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Guido Reifenberger, Hans-Georg Wirsching, Christiane B. Knobbe-Thomsen, Michael Weller

Institutions: University of Düsseldorf, University of Zurich

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Advances in the molecular genetics of gliomas — implications for classification and therapy

Guido Reifenberger[1,2], Hans-Georg Wirsching[3], Christiane B. Knobbe-Thomsen[1], Michael Weller[3]*

[1]Department of Neuropathology, Heinrich Heine University Düsseldorf, Moorenstrasse. 5, D-40225 Düsseldorf, Germany.

[2]German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ) Heidelberg, partner site Essen/Düsseldorf, Moorenstrasse. 5, D-40225 Düsseldorf, Germany.

[3]Department of Neurology and Brain Tumour Centre, Cancer Centre Zürich, University Hospital and University of Zürich, Frauenklinikstrasse 26, CH-8091 Zürich, Switzerland.

*Current address: Fred Hutchinson Cancer Research Center, Human Biology Division, 1100 Fairview Avenue North, C3-111, P.O. Box 19024, Seattle, Washington 98109-1024, USA.

Correspondence to G.R.

reifenberger@med.uni-duesseldorf.de

Abstract | Genome-wide molecular profiling studies have revealed characteristic genetic alterations and epigenetic profiles associated with different types of gliomas. These molecular characteristics can be used to refine glioma classification, to improve prediction of patient outcomes, and to guide individualized treatment. Thus, the WHO Classification of Tumours of the Central Nervous System was revised in 2016 to incorporate molecular biomarkers — together with classic histological features — in an integrated diagnosis, in order to define distinct glioma entities as precisely as possible. This paradigm shift is markedly changing glioma diagnostics, and has important implications for future clinical trials and patient management in daily practice. Herein, we highlight the developments in our

understanding of the molecular genetics of gliomas, and review the current landscape of clinically relevant molecular biomarkers for use in classification of gliomas. Novel approaches to the genetic characterization of gliomas based on large-scale DNA-methylation profiling and next-generation sequencing are also discussed. In addition, we illustrate how advances in the molecular genetics of gliomas can promote the development and clinical translation of novel pathogenesis-based therapeutic approaches, thereby paving the way towards precision medicine in neuro-oncology.

Malignant tumours of the central nervous system (CNS) are among the cancers with the poorest prognosis, as indicated by the association of brain tumours with the highest estimated number of years (mean: ~20 years) of potential life lost owing to any cancer[1]. Gliomas are the most common primary CNS tumours, with an estimated annual incidence of 6.6 per 100,000 individuals in the USA[2]. About half of all newly diagnosed gliomas correspond to glioblastoma, which is the most malignant type of brain cancer — with median patient survival durations of approximately 14–17 months in contemporary clinical trials[3-5] and ~12 months in population-based studies[2,6].

Studies in transgenic mice indicate that gliomas can arise from a range of cell types, including neural stem cells, astrocytes, or oligodendroglial progenitor cells[7]. Genome-wide molecular-profiling studies have revealed comprehensive mutational landscapes for all major types of human gliomas occurring in adults[8-12] and children[13-21]. These developments have markedly advanced our mechanistic understanding of glioma tumorigenesis, and have identified novel biomarkers for improved tumour classification, as well as promising new therapeutic targets.

Before publication of the revised WHO Classification of Tumours of the CNS in 2016[22], gliomas were exclusively classified using light microscopy according to histological criteria defined in the 2007 WHO Classification[23]. In addition to histological tumour typing, each tumour is assigned to a histological grade based on the degree of anaplasia, from WHO grade I to IV. This WHO grading system reflects tumour malignancy and presumed natural disease course, with WHO grade I indicating a slow-growing lesion usually associated with favourable prognosis, whereas WHO grade IV is assigned to highly malignant tumours. Histological classification has for many decades served as the ‘gold-standard’ for glioma diagnostics, but is associated with considerable interobserver variability, particularly in the context of diffusely infiltrating gliomas[24]. Studies have revealed that molecular classifications of gliomas correlate better with clinical outcome than histological classification[10,11,25,26]. Moreover, certain histological entities, such as glioblastoma, encompass a spectrum of biologically distinct tumour groups associated with differences in age at onset, tumour

location, and prognosis[8,9,12,21]. In addition, some traditional glioma categories, including oligoastrocytoma and gliomatosis cerebri, lack disease-specific genetic profiles, and consist of diverse astrocytic and oligodendroglial entities[27,28]. In the revised 2016 WHO Classification of Tumours of the CNS[22], the advances in our molecular understanding of gliomas are leveraged in a novel, multilayered approach to disease categorization incorporating both histological and molecular information in an 'integrated diagnosis' (BOX 1).[29-30]

In this Review, we highlight advances in the molecular genetics of gliomas, with a particular focus on diagnostically relevant alterations. In addition, we address the role of predictive biomarkers and novel high-throughput molecular testing in glioma diagnostics, and discuss the implications of these advances on the clinical management of patients with glioma, as well as the design of future clinical trials.

[H1] Molecular genetics of adult gliomas

A major improvement in the 2016 WHO classification of gliomas, compared with the preceding 2007 classification, is the distinction of different glioma entities according to isocitrate dehydrogenase 1 or 2 (*IDH*) mutation status (TABLE 1). The discovery of *IDH* mutations in most WHO grade II and III gliomas constituted a key breakthrough in understanding the disease[31-33]. Numerous studies have revealed that the presence of *IDH* mutations distinguishes gliomas with distinct biologies and clinical behaviours[34]. Mechanistically, mutant IDH proteins acquire a neomorphic enzymatic activity that results in conversion of α-ketoglutarate (α-KG) to D-2-hydroxyglutarate (D-2-HG), which in turn inhibits α-KG-dependent dioxygenases, such as ten-eleven translocation (TET) family 5-methylcytosine hydroxylases and the Jumonji-C-domain-containing histone lysine demethylases[35]. Thereby, *IDH* mutation causes aberrant DNA and histone methylation, eventually leading to widespread hypermethylation of CpG islands, a phenomenon termed the 'glioma CpG-island methylator phenotype' (G-CIMP)[36]. Diagnostic testing for *IDH* mutations usually involves immunostaining with an antibody to IDH1 R132H protein[37], which detects the most common missense mutation in gliomas present in approximately 90% of the

cases and has proved reliable across different laboratories[38]; however, immunonegative tumours require additional molecular testing — for example, by DNA sequencing — to rule out the presence of other *IDH1* or *IDH2* mutations[22,30]. In the following sections, we briefly summarize the genetic alterations most commonly associated with the prototypic glioma entities defined in the 2016 WHO classification (TABLE 1).

[H2] Diffuse astrocytic and oligodendroglial tumours

The diffuse astrocytic and oligodendroglial tumour category of brain cancers comprises diverse glioma subtypes. The main disease entities included in this group are *IDH*-mutant astrocytic gliomas of WHO grades II–IV, *IDH*-mutant and 1p/19q-codeleted oligodendroglial tumours of WHO grades II–III, *IDH*-wild-type glioblastomas of WHO grade IV, and a newly introduced class of histone H3 K27M (H3-K27M)-mutant diffuse midline gliomas of WHO grade IV (TABLE 2; FIG. 1)[22]. *IDH*-wild-type diffuse and anaplastic astrocytomas (WHO grades II and III, respectively) are provisional categories in the 2016 WHO classification (TABLE 2); in adults, most of these tumours are associated with a poor prognosis and often harbour genetic aberrations that are detected in *IDH*-wild-type glioblastomas, indicating that such cases might reflect underestimation of malignancy grade based on histology[25,39]. Importantly, however, a subset of *IDH*-wild-type diffuse astrocytomas share molecular similarities with pilocytic astrocytoma and are associated with favourable survival[12]. Thus, in patients with disease initially classified as *IDH*-wild-type diffuse or anaplastic astrocytoma, additional molecular testing for genetic aberrations associated with either *IDH*-wild-type glioblastoma, (for example, *TERT*-promoter mutations, *EGFR* amplification, loss of chromosome 10 and gain of chromosome 7), or pilocytic astrocytoma (such as *KIAA11549–BRAF* fusion; TABLE 2) may provide diagnostically helpful information[40].

[H3] IDH-mutant astrocytic gliomas. *IDH* mutation is probably among the earliest genetic aberrations that occur during the development of these tumours[11]; however, findings in mice indicate that *IDH* mutation alone is not sufficient for tumorigenesis[41]. Indeed, *IDH*-mutant astrocytomas commonly carry additional mutations in *TP53* and *ATRX*[10,11], indicating that

IDH-mutant astrocytoma development requires multiple genetic 'hits'. *ATRX* mutations lead to immunohistochemically detectable loss of nuclear expression of the transcriptional regulator ATRX that is important in chromatin remodeling and regulation of telomere length[42] (FIG. 2a). Genetic alterations associated with progression from diffuse (WHO grade II) to anaplastic (WHO grade III) astrocytoma and eventually *IDH*-mutant (secondary) glioblastoma are variable and include chromosomal 9p21 deletions involving *CDKN2A (*encoding both cyclin-dependent kinase inhibitor 2A, also known as $p16^{INK4A}$, and ARF, also known as $p14^{ARF}$) and *CDKN2B* (encoding cyclin-dependent kinase 4 inhibitor B, also known as $p15^{INK4B}$), deletion of 19q, and a variety of other chromosomal imbalances[40]. In addition, activation of MYC and the receptor tyrosine kinase (RTK)/RAS/PI3K pathway, upregulation of forkhead box protein M1 (FOXM1) expression and E2F2-dependent cell-cycle progression, and epigenetic silencing of developmental transcription factor genes regulated by the polycomb repressive complex 2 have been implicated in progression of *IDH*-mutant gliomas[43]. Accumulation of somatic mutations in inhibitors of the G1/S cell-cycle checkpoint, including members of the retinoblastoma (RB) pathway, and low levels of methylation at CpG sites in regulatory regions of genes involved in cell-cycle progression, e.g. *TP73*, further implicate dysregulated cell division as a convergence point of molecular events driving progression[12,44].

[H3] IDH-mutant and 1p/19q-codeleted oligodendroglial tumours. Oligodendrogliomas are genetically defined by coexistent *IDH* mutation and whole-arm codeletion of chromosome 1p and 19q (FIG. 2b) — the latter aberration is caused by an unbalanced t(1;19)(q10;p10) translocation[45-46]. Activating mutations in the *TERT* promoter (which lead to aberrant expression of telomerase reverse transcriptase)[47] are present in >95% of oligodendroglial tumours and *CIC* mutation (resulting in inactivation of the protein homologue of *Drosophila* capicua, a transcriptional repressor) is detectable in more than two thirds of the cases[48]. Mutations in *FUBP1* (encoding far upstream element-binding protein 1, which is involved in regulating MYC expression) are found in approximately one third of

oligodendroglial tumours (TABLE 2)[48]. Less common alterations affect developmental pathway genes, predominantly *NOTCH1*; genes encoding epigenetic regulators, such as *SETD2*; and PI3K pathway genes, for example, *PIK3CA*[10,11]. Genetic alterations that have been linked to a more-aggressive disease phenotype include 9p21 deletion[49], mutations in the transcription factor 12 gene (*TCF12*)[50], and aberrations resulting in activation of MYC signalling[51].

[H3] Oligoastrocytic gliomas. In the 2016 WHO classification, oligoastrocytomas are no longer considered as separate entities because they lack a distinctive genetic profile, and instead have either astrocytic or oligodendroglial genotypes[10,11,27]. Thus, testing for *IDH* mutation and 1p/19q codeletion is required[22]. Classification as oligoastrocytoma (or anaplastic oligoastrocytoma), not otherwise specified (NOS) is restricted to rare instances in which molecular testing remains inconclusive or could not be properly performed (TABLE 2)[22]. Individual cases of *IDH*-mutant 'oligoastrocytoma' consisting of spatially and genetically distinct populations of oligodendroglial cells with 1p/19q codeletion and astrocytic cells without 1p/19q codeletion but loss of nuclear ATRX expression have been reported[52] but are not recognized as separate disease entity in the WHO classification[22].

