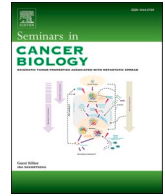




Contents lists available at ScienceDirect

## Seminars in Cancer Biology

journal homepage: [www.elsevier.com/locate/semcancer](http://www.elsevier.com/locate/semcancer)

## Cancer cell heterogeneity &amp; plasticity in glioblastoma and brain tumors

Adam Lauko<sup>a,b,c</sup>, Alice Lo<sup>a</sup>, Manmeet S. Ahluwalia<sup>d,e</sup>, Justin D. Lathia<sup>a,b,c,d,e,\*</sup><sup>a</sup> Department of Cardiovascular & Metabolic Sciences, Lerner Research Institute, Cleveland Clinic, Cleveland, OH, United States<sup>b</sup> Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, United States<sup>c</sup> Medical Scientist Training Program, Case Western Reserve University School of Medicine, Cleveland, OH, United States<sup>d</sup> Rose Ella Burkhardt Brain Tumor and Neuro-Oncology Center, Cleveland Clinic, Cleveland, OH, United States<sup>e</sup> Case Comprehensive Cancer Center, Cleveland, OH, United States

## ARTICLE INFO

## Keywords:

Glioblastoma  
Heterogeneity  
Plasticity  
Epigenetics  
Tumor microenvironment

## ABSTRACT

Brain tumors remain one of the most difficult tumors to treat and, depending on the diagnosis, have a poor prognosis. Of brain tumors, glioblastoma (GBM) is the most common malignant glioma and has a dismal prognosis, with only about 5% of patients alive five years after diagnosis. While advances in targeted therapies and immunotherapies are rapidly improving outcomes in a variety of other cancers, the standard of care for GBM has largely remained unaltered since 2005. There are many well-studied challenges that are either unique to brain tumors (i.e., blood-brain barrier and immunosuppressive environment) or amplified within GBM (i.e., tumor heterogeneity at the cellular and molecular levels, plasticity, and cancer stem cells) that make this disease particularly difficult to treat. While we touch on all these concepts, the focus of this review is to discuss the immense inter- and intra-tumoral heterogeneity and advances in our understanding of tumor cell plasticity and epigenetics in GBM. With each improvement in technology, our understanding of the complexity of tumoral heterogeneity and plasticity improves and we gain more clarity on the causes underlying previous therapeutic failures. However, these advances are unlocking new therapeutic opportunities that scientists and physicians are currently exploiting and have the potential for new breakthroughs.

## 1. Introduction

A study published in 2017 by the National Cancer Institute found across-the-board improvements in 5-year survival for patients with cancer from the 1970s to 2013 (except for cervical and uterine cancer) [1]. However, even with this improvement in survival, patients with brain tumors still have only a 30 % survival at 5 years after diagnosis, and this number is far worse for some tumors types, such as glioblastoma

(GBM) in adults and diffuse intrinsic pontine glioma (DIPG) in children.

The brain and spinal cord are the most well-protected organs in the body and are evolutionarily designed to be protected from damage as much as possible throughout one's lifetime. In addition, each region of the brain has a specific function and can cause serious physical or mental disability when perturbed. These truths lead to various challenges that are not seen in other regions of the body when attempting to treat tumors within the central nervous system (CNS). An obvious example of

**Abbreviations:** CNS, Central nervous system; GBM, glioblastoma; DIPG, diffuse intrinsic pontine glioma; WHO, World Health Organization; TMZ, temozolomide; TTF, tumor-treating fields; CSCs, cancer stem cells; WGA, whole genome amplification; EGFR, epidermal growth factor receptor; IDH1, isocitrate dehydrogenase; MDM, murine double minute; TERT, telomerase reverse transcriptase; PDGFRA, platelet-derived growth factor receptor alpha; PTEN, phosphatase and tensin homolog; ATRX, ATRX chromatin remodeler; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; CDK4, cyclin-dependent kinase 4; TCGA, The Cancer Genome Atlas; CAN, copy number alteration; Ivy GAP, IVY Glioblastoma Atlas Project; NPC, neural-progenitor cell; OPC, oligodendrocyte progenitor cell; AC, astrocyte; G-CIMP, glioma-CpG island methylator phenotype; N6-ma, N6-methyladenine; m6A, N6-methyladenosine; ALKBH1, AlkB homolog 1, histone H2A dioxygenase; HIFs, hypoxia-inducible factors; VEGF, vascular endothelial growth factor; TME, tumor microenvironment; SDF-1, stromal derived factor-1; TGF- $\beta$ , transforming growth factor- $\beta$ ; FGF2, fibroblastic growth factor 2; NLGN3, neuroligin-3; NMDARs, N-methyl-D-aspartate receptors; AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; RANKL, receptor activator of nuclear factor kappa-B ligand; GDF-15, growth/differentiation factor 15; CTGF, connective tissue growth factor; TAMs, tumor-associated macrophages; TILs, tumor-infiltrating lymphocytes; MIF, macrophage migration inhibitory factor; MDSCs, myeloid-derived suppressor cells; mMDSCs, monocytic MDSCs; gMDSCs, granulocytic MDSCs; CSF-1R, colony-stimulating factor-1R; JAM-A, junctional adhesion molecule-A.

\* Corresponding author at: Department of Cardiovascular & Metabolic Sciences, Lerner Research Institute, 9500 Euclid Ave., NC10, Cleveland, OH, 44195, United States.

E-mail address: [lathiaj@ccf.org](mailto:lathiaj@ccf.org) (J.D. Lathia).

<https://doi.org/10.1016/j.semcan.2021.02.014>

Received 1 December 2020; Accepted 22 February 2021

Available online 25 February 2021

1044-579X/© 2021 Elsevier Ltd. All rights reserved.

this is that neurosurgeons must first maneuver through the skull and, when in the parenchyma, must resect the tumor with as narrow margins as possible to limit debilitating comorbidities.

This review will discuss the many ways by which our understanding of brain tumors has evolved and prevented scientists and clinician from developing treatments that improve long-term survival. We will focus on the high levels of heterogeneity and plasticity in GBM and other brain tumors as well as discuss new therapeutic avenues that have been exposed due to the exponential growth in our understanding of these tumors.

## 2. Overview of brain tumors

It is estimated that between 10–20 % of patients with cancer will be diagnosed with a brain metastasis, and autopsy studies have reported a true incidence of 30–40 % in all patients with cancer [2–5]. A total of 67–80 % of brain metastases come from either lung, breast, or melanoma primary tumors. In all three of these tumors, the presence of brain metastases portends a poor survival. The median survival of patients with brain metastasis has been observed to be 13 months [6], and even in patients with the best prognostic factors, the most recent data demonstrate a median survival of only 33 months after the diagnosis of a brain metastasis [6]. While more recent studies have identified genetic differences between the primary tumor and brain metastases [7], the intratumoral heterogeneity of brain metastases has not been investigated in great detail.

Gliomas are a group of brain tumors that were once thought to derive from mature glial cells. However, more recent evidence points towards progenitor cells as the possible cells of origin, including neural and glial progenitor cells within the brain [10–12]. Although it should be noted that the exact cell of origin is still debated. The latest WHO classification system from 2016 integrates a number of characteristics into the diagnostic strategy [13]. Grade I tumors are non-diffuse and surgically-curable and make up only a small fraction of adult tumors. Diffuse gliomas are then stratified based on grade, *isocitrate dehydrogenase (IDH1/2)*, mutation status, and 1p/19q codeletion. Grade II tumors have a relatively favorable prognosis of around 10 years depending on the histological subtype. Most of these patients have a mutation to the *IDH1/2* genes, which leads to the generation of the oncometabolite 2-hydroxyglutarate. The natural course of some low-grade gliomas is to progress to high-grade gliomas, and therefore, maximal safe surgical resection of these tumors is the primary treatment. In some instances, these patients will also receive radiation or the chemotherapy temozolomide (TMZ). However, a recent retrospective study identified TMZ as a modifiable risk factor that contributes to malignant transformation along with wild-type *IDH* or mutated *IDH* with intact 1p/19q [14].

Pediatric brain tumors are now the number one cause of cancer-related death in children. These pediatric tumors include a wide range of benign and malignant tumors with various prognostic indications. These tumors include medulloblastoma, a primitive neuroectodermal tumor that accounts for approximately 20 % of childhood brain tumors [15]. Medulloblastoma comprises a biologically heterogeneous group of embryonal tumors that have been subgrouped into the following four types: WNT, Sonic Hedgehog, Group 3 and Group 4 [16–19]. As a result of its prevalence, the genetic profile of medulloblastoma has been well characterized, and the cell heterogeneity and plasticity of these tumors have been investigated. However, for the purposes of this review, we will focus on adult GBM as a paradigm for cellular heterogeneity and plasticity.

Finally, WHO grade IV glioma is known as GBM. It was acknowledged in the 2016 report that IDH mutant and wild-type GBM were different diseases but they both maintained the GBM label. However, since its publication, these differences have only been highlighted further and in the yet to be released WHO 2021 classification system, IDH mutant grade IV tumors will be renamed “IDH-mutant grade 4 astrocytoma” [188]. Throughout the review, IDH mutant status of patients

in studies are noted when appropriate. Treatment for GBM includes maximal safe surgical resection, radiation, TMZ, and the recently approved tumor-treating fields (TTF), an antimetabolic treatment that delivers alternating electric fields to the scalp [20,21]. Despite these therapies, the latest clinical trials have demonstrated a median survival of only 20.9 months, with a median survival outside clinical trials thought to be around 15 months [20]. There are a multitude of hypotheses for the poor prognosis of patients with GBM, including a failure of drugs to penetrate the blood-brain barrier [22], tumor cell invasion into the brain parenchyma, a hypoxic microenvironment [23], cancer stem cells (CSCs) [24] and tumor cell heterogeneity and plasticity [25].

Here, we will focus primarily on GBM and the heterogeneity observed within individual tumors and between tumors. We will investigate how advances in single-cell sequencing and epigenetic profiling are providing new insights into the complexity of the disease and discuss how CSCs can interact with other cells in the tumor microenvironment and drive treatment resistance, tumor recurrence and tumor cell plasticity. Finally, we discuss the ways that immune cells, endothelial cells, astrocytes, and neurons support the tumor microenvironment and may provide novel therapeutic targets.

## 3. Molecular heterogeneity of glioblastoma

It has long been observed that GBM possesses extensive inter- and intra-tumoral heterogeneity. Early histological studies of GBM focused on the extent of necrosis, nuclear size, astrocytic differentiation, cell size, number of mitotic cells, distribution of cell density and vascularization [26]. Through this approach and the sampling of various locations within the tumor, scientists and pathologists were able to observe significant histological variations even within the same tumor. This phenomenon can be demonstrated *ex vivo*, as well. In one example of this, five subclones were isolated from a single tumor, and after orthotopic xenotransplantation, each subclone had a distinct histology and tumorigenicity [27].

### 3.1. Genetic Heterogeneity

One of the first technological advancements that furthered our understanding of GBM heterogeneity was the development of whole genome amplification (WGA) methods, which enable the identification of chromosomal imbalances. Nobusawa et al. utilized WGA on 14 different primary GBM samples from two to five locations within each tumor and identified some alterations that were common among all locations and others that were region specific [28]. In another study, single molecule molecular inversion probes targeting 33 cancer genes were used to assay point mutations and gene amplifications. The authors found evidence of regional mutational heterogeneity even within the same tumor [29]. Some commonly altered genes among GBM patients include epidermal growth factor receptor (*EGFR*), telomerase reverse transcriptase (*TERT*), platelet-derived growth factor receptor alpha (*PDGFRA*), cyclin-dependent kinase 4 (*CDK4*), murine double minute-2 (*MDM2*), *MDM4*, and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) [30]. Other common mutations or deletions of tumor suppressors include phosphatase and tensin homolog (*PTEN*), ATRX chromatin remodeler (*ATRX*), *IDH1*, and *TP53* [31,32].

Amplification of the genes encoding receptor tyrosine kinases (RTKs), especially *EGFR* and *PDGFRA*, has been well documented in GBM. Snurderl et al. demonstrated that amplification of different RTKs were rarely found in the same region of the tumor, however, different RTKs were amplified in distinct subpopulations of cells [33]. Spatially distinct RTK amplifications were then verified by two additional studies, further emphasizing the intra-tumoral heterogeneity [34,35]. Finally, extrachromosomal DNA can lead to oncogene amplification that drives cancer growth [64]. In GBM, this extrachromosomal DNA is unevenly inherited by offspring cells and impacts the oncogenic potential of the daughter cells [65]. Furthermore, *EGFR* amplification occurs in circular

extrachromosomal DNA, and these circular pieces of DNA tend to harbor epigenetic enhancer regions that topologically interact with the EGFR locus to increase EGFR gene expression [66]. Studies such as these may help to explain the lack of efficacy thus far of EGFR targeted therapies in GBM [36–39]. This lack of efficacy is in contrast to the use of EGFR inhibitors in EGFR-mutant lung cancer with CNS metastases, where one study found a 91 % CNS objective response rate in patients treated with the EGFR inhibitor osimertinib [40,41]. As a result of these studies demonstrating heterogeneity within individual tumors and the lack of efficacy of RTK inhibitor monotherapy, the field has largely moved away from the use of these drugs. However, there is ongoing research on the use of RTK inhibitors as a combination therapy as well as on developing immunotherapies that target cells with high RTK expression.

### 3.2. Expression Heterogeneity

Advances in sequencing technology and the initiation of The Cancer Genome Atlas (TCGA) led to a major shift in our classification of GBM. Bioinformatics analysis of gene expression profiles of tumors identified four distinct molecular subtypes of GBM: proneural, mesenchymal, classical and neural (the neural subtype is excluded from most in follow-up classifications as the neural subtype was thought to be a contamination from normal neural tissue) [42,43]. While this characterization of bulk tumor RNA revolutionized the classification of GBM, heterogeneity and plasticity within these groups were quickly identified. In the earliest challenge to this classification scheme, Sottoriva et al. sampled spatially distinct tumor fragments from 11 patients with GBM and found that within the same patient, multiple molecular subtypes could be identified [44]. The authors also found heterogeneous driver aberrations and copy number alterations (CNA) within the same tumor. In addition, by comparing CNA data and measuring mitotic distances among cells of a tissue fragment, the investigators reconstructed the phylogeny and relationships among subclones. Through this method, the authors found that loss of cyclin-dependent kinase inhibitor 2A (*CDKN2A/B*) and amplification of *EGFR*, *CDK6* and *MET* occur early in tumor development, while alterations in *PDGFRA*, *PTEN* and *TP53* are later malignant events [44]. A later study utilizing 127 multi-sector or longitudinal specimens from 52 patients confirmed these findings [45]. These authors also conducted a chemical screen of patient-derived cells and found that targeting truncal events is more efficacious in reducing tumor burden [45]. Finally, viewing GBM from a temporal standpoint, Wang et al. found that 66 % of patients undergo a subtype switch at recurrence, highlighting the plasticity of these expression states [46].

Gill et al. performed RNA-seq on biopsies taken from within the contrast-enhancing core and the non-enhancing margins of tumors. The contrast-enhancing regions resembled proneural, classical, or mesenchymal subtypes, whereas the non-enhancing margin resembled a neural subtype [47]. The authors also found that the expression pattern of the non-enhancing region was influenced by non-neoplastic brain cells, which will be discussed in greater detail in later sections. Finally, patterns of cell type alterations varied among the GBM subtypes, as the non-enhancing regions of proneural tumors were enriched with oligodendrocyte progenitor genes, whereas mesenchymal tumors were enriched for astrocytic and microglial genes [47]. The importance of molecular subtyping for laboratory studies cannot be overstated; however, almost ten years after its initial development, molecular subtyping has had almost no translational impact on the clinical setting.

Another attempt to further our understanding of the spatial heterogeneity of GBM was the development of the Ivy Glioblastoma Atlas Project (Ivy GAP) [48]. The Ivy GAP database includes a comprehensive pathology-molecular map of GBM that enables the unbiased study of molecular alterations of known anatomical features. The authors utilized laser microdissection and RNA-seq in 41 patients, dissecting out the leading edge, infiltrating tumor, cellular tumor, pseudopalisading cells around necrotic regions, and microvascular proliferation regions for each tumor. In general, samples from the same anatomical feature

were more like each other than like other samples from the same tumor. However, even with this complex analysis, there was no mutation associated with a particular anatomic feature that predicted overall survival better than O6-methylguanine–DNA methyltransferase (*MGMT*) promoter methylation status of the bulk tumor, suggesting that this known adversary remains a significant and predictive hurdle [48]. The creators of the Ivy GAP platform have deposited their data online (<http://glioblastoma.alleninstitute.org/>), and this data have already led to numerous additional publications [49–52].

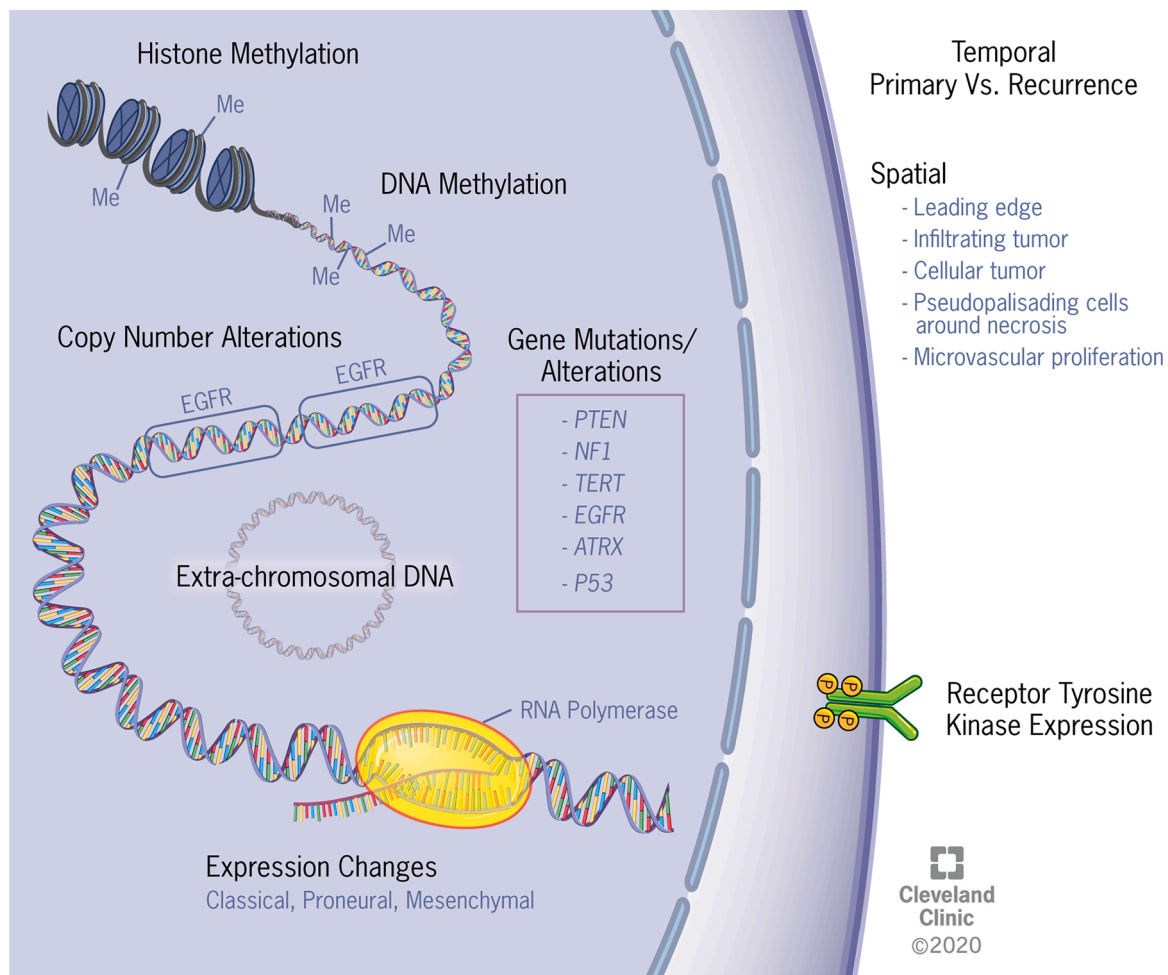
The next technological leap that added to our understanding of intratumor heterogeneity was single-cell sequencing. In the first of these studies, Patel et al. performed single-cell RNA-seq on five different tumors. The authors confirmed previous finding by showing that GBM subtype classifiers are variably expressed across individual cells within a single tumor [53]. A more recent study combined single-cell RNA-seq, analysis of the TCGA, and experimental models to argue that malignant cells in GBM exist in four main cellular states that are reminiscent of canonical neurodevelopmental cell types. This includes astrocyte (AC)-like, oligodendrocyte progenitor cell (OPC)-like, neural progenitor cell (NPC)-like and mesenchymal (MES)-like states. Single-cell lineage tracing experiments found plasticity between these four states. The NPC-like, OPC-like, and AC-like transcriptional states correlated with copy number aberrations in specific loci: *CDK4*, *PDGFRA*, and *EGFR*, respectively [54].

Another question that remains to be completely resolved is treatment-induced plasticity and temporal heterogeneity. In 2014, Johnson et al. sequenced the exomes of 23 initial low-grade *IDH* mutant gliomas and recurrent tumors from the same patient [55]. In 43 % of cases, at least half of the mutations in the initial tumor were undetected at recurrence. Additionally, 6 of 10 patients treated with TMZ at recurrence were hypermutated and harbored driver mutations in retinoblastoma and Akt-mTOR pathway genes [55]. However, more recently, the Glass consortium assembled a database of initial and recurrence samples from 222 patients. In this study, 35 patients exhibited treatment-related hypermutation at recurrence, and 70 % of the cohort had an increased mutational burden after recurrence. However, the authors found that driver genes detected at the initial disease were retained at recurrence and there was little evidence of recurrence-specific gene alterations. Only 16 % of *IDH* wild-type patients exhibited treatment-related hypermutation, similar to the 17 % estimate in Wang et al. [46]. Therefore, the authors concluded that the strongest selective pressures occur early in glioma development and that current therapies shape this evolution in a stochastic manner [56]. An important difference between the two studies is that in Johnson et al. all 23 patients were *IDH* mutant, while only 88/222 patients in Barthel et al. were *IDH* mutant. Additionally, within *IDH*-mutant tumors without 1p19q deletion, 47 % of patients had treatment-associated hypermutation, suggesting that treatment-related mutations may be dependent on *IDH* mutation status [56].

Study after study in GBM consistently observe a high level of intratumoral heterogeneity. Early pathologists observed this heterogeneity through histology, and with each advance in genomic technology, the heterogeneity of GBM has been further clarified on multiple molecular levels (Fig. 1). In the next section, we will discuss more recent studies on the epigenetics of GBM that are providing additional insights into the intra-tumoral heterogeneity and plasticity of these cells.

## 4. Epigenetic heterogeneity in glioblastoma

While advances in our understanding of the genetics and expression patterns of GBM have enabled vertical advancements in the field, these advances have yet to result in changes in the clinical management of GBM, and the five-year survival remains around 5% [57]. Additionally, the GLASS consortium finding that mutations at recurrence are largely random suggests that mutational evolution is not driving therapeutic resistance and plasticity in GBM. This has led many in the field to



**Fig. 1.** Glioblastoma exhibits inter- and intratumoral heterogeneity at multiple levels. Schematic depicting inter- and intratumoral heterogeneity ranging from epigenetic changes to the enhancer landscape to amplification of receptor tyrosine kinases.

investigate potential epigenetic pathways that may be contributing to therapeutic resistance and recurrence.

The most established of these epigenetic changes is *MGMT* promoter methylation [58]. The *MGMT* gene encodes a DNA repair protein that removes alkyl groups from the O<sup>6</sup> position of guanine, an important site of DNA alkylation. Therefore, patients with a hypomethylated *MGMT* promoter have elevated levels of the MGMT protein and are more resistant to alkylating agents such as TMZ. *MGMT* methylation status remains one of the best predictors of survival in GBM.

In the first attempt at measuring global methylation patterns in GBM, researchers utilized TCGA data and observed a subset of patients with hypermethylation at a large number of loci. This study then classified a subset of patients as having a glioma-CpG island methylator phenotype (G-CIMP). These patients are generally younger and experience significantly improved outcomes [59]. To investigate intratumoral heterogeneity in methylation, Wenger et al. took three to four spatially separated biopsies from 12 GBM patients and performed genome-wide DNA methylation analysis [60]. As Sottoriva et al. found using transcriptional data, when GBM subtype was determined by methylation status, 5 of 12 patients had cells from multiple subtypes [44,60]. Ultimately, the authors concluded that GBM contains a significant variety of intratumoral DNA methylation patterns. In another study, methylation changes over time were investigated using formalin-fixed paraffin-embedded samples and bisulfate sequencing of 112 patient samples. DNA methylation was predictive of immune cell infiltration, extent of necrosis, and shape of tumor cell nuclei. Interestingly, DNA methylation erosion and subclonal heterogeneity were variable across patients but similar between primary

and recurring tumors, which argues against a therapy-induced increase in epigenetic heterogeneity, as well as against a decrease in epigenetic heterogeneity due to strong selective sweeps driven by therapeutically resistant subclones [61].

Another epigenetic modification that has been identified as markedly upregulated in GBM is N6-methyladenine (N6-ma). N6-ma levels are dynamically regulated by ALKBH1, and depletion of ALKBH1 led to the transcriptional silencing of numerous oncogenic pathways by decreasing chromatin accessibility. In another study from this group, N6-methyladenosine (m6A) mRNA modifications were found to be upregulated in CSCs. The m6A reader YTHDF2 then stabilizes this modified mRNA, including the mRNA encoding *MYC* and *VEGFA* [62]. Another study investigated the super-enhancer landscape of 44 patient-derived GBM stem cells, 50 primary tumors and 10 neural stem cells. The authors found two highly distinct super-enhancer states that showed opposing patterns of H3K27ac. Group 2 was highly associated with mesenchymal features, while group 1 exhibited proneural, classical and proliferative features [63].

As a whole, the field of GBM epigenetics is relatively young, and there is still much to learn about how epigenetic changes at all levels impact tumor cell behavior. The use of drugs targeting epigenetic modifications either by histone deacetylase (HDAC) inhibitors, bromodomain & extra-terminal domain (BET) inhibitors, or other mechanisms was recently reviewed [25]. However, to date, only slightly more than 20 clinical trials have been initiated in the epigenetics field, and this number pales in comparison to the number of trials using chemotherapies, anti-angiogenic agents and immunotherapies. Therefore, while



there is still much to learn, targeting epigenetic changes in GBM is an exciting path moving forward.

## 5. Cancer stem cells

Any discussion of GBM heterogeneity and plasticity is not complete without a discussion of the CSC population. CSCs are a population of tumor cells that are defined by their functional ability to self-renew, initiate tumors, and undergo persistent proliferation [31,67]. There is no single marker of CSCs in GBM, however the glycoprotein CD133 was initially found to mark a CSC population and is widely used for sorting tumor cells today [68,69]. Furthermore, it was discovered that those with a higher proportion of CD133<sup>+</sup> cells present correlated with shorter survival [70,71]. CD133 has been shown to signal through AKT and Wnt to drive the CSC state [72]. However, it has been well documented that subgroups within the CD133<sup>+</sup> population can also exhibit CSC functional characteristics [73,74]. To date, various intracellular proteins (SOX2, MYC, and NESTIN) and cell surface markers (CD133, CD15, CD49f, L1CAM and CD44) have been identified as markers of CSCs in GBM [75–82]. The multiple, not necessarily overlapping, CSC markers begins to describe the vast amount of heterogeneity among the CSC population itself [74]. This was recently demonstrated by a series on single cell analyses that revealed CSC were subject to extensive plasticity and could be modeled on a spectrum of cell states (development/injury response) or metabolic dependencies [182–184], and may be better modeled in a multi-dimensional state, taking into account multiple ends of phenotypic [185].

### 5.1. Drivers of the cancer stem cell state

While the CSC state has been widely studied and numerous factors have been shown to drive cells into a stem cell-like phenotype or to be necessary for CSC maintenance including microRNAs [83,84] and metabolism [85] here we will highlight two of these factors, hypoxia and treatment resistance. Hypoxia is a hallmark of the GBM microenvironment and leads to phenotypic changes [86]. Hypoxia promotes growth of CSCs and increases the expression of hypoxia-inducible factors (HIFs) and vascular endothelial growth factor (VEGF) [86–89]. In chronic hypoxic conditions, HIFs can upregulate stem cell transcription factors including KLF4, SOX2, and OCT4 [88,90] that in turn affect downstream pathways. Furthermore, HIF1 $\alpha$  can directly activate Notch and WNT signaling pathways [91]. Thus, the hypoxic conditions present in GBM can induce a more stem cell-like phenotype that leads to further propagation of tumor growth.

Current standard of care for GBM follows what is known as the Stupp protocol. This includes a gross total surgical resection followed by radiotherapy and chemotherapy using the alkylating agent TMZ and, more recently, TTF [20,21,92,93]. However, both chemotherapy and radiotherapy can cause changes to CSC phenotypes, and CSCs have elevated levels of resistance to these therapies [94,95]. CSCs contribute to the radiation resistance through increased use of DNA repair mechanisms [94]. A recent study found that exposure to ionizing radiation leads to CSCs that were initially enriched for a CD133<sup>+</sup> proneural signature to transition into a CD109<sup>+</sup> mesenchymal subtype. This shift to CD109 positivity often leads to a mesenchymal recurrence [96].

As previously discussed, TMZ functions by adding alkyl groups to thymine and guanine, which causes DNA damage and initiates apoptosis. However, TMZ does not work on all CSC populations, leading to divergent CSC phenotypes [95]. Further studies have also shown that after exposure to TMZ, the CSC pool increases both *in vitro* and *in vivo*. Lineage-tracing analysis demonstrated that the CSC pool had shifted toward increased expression of stem markers such as CD133, SOX2, and OCT4 [30,97]. These studies highlight how ionizing radiation and TMZ treatment can induce plasticity within the CSC population and drive post-treatment changes.

## 5.2. Models of glioblastoma heterogeneity

Three primary models have been formulated to explain the heterogeneity within GBM: the clonal evolution model, the CSC model, and the plasticity model. The clonal evolution model suggests that certain CSCs are selected over time based on factors such as their genetic, epigenetic, and tumor microenvironment (TME) interactions. The selection of these cellular traits drives progression and increases heterogeneity, as the selective pressures are temporally and spatially distinct [98–101]. Therefore, certain CSCs evolve to be more fit to survive in hypoxic environments, while others may grow in more nutrient-dense regions [99]. The second model is the CSC model, which suggests that a small sub-population of cells is predisposed to drive tumor progression, invasiveness, and therapeutic resistance. Thus, the observed heterogeneity is a result of the differentiation of the CSC population, which gives rise to intermediate progenitor and terminally differentiated progeny [31,100,101]. Therefore, in this model, CSCs are the source of tumor initiation and heterogeneity. Lastly, the plasticity model builds upon the CSC model and states that CSCs can interconvert between stem cell and differentiated states. Unlike the CSC model, the plasticity model suggests that even upon differentiation, the differentiated cells can convert back into CSCs [49,54,86]. Two recently published studies in GBM provide strong evidence that the behavior of CSCs is best described by the plasticity model. Dirkse et al. demonstrated that the phenotypic heterogeneity observed in GBM arises from non-hierarchical, reversible state transitions that are driven by the microenvironment [86]. In another study, Neftel et al. utilized single-cell RNA-sequencing, bulk genetics and the TCGA and found that malignant cells exist in four main cellular states that each exhibit a high level of plasticity [54]. Together, these studies and others are slowly shifting the focus of the field from identifying therapies that target CSCs to identifying therapies that target the plasticity of GBM.

## 6. Glioblastoma interaction with non-tumor cells

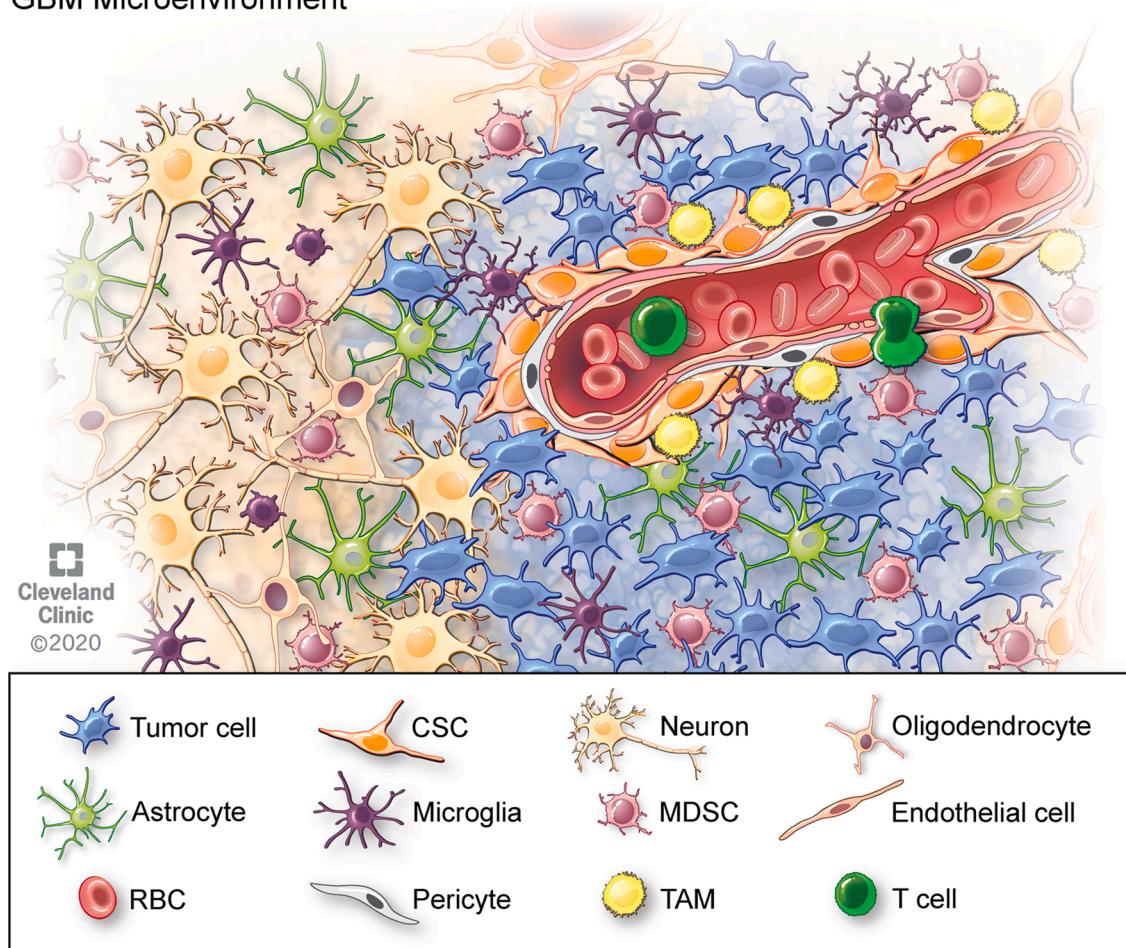
One fact that has been understood for many years is that the GBM microenvironment consists of more than merely tumor cells. Numerous other cell populations within the brain and the immune system contribute to the conditions that lead to tumor growth. These populations include endothelial cells, microglia, astrocytes, neurons, and immune cells. The failure of many tumor cell targeting therapies has led scientists and physicians to investigate the potential of targeting other cells within the tumor microenvironment (Fig. 2).

### 6.1. Endothelial cells

It has long been understood that malignant cells require oxygen, nutrients, and the removal of waste to proliferate and survive. Brain tumors expand their vasculature through a process known as tumor angiogenesis, where endothelial cells proliferate or are recruited from the bone marrow. In addition, endothelial cells also play an essential role in what is known as the blood-brain barrier, which functions to closely monitor and control which chemicals and cells come into and out of the brain [102]. Tumor cells and endothelial cells can interact through three primary mechanisms. First, tumor cells secrete numerous growth factors including stromal-derived factor-1 (SDF-1), platelet-derived growth factor (PDGF), transforming growth factor (TGF- $\beta$ ), fibroblastic growth factor 2 (FGF2) and the most well studied, VEGF [103]. Secondly, the two cell types communicate via direct contact through gap junctions, in particular through connexin 43 [104–106]. Finally, indirect interactions via intermediate cells such as pericytes, astrocytes and neurons also occur. The complex relationship between endothelial cells and tumor cells was recently reviewed in further detail [103].

Brain tumors are some of the most vascularized solid tumors found in humans, and the targeting of endothelial cells in GBM was a leading

## GBM Microenvironment



**Fig. 2.** The tumor microenvironment of glioblastoma includes various other cells types in addition to tumor cells. Schematic depicting the crosstalk between cell types in the tumor microenvironment that drive glioblastoma progression and recurrence.

effort in clinical trials in the late 2000s [107]. In 2009, after a phase II trial that demonstrated a dramatic overall radiographic response rate, the FDA granted accelerated approval to bevacizumab, a monoclonal antibody against VEGF-A [108,109]. However, two phase III trials in patients with newly diagnosed GBM failed to demonstrate any improvement in overall survival [110,111]. Numerous follow-up studies that combined bevacizumab with various chemotherapies and targeted therapies have also failed to demonstrate any overall survival benefit [112–123]. Only one phase II study of a combination of bevacizumab and lomustine in patients with recurrent GBM demonstrated a survival benefit at 9 months; however, the subsequent phase III trial did not show any improvement in survival [124]. Additionally, a multitude of other studies has investigated additional anti-angiogenic agents, but none of these treatments have led to improved overall survival, and as a result, the field has slowly shifted away from anti-angiogenic treatments [125–135]. While the endothelial-tumor cell interaction remains an active area of research, clinical efforts to target angiogenesis in GBM have for the most part come to a halt. However, the use of bevacizumab as an alternative to steroids in the setting of immunotherapy has gained traction (NCT03452579).

## 6.2. Neurons

The first known observation of an interaction between GBM cells and neurons came in 1938 by the Belgian pathologist Dr. H. J. Scherer. He observed that glioma cells had a tendency to grow along and around

normal neurons, and he described this observation as “precocious perineural growth” [136]. Since that time, determining whether invasive tumor cells were merely traveling along a path of least resistance or whether the two cell types are actively engaging in bidirectional signaling has been the goal of numerous neuroscientists and cancer biologists [137]. When considering the latest evidence for neuron-tumor cell relationships, it is worth remembering that there is ample evidence for a close, bidirectional relationship between glial cells and neurons in normal physiology.

The first molecule that was uncovered as a pro-tumorigenic neuronal molecule is the synaptic cell adhesion protein neuroligin-3 (NLGN3). NLGN3 is proteolytically cleaved at the cell surface, releasing the N-terminal ectodomain, which in turn leads to tumor cell proliferation [138]. In mice with *NLGN3* knocked out, intracranial injection of xenografts leads to failure of the tumor to progress. While this was true across numerous molecularly distinct gliomas, xenografts of breast cancer brain metastases were not impacted by *NLGN3* knockout [139].

The role of neurotransmitters in glioma progression has been of interest to clinicians and scientists since the observation that the use of tricyclic antidepressants reduces the odds of developing glioma in a dose- and time-dependent manner [140]. Because tricyclic antidepressants are classically thought to act via the inhibition of serotonin and norepinephrine re-uptake, the role of neurotransmitters in glioma development is of great interest. Glioma cells have been shown express dopamine, glutamate, GABA and serotonin receptors [141–145].

In 2019, three ground-breaking studies were published that further

characterized the interaction between neurons and tumor cells within the brain and the importance of glutamate. Venkataramani et al. were able to demonstrate functional bona fide chemical synapses between presynaptic neurons and postsynaptic glioma tumor cells [146]. These synapses formed with typical synaptic ultrastructure and produced postsynaptic currents via glutamate receptors of the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtype. Furthermore, tumor growth and invasion were reduced by anesthesia or the AMPA receptor antagonist perampanel. Venkatesh et al. observed a similar finding mediated through AMPA receptor-dependent neuron-glioma synapses. They also found that neuronal activity evokes non-synaptic activity-dependent potassium currents that are amplified by gap junction-mediated tumor interconnections [147]. Finally, Zeng et al. investigated breast-to-brain metastasis and identified activation of N-methyl-D-aspartate receptors (NMDARs) by glutamate ligands as essential. Pseudo-tripartite synapses between cancer cells and glutamatergic neurons were responsible for stimulating the NMDARs [148].

Uncovering the interactions between neurons and tumor cells has unlocked an entirely new avenue of therapeutic options. One method for targeting this interaction is the utilization of neurotransmitter-blocking drugs that may mimic the phenotype observed with tricyclic antidepressants [140]. Another innovative approach that has been discussed is the implantation of electrodes into the resection cavity during craniotomy to disrupt neuron-tumor cell interactions. This would be an additional electromagnetic treatment option for GBM (in addition to TTF [20]).

### 6.3. Astrocytes

Tumor-associated astrocytes directly interact with GBM cells, leading to the formation of reactive astrocytes. These astrocytes facilitate tumor progression, proliferation and migration through multiple mechanisms [149]. The signaling between astrocytes and GBM tumor cells is bidirectional. Tumor cells have been shown to activate astrocytes through receptor activator of nuclear factor kappa-B ligand (RANKL), extracellular vesicles, and connexin 43 transmission of cGAMP, whereas astrocytes promote tumor growth through the secretion of IL-6, VEGF, TGF- $\beta$ , growth/differentiation factor 15 (GDF-15), glutamine, tumor necrosis factor (TNF) and numerous other cytokines [149]. As evidenced by many of the anti-inflammatory molecules just mentioned, astrocytes play a significant role in driving an immunosuppressive microenvironment in GBM [150]. Astrocytes have also been shown to play a role in assisting tumor cell migration and infiltration. Connective tissue growth factor (CTGF) secreted by astrocytes mediates GBM cell infiltration and is a potential therapeutic target in GBM [151]. While less is known about astrocytes compared to other cell populations within the microenvironment, targeting astrocytes is an underexplored area and remains a viable option for GBM therapies due to their central role in regulating other cell populations.

### 6.4. Immune cells

Two lines of thinking have long driven the dogma that the brain is an “immunologically privileged” organ: the existence of the blood-brain barrier and the lack of a conventional lymphatic system [152]. However, evidence is rapidly accumulating that emphasizes the importance of immune cells within the tumor microenvironment for tumor progression and the potential for immune-stimulating therapies to be the treatments of the future. However, it is worth remembering that the immune checkpoint (PD-1 and CTLA-4) inhibitors that have shown field-altering efficacy in tumors including melanoma [153] and lung cancer [154] have thus far failed to demonstrate any efficacy in GBM [155].

The different GBM subtypes outlined above have been shown to have heterogeneity within their immune populations. Utilizing the CIBERSORT method, which characterizes the cellular composition of a

complex tissue from gene expression profiles [156], Wang et al. found that tumor-associated macrophages (TAMs), neutrophils and CD4<sup>+</sup> T cells are increased in tumors enriched in the mesenchymal signature, whereas an activated dendritic cell signature was found more in the “classical” tumors [51]. Another group utilized a tissue microarray to investigate the immune cell composition of 98 tumors. Microglia and TAMs were the most prevalent cells in all four subtypes, and CD3<sup>+</sup> cells made up only 1% of cells within the tumor, independent of the subtype [157]. Additionally, tumor-infiltrating lymphocytes (TILs) generally displayed an exhausted phenotype, and NK cells expressed reduced levels of activating receptors, highlighting the challenges of T cell- and NK cell-targeted therapies in GBM [158,159]. Studies such as these highlight the low frequency of TILs, which may account for the lack of efficacy of immune checkpoint inhibitors thus far. Currently, scientists are developing chimeric antigen receptor (CAR)-T cell technology, which has been exceedingly successful in liquid tumors, for the targeting of GBM [186]. For example, some groups are utilizing GBM-binding potential of chlorotoxin while others have developed CAR-T cells directed at EGFRVIII [187].

The exhausted phenotype of TILs in GBM is in part due to the highly immunosuppressive tumor microenvironment of GBM. The secretion of cytokines including TGF- $\beta$ , IL-6, IL-10, macrophage migration inhibitory factor (MIF) and numerous others has been demonstrated to cause a local immunosuppressive phenotype [160–163]. In addition to soluble factors, direct cell-cell communication through PD-L1, tolerogenic HLA, the apoptosis-inducing receptor FAS, and changes in glycosylation can all cause immune suppression [164–167].

Another type of immune cell found in GBM is myeloid-derived suppressor cells (MDSCs). MDSCs are a population of immune cells that have a remarkable ability to suppress T cells through a variety of mechanisms and are subdivided into monocytic (mMDSC) and granulocytic (gMDSC) subsets. MIF secreted by CSCs within the tumor leads to increased MDSCs, and targeting this cell population with low-dose capecitabine has been demonstrated to be a potential therapeutic option in clinical trials [168,169]. Additionally, it was recently observed that there is a sex difference in MDSC populations in GBM, with gMDSCs elevated in the blood of females and mMDSCs enriched in the tumors of males [170].

Finally, microglia are the resident immune cells within the brain. Numerous studies have demonstrated that microglia within GBM predominantly exhibit anti-inflammatory M2 polarization [171,172]. Microglia and tumor cells cross-talk through the secretion of EGF, colony-stimulating factor-1R (CSF-1R), TGF- $\beta$  and IL-10 [173]. Microglia can facilitate the invasion of GBM cells through the upregulation of matrix metalloproteinase 14 [174]. GBM cells also upregulate CD47 to prevent phagocytosis by microglia, and work is being done to block CD47 [175]. Finally, a recent study demonstrated that microglia function in a sex-specific manner, as female microglia that were deficient in junctional adhesion molecule-A (JAM-A) succumbed to GBM more rapidly than WT females, whereas the same phenotype was not observed in males [176]. This study combined with the MDSC data above highlights the heterogeneity between males and females and opens up the prospect of sex-specific treatments [177].

While the clinical data for therapies targeting the immune system thus far have shown little efficacy, our understanding of the immune compartment of the GBM tumor microenvironment has grown since the initiation of these trials. In addition to the studies mentioned above, the role of dendritic cells [178] and B cells is actively being researched [179]. Currently, there are at least 60 active immunotherapy clinical trials for GBM that are outlined in a recent review [180]. These trials range from novel checkpoint inhibitors, vaccines targeting specific antigens or patient tumor lysates, chimeric antigen receptor (CAR)-T and CAR-NK cell therapies, oncolytic viruses, and macrophage-based immunotherapies. Studying the many ways immune cells interact with tumor cells is an active area of research and may be the foundation of future therapies.



## 7. Conclusion

Brain tumors are among the most heterogeneous tumors to have been characterized. From early histological studies that documented regions of necrosis to more recent single-cell RNA sequencing and methylation profiling, GBM is a tumor with resounding intra- and inter-tumoral heterogeneity. In addition, heterogeneity at the level of cell type, the sex and age of the patient, and plasticity over time all add to the complexity of the disease. Unfortunately, this lack of homogeneity or single driver mutations has left GBM patients without the targeted therapies that have revolutionized treatments for tumors such as breast cancer, melanoma, lung cancer and chronic myeloid leukemia.

With each new technological advancement, new research emerges, at a significant financial price, that verifies what pathologists have known for well over 50 years. This leaves physicians and scientists with the challenge of translating laboratory advancements into changes in clinical care. Our understanding of *MGMT* promotor methylation and *IDH* mutation status has revolutionized prognostic outlook for patients, and these characteristics are utilized in the design of clinical trials. In contrast, laboratory advancements such as tumor subtyping (classical, proneural and mesenchymal) and methylation profiles have for the most part remained an academic exercise. The goal moving forward must be the integration of these advancements into clinical trial designs that will match treatment approaches with the patients most likely to benefit.

With recent findings, three topics emerge as areas that require further research and are most likely to lead to future therapies. First, a move toward growing our understanding of the plasticity of CSCs and the epigenetics of GBM as a whole is shifting our understanding of the disease. To date, CSC targeted therapies have not panned out as viable treatment opportunities; however, focusing on the plasticity of GBM and the epigenetic changes that drive this plasticity may unlock unexpected therapeutic avenues. The second area is furthering our understanding of the communication between GBM cells and other cells within the tumor microenvironment. In particular, the interaction between neurons and tumor cells provides an opportunity for pharmacologic inhibitors, as well as surgical and electromagnetic techniques, that could revolutionize the treatment of brain tumors. Finally, it is impossible to ignore the success of immunotherapy in other cancers, and therefore, furthering our understanding of the immunosuppressive microenvironment in GBM has the potential to usher in the next generation of immune-stimulating therapies.

The efficacy of drugs such as osimertinib [40] (an EGFR inhibitor) and nivolumab/ipilimumab [181] in the setting of brain metastases has demonstrated that targeted therapies and immunotherapies have the potential to reach tumors within the brain. For the reasons outlined above, GBM heterogeneity adds additional layers of complexity that must be overcome for these and other drugs to improve survival in GBM patients. However, due to advances in our understanding of the genetics and epigenetics of tumor cells as well as of the cell populations that tumor cells rely on, the prospect of making the first new meaningful developments in clinical care for only the second time since 2005 remains high [20,21].

## Funding source

Work in the Lathia laboratory is supported by the Cleveland Clinic, Case Comprehensive Cancer Center, the American Brain Tumor Association, National Brain Tumor Society, and NIH R01 NS109742, R01 NS117104, and P01 CA245705.

Alice is supported by a summer research scholarship provided by CWRU SOURCE with additional funding provided by the Women in Science and Engineering Roundtable.

## Declaration of Competing Interest

Manmeet Ahluwalia declares the following: grants/research support-

Astrazeneca, Abbvie, BMS, Bayer, Incyte, Pharmacyclics, Novocure, Mimivax, Merck. Receipt of honoraria or consulting fees: Elsevier, Wiley, VBI, Vaccines, Bayer, Tocagen, Novocure. Shareholder: Doctible, Mimivax, Cytodyn.

## Acknowledgements

We regret that we were unable to include all work on glioblastoma, cancer stem cells, cellular heterogeneity, and plasticity in this review due to spatial limitations. We would like to thank the members of the Lathia laboratory for insightful discussions. We thank Ms. Amanda Mendelsohn for illustration assistance and Dr. Erin Mulkearns-Hubert for editorial assistance. Work in the Lathia laboratory is supported by the Cleveland Clinic, Case Comprehensive Cancer Center, the American Brain Tumor Association, National Brain Tumor Society, and NIH R01 NS109742, R01 NS117104, and P01 CA245705. Alice Lo work was supported by a summer research scholarship provided by CWRU SOURCE with additional funding provided by the Women in Science and Engineering Roundtable.

## References

- [1] A. Jemal, E.M. Ward, C.J. Johnson, K.A. Cronin, J. Ma, A.B. Ryerson, A. Mariotto, A.J. Lake, R. Wilson, R.L. Sherman, R.N. Anderson, S.J. Henley, B.A. Kohler, L. Penberthy, E.J. Feuer, H.K. Weir, Annual report to the nation on the status of Cancer, 1975–2014, featuring survival, *J. Natl. Cancer Inst.* 109 (2017), <https://doi.org/10.1093/jnci/djx030>.
- [2] E. Tabouret, O. Chinot, P. Metellus, A. Tallet, P. Viens, A. Gonçalves, Recent trends in epidemiology of brain metastases: an overview, *Anticancer Res.* 32 (2012) 4655–4662.
- [3] W.A. Hall, H.R. Djalilian, E.S. Nussbaum, K.H. Cho, Long-term survival with metastatic cancer to the brain, *Med. Oncol.* 17 (2000) 279–286.
- [4] A.K. Percy, L.R. Elveback, H. Okazaki, L.T. Kurland, Neoplasms of the central nervous system. Epidemiologic considerations, *Neurology* 22 (1972) 40–48, <https://doi.org/10.1212/wnl.22.1.40>.
- [5] Y. Tsukada, A. Fouad, J.W. Pickren, W.W. Lane, Central nervous system metastasis from breast carcinoma. Autopsy study, *Cancer* 52 (1983) 2349–2354.
- [6] P.W. Sperduto, S. Mesko, J. Li, D. Cagney, A. Aizer, N.U. Lin, E. Nesbit, T. J. Kruser, J. Chan, S. Braunstein, J. Lee, J.P. Kirkpatrick, W. Breen, P.D. Brown, D. Shi, H.A. Shih, H. Soliman, A. Sahgal, R. Shanley, W.A. Sperduto, E. Lou, A. Everrett, D.H. Boggs, L. Masucci, D. Roberge, J. Remick, K. Plichta, J.M. Buatti, S. Jain, L.E. Gaspar, C.-C. Wu, T.J.C. Wang, J. Bryant, M. Chuong, Y. An, V. Chiang, T. Nakano, H. Aoyama, M.P. Mehta, Survival in patients with brain metastases: summary report on the updated diagnosis-specific graded prognostic assessment and definition of the eligibility quotient, *J. Clin. Oncol.* (2020), <https://doi.org/10.1200/JCO.20.01255>. JCO2001255.
- [7] P.K. Bastianios, S.L. Carter, S. Santagata, D.P. Cahill, A. Taylor-Weiner, R. T. Jones, E.M. Van Allen, M.S. Lawrence, P.M. Horowitz, K. Cibulskis, K.L. Ligon, J. Taberner, J. Seoane, E. Martinez-Saez, W.T. Curry, I.F. Dunn, S.H. Paek, S.-H. Park, A. McKenna, A. Chevalier, M. Rosenberg, F.G. Barker, C.M. Gill, P. Van Hummelen, A.R. Thorner, B.E. Johnson, M.P. Hoang, T.K. Choueiri, S. Signoretti, C. Sougnez, M.S. Rabin, N.U. Lin, E.P. Winer, A. Stemmer-Rachamimov, M. Meyerson, L. Garraway, S. Gabriel, E.S. Lander, R. Beroukhim, T.T. Batchelor, J. Baselga, D.N. Louis, G. Getz, W.C. Hahn, Genomic characterization of brain metastases reveals branched evolution and potential therapeutic targets, *Cancer Discov.* 5 (2015) 1164–1177, <https://doi.org/10.1158/2159-8290.CD-15-0369>.
- [10] Y. Zhu, F. Guignard, D. Zhao, L. Liu, D.K. Burns, R.P. Mason, A. Messing, L. F. Parada, Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma, *Cancer Cell* 8 (2005) 119–130, <https://doi.org/10.1016/j.ccr.2005.07.004>.
- [11] L.M.L. Chow, R. Endersby, X. Zhu, S. Rankin, C. Qu, J. Zhang, A. Broniscer, D. W. Ellison, S.J. Baker, Cooperativity within and among Pten, p53, and Rb pathways induces high-grade astrocytoma in adult brain, *Cancer Cell* 19 (2011) 305–316, <https://doi.org/10.1016/j.ccr.2011.01.039>.
- [12] C. Liu, J.C. Sage, M.R. Miller, R.G.W. Verhaak, S. Hippenmeyer, H. Vogel, O. Foreman, R.T. Bronson, A. Nishiyama, L. Luo, H. Zong, Mosaic analysis with double markers reveals tumor cell of origin in glioma, *Cell* 146 (2011) 209–221, <https://doi.org/10.1016/j.cell.2011.06.014>.
- [13] D.N. Louis, A. Perry, G. Reifenberger, A. von Deimling, D. Figarella-Branger, W. K. Cavenee, H. Ohgaki, O.D. Wiestler, P. Kleihues, D.W. Ellison, The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary, *Acta Neuropathol.* 131 (2016) 803–820, <https://doi.org/10.1007/s00401-016-1545-1>.
- [14] M.C. Tom, D.Y.J. Park, K. Yang, C.M. Leyrer, W. Wei, X. Jia, V. Varra, J.S. Yu, S. T. Chao, E.H. Balagamwala, J.H. Suh, M.A. Vogelbaum, G.H. Barnett, R. A. Prayson, G.H.J. Stevens, D.M. Peereboom, M.S. Ahluwalia, E.S. Murphy, Malignant transformation of molecularly classified adult low-grade glioma, *Int. J. Radiat. Oncol. Biol. Phys.* 105 (2019) 1106–1112, <https://doi.org/10.1016/j.ijrobp.2019.08.025>.



- [15] J. Wang, A. Garancher, V. Ramaswamy, R.J. Wechsler-Reya, Medulloblastoma: From Molecular Subgroups to Molecular Targeted Therapies, *Annu. Rev. Neurosci.* 41 (2018) 207–232, <https://doi.org/10.1146/annurev-neuro-070815-013838>.
- [16] P.A. Northcott, I. Buchhalter, A.S. Morrissy, V. Hovestadt, J. Weischenfeldt, T. Ehrenberger, S. Gröbner, M. Segura-Wang, T. Zichner, V.A. Rudneva, H.-J. Warnatz, N. Sidiropoulos, A.H. Phillips, S. Schumacher, K. Kleinheinz, S. M. Waszak, S. Erkek, D.T.W. Jones, B.C. Worst, M. Kool, M. Zapatka, N. Jäger, L. Chavez, B. Hutter, M. Bieg, N. Paramasivam, M. Heinold, Z. Gu, N. Ishaque, C. Jäger-Schmidt, C.D. Imbusch, A. Jugold, D. Hübschmann, T. Risch, V. Amstislavskiy, F.G.R. Gonzalez, U.D. Weber, S. Wolf, G.W. Robinson, X. Zhou, G. Wu, D. Finkelstein, Y. Liu, F.M.G. Cavalli, B. Luu, V. Ramaswamy, X. Wu, J. Koster, M. Ryzhova, Y.-J. Cho, S.L. Pomeroy, C. Herold-Mende, M. Schuhmann, M. Ebinger, L.M. Liau, J. Mora, R.E. McLendon, N. Jabado, T. Kumabe, E. Chuah, Y. Ma, R.A. Moore, A.J. Mungall, K.L. Mungall, N. Thiessen, K. Tse, T. Wong, S.J. M. Jones, O. Witt, T. Milde, A. Von Deimling, D. Capper, A. Korshunov, M.-L. Yaspo, R. Kriwacki, A. Gajjar, J. Zhang, R. Beroukhi, E. Fraenkel, J.O. Korbel, B. Brors, M. Schlesner, R. Eils, M.A. Marra, S.M. Pfister, M.D. Taylor, P. Lichter, The whole-genome landscape of medulloblastoma subtypes, *Nature* 547 (2017) 311–317, <https://doi.org/10.1038/nature22973>.
- [17] P.A. Northcott, G.W. Robinson, C.P. Kratz, D.J. Mabbott, S.L. Pomeroy, S. C. Clifford, S. Rutkowski, D.W. Ellison, D. Malkin, M.D. Taylor, A. Gajjar, S. M. Pfister, Medulloblastoma, *Nat. Rev. Dis. Primers* 5 (2019) 1–20, <https://doi.org/10.1038/s41572-019-0063-6>.
- [18] M.D. Taylor, P.A. Northcott, A. Korshunov, M. Remke, Y.-J. Cho, S.C. Clifford, C. G. Eberhart, D.W. Parsons, S. Rutkowski, A. Gajjar, D.W. Ellison, P. Lichter, R. J. Gilbertson, S.L. Pomeroy, M. Kool, S.M. Pfister, Molecular subgroups of medulloblastoma: the current consensus, *Acta Neuropathol.* 123 (2012) 465–472, <https://doi.org/10.1007/s00401-011-0922-z>.
- [19] B. Manoranjan, C. Venugopal, D. Bakshinyan, A.A. Adile, L. Richards, M. M. Kameda-Smith, O. Whitley, A. Dvorkin-Gheva, M. Subapanditha, N. Savage, N. Tatari, D. McKenna, B. Bassy-Archibong, N. Winegarden, R. Hallett, J. P. Provias, B. Yarascavitch, O. Ajani, A. Fleming, G.D. Bader, T.J. Pugh, B. W. Doble, S.K. Singh, Wnt activation as a therapeutic strategy in medulloblastoma, *Nat. Commun.* 11 (2020) 4323, <https://doi.org/10.1038/s41467-020-17953-4>.
- [20] R. Stupp, S. Taillibert, A. Kanner, W. Read, D.M. Steinberg, B. Lhermitte, S. Toms, A. Idubai, M.S. Ahluwalia, K. Fink, F.D. Meco, F. Lieberman, J.-J. Zhu, G. Stragliotto, D.D. Tran, S. Brem, A.F. Hottinger, E.D. Kirson, G. Lavy-Shahaf, U. Weinberg, C.-Y. Kim, S.-H. Paek, G. Nicholas, J. Bruna, H. Hirte, M. Weller, Y. Palti, M.E. Hegi, Z. Ram, Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial, *JAMA* 318 (2017) 2306–2316, <https://doi.org/10.1001/jama.2017.18718>.
- [21] R. Stupp, W.P. Mason, M.J. van den Bent, M. Weller, B. Fisher, M.J.B. Taphoorn, K. Belanger, A.A. Brandes, C. Marosi, U. Bogdahn, J. Curschmann, R.C. Janzer, S. K. Ludwin, T. Gorlia, A. Allgeier, D. Lacombe, J.G. Cairncross, E. Eisenhauer, R. O. Mirimanoff, European organisation for research and treatment of Cancer brain tumor and radiotherapy groups, national Cancer institute of Canada clinical trials group, radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma, *N. Engl. J. Med.* 352 (2005) 987–996, <https://doi.org/10.1056/NEJMoa043330>.
- [22] B.G. Harder, M.R. Blomquist, J. Wang, A.J. Kim, G.F. Woodworth, J.A. Winkles, J. C. Loftus, N.L. Tran, Developments in blood-brain barrier penetration and drug repurposing for improved treatment of glioblastoma, *Front. Oncol.* 8 (2018) 462, <https://doi.org/10.3389/fonc.2018.00462>.
- [23] N. Colwell, M. Larion, A.J. Giles, A.N. Seldomridge, S. Sizdahkhani, M.R. Gilbert, D.M. Park, Hypoxia in the glioblastoma microenvironment: shaping the phenotype of cancer stem-like cells, *Neuro Oncol.* 19 (2017) 887–896, <https://doi.org/10.1093/neuonc/now258>.
- [24] R.C. Gimple, S. Bhargava, D. Dixit, J.N. Rich, Glioblastoma stem cells: lessons from the tumor hierarchy in a lethal cancer, *Genes Dev.* 33 (2019) 591–609, <https://doi.org/10.1101/gad.324301.119>.
- [25] R. Suter, J. Rodriguez-Blanco, N.G. Ayad, Epigenetic pathways and plasticity in brain tumors, *Neurobiol. Dis.* (2020) 105060, <https://doi.org/10.1016/j.nbd.2020.105060>.
- [26] P.C. Burger, S.B. Green, Patient age, histologic features, and length of survival in patients with glioblastoma multiforme, *Cancer* 59 (1987) 1617–1625, [https://doi.org/10.1002/1097-0142\(19870501\)59:9<1617::aid-cnrcr2820590916>3.0.co;2-x](https://doi.org/10.1002/1097-0142(19870501)59:9<1617::aid-cnrcr2820590916>3.0.co;2-x).
- [27] R. Reinartz, S. Wang, S. Kebir, D.J. Silver, A. Wieland, T. Zheng, M. Küpper, L. Rauschenbach, R. Fimmers, T.M. Shepherd, D. Trageser, A. Till, N. Schäfer, M. Glas, A.M. Hillmer, S. Cichon, A.A. Smith, T. Pietsch, Y. Liu, B.A. Reynolds, A. Yachnis, D.W. Pincus, M. Simon, O. Brüstle, D.A. Steindler, B. Scheffler, Functional subclone profiling for prediction of treatment-induced intratumor population shifts and discovery of rational drug combinations in human glioblastoma, *Clin. Cancer Res.* 23 (2017) 562–574, <https://doi.org/10.1158/1078-0432.CCR-15-2089>.
- [28] S. Nobusawa, J. Lachuer, A. Wierinckx, Y.H. Kim, J. Huang, C. Legras, P. Kleihues, H. Ohgaki, Intratumoral patterns of genomic imbalance in glioblastomas, *Brain Pathol.* 20 (2010) 936–944, <https://doi.org/10.1111/j.1750-3639.2010.00395.x>.
- [29] A. Kumar, E.A. Boyle, M. Tokita, A.M. Mikheev, M.C. Sanger, E. Girard, J. R. Silber, L.F. Gonzalez-Cuyar, J.B. Hiatt, A. Adey, C. Lee, J.O. Kitzman, D. E. Born, D.L. Silbergeld, J.M. Olson, R.C. Rostomily, J. Shendure, Deep sequencing of multiple regions of glial tumors reveals spatial heterogeneity for mutations in clinically relevant genes, *Genome Biol.* 15 (2014) 530, <https://doi.org/10.1186/s13059-014-0530-z>.
- [30] A.R. Safa, M.R. Saadatzadeh, A.A. Cohen-Gadol, K.E. Pollok, K. Bijangi-Vishehsaraei, Glioblastoma stem cells (GSCs) epigenetic plasticity and interconversion between differentiated non-GSCs and GSCs, *Genes Dis.* 2 (2015) 152–163, <https://doi.org/10.1016/j.gendis.2015.02.001>.
- [31] J.D. Lathia, S.C. Mack, E.E. Mulkearns-Hubert, C.L.L. Valentim, J.N. Rich, Cancer stem cells in glioblastoma, *Genes Dev.* 29 (2015) 1203–1217, <https://doi.org/10.1101/gad.261982.115>.
- [32] K. Ludwig, H.I. Kornblum, Molecular markers in glioma, *J. Neurooncol.* 134 (2017) 505–512, <https://doi.org/10.1007/s11060-017-2379-y>.
- [33] M. Snuderl, L. Fazlollahi, L.P. Le, M. Nitta, B.H. Zhelyazkova, C.J. Davidson, S. Akhavanfard, D.P. Cahill, K.D. Aldape, R.A. Betensky, D.N. Louis, A.J. Iafrate, Mosaic amplification of multiple receptor tyrosine kinase genes in glioblastoma, *Cancer Cell* 20 (2011) 810–817, <https://doi.org/10.1016/j.ccr.2011.11.005>.
- [34] S.E. Little, S. Popov, A. Jury, D.A. Bax, L. Doey, S. Al-Sarraj, J.M. Jurgensmeier, C. Jones, Receptor tyrosine kinase genes amplified in glioblastoma exhibit a mutual exclusivity in variable proportions reflective of individual tumor heterogeneity, *Cancer Res.* 72 (2012) 1614–1620, <https://doi.org/10.1158/0008-5472.CAN-11-4069>.
- [35] N.J. Szerlip, A. Pedraza, D. Chakravarty, M. Azim, J. McGuire, Y. Fang, T. Ozawa, S. Akhavanfard, D.P. Cahill, K.D. Aldape, R.A. Betensky, D.N. Louis, A.J. Iafrate, Intratumoral heterogeneity of receptor tyrosine kinases EGFR and PDGFRA amplification in glioblastoma defines subpopulations with distinct growth factor response, *Proc. Natl. Acad. Sci. U.S.A.* 109 (2012) 3041–3046, <https://doi.org/10.1073/pnas.1114033109>.
- [36] M. Weller, N. Butowski, D.D. Tran, L.D. Recht, M. Lim, H. Hirte, L. Ashby, L. Mechtler, S.A. Goldlust, F. Iwamoto, J. Drappatz, D.M. O'Rourke, M. Wong, M. G. Hamilton, G. Finocchiaro, J. Perry, W. Wick, J. Green, Y. He, C.D. Turner, M. J. Yellin, T. Keler, T.A. Davis, R. Stupp, J.H. Sampson, ACT IV trial investigators, Rindopipemut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial, *Lancet Oncol.* 18 (2017) 1373–1385, [https://doi.org/10.1016/S1470-2045\(17\)30517-X](https://doi.org/10.1016/S1470-2045(17)30517-X).
- [37] M.E. Hegi, A.-C. Diserens, P. Bady, Y. Kamoshima, M.C.M. Kouwenhoven, M. Delorenzi, W.L. Lambiv, M.-F. Hamou, M.S. Matter, A. Koch, F.L. Heppner, Y. Yonekawa, A. Merlo, K. Frei, L. Mariani, S. Hofer, Pathway analysis of glioblastoma tissue after preoperative treatment with the EGFR tyrosine kinase inhibitor gefitinib—a phase II trial, *Mol. Cancer Ther.* 10 (2011) 1102–1112, <https://doi.org/10.1158/1535-7163.MCT-11-0048>.
- [38] A. Chakravarti, M. Wang, H.I. Robins, T. Lautenschlaeger, W.J. Curran, D. G. Brachman, C.J. Schultz, A. Choucair, M. Dolled-Filhart, J. Christiansen, M. Gustavson, A. Molinaro, P. Mischel, A.P. Dick, M. Bredel, M. Mehta, RTOG 0211: a phase 1/2 study of radiation therapy with concurrent gefitinib for newly diagnosed glioblastoma patients, *Int. J. Radiat. Oncol. Biol. Phys.* 85 (2013) 1206–1211, <https://doi.org/10.1016/j.ijrobp.2012.10.008>.
- [39] D.A. Haas-Kogan, M.D. Prados, T. Tihan, D.A. Eberhard, N. Jelluma, N.D. Arvold, R. Baumber, K.R. Lamborn, A. Kapadia, M. Malec, M.S. Berger, D. Stokoe, Epidermal growth factor receptor, protein kinase B/Akt, and glioma response to erlotinib, *J. Natl. Cancer Inst.* 97 (2005) 880–887, <https://doi.org/10.1093/jnci/dji161>.
- [40] T. Reungwetwattana, K. Nakagawa, B.C. Cho, M. Cobo, E.K. Cho, A. Bertolini, S. Bohnet, C. Zhou, K.H. Lee, N. Nogami, I. Okamoto, N. Leighl, R. Hodge, A. McKeown, A.P. Brown, Y. Rukazekov, S.S. Ramalingam, J. Vansteenkiste, CNS Response to Osimertinib Versus Standard Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Patients With Untreated EGFR-Mutated Advanced Non-Small-Cell Lung Cancer, *J. Clin. Oncol.* (2018), <https://doi.org/10.1200/JCO.2018.78.3118>, JCO2018783118.
- [41] J.-C. Soria, Y. Ohe, J. Vansteenkiste, T. Reungwetwattana, B. Chewakulyong, K. H. Lee, A. Dechaphunkul, F. Imamura, N. Nogami, T. Kurata, I. Okamoto, C. Zhou, B.C. Cho, Y. Cheng, E.K. Cho, P.J. Voon, D. Planchard, W.-C. Su, J.E. Gray, S.-M. Lee, R. Hodge, M. Marotti, Y. Rukazekov, S.S. Ramalingam, Osimertinib in untreated EGFR-Mutated advanced non-Small-Cell lung Cancer, *N. Engl. J. Med.* (2017), <https://doi.org/10.1056/NEJMoa1713137>.
- [42] R. Shen, Q. Mo, N. Schultz, V.E. Seshan, A.B. Olshen, J. Huse, M. Ladanyi, C. Sander, Integrative subtype discovery in glioblastoma using iCluster, *PLoS One* 7 (2012) e35236, <https://doi.org/10.1371/journal.pone.0035236>.
- [43] R.G.W. Verhaak, K.A. Hoadley, E. Purdom, V. Wang, Y. Qi, M.D. Wilkerson, C. R. Miller, L. Ding, T. Golub, J.P. Mesirov, G. Alexe, M. Lawrence, M. O'Kelly, P. Tamayo, B.A. Weir, S. Gabriel, W. Winckler, S. Gupta, L. Jakkula, H.S. Feiler, J. G. Hodgson, C.D. James, J.N. Sarkaria, C. Brennan, A. Kahn, P.T. Spellman, R. K. Wilson, T.P. Speed, J.W. Gray, M. Meyerson, G. Getz, C.M. Perou, D.N. Hayes, Cancer Genome Atlas Research Network, Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1, *Cancer Cell* 17 (2010) 98–110, <https://doi.org/10.1016/j.ccr.2009.12.020>.
- [44] A. Sottoriva, I. Spiteri, S.G.M. Piccirillo, A. Touloumis, V.P. Collins, J.C. Marioni, C. Curtis, C. Watts, S. Tavaré, Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 4009–4014, <https://doi.org/10.1073/pnas.1219747110>.
- [45] J.-K. Lee, J. Wang, J.K. Sa, E. Ladewig, H.-O. Lee, I.-H. Lee, H.J. Kang, D. S. Rosenbloom, P.G. Camara, Z. Liu, P. van Nieuwenhuizen, S.W. Jung, S.W. Choi, J. Kim, A. Chen, K.-T. Kim, S. Shin, Y.J. Seo, J.-M. Oh, Y.J. Shin, C.-K. Park, D.-S. Kong, H.J. Seol, A. Blumberg, J.-I. Lee, A. Iavarone, W.-Y. Park, R. Rabadan, D.-H. Nam, Spatiotemporal genomic architecture informs precision oncology in glioblastoma, *Nat. Genet.* 49 (2017) 594–599, <https://doi.org/10.1038/ng.3806>.

- [46] J. Wang, E. Cazzato, E. Ladewig, V. Frattini, D.I.S. Rosenbloom, S. Zairis, F. Abate, Z. Liu, O. Elliott, Y.-J. Shin, J.-K. Lee, I.-H. Lee, W.-Y. Park, M. Eoli, A. J. Blumberg, A. Lasorella, D.-H. Nam, G. Finocchiaro, A. Iavarone, R. Rabadan, Clonal evolution of glioblastoma under therapy, *Nat. Genet.* 48 (2016) 768–776, <https://doi.org/10.1038/ng.3590>.
- [47] B.J. Gill, D.J. Pisapia, H.R. Malone, H. Goldstein, L. Lei, A. Sonabend, J. Yun, J. Samanamud, J.S. Sims, M. Banu, A. Dovas, A.F. Teich, S.A. Sheth, G. M. McKhann, M.B. Sisti, J.N. Bruce, P.A. Sims, P. Canoll, MRI-localized biopsies reveal subtype-specific differences in molecular and cellular composition at the margins of glioblastoma, *Proc. Natl. Acad. Sci. U.S.A.* 111 (2014) 12550–12555, <https://doi.org/10.1073/pnas.1405839111>.
- [48] R.B. Puchalski, N. Shah, J. Miller, R. Dalley, S.R. Nomura, J.-G. Yoon, K.A. Smith, M. Lanterovich, D. Bertagnoli, K. Bickley, A.F. Boe, K. Brouner, S. Butler, S. Caldejon, M. Chapin, S. Datta, N. Dee, T. Desta, T. Dolbeare, N. Dotson, A. Ebbert, D. Feng, X. Feng, M. Fisher, G. Gee, J. Goldy, L. Gourley, B.W. Gregor, G. Gu, N. Hejazinia, J. Hohmann, P. Hothi, R. Howard, K. Joines, A. Kriedberg, L. Kuan, C. Lau, F. Lee, H. Lee, T. Lemon, F. Long, N. Mastan, E. Mott, C. Murthy, K. Ngo, E. Olson, M. Reding, Z. Riley, D. Rosen, D. Sandman, N. Shapovalova, C. R. Slaughterbeck, A. Sodt, G. Stockdale, A. Szafer, W. Wakeman, P.E. Wahnoutka, S.J. White, D. Marsh, R.C. Rostomily, L. Ng, C. Dang, A. Jones, B. Keogh, H. R. Gittleman, J.S. Barnholtz-Sloan, P.J. Cimino, M.S. Uppin, C.D. Keene, F. R. Farrokhi, J.D. Lathia, M.E. Berens, A. Iavarone, A. Bernard, E. Lein, J. W. Phillips, S.W. Rostad, C. Cobbs, M.J. Hawrylycz, G.D. Foltz, An anatomic transcriptional atlas of human glioblastoma, *Science* 360 (2018) 660–663, <https://doi.org/10.1126/science.aaf2666>.
- [49] T.E. Miller, B.B. Liao, L.C. Wallace, A.R. Morton, Q. Xie, D. Dixit, D.C. Factor, L.J. Y. Kim, J.J. Morrow, Q. Wu, S.C. Mack, K.C. G. Hubert, S.M. Gillespie, J. A. Flavan, T. Hoffmann, R. Thummalaipalli, M.T. Hemann, P.J. Paddison, C. M. Horbinski, J. Zuber, P.C. Scacheri, B.E. Bernstein, P.J. Tesar, J.N. Rich, Transcription elongation factors represent in vivo cancer dependencies in glioblastoma, *Nature* 547 (2017) 355–359, <https://doi.org/10.1038/nature23000>.
- [50] D. Yu, O.F. Khan, M.L. Suvà, B. Dong, W.K. Panek, T. Xiao, M. Wu, Y. Han, A. U. Ahmed, I.V. Balyasnikova, H.F. Zhang, C. Sun, R. Langer, D.G. Anderson, M. S. Lesniak, Multiplexed RNAi therapy against brain tumor-initiating cells via lipopolymeric nanoparticle infusion delays glioblastoma progression, *Proc. Natl. Acad. Sci. U. S. A.* 114 (2017) E6147–E6156, <https://doi.org/10.1073/pnas.1701911114>.
- [51] Q. Wang, B. Hu, X. Hu, H. Kim, M. Squatrito, L. Scarpace, A.C. deCarvalho, S. Lyu, P. Li, Y. Li, F. Barthel, H.J. Cho, Y.-H. Lin, N. Satani, E. Martinez-Ledesma, S. Zheng, E. Chang, C.-E.G. Sauvè, A. Olar, Z.D. Lan, G. Finocchiaro, J.J. Phillips, M.S. Berger, K.R. Gabrusiewicz, G. Wang, E. Eskilsson, J. Hu, T. Mikkelsen, R. A. DePinho, F. Muller, A.B. Heimberger, E.P. Sulman, D.-H. Nam, R.G. W. Verhaak, Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment, *Cancer Cell* 32 (2017) 42–56, <https://doi.org/10.1016/j.ccr.2017.06.003>, e6.
- [52] O. Celiku, M.R. Gilbert, O. Lavi, Computational modeling demonstrates that glioblastoma cells can survive spatial environmental challenges through exploratory adaptation, *Nat. Commun.* 10 (2019) 5704, <https://doi.org/10.1038/s41467-019-13726-w>.
- [53] A.P. Patel, I. Tirosh, J.J. Trombetta, A.K. Shalek, S.M. Gillespie, H. Wakimoto, D. P. Cahill, B.V. Nahed, W.T. Curry, R.L. Martuza, D.N. Louis, O. Rozenblatt-Rosen, M.L. Suvà, A. Regev, B.E. Bernstein, Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma, *Science* 344 (2014) 1396–1401, <https://doi.org/10.1126/science.1254257>.
- [54] C. Neftel, J. Laffy, M.G. Filbin, T. Hara, M.E. Shore, G.J. Rahme, A.R. Richman, D. Silverbush, M.L. Shaw, C.M. Hebert, J. Dewitt, S. Gritsch, E.M. Perez, L. N. Gonzalez Castro, X. Lan, N. Druck, C. Rodman, D. Dionne, A. Kaplan, M. S. Bertalan, J. Small, K. Pelton, S. Becker, D. Bonal, Q.-D. Nguyen, R.L. Servis, J. M. Fung, R. Mylvaganam, L. Mayr, J. Gojo, C. Haberler, R. Geyeregger, T. Czech, I. Slave, B.V. Nahed, W.T. Curry, B.S. Carter, H. Wakimoto, P.K. Brastianos, T. T. Batchelor, A. Stemmer-Rachamimov, M. Martinez-Lage, M.P. Froesch, I. Stamenkovic, N. Riggi, E. Rheinbay, M. Monje, O. Rozenblatt-Rosen, D. P. Cahill, A.P. Patel, T. Hunter, I.M. Verma, K.L. Ligon, D.N. Louis, A. Regev, B. E. Bernstein, I. Tirosh, M.L. Suvà, An integrative model of cellular states, plasticity, and genetics for glioblastoma, *Cell* 178 (2019) 835–849, <https://doi.org/10.1016/j.cell.2019.06.024>, e21.
- [55] B.E. Johnson, T. Mazar, C. Hong, M. Barnes, K. Aihara, C.Y. McLean, S.D. Fouse, S. Yamamoto, H. Ueda, K. Tatsuno, S. Asthana, L.E. Jalbert, S.J. Nelson, A. W. Bollen, W.C. Gustafson, E. Charron, W.A. Weiss, I.V. Smirnov, J.S. Song, A. B. Olshen, S. Cha, Y. Zhao, R.A. Moore, A.J. Mungall, S.J.M. Jones, M. Hirst, M. A. Marra, N. Saito, H. Aburatani, A. Mukasa, M.S. Berger, S.M. Chang, B.S. Taylor, J.F. Costello, Mutational analysis reveals the origin and therapy-driven evolution of recurrent glioma, *Science* 343 (2014) 189–193, <https://doi.org/10.1126/science.1239947>.
- [56] F.P. Barthel, K.C. Johnson, F.S. Varn, A.D. Moskalik, G. Tanner, E. Kocakavuk, K. J. Anderson, O. Abiola, K. Aldape, K.D. Alfaro, D. Alpar, S.B. Amin, D.M. Ashley, P. Bandopadhyay, J.S. Barnholtz-Sloan, R. Beroukhim, C. Bock, P.K. Brastianos, D.J. Brat, A.R. Brodbelt, A.F. Bruns, K.R. Bulsara, A. Chakrabarty, A. Chakravarti, J.H. Chuang, E.B. Claus, E.J. Cochran, J. Connelly, J.F. Costello, G. Finocchiaro, M.N. Fletcher, P.J. French, H.K. Gan, M.R. Gilbert, P.V. Gould, M.R. Grimmer, A. Iavarone, A. Ismail, M.D. Jenkinson, M. Khasraw, H. Kim, M.C. M. Kouwenhoven, P.S. LaViolette, M. Li, P. Lichter, K.L. Ligon, A.K. Lowman, T. M. Malta, T. Mazar, K.L. McDonald, A.M. Molinaro, D.-H. Nam, N. Nayyar, H. K. Ng, C.Y. Ngan, S.P. Niclou, J.M. Niers, H. Noushmehr, J. Noorbakhsh, D. R. Ormond, C.-K. Park, L.M. Poisson, R. Rabadan, B. Radlwimmer, G. Rao, G. Reifenberger, J.K. Sa, M. Schuster, B.L. Shaw, S.C. Short, P.A.S. Smitt, A. E. Sloan, M. Smits, H. Suzuki, G. Tabatabai, E.G. Van Meir, C. Watts, M. Weller, P. Wesseling, B.A. Westerman, G. Widhalm, A. Woehrer, W.K.A. Yung, G. Zadeh, J.T. Huse, J.F. De Groot, L.F. Stead, R.G.W. Verhaak, GLASS Consortium, Longitudinal molecular trajectories of diffuse glioma in adults, *Nature* 576 (2019) 112–120, <https://doi.org/10.1038/s41586-019-1775-1>.
- [57] P.D. Delgado-López, E.M. Corrales-García, Survival in glioblastoma: a review on the impact of treatment modalities, *Clin. Transl. Oncol.* 18 (2016) 1062–1071, <https://doi.org/10.1007/s12094-016-1497-x>.
- [58] M.E. Hegi, A.-C. Diserens, T. Gorlia, M.-F. Hamou, N. de Tribolet, M. Weller, J. M. Kros, J.A. Hainfellner, W. Mason, L. Mariani, J.E.C. Bromberg, P. Hau, R. O. Mirimanoff, J.G. Cairncross, R.C. Janzer, R. Stupp, MGMT gene silencing and benefit from temozolomide in glioblastoma, *N. Engl. J. Med.* 352 (2005) 997–1003, <https://doi.org/10.1056/NEJMoa043331>.
- [59] H. Noushmehr, D.J. Weisenberger, K. Diefes, H.S. Phillips, K. Pujara, B.P. Berman, F. Pan, C.E. Pelloski, E.P. Sulman, K.P. Bhat, R.G.W. Verhaak, K.A. Hoadley, D. N. Hayes, C.M. Perou, H.K. Schmidt, L. Ding, R.K. Wilson, D. Van Den Berg, H. Shen, H. Bengtsson, P. Neuvial, L.M. Cope, J. Buckley, J.G. Herman, S. B. Baylin, P.W. Laird, K. Aldape, Identification of a CpG island methylator phenotype that defines a distinct subgroup of glioma, *Cancer Cell* 17 (2010) 510–522, <https://doi.org/10.1016/j.ccr.2010.03.017>.
- [60] A. Wenger, S. Ferreyra Vega, T. Kling, T.O. Bontell, A.S. Jakola, H. Carén, Intratumor DNA methylation heterogeneity in glioblastoma: implications for DNA methylation-based classification, *Neuro-Oncol.* 21 (2019) 616–627, <https://doi.org/10.1093/neuonc/noz011>.
- [61] J. Klughammer, B. Kiesel, T. Roetzer, N. Fortelny, A. Nemc, K.-H. Nennig, J. Furtner, N.C. Sheffield, P. Datlinger, N. Peter, M. Nowosielski, M. Augustin, M. Mischkulnig, T. Ströbel, D. Alpar, B. Ergüner, M. Senekowitsch, P. Moser, C. F. Freyschlag, J. Kerschbaumer, C. Thomé, A.E. Grams, G. Stockhammer, M. Kitzwoegerer, S. Oberndorfer, F. Marhold, S. Weis, J. Trenkler, J. Buchthoerner, J. Pichler, J. Haybaeck, S. Krassnig, K. Mahdy Ali, G. von Campe, F. Payer, C. Sherif, J. Preiser, T. Hauser, P.A. Winkler, W. Kleindienst, F. Würtz, T. Brandner-Kokalj, M. Stultschnig, S. Schweiger, K. Dieckmann, M. Preusser, G. Langs, B. Baumann, E. Knosp, G. Widhalm, C. Marosi, J.A. Hainfellner, A. Woehrer, C. Bock, The DNA methylation landscape of glioblastoma disease progression shows extensive heterogeneity in time and space, *Nat. Med.* 24 (2018) 1611–1624, <https://doi.org/10.1038/s41591-018-0156-x>.
- [62] D. Dixit, B.C. Prager, R.C. Gimple, H.X. Poh, Y. Wang, Q. Wu, Z. Qiu, R.L. Kidwell, L.J.Y. Kim, Q. Xie, K. Vitting-Seerup, S. Bhargava, Z. Dong, L. Jiang, Z. Zhu, P. Hamerlik, S.R. Jaffrey, J.C. Zhao, X. Wang, J.N. Rich, The RNA m6A reader YTHDF2 maintains oncogene expression and is a targetable dependency in glioblastoma stem cells, *Cancer Discov.* (2020), <https://doi.org/10.1158/2159-8290.CD-20-0331>.
- [63] S.C. Mack, K.W. Pajitler, L. Chavez, K. Okonechnikov, K.C. Bertrand, X. Wang, S. Erkek, A. Federation, A. Song, C. Lee, X. Wang, L. McDonald, J.J. Morrow, A. Saiahkova, P. Sin-Chan, Q. Wu, K.A. Michaelraj, T.E. Miller, C.G. Hubert, M. Ryzhova, L. Garzia, L. Donovan, S. Dombrowski, D.C. Factor, B. Luu, C.L. L. Valentim, R.C. Gimple, A. Morton, L. Kim, B.C. Prager, J.J.Y. Lee, X. Wu, J. Zuccaro, Y. Thompson, B.L. Holgado, J. Reimand, S.Q. Ke, A. Tropper, S. Lai, S. Vijayarajah, S. Doan, V. Mahadev, A.F. Miñan, S.N. Gröbner, M. Lienhard, M. Zapatka, Z. Huang, K.D. Aldape, A.M. Carcaboso, P.J. Houghton, S.T. Keir, T. Milde, H. Witt, Y. Li, C.-J. Li, X.-W. Bian, D.T.W. Jones, I. Scott, S.K. Singh, A. Huang, P.B. Dirks, E. Bouffett, J.E. Bradner, V. Ramaswamy, N. Jabado, J. T. Rutka, P.A. Northcott, M. Lupien, P. Lichter, A. Korshunov, P.C. Scacheri, S. M. Pfister, M. Kool, M.D. Taylor, J.N. Rich, Therapeutic targeting of ependymoma as informed by oncogenic enhancer profiling, *Nature* 553 (2018) 101–105, <https://doi.org/10.1038/nature25169>.
- [64] H. Kim, N.-P. Nguyen, K. Turner, S. Wu, A.D. Gujar, J. Luebeck, J. Liu, V. Deshpande, U. Rajkumar, S. Namburi, S.B. Amin, E. Yi, F. Menghi, J. H. Schulte, A.G. Henssen, H.Y. Chang, C.R. Beck, P.S. Mischel, V. Bafna, R.G. W. Verhaak, Extrachromosomal DNA is associated with oncogene amplification and poor outcome across multiple cancers, *Nat. Genet.* 52 (2020) 891–897, <https://doi.org/10.1038/s41588-020-0678-2>.
- [65] A.C. deCarvalho, H. Kim, L.M. Poisson, M.E. Winn, C. Mueller, D. Cherba, J. Koeman, S. Seth, A. Protopopov, M. Felicella, S. Zheng, A. Multani, Y. Jiang, J. Zhang, D.-H. Nam, E.F. Petricoin, L. Chin, T. Mikkelsen, R.G.W. Verhaak, Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma, *Nat. Genet.* 50 (2018) 708–717, <https://doi.org/10.1038/s41588-018-0105-0>.
- [66] A.R. Morton, N. Dogan-Artun, Z.J. Faber, G. MacLeod, C.F. Bartels, M.S. Piazza, K. C. Allan, S.C. Mack, X. Wang, R.C. Gimple, Q. Wu, B.P. Rubin, S. Shetty, S. Angers, P.B. Dirks, R.C. Sallari, M. Lupien, J.N. Rich, P.C. Scacheri, Functional enhancers shape extrachromosomal oncogene amplifications, *Cell* 179 (2019) 1330–1341, <https://doi.org/10.1016/j.cell.2019.10.039>, e13.
- [67] F. Papaccio, F. Paino, T. Regad, G. Papaccio, V. Desiderio, V. Tirino, Concise review: cancer cells, Cancer stem cells, and mesenchymal stem cells: influence in Cancer development, *Stem Cells Transl. Med.* 6 (2017) 2115–2125, <https://doi.org/10.1002/sctm.17-0138>.
- [68] R. Galli, E. Binda, U. Orfanelli, B. Cipelletti, A. Gritti, S. De Vitis, R. Fiocco, C. Foroni, F. Dimeco, A. Vescevi, Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma, *Cancer Res.* 64 (2004) 7011–7021, <https://doi.org/10.1158/0008-5472.CAN-04-1364>.
- [69] S.K. Singh, C. Hawkins, I.D. Clarke, J.A. Squire, J. Bayani, T. Hide, R. M. Henkelman, M.D. Cusimano, P.B. Dirks, Identification of human brain tumour initiating cells, *Nature* 432 (2004) 396–401, <https://doi.org/10.1038/nature03128>.

- [70] R. Pallini, L. Ricci-Vitiani, G.L. Banna, M. Signore, D. Lombardi, M. Todaro, G. Stassi, M. Martini, G. Maira, L.M. Larocca, R.D. Maria, Cancer stem cell analysis and clinical outcome in patients with glioblastoma multiforme, *Clin. Cancer Res.* 14 (2008) 8205–8212, <https://doi.org/10.1158/1078-0432.CCR-08-0644>.
- [71] F. Zeppernick, R. Ahmadi, B. Campos, C. Dictus, B.M. Helmke, N. Becker, P. Lichter, A. Unterberg, B. Radlwimmer, C.C. Herold-Mende, Stem cell marker CD133 affects clinical outcome in glioma patients, *Clin. Cancer Res.* 14 (2008) 123–129, <https://doi.org/10.1158/1078-0432.CCR-07-0932>.
- [72] B. Manoranjan, C. Chokshi, C. Venugopal, M. Subapanditha, N. Savage, N. Tatari, J.P. Provias, N.K. Murty, J. Moffat, B.W. Doble, S.K. Singh, A CD133-AKT-Wnt signaling axis drives glioblastoma brain tumor-initiating cells, *Oncogene* 39 (2020) 1590–1599, <https://doi.org/10.1038/s41388-019-1086-x>.
- [73] D. Beier, P. Hau, M. Proescholdt, A. Lohmeier, J. Wischhusen, P.J. Oefner, L. Aigner, A. Brawanski, U. Bogdahn, C.P. Beier, CD133+ and CD133– Glioblastoma-Derived Cancer Stem Cells Show Differential Growth Characteristics and Molecular Profiles, *Cancer Res.* 67 (2007) 4010–4015, <https://doi.org/10.1158/0008-5472.CAN-06-4180>.
- [74] R. Chen, M.C. Nishimura, S.M. Bumbaca, S. Kharbanda, W.F. Forrest, I. M. Kasman, J.M. Greve, R.H. Soriano, L.L. Gilmour, C.S. Rivers, Z. Modrusan, S. Nacu, S. Guerrero, K.A. Edgar, J.J. Wallin, K. Lamszus, M. Westphal, S. Heim, C.D. James, S.R. VandenBerg, J.F. Costello, S. Moorefield, C.J. Cowdrey, M. Prados, H.S. Phillips, A hierarchy of self-renewing tumor-initiating cell types in glioblastoma, *Cancer Cell* 17 (2010) 362–375, <https://doi.org/10.1016/j.ccr.2009.12.049>.
- [75] H.D. Hemmati, I. Nakano, J.A. Lazareff, M. Masterman-Smith, D.H. Geschwind, M. Bronner-Fraser, H.I. Kornblum, Cancerous stem cells can arise from pediatric brain tumors, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 15178–15183, <https://doi.org/10.1073/pnas.2036535100>.
- [76] I. Ben-Porath, M.W. Thomson, V.J. Carey, R. Ge, G.W. Bell, A. Regev, R. A. Weinberg, An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors, *Nat. Genet.* 40 (2008) 499–507, <https://doi.org/10.1038/ng.127>.
- [77] M.L. Suvà, E. Rheinbay, S.M. Gillespie, A.P. Patel, H. Wakimoto, S.D. Rabkin, N. Riggi, A.S. Chi, D.P. Cahill, B.V. Nahed, W.T. Curry, R.L. Martuza, M.N. Rivera, N. Rossetti, S. Kasif, S. Beik, S. Kadri, I. Tirosh, I. Wortman, A.K. Shalek, O. Rozenblatt-Rosen, A. Regev, D.N. Louis, B.E. Bernstein, Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells, *Cell* 157 (2014) 580–594, <https://doi.org/10.1016/j.cell.2014.02.030>.
- [78] K.L. Ligon, E. Huillard, S. Mehta, S. Kesari, H. Liu, J.A. Alberta, R.M. Bachoo, M. Kane, D.N. Louis, R.A. Depinho, D.J. Anderson, C.D. Stiles, D.H. Rowitch, Olig2-regulated lineage-restricted pathway controls replication competence in neural stem cells and malignant glioma, *Neuron* 53 (2007) 503–517, <https://doi.org/10.1016/j.neuron.2007.01.009>.
- [79] J. Kim, A.J. Woo, J. Chu, J.W. Snow, Y. Fujiwara, C.G. Kim, A.B. Cantor, S. H. Orkin, A Myc network accounts for similarities between embryonic stem and cancer cell transcription programs, *Cell* 143 (2010) 313–324, <https://doi.org/10.1016/j.cell.2010.09.010>.
- [80] J.D. Lathia, J. Gallagher, J.M. Heddleston, J. Wang, C.E. Eyler, J. MacSwords, Q. Wu, A. Vasanji, R.E. McLendon, A.B. Hjelmeland, J.N. Rich, Integrin alpha 6 regulates glioblastoma stem cells, *Cell Stem Cell* 6 (2010) 421–432, <https://doi.org/10.1016/j.stem.2010.02.018>.
- [81] S. Bao, Q. Wu, Z. Li, S. Sathornsumetee, H. Wang, R.E. McLendon, A. B. Hjelmeland, J.N. Rich, Targeting cancer stem cells through LICAM suppresses glioma growth, *Cancer Res.* 68 (2008) 6043–6048, <https://doi.org/10.1158/0008-5472.CAN-08-1079>.
- [82] J. Anido, A. Sáez-Borderías, A. González-Juncá, L. Rodón, G. Folch, M. A. Carmona, R.M. Prieto-Sánchez, I. Barba, E. Martínez-Sáez, L. Prudkin, I. Cuartas, C. Raventós, F. Martínez-Ricarte, M.A. Poca, D. García-Dorado, M. M. Lahn, J.M. Yingling, J. Rodón, J. Sahuquillo, J. Baselga, J. Seoane, TGF- $\beta$  Receptor Inhibitors Target the CD44(high)/1d1(high) Glioma-Initiating Cell Population in Human Glioblastoma, *Cancer Cell* 18 (2010) 655–668, <https://doi.org/10.1016/j.ccr.2010.10.023>.
- [83] B.M. Alrfaei, P. Clark, R. Vemuganti, J.S. Kuo, MicroRNA miR-100 decreases glioblastoma growth by targeting SMARCA5 and ErbB3 in tumor-initiating cells, *Technol. Cancer Res. Treat.* 19 (2020), <https://doi.org/10.1177/1533033820960748>, 1533033820960748.
- [84] A.G. Alvarado, S.M. Turaga, P. Sathyan, E.E. Mulkearns-Hubert, B. Otvos, D. J. Silver, J.S. Hale, W.A. Flavahan, P.O. Zinn, M. Sinyuk, M. Li, M.R. Guda, K. K. Velpula, A.J. Tsung, I. Nakano, M.A. Vogelbaum, S. Majumder, J.N. Rich, J. D. Lathia, Coordination of self-renewal in glioblastoma by integration of adhesion and microRNA signaling, *Neuro-Oncology* 18 (2016) 656–666, <https://doi.org/10.1093/neuonc/nov196>.
- [85] W. Zhou, Y. Yao, A.J. Scott, K. Wilder-Romans, J.J. Dresser, C.K. Werner, H. Sun, D. Pratt, P. Sajjakulnukit, S.G. Zhao, M. Davis, B.S. Nelson, C.J. Hallbrook, L. Zhang, F. Gatto, Y. Umemura, A.K. Walker, M. Kachman, J.N. Sarkaria, J. Xiong, M.A. Morgan, A. Rehemtulla, M.G. Castro, P. Lowenstein, S. Chandrasekaran, T.S. Lawrence, C.A. Lyssiotis, D.R. Wahl, Purine metabolism regulates DNA repair and therapy resistance in glioblastoma, *Nat. Commun.* 11 (2020), <https://doi.org/10.1038/s41467-020-17512-x>.
- [86] A. Dirkse, A. Golebiewska, T. Buder, P.V. Nazarov, A. Muller, S. Poovathingal, N. H.C. Brons, S. Leite, N. Sauvageot, D. Sarkisjan, M. Seyfrid, S. Fritah, D. Stieber, A. Michelucci, F. Hertel, C. Herold-Mende, F. Azaue, A. Skupin, R. Bjerkvig, A. Deutsch, A. Voss-Böhme, S.P. Niclou, Stem cell-associated heterogeneity in Glioblastoma results from intrinsic tumor plasticity shaped by the microenvironment, *Nat. Commun.* 10 (2019) 1787, <https://doi.org/10.1038/s41467-019-09853-z>.
- [87] F. Pistollato, H.-L. Chen, B.R. Rood, H.-Z. Zhang, D. D’Avella, L. Denaro, M. Gardiman, G. te Kronnie, P.H. Schwartz, E. Favaro, S. Indraccolo, G. Basso, D. M. Panchision, Hypoxia and HIF1 $\alpha$  repress the differentiative effects of BMPs in high-grade glioma, *Stem Cells* 27 (2009) 7–17, <https://doi.org/10.1634/stemcells.2008-0402>.
- [88] B.C. Prager, Q. Xie, S. Bao, J.N. Rich, Cancer stem cells: the architects of the tumor ecosystem, *Cell Stem Cell* 24 (2019) 41–53, <https://doi.org/10.1016/j.stem.2018.12.009>.
- [89] A. Soeda, M. Park, D. Lee, A. Mintz, A. Androutsellis-Theotokis, R.D. McKay, J. Engh, T. Iwama, T. Kunisada, A.B. Kassam, I.F. Pollack, D.M. Park, Hypoxia promotes expansion of the CD133-positive glioma stem cells through activation of HIF-1 $\alpha$ , *Oncogene* 28 (2009) 3949–3959, <https://doi.org/10.1038/onc.2009.252>.
- [90] J. Mathieu, Z. Zhang, W. Zhou, A.J. Wang, J.M. Heddleston, C.M.A. Pinna, A. Hubaud, B. Stadler, M. Choi, M. Bar, M. Tewari, A. Liu, R. Vessella, R. Rostomily, D. Born, M. Horwitz, C. Ware, C.A. Blau, M.A. Cleary, J.N. Rich, H. Ruohola-Baker, HIF Induces Human Embryonic Stem Cell Markers in Cancer Cells, *Cancer Res.* 71 (2011) 4640–4652, <https://doi.org/10.1158/0008-5472.CAN-10-3320>.
- [91] J. Man, X. Yu, H. Huang, W. Zhou, C. Xiang, H. Huang, L. Miele, Z. Liu, G. Bebek, S. Bao, J.S. Yu, Hypoxic induction of vasorin regulates Notch1 turnover to maintain glioma stem-like cells, *Cell Stem Cell* 22 (2018) 104–118, <https://doi.org/10.1016/j.stem.2017.10.005>, e6.
- [92] R. Stupp, M.E. Hegi, W.P. Mason, M.J. van den Bent, M.J.B. Taphoorn, R. C. Janzer, S.K. Ludwin, A. Allgeier, B. Fisher, K. Belanger, P. Hau, A.A. Brandes, J. Gijtenbeek, C. Marosi, C.J. Vecht, K. Mokhtari, P. Wesseling, S. Villa, E. Eisenhauer, T. Gorlia, M. Weller, D. Lacombe, J.G. Cairncross, R.-O. Mirimanoff, European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups, National Cancer Institute of Canada Clinical Trials Group, Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial, *Lancet Oncol.* 10 (2009) 459–466, [https://doi.org/10.1016/S1470-2045\(09\)70025-7](https://doi.org/10.1016/S1470-2045(09)70025-7).
- [93] R. Stupp, M.E. Hegi, M.R. Gilbert, A. Chakravarti, Chemoradiotherapy in malignant glioma: standard of care and future directions, *JCO* 25 (2007) 4127–4136, <https://doi.org/10.1200/JCO.2007.11.8554>.
- [94] S. Bao, Q. Wu, R.E. McLendon, Y. Hao, Q. Shi, A.B. Hjelmeland, M.W. Dewhirst, D.D. Bigner, J.N. Rich, Glioma stem cells promote radioresistance by preferential activation of the DNA damage response, *Nature* 444 (2006) 756–760, <https://doi.org/10.1038/nature05236>.
- [95] G. Liu, X. Yuan, Z. Zeng, P. Tunic, H. Ng, I.R. Abdulkadir, L. Lu, D. Irvin, K. L. Black, J.S. Yu, Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma, *Mol. Cancer* 5 (2006) 67, <https://doi.org/10.1186/1476-4598-5-67>.
- [96] M. Minata, A. Audia, J. Shi, S. Lu, J. Bernstock, M.S. Pavlyukov, A. Das, S.-H. Kim, Y. J. Shin, Y. Lee, H. Koo, K. Snigda, I. Waghmare, X. Guo, A. Mohyeldin, D. Gallego-Perez, J. Wang, D. Chen, P. Cheng, F. Mukheef, M. Contreras, J. F. Reyes, B. Vaillant, E.P. Sulman, S.-Y. Cheng, J.M. Markert, B.A. Tannous, X. Lu, M. Kango-Singh, L.J. Lee, D.-H. Nam, I. Nakano, K.P. Bhat, Phenotypic Plasticity of Invasive Edge Glioma Stem-like Cells in Response to Ionizing Radiation, *Cell Rep.* 26 (2019) 1893–1905, <https://doi.org/10.1016/j.celrep.2019.01.076>, e7.
- [97] B. Auffinger, A.L. Tobias, Y. Han, G. Lee, D. Guo, M. Dey, M.S. Lesniak, A. U. Ahmed, Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy, *Cell Death Differ.* 21 (2014) 1119–1131, <https://doi.org/10.1038/cdd.2014.31>.
- [98] N. McGranahan, C. Swanton, Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future, *Cell* 168 (2017) 613–628, <https://doi.org/10.1016/j.cell.2017.01.018>.
- [99] P.R. Prasetyanti, J.P. Medema, Intra-tumor heterogeneity from a cancer stem cell perspective, *Mol. Cancer* 16 (2017) 41, <https://doi.org/10.1186/s12943-017-0600-4>.
- [100] J.N. Rich, Cancer stem cells: understanding tumor hierarchy and heterogeneity, *Medicine* 95 (2016) S2, <https://doi.org/10.1097/MD.00000000000004764>.
- [101] A.P. Thankamony, K. Saxena, R. Murali, M.K. Jolly, R. Nair, Cancer stem cell plasticity – a deadly deal, *Front. Mol. Biosci.* 7 (2020), <https://doi.org/10.3389/fmolb.2020.00079>.
- [102] R. Daneman, A. Prat, The blood–brain barrier, *Cold Spring Harb. Perspect. Biol.* 7 (2015), <https://doi.org/10.1101/cshperspect.a020412>.
- [103] M. Peleli, A. Moustakas, A. Papapetropoulos, Endothelial-tumor cell interaction in brain and CNS malignancies, *Int. J. Mol. Sci.* 21 (2020), <https://doi.org/10.3390/ijms21197371>.
- [104] J.H.-C. Lin, T. Takano, M.L. Cotrina, G. Arcuino, J. Kang, S. Liu, Q. Gao, L. Jiang, F. Li, H. Lichtenberg-Frate, S. Haubrich, K. Willecke, S.A. Goldman, M. Nedergaard, Connexin 43 enhances the adhesivity and mediates the invasion of malignant glioma cells, *J. Neurosci.* 22 (2002) 4302–4311, <https://doi.org/10.1523/JNEUROSCI.22-11-04302.2002>.
- [105] D. Thuringer, J. Boucher, G. Jego, N. Pernet, L. Cronier, A. Hammann, E. Solary, C. Garrido, Transfer of functional microRNAs between glioblastoma and microvascular endothelial cells through gap junctions, *Oncotarget* 7 (2016) 73925–73934, <https://doi.org/10.18632/oncotarget.12136>.
- [106] W. Zhang, J.A. DeMattia, H. Song, W.T. Coulwood, Communication between malignant glioma cells and vascular endothelial cells through gap junctions, *J. Neurosurg.* 98 (2003) 846–853, <https://doi.org/10.3171/jns.2003.98.4.0846>.



- [107] T.T. Batchelor, D.A. Reardon, J.F. de Groot, W. Wick, M. Weller, Antiangiogenic therapy for glioblastoma: current status and future prospects, *Clin. Cancer Res.* 20 (2014) 5612–5619, <https://doi.org/10.1158/1078-0432.CCR-14-0834>.
- [108] T.N. Kreisl, L. Kim, K. Moore, P. Duic, C. Royce, I. Stroud, N. Garren, M. Mackey, J.A. Butman, K. Camphausen, J. Park, P.S. Albert, H.A. Fine, Phase II trial of single-agent bevacizumab followed by Bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma, *J. Clin. Oncol.* 27 (2009) 740–745, <https://doi.org/10.1200/JCO.2008.16.3055>.
- [109] H.S. Friedman, M.D. Prados, P.Y. Wen, T. Mikkelsen, D. Schiff, L.E. Abrey, W.K. A. Yung, N. Paleologos, M.K. Nicholas, R. Jensen, J. Vredenburgh, J. Huang, M. Zheng, T. Cloughesy, Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma, *J. Clin. Oncol.* 27 (2009) 4733–4740, <https://doi.org/10.1200/JCO.2008.19.8721>.
- [110] M.R. Gilbert, J.J. Dignam, T.S. Armstrong, J.S. Wefel, D.T. Blumenthal, M. A. Vogelbaum, H. Colman, A. Chakravarti, S. Pugh, M. Won, R. Jeraj, P.D. Brown, K.A. Jaeckle, D. Schiff, V.W. Stieber, D.G. Brachman, M. Werner-Wasik, I. W. Tremont-Lukats, E.P. Sulman, K.D. Aldape, W.J. Curran, M.P. Mehta, A randomized trial of bevacizumab for newly diagnosed glioblastoma, *N. Engl. J. Med.* 370 (2014) 699–708, <https://doi.org/10.1056/NEJMoa1308573>.
- [111] O.L. Chinot, W. Wick, W. Mason, R. Henriksson, F. Saran, R. Nishikawa, A. F. Carpentier, K. Hoang-Xuan, P. Kavan, D. Cernea, A.A. Brandes, M. Hilton, L. Abrey, T. Cloughesy, Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma, *N. Engl. J. Med.* 370 (2014) 709–722, <https://doi.org/10.1056/NEJMoa1308345>.
- [112] A.B. Francesconi, S. Dupre, M. Matos, D. Martin, B.G. Hughes, D.K. Wyld, J. D. Lickliter, Carboplatin and etoposide combined with bevacizumab for the treatment of recurrent glioblastoma multiforme, *J. Clin. Neurosci.* 17 (2010) 970–974, <https://doi.org/10.1016/j.jocn.2009.12.009>.
- [113] D.A. Reardon, A. Desjardins, K.B. Peters, S. Gururangan, J.H. Sampson, R. E. McLendon, J.E. Herndon, A. Bulusu, S. Threath, A.H. Friedman, J. J. Vredenburgh, H.S. Friedman, Phase II study of carboplatin, irinotecan, and bevacizumab for bevacizumab naïve, recurrent glioblastoma, *J. Neurooncol.* 107 (2012) 155–164, <https://doi.org/10.1007/s11060-011-0722-2>.
- [114] D.A. Reardon, A. Desjardins, J.J. Vredenburgh, S. Gururangan, J.H. Sampson, S. Sathornsumetee, R.E. McLendon, J.E. Herndon, J.E. Marcello, J. Norfleet, A. H. Friedman, D.D. Bigner, H.S. Friedman, Metronomic chemotherapy with daily, oral etoposide plus bevacizumab for recurrent malignant glioma: a phase II study, *Br. J. Cancer* 101 (2009) 1986–1994, <https://doi.org/10.1038/sj.bjc.6605412>.
- [115] S.A. Ali, W.M. McHayleh, A. Ahmad, R. Sehgal, M. Braffet, M. Rahman, G. Bejjani, D.M. Friedland, Bevacizumab and irinotecan therapy in glioblastoma multiforme: a series of 13 cases, *J. Neurosurg.* 109 (2008) 268–272, <https://doi.org/10.3171/JNS/2008/109/8/0268>.
- [116] F. Bokstein, S. Shpigel, D.T. Blumenthal, Treatment with bevacizumab and irinotecan for recurrent high-grade glial tumors, *Cancer* 112 (2008) 2267–2273, <https://doi.org/10.1002/cncr.23401>.
- [117] T.Y. Kang, T. Jin, H. Elinzano, D. Peereboom, Irinotecan and bevacizumab in progressive primary brain tumors, an evaluation of efficacy and safety, *J. Neurooncol.* 89 (2008) 113–118, <https://doi.org/10.1007/s11060-008-9599-0>.
- [118] R.M. Zuniga, R. Torcuator, R. Jain, J. Anderson, T. Doyle, S. Ellika, L. Schultz, T. Mikkelsen, Efficacy, safety and patterns of response and recurrence in patients with recurrent high-grade gliomas treated with bevacizumab plus irinotecan, *J. Neurooncol.* 91 (2009) 329–336, <https://doi.org/10.1007/s11060-008-9718-y>.
- [119] B. Hasselbalch, J.G. Eriksen, H. Broholm, L.J. Christensen, K. Grunnet, M. R. Horsman, H.S. Poulsen, M.-T. Stockhausen, U. Lassen, Prospective evaluation of angiogenic, hypoxic and EGFR-related biomarkers in recurrent glioblastoma multiforme treated with cetuximab, bevacizumab and irinotecan, *APMIS* 118 (2010) 585–594, <https://doi.org/10.1111/j.1600-0463.2010.02631.x>.
- [120] A. Desjardins, D.A. Reardon, A. Coan, J. Marcello, J.E. Herndon, L. Bailey, K. B. Peters, H.S. Friedman, J.J. Vredenburgh, Bevacizumab and daily temozolomide for recurrent glioblastoma, *Cancer* 118 (2012) 1302–1312, <https://doi.org/10.1002/cncr.26381>.
- [121] J. Drappatz, E.Q. Lee, S. Hammond, S.A. Grimm, A.D. Norden, R. Beroukhim, M. Gerard, D. Schiff, A.S. Chi, T.T. Batchelor, L.M. Doherty, A.S. Ciampa, D. C. Lafrankie, S. Ruland, S.M. Snodgrass, J.J. Raizer, P.Y. Wen, Phase I study of panobinostat in combination with bevacizumab for recurrent high-grade glioma, *J. Neurooncol.* 107 (2012) 133–138, <https://doi.org/10.1007/s11060-011-0717-z>.
- [122] K.C. Cuneo, J.J. Vredenburgh, J.H. Sampson, D.A. Reardon, A. Desjardins, K. B. Peters, H.S. Friedman, C.G. Willett, J.P. Kirkpatrick, Safety and efficacy of stereotactic radiosurgery and adjuvant bevacizumab in patients with recurrent malignant gliomas, *Int. J. Radiat. Oncol. Biol. Phys.* 82 (2012) 2018–2024, <https://doi.org/10.1016/j.ijrobp.2010.12.074>.
- [123] W. Taal, H.M. Oosterkamp, A.M.E. Walenkamp, H.J. Dubbink, L.V. Beerepoot, M. C.J. Hanse, J. Buter, A.H. Honkoop, D. Boerman, F.Y.F. de Vos, W.N.M. Dinjens, R.H. Enting, M.J.B. Taphoorn, F.W.P.J. van den Berkmortel, R.L.H. Jansen, D. Brandsma, J.E.C. Bromberg, I. van Heuvel, R.M. Vernhout, B. van der Holt, M. J. van den Bent, Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial, *Lancet Oncol.* 15 (2014) 943–953, [https://doi.org/10.1016/S1470-2045\(14\)70314-6](https://doi.org/10.1016/S1470-2045(14)70314-6).
- [124] W. Wick, T. Gorlia, M. Bendzus, M. Taphoorn, F. Sahn, I. Harting, A.A. Brandes, W. Taal, J. Domont, A. Idbaih, M. Campone, P.M. Clement, R. Stupp, M. Fabbro, E. Le Rhun, F. Dubois, M. Weller, A. von Deimling, V. Gollinopoulos, J. C. Bromberg, M. Platten, M. Klein, M.V. Van Den Bent, Lomustine and Bevacizumab in progressive glioblastoma, *N. Engl. J. Med.* 377 (2017) 1954–1963, <https://doi.org/10.1056/NEJMoa1707358>.
- [125] D.A. Reardon, J.J. Vredenburgh, A. Desjardins, K. Peters, S. Gururangan, J. H. Sampson, J. Marcello, J.E. Herndon, R.E. McLendon, D. Janney, A. H. Friedman, D.D. Bigner, H.S. Friedman, Effect of CYP3A-inducing anti-epileptics on sorafenib exposure: results of a phase II study of sorafenib plus daily temozolomide in adults with recurrent glioblastoma, *J. Neurooncol.* 101 (2011) 57–66, <https://doi.org/10.1007/s11060-010-0217-6>.
- [126] E. Pan, D. Yu, B. Yue, L. Potthast, S. Chowdhary, P. Smith, M. Chamberlain, A prospective phase II single-institution trial of sunitinib for recurrent malignant glioma, *J. Neurooncol.* 110 (2012) 111–118, <https://doi.org/10.1007/s11060-012-0943-z>.
- [127] E.R. Gerstner, A.F. Eichler, S.R. Plotkin, J. Drappatz, C.L. Doyle, L. Xu, D.G. Duda, P.Y. Wen, R.K. Jain, T.T. Batchelor, Phase I trial with biomarker studies of vatalanib (PTK787) in patients with newly diagnosed glioblastoma treated with enzyme inducing anti-epileptic drugs and standard radiation and temozolomide, *J. Neurooncol.* 103 (2011) 325–332, <https://doi.org/10.1007/s11060-010-0390-7>.
- [128] A.A. Brandes, R. Stupp, P. Hau, D. Lacombe, T. Gorlia, A. Tosoni, R. O. Mirimanoff, J.M. Kros, M.J. van den Bent, EORTC study 26041-22041: phase I/II study on concomitant and adjuvant temozolomide (TMZ) and radiotherapy (RT) with PTK787/ZK222584 (PTK/ZK) in newly diagnosed glioblastoma, *Eur. J. Cancer* 46 (2010) 348–354, <https://doi.org/10.1016/j.ejca.2009.10.029>.
- [129] J.D. Hainsworth, T. Ervin, E. Friedman, V. Priego, P.B. Murphy, B.L. Clark, R. E. Lamar, Concurrent radiotherapy and temozolomide followed by temozolomide and sorafenib in the first-line treatment of patients with glioblastoma multiforme, *Cancer* 116 (2010) 3663–3669, <https://doi.org/10.1002/cncr.25275>.
- [130] T.N. Kreisl, P. Smith, J. Sul, C. Salgado, F.M. Iwamoto, J.H. Shih, H.A. Fine, Continuous daily sunitinib for recurrent glioblastoma, *J. Neurooncol.* 111 (2013) 41–48, <https://doi.org/10.1007/s11060-012-0988-z>.
- [131] J. Drappatz, A.D. Norden, E.T. Wong, L.M. Doherty, D.C. Lafrankie, A. Ciampa, S. Kesari, C. Sceppe, M. Gerard, P. Phan, D. Schiff, T.T. Batchelor, K.L. Ligon, G. Young, A. Muzikansky, S.E. Weiss, P.Y. Wen, Phase I study of vandetanib with radiotherapy and temozolomide for newly diagnosed glioblastoma, *Int. J. Radiat. Oncol. Biol. Phys.* 78 (2010) 85–90, <https://doi.org/10.1016/j.ijrobp.2009.07.1741>.
- [132] D.A. Reardon, M.J. Egorin, A. Desjardins, J.J. Vredenburgh, J.H. Beumer, T. F. Lagattuta, S. Gururangan, J.E. Herndon, A.J. Salvado, H.S. Friedman, Phase I pharmacokinetic study of the vascular endothelial growth factor receptor tyrosine kinase inhibitor vatalanib (PTK787) plus imatinib and hydroxyurea for malignant glioma, *Cancer* 115 (2009) 2188–2198, <https://doi.org/10.1002/cncr.24213>.
- [133] D.A. Reardon, J.J. Vredenburgh, A. Coan, A. Desjardins, K.B. Peters, S. Gururangan, S. Sathornsumetee, J.N. Rich, J.E. Herndon, H.S. Friedman, Phase I study of sunitinib and irinotecan for patients with recurrent malignant glioma, *J. Neurooncol.* 105 (2011) 621–627, <https://doi.org/10.1007/s11060-011-0631-4>.
- [134] D.A. Reardon, M.D. Groves, P.Y. Wen, L. Nabors, T. Mikkelsen, S. Rosenfeld, J. Raizer, J. Barriuso, R.E. McLendon, A.B. Suttle, B. Ma, C.M. Curtis, M.M. Dar, J. de Bono, A phase I/II trial of pazopanib in combination with lapatinib in adult patients with relapsed malignant glioma, *Clin. Cancer Res.* 19 (2013) 900–908, <https://doi.org/10.1158/1078-0432.CCR-12-1707>.
- [135] A. Muhic, H.S. Poulsen, M. Sorensen, K. Grunnet, U. Lassen, Phase II open-label study of nintedanib in patients with recurrent glioblastoma multiforme, *J. Neurooncol.* 111 (2013) 205–212, <https://doi.org/10.1007/s11060-012-1009-y>.
- [136] H. Scherer, Structural development in gliomas, *Am. J. Cancer* 34 (1938) 333–351.
- [137] S. Gillespie, M. Monje, An active role for neurons in glioma progression: making sense of Scherer's structures, *Neuro Oncol.* 20 (2018) 1292–1299, <https://doi.org/10.1093/neuonc/nyo083>.
- [138] H.S. Venkatesh, T.B. Johung, V. Caretti, A. Noll, Y. Tang, S. Nagaraja, E. M. Gibson, C.W. Mount, J. Polepalli, S.S. Mitra, P.J. Woo, R.C. Malenka, H. Vogel, M. Bredel, P. Mallick, M. Monje, Neuronal activity promotes glioma growth through Neuroligin-3 secretion, *Cell* 161 (2015) 803–816, <https://doi.org/10.1016/j.cell.2015.04.012>.
- [139] H.S. Venkatesh, L.T. Tam, P.J. Woo, J. Lennon, S. Nagaraja, S.M. Gillespie, J. Ni, D.Y. Duveau, P.J. Morris, J.J. Zhao, C.J. Thomas, M. Monje, Targeting neuronal activity-regulated neuroligin-3 dependency in high-grade glioma, *Nature* 549 (2017) 533–537, <https://doi.org/10.1038/nature24014>.
- [140] A.J. Walker, T. Card, