Glioblastoma (GBM) is the most common primary malignant tumor of the central nervous system. The current standard of care for GBM is maximal resection followed by postoperative radiation with concomitant and adjuvant temozolomide. Despite this multimodality treatment, the median survival for GBM remains marginally better than 1 year. In the past decade, genome-wide analyses have uncovered new molecular features of GBM that have refined its classification and provided new insights into the molecular basis for GBM pathogenesis. Here, we review these molecular features and discuss major clinical trials that have recently defined the field. We describe genetic alterations in isocitrate dehydrogenase, ATRX, the telomerase promoter, and histone H3 variants that promote GBM tumorigenesis and have altered GBM categorization. We also discuss intratumoral genetic heterogeneity as one explanation for therapeutic failures and explain how ultra-long extensions of glioma cells, called tumor microtubes, mediate therapeutic resistance. These findings provide new insights into GBM biology and offer hope for the development of next-generation therapies.

Most cases of GBM occur in the absence of identifiable risk factors. Prior cranial irradiation is the only well-established environmental risk factor. Hereditary cancer susceptibility syndromes are present in <5% of GBMs and include disorders such as Li-Fraumeni syndrome (TP53 mutation), Turcot syndrome (biallelic mutation of mismatch repair genes), and neurofibromatosis type 1 (NF1 mutation). Germline risk loci identified through genome-wide association studies account for <30% of GBM risk and include polymorphisms in the genes TP53, TERT, EGFR, CDKN2B-AS1, and RTEL1. Genomic analyses of tumors over the past decade have uncovered mutational, copy number, gene expression, and epigenetic alterations in GBMs. Integration of these data provides deeper understanding of GBM pathogenesis and has led to...
the identification of new molecular subtypes whose evaluation has become routine in GBM diagnosis. Such advances hold promise for the development of new therapeutic strategies in GBM.

**Standard Therapy for Glioblastoma**

The current standard of care for newly diagnosed GBM patients is resection followed by postoperative radiation therapy (RT) with concurrent and adjuvant temozolomide. Surgery serves to alleviate symptoms of mass effect, reduce tumor burden, and provide adequate tissue for diagnosis and molecular profiling. Although randomized evidence for the efficacy of maximal surgical resection is limited, numerous retrospective studies and a meta-analysis of 41,117 patients have found that gross total resection improves overall and progression-free survival relative to subtotal resection. Furthermore, fluorescence-guided surgery using 5-aminolevulinic acid, which allows for more complete resection, was found to improve progression-free survival in a randomized trial. Based on this and other evidence, maximal safe resection is considered the current standard for GBM treatment.

Postoperative radiation was established as the cornerstone of adjuvant therapy for GBM by the Brain Tumor Study Group (BTSG) 69–01 trial, which showed that whole brain radiation improved overall survival (median 35 versus 14 weeks). Radiation dose was subsequently examined in a pooled analysis of BTSG trials showing longer survival for 60 Gy compared with 45 Gy, which was confirmed in a subsequent randomized trial by the Medical Research Council, making 60 Gy the standard of care. Further dose escalation to 70 Gy failed to improve survival, and dose dense radiation with or without concurrent and adjuvant temozolomide. Temozolomide was given at 75 mg/m² daily during radiation and then at 150 to 200 mg/m² during the first 5 days of a 4-week cycle for a total of six cycles. The addition of temozolomide improved median survival from 12.1 to 14.6 months and 5-year overall survival from 1.9% to 9.8. The relative benefit from concomitant versus adjuvant temozolomide remains unclear. Notably, intensifying postradiation temozolomide dose in another randomized trial, RTOG 0525, failed to improve survival further. Contemporary randomized trials have utilized postoperative radiation with concurrent and adjuvant temozolomide as a backbone on which to add investigational therapies, and these trials are summarized in Table 1.

**Other Therapies in the Primary Setting**

GBMs are highly vascular tumors, which has led to the study of angiogenesis inhibition as a treatment strategy. Bevacizumab is a humanized monoclonal antibody against vascular endothelial growth factor A that showed progression-free survival benefit but not overall survival benefit in two phase III randomized trials. In one trial, RTOG 0825, bevacizumab was added to the standard regimen of radiation with concurrent and adjuvant temozolomide. There was no difference in overall survival between the group that received bevacizumab and the group that did not. A progression-free survival benefit was noted in the bevacizumab group (median 10.7 versus 7.3 months), which did not meet the prespecified efficacy target. In the other randomized trial, AVAglio, the addition of bevacizumab to radiation and temozolomide did not confer an advantage in terms

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Table 1 Contemporary phase III trials using temozolomide in newly diagnosed GBM

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Treatment arm</th>
<th>n</th>
<th>OS (months)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stupp et al (2005), EORTC/NCIC</td>
<td>RT control  TMZ/RT</td>
<td>286</td>
<td>12.1</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>287</td>
<td>14.6 (# &lt; 0.0001)</td>
<td></td>
</tr>
<tr>
<td>Gilbert et al (2013), RTOG 0525</td>
<td>TMZ/RT standard dose  TMZ/RT dose dense</td>
<td>411 422</td>
<td>16.6 14.9 (# ≤ 0.63)</td>
<td>18</td>
</tr>
<tr>
<td>Gilbert et al (2014), RTOG 0825</td>
<td>TMZ/RT  TMZ/RT/Bev</td>
<td>309 312</td>
<td>16.1 15.7 (# ≤ 0.21)</td>
<td>19</td>
</tr>
<tr>
<td>Chinot et al (2014), AVAglio</td>
<td>TMZ/RT  TMZ/RT/Bev</td>
<td>463 458</td>
<td>16.7 16.8 (# ≤ 0.10)</td>
<td>20</td>
</tr>
<tr>
<td>Stupp et al (2014), CENTRIC</td>
<td>TMZ/RT  TMZ/RT/cilengitide</td>
<td>273 272</td>
<td>26.3 26.3 (# ≤ 0.86)</td>
<td>21</td>
</tr>
<tr>
<td>Stupp et al (2017), EF-14</td>
<td>TMZ/RT  TMZ/RT/TTF</td>
<td>229 466</td>
<td>16.0 20.9 (# &lt; 0.001)</td>
<td>24</td>
</tr>
</tbody>
</table>

Abbreviations: Bev, bevacizumab; GBM, glioblastoma; OS, overall survival; RT, radiation therapy; TMZ, temozolomide; TTF, tumor treating fields. *All tumors in this trial were MGMT methylated.
of overall survival, but there was a statistically significant increase in progression-free survival from 6.2 to 10.6 months.20 These two trials highlighted several hallmarks of bevacizumab therapy: (1) the agent reduces vascular permeability and alters contrast-enhancement in MR imaging, confounding interpretation of surveillance scans and raising problems with progression-free survival endpoints that may be driven by imaging findings; (2) there is a slight increase in the rate of adverse events, especially thromboembolic and hemorrhagic events, among patients taking bevacizumab. These and other data have relegated bevacizumab to use in carefully selected patients in the newly diagnosed setting. The integrin inhibitor cilengitide is another agent with antiangiogenic activity that failed to improve overall survival when added to standard therapy in the CENTRIC trial (►Table 1). Cilengitide was administered intravenously on a biweekly schedule with both radiation and temozolomide and then administered again after radiation for up to 18 months, with no improvement in survival.21 Two adjuvant therapies have resulted in increased overall survival but have not been routinely adopted in clinical practice. Carmustine wafers (Gliadel) implanted in the resection cavity at the time of surgery to deliver local adjuvant chemotherapy have demonstrated an overall survival benefit in two randomized trials (median 13.9 versus 11.6 months in one trial).22,23 However, when non-GBM cases were removed from the analysis in one of these trials, the benefit was no longer statistically significant.23 Concern regarding adverse effects, including cerebrospinal fluid leakage and intracranial hypertension, and challenges in interpretation of imaging findings after wafer placement have prevented wide adoption of this adjuvant therapy. Tumor-treating fields (TTFs) have also emerged as a novel therapy, with randomized evidence supporting an overall survival benefit. TTFs involve placement of a specialized helmet worn for >18 hours/day that delivers continuous alternating electric fields via transducers placed on the shaved scalp. The EF-14 phase III trial randomized patients to standard radiation with concurrent and adjuvant temozolomide with or without TTF maintenance treatment following radiation completion. The trial was closed early after an overall survival benefit was observed at the first interim analysis and ultimately yielded a benefit of 20.9 versus 16.0 months in the final analysis.24 The therapy has subsequently gained in popularity, but some authorities have raised concern over the lack of a sham placebo device in the trial, the invasive nature of the device in terms of the patient experience, and the incompletely understood antitumor mechanism.25

### Elderly Patients

Nearly half of patients with GBM are diagnosed over the age of 65.1 Due to upper age limits or poor accrual of such patients in clinical trials, randomized evidence for the elderly population was relatively lacking until recently. To determine whether elderly patients should be treated with the same intensity as younger patients, several studies initially examined whether surgery or radiation provides a survival benefit in the elderly. A Finnish study confirmed that surgical debulking was associated with improved overall survival compared with biopsy alone in patients >65 years old.26 Subsequently, a French trial demonstrated that 50 Gy adjuvant radiation improved overall survival relative to supportive care alone in GBM patients >70 years old with good performance status.27 Together, these studies support intervention with surgery and radiation rather than supportive care alone in the elderly. With randomized evidence supporting radiation in the elderly, subsequent trials have focused on abbreviating radiation regimens and examining the role of chemotherapy (►Table 2). In one trial, GBM patients ≥60 years old were randomized to conventional 60 Gy in 30 fractions versus hypofractionated 40 Gy in 15 fractions, and no difference in overall survival between the two regimens was observed (44.7% versus 41.7% at 6 months).28 In a subsequent randomized trial, an extremely hypofractionated regimen of 25 Gy in 5 fractions was explored in a population of elderly (>65) and/or frail (age >50 and KPS 50–70) GBM patients.29 While this regimen had survival equivalent to that of 40 Gy in 15 fractions, it is likely to have adverse effects on neurocognitive function, which may preclude its adoption. In the German

### Table 2 Randomized clinical trials for GBM in the elderly

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Treatment arm</th>
<th>N</th>
<th>OS (months)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keime–Guibert et al (2007), ANOCEF</td>
<td>BSC/BSC/RT (50.4 Gy in 28 fx)</td>
<td>42</td>
<td>3.9</td>
<td>27</td>
</tr>
<tr>
<td>Wick et al (2012), NOA-08</td>
<td>RT (60 Gy in 30 fx)</td>
<td>47</td>
<td>5.1</td>
<td>28</td>
</tr>
<tr>
<td>Wick et al (2012), NOA-08</td>
<td>RT (40 Gy in 15 fx)</td>
<td>48</td>
<td>5.6 (p = 0.57)</td>
<td>28</td>
</tr>
<tr>
<td>Malmström et al (2012), NORDIC</td>
<td>RT (60 Gy in 30 fx)</td>
<td>178</td>
<td>9.6</td>
<td>30</td>
</tr>
<tr>
<td>Malmström et al (2012), NORDIC</td>
<td>RT (34 Gy in 10 fx)</td>
<td>100</td>
<td>6.0</td>
<td>31</td>
</tr>
<tr>
<td>Malmström et al (2012), NORDIC</td>
<td>RT (40 Gy in 15 fx)</td>
<td>98</td>
<td>7.5</td>
<td>31</td>
</tr>
<tr>
<td>Malmström et al (2012), NORDIC</td>
<td>RT (40 Gy in 15 fx)/TMZ</td>
<td>93</td>
<td>8.3*</td>
<td>31</td>
</tr>
<tr>
<td>Perry et al (2017), NCIC CE.6/EORTC 26062</td>
<td>RT (40 Gy in 15 fx)</td>
<td>281</td>
<td>7.6</td>
<td>32</td>
</tr>
<tr>
<td>Perry et al (2017), NCIC CE.6/EORTC 26062</td>
<td>RT (40 Gy in 15 fx)/TMZ</td>
<td>281</td>
<td>9.3 (p &lt; 0.001)</td>
<td>32</td>
</tr>
</tbody>
</table>

Abbreviations: BSC, best supportive care; GBM, glioblastoma; OS, overall survival; RT, radiation therapy; TMZ, temozolomide.

*p = 0.01 (versus 60 Gy in 30 fx), p = 0.24 (versus 34 Gy in 10 fx).
Salvage Therapies for Recurrent GBM

Treatment options for recurrent GBM include resection, reirradiation, and systemic therapies. Despite numerous clinical trials, no standard regimen has been established for recurrent GBM, due to a paucity of randomized evidence and disease heterogeneity in single-arm prospective studies. Reresection and reirradiation are controversial but may be appropriate for select patients with good performance status, small tumor volume, and no involvement of critical brain structures, with radiation often involving hypofractionated stereotactic approaches. Nitrosoureas, temozolomide, and bevacizumab represent the most widely accepted systemic therapies. Temozolomide rechallenge appears most beneficial for patients with MGMT (O6-methylguanine methyltransferase) promoter methylation. Bevacizumab efficacy was first demonstrated in two phase II trials, which led to its approval as monotherapy in 2009 and subsequent widespread adoption in clinical practice. It has since been tested in combination with numerous agents including nitrosoureas. Notably, addition of lomustine to bevacizumab improved overall survival in the BELOB phase II randomized trial but failed to confer a survival benefit in a subsequent phase III trial. No targeted agents have demonstrated efficacy superior to that of alkylating chemotherapy, with notable examples including the kinase inhibitor enzastaurin and the VEGFR inhibitor cediranib, both studied in randomized phase III trials. Given multiple treatment options, therapy selection in recurrent GBM should be individualized, taking into account patient fitness, neurological symptoms, volume of disease, involvement of critical brain structures, and overlap with prior radiation fields. Regardless of therapy choice, overall survival is dismal at ~6 to 12 months after recurrence.

Molecular Features

Integrated Genomic Analyses

Integrated genomic analyses in the past decade have uncovered mutational, copy number, gene expression, and epigenetic alterations in GBM. The most frequent somatic alterations in GBMs include point mutations in the promoter of the telomerase catalytic subunit TERT (in 60–80% of GBMs), deletion of CDKN2A/B (~50%), amplification or mutation of the receptor tyrosine kinase EGFR (~40%), inactivation of the tumor suppressors TP53 (~30%) and PTEN (~30%), alterations in PIK3CA or PIK3R1 (~20%), CDK4 amplification (~15%), RB1 inactivation (~10%), NF1 mutation (~10%), and IDH1/2 mutation (5–10%). Uncommon activating mutations of the kinase BRAF (1–2%) occur in a new 2016 WHO-defined entity called "epithelioid glioblastoma" and are notable given the activity of BRAF-targeted therapies in other cancer types. Most EGFR-amplified GBMs contain additional activating mutations or rearrangements of EGFR, such as the EGFR vIII deletion which is present in ~30% of GBMs. Pathway analysis has revealed that most GBMs contain alterations in the TP53, RB, and RTK/PI3K/RAS pathways, and that genes in each pathway tend to be altered in a mutually exclusive manner. For instance, most tumors contain an alteration of at least one RB pathway gene (CDK4, CDK6, CCND2, CDKN2A/B, or RB1). The IDH and TERT mutations have helped to refine the classification of WHO grade II to IV adult diffuse gliomas, as most primary GBMs are IDH wild-type with TERT mutations, while grade II to III astrocytomas and secondary GBMs are IDH mutant without TERT mutations. Oligodendrogliomas contain both IDH mutations and TERT mutations, in addition to characteristic loss of heterozygosity of 1p/19q.
The Cancer Genome Atlas and other groups have proposed classification systems for GBM based on bulk tumor mRNA expression patterns. This has been used to classify tumors as proneural, neural, mesenchymal, or classical subtypes, which associate with specific genetic events and clinical profiles. For example, the proneural subgroup contains the IDH-mutant GBMs; EGFR alterations are enriched in the classical subgroup, and NF1 alterations are enriched in the mesenchymal subgroup. Furthermore, the mesenchymal subtype has been associated with radioresistance, while the proneural subtype was linked to an overall survival benefit from bevacizumab in the AVAglio trial. However, single-cell RNA sequencing has revealed that most GBMs comprise tumor cell subpopulations exhibiting most or all of these expression subtypes, calling into question their clinical utility. DNA methylation signatures have also been proposed to classify GBM, and a glioma CpG island methylator phenotype (G-CIMP) was identified that tightly associates with IDH-mutant GBM. The complexity of these classification schemes and the problem with tumor heterogeneity has limited their translation to the clinic.

### MGMT

The DNA repair enzyme MGMT protects against the cytotoxic effect of temozolomide by removing methyl adducts deposited on guanine bases by the drug. Epigenetic silencing of MGMT by methylation of its promoter remains an important GBM prognostic marker and predictor of temozolomide response. In EORTC26981/22981 NCIC CE.3, MGMT promoter methylation was an independent favorable prognostic factor for overall survival. Among patients with MGMT promoter methylation, a survival benefit was observed for those who received temozolomide compared with those who did not (21.7 versus 15.3 months). In the elderly NOA-08 and Nordic trials, temozolomide benefit was similarly observed in patients with MGMT promoter methylation but not in patients without it. In the elderly EORTC 26062/NCIC CE.6 trial, however, a strong trend toward temozolomide benefit was observed in patients whose tumors lacked MGMT promoter methylation. MGMT status may influence the decision to withhold temozolomide or the duration of temozolomide treatment in the elderly (►Fig. 1), although further research is needed given results from the EORTC 26062/NCIC CE.6 trial. Notably, the G-CIMP phenotype partially overlaps with MGMT promoter methylation, which occurs in 79% of G-CIMP tumors yet only 46% of non-G-CIMP tumors. Lastly, in contrast to MGMT promoter methylation which increases temozolomide sensitivity, mutation of mismatch repair (MMR) genes confers temozolomide resistance in cellular models and is associated with GBM recurrence after temozolomide treatment in patients.

### IDH Mutations

Perhaps the most important molecular marker for grade II to IV gliomas, mutations in isocitrate dehydrogenases 1 and 2 (IDH1 and IDH2, collectively referred to as IDH) have made their way into widespread clinical practice since their discovery in a genome-wide analysis in 2008. Mutations in IDH1 and less commonly IDH2 are found in almost all grade II to III astrocytomas, grade II to III oligodendrogliomas, and grade II to III anaplastic astrocytomas.

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**Fig. 2** IDH, TERT, and ATRX alterations refine the classification of adult diffuse gliomas. The combination of IDH and TERT genotyping aids in the classification of diffuse gliomas. Most primary glioblastomas (GBMs) contain TERT mutations but not IDH mutations; grade II to III astrocytomas and secondary GBMs contain IDH mutations but not TERT mutations; and grade II to III oligodendrogliomas contain both TERT and IDH mutations. Also shown are TP53 and ATRX mutations commonly found in astrocytic tumors and 1p/19q loss of heterozygosity found in grade II to III oligodendrogliomas.
and secondary GBMs that have transformed from lower grade gliomas, while 90% of primary GBMs are wild-type for IDH\(^55\) (\(-\text{Fig. 2}\)). The current WHO classification now categorizes IDH wild-type and IDH-mutant GBM as distinct entities. Among GBM patients, IDH mutation is associated with a younger median age at diagnosis, a longer median overall survival, a predilection for the frontal lobes, and less necrosis and contrast-enhancement on MRI\(^55,57\) (\(-\text{Fig. 3}\)). IDH mutational status can be routinely assessed by immunohistochemistry for the IDH1-R132H mutation, which accounts for \(-90\%\) of all IDH mutations, or by sequencing-based analyses. Mechanistic studies have demonstrated that IDH mutations result in a gain of function that produces the oncometabolite 2-hydroxyglutarate (2HG).\(^58\) This metabolite promotes epigenetic silencing through inhibition of \(\alpha\)-KG-dependent dioxygenases, which include Jumonji histone lysine demethylases and TET DNA hydroxylases that promote histone and DNA demethylation, respectively\(^59,60\) (\(-\text{Fig. 4}\)). In addition, 2HG can activate EGLN prolyl hydroxylases to promote HIF1 degradation and cellular transformation.\(^60\) Notably, IDH mutation alone is not sufficient for tumorigenesis but requires additional genetic alterations.\(^59,61\) These studies have led to the development of small molecule IDH inhibitors, which are in early clinical trials, and synthetic lethal approaches to target IDH-mutant tumors.\(^59\)

**Telomere Maintenance—TERT and ATRX**

Telomeres are incompletely replicated during the cell cycle and thus shorten at each cell division, eventually causing cellular senescence. To prevent senescence, most GBMs upregulate the enzyme telomerase (TERT), which can extend chromosome ends. In up to 83% of primary GBMs, upregulation is accomplished through TERT promoter mutations,\(^43\) which can aberrantly recruit the ETS transcription factor GABP\(^62\) (\(-\text{Fig. 4}\)). A subset of GBMs maintains telomere length through another pathway called alternative lengthening of telomeres (ALT) that is associated with better overall survival\(^63\) and transformation from lower grade astrocytomas. Inactivation of the helicase and histone chaperone ATRX is associated with the establishment of ALT.\(^64\) Recently, ATRX mutations have been discovered in 85% of grade II to III astrocytomas,\(^65\) in the majority of secondary GBMs,\(^66\) and in 30% of pediatric GBMs\(^67\) and correlate with the ALT phenotype in these tumors. ATRX may inhibit ALT by protecting against replication fork stalling at telomeres and thereby minimizing telomeric homologous recombination which is necessary for ALT\(^64\) (\(-\text{Fig. 4}\)). ATRX may reduce fork stalling by unwinding quadruplex DNA at telomeres and by promoting telomeric deposition of the histone H3.3.\(^64\) Notably, TERT and ATRX mutations are mutually exclusive in GBM and therefore define GBM subsets with distinct pathways for maintaining telomere length (\(-\text{Fig. 2}\)). Interestingly, ALT cells are sensitive to inhibition of the replication stress kinase ATR,\(^68\) making this kinase a potential target for the treatment of gliomas with inactivation of ATRX.

**Histone H3 Mutations—K27M and G34R/V**

Recently, genome-wide sequencing studies have discovered mutations in histone H3 genes in GBM. Specifically, the mutation K27M occurring in the histone variants H3.3 and H3.1 has been identified in 20% of pediatric GBMs\(^67,69\) and nearly 80% of pediatric diffuse intrinsic pontine gliomas (DIPGs).\(^69\) This mutation is present in not only pediatric tumors but can also occur in GBMs in young adults.\(^70,71\) Importantly, K27M tumors tend to be centered in midline...
structures (such as the pons, midbrain, and thalamus) (Fig. 3), which has led to the definition of “diffuse midline glioma, H3 K27M-mutant” as a new grade IV entity in the 2016 WHO classification. Mechanistically, K27M mutation leads to global reduction in histone H3 K27 trimethylation through impaired recruitment of PRC2 and inhibition of the K27 methylase EZH2 within the PRC2 complex (Fig. 4). Consistent with this mechanism of pathogenesis, targeted increase in global H3 K27M trimethylation levels using demethylase or deacetylase inhibitors inhibits growth of K27M tumors in xenograft studies. Additional therapeutic targets for K27M tumors include the activin receptor ACVR1, which harbors stimulating mutations in 20% of K27M DIPGs, and the p53 phosphatase PPM1D, which is mutated in nearly 40% of K27M brainstem gliomas in children and adults.

In addition to K27M, recurrent mutations in Gly34 of histone H3.3 (G34R/V) have been identified in GBMs. While the mutations are just a few amino acids apart in histone H3.3, tumors with G34R/V mutation are clinically and biologically distinct from those with K27M mutation. G34R/V tumors tend to occur in the cerebral hemispheres, follow a more favorable clinical course, and have a different epigenetic signature compared with K27M tumors (Fig. 3). Like IDH-mutated GBMs, the G34 GBMs almost invariably contain ATRX mutations. The G34 tumors also appear to comprise a more heterogeneous group, with histopathologic features typical of either GBM or PNETs (primitive neuroectodermal tumors), which has led to the proposal that they be considered a single clinical entity.

Proposed mechanisms of oncogenesis for the histone 3 mutations and for other emerging molecular alterations detailed above are shown in Fig. 4.

**Intratumoral Heterogeneity**

Molecular intratumoral heterogeneity occurs commonly in GBMs and can manifest as the presence of different subclones in the same tumor. For example, subclones with mutually exclusive amplifications of different receptor tyrosine kinases (EGFR, PDGFR, or MET) have been found intermingled within the same tumor. Intratumoral heterogeneity has also been observed for PTEN deletion.
and for gene expression signatures identified by single cell RNA-sequencing.\textsuperscript{50} Tyrosine kinase inhibitors targeting EGFR and a vaccine against the constitutively active EGFR vIII mutant have demonstrated limited success in clinical trials,\textsuperscript{81–83} possibly because EGFR alterations affect only a subclonal portion of tumor cells and are late events.\textsuperscript{57,78,79} In contrast, IDH and histone H3 mutations harbor characteristics that suggest they are initiating events. For instance, IDH mutations in secondary GBMs appear at the earliest biopsy of the disease,\textsuperscript{54,55} are generally stable throughout tumor development,\textsuperscript{54,55} and have a mutational signature consistent with appearance before or concurrent with p53 mutation.\textsuperscript{57} Similarly, histone H3 K27M mutations in DIPGs are homogeneous throughout tumor tissue.\textsuperscript{84} These potentially early driver alterations, which define GBMs with distinct clinical features including prognosis, patient age, tumor location, and other genetic features (\textsuperscript{\textbullet} Fig. 3), may represent promising therapeutic targets. Notably, founder events in IDH-WT GBMs, which account for the majority of adult GBMs and which can arise within months, remain to be more clearly identified.\textsuperscript{78,85} Ultimately, given the diversity of pathways involved in GBM pathogenesis, combination of molecular therapies will likely be necessary.\textsuperscript{57}

**Tumor Microtubes as a Novel Mechanism for GBM Progression and Resistance**

The recent discovery of ultra-long membranous protrusions that extend from astrocytoma cells, called tumor microtubes (TM), has changed our understanding of GBM\textsuperscript{86,87} (\textsuperscript{\textbullet} Fig. 5). TMs interconnect individual GBM cells in a single communicating syncytium with extensions that invade and colonize the brain by sending nuclear material to distant locations.\textsuperscript{86}

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**Fig. 5** Tumor microtube (TM) functions during glioma progression. Overview of the four known molecular functions of TMs, whose development depends on intact 1p/19q status. While GAP-43 is necessary for all four functions (invasion, proliferation, interconnection, and network formation), TTYH1 appears to promote only invasion and tumor cell proliferation. Cx43, connexin 43 gap junctions; ER, endoplasmic reticulum; MT, microtubules; Mito, mitochondria; ICW, intercellular calcium waves; TM, tumor microtube. Adapted from prior reference.\textsuperscript{86}
Multiple characteristics of TMs have translational importance. First, approximately half of the cells in a malignant astrocytoma are connected through the TM network. Importantly, these cells are resistant to the cytotoxic effects of both radiotherapy and temozolomide. Second, TMs extend to surgical lesions in a mouse model, which may contribute to local recurrence after resection. Third, GBM cells hijack CNS developmental programs, involving factors such as GAP-43 and Ttyh1, to promote TM formation (Fig. 5), making these pathways promising therapeutic targets despite their attenuated function in the adult CNS. Fourth, development of functional TMs depends on intact 1p/19q status, which may be due to the presence of multiple neurotrophic factors, such as Ttyh1, on the 1p and 19q arms (Fig. 5). This point is interesting because it may explain why patients with 1p/19q codeleted gliomas have better survival independent of IDH mutation. Finally, communication via the TM network, which is enabled by Cx43 gap junctions between cells, can be blocked by gap junction inhibitors and other means to impair TM function. In summary, gliomas appear to be organized as a single functional unit with syncytial connections between the glioma core and extensions that invade adjacent brain tissue. Research in this area is likely to shed further light on pathways regulating TM formation and on oncogenic consequences of TM function, which may lead to novel therapies that inhibit this important mechanism of GBM resistance.

**Future Directions**

Future advances in GBM treatment will be facilitated by increased understanding of GBM pathogenesis and innovative approaches to exploiting tumor vulnerabilities. Given problems with intratumoral heterogeneity, the most promising targets may be genes whose alterations serve as clonal initiating events. EGFR inhibitors have had limited success in clinical trials possibly because EGFR alterations represent late events that occur in only a fraction of tumor cells. Numerous therapeutic approaches have been proposed to exploit dependencies in IDH-mutant tumors. One interesting strategy involves depletion of NAD+ by NAMPT inhibitors, in IDH-mutant tumors have downregulated NAD+ salvage pathways, making them sensitive to reductions in NAD+ levels. Data are also emerging from early-phase trials for the treatment of IDH-mutant tumors with IDH inhibitors. The discovery of hotspot mutations in the TERT promoter and in histone tails raises the question as to whether dependencies in tumors with these alterations can be therapeutically exploited as well. For example, tumors with TERT promoter mutations may develop addictions to pathways regulating transcription factors aberrantly recruited to mutated TERT promoters. Promising therapeutic approaches for histone H3 K27M tumors may involve agents that regulate epigenetic pathways as described above. In addition to intratumoral heterogeneity, escape mechanisms are likely responsible for the failure of targeted agents in clinical trials. Such mechanisms allow continued flux through a pathway and can include the activation of downstream effectors by alternative inputs or the loss of pathway inhibitors such as PTEN. An additional reason for the limited efficacy of targeted approaches may be inadequate target suppression as observed for EGFR inhibitors. This finding highlights the importance of assessing pharmacodynamic end points in clinical trials and continued research on strategies to penetrate the blood–brain barrier. Ultimately, difficulties with intratumoral heterogeneity and tumor evolution will likely require combination treatments that target multiple pathways simultaneously and that counter anticipated escape mechanisms. Such combination strategies will benefit from sequential biopsies to assist with therapy selection upon tumor evolution.

Immunotherapeutic approaches represent an additional line of promising investigation. GBMs are immunosuppressive due to numerous mechanisms, including decreased MHC expression, increased PD-L1 expression, secretion of anti-inflammatory IL-10, and restriction of immune cell infiltration by the blood–brain barrier. Multiple strategies to enhance immune targeting of GBMs are under investigation, including immune checkpoint inhibitors, chimeric antigen receptor (CAR) T cells, and vaccines. For example, the checkpoint inhibitors nivolumab and pembrolizumab are currently being tested in newly diagnosed and recurrent GBM patients in at least 15 clinical trials. CAR T cells targeting the tumor antigens EGFR vIII and HER2 are also in early clinical trials. While newly diagnosed GBMs have generally low mutational loads, recurrent tumors exposed to temozolomide are often highly mutated and may represent attractive targets for immunotherapeutic agents. Immunotherapy for gliomas is comprehensively reviewed in another article in this issue.

Translating molecular advances into clinical progress will depend on recognizing limitations in preclinical models and clinical trial design. Using patient-derived preclinical models rather than artificial cell culture systems may improve the identification and validation of therapeutic targets. Molecular stratification in clinical trials is important for targeting therapies to patients with appropriate molecular alterations. Additionally, innovative approaches to clinical trial design may accelerate the testing of new agents. Such approaches include umbrella trials, which assign agents to patients based on molecular alterations, and platform trials, which can adaptively add or remove treatment arms. Together these new approaches may help to translate new therapies targeted at telomere biology, histone regulatory networks, tumor metabolism, immunotherapy, and TM functionality to the clinic.

**Acknowledgments**

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