[H3] IDH-wild-type glioblastoma. *IDH*-wild-type glioblastomas WHO grade IV can arise in individuals of any age, but preferentially manifest in patients >50 years of age. These tumours typically manifest as 'primary glioblastomas' — that is, glioblastomas that present with a short clinical history of usually less than 3 months before diagnosis and without a pre-existing lower-grade glioma[22]. Glioblastomas that develop *de novo* in non-midline locations in patients ≥55 years of age can be diagnosed as *IDH*-wild-type glioblastoma when immunohistochemical staining for IDH1 R132H is negative[22]. In patients aged <55 years and in patients with clinical evidence of pre-existing lower-grade glioma, exclusion of other *IDH* mutations is required — for example, via DNA sequencing — to fully rule out *IDH*-mutant glioblastoma (see below)[22]. *IDH*-wild-type glioblastomas in adults are characterized by

frequent gain of chromosome 7, monosomy of chromosome 10, mutation or homozygous deletion of *PTEN*, homozygous deletion of *CDKN2A* and *CDKN2B*, and *TERT*-promoter mutations (TABLE 1)[53]; other less-common alterations include mutations in *TP53, PIK3CA, PIK3R1* (encoding PI3K-regulatory subunit 1), and *NF1* (encoding neurofibromatosis type 1)[9]. Gene amplifications are commonly detected in *IDH*-wild-type glioblastomas, and involve the *EGFR*, *PDGFRA*, and *MET* genes encoding mitogenic RTKs; the cyclin-dependent kinases genes *CDK4* and *CDK6* that mediate transition from G1 to S phase of the cell cycle; and *MDM2* and *MDM4*, which encode proteins that inhibit the activity of p53 (TABLE 1)[53]. *EGFR* amplification is detectable in about 40% of *IDH*-wild-type glioblastomas, with half of these tumours also harbouring a genetic rearrangement that results in deletion of *EGFR* exons 2–7 [34,53]. This aberration leads to expression of EGFR variant III (EGFRvIII; FIG. 2c), which lacks the extracellular ligand-binding region encoded by the deleted exons, but is constitutively active[53]. *BRAF*^{V600E} is a rare, but druggable mutation in *IDH*-wild-type glioblastoma that is detectable in approximately 50% of epithelioid glioblastomas (FIG. 2d)[54], a newly described provisional variant of *IDH*-wild-type glioblastoma. Other histological variants are giant cell glioblastoma and gliosarcoma (TABLE 2)[22].

[H3] IDH-mutant glioblastoma. The *IDH*-mutant glioblastoma subtype accounts for <10% of all glioblastomas and typically manifest in young adults[22]. These tumours include almost all secondary glioblastomas that develop via progression from pre-existing lower-grade gliomas. Consequently, the molecular profile associated with this class of gliomas is similar to that of *IDH*-mutant astrocytomas, including frequent *TP53* and *ATRX* mutation alongside a G-CIMP phenotype[53]. Lower DNA-methylation levels as usually present in *IDH*-mutant and G-CIMP-positive astrocytic gliomas have been detected in a subset of patients and are associated with less favourable outcome[12]. However, the prognosis of patients with *IDH*-mutant glioblastoma is typically better — with a greater likelihood of long-term survival — than that of patients with *IDH*-wild-type glioblastoma due to younger mean age at diagnosis, higher

frequency of *MGMT*-promoter methylation (see below) and other, yet to be identified factors[34,53].

[H3] Molecular subgroups of adult glioblastoma. mRNA-expression analyses have revealed four distinct subtypes of glioblastoma: proneural, neural, classic and mesenchymal[55]. The clinical utility of stratifying patients according to these expression signatures is limited, however, as they can be heterogeneous within a given tumour and can change in response to external stimuli, including therapy[56,57]. Nevertheless, the mesenchymal expression signature has been linked to radioresistance and poor survival[54], whereas the proneural signature has been associated with a benefit from antiangiogenic treatment in patients with *IDH*-wild-type glioblastoma.[58]

DNA-methylation profiles can be used to robustly distinguish glioblastoma subgroups associated with specific epigenetic patterns and gene-expression profiles[8,9]. Four major subgroups of adult glioblastoma have been identified, including an *IDH*-mutant, G-CIMP-positive and typically *MGMT*-promoter-methylated subgroup with proneural gene-expression profile, and three subgroups of *IDH*-wild-type glioblastoma (FIG. 3). Among the *IDH*-wild-type glioblastoma subgroups, 'receptor tyrosine kinase I' (RTK I) glioblastomas predominantly occur in adolescents and young adults, and are characterized by *PDGFRA* amplification and a proneural gene-expression profile. The 'receptor tyrosine kinase II' (RTK II) and the 'mesenchymal' *IDH*-wild-type glioblastoma subtypes predominate in patients older than 50 years of age, and are distinguished by different DNA-methylation profiles, with fewer copy number variations and a mesenchymal versus classic gene-expression signature in mesenchymal glioblastoma (FIG. 3)[8].

[H1] Molecular genetics of paediatric gliomas

Paediatric gliomas comprise three major disease groups: firstly, tumours with circumscribed growth that often harbour *BRAF* aberrations; second, tumours with diffuse growth and frequent alterations in *FGFR1* or rearrangement of *MYB,* or the *MYBL* genes; third, a

heterogeneous group of malignant gliomas, including tumours with mutations in histone-H3-family genes.

[H2] Gliomas with circumscribed growth

[H3] Pilocytic astrocytoma. The most common form of glioma with circumscribed growth, pilocytic astrocytoma (WHO grade I), is characterized by genetic alterations that result in activation of MAPK signalling (TABLE 1)[15]; fusion of the *BRAF* and *KIAA1549* genes on chromosome 7q is common, particularly in cerebellar tumours (FIG. 2e), and has been associated with favourable patient outcome[59]. Subsets of pilocytic astrocytomas carry fusions involving different MAPK-pathway genes, such as *RAF1*, *PTPN11,* or *NTRK2,* or harbour mutations in *BRAF*, *KRAS*, *FGFR1,* or *NF1*[15]. Mutations in non-MAPK-pathway genes are usually absent, making pilocytic astrocytoma a 'single-pathway disease'[15]. Pilomyxoid astrocytoma is a rare, histological variant of pilocytic astrocytoma associated with a higher likelihood of local recurrence and cerebrospinal spread (TABLE 2)[22].

[H3] Pleomorphic xanthoastrocytoma. Pleomorphic xanthoastrocytoma (PXA) is typically associated with $BRAF^{V600E}$ mutation, often occurring together with homozygous deletion of *CDKN2A* and loss of $p16^{INK4A}$ expression (TABLE 2)[60-62]. Genetic alterations that drive progression of WHO grade II PXA towards WHO grade III anaplastic PXA are poorly defined, as is the relationship between anaplastic PXA and epithelioid glioblastoma, which also harbour $BRAF^{V600E}$ mutations[63].

[H3] Subependymal giant-cell astrocytoma. The development of subependymal giant-cell astrocytoma (WHO grade I) is closely linked to aberrations affecting the tuberous sclerosis complex (TSC). The typical genetic changes are mutation and allelic losses leading to loss of either hamartin (*TSC1)* or tuberin (*TSC2)* expression (TABLE 1), which results in activation of the mTOR-signalling pathway[64].

[H2] Well-differentiated diffuse gliomas

Unlike their adult counterparts, diffuse gliomas in children usually lack *IDH* mutation and 1p/19q codeletion[16,65]. Subsets of paediatric diffuse gliomas harbour *FGFR1* alterations, rearrangements of *MYB* or the *MYBL* genes, or *BRAF* aberrations (TABLE 1)[16,66]. However, these tumour groups are not yet considered as distinct entities or variants in the 2016 WHO classification[22]. They typically portend a favourable prognosis and malignant progression is uncommon. Angiocentric glioma, a rare WHO grade I glioma with infiltrative growth in children and young adults, is characterized by *MYB–QKI* fusion rearrangements that may promote tumorigenesis via three different mechanisms: *MYB* activation by truncation, enhancer translocation driving aberrant MYB-QKI expression, and hemizygous loss of the tumor suppressor *QKI*[67].

[H2] Malignant gliomas and glioblastomas

H3-K27M mutant diffuse midline glioma is a WHO grade IV glioma typically located in the thalamus, brain stem or spinal cord[22]. This disease entity includes more than 70% of diffuse intrinsic pontine gliomas (DIPGs) in children[14]. The genetic hallmark of these tumour types, K27M mutation in the histone H3-encoding genes *H3F3A* or *HIST1H3B/C*[13,14], leads to global reduction of cellular histone H3 lysine 27 (H3-K27) trimethylation (FIG. 2f) via impaired recruitment of the polycomb repressive complex 2 and inhibition of the histone-lysine *N*-methyltransferase EZH2[68,69]. Of note, findings from preclinical studies with GSK-J4, which inhibits lysine-specific demethylase 6B (a Jumonji-C-domain-containing histone-lysine demethylase), and panobinostat, a pan-histone-deacetylase inhibitor, suggest a potential for epigenetic therapy in the treatment of H3-K27M-mutant gliomas[70,71].

H3-K27M-mutant gliomas frequently harbour mutations in *TP53* and/or *PPM1D* (encoding magnesium-dependent protein phosphatase 1D); amplification of proto-oncogenes, such as *PDGFRA*, *MYC, MYCN, CDK4*, *CDK6,* or *CCND1-3* (encoding cyclin D1–3), *ID2,* and *MET* is also common[13,14,18] (TABLE 1; FIG. 3). About 20% of DIPGs carry activin receptor 1 gene (*ACVR1*) mutations, whereas *FGFR1* alterations are mostly associated with thalamic

tumours[14,18]. *H3F3A*^{K27M}-mutant DIPGs differ from their *HIST1H3B*^{K27M}-mutant counterparts by a proneural versus a mesenchymal gene-expression profile, and the former have been associated with a less-favourable outcome[72].

Hemispheric malignant gliomas in children comprise different molecular subgroups, including *H3F3A*^{G34}-mutant tumours and a small fraction (~6% of paediatric glioblastomas in one series)[21] of *IDH*-mutant glioblastomas (FIG. 3). *IDH*-wild-type glioblastomas with genetic profiles similar to those of *IDH*-wild-type glioblastomas in adults also occur in children, and are associated with poor prognosis[21]; however, approximately 20% of paediatric glioblastomas have prognostically favourable epigenetic profiles related to PXA or well-differentiated (low-grade) paediatric gliomas[21].

[H1] Molecular genetics of ependymal tumours

A study published in 2015 revealed nine distinct biological subgroups of ependymomas, consisting of three subgroups each among spinal, posterior fossa, and supratentorial tumours[20]. Prognostically favourable tumours with subependymoma-like molecular profiles can occur at each of these anatomical sites.

More than two thirds of supratentorial ependymomas in children carry gene rearrangements that result in fusion proteins involving the NF-κB subunit RELA and C11orf95 (TABLE 1)[19,20]. These RELA-fusion-positive tumours, which have an aberrant NF-κB transcriptional programme and an unfavourable prognosis, constitute a novel entity in the 2016 WHO classification (TABLE 2)[22]. Less commonly, supratentorial ependymomas harbour YES-associated protein 1 (*YAP1*) fusions (TABLE 1), and these tumours are associated with more-favourable outcomes[20].

Among posterior fossa ependymomas, a prognostically unfavourable subgroup (PF-A), is characterized by genomic stability, and tumours in this subgroup are probably mainly driven by epigenetic mechanisms as recurrent genetic alterations have not been identified (TABLE 1)[20]. Another subgroup (PF-B) has chromosomal instability, but a clinically less-aggressive phenotype[20,73].

Spinal ependymomas are mostly indolent tumours, with intramedullary ependymomas frequently demonstrating *NF2* mutations (TABLE 1), while myxopapillary ependymomas of the filum terminale often show multiple numerical chromosome aberrations[20]. Among all ependymoma subgroups, *RELA*-fusion-positive supratentorial ependymomas and PF-A ependymomas are associated with the worst outcomes as indicated by a 10-year overall survival rate of approximately 50% compared to 88-100% in the other subgroups in a large restrospective cohort[20].

[H1] Glioma biomarkers

[H2] Entity-defining molecular biomarkers

The 2016 WHO classification of gliomas incorporates *IDH* mutation, 1p/19q codeletion, H3-K27M mutation and *C11orf95–RELA* fusion as diagnostic biomarkers that define distinct glioma entities (TABLE 2). Other biomarkers can provide additional diagnostic information, including loss of nuclear ATRX expression, *TERT*-promoter mutation, *BRAF* mutation or fusion, and H3-G34 mutation[30,40]. If molecular testing cannot be performed, or the results remain inconclusive, the term 'NOS' (not otherwise specified) has been introduced to indicate that the diagnosis is based on histology only — that is, information on the relevant biomarker(s) was not available for an integrated diagnosis (TABLE 1)[22,30].

[H2] Predictive molecular biomarkers

The number of biomarkers of predictive significance for guiding the post-surgery treatment of patients with glioma is increasing[74]**.** Among these, the presence of *MGMT*-promoter methylation is predictive of benefit from alkylating agent chemotherapy in patients with *IDH*-wild-type glioma, particularly in elderly patients[75,76]. Long-term follow-up studies of two phase III trials in patients with anaplastic glioma have revealed that 1p/19q codeletion is a predictive marker for benefit from upfront combined radiotherapy and chemotherapy with procarbazine, lomustine, and vincristine (PCV)[77,78]. Other genetic aberrations are emerging as potential predictive biomarkers of response to glioma therapy[40] (TABLE 3).

[H3] MGMT-promoter methylation. The standard drug used in concomitant radiochemotherapy and maintenance chemotherapy for glioblastoma is the DNA-alkylating agent temozolomide[79]. A major temozolomide-induced DNA-adduct, 6-*O*-methylguanine, is effectively repaired by 6-*O*-methylguanine-DNA methyltransferase (MGMT; also known as methylated-DNA--protein-cysteine methyltransferase), via alkylation of the enzyme itself, followed by ubiquitylation and subsequent proteasomal degradation of the enzyme[80]. Thus, MGMT expression levels correspond to the cellular 6-*O*-methylguanine-repair capacity, and tumour cells with low or absent MGMT expression are rendered more sensitive to temozolomide[80]. Approximately 40% of *IDH*-wild-type glioblastomas demonstrate hypermethylation of a *MGMT*-associated 5'-CpG island, which results in transcriptional repression and reduced MGMT expression[80]. This epigenetic change is known as *MGMT*-promoter methylation, and has been closely linked to benefit from temozolomide therapy and prolonged survival of patients with glioblastoma[3,81]. Moreover, phase III trials in glioblastoma patients aged >65 years revealed that *MGMT*-promoter methylation is a highly relevant biomarker for guiding treatment decisions between radiotherapy or temozolomide chemotherapy[75,76]. The predictive role of *MGMT*-promoter methylation for response to temozolomide might be restricted to *IDH*-wild-type gliomas, however. *MGMT*-promoter methylation is present in most *IDH*-mutant and G-CIMP-positive gliomas and portends a favourable prognosis in these settings, but is not linked to differential benefit from either temozolomide or radiotherapy[82].

MGMT-promoter methylation usually occurs homogeneously within different regions of an individual glioma[83], and remains stable over the course of disease[84]. Nevertheless, tumours with *MGMT*-promoter methylation can acquire secondary temozolomide resistance owing to mutations that drive clonal evolution and tumour recurrence. For example, mutations in DNA mismatch repair genes can cause a hypermutator genotype[85]. Despite the clinical significance of *MGMT*-promoter methylation, diagnostic testing remains challenging owing to heterogeneous methylation of *MGMT*-associated CpG sites between different tumours, unclear thresholds for defining ‘positivity’ in tumours with weakly or borderline detectable

MGMT-promoter methylation, and the use of diverse, nonstandardized testing methods[40,80]. Thus, harmonization of test protocols, and establishment of internal and external quality assessments are important to assure robust diagnostic results.

[H3] 1p and 19q codeletion. 1p/19q codeletion has been implicated as independent predictive biomarker of benefit from the addition of PCV chemotherapy to upfront treatment with irradiation in patients with anaplastic glioma[77,78]. The mechanisms underlying the favourable treatment responses and long-term survival of patients with *IDH*-mutant and 1p/19q-codeleted gliomas (median overall survival of >10 years) are poorly understood. Of note, only whole-arm 1p/19q codeletion combined with *IDH* mutation is prognostically favourable; partial deletions on either chromosome arm, which can occur in *IDH*-wild-type glioblastomas, are associated with poor patient outcomes[86].

[H3] Emerging predictive biomarkers. *BRAF*^{V600E} mutation has emerged as a promising predictive biomarker of response to BRAF inhibitors in patients with glioma.[87] Similarly, detection of *IDH* mutation is important to identify patients suitable for evaluation of treatments with inhibitors of mutant IDH[88], or peptide-based vaccination targeting IDH1 R132H[89] in clinical trials. Likewise, treatments targeted at EGFR or EGFRvIII would require predictive testing for *EGFR* amplification or EGFRvIII positivity[90-94]. Another potential predictive biomarker is the presence of *FGFR–TACC* fusions, which may identify patients with glioblastoma who are potentially eligible for FGFR-inhibitor therapy[95,96].

[H2] Novel molecular diagnostics approaches

Each of the biomarkers we have discussed can be assessed using tests predicated on a single protein or gene, involving immunohistochemistry, fluorescence *in situ* hybridisation (FISH), DNA sequencing, or other methods[40]. However, the advent of high-throughput technologies for molecular testing, including microarray-based procedures and next-generation sequencing (NGS), provides promising opportunities for the development of novel

diagnostics (BOX 2). At present, application of whole-exome or whole-genome sequencing is mostly restricted to research projects and selected clinical trials, e.g. the INFORM trial in paediatric patients[97] or the NCT Neuro Master Match (N^2M^2) and NCI-MATCH trials in adult patients[98,99]. However, NGS of brain-tumour-tailored gene panels is already performed in clinical practice in some centres, typically covering between ~20 to ~150 genes known to be mutated in gliomas and/or other types of brain tumours[100-103]. This technique can be applied to routinely processed tissue samples, and enables sequencing of diagnostically relevant genes, as well as identification of actionable mutations at high sensitivity and specificity. Thereby, fast and robust parallel analysis of multiple markers can be achieved at affordable costs. Another complementary approach involves DNA-methylation profiling using microarray technology, which has revealed distinct molecular subgroups among anaplastic gliomas[25], glioblastomas[8,21], and ependymal tumours[20] as described in previous sections of this Review. Use of this approach has also revealed that gliomatosis cerebri is not a distinct entity[28]. Moreover, copy-number changes across all chromosomes and *MGMT*-promoter methylation status can be assessed in parallel with the DNA methylation profiles using special bioinformatics algorithms for analysis of the microarray data [25,104]. The use of these and other advanced molecular assays will soon become a widespread practice.

The current WHO classification of glioma relies exclusively on assessments of tumour tissue; however, 'liquid biopsy' approaches using ultra-deep sequencing of DNA from cerebrospinal fluid or plasma, for example, hold promise for non-invasive diagnostics and disease monitoring of various cancers, including gliomas[105-107]. Glioma-associated micro-RNAs, such as *miR-21*, can also be detected in cerebrospinal fluid, and may potentially serve as diagnostic, prognostic, and/or predictive biomarkers[108,109].

[H1] Current multimodal therapy of gliomas

Despite tremendous advances in understanding glioma genetics, molecularly targeted therapies have, to date, failed in phase III trials in patients with this disease, and the classic treatment modalities of surgery, radiotherapy and/or chemotherapy remain the mainstay of

therapy[34,74]. Surgery can be curative in patients with circumscribed gliomas, such as pilocytic astrocytoma, PXA, and subependymal giant-cell astrocytoma (FIG. 4). Most ependymomas also have circumscribed growth, but local brain invasion is common and the risk of dissemination within the CNS increases with the duration of the disease; therefore, complete resection is prognostically favourable, but consolidating radiotherapy is often required for prolonged tumour control in patients with anaplastic ependymoma and following incomplete resection of WHO grade II ependymoma[110].

Gross total resection is also associated with improved survival of patients with diffuse gliomas, including glioblastoma[111,112], not excluding the growing group of elderly patients[75,113]. Diffuse gliomas generally recur after resection; therefore, additional treatment aimed at prolonging survival while maintaining quality of life is the standard of care. The choice and timing of the various available treatments depend on patient age, clinical performance status, tumour entity, and molecular biomarkers (FIG. 4). Postsurgical radiotherapy provides improved local control in patients with diffuse gliomas of any grade when compared with surgery alone. Furthermore, radiotherapy prolongs survival in patients with WHO grade III and IV gliomas and is therefore standard of care.[74] Delaying radiotherapy until recurrence progression after surgery does not compromise the overall survival in patients with WHO grade II gliomas[114], but such 'watchful waiting' strategies should only be considered for patients aged <40 years and with favourable prognostic factors[115,116] (FIG. 4). The RTOG 9802 trial[117] results demonstrated that patients with WHO grade II diffuse gliomas considered to require treatment beyond surgery experienced prolonged survival when PCV polychemotherapy was added to radiotherapy. In contrast, the EORTC 22033-26033 trial that compared radiotherapy alone with chemotherapy alone did not result in the identification of any subgroup of patients with a clear benefit from either treatment over the other[118].

1p/19q codeletion is a predictive biomarker of long-term benefit from PCV polychemotherapy administered immediately before or after radiotherapy in patients with anaplastic glioma[77,78] (FIG. 4a). Whether the PCV regimen can be substituted by temozolomide will be explored in the new CODEL trial[119]. Whether the benefit from adding

PCV to radiotherapy observed in a subset of patients with tumours lacking 1p/19q codeletion is linked more closely to *IDH* mutation[120] or *MGMT*-promoter methylation[121] remains controversial. Nevertheless, preliminary data from the CATNON trial[122] have confirmed a role of early maintenance alkylating chemotherapy with temozolomide after radiotherapy in patients with anaplastic glioma lacking 1p/19q codeletion.

The standard of care for patients with glioblastoma is postoperative chemoradiotherapy, with 60 Gy in 30 fractions and concomitant daily temozolomide, followed by six cycles of maintenance temozolomide (TMZ/RT→TMZ; FIG. 4b)[74,79]. This treatment approach was established based on the results of a trial that did not include patients aged >70 years[79], and subgroup analyses have suggested only limited benefit from addition of temozolomide to radiotherapy in patients aged 65–70 years[123]. Trials in elderly glioblastoma patients (variably defined as patients aged ≥60-70 years of age), who have a particularly poor prognosis, demonstrated the efficacy of shorter, hypofractionated radiotherapy regimens in this population[123,124], and a predictive role of *MGMT*-promoter methylation for benefit from first-line temozolomide alone[75,76]. Nonetheless, elderly patients with *MGMT*-promoter-methylated tumours who are eligible for combined modality treatment can benefit from the TMZ/RT→TMZ regimen[125].

The definition of standards of care at glioma recurrence remains challenging. Gross total resection is associated with prolonged survival in patients with recurrent glioblastoma[126], but the value of repeat surgery is less clear in patients with other gliomas. Surgery merely to reduce tumour volume — rather than with the aim of achieving gross tumour resection — is under debate because the validity of the retrospective studies suggesting beneficial effects, compared with no repeat surgery, is challenged by major imbalances in prognostic factors between the treatment cohorts[74]. Radiotherapy at recurrence is an option for patients who did not receive first-line radiotherapy, but is limited by neurotoxic dose-accumulation effects that may cause radionecrosis[127]. Systemic treatments that can be used at disease recurrence include alkylating agent chemotherapy, mostly with lomustine and/or CCNU[128,129], or re-challenge with temozolomide in patients with *MGMT*-promoter methylation and

apparent benefit from first-line treatment with this agent[130,131]; however, effects on survival are moderate at best. In the USA and some other countries, antiangiogenic therapy with bevacizumab was approved for the treatment of recurrent glioblastoma on the basis of radiographic response rates in the range of 20-40% — and presumed clinical benefit — in two uncontrolled trials[132,133]; approval was conditional on the subsequent demonstration of an overall survival benefit, which was achieved neither in studies in patients with newly diagnosed glioblastomas[4,5], nor when bevacizumab was combined with CCNU in the treatment of patients with recurrent disease[134]. Application of 'tumour treating fields' (TTF), that is, alternating electric fields that are applied via skin electrodes placed on the shaved scalp, prolonged the survival of patients with newly diagnosed glioblastoma in a phase III trial[135]; however, skepticism surrounds this approach, owing to trial design issues, the uniformity of results across subgroups, and a small effect magnitude relative to the potentially major adverse effects on quality of life[136].

[H1] Mechanisms of progression after therapy

Gliomas are characterized by intratumoural heterogeneity of cells with distinct profiles of molecular aberrations, which might facilitate malignant progression and therapy resistance. *IDH* mutations are generally retained in recurrent tumours[43,137], thus underscoring the role of these alterations as tumour-initiating events (that is, 'driver' mutations) and a potential therapeutic target. The *IDH* mutation-associated G-CIMP is usually also maintained at disease recurrence[44]; however, malignant progression can be accompanied by a reduction in the frequency of methylated CpG sites, and rare tumours show loss of G-CIMP at recurrence, which has been associated with poor outcomes[12].

By contrast, increased DNA methylation upon glioma progression has been observed at a small fraction of CpG sites, particularly those in genes encoding developmental transcription factor families with key roles in pattern formation and cell-fate determination. Notably, patterns of hypermethylated CpGs required for maintenance of the human embryonal stem-cell (hESC) phenotype are enriched upon progression towards glioblastoma, including the

promoters of genes that are strongly silenced in hESC as a result of H3-K27 trimethylation[43]. Thus, a high grade of histological malignancy is associated with stem-like features, a finding that supports the hypothesis that stem-like cells mediate resistance to chemotherapy and/or radiotherapy as derived mostly from studies of *IDH*-wild-type glioblastomas[138-140]. The lack of a defining molecular marker profile challenges the prospective identification and *in situ* characterization of such stem-like cells. Instead, models of glioma stem-like cells rely on enrichment strategies based on single or multiple marker-based segregation using e.g. CD15, CD44, or CD133[141]. Marker-positive cells are then propagated in the brains of immune-compromised mice or *in vitro* under stem-cell versus non-stem-cell promoting growth conditions to retrospectively confirm enrichment of stem-like traits; however, selection and epigenetic biases must be considered when interpreting data from these models[56]. Genetic differences between glioma stem-like and non-stem-like cells have not been defined, although an epigenetic pattern resembling that of paediatric glioblastomas with *H3F3A* mutation is characteristic of CD133-positive stem cells[142]. Single-cell mRNA-expression studies have revealed a dynamic distribution of stem-like versus more-differentiated traits, contributing to glioblastoma intratumour heterogeneity[57]. Depletion of glioma stem-like cells, via targeted disruption of CD44 or the nuclear receptor tailless (Tlx), improved survival in preclinical models[143,144]; however, early clinical trials evaluating therapies targeting glioma stem-like cells, e.g., utilizing the sonic hedgehog signaling inhibitor GDC0449 (vismodegib) or the notch signaling inhibitor RO4929097, failed to induce durable tumor responses[145,146].

Clonal selection during malignant progression can favour cells that lack mutations present in the initially predominant clones (including oncogenic drivers, such as *TP53*, *ATRX*, *FUBP1*, *SMARCA4* and BRAF)[43,137], indicating that alternative pathways of progression can diverge at early stages of tumour evolution, even without the selective pressure inferred by anticancer treatments. Temozolomide chemotherapy can, however, contribute to malignant progression by inducting a hypermutation state that is associated with driver mutations in components of the RB1 and mTOR pathways[137]. Defects in genes encoding DNA mismatch repair proteins are thought to underlie the hypermutation state, and could potentially be used

to identify patients at risk of therapy-associated malignant progression[147]. Indeed, the *MGMT*-promoter methylation status is essentially unaffected by progression of glioblastomas after treatment with the TMZ/RT→TMZ regimen[82], thus implicating alternative mechanisms of temozolomide-resistance including DNA mismatch repair deficiency leading to DNA hypermutation[84,85,137].

Resistance of diffuse gliomas to radiotherapy can be mediated by activation of the DNA-damage response[139,148], and a rapid radiotherapy-induced switch towards a mesenchymal gene-expression pattern that results in enhanced pro-survival signalling via NF-κB to counteract proapoptotic signals[56,149]. Formation of an interconnecting microtubule network between glioma cells has been proposed as a new mechanism of radioresistance[150]. Ultimately large sets of well-annotated, matched samples from individual patients collected before and after therapy will be required to characterize the molecular basis of glioma chemotherapy and radiotherapy resistance.

[H1] Novel pathogenesis-based treatments

[H2] Targeting oncogenic signalling pathways

Improved understanding of the molecular mechanisms that underlie gliomagenesis has prompted attempts to target identified drivers of the disease. Such approaches are particularly promising in circumscribed gliomas that are driven by activation of a single pathway. For example, treatment with the mTOR-inhibitor everolimus is efficacious in patients with subependymal giant-cell astrocytomas, in terms of reductions in tumour volumes and seizure frequencies[151,152], and responses to the BRAF inhibitor vemurafenib have been reported in patients with *BRAF*^{V600E}-positive recurrent PXA[153,154]. A clinical trial of vemurafenib is ongoing in paediatric patients with *BRAF*^{V600E}-positive recurrent malignant gliomas[155]. A recent phase 1/2 trial of the mutant BRAF inhibitor dabrafenib in pediatric patients with BRAF^{V600}–mutant relapsed or refractory low-grade gliomas revealed promising activity as indicated by an objective response rate of 41%[156]. By contrast, preclinical studies in *KIAA1549–BRAF*-fusion-positive pilocytic astrocytomas revealed that PLX4720, an

analogue of vemurafenib, induced paradoxical activation of MAPK signalling[157]. Similarly, sorafenib treatment resulted in paradoxical MAPK-pathway activation and accelerated tumour growth in patients with pilocytic astrocytoma[158]. Use of the MAPK-pathway inhibitor selumetinib might circumvent this limitation, and this agent is currently under clinical investigation for treatment of patients with pilocytic astrocytomas[159]. The more-diverse biology of ependymomas suggests that similarly straight-forward treatment approaches might not be effective in this disease, but trials of everolimus, or hormone-receptor blockade in HER2-positive ependymoma, are ongoing[34].

In glioblastoma, complete response of a paediatric patient with *BRAF*^{V600E}-positive glioblastoma to vemurafenib provides further proof of principle for targeting aberrant signalling pathways[160]. Other examples include the association of benefit from therapy using the mTOR inhibitor temsirolimus with phosphorylation of the downstream mTOR target ribosomal protein S6 kinase β1 (S6K1) detected in tumour samples from patients with recurrent glioblastoma[161], and with mTOR S2448 phosphorylation detected in tumour specimens from those with newly diagnosed *MGMT*-unmethylated glioblastoma (in *post-hoc* analyses)[162]. Efficacy studies using these molecular entry criteria are, however, not underway. Overall, the targeted inhibition of oncogenic signalling has not proven effective in randomized controlled trials involving patients with glioblastoma [129,162,163,164] Several early trials were performed to evaluate the efficacy of EGFR inhibitors in patients with diffuse gliomas, but none monitored for the presence of *EGFR* amplification in tumour cells as a potential predictive biomarker of response[165]. A recent promising approach exploits *EGFR* amplification to deliver the microtubule toxin monomethyl auristatin F through a drug-conjugated antibody directed specifically to the activated conformation of EGFR[166].

Resistance to oncogenic signalling inhibitors is related to clonal diversity, which can be bypassed by targeting aberrations that occurred early in tumourigenesis and thus are shared by all (or almost all) tumour cells. *IDH* mutation is a key example of this type of oncogenic aberration. Indeed, preclinical data indicate that small-molecule inhibitors of mutant IDH proteins can reverse the G-CIMP signature, induce glial differentiation, and inhibit the growth

of *IDH*-mutant glioma cells[88]. Early stage clinical trials of such agents are ongoing in advanced *IDH*-mutant malignancies, including gliomas[167-169]. The concept of epigenomic reshaping utilizing inhibitors of H3-K27 demethylase and histone deacetylases has also been explored with promising results in preclinical models of H3-K27M-mutant paediatric brain stem gliomas[70,71]. In *IDH*-wild-type glioblastomas, EGFRvIII has been identified as a driver of epigenetic remodelling that promotes tumour growth[170], but EGFRvIII is expressed in only subsets of tumour cells and is relatively resistant to inhibitors of EGFR signalling[171], thus presenting challenges for therapeutic targeting of this aberration.

The extensive vascularity of glioblastoma has prompted investigations of several antiangiogenic approaches to therapy, including the use of small-molecule inhibitors of VEGF signalling, the anti-VEGF antibody bevacizumab, and inhibition of integrins by a cyclic peptide (cilengitide); however, enthusiasm for such strategies has been dampened by reports of several negative phase III trials[4,5,134,164]. Nevertheless, subgroup analyses of AVAGlio[4], a placebo-controlled phase III trial designed to assess the addition of bevacizumab to standard first-line chemoradiotherapy in patients with newly diagnosed glioblastoma, indicate that a proneural gene-expression signature might be a biomarker for benefit from antiangiogenic therapy[58].

In spite of the potential to identify disease drivers and the available means to target these aberrations in diffuse gliomas, mosaicism of genomic alterations[172], clonal selection, and treatment-induced evolution, as well as dose-limiting toxicities, and other pharmacokinetic and pharmacodynamic issues complicate clinical translation of molecularly targeted treatments[173]. Combinations of the different treatment approaches individualized according to molecular features and the clonal composition of each tumour, and possibly involving sequential biopsies, might be required to overcome spatial and temporal heterogeneity of diffuse gliomas.

[H2] Immunotherapy approaches

Gliomas are not considered highly immunogenic because, in contrast to tumours against

which immunotherapy has proven effectiveness (such as melanoma or non-small-cell lung cancer), mutational loads are typically low. Moreover, gliomas are characterized by profound immunosuppression mediated by secreted (TGF-β, IL-10) and cell surface (CD95L, PD-L1) immunosuppressive factors, by infiltration of immune inhibitory cells in the tumor microenvironment and by anatomical peculiarities of the brain such as the blood brain barrier and paucity of lymphatic vessels[174,175]. Nevertheless, multiple strategies to overcome this immunosuppression and exploit antitumour immune responses have been pursued in patients with gliomas. Firstly, vaccination, that is, immunization with tumour-specific or tumour-associated peptides, or by application of autologous antigen-presenting cells boosted with such peptides. Secondly, immunomodulatory drugs that target T-cell inhibitory signalling to enhance physiological antitumour responses. Finally, adoptive T-cell transfer, which involves *in vitro* clonal expansion or genetic engineering of T-cells with high avidity for tumour-specific or tumour-associated epitopes[176].

[H3] Vaccination. EGFRvIII has been considered a promising target yielding tumour-specific epitopes for boosting antitumour adaptive immune responses with minimal risk of cross-reactivity against nontumour cells. In combination with bevacizumab therapy, vaccination with an EGFRvIII-specific peptide, rindopepimut (also known as CDX-110), seemed to prolong survival of patients with EGFRvIII-positive recurrent glioblastoma in an exploratory randomized phase II trial[94]. In patients with newly diagnosed EGFRvIII-positive glioblastoma, however, overall survival was unaffected by addition of rindopepimut to standard temozolomide maintenance therapy in the double-blind, placebo-controlled, phase III ACT-IV trial[177]. Despite this setback, EGFRvIII remains an attractive treatment target in patients with glioblastoma and continues to be explored in this context. R132H-mutant IDH is another potential target for single-peptide vaccination approaches. Epitopes containing the mutated portion of this protein are presented on MHC class II molecules and induce mutation-specific $CD4^+$ T-cell responses, anti-IDH1-R132H $CD4^+$ T cells and antibodies occur spontaneously in patients with *IDH1*^{R132H}-mutant glioma. and this vaccination approach evoked durable

anticancer responses in a preclinical glioma model[89].

The transfer of autologous dendritic cells (DCs) pulsed *ex vivo* with tumour-associated peptides (or RNAs encoding such peptides) is currently being explored as an alternative vaccination strategy. For example, in 12 patients with glioblastoma, immunization with DCs pulsed with the cytomegalovirus (CMV) antigen pp65 RNA, which is expressed in >90% of glioblastomas, but not in surrounding nonmalignant brain tissue, triggered antitumour T-cell responses that were reinforced by preconditioning of the vaccine site with the recall antigen tetanus/diphtheria toxoid[178]. Moreover, prolongation of progression-free survival by 2.4 months was reported in a placebo-controlled phase II trial exploring addition of the DC vaccine ICT-107 to maintenance therapy for patients with newly diagnosed glioblastoma[179]. ICT-107 comprises autologous DCs pulsed with six synthetic peptides from glioma-associated antigens MAGE-1, HER-2, AIM-2, TRP-2, gp100, and IL-13Rα2; in this trial unpulsed DCs were used as the placebo[179]. A pivotal phase III trial has been launched to further evaluate this approach[180]. The use of a DC vaccine in addition to standard therapy for patients with newly diagnosed glioblastoma has also been explored in the phase III DCVax trial[181], wherein autologous DCs were pulsed with tumour lysates derived from the same patient; however, results of this trial have not been released yet.

[H3] Immune-checkpoint blockade. The immunosuppressive microenvironment of gliomas is a major caveat that can limit the effectiveness of immunotherapy[182]. Monoclonal antibodies directed at the inhibitory immune checkpoints mediated by the T-cell receptors cytotoxic T-lymphocyte-associated protein 4 (CTLA-4; ipilimumab) or programmed cell-death protein 1 (PD-1; nivolumab and pembrolizumab) have entered clinical practice, notably as treatments of metastatic melanoma and non-small-cell lung cancers[183-186]. Furthermore, antibodies to PD-1 ligand 1 (PD-L1) are being developed — and one such agent, atezolizumab, has been approved by the FDA for the treatment of bladder cancer. In addition, antibodies neutralizing other immunoreceptors, such as killer-cell immunoglobulin-like receptors (KIR) or lymphocyte activation gene 3 (LAG-3) have been developed[182]. Interestingly, immune-checkpoint

blockade resulted in tumour eradication in an immunocompetent glioblastoma model[187], and impressive clinical responses to nivolumab have been observed in two siblings with recurrent multifocal, DNA-mismatch-repair-deficient glioblastoma[188]. Current trials being conducted to evaluate immunomodulatory drugs in glioma include the phase III CheckMate-143 trial of nivolumab and bevacizumab in patients with recurrent glioblastoma[189], the double-blind phase II CheckMate-548 trial of nivolumab therapy as an adjunct to standard chemoradiotherapy in patients with newly diagnosed *MGMT*-methylated glioblastoma[190], and the open-label phase III CheckMate-498 trial assessing the efficacy of nivolumab as an alternative to temozolomide in patients with newly diagnosed *MGMT*-unmethylated glioblastoma[191].

[H3] CAR T-cell therapy. Another immunotherapeutic concept involves adoptive transfer of genetically engineered chimeric antigen receptor (CAR) T cells, which have been modified to express binding domains with high affinity for tumour antigens linked to intracellular signalling domains that trigger T-cell activation, proliferation, and persistence, similar to normal T-cell receptors[192]. Severe 'on-tumour' toxicity, attributable to cytokine release by activated CAR T cells, has been associated with this therapeutic approach, for example, in patients with haematological malignancies[193]; however, 'on-target, off-tumour' cross-reactivity can also result in severe organ-specific toxicity, such as neurological toxicity owing to targeting of neurons expressing melanoma-associated antigen A (MAGE-A) proteins by autologous anti-MAGE-A3 CAR T cells[194]. Nevertheless, this treatment paradigm holds promise for glioma therapy. For instance, treatment with CAR T cells directed at EGFRvIII[195] or podoplanin[196] reduced glioma growth in mouse models. Early clinical trials using CAR T-cells targeting EGFRvIII[197] or HER2[198] are ongoing in patients with glioblastoma. HER2 expression on cardiomyocytes warrants close monitoring of cardiac function in patients who receive HER2-targeting CAR T cells; however, cardiac failure was not observed in 19 patients with sarcoma treated with such cells[199].

[H1] Innovative clinical trial design

The low incidence rates of various gliomas challenge patient accrual to sufficiently powered clinical trials. Generally, the number of events and thus statistical power of oncology trials can be increased by creating national and international networks to maximize patient recruitment, by minimizing trial durations, by inclusion of composite or continuous outcome measures, such as quality-of-life outcomes, and by minimizing 'noise' by ensuring high-data quality[200]. Historical controls have also been used to reduce sample size in past single-arm phase II trials in patients with glioma, but a series of phase III trials with a rationale built on such trials had negative results[128,129,164], thus raising questions regarding this approach. Of note, the overall survival of patients with glioblastoma treated in the standard chemoradiotherapy control arms of clinical trials has improved substantially during the past decade, ranging from 14.6 months in the pivotal EORTC/NCIC trial[79] to approximately 17 months in contemporary phase III trials[3-5,135], probably owing to improved surgical technique, more-aggressive treatment of recurrent disease, and better management of treatment-related complications. Importantly, these improvements in care can distort the outcome measures of single-arm trials in which the survival of contemporary cohorts is compared to that of historical cohorts[201].

Molecular stratification of gliomas augments the challenge of patient accrual, but might increase the yield of a study by accurately selecting the 'right' patients for treatment with the 'right' molecularly targeted therapies. A constant increase in knowledge of the molecular mechanisms that drive gliomagenesis and the failure of previous trials of molecular targeted therapies for gliomas underscore the need for a step back towards hypothesis-generating phase II trial designs, because only combination treatments that counter anticipated escape mechanisms will ultimately overcome treatment resistance. Two-step designs, such as adaptive and crossover trials including repeat biopsy sampling to enable the use of molecular diagnostics, have been advocated for this purpose in the setting of other rare cancers[200]; however, such designs might be difficult to apply in glioma trials owing to the costs and risks associated with cranial surgery, and because clinical deterioration at tumour progression can

preclude further trial participation in a substantial proportion of patients. Nevertheless, comparison of molecular tumour profiles before and after therapy, as well as examination of tissue from 'responders' versus 'non-responders' are important in generating hypotheses for combination treatments to overcome resistance, and might yield novel biomarkers for prediction of escape mechanisms in individual patients.

Classical one-fits-all trial designs whereby randomization is based on histology alone are outdated (FIG. 5a), and biomarker-based entry criteria are increasingly applied. Different treatment options can then be randomly allocated to the biomarker-positive versus the biomarker-negative patients, in order to maximize recruitment (FIG. 5b). In trials involving patients with diffuse glioma, *IDH* mutation, 1p/19q codeletion and *MGMT*-promoter methylation are commonly determined at diagnosis and are candidates for biomarker-based stratification. Several ongoing trials are incorporating more-specific molecular entry criteria. For example, assessment of *EGFR* amplification is being performed in the clinical development programme of the anti-EGFR antibody–drug conjugate ABT-414[202,203] and in an uncontrolled trial of the small-molecule EGFR-inhibitor dacomitinib[204] which is anticipated to cross the blood–brain barrier more readily than previously evaluated EGFR inhibitors.[92,93]. Moreover, assessment of rare fusion proteins and activating mutations involving the *FGFR* gene[95,96] is being incorporated in a trial of a novel FGFR-inhibitor (BGJ398)[205]. Basket trials extend biomarker-based patient recruitment according to the rationale that a particular molecular alteration validates a clinical target for which a specific inhibitor is available, independent of disease site or histology. By contrast, umbrella trials offer the opportunity for maximizing recruitment by enabling patient stratification using multiple molecular markers, for example, particular genetic alterations detected in subsets of patients with the same cancer type that can be targeted with different inhibitors (FIG. 5c). In basket and umbrella trials, the intent is not to compare the outcomes of patients in the different treatment arms. Instead, these designs can be viewed as incorporating several separate phase II trials — enabling investigation of the efficacy of either a single agent in the different diseases included in the 'basket' of a common molecular target, or multiple different therapies allocated based on

molecular profiles under the ‘umbrella’ of a common disease. Randomization of patients with rare cancers to a control arm is not feasible and, therefore, response assessment is the primary outcome measure used in these trials. The key strength of basket and umbrella trials is the prospective annotation of clinical and molecular data to generate hypotheses for testing in future randomized trials; thus, repeated biopsy sampling at disease progression should be included in such designs.

Tremendous technological progress in microarray-based and NGS-based diagnostics poses novel opportunities for such innovative trial approaches, although molecular stratification based on high-throughput technologies is challenged by logistical issues, such as availability of sufficient amounts of tissue, insufficient depths of sequencing to detect clones of low abundance, consumption of time and resources, and a limited consensus on data interpretation[206]. Moreover, basket and umbrella trials require a functional molecular screening platform and potentially the involvement of multiple pharmaceutical companies in order to obtaining a reasonable assortment of drugs with which to target the detected molecular aberrations, but are, nevertheless, the most-promising approach to developing a precision-medicine strategy for patients with glioma. Ongoing or upcoming prospective studies using NGS-based diagnostics include the INFORM registry basket trial in paediatric patients with recurrent cancers including gliomas[97], the NCT Neuro Master Match (N^2M^2) umbrella trial in patients newly diagnosed *MGMT*-promoter-unmethylated glioblastoma[98], and the NCI-MATCH trial in adult patients with any type of advanced-stage cancer including gliomas[99].

[H1] Conclusions

Advances in molecular profiling technologies have enabled the characterization of genetic and epigenetic changes in gliomas at a hitherto unprecedented comprehensiveness. New biomarkers have been identified that can improve diagnostic accuracy and guide individualized treatment. These developments have led to the 2016 update of the WHO Classification of Tumours of the CNS that breaks with the traditional approach of purely

histology-based glioma diagnostics by incorporating molecular biomarkers in an integrated diagnosis. In parallel, improved knowledge of glioma biology has provided opportunities for novel pathogenesis-based pharmacological treatments and innovative immunotherapeutic strategies; for example, new strategies for targeting tumour-associated mutant proteins or immune checkpoints have emerged. Moreover, innovative trial concepts have been initiated that involve predictive molecular profiling followed by individualized therapy specifically tailored to the characteristics of each tumour. Thus, the time has come for expand the implementation of precision medicine in neuro-oncology.

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Author contributions
The concept of the article was developed by G.R. and M.W. All authors contributed to the research of data for the article, made substantial contributions to the discussion of its content, wrote parts of the manuscript, designed figures or tables, and reviewed and edited the final manuscript prior to submission for publication.

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G.R. has received research grants from Merck Serono and Roche, as well as honoraria for advisory boards or lectures from Amgen and Celldex. M.W. has received research funding from Merck Serono, MSD and Novocure, as well as honoraria from Merck Serono and MSD, and has consultancy relationships with BMS, Celldex, Genentech/Roche, Merck Serono, MSD, and Novocure. H.-G.W. and C.B.K.-T. declare no competing interests.

FURTHER INFORMATION
The Cancer Genome Atlas: http://cancergenome.nih.gov

The US NIH ClinicalTrials.gov database: www.clinicaltrials.gov
The European Association of Neuro-Oncology: www.eano.eu
The European Organisation for Research and Treatment of Cancer Brain Tumour Group: www.eortc.org/research-groups/brain-tumour-group
WHO Classification of Tumours: www.pubcan.org

Key points

The 2016 WHO Classification of Tumours of the Central Nervous System represents a paradigm shift, replacing traditional histology-based glioma diagnostics with an integrated histological and molecular classification system that enables more-precise tumour categorization

- The requisite diagnostic biomarkers for the 2016 WHO classification of gliomas are *IDH1/2* mutations, 1p/19q codeletion, *H3F3A* or *HIST1H3B/C* K27M (H3-K27M) mutations and *C11orf95–RELA* fusions

- Additional diagnostically relevant biomarkers include loss of nuclear ATRX expression, *TERT*-promoter mutations, *KIAA1549–BRAF* fusion, $BRAF^{V600E}$ mutation, H3-G34 mutation, and several other alterations associated with rare glioma entities

- *MGMT*-promoter methylation is predictive of benefit from alkylating chemotherapy, particularly in elderly patients with *IDH*-wild-type glioblastoma; predictive biomarkers for targeted therapies, such as *IDH1* and *BRAF* mutations, are also emerging

- Novel methods for large-scale DNA-methylation, copy-number and mutational profiling will further advance the assessment of glioma-associated molecular biomarkers

- Clinical trials require assessment of molecular biomarkers as criteria for study entry and/or patient stratification; predictive DNA sequencing followed by targeted therapy will support the implementation of precision medicine in neuro-oncology

BOX 1 | **The integrated diagnosis concept used in the 2016 WHO Classification of Tumours of the Central Nervous System (CNS)**

The 2016 WHO Classification of Tumours of the CNS[22] follows a multilayered approach for glioma classification, combining three layers of information derived from the tumour to determine the final ('top layer') integrated diagnosis, as follows:

- **Top layer:** Final (integrated) diagnosis incorporating all tissue-based information
- **Layer 3:** Molecular information (results of molecular testing for diagnostic biomarkers)
- **Layer 2:** Histological tumour grade (WHO grade)
- **Layer 1:** Histological tumour type

For example, tumours of the histological 'oligodendroglioma' subtype (layer 1) and WHO grade II (layer 2) that harbour an *IDH1* mutation and 1p/19q codeletion (layer 3) have a final (top layer) integrated diagnosis of 'oligodendroglioma, *IDH1*-mutant and 1p/19q-codeleted, WHO grade II'.
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BOX 2 | **Novel high-throughput technologies for the molecular diagnosis of glioma**

Microarray-based DNA-methylation profiling
Can provide information on diagnostic DNA-methylation profiles and DNA copy-number alterations (CNAs).

- Advantages: applicable to formalin-fixed paraffin-embedded (FFPE) material; no blood-cell-derived DNA required as a reference; and is relatively inexpensive
- Limitations: cannot detect subtle alterations, such as point mutations

Sequencing of glioma-associated gene panels
Provides information on mutations and CNAs of selected cancer-related genes.

- Advantages: applicable to FFPE material; small gene panels might not require analysis of blood-cell-derived DNA as a reference; enables high sequence coverage from very low amounts of DNA; and is relatively inexpensive
- Limitations: cannot detect DNA-methylation changes, such as *MGMT*-promoter methylation

Diagnostic whole-exome or whole-genome sequencing
Provides information on mutational profiles across all coding exons or the entire genome of a given tumour.

- Advantages: provides a comprehensive overview of mutations, CNAs and complex chromosomal rearrangements
- Limitations: requires blood-cell-derived DNA for reference; requires bioinformatics expertise; relatively expensive; and cannot detect DNA-methylation changes, such as *MGMT*-promoter methylation

Figure 1 | **Diagnostic approach for integrated histological–molecular classification of diffuse gliomas according to the 2016 WHO Classification of Tumours of the Central Nervous System**[22]. In addition to histological typing and grading, diffuse gliomas are evaluated for *IDH1/IDH2* (*IDH*) mutation status. Nuclear ATRX expression is determined by immunohistochemistry. Testing for 1p/19q codeletion is performed in patients with *IDH*-mutant tumours with retained nuclear ATRX expression to further refine the classification of these tumours. *IDH*-wild-type gliomas located in midline structures (thalamus, brain stem, or spinal cord) are additionally tested for H3-K27M mutation. Dashed lines indicate small subgroups of tumours with the respective diagnoses. *Nuclear ATRX expression retained in most *IDH*-wild-type WHO grade II/III astrocytic tumours. ‡*IDH*-wild-type WHO grade II/III astrocytoma is considered a provisional entity in the 2016 WHO Classification of Tumours of the Central Nervous System.

Figure 2 | **Histological and molecular features of selected glioma entities. a** | This tumour was identified as an anaplastic astrocytic glioma on hematoxylin and eosin (HE) staining and had immunopositivity for IDH1 R132H and loss of nuclear ATRX expression on immunohistochemical (IHC) analysis, leading to a diagnosis of anaplastic astrocytoma, *IDH*-mutant (WHO grade III). **b** | HE staining revealed a cellular glioma composed of tumour cells with round nuclei and a clear cytoplasm; DNA pyrosequencing revealed an *IDH2*^{R172K} mutation (c.515G>A, shown is the sequence of the reverse strand) and microsatellite analysis demonstrated loss of heterozygosity at the *D1S468* (1p) and *D19S219* (19q) loci — arrows indicate alleles lost in tumour DNA (T) relative to blood DNA (B). These features enabled a diagnosis of anaplastic oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted (WHO grade III). **c** | These photomicrographs indicate features of glioblastoma, *IDH* wild type (WHO grade IV). Note microvascular proliferation visible on HE staining (arrow), the lack of IDH1 R132H immunostaining and expression of EGFR variant III (EGFRvIII) on IHC analysis. **d** | HE staining revealed a cellular tumour composed of epithelioid cells, with focal glial fibrillary acidic protein (GFAP) expression detected on IHC analysis; DNA sequencing of the

tumour revealed a *BRAF*^{V600E} mutation (c.T1799A, shown is the sequence of the reverse strand). These features enabled a diagnosis of glioblastoma, *IDH*-wild-type, epithelioid variant (WHO grade IV). **e** | An example of a pilocytic astrocytoma (WHO grade I): a spindle-cell astrocytoma with biphasic growth pattern on HE staining, immunopositivity for OLIG2, and expression of a *KIAA1549–BRAF* fusion transcript detected by reverse transcription-PCR (row A: PCR products for *KIAA1549* exon *15/BRAF* exon 9 fusion transcripts; row B: PCR products for *BRAF*-wild-type transcripts; lane 1: PCR bands obtained for the depicted tumour sample revealing *KIAA1549–BRAF* positivity; lane 2: *KIAA1549–BRAF*-negative control; lane 3: *KIAA1549–BRAF*-positive control; lane 4: no template control). **f** | Characteristics of a diffuse midline glioma, H3-K27M-mutant (WHO grade IV). Histological analyses revealed a diffuse astrocytic glioma (HE staining) with nuclear immunopositivity for K27M-mutated histone H3 (H3-K27M), and loss of nuclear positivity on IHC staining for trimethylated lysine 27 of histone 3 (H3K27me3). Specific immunoreactivity in the IHC photomicrographs is indicated by 3,3-diaminobencidine staining (brown); IHC sections are counterstained with hemalum (light blue).

Figure 3 | **Molecular subgroups of glioblastoma, as defined by distinct genetic and epigenetic profiles**[8,21,40]. Among the glioblastoma subtypes predominantly found in children below 18 years of age, tumours with pleomorphic xanthoastrocytoma (PXA)-like or low-grade glioma (LGG)-like molecular profiles are associated with favourable outcomes, whereas prognosis for patients with H3-K27M-mutant diffuse midline gliomas is unfavourable. The H3-G34-mutant subgroup most commonly develops in children and young adults before 30 years of age. *IDH*-mutant glioblastomas most commonly manifest in young adults between 20 and 50 years of age and hold the best prognosis of all the adult glioblastoma types. Receptor tyrosine kinase I (RTK I) tumours also tend to occur in young adults. RTK II (classic) and mesenchymal glioblastomas are the most-common glioblastoma types in patients older than 50 years of age and are associated with poor prognosis. Genes mutated in each subgroup are indicated, as is the approximate percentage of patients with *MGMT*-

promoter-methylated tumours in each group. Chr., chromosome; G-CIMP, glioma-associated CpG-island methylator phenotype; OS, overall survival. *$BRAF^{V600E}$ mutation detectable in a minor fraction of tumours; ‡Corresponds to diffuse midline glioma, H3-K27M-mutant; §Mutated in a subset of diffuse intrinsic pontine gliomas; ||Mutated in a subset of H3-K27M-mutant tumours; Modified with permission from Elsevier B.V.© Masui K., Mischel, P.S. & Reifenberger, G. *Handb. Clin. Neurol*. **134**, 97–120 (2016).

Figure 4 | **Current post-surgery treatment strategies for major glioma entities classified according to the 2016 WHO Classification of Tumours of the Central Nervous System**[22]. **a** | Standard post-surgery treatments of *IDH*-mutant adult gliomas. **b** | Standard post-surgery treatments of *IDH*-wild-type adult gliomas. **c** | Standard post-surgery treatments of common paediatric glioma entities. Dashed lines indicate smaller subgroups of patients with the respective diagnoses. PCV, procarbazine, CCNU (lomustine), and vincristine; RT, radiotherapy; SEGA, subependymal giant-cell astrocytoma; TMZ, temozolomide; TMZ/RT → TMZ, radiotherapy with concomitant and maintainance temozolomide. * Diffuse astrocytoma, *IDH*-wild-type (WHO grade II) and anaplastic astrocytoma, *IDH*-wild-type (WHO grade III) are provisional entities according to the 2016 WHO classification.

Figure 5 | **Schematic illustration of different clinical trial designs. a** | Conventional clinical trials usually randomly allocate patients into an experimental (Exp) arm and a standard treatment arm for reference. **b** | Trial design with stratification of patients into different treatment arms based on individual biomarkers and comparison to standard treatments. **c** | Umbrella trial design to compare different types of targeted treatment for a particular cancer type based on molecular profiling of the tumour in each patient followed by allocation of individualized treatment; patients with tumours that lack actionable mutations are assigned to the standard-of-care arm.

TABLE 1 | **Common genetic, epigenetic and chromosomal aberrations associated with the major glioma entities**[40]

Glioma entity	Genetic	Epigenetic	Chromosomal
Diffuse astrocytic and oligodendroglial tumours			
Diffuse astrocytoma, *IDH*-mutant	*IDH1* or *IDH2*, *TP53*, *ATRX* mutation	G-CIMP	Trisomy 7 or 7q gain; LOH 17p
Anaplastic astrocytoma, *IDH*-mutant	*IDH1* or *IDH2*, *TP53*, *ATRX* mutation	G-CIMP	Trisomy 7 or 7q gain; LOH 17p
Oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted	*IDH1* or *IDH2*, *TERT*, *CIC*, *FUBP1* mutation	G-CIMP	1p/19q codeletion
Anaplastic oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted	*IDH1* or *IDH2*, *TERT*, *CIC*, *FUBP1, TCF12* mutation; *CDKN2A* deletion	G-CIMP	1p/19q codeletion
Glioblastoma, *IDH*-mutant	*IDH1* or *IDH2*, *TP53*, *ATRX* mutation; *CDKN2A* homozygous deletion	G-CIMP	Trisomy 7 or 7q gain; LOH 17p; 10q deletion
Glioblastoma, *IDH*-wild-type*	*TERT*, *PTEN*, *TP53*, *PIK3CA*, *PIK3R1*, *NF1*, *H3F3A*^{G34} mutation; *CDKN2A, PTEN* homozygous deletion; *EGFR, PDGFRA, MET, CDK4, CDK6, MDM2 MDM4* amplification; *EGFRvIII* rearrangement	*MGMT*-promoter methylation	Trisomy 7 or 7q gain; monosomy 10; double minute chromosomes
Diffuse midline glioma, H3-K27M-mutant‡	*H3F3A*^{K27M} or *HIST1H3B/C*^{K27M}, *TP53*, *PPMD1*, *ACVR1, FGFR1* mutation, *PDGFRA, MYC, MYCN, CDK4, CDK6, CCND1-3, ID2, MET* amplification	Loss of histone H3 lysin trimethylation	-
Well-differentiated paediatric diffuse glioma§	*MYB* or *MYBL* rearrangement; *FGFR1* duplication	-	-
Other (astrocytic) gliomas			
Pilocytic astrocytoma	*BRAF*, *RAF1*, *NTRK2* gene fusions; *BRAF*^{V600E}, *NF1, KRAS, FGFR1, PTPN11* mutation	-	-
Pleomorphic xanthoastrocytoma	*BRAF*^{V600E} mutation, *CDKN2A/p14*^{ARF} homozygous deletion	-	-
Subependymal giant cell astrocytoma	*TSC1* or *TSC2* mutation and LOH	-	-
Angiocentric glioma	*MYB–QKI* gene fusions/rearrangements	-	-
Supratentorial ependymal tumours			
Ependymoma, *RELA*-fusion positive‖	*C11orf95–RELA* fusion	-	11q aberrations
Ependymoma‖	*YAP1* gene fusions	-	11q aberrations
Posterior fossa (PF) ependymal tumours			
Ependymoma PF-A‖	-	PF-A DNA methylation profile with global hypermethylation	Stable genotype
Ependymoma PF-B‖	-	PF-B DNA methylation profile	Multiple copy-number imbalances (CIN)
Spinal intramedullary ependymal tumours			
Ependymoma	*NF2* mutation	-	22q deletion,

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TABLE 2 I **2016 WHO classification of gliomas**[22]

Tumour classification	**WHO grade**
Diffuse astrocytic and oligodendroglial tumours	
Diffuse astrocytoma, *IDH*-mutant	II
• Gemistocytic astrocytoma, *IDH*-mutant	II
Diffuse astrocytoma, *IDH*-wild-type*	II
Diffuse astrocytoma, NOS	II
Anaplastic astrocytoma, *IDH*-mutant	III
Anaplastic astrocytoma, *IDH*-wild-type*	III
Anaplastic astrocytoma, NOS	III
Glioblastoma, *IDH*-wild-type	IV
• Giant-cell glioblastoma	IV
• Gliosarcoma	IV
• Epithelioid glioblastoma*	IV
Glioblastoma, *IDH*-mutant	IV
Glioblastoma, NOS	IV
Diffuse midline glioma, H3-K27M-mutant	IV
Oligodendroglioma, *IDH*-mutant and 1p/19q-codeleted	II
Oligodendroglioma, NOS	II
Anaplastic oligodendroglioma, *IDH* mutant and 1p/19q-codeleted	III
Anaplastic oligodendroglioma, NOS	III
Oligoastrocytoma, NOS‡	II
Anaplastic oligoastrocytoma, NOS‡	III
Other astrocytic tumours	
Pilocytic astrocytoma	I
• Pilomyxoid astrocytoma	-
Subependymal giant cell astrocytoma	I
Pleomorphic xanthoastrocytoma	II
Anaplastic pleomorphic xanthoastrocytoma	III
Ependymal tumours	
Subependymoma	I
Myxopapillary ependymoma	I
Ependymoma	II
• Clear cell ependymoma	II
• Papillary ependymoma	II
• Tanicytic ependymoma	II
Ependymoma, *RELA*-fusion-positive	II or III
Anaplastic ependymoma	III
Other gliomas	
Chordoid glioma of the third ventricle	II
Angiocentric glioma	I
Astroblastoma	-

NOS categories are reserved for the rare instancies that a tumour cannot be molecularly tested or that test results remain inconclusive[22]. H3-K27M, K27M-mutated histone H3; NOS, not otherwise specified. *Provisional tumour entities or variants. ‡The diagnosis of 'oligoastrocytoma, NOS' or 'anaplastic oligoastrocytoma; NOS' is discouraged in the 2016 WHO classification of gliomas[22]: oligoastrocytic (mixed) gliomas should be assigned either to an astrocytic or an oligodendroglial tumour entity via appropriate molecular testing for *IDH* mutation and 1p/19q codeletion.

TABLE 3 I **Predictive molecular biomarkers relevant to gliomas**

Biomarker	Application	References
Predictive biomarkers in clinical use		
MGMT promoter methylation	Prediction of benefit from alkylating chemotherapy in patients with *IDH*-wild-type gliomas, in particular elderly patients with glioblastoma	[75,76]
1p/19q codeletion	Prediction of benefit from upfront radiotherapy and PCV as opposed to radiotherapy alone in patients with anaplastic glioma	[77,78]
Examples of emerging novel predictive biomarkers		
BRAF mutation	Identification of patients with *BRAF*^{V600}-mutant gliomas eligible for BRAF-inhibitor therapy	[87,153–156,160]
IDH1/IDH2 mutation	Identification of patients with *IDH*-mutant diffuse gliomas eligible for peptide-based vaccination or mutant-IDH inhibitors	[88,89]
EGFRvIII expression	Identification of patients with EGFRvIII-positive glioblastomas eligible for EGFRvIII-peptide-based vaccination	[90,91,94]
EGFR amplification	Identification of patients with *EGFR*-amplified glioblastomas eligible for treatment with anti-EGFR antibodies	[92,93]
FGFR–TACC fusion	Identification of patients with *FGFR–TACC*-positive glioblastomas eligible for FGFR-inhibitor therapy	[95,96]

EGFRvIII, *EGFR* variant III; PCV, procarbazine, CCNU (lomustine), and vincristine.

Biographies

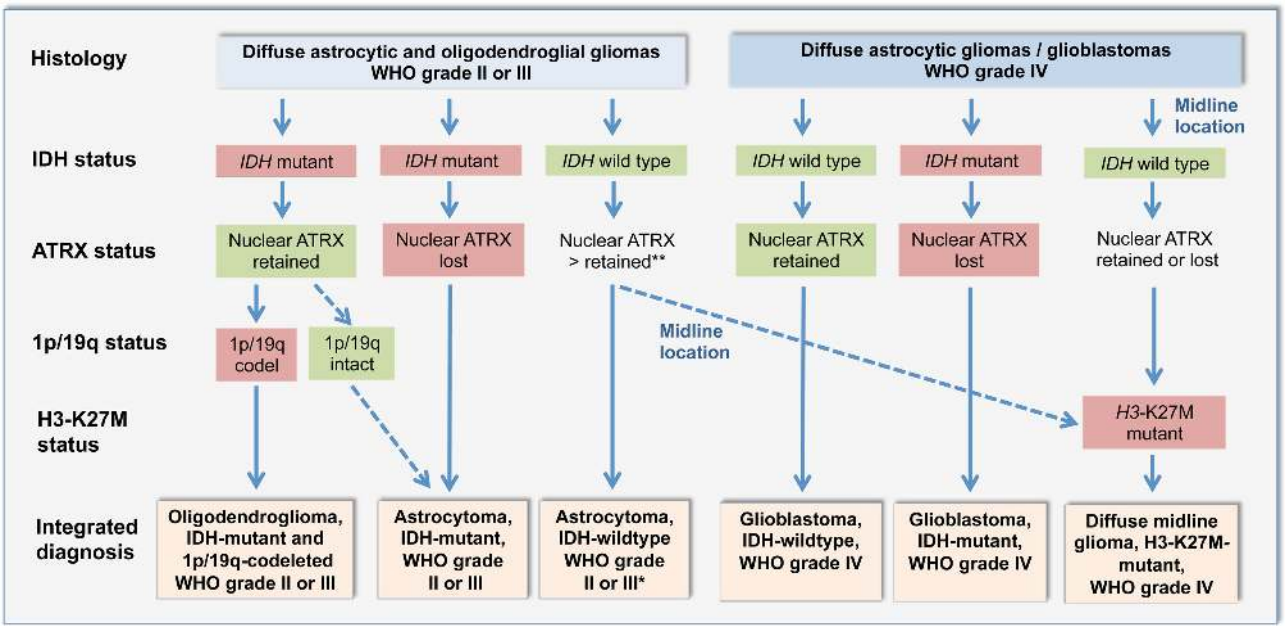
Guido Reifenberger is Professor and Chairman of the Department of Neuropathology, Heinrich Heine University Düsseldorf, Germany. He received an MD degree from the Heinrich Heine University and a PhD degree from the Karolinska Institute, Stockholm, Sweden. He was trained in neuropathology at Heinrich Heine University Düsseldorf and spent 2 years as a visiting scientist at the Sahlgrenska University Hospital in Gothenburg, Sweden. From 1997–2000 he was associate professor for molecular neurooncology at the University of Bonn, Germany. He has chaired the Department of Neuropathology at the Heinrich Heine University Düsseldorf since 2000. His research is focused on the molecular genetics and diagnostics of brain tumours, in particular, gliomas. He serves as co-chair of the Brain Tumour Reference Centre of the German Society for Neuropathology and Neuroanatomy, and was a member of the editorial advisory team for the 2016 WHO Classification of Tumours of the Central Nervous System.

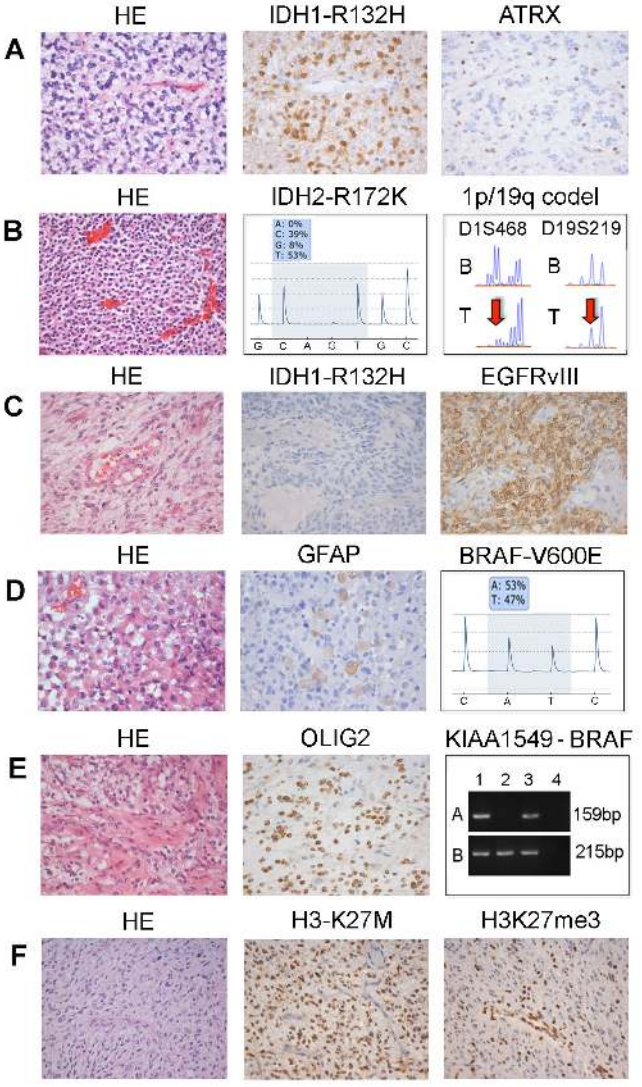
Hans-Georg Wirsching is a Research Fellow at the Fred Hutchinson Cancer Research Center in Seattle, Washington, USA. He received an MD degree from the Albert Ludwigs University, Freiburg, Germany, and trained at the Department of Neurology, University Hospital and University of Zurich between 2010–2015. He received the Investigator Award from the European Academy of Neurology in 2016. His research interests are focused on molecular targeted approaches to overcome the clonal diversity of gliomas, in particular, redirection of immune responses against such tumours.

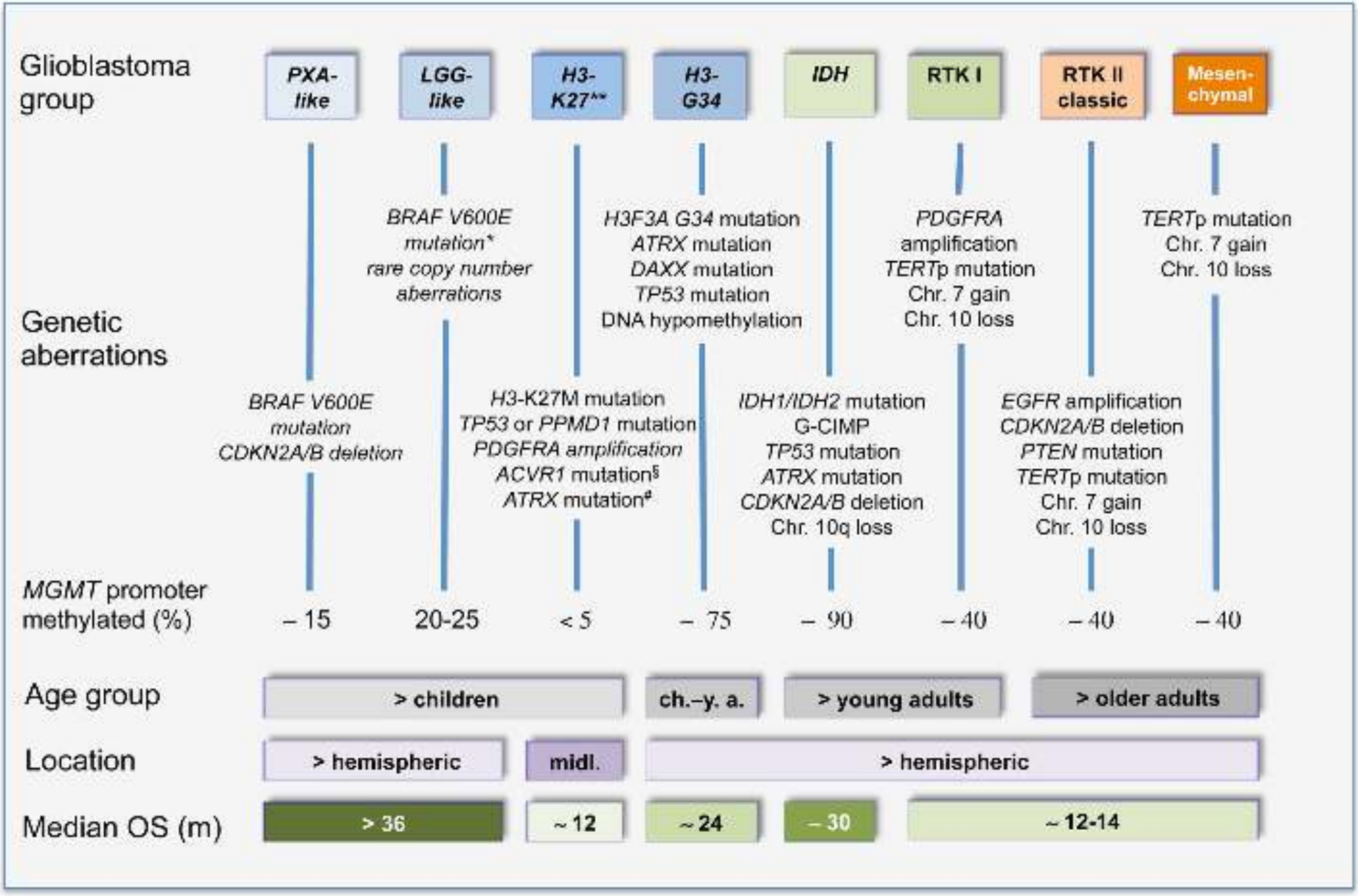
Christiane B. Knobbe-Thomsen is a neuropathologist at the Department of Neuropathology, Heinrich Heine University Düsseldorf, Germany. She received her MD degree from the Heinrich Heine University Düsseldorf. After 2.5 years of neuropathology training in Düsseldorf she went on to work as a postdoctoral research fellow at the Ontario Cancer Institute, Toronto, Canada. In 2012, she returned to Heinrich Heine University Düsseldorf and since 2014 she has been a Max-Eder-Junior-Research Group Leader at the Department of Neuropathology. Her research

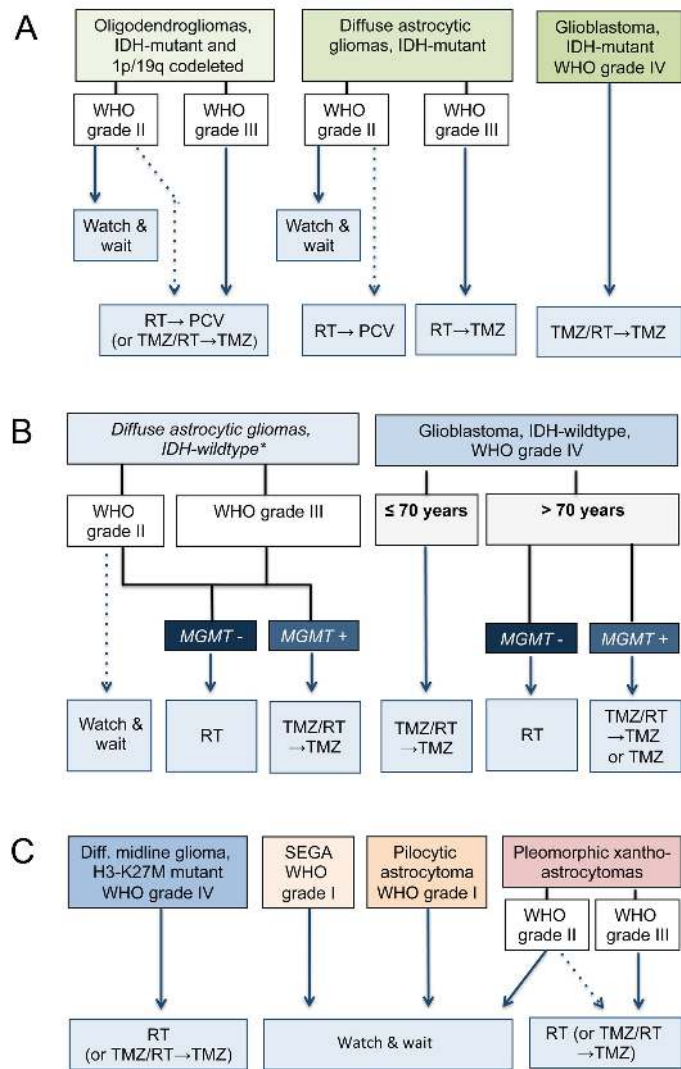
focus is on molecular pathomechanisms in primary brain tumours, in particular, *IDH*-mutant gliomas.

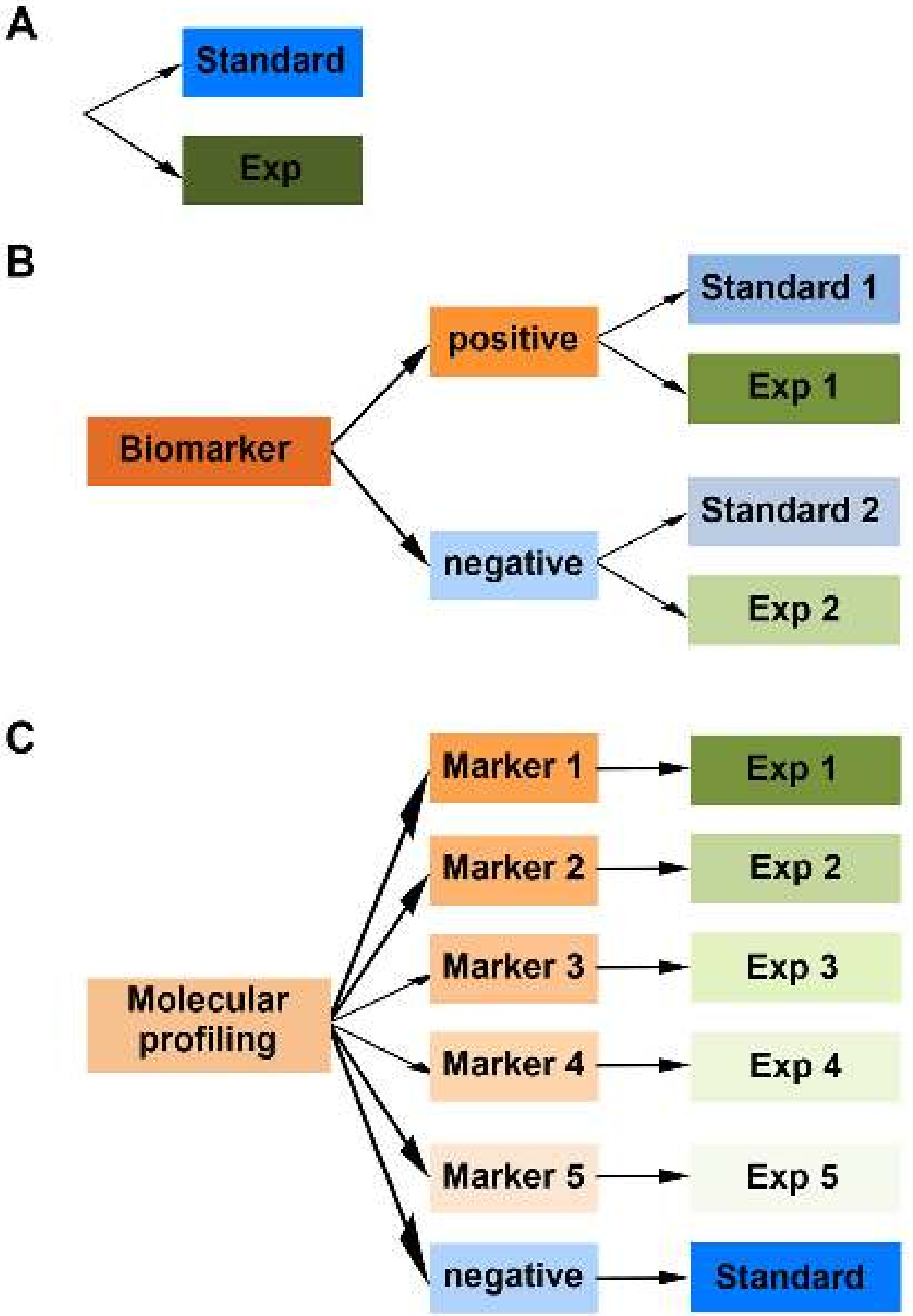
Michael Weller is a professor and Chairman of the Department of Neurology, University Hospital and University of Zurich, Switzerland. He received his MD degree from the University of Cologne, Germany (1989), and was trained at the Universities of Tübingen and Würzburg in Germany (1990–1991 and 1995-1996), the National Institute of Mental Health, Bethesda, Maryland, USA (1992), and the University Hospital Zurich (1993–1994). He was the Chairman of the German Neuro-Oncology Group from 2001–2008, and serves has served as the Chairman of the German Glioma Network since 2004. He has been President of the European Association for Neuro-Oncology (EANO) from 2014 to 2016, and serves as Chairman of the European Organisation for Research and Treatment of Cancer (EORTC) Brain Tumour Group since 2015. His research interests are focused on the development and treatment of brain tumours.


Histology
Diffuse astrocytic and oligodendroglial gliomas WHO grade II or III
Diffuse astrocytic gliomas / glioblastomas WHO grade IV
Midline location
IDH status
IDH mutant
IDH mutant
IDH wild type
IDH wild type
IDH mutant
IDH wild type
ATRX status
Nuclear ATRX retained
Nuclear ATRX lost
Nuclear ATRX > retained**
Nuclear ATRX retained
Nuclear ATRX lost
Nuclear ATRX retained or lost
1p/19q status
1p/19q codel
1p/19q intact
Midline location
H3-K27M status
H3-K27M mutant
Integrated diagnosis
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted WHO grade II or III
Astrocytoma, IDH-mutant, WHO grade II or III
Astrocytoma, IDH-wildtype WHO grade II or III*
Glioblastoma, IDH-wildtype, WHO grade IV
Glioblastoma, IDH-mutant, WHO grade IV
Diffuse midline glioma, H3-K27M-mutant, WHO grade IV


HE
IDH1-R132H
ATRX
A
HE
IDH2-R172K
1p/19q codel
B
A: 0%
C: 39%
G: 8%
T: 53%
G C A C T G C
D1S468
D19S219
B
T
HE
IDH1-R132H
EGFRvIII
C
HE
GFAP
BRAF-V600E
D
A: 53%
T: 47%
C A T C
HE
OLIG2
KIAA1549 - BRAF
E
1 2 3 4
A
B
159bp
215bp
HE
H3-K27M
H3K27me3
F


Glioblastoma group
PXA-like
LGG-like
H3-K27**
H3-G34
IDH
RTK I
RTK II classic
Mesen-chymal
Genetic aberrations
BRAF V600E mutation
CDKN2A/B deletion
BRAF V600E mutation*
rare copy number aberrations
H3-K27M mutation
TP53 or PPMD1 mutation
PDGFRA amplification
ACVR1 mutation§
ATRX mutation#
H3F3A G34 mutation
ATRX mutation
DAXX mutation
TP53 mutation
DNA hypomethylation
IDH1/IDH2 mutation
G-CIMP
TP53 mutation
ATRX mutation
CDKN2A/B deletion
Chr. 10q loss
PDGFRA amplification
TERTp mutation
Chr. 7 gain
Chr. 10 loss
EGFR amplification
CDKN2A/B deletion
PTEN mutation
TERTp mutation
Chr. 7 gain
Chr. 10 loss
TERTp mutation
Chr. 7 gain
Chr. 10 loss
MGMT promoter methylated (%)
– 15
20-25
< 5
– 75
– 90
– 40
– 40
– 40
Age group
> children
ch.–y. a.
> young adults
> older adults
Location
> hemispheric
midl.
> hemispheric
Median OS (m)
> 36
~ 12
~ 24
– 30
~ 12-14


A
Oligodendrogliomas, IDH-mutant and 1p/19q codeleted
Diffuse astrocytic gliomas, IDH-mutant
Glioblastoma, IDH-mutant WHO grade IV
WHO grade II
WHO grade III
WHO grade II
WHO grade III
Watch & wait
Watch & wait
RT→ PCV (or TMZ/RT→TMZ)
RT→ PCV
RT→TMZ
TMZ/RT→TMZ
B
Diffuse astrocytic gliomas, IDH-wildtype*
Glioblastoma, IDH-wildtype, WHO grade IV
WHO grade II
WHO grade III
≤ 70 years
> 70 years
MGMT -
MGMT +
MGMT -
MGMT +
Watch & wait
RT
TMZ/RT →TMZ
TMZ/RT →TMZ
RT
TMZ/RT →TMZ or TMZ
C
Diff. midline glioma, H3-K27M mutant WHO grade IV
SEGA WHO grade I
Pilocytic astrocytoma WHO grade I
Pleomorphic xantho-astrocytomas
WHO grade II
WHO grade III
RT (or TMZ/RT→TMZ)
Watch & wait
RT (or TMZ/RT →TMZ)


A
Standard
Exp
B
Biomarker
positive
negative
Standard 1
Exp 1
Standard 2
Exp 2
C
Molecular profiling
Marker 1
Marker 2
Marker 3
Marker 4
Marker 5
negative
Exp 1
Exp 2
Exp 3
Exp 4
Exp 5
Standard