125}. 2-HG has been shown to inhibit a variety of 2-oxoglutarate-dependent dioxygenases, including key epigenetic regulators such as the DNA demethylating enzymes of the TET¹²⁶ or Jumonji domain families^{127, 128} (Figure 2). Hence, the methylome of IDH-mutant GBM is dominated by widespread DNA hypermethylation at gene promoters (G-CIMP)¹¹⁸, irrespective of patient age⁴⁰. The interaction of TET2 with particular transcription factors, such as EBF1, might result in specific rather than random gene promoter hypermethylation¹²⁹. Although there is a strong association of IDH mutation with G-CIMP, early passage neurosphere cultures from IDH wild-type GBM can show a G-CIMP-like phenotype, suggesting intratumoural heterogeneity and/or the involvement of other, as yet unknown, factors³⁴.

The cells of origin of glioblastoma

Although multiple genetic and epigenetic alterations are known to initiate or promote gliomagenesis, answers as to the cell of origin that acquires the first tumour-promoting mutation remain elusive. Several cell types including NSCs^{130, 131}, more differentiated progeny such as oligodendrocyte precursors^{132, 133}, or cells of the astrocytic lineage are discussed as putative glioma-initiating cells. Indeed, all these cell types can give rise to glioma if genetically manipulated (Box 2). Interestingly, malignant transformation seems not to be limited to undifferentiated cell types – transformation of mature neurons or astrocytes by depletion of *Nf1* and *Trp53* has also been shown to generate malignant gliomas *in vivo*¹³⁴. Various pieces of evidence, including differences in tumour location, patient age, mutational spectrum or expression of neuronal lineage markers have led to growing speculation that in addition to acquiring distinct genetic events, at least some of the GBM subgroups described above also have unique cellular origins. This hypothesis might especially prove true for the tight correlations between molecular and clinical characteristics observed in DIPG and midline GBM, which possibly originate from recently identified pontine precursor-like cells¹³⁵.

Box 2

In vivo tumour models of glioblastoma

In vivo tumour models provide an indispensable tool to study the contribution of cancer-related genes or develop and evaluate novel treatment options. Strategies to reproduce glioblastoma (GBM) tumours in animals include xenograft transplantation models, germline modification of mouse strains and/or somatic gene transfer^{192, 193}. Orthotopic injection of primary human GBM cells into immunocompromised mice gives rise to tumours partially retaining histological features and key molecular characteristics of human GBM, but with the disadvantages of altered tumour immunology and absence of a natural microenvironment^{194–197}. To recapitulate the diversity of human GBM subgroups^{23, 25, 40}, genetically engineered mouse models allowing for spontaneous tumour growth have been generated by adopting key glioma signature mutations in

receptor tyrosine kinase (RTK) genes, *TP53*, *PTEN*, *RB*, *NF1* and in cell-cycle pathways^{192, 193, 198, 199}. While overexpression of epidermal growth factor receptor (EGFR) alone or EGFRvIII alone is insufficient in effectively promoting tumour formation from neuroglial precursors²⁰⁰ or astrocytic cells^{200, 201}, concomitant overexpression of EGFRvIII (and less efficiently wild-type EGFR) with loss of the cyclin dependent kinase inhibitor 2A (*Cdkn2a*) and/or *Pten* and *Trp53* loci is capable of inducing GBM^{202–205} that might resemble human tumours of the classical transcriptome subtype^{25, 40}. Similarly, PDGF-driven gliomagenesis recapitulates GBM formation in mice when combined with germline loss of *Cdkn2a*, *Pten*, or *Trp53*^{206–208}. This model has been successfully used to study paediatric and adult GBM biology^{192, 206, 209, 210}, and is significantly enriched in proneural and RTK I GBM subtypes^{23, 25, 40}. The mesenchymal subtype of GBM might be best investigated using established models based on loss-of-function of *Nf1*, which cooperates with early *Trp53* mutations (and *Pten* haploinsufficiency) to induce astrocytomas of different grades^{131, 211–215}. Although molecular profiling of genetically engineered GBM mouse models suggests significant similarities with the adult human disease¹⁹⁹, they may not reflect intratumoural heterogeneity to the same degree observed in primary tumours, and many novel findings of recent genome-wide studies have not yet been successfully transferred into *in vivo* models, including recurrent mutations in isocitrate dehydrogenase 1 (*IDH1*)²¹⁶, *H3F3A*, *HIST1H3B*¹⁰⁶, *ATRX* and others. These are now being assessed in the context of reasonable co-oncogenic alterations in order to complement the currently available repertoire of GBM *in vivo* models.

It is widely thought that *IDH1*-mutant GBM might represent a distinct disease entity that probably arises from a different cellular origin compared with GBM in which IDH is not mutated¹³⁶. Based on a current model, mutations of *IDH1* represent an early event in gliomagenesis, priming quiescent neural progenitor cells for additional tumour-promoting genetic hits such as *TP53* mutations or 1p/19q co-deletions, leading to the manifestation of WHO grade II/III gliomas. Subsequent malignant transformation into grade IV GBM is mediated by additional key genomic alterations (such as RTK pathway activation or *PTEN* mutations)¹³⁶. This stepwise progression of secondary, *IDH1*-mutant GBM, with preservation of a proneural expression pattern, substantially differs from *de novo* GBM, which seem to originate as the result of genetic hits in a distinct cell population more closely resembling a NSC¹³⁶.

Lineage tracing experiments have indicated that oligodendrocyte precursor cells (OPCs) can be considered as cells of origin for *Nf1* and *Trp53*-mutant GBM, although identical genetic hits can also give rise to glioma when introduced into NSCs¹³². In a premalignant state, *in vivo* proliferation of *Nf1*/*Trp53*-mutant cells was found to be dramatically increased only in the OPC lineage and not NSCs or progeny lineages of astrocytes, neurons or oligodendrocytes. Fascinatingly, direct introduction of *Nf1* and *Trp53* mutations into OPCs led to the generation of gliomas that molecularly and histologically resembled tumours initiated from NSCs, with transcriptomes of both models corresponding well with the proneural subtype¹³².

Assuming that tumours partially retain the gene expression signature of their cellular origin, transcriptional profiles of *H3F3A* K27- and G34-mutant GBM were found to resemble those of distinct anatomical regions at different stages in the developing human brain^{40, 137}. Expression of *OLIG1* and *OLIG2* was originally thought to be a common feature of human gliomas^{138–140} and *OLIG2* was considered essential for promoting gliomagenesis *in vivo*^{141, 142}. However, *H3F3A* G34-mutant GBM presumably arise from NSCs or undifferentiated progenitor cells (not of oligodendroglial lineage) which epigenetically repress *OLIG1* and *OLIG2* expression⁴⁰ in order to preserve multipotency, unlike other glioma subtypes initiated from *OLIG2*-positive progenitor-like cells of the subventricular zone¹⁴³.

Novel strategies in glioblastoma therapy

Current postoperative standard treatment for GBM patients is mainly based on unselective induction of DNA damage *via* radiotherapy and alkylating agents such as TMZ⁸. The majority of drugs specifically targeting key signalling pathways and mechanisms of gliomagenesis, such as RTK signalling (erlotinib^{144–147}, gefitinib^{147, 148}, cetuximab¹⁴⁹ and imatinib^{150–153}) or angiogenesis (bevacizumab^{154, 155} and cediranib¹⁵⁶) do not provide a significant survival benefit when tested alone or in combination with other therapies (reviewed in¹⁵⁷). Comparable results were obtained when combining these drugs with pharmacological histone deacetylase (HDAC) inhibitors (such as panobinostat¹⁵⁸ and vorinostat^{158, 159}), although the use of an HDAC inhibitor such as valproic acid in addition to radiotherapy was associated with a better outcome in patients with GBM^{160, 161}. It should be noted, however, that efforts to identify molecularly defined patient subgroups for targeted therapies have generally been lacking, and it is possible that within the context of a negative clinical trial, subgroups of patients could be defined that would benefit from such a specific therapeutic approach. Indeed, the identification of several different molecular subgroups^{25, 27, 40} would argue against the use of one standardised therapy for GBM and the increasing knowledge about the genetic and/or epigenetic events driving these subgroups offers the opportunity to design innovative patient-tailored treatment protocols.

Given the high prevalence of IDH mutations in GBM and other malignancies such as acute myeloid leukaemia (AML), several studies have sought to therapeutically target the neomorphic enzymatic activity of mutant IDH1 and IDH2 using specific inhibitors^{162–164}. Recently, the small molecule AGI-5198 was shown to inhibit R132H-mutant IDH1, leading to reduced levels of 2-HG and substantial growth reduction of glioma cells *in vitro* and human glioma xenografts *in vivo*¹⁶². Interestingly, pharmacological blockade of mutant IDH1 did not reverse the G-CIMP in glioma xenografts, but induced expression of genes associated with astrocytic and oligodendrocytic differentiation¹⁶². Although not tested in GBM yet, a recent study has reported on the successful inhibition of mutant IDH2 by a small molecule (AGI-6780) in AML¹⁶⁴. This encouraging data demonstrates that these new drugs might represent an effective treatment option for patients with an IDH-mutant GBM.

In contrast, tumour growth and disease progression of GBM lacking mutations of *IDH1* or *IDH2* have recently been shown to utilize alternative energy resources such as branched-chain amino acids (BCAA) to compensate for increased metabolic demand. Expression of

branched-chain amino acid transaminase 1 (*BCAT1*) was found to be highly dependent on intracellular levels of 2-OG, which is mainly produced by (wild-type) IDH1¹⁶⁵. Impaired BCAA metabolism via knockdown of *BCAT1* or overexpression of mutant IDH1 resulted in reduced cell proliferation¹⁶⁵, rendering the reprogramming of energy metabolism an attractive target for innovative therapeutic approaches in GBM. Interestingly, the metabolic enzyme pyruvate kinase M2 (PKM2) was shown to have a non-metabolic function as a key regulator of histone phosphorylation and acetylation. Activation of EGFR (and also of PDGFR) results in activation of ERK2, which was found to phosphorylate PKM2 leading to nuclear translocation and promotion of the Warburg effect¹⁶⁶. Nuclear PKM2 was found to phosphorylate histone H3 at threonine 11, causing dissociation of HDAC3 from the *CCND1* and *MYC* gene promoters¹⁶⁷ (Figure 2). Subsequent expression of cyclin D1 and MYC due to increased acetylation of histone H3 at lysine 9 was found to induce cell proliferation and gliomagenesis¹⁶⁶.

The identification of histone mutations in a large proportion of HGG from paediatric and adolescent patients^{41, 101} has raised expectations for targeted treatment approaches that tackle these tumours^{40, 43}. Although the precise mechanism of K27M-mediated PRC2 inhibition is not fully understood, pharmacological intervention targeting K27M-mutant H3.3 or downstream consequences of this change might represent the ultimate therapeutic goal. Care must be taken here, however, since the role of the PRC2 complex in oncogenesis seems to be highly context-dependent, and unspecific pharmacological H3K27me3 upregulation in K27M-mutant tumours could in fact promote disease progression, as has been shown for other malignancies harbouring EZH2 activating mutations^{168–170}. The existence of ALT as the predominant telomere maintenance mechanism in the majority of H3-mutant HGG (especially those with G34R and G34V alterations)⁴¹ might also represent a promising biological target for the development of more powerful drugs in the future, as we begin to understand the underlying molecular details. In contrast, pharmacological inhibition of telomerase activity might be more effective in GBM in adult patients, given their dependence on high levels of telomerase expression¹⁷¹ (Figure 3). Despite the manifold differences between GBM subtypes, the evolving overall theme of epigenetic deregulation holds promise for epigenetic modulators to be active across subsets of epigenetically distinct tumours, as has recently been shown for a new class of agents targeting the BET domain proteins¹⁷².

Despite the identification of these multiple new targets in certain patient subgroups, bridging the gap between identification of recurrent genomic drivers, such as *EGFR*, and successful targeted therapies remains a considerable challenge, as indicated by the above-mentioned failures of many of the agents tested to date. The development of novel drugs requires accurate assessment of pharmacodynamic and pharmacokinetic parameters of drug activity in preclinical and clinical trials, and penetrance of the blood brain barrier is an additional challenge (although the integrity of this barrier in GBM is debatable). Although some drugs show detectable activity both *in vitro* and *in vivo*, downstream signal transducers may remain unaffected¹⁷³, and even for drugs able to induce a clinical response in GBM patients, treated tumours ultimately progress. One reason for the failure of early clinical trials may be a lack of patient selection for a specific therapy, implying that molecular determinants of the

response to a chosen intervention are needed¹⁷⁴. Another could be pre-existing¹⁷⁵ or emerging tumour resistance mechanisms in response to treatment, for example through activation of alternative RTKs as a result of EGFR inhibition¹⁷⁶.

Due to the inter- and intratumoural molecular heterogeneity of GBM, it is likely that only a small proportion of patients will benefit from therapeutic interventions aimed at any one target, but synergistic combination approaches may prove more effective^{177–179}. This likelihood raises the need for prospective tissue collection in order to maximize information gained from clinical trials. Molecular pathological stratification criteria are needed in this and other cancers to optimize enrolment in the therapeutic arm most adapted to tumour biology, and efficacy of a specific agent should be considered in the context of known and clinically relevant biological subsets of glioma.

Summary

Recent large-scale profiling studies have revealed a number of novel findings that have changed the way we look at the genomic and epigenomic landscape of GBM. The community is slowly gaining insight into the complex interactions between genetic alterations and changes in DNA methylation, histone modifications, chromatin remodelling and gene expression, and how deregulation on different levels might contribute to GBM pathogenesis. IDH and H3-mutated GBM are outstanding examples whereby disrupted epigenetic mechanisms due to genetic mutations result in the establishment of mutation-specific epigenetic and transcriptional programs. These and other recurrent genetic aberrations occur in a specific context of cellular origin, co-oncogenic hits and epigenetic phenomena, and are present in distinct patient populations. The biological discrimination of GBM subgroups should therefore guide the design of future clinical trials. In addition, the complex interplay of ‘hard-wired’ genetic events and potentially reversible alterations of the GBM epigenome offers novel opportunities for the development of molecularly targeted therapies, which might represent a promising strategy to tackle this deadly brain tumour.

Glossary

WHO Classification of tumours of the central nervous system	Classification system where histological grading is applied as a means of predicting the biological behaviour of a tumour. It ranges from benign tumours (grade I) to highly aggressive, rapidly progressing tumours with frequently fatal outcome (grade IV)
Glioma	Glioma refers to tumours that have histologic features similar to normal glial cells, i.e. astrocytes (astrocytoma), oligodendrocytes (oligodendroglioma), or ependymal cells (ependymoma), but is often used to imply only astrocytic or oligodendroglial tumours
Diffuse intrinsic pontine glioma (DIPG)	Highly infiltrative glial tumour arising in the pons. Occurs almost exclusively in children, with a peak age at diagnosis of between 5 and 9 years

Temozolomide (TMZ)	Alkylating chemotherapeutic agent used for the treatment of GBM. Triggers tumour cell death through extensive DNA damage
CpG Island Methylator Phenotype (CIMP)	DNA methylation pattern of widespread CpG island promoter methylation. CIMP is frequently reported to be associated with distinct tumour subgroups, patient prognosis and response to treatment
Chromothripsis	Clustered chromosomal rearrangements in one or a few chromosomes during cancer development, thought to occur through a one-step catastrophic genomic event
High amplitude focal copy-number alteration	Small fragment (typically ~ 3 Mb or smaller in size) of amplified or homozygously deleted DNA, often resulting in numerous copies of oncogenes or deletion of both copies of tumour suppressor genes
Double minute chromosomes	Small circular fragment of extrachromosomal DNA frequently harbouring one or more oncogenes
Alternative lengthening of telomeres (ALT)	One or more mechanism(s) by which 5–10% of human cancers maintain or increase the overall length of their telomeres without the need of increased telomerase activity. The exact molecular mechanism(s) of ALT remain elusive, but may rely on recombination-mediated elongation
Warburg effect	Predominant production of energy by a high rate of glycolysis followed by lactic acid fermentation in the cytosol observed in most cancer cells in the presence of oxygen
Polycomb repressive complex 2 (PRC2)	One of two classes of polycomb-group proteins. PRC2 has methyltransferase activity and primarily trimethylates histone H3 on lysine 27 (i.e. H3K27me3), a mark of transcriptionally silent chromatin

References

1. Louis, DN.; Ohgaki, H.; Wiestler, OD.; Cavenee, WK. WHO Classification of tumors of the central nervous system. IARC; Lyon: 2007.
2. Dolecek T, Propp J, Stroup N, Kruchko C. CBTRUS statistical report: primary brain and central nervous system tumors diagnosed in the United States in 2005–2009. *Neuro-oncology*. 2012; 14 (Suppl 5):49.
3. Ohgaki H, Kleihues P. Genetic pathways to primary and secondary glioblastoma. *Am J Pathol*. 2007; 170:1445–1453. [PubMed: 17456751]
4. Puget S, et al. Mesenchymal transition and PDGFRA amplification/mutation are key distinct oncogenic events in pediatric diffuse intrinsic pontine gliomas. *PLoS One*. 2012; 7:e30313. [PubMed: 22389665]
5. Khuong-Quang DA, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. *Acta Neuropathol*. 2012; 124:439–47. [PubMed: 22661320]

6. Warren K. Diffuse intrinsic pontine glioma: poised for progress. *Front Oncol.* 2012; 2:205. [PubMed: 23293772]
7. Cage T, et al. Feasibility, safety, and indications for surgical biopsy of intrinsic brainstem tumors in children. *Child Nerv Syst.* 2013; 29:1313–9.
8. Stupp R, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005; 352:987–996. This was the first study to show that the addition of temozolomide to radiotherapy results in a statistically significant survival benefit for newly diagnosed glioblastoma patients. [PubMed: 15758009]
9. Stupp R, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 2009; 10:459–466. [PubMed: 19269895]
10. Cohen KJ, et al. Temozolomide in the treatment of high-grade gliomas in children: a report from the Children’s Oncology Group. *Neuro-Oncology.* 2011; 13:317–23. [PubMed: 21339192]
11. Hargrave D, Bartels U, Bouffet E. Diffuse brainstem glioma in children: critical review of clinical trials. *Lancet Oncol.* 2006; 7:241–248. [PubMed: 16510333]
12. Janssens G, et al. Hypofractionation vs conventional radiation therapy for newly diagnosed diffuse intrinsic pontine glioma: a matched-cohort analysis. *Int J Radiat Oncol.* 2013; 85:315–320.
13. Jansen MH, van Vuurden DG, Vandertop WP, Kaspers GJ. Diffuse intrinsic pontine gliomas: a systematic update on clinical trials and biology. *Cancer Treat Rev.* 2012; 38:27–35. [PubMed: 21764221]
14. Godard S, et al. Classification of human astrocytic gliomas on the basis of gene expression: a correlated group of genes with angiogenic activity emerges as a strong predictor of subtypes. *Cancer Res.* 2003; 63:6613–6625. [PubMed: 14583454]
15. Shai R, et al. Gene expression profiling identifies molecular subtypes of gliomas. *Oncogene.* 2003; 22:4918–4923. [PubMed: 12894235]
16. Maher E, et al. Marked genomic differences characterize primary and secondary glioblastoma subtypes and identify two distinct molecular and clinical secondary glioblastoma entities. *Cancer Res.* 2006; 66:11502–11513. [PubMed: 17114236]
17. Tso CL, et al. Distinct transcription profiles of primary and secondary glioblastoma subgroups. *Cancer Res.* 2006; 66:159–167. [PubMed: 16397228]
18. Faury D, et al. Molecular profiling identifies prognostic subgroups of pediatric glioblastoma and shows increased YB-1 expression in tumors. *J Clin Oncol.* 2007; 25:1196–208. [PubMed: 17401009]
19. Nutt C, et al. Gene expression-based classification of malignant gliomas correlates better with survival than histological classification. *Cancer Res.* 2003; 63:1602–1607. [PubMed: 12670911]
20. Freije W, et al. Gene expression profiling of gliomas strongly predicts survival. *Cancer Res.* 2004; 64:6503–6510. [PubMed: 15374961]
21. Shirahata M, et al. Gene expression-based molecular diagnostic system for malignant gliomas is superior to histological diagnosis. *Clin Cancer Res.* 2007; 13:7341–7356. [PubMed: 18094416]
22. Liang Y, et al. Gene expression profiling reveals molecularly and clinically distinct subtypes of glioblastoma multiforme. *P Natl Acad Sci USA.* 2005; 102:5814–5819.
23. Phillips H, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer cell.* 2006; 9:157–173. One of the first studies to classify glioblastoma into three distinct molecular subclasses based on gene expression profiling signatures associated with differences in patient survival. [PubMed: 16530701]
24. Gravendeel L, et al. Intrinsic gene expression profiles of gliomas are a better predictor of survival than histology. *Cancer Res.* 2009; 69:9065–9072. [PubMed: 19920198]
25. Verhaak RG, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. *Cancer Cell.* 2010; 17:98–110. This TCGA study discovered four distinct subtypes of glioblastoma distinguished by gene expression patterns associated with distinct genetic aberrations and clinical characteristics. [PubMed: 20129251]

26. Brennan CW, et al. The somatic genomic landscape of glioblastoma. *Cell*. 2013; 155:462–77. This most recent TCGA study describes the landscape of somatic genomic alterations based on multi-dimensional and comprehensive characterization of more than 500 glioblastoma tumors. [PubMed: 24120142]
27. Noushmehr H, et al. Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma. *Cancer Cell*. 2010; 17:510–22. This was the first TCGA study to apply genome-wide DNA methylation profiling and identified a Glioma-CpG Island Methylator Phenotype (G-CIMP) to be linked to less severe outcome. [PubMed: 20399149]
28. Huse J, Phillips H, Brennan C. Molecular subclassification of diffuse gliomas: seeing order in the chaos. *Glia*. 2011; 59:1190–1199. [PubMed: 21446051]
29. Zheng S, Chheda MG, Verhaak RG. Studying a complex tumor: potential and pitfalls. *Cancer J*. 2012; 18:107–14. [PubMed: 22290264]
30. Carro MS, et al. The transcriptional network for mesenchymal transformation of brain tumours. *Nature*. 2010; 463:318–25. [PubMed: 20032975]
31. Bhat K, et al. The transcriptional coactivator TAZ regulates mesenchymal differentiation in malignant glioma. *Genes Dev*. 2011; 25:2594–2609. [PubMed: 22190458]
32. Frattini V, et al. The integrated landscape of driver genomic alterations in glioblastoma. *Nat Genet*. 2013; 45:1141–9. This manuscript reports the genomic landscape of driver genes targeted by both mutations and copy-number aberrations through an integrated computational and experimental pipeline. It also reports the landscape of gene fusions in GBM. [PubMed: 23917401]
33. Danussi C, et al. RHPN2 Drives Mesenchymal Transformation in Malignant Glioma by Triggering RhoA Activation. *Cancer Res*. 2013; 73:5140–5150. [PubMed: 23774217]
34. Bhat KP, et al. Mesenchymal differentiation mediated by NF-kappaB promotes radiation resistance in glioblastoma. *Cancer Cell*. 2013; 24:331–46. [PubMed: 23993863]
35. Haque T, et al. Gene expression profiling from formalin-fixed paraffin-embedded tumors of pediatric glioblastoma. *Clin Cancer Res*. 2007; 13:6284–6292. [PubMed: 17975139]
36. Paugh BS, et al. Integrated molecular genetic profiling of pediatric high-grade gliomas reveals key differences with the adult disease. *J Clin Oncol*. 2010; 28:3061–8. [PubMed: 20479398]
37. Paugh BS, et al. Genome-wide analyses identify recurrent amplifications of receptor tyrosine kinases and cell-cycle regulatory genes in diffuse intrinsic pontine glioma. *J Clin Oncol*. 2011; 29:3999–4006. [PubMed: 21931021]
38. Bax DA, et al. A distinct spectrum of copy number aberrations in paediatric high grade gliomas. *Clin Cancer Res*. 2010; 16:3368–3377. [PubMed: 20570930]
39. Qu HQ, et al. Genome-wide profiling using single-nucleotide polymorphism arrays identifies novel chromosomal imbalances in pediatric glioblastomas. *Neuro-Oncology*. 2010; 12:153–63. [PubMed: 20150382]
40. Sturm D, et al. Hotspot Mutations in H3F3A and IDH1 Define Distinct Epigenetic and Biological Subgroups of Glioblastoma. *Cancer Cell*. 2012; 22:425–37. This study applied genome-wide DNA methylation profiling to a combined cohort of paediatric and adult patients and described biological subgroups of glioblastomas associated with distinct molecular aberrations and clinical characteristics. [PubMed: 23079654]
41. Schwartzentruber J, et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. *Nature*. 2012; 482:226–31. Together with Wu *et al.* 2012, these were the first studies to identify recurrent *H3F3A/HIST1H3B* mutations in paediatric high-grade gliomas and the first reports on somatic histone mutations in human cancer. [PubMed: 22286061]
42. Fontebasso A, Liu XY, Sturm D, Jabado N. Chromatin remodeling defects in pediatric and young adult glioblastoma: a tale of a variant histone 3 tail. *Brain Pathol*. 2013; 23:210–216. [PubMed: 23432647]
43. Bjerke L, et al. Histone H3.3 Mutations Drive Pediatric Glioblastoma through Upregulation of MYCN. *Cancer Discov*. 2013; 3 (5):512–519.
44. Bender S, et al. Reduced H3K27me3 and DNA Hypomethylation Are Major Drivers of Gene Expression in K27M Mutant Pediatric High-Grade Gliomas. *Cancer cell*. 2013; 24:660–72. [PubMed: 24183680]

45. Costello J, Berger M, Huang H, Cavenee W. Silencing of p16/CDKN2 expression in human gliomas by methylation and chromatin condensation. *Cancer Res.* 1996; 56:2405–2410. [PubMed: 8625319]
46. Baeza N, Weller M, Yonekawa Y, Kleihues P, Ohgaki H. PTEN methylation and expression in glioblastomas. *Acta Neuropathol.* 2003; 106:479–485. [PubMed: 12904991]
47. Nakamura M, Yonekawa Y, Kleihues P, Ohgaki H. Promoter hypermethylation of the RB1 gene in glioblastomas. *Lab Invest.* 2001; 81:77–82. [PubMed: 11204276]
48. Amatya V, Naumann U, Weller M, Ohgaki H. TP53 promoter methylation in human gliomas. *Acta Neuropathol.* 2005; 110:178–184. [PubMed: 16025287]
49. Alaminos M, et al. EMP3, a myelin-related gene located in the critical 19q13.3 region, is epigenetically silenced and exhibits features of a candidate tumor suppressor in glioma and neuroblastoma. *Cancer Res.* 2005; 65:2565–2571. [PubMed: 15805250]
50. Bruna A, et al. High TGFbeta-Smad activity confers poor prognosis in glioma patients and promotes cell proliferation depending on the methylation of the PDGF-B gene. *Cancer cell.* 2007; 11:147–160. [PubMed: 17292826]
51. Waha A, et al. Epigenetic silencing of the protocadherin family member PCDH-gamma-A11 in astrocytomas. *Neoplasia.* 2005; 7:193–199. [PubMed: 15799819]
52. Zhou H, et al. Reciprocal regulation of SOCS 1 and SOCS3 enhances resistance to ionizing radiation in glioblastoma multiforme. *Clin Cancer Res.* 2007; 13:2344–2353. [PubMed: 17438093]
53. Zardo G, et al. Integrated genomic and epigenomic analyses pinpoint biallelic gene inactivation in tumors. *Nat Genet.* 2002; 32:453–458. [PubMed: 12355068]
54. Esteller M, Hamilton S, Burger P, Baylin S, Herman J. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. *Cancer Res.* 1999; 59:793–797. First study to show inactivation by promoter hypermethylation of the *MGMT* gene in glioblastoma patient samples. [PubMed: 10029064]
55. Felsberg J, et al. Promoter methylation and expression of MGMT and the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2 in paired primary and recurrent glioblastomas. *Int J Cancer.* 2011; 129:659–670. [PubMed: 21425258]
56. Wick W, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: the NOA-08 randomised, phase 3 trial. *Lancet Oncol.* 2012; 13:707–715. [PubMed: 22578793]
57. Malmström A, et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. *Lancet Oncol.* 2012; 13:916–926. References 56 and 57 demonstrated *MGMT* promoter methylation to be a predictive biomarker for the benefit from alkylating agent chemotherapy particularly in elderly glioblastoma patients. [PubMed: 22877848]
58. Costello J, Futscher B, Tano K, Graunke D, Pieper R. Graded methylation in the promoter and body of the O6-methylguanine DNA methyltransferase (*MGMT*) gene correlates with *MGMT* expression in human glioma cells. *J Biol Chem.* 1994; 269:17228–17237. First study to report on hypermethylation of the *MGMT* gene in human glioma cells. [PubMed: 8006031]
59. Costello J, Futscher B, Kroes R, Pieper R. Methylation-related chromatin structure is associated with exclusion of transcription factors from and suppressed expression of the O-6-methylguanine DNA methyltransferase gene in human glioma cell lines. *Mol Cell Biol.* 1994; 14:6515–6521. [PubMed: 7523853]
60. Esteller M, et al. Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *N Engl J Med.* 2000; 343:1350–1354. [PubMed: 11070098]
61. Hegi ME, et al. *MGMT* gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med.* 2005; 352:997–1003. This study demonstrated that the presence of an inactivated *MGMT* gene confers benefit from alkylating agent chemotherapy using Temozolomide. [PubMed: 15758010]

62. Olson R, Brastianos P, Palma D. Prognostic and predictive value of epigenetic silencing of MGMT in patients with high grade gliomas: a systematic review and meta-analysis. *J Neuro-Oncol.* 2011; 105:325–335.
63. Donson A, Addo-Yobo S, Handler M, Gore L, Foreman N. MGMT promoter methylation correlates with survival benefit and sensitivity to temozolomide in pediatric glioblastoma. *Pediatr Blood Cancer.* 2007; 48:403–407. [PubMed: 16609952]
64. Buttarelli F, et al. Evaluation status and prognostic significance of O6-methylguanine-DNA methyltransferase (MGMT) promoter methylation in pediatric high grade gliomas. *Child Nerv Syst.* 2010; 26:1051–1056.
65. Srivastava A, et al. MGMT gene promoter methylation in pediatric glioblastomas. *Child Nerv Syst.* 2010; 26:1613–1618.
66. Lee J, et al. MGMT promoter gene methylation in pediatric glioblastoma: analysis using MS-MLPA. *Child Nerv Syst.* 2011; 27:1877–1883.
67. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature.* 2008; 455:1061–8. This first comprehensive TCGA study on 206 glioblastoma samples reported three core signalling pathways (RTK/RAS/PI-3K, P53, and RB) implicated in glioblastoma development and treatment resistance. [PubMed: 18772890]
68. Cadieux B, Ching TT, VandenBerg S, Costello J. Genome-wide hypomethylation in human glioblastomas associated with specific copy number alteration, methylenetetrahydrofolate reductase allele status, and increased proliferation. *Cancer Res.* 2006; 66:8469–8476. [PubMed: 16951158]
69. Hovestadt V, et al. Robust molecular subgrouping and copy-number profiling of medulloblastoma from small amounts of archival tumour material using high-density DNA methylation arrays. *Acta neuropathologica.* 2013; 125:913–916. [PubMed: 23670100]
70. Malhotra A, et al. Breakpoint profiling of 64 cancer genomes reveals numerous complex rearrangements spawned by homology-independent mechanisms. *Genome Res.* 2013; 23:762–776. [PubMed: 23410887]
71. Bozdag S, et al. Age-specific signatures of glioblastoma at the genomic, genetic, and epigenetic levels. *PloS One.* 2013; 8:e62982. [PubMed: 23658659]
72. Jones C, Perryman L, Hargrave D. Paediatric and adult malignant glioma: close relatives or distant cousins? *Nat Rev Clin Oncol.* 2012; 9:400–413. [PubMed: 22641364]
73. Zheng S, et al. A survey of intragenic breakpoints in glioblastoma identifies a distinct subset associated with poor survival. *Genes Dev.* 2013; 27:1462–1472. [PubMed: 23796897]
74. Bax D, et al. EGFRvIII deletion mutations in pediatric high-grade glioma and response to targeted therapy in pediatric glioma cell lines. *Clin Cancer Res.* 2009; 15:5753–5761. [PubMed: 19737945]
75. Cho J, et al. Glioblastoma-derived epidermal growth factor receptor carboxyl-terminal deletion mutants are transforming and are sensitive to EGFR-directed therapies. *Cancer Res.* 2011; 71:7587–7596. [PubMed: 22001862]
76. Gan H, Cvrljevic A, Johns T. The epidermal growth factor receptor variant III (EGFRvIII): where wild things are altered. *FEBS J.* 2013; 280:5350–70. [PubMed: 23777544]
77. Biernat W, Huang H, Yokoo H, Kleihues P, Ohgaki H. Predominant expression of mutant EGFR (EGFRvIII) is rare in primary glioblastomas. *Brain Pathol.* 2004; 14:131–136. [PubMed: 15193025]
78. Ozawa T, et al. PDGFRA gene rearrangements are frequent genetic events in PDGFRA-amplified glioblastomas. *Genes Dev.* 2010; 24:2205–2218. [PubMed: 20889717]
79. Paugh B, et al. Novel Oncogenic PDGFRA Mutations in Pediatric High-Grade Gliomas. *Cancer Res.* 2013; 73:6219–29. [PubMed: 23970477]
80. Sanborn J, et al. Double minute chromosomes in glioblastoma multiforme are revealed by precise reconstruction of oncogenic amplicons. *Cancer Res.* 2013; 73:6036–45. [PubMed: 23940299]
81. Vogt N, et al. Molecular structure of double-minute chromosomes bearing amplified copies of the epidermal growth factor receptor gene in gliomas. *P Natl Acad Sci USA.* 2004; 101:11368–73.
82. Singh D, et al. Transforming fusions of FGFR and TACC genes in human glioblastoma. *Science.* 2012; 337:1231–5. The first report of a recurrent gene fusion (*FGFR-TACC*) in GBM. This study

- has paved the way to a personalized tailored therapeutic approach with FGFR inhibitors for GBM harbouring *FGFR-TACC* fusions. [PubMed: 22837387]
83. Parker B, et al. The tumorigenic FGFR3-TACC3 gene fusion escapes miR-99a regulation in glioblastoma. *J Clin Invest.* 2013; 123:855–865. [PubMed: 23298836]
 84. Phillips J, et al. PDGFRA Amplification is Common in Pediatric and Adult High-Grade Astrocytomas and Identifies a Poor Prognostic Group in IDH1 Mutant Glioblastoma. *Brain Pathol.* 2013; 23:565–73. [PubMed: 23438035]
 85. Zarghooni M, et al. Whole-Genome Profiling of Pediatric Diffuse Intrinsic Pontine Gliomas Highlights Platelet-Derived Growth Factor Receptor {alpha} and Poly (ADP-ribose) Polymerase As Potential Therapeutic Targets. *J Clin Oncol.* 2010; 28:1337–44. [PubMed: 20142589]
 86. Parsons DW, et al. An integrated genomic analysis of human glioblastoma multiforme. *Science.* 2008; 321:1807–12. This comprehensive analysis discovered of a variety of genes that were not known to be altered in glioblastoma, most notably recurrent hotspot mutations in *IDH1*. [PubMed: 18772396]
 87. Liu XY, et al. Frequent ATRX mutations and loss of expression in adult diffuse astrocytic tumors carrying IDH1/IDH2 and TP53 mutations. *Acta Neuropathol.* 2012; 124:615–625. [PubMed: 22886134]
 88. Jiao Y, et al. Frequent ATRX, CIC, FUBP1 and IDH1 mutations refine the classification of malignant gliomas. *Oncotarget.* 2012; 3:709–722. [PubMed: 22869205]
 89. Dawson M, Kouzarides T. Cancer epigenetics: from mechanism to therapy. *Cell.* 2012; 150:12–27. [PubMed: 22770212]
 90. Shen H, Laird P. Interplay between the cancer genome and epigenome. *Cell.* 2013; 153:38–55. [PubMed: 23540689]
 91. Killela P, et al. TERT promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal. *P Natl Acad Sci USA.* 2013; 110:6021–6026. This study was the first to report recurrent somatic mutations in the *TERT* gene promoter as a likely mechanism of telomerase activation in a large proportion of glioblastomas.
 92. Arita H, et al. Upregulating mutations in the TERT promoter commonly occur in adult malignant gliomas and are strongly associated with total 1p19q loss. *Acta Neuropathol.* 2013; 126:267–276. [PubMed: 23764841]
 93. Nonoguchi N, et al. TERT promoter mutations in primary and secondary glioblastomas. *Acta Neuropathol.* 2013; 126:931–7. [PubMed: 23955565]
 94. Vinagre J, et al. Frequency of TERT promoter mutations in human cancers. *Nat Commun.* 2013; 4:2185. [PubMed: 23887589]
 95. Koelsche C, et al. Distribution of TERT promoter mutations in pediatric and adult tumors of the nervous system. *Acta Neuropathol.* 2013; 126:907–15. [PubMed: 24154961]
 96. Boldrini L, et al. Telomerase activity and hTERT mRNA expression in glial tumors. *Int J Oncol.* 2006; 28:1555–1560. [PubMed: 16685456]
 97. Heaphy C, et al. Altered telomeres in tumors with ATRX and DAXX mutations. *Science.* 2011; 333(425) This was the first study to report on the presence of an alternative lengthening of telomeres (ALT) phenotype occurring in tumours with mutation in *ATRX* or *DAXX*.
 98. Hunter C, et al. A hypermutation phenotype and somatic MSH6 mutations in recurrent human malignant gliomas after alkylator chemotherapy. *Cancer Res.* 2006; 66:3987–3991. [PubMed: 16618716]
 99. Cahill D, et al. Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. *Clin Cancer Res.* 2007; 13:2038–2045. [PubMed: 17404084]
 100. Yip S, et al. MSH6 mutations arise in glioblastomas during temozolomide therapy and mediate temozolomide resistance. *Clin Cancer Res.* 2009; 15:4622–4629. [PubMed: 19584161]
 101. Wu G, et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet.* 2012; 44:251–3. Together with Schwartzentruber *et al.* 2012, these were the first studies to identify recurrent *H3F3A/HIST1H3B* mutations in paediatric high-grade gliomas and the first reports on somatic histone mutations in human cancer. [PubMed: 22286216]

102. Downing J, et al. The Pediatric Cancer Genome Project. *Nat Genet.* 2012; 44:619–622. [PubMed: 22641210]
103. Zhang J, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. *Nat Genet.* 2013; 45:602–612. [PubMed: 23583981]
104. Jones DTW, et al. Recurrent somatic alterations of *FGFR1* and *NTRK2* in pilocytic astrocytoma. *Nat Genet.* 2013; 45:927–932. [PubMed: 23817572]
105. Venneti S, et al. Evaluation of Histone 3 Lysine 27 Trimethylation (H3K27me3) and Enhancer of Zest 2 (EZH2) in Pediatric Glial and Glioneuronal Tumors Shows Decreased H3K27me3 in H3F3A K27M Mutant Glioblastomas. *Brain Pathol.* 2013; 23:558–564. [PubMed: 23414300]
106. Lewis P, et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science.* 2013; 340:857–861. This was the first study to show the functional inhibition of PRC2 by a gain-of-function mutation at position K27 of histone variant H3.3, leading to globally reduced levels of H3K27me3. [PubMed: 23539183]
107. Chan KM, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. *Genes Dev.* 2013; 27:985–990. [PubMed: 23603901]
108. Swartling FJ, et al. Distinct neural stem cell populations give rise to disparate brain tumors in response to N-MYC. *Cancer Cell.* 2012; 21:601–13. [PubMed: 22624711]
109. Puissant A, et al. Targeting MYCN in neuroblastoma by BET bromodomain inhibition. *Cancer discovery.* 2013; 3:308–323. [PubMed: 23430699]
110. Fontebasso A, et al. Mutations in SETD2 and genes affecting histone H3K36 methylation target hemispheric high-grade gliomas. *Acta Neuropathol.* 2013; 125:659–669. [PubMed: 23417712]
111. Li F, et al. The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutS α . *Cell.* 2013; 153:590–600. [PubMed: 23622243]
112. Chen YJ, et al. Association of mutant TP53 with alternative lengthening of telomeres and favorable prognosis in glioma. *Cancer Res.* 2006; 66:6473–6476. [PubMed: 16818615]
113. Yan H, et al. IDH1 and IDH2 mutations in gliomas. *N Engl J Med.* 2009; 360:765–73. This study identified *IDH1/2* mutations in the majority of lower grade gliomas and secondary glioblastomas associated with better outcome than those with wild-type *IDH* genes. [PubMed: 19228619]
114. Ichimura K, et al. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. *Neuro-Oncology.* 2009; 11:341–7. [PubMed: 19435942]
115. Zhang C, Moore L, Li X, Yung W, Zhang W. IDH1/2 mutations target a key hallmark of cancer by deregulating cellular metabolism in glioma. *Neuro-oncology.* 2013; 15:1114–26. [PubMed: 23877318]
116. Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. *Clin Cancer Res.* 2013; 19:764–772. [PubMed: 23209033]
117. Larjavaara S, et al. Incidence of gliomas by anatomic location. *Neuro-oncology.* 2007; 9:319–325. [PubMed: 17522333]
118. Turcan S, et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature.* 2012; 483:479–83. This study was the first to show a causal link between mutations in *IDH1* and the Glioma-CpG Island Methylator Phenotype (G-CIMP). [PubMed: 22343889]
119. Kim W, Liao L. IDH mutations in human glioma. *Neurosurg Clin N Am.* 2012; 23:471–480. [PubMed: 22748659]
120. Ichimura K. Molecular pathogenesis of IDH mutations in gliomas. *Brain Tumor Pathol.* 2012; 29:131–139. [PubMed: 22399191]
121. Lass U, et al. Clonal analysis in recurrent astrocytic, oligoastrocytic and oligodendroglial tumors implicates IDH1- mutation as common tumor initiating event. *PLoS One.* 2012; 7:e41298. [PubMed: 22844452]
122. Hartmann C, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. *Acta Neuropathol.* 2010; 120:707–718. [PubMed: 21088844]
123. Hartmann C, et al. Longterm survival in primary glioblastoma with versus without isocitrate dehydrogenase mutations. *Clin Cancer Res.* 2013; 19:5146–57. [PubMed: 23918605]

124. Ward PS, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. *Cancer Cell*. 2010; 17:225–34. [PubMed: 20171147]
125. Dang L, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature*. 2009; 462:739–744. [PubMed: 19935646]
126. Figueroa M, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer cell*. 2010; 18:553–567. [PubMed: 21130701]
127. Xu W, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. *Cancer Cell*. 2011; 19:17–30. [PubMed: 21251613]
128. Chowdhury R, et al. The oncometabolite 2-hydroxyglutarate inhibits histone lysine demethylases. *EMBO Rep*. 2011; 12:463–9. [PubMed: 21460794]
129. Guilhamon P, et al. Meta-analysis of IDH-mutant cancers identifies EBF1 as an interaction partner for TET2. *Nat Commun*. 2013; 4:2166. [PubMed: 23863747]
130. Koso H, et al. Transposon mutagenesis identifies genes that transform neural stem cells into glioma-initiating cells. *P Natl Acad Sci USA*. 2012; 109:E2998–3007.
131. Alcantara Llaguno S, et al. Malignant astrocytomas originate from neural stem/progenitor cells in a somatic tumor suppressor mouse model. *Cancer cell*. 2009; 15:45–56. [PubMed: 19111880]
132. Liu C, et al. Mosaic analysis with double markers reveals tumor cell of origin in glioma. *Cell*. 2011; 146:209–21. [PubMed: 21737130]
133. Sugiarto S, et al. Asymmetry-defective oligodendrocyte progenitors are glioma precursors. *Cancer cell*. 2011; 20:328–340. [PubMed: 21907924]
134. Friedmann-Morvinski D, et al. Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. *Science*. 2012; 338:1080–4. [PubMed: 23087000]
135. Monje M, et al. Hedgehog-responsive candidate cell of origin for diffuse intrinsic pontine glioma. *P Natl Acad Sci USA*. 2011; 108:4453–4458.
136. Lai A, et al. Evidence for sequenced molecular evolution of IDH1 mutant glioblastoma from a distinct cell of origin. *J Clin Oncol*. 2011; 29:4482–90. [PubMed: 22025148]
137. Kang H, et al. Spatio-temporal transcriptome of the human brain. *Nature*. 2011; 478:483–489. [PubMed: 22031440]
138. Ohnishi A, et al. Expression of the oligodendroglial lineage-associated markers Olig1 and Olig2 in different types of human gliomas. *J Neuropathol Exp Neurol*. 2003; 62:1052–9. [PubMed: 14575240]
139. Lu QR, et al. Oligodendrocyte lineage genes (OLIG) as molecular markers for human glial brain tumors. *P Natl Acad Sci USA*. 2001; 98:10851–6.
140. Marie Y, et al. OLIG2 as a specific marker of oligodendroglial tumour cells. *Lancet*. 2001; 358:298–300. [PubMed: 11498220]
141. Barrett LE, et al. Self-renewal does not predict tumor growth potential in mouse models of high-grade glioma. *Cancer Cell*. 2012; 21:11–24. [PubMed: 22264785]
142. Appolloni I, et al. Antagonistic modulation of gliomagenesis by Pax6 and Olig2 in PDGF-induced oligodendrogloma. *Int J Cancer*. 2012; 131:E1078–87. [PubMed: 22514120]
143. Wang Y, et al. Expression of mutant p53 proteins implicates a lineage relationship between neural stem cells and malignant astrocytic glioma in a murine model. *Cancer cell*. 2009; 15:514–526. [PubMed: 19477430]
144. Prados MD, et al. Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. *J Clin Oncol*. 2009; 27:579–84. [PubMed: 19075262]
145. Brown PD, et al. Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. *J Clin Oncol*. 2008; 26:5603–9. [PubMed: 18955445]
146. van den Bent MJ, et al. Randomized phase II trial of erlotinib versus temozolomide or carmustine in recurrent glioblastoma: EORTC brain tumor group study 26034. *J Clin Oncol*. 2009; 27:1268–74. [PubMed: 19204207]

147. Lassman AB, et al. Molecular study of malignant gliomas treated with epidermal growth factor receptor inhibitors: tissue analysis from North American Brain Tumor Consortium Trials 01-03 and 00-01. *Clin Cancer Res.* 2005; 11:7841–50. [PubMed: 16278407]
148. Franceschi E, et al. Gefitinib in patients with progressive high-grade gliomas: a multicentre phase II study by Gruppo Italiano Cooperativo di Neuro-Oncologia (GICNO). *Br J Cancer.* 2007; 96:1047–51. [PubMed: 17353924]
149. Neyns B, et al. Stratified phase II trial of cetuximab in patients with recurrent high-grade glioma. *Ann Oncol.* 2009; 20:1596–603. [PubMed: 19491283]
150. Raymond E, et al. Phase II study of imatinib in patients with recurrent gliomas of various histologies: a European Organisation for Research and Treatment of Cancer Brain Tumor Group Study. *J Clin Oncol.* 2008; 26:4659–65. [PubMed: 18824712]
151. Wen PY, et al. Phase I/II study of imatinib mesylate for recurrent malignant gliomas: North American Brain Tumor Consortium Study 99-08. *Clin Cancer Res.* 2006; 12:4899–907. [PubMed: 16914578]
152. Reardon DA, et al. Multicentre phase II studies evaluating imatinib plus hydroxyurea in patients with progressive glioblastoma. *Br J Cancer.* 2009; 101:1995–2004. [PubMed: 19904263]
153. Dresemann G, et al. Imatinib in combination with hydroxyurea versus hydroxyurea alone as oral therapy in patients with progressive pretreated glioblastoma resistant to standard dose temozolomide. *J Neurooncol.* 2010; 96:393–402. [PubMed: 19688297]
154. Friedman HS, et al. Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. *J Clin Oncol.* 2009; 27:4733–40. [PubMed: 19720927]
155. Lai A, et al. Phase II study of bevacizumab plus temozolomide during and after radiation therapy for patients with newly diagnosed glioblastoma multiforme. *J Clin Oncol.* 2011; 29:142–8. [PubMed: 21135282]
156. Batchelor TT, et al. Phase III Randomized Trial Comparing the Efficacy of Cediranib As Monotherapy, and in Combination With Lomustine, Versus Lomustine Alone in Patients With Recurrent Glioblastoma. *J Clin Oncol.* 2013; 31:3212–8. [PubMed: 23940216]
157. Tanaka S, Louis DN, Curry WT, Batchelor TT, Dietrich J. Diagnostic and therapeutic avenues for glioblastoma: no longer a dead end? *Nat Rev Clin Oncol.* 2013; 10:14–26. [PubMed: 23183634]
158. Drappatz J, et al. Phase I study of panobinostat in combination with bevacizumab for recurrent high-grade glioma. *J Neurooncol.* 2012; 107:133–8. [PubMed: 21984064]
159. Friday BB, et al. Phase II trial of vorinostat in combination with bortezomib in recurrent glioblastoma: a north central cancer treatment group study. *Neuro-Oncology.* 2012; 14:215–21. [PubMed: 22090453]
160. Barker CA, Bishop AJ, Chang M, Beal K, Chan TA. Valproic acid use during radiation therapy for glioblastoma associated with improved survival. *Int J Radiat Oncol.* 2013; 86:504–9.
161. Weller M, et al. Prolonged survival with valproic acid use in the EORTC/NCIC temozolomide trial for glioblastoma. *Neurology.* 2011; 77:1156–64. [PubMed: 21880994]
162. Rohle D, et al. An inhibitor of mutant IDH1 delays growth and promotes differentiation of glioma cells. *Science.* 2013; 340:626–30. [PubMed: 23558169]
163. Davis, M., et al. Probe Reports from the NIH Molecular Libraries Program. Bethesda (MD): 2010.
164. Wang F, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. *Science.* 2013; 340:622–6. [PubMed: 23558173]
165. Tonjes M, et al. BCAT1 promotes cell proliferation through amino acid catabolism in gliomas carrying wild-type IDH1. *Nat Med.* 2013; 19:901–8. [PubMed: 23793099]
166. Yang W, et al. ERK1/2-dependent phosphorylation and nuclear translocation of PKM2 promotes the Warburg effect. *Nat Cell Biol.* 2012; 14:1295–304. [PubMed: 23178880]
167. Yang W, et al. PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. *Cell.* 2012; 150:685–696. [PubMed: 22901803]
168. McCabe MT, et al. Mutation of A677 in histone methyltransferase EZH2 in human B-cell lymphoma promotes hypertrimethylation of histone H3 on lysine 27 (H3K27). *P Natl Acad Sci USA.* 2012; 109:2989–94.

169. Ryan RJ, et al. EZH2 codon 641 mutations are common in BCL2-rearranged germinal center B cell lymphomas. *PLoS One*. 2011; 6:e28585. [PubMed: 22194861]
170. Sneeringer CJ, et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. *Proc Natl Acad Sci USA*. 2010; 107:20980–5.
171. Marian C, et al. The telomerase antagonist, imetelstat, efficiently targets glioblastoma tumor-initiating cells leading to decreased proliferation and tumor growth. *Clin Cancer Res*. 2010; 16:154–163. [PubMed: 20048334]
172. Cheng Z, et al. Inhibition of BET bromodomain targets genetically diverse glioblastoma. *Clin Cancer Res*. 2013; 19:1748–1759. [PubMed: 23403638]
173. Hegi M, et al. Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib—a phase II trial. *Mol Cancer Ther*. 2011; 10:1102–1112. [PubMed: 21471286]
174. Mellinghoff I, et al. Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. *N Engl J Med*. 2005; 353:2012–2024. [PubMed: 16282176]
175. Fenton T, et al. Resistance to EGF receptor inhibitors in glioblastoma mediated by phosphorylation of the PTEN tumor suppressor at tyrosine 240. *Proc Natl Acad Sci USA*. 2012; 109:14164–14169.
176. Jun H, Bronson R, Charest A. Inhibition of EGFR induces a c-MET driven stem cell population in Glioblastoma. *Stem cells* 2013. 2013 Oct 1. Epub ahead of print. 10.1002/stem.1554
177. Bielen A, et al. Enhanced efficacy of IGF1R inhibition in pediatric glioblastoma by combinatorial targeting of PDGFR α / β . *Mol Cancer Ther*. 2011; 10:1407–1418. [PubMed: 21659463]
178. Szerlip N, et al. Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response. *Proc Natl Acad Sci USA*. 2012; 109:3041–3046.
179. Little S, et al. Receptor tyrosine kinase genes amplified in glioblastoma exhibit a mutual exclusivity in variable proportions reflective of individual tumor heterogeneity. *Cancer Res*. 2012; 72:1614–1620. [PubMed: 22311673]
180. Marusyk A, Polyak K. Tumor heterogeneity: causes and consequences. *Biochimica et biophysica acta*. 2010; 1805:105–117. [PubMed: 19931353]
181. Harada K, et al. Intratumoral cytogenetic heterogeneity detected by comparative genomic hybridization and laser scanning cytometry in human gliomas. *Cancer Res*. 1998; 58:4694–4700. [PubMed: 9788624]
182. Jung V, et al. Evidence of focal genetic microheterogeneity in glioblastoma multiforme by area-specific CGH on microdissected tumor cells. *J Neuropath Exp Neur*. 1999; 58:993–999. [PubMed: 10499441]
183. Nobusawa S, et al. Intratumoral patterns of genomic imbalance in glioblastomas. *Brain Pathol*. 2010; 20:936–944. [PubMed: 20406234]
184. Ren ZP, et al. Molecular genetic analysis of p53 intratumoral heterogeneity in human astrocytic brain tumors. *J Neuropath Exp Neur*. 2007; 66:944–954. [PubMed: 17917588]
185. Hegi M, et al. Correlation of O6-methylguanine methyltransferase (MGMT) promoter methylation with clinical outcomes in glioblastoma and clinical strategies to modulate MGMT activity. *J Clin Oncol*. 2008; 26:4189–4199. [PubMed: 18757334]
186. Sottoriva A, et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. *Proc Natl Acad Sci USA*. 2013; 110:4009–4014.
187. Snuderl M, et al. Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma. *Cancer cell*. 2011; 20:810–817. [PubMed: 22137795]
188. Inda, M-d-M., et al. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. *Genes Dev*. 2010; 24:1731–1745. [PubMed: 20713517]
189. Stommel J, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science*. 2007; 318:287–290. [PubMed: 17872411]
190. Greaves M, Maley C. Clonal evolution in cancer. *Nature*. 2012; 481:306–313. [PubMed: 22258609]

191. Jordan C, Guzman M, Noble M. Cancer stem cells. *N Engl J Med.* 2006; 355:1253–1261. [PubMed: 16990388]
192. Hambardzumyan D, Parada L, Holland E, Charest A. Genetic modeling of gliomas in mice: new tools to tackle old problems. *Glia.* 2011; 59:1155–1168. [PubMed: 21305617]
193. Chen L, Zhang Y, Yang J, Hagan J, Li M. Vertebrate animal models of glioma: Understanding the mechanisms and developing new therapies. *Biochimica et biophysica acta.* 2013; 1836:158–165. [PubMed: 23618720]
194. Lee J, et al. Tumor stem cells derived from glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors than do serum-cultured cell lines. *Cancer cell.* 2006; 9:391–403. [PubMed: 16697959]
195. Radaelli E, et al. Immunohistopathological and neuroimaging characterization of murine orthotopic xenograft models of glioblastoma multiforme recapitulating the most salient features of human disease. *Histol Histopathol.* 2009; 24:879–891. [PubMed: 19475534]
196. Zhao Y, et al. An extensive invasive intracranial human glioblastoma xenograft model: role of high level matrix metalloproteinase 9. *Am J Pathol.* 2010; 176:3032–3049. [PubMed: 20413683]
197. Luchman H, et al. An in vivo patient-derived model of endogenous IDH1-mutant glioma. *Neuro-oncology.* 2012; 14:184–191. [PubMed: 22166263]
198. Jacques T, et al. Combinations of genetic mutations in the adult neural stem cell compartment determine brain tumour phenotypes. *EMBO J.* 2010; 29:222–235. [PubMed: 19927122]
199. Chow L, et al. Cooperativity within and among Pten, p53, and Rb pathways induces high-grade astrocytoma in adult brain. *Cancer cell.* 2011; 19:305–316. [PubMed: 21397855]
200. Holland E, Hively W, DePinho R, Varmus H. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. *Genes Dev.* 1998; 12:3675–3685. [PubMed: 9851974]
201. Ding H, et al. Oligodendrogliomas result from the expression of an activated mutant epidermal growth factor receptor in a RAS transgenic mouse astrocytoma model. *Cancer Res.* 2003; 63:1106–1113. [PubMed: 12615729]
202. Holland E. A mouse model for glioma: biology, pathology, and therapeutic opportunities. *Toxicol Pathol.* 2000; 28:171–177. [PubMed: 10669005]
203. Weiss W, et al. Genetic determinants of malignancy in a mouse model for oligodendroglioma. *Cancer Res.* 2003; 63:1589–1595. [PubMed: 12670909]
204. Wei Q, et al. High-grade glioma formation results from postnatal pten loss or mutant epidermal growth factor receptor expression in a transgenic mouse glioma model. *Cancer Res.* 2006; 66:7429–7437. [PubMed: 16885338]
205. Zhu H, et al. Oncogenic EGFR signaling cooperates with loss of tumor suppressor gene functions in gliomagenesis. *P Natl Acad Sci USA.* 2009; 106:2712–2716.
206. Hambardzumyan D, Amankulor NM, Helmy KY, Becher OJ, Holland EC. Modeling Adult Gliomas Using RCAS/t-va Technology. *Transl Oncol.* 2009; 2:89–95. [PubMed: 19412424]
207. Hede SM, et al. GFAP promoter driven transgenic expression of PDGFB in the mouse brain leads to glioblastoma in a Trp53 null background. *Glia.* 2009; 57:1143–1153. [PubMed: 19115382]
208. Lei L, et al. Glioblastoma models reveal the connection between adult glial progenitors and the proneural phenotype. *PLoS One.* 2011; 6:e20041. [PubMed: 21625383]
209. Becher O, Holland E. Genetically engineered models have advantages over xenografts for preclinical studies. *Cancer Res.* 2006; 66:3355. [PubMed: 16585152]
210. Becher O, et al. Preclinical evaluation of radiation and perfosine in a genetically and histologically accurate model of brainstem glioma. *Cancer Res.* 2010; 70:2548–2557. [PubMed: 20197468]
211. Reilly K, Loisel D, Bronson R, McLaughlin M, Jacks T. Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. *Nat Genet.* 2000; 26:109–113. [PubMed: 10973261]
212. Bajenaru M, et al. Astrocyte-specific inactivation of the neurofibromatosis 1 gene (NF1) is insufficient for astrocytoma formation. *Mol Cell Biol.* 2002; 22:5100–5113. [PubMed: 12077339]

213. Reilly K, et al. Susceptibility to astrocytoma in mice mutant for Nf1 and Trp53 is linked to chromosome 11 and subject to epigenetic effects. *P Natl Acad Sci USA*. 2004; 101:13008–13013.
214. Zhu Y, et al. Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer cell*. 2005; 8:119–130. [PubMed: 16098465]
215. Kwon CH, et al. Pten haploinsufficiency accelerates formation of high-grade astrocytomas. *Cancer Res*. 2008; 68:3286–3294. [PubMed: 18451155]
216. Sasaki M, et al. D-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. *Genes Dev*. 2012; 26:2038–2049. [PubMed: 22925884]
217. Barrow J, et al. Homozygous loss of ADAM3A revealed by genome-wide analysis of pediatric high-grade glioma and diffuse intrinsic pontine gliomas. *Neuro-oncology*. 2011; 13:212–222. [PubMed: 21138945]
218. Raffel C, et al. Analysis of oncogene and tumor suppressor gene alterations in pediatric malignant astrocytomas reveals reduced survival for patients with PTEN mutations. *Clin Cancer Res*. 1999; 5:4085–4090. [PubMed: 10632344]
219. Pollack IF, et al. Rarity of PTEN deletions and EGFR amplification in malignant gliomas of childhood: results from the Children’s Cancer Group 945 cohort. *J Neurosurg*. 2006; 105:418–24. [PubMed: 17328268]
220. Pollack I, et al. Age and TP53 mutation frequency in childhood malignant gliomas: results in a multi-institutional cohort. *Cancer Res*. 2001; 61:7404–7407. [PubMed: 11606370]
221. Pollack I, et al. IDH1 mutations are common in malignant gliomas arising in adolescents: a report from the Children’s Oncology Group. *Child Nerv Syst*. 2011; 27:87–94.
222. Gallia G, et al. PIK3CA gene mutations in pediatric and adult glioblastoma multiforme. *Mol Cancer Res*. 2006; 4:709–714. [PubMed: 17050665]
223. Schiffman J, et al. Oncogenic BRAF mutation with CDKN2A inactivation is characteristic of a subset of pediatric malignant astrocytomas. *Cancer Res*. 2010; 70:512–519. [PubMed: 20068183]
224. Nicolaides T, et al. Targeted therapy for BRAFV600E malignant astrocytoma. *Clin Cancer Res*. 2011; 17:7595–7604. [PubMed: 22038996]

Biographies

Dominik Sturm

Dominik Sturm is a physician scientist in the Division of Pediatric Neurooncology at the German Cancer Research Center (DKFZ), Heidelberg, Germany. His recent research activities focused on elucidating the molecular biology underlying childhood high-grade gliomas and diffuse intrinsic pontine gliomas, and applying novel molecular diagnostic tools to improve childhood brain tumour classification. Having received his MD in 2011, he is currently in clinical training as a paediatrician at the Department of Pediatric Oncology and Hematology at the Heidelberg University Medical Center for Children and Adolescents.

Sebastian Bender

Sebastian Bender is a postdoctoral research fellow in S.M.P.’s laboratory at the German Cancer Research Center (DKFZ), Heidelberg, Germany. He received his PhD from the University of Heidelberg, Germany, in 2013 for studying the molecular consequences of histone H3.3 mutations in paediatric high-grade gliomas. His main research focus is the characterization of molecular mechanisms driving tumourigenesis in childhood brain tumours.

David T.W. Jones

David Jones is a senior postdoctoral scientist at the DKFZ, where he has played a major part in the International Cancer Genome Consortium (ICGC) PedBrain Tumour sequencing project. He received his PhD from the University of Cambridge in 2009, working in the group of V. Peter Collins. In 2008, he was the first to describe a highly recurrent *BRAF* fusion gene occurring in two-thirds of the most common childhood brain tumour (pilocytic astrocytoma). His primary research focus is the application of cutting-edge genomics techniques to identify new diagnostic, prognostic and therapeutic targets in the field of neurooncology.

Peter Lichter

Peter Lichter pioneered the development of technologies to delineate almost any chromosomal region by fluorescence *in situ* hybridization (FISH) and to detect DNA copy-number alterations *via* high resolution comparative genomic hybridization (arrayCGH). Applying these, as well as next-generation DNA sequencing approaches, he made major contributions to decipher the higher order genome organization and to elucidate pathomechanisms of tumour aetiology and progression, including the description of novel prognostic or predictive gene signatures.

Jacques Grill

Jacques Grill is the Head of the Pediatric Brain Tumour Program at Gustave Roussy, Villejuif, France, and Principal Investigator of Several International Trials including HERBY, a randomized phase II study for paediatric non-brainstem high-grade gliomas and BIOMEDE, a biology-driven trial for diffuse intrinsic pontine glioma. He is the group leader of a research team of the CNRS Unit 8203 dedicated to the discovery of new biomarkers and therapeutics targets in glial tumours.

Oren Becher

Oren Becher is an assistant professor in the Departments of Pediatrics and Pathology at Duke University. His laboratory is focused on the development of genetically engineered Diffuse Intrinsic Pontine Glioma (DIPG) mouse models, dissection of the function of novel genetic alterations in DIPG, and the evaluation of the novel therapeutics.

Cynthia Hawkins

Cynthia Hawkins is a paediatric neuropathologist and scientist at The Hospital for Sick Children (SickKids) in Toronto, Canada. Her clinical practice includes diagnosis of paediatric brain tumours and she serves as a central pathology reviewer for several national and international paediatric brain tumour clinical trials. The Hawkins laboratory is focused on the study of paediatric high grade glioma, in particular diffuse intrinsic pontine glioma. Her lab uses cancer genetic techniques in combination with *in vitro* and *in vivo* models to better understand what cellular processes underlie paediatric high grade glioma and how we can use this information to improve our ability to diagnose and treat children with brain tumours.

Jacek Majewski

Dr. Jacek Majewski obtained his BS in Physics and MS in Electrical Engineering from Stanford, and PhD in Biology from Wesleyan University. Having vowed never to live in New York, he nevertheless followed his fiancée to the Rockefeller University as a post-doctoral fellow in statistical genetics. He is currently a Canada Research Chair and Associate Professor at the Department of Human Genetics at McGill University. His research involves genomics and analysis of high throughput data aimed at understanding human genetic disorders. The approaches include RNA sequencing, exome, and whole genome sequencing, with specific applications to monogenic disorders and cancer.

Chris Jones

Chris Jones is a research team leader at in the Divisions of Molecular Pathology and Cancer Therapeutics at The Institute of Cancer Research (ICR) in London, UK. He completed his first degree in toxicology and pharmacology and later a PhD in molecular biology at the School of Pharmacy, University of London, UK. He moved to the ICR in 2001 and took up his post focusing on the genomics of childhood cancer in 2003. He is a Fellow of the Royal College of Pathologists by published works and is active in international paediatric brain tumour collaborative groups and clinical trials.

Joseph F. Costello

Dr. Costello is a Professor of Neurosurgery at UCSF, holds the Karen Osney Brownstein Endowed Chair in Neuro-oncology and serves as the Director of the UCSF-based NIH Roadmap Epigenome Mapping Center. Dr. Costello's laboratory investigates how genetic and epigenetic mechanisms interact in the control of gene expression. His research includes epigenetic technology development and its application to human stem cells and tissues from different developmental stages. His translational research investigates tumour genome and epigenome evolution by integrating treatment, advanced imaging and histologic analyses with patterns of mutations and epimutations.

Antonio Iavarone

Antonio Iavarone received his MD at the Catholic University of Rome, where he also received the Residency degree in Paediatrics and performed a fellowship training in Paediatric Oncology. He worked at UCSF, Memorial Sloan Kettering Cancer Center and Albert Einstein College of Medicine in New York. He is Professor of Neurology and Pathology at Columbia University. One of the first discoveries made by his group is the now widely accepted notion that the most aggressive forms of brain tumours are invariably associated with loss of differentiated features. In more recent years his laboratory identified the master regulators and key genomic drivers (gene fusions and mutations) responsible for initiation, progression and maintenance of malignant glioma.

Kenneth Aldape

Kenneth Aldape is a neuropathologist with an interest in basic and translational research in brain tumours. He has a particular interest in molecular classification of glioma. His research has encompassed expression profiling and use of other genome-wide platforms to identify subclasses of these tumours. He is particularly interested in clinically relevant molecular alterations that can inform treatment and potentially lead to precision medicine for patients with primary and metastatic brain tumours.

Cameron W. Brennan

Cameron Brennan is an oncologic neurosurgeon and a laboratory investigator focused on tumour molecular profiling and brain tumour drug responses. He received his MD and neurosurgery training at New York Hospital – Cornell Medical Center, followed by postdoctoral work in Ronald DePinho’s laboratory at the Dana-Farber Cancer Institute. He joined Memorial Sloan-Kettering Cancer Center in 2004 and opened a laboratory in 2009 within the Human Oncology and Pathogenesis Program. He has been a part of The Cancer Genome Atlas from its inception, initially developing methods to identify DNA structural rearrangements and recently as external co-chair of the glioblastoma initiative.

Nada Jabado

Nada Jabado is an Associate Professor with tenure in the Department of Paediatrics and an associate member of the departments of Human Genetics, Oncology, and Experimental Medicine at McGill University. She obtained her MD with a specialization in Paediatrics and a PhD in Immunology from the Université de Paris VI in France. Her work aims to identify molecular alterations underlying paediatric gliomas. She has authored/co-authored 99 peer-reviewed papers in her career and received several awards recognizing her contribution to the field of cancer including the William E. Rawl young investigator award from the National Canadian Cancer Research Council in 2012.

Stefan M. Pfister

Stefan M. Pfister was appointed Head of the Division of Pediatric Neurooncology at the DKFZ in 2012. He is a paediatrician by training, and received his MD from Tübingen University, Germany, and his clinical education at Mannheim and Heidelberg University Hospitals. As a physician scientist, he completed postdoctoral fellowships with Christopher Rudd at the Dana-Faber Cancer Institute/Harvard Medical School, Boston, USA, and with Peter Lichter at the DKFZ, Division of Molecular Genetics, Heidelberg, Germany. His research is focused on the genetic characterization of childhood brain tumours through the application of next-generation profiling methods and subsequently translating novel findings into a clinical context.

Key points

- Glioblastoma is the most frequent and most aggressive malignant primary brain tumour and remains almost universally incurable in both children and adults.
- Comprehensive molecular profiling studies have greatly broadened our knowledge of the underlying genomic and epigenomic aberrations associated with glioblastoma initiation and progression.
- Genetic lesions result in disrupted epigenetic control mechanisms by altering histone modifications, DNA methylation and gene expression patterns in a large proportion of glioblastomas.
- Based on recurrent combinations of genomic and/or epigenomic features with distinct patient characteristics, glioblastomas across all ages are being dissected into meaningful biological subgroups, which are likely to guide future clinical trial design.
- The complex interplay between the glioblastoma genome and epigenome opens the avenue for the development of novel innovative therapeutic strategies that are urgently needed to tackle this deadly brain tumour.

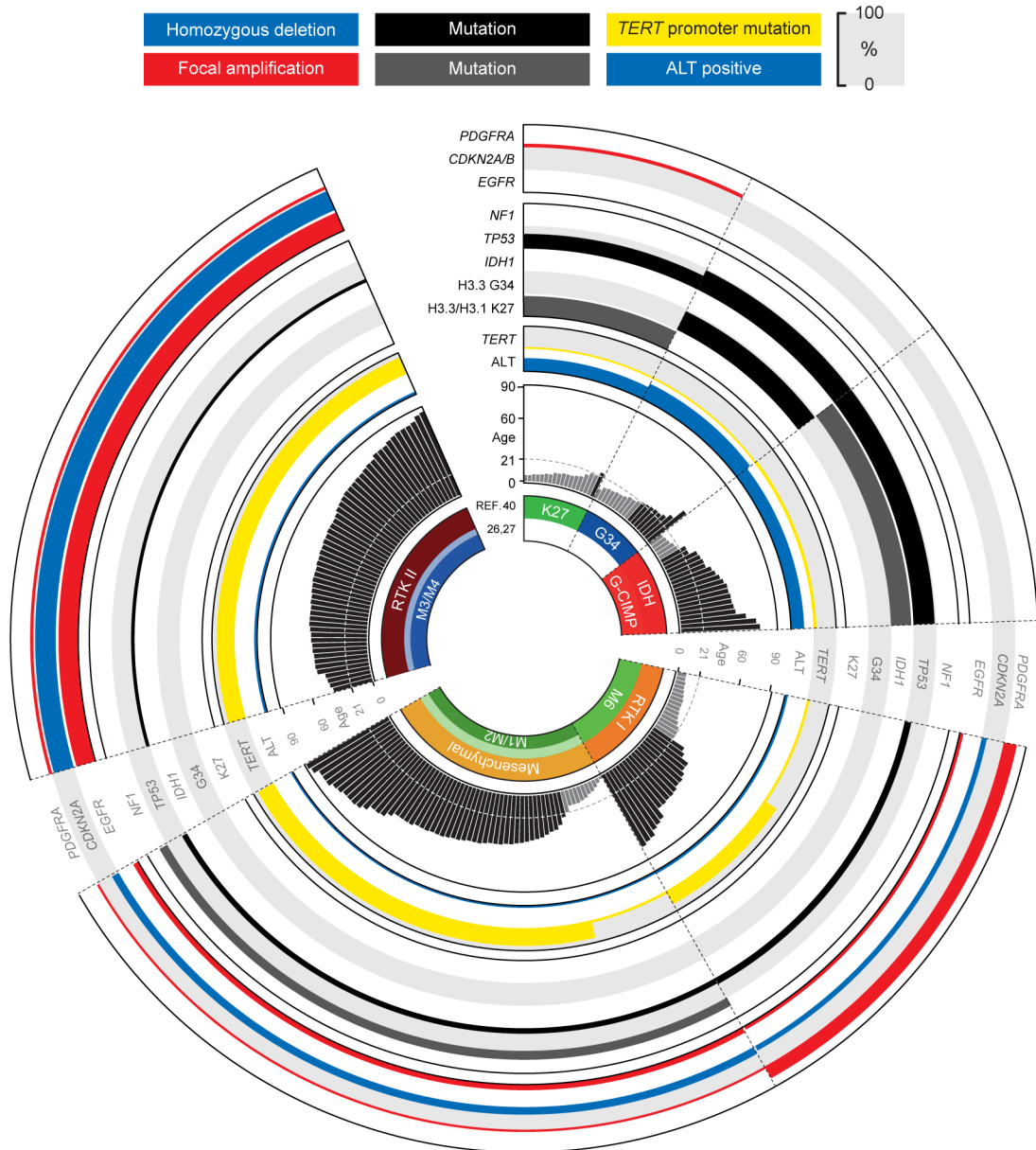


Figure 1. Age-based genomic and epigenomic features of biological glioblastoma subgroups

Simplified schematic overview of glioblastoma subgroups depicting recurrent associations between genomic and epigenomic features: (from inside to outside) DNA methylation subclass affiliation (TCGA methylation [REFS^{26, 27}], and dkfz methylation [REF.⁴⁰]), patient age (years), telomere maintenance mechanisms, mutational status (H3.3 and H3.1 K27, H3.3 G34, *IDH1*, *TP53* and *NF1*), and copy-number aberrations, grouped by biological subgroup and sorted by patient age. Copy-number states, presence of mutations and ALT positivity are represented by different colours as indicated. Height of bars represents an estimated percentage of cases positive for a specific feature. ALT, alternative lengthening of telomeres; *CDKN2A* and *B*, cyclin-dependent kinase inhibitor 2A and 2B; *EGFR*, epidermal growth factor receptor; G-CIMP, glioma-CpG island methylator phenotype; *IDH1*, isocitrate dehydrogenase 1; *NF1*, neurofibromin 1; *PDGFRA*, platelet-derived growth factor receptor type alpha; RTK, receptor tyrosine kinase; *TERT*, telomerase reverse transcriptase.

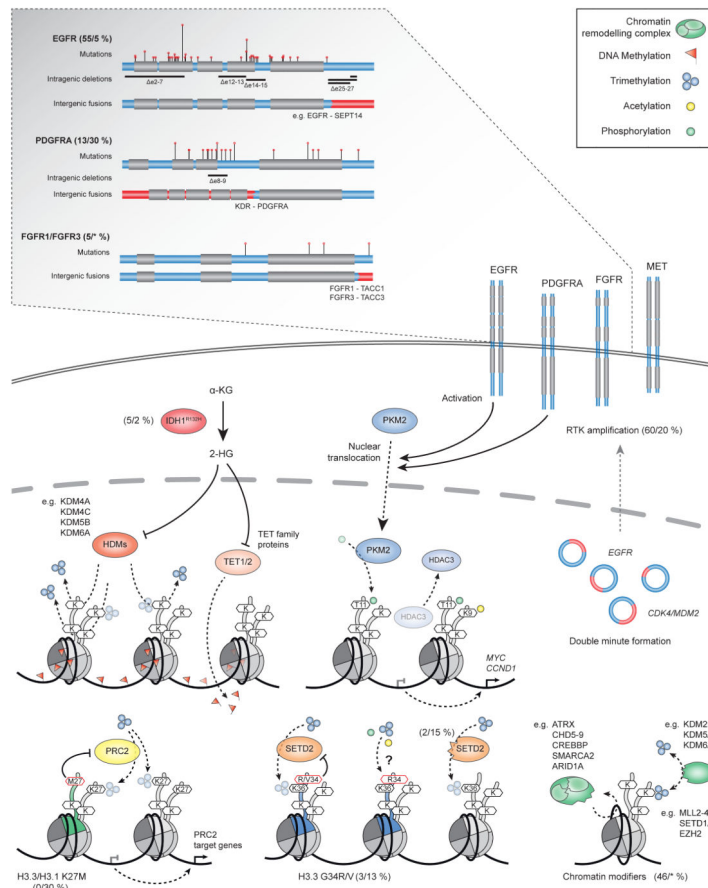


Figure 2. Interplay between the glioblastoma genome and epigenome

Frequent genomic alterations in GBM impacting the epigenomic machinery. Receptor tyrosine kinases (RTKs) are commonly activated by somatic mutations or structural variations (see magnification), leading to PKM2 nuclear translocation and expression of *MYC* and *CCND1*. Recurrent somatic mutations in *IDH1*, *IDH2*, histone proteins, *SETD2* and various other chromatin modifying proteins result in the disruption of multiple epigenetic regulatory processes by affecting histone modification, DNA methylation and chromatin remodelling. Numbers in brackets represent estimated frequencies of alterations observed in GBM of adults and childhood HGG, respectively (see Table 2 for exact numbers; *unknown). 2-HG, 2-hydroxyglutarate; α -KG, α -ketoglutarate; ARID1A, AT-rich interactive domain-containing protein 1A; ATRX, alpha thalassemia/mental retardation syndrome X-linked; CDK4, cyclin-dependent kinase 4; CHD, chromodomain helicase DNA binding protein; CREBBP, CREB-binding protein; EGFR, epidermal growth factor receptor; EZH2, enhancer of zeste homolog 2; FGFR, fibroblast growth factor receptor; HDM, histone demethylase; *IDH1*, isocitrate dehydrogenase 1; KDM, lysine (K)-specific demethylase; KDR, kinase insert domain receptor; MDM2, mouse double minute 2 homolog; MLL, mixed-lineage leukemia; PDGFRA, platelet-derived growth factor receptor type alpha; PKM2, pyruvate kinase muscle isozyme; PRC2, polycomb repression complex 2; RTK, receptor tyrosine kinase; SEPT14, septin 14; SETD1A, SET domain containing 1A; SETD2, SET domain containing 2; SMARCA2, SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2; TACC, transforming acidic coiled-coil.

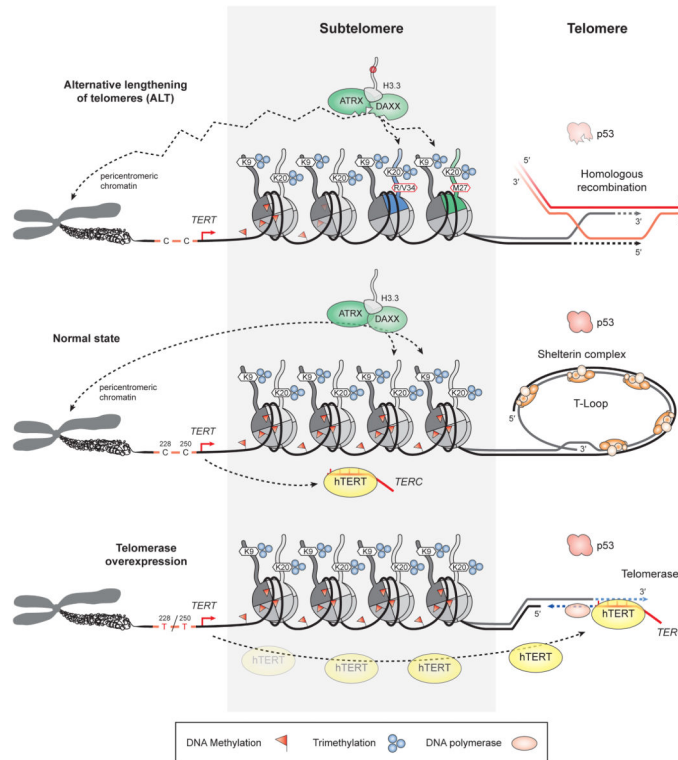


Figure 3. Telomere maintenance mechanisms in glioblastoma

Maintenance of telomere length is accomplished by different mechanisms in GBM. In normal cells (middle panel), chromosome ends are protected from undergoing non-homologous end joining or homologous recombination by forming a T-Loop structure.

Alternative lengthening of telomeres (ALT), thought to be mediated by homologous recombination, is more prevalent in the paediatric setting (upper panel). While mutations in ATRX or DAXX (mediating incorporation of histone H3.3 into pericentromeric and subtelomeric regions) are known to promote ALT, the contribution of p53 loss, H3.3 mutations and/or subtelomeric DNA hypomethylation (such as in GBM harbouring H3.3 G34R/V mutation) needs further elucidation. Telomere length in adult GBM (lower panel) in the presence of functional p53 is mainly maintained by upregulated telomerase (hTERT) expression as a consequence of *TERT* promoter hotspot mutations C228T or C250T, for example. ATRX, alpha thalassemia/mental retardation syndrome X-linked; DAXX, death-domain associated protein; T-Loop, telomere loop; TERC, telomerase RNA component; *TERT*, telomerase reverse transcriptase.

Table 1

Frequent chromosomal copy-number alterations in glioblastoma

Genomic region	Copy-number change	Frequency (%) ^{adult GBM*}	Frequency (%) ^{childhood HG-G^{††}}	Subtype enrichment	References
Chromosome 1q	Single copy gain	9	19–29	G34	26, 36, 38
Chromosome 6q	Single copy loss	22	4–5	non-H3/non-G-CIMP	26, 36, 38
Chromosome 7	Single copy gain	74–83	13–19	non-H3/non-G-CIMP	26, 36, 38, 40
Chromosome 9p	Single copy loss	33–38	11–18	non-K27	26, 36, 38
Chromosome 10	Single copy loss	80–86	16–38	non-H3/non-G-CIMP	26, 36, 38, 40
Chromosome 13q	Single copy loss	31–33	24–34	G34, RTK I	26, 36, 38
Chromosome 14q	Single copy loss	26–27	16–29	RTK I	26, 36, 38
Chromosome 19	Single copy gain	35–40	1–13	Classical, RTK II	26, 36, 38
Chromosome 20	Single copy gain	39	3–6	Classical, RTK II	26, 36, 38
Chromosome 22q	Single copy loss	33	1–16	G-CIMP, MES, RTK II	26, 36, 38

* 21 years;

^{††} < 21 years, diffuse intrinsic pontine glioma (DIPG), anaplastic astrocytoma WHO grade III, GBM; G-CIMP, glioma-CpG island methylator phenotype; RTK, receptor tyrosine kinase.

Table 2

Recurrent somatic alterations in glioblastoma

Target gene(s)	Alteration type	Frequency (%)/adult GBM*	Frequency (%)/childhood HGG [†]	Subtype enrichment	References
EGFR	Focal amplification	43	1–11	Classical, Neural, RTK II	25, 26, 36, 38, 40, 67, 74
	Point mutation	18–26	4	Classical	26, 27, 41
	Intragenic deletion (e2-7; EGFRvIII)	10–64	17	Classical	26, 74, 76
	Intragenic deletion (e25-27)	3–9	NA	Classical	26
	Intragenic deletion (e12-13/ e14-15)	29/3	NA	Classical	26
PDGFRA	Fusion	8	NA	Classical, Mesenchymal	26, 32
	Focal amplification	11–26	8–39	Proneural non-G-CIMP, RTK I	25, 26, 36, 38, 40, 84, 217
PTEN	Mutation	3–4	4–9		4, 26, 41, 79
	Intragenic deletion	7–18	4		26, 78, 79
	Focal deletion	8–10	0–1		26, 36, 38
NFI	Mutation	23–31	1–12	non-G-CIMP	26, 41, 218, 219
	Focal deletion	1	0–1	Mesenchymal	25, 26, 36, 38
	Mutation	9–10	24	Mesenchymal	25, 26, 41
RBI	Focal deletion	3–4	0–3		26, 36, 38
	Mutation	7–9	10	Mesenchymal	26, 41
CDKN2A and CDKN2B	Focal deletion	62	10–19	non-H3/non-G-CIMP	25, 26, 36, 38, 217
	Promoter mutation	55–83	3–11	non-H3/non-G-CIMP	26, 91–93, 95
TP53	Mutation	20–29	34–37	K27, G34, G-CIMP	25–27, 72, 220

Target gene(s)	Alteration type	Frequency (%adult GBM)*	Frequency (%childhood HGG)†	Subtype enrichment	References
<i>CDK4</i>	Focal amplification	13–18	3–4	Proneural non-G-CIMP	26, 36, 38
<i>FGFR1</i> or <i>FGFR3</i>	Fusion	3–8	NA		82, 83
<i>IDH1</i>	Mutation	5–12	0–16	G-CIMP	25–27, 36, 40, 41, 86, 113, 221
<i>PIK3CA</i>	Mutation	7–21	8–20		26, 41, 222, §
<i>PIK3RI</i>	Mutation	6–11	10–12		26, 41, §
<i>H3F3A G34R/V</i>	Mutation	0–3	12–14	G34	26, 40, 41, 101
<i>H3F3A K27M</i>	Mutation	0–1	23–43	K27	26, 40, 41, 101, §, ‡
<i>HIST1H3B K27M</i> or <i>HIST1H3C K27M</i>	Mutation	0	12–31‡		26, 67, 101, §, ‡
<i>ATRX</i>	Mutation	6	14–29	G34, G-CIMP	26, 41, 97
<i>SETD2</i>	Mutation	1–2	15		26, 110
<i>ACVRI</i>	Mutation	0	20–22‡		26, §, ‡
<i>MYCN</i>	Focal amplification	3	3–5		26, 36, 38, 217
<i>MYC</i>	Focal amplification	2	3–4	G-CIMP	26, 36, 38
<i>BRAFV600E</i>	Mutation	2	10–25		26, 41, 223, 224

* 21 years;

† < 21 years, diffuse intrinsic pontine glioma (DIPG), anaplastic astrocytoma WHO grade III, GBM; *ACVRI*, activin a receptor type I; *ATRX*, alpha thalassemia/mental retardation syndrome X-linked; *CDK4*, cyclin-dependent kinase 4; *CDKN2A* and *B*, cyclin-dependent kinase inhibitor 2A and 2B; *EGFR*, epidermal growth factor receptor; *FGFR*, fibroblast growth factor receptor; G-CIMP, glioma-CpG island methylator phenotype; *IDH1*, isocitrate dehydrogenase 1; *NFI*, neurofibromin 1; *PDGFRA*, platelet-derived growth factor receptor type alpha; *PIK3CA*, phosphatidylinositol 3-kinase catalytic subunit alpha; *PIK3RI*, phosphatidylinositol 3-kinase regulatory subunit alpha; *P TEN*, phosphatase and tensin homolog; *RBI*, retinoblastoma 1; RTK, receptor tyrosine kinase; *SETD2*, SET domain containing 2; *TERT*, telomerase reverse transcriptase;

‡ DIPG only;

§ C.J., J.G., O.B., C.H., J.M., N.J., unpublished observations;

‡ Suzanne J. Baker, personal communication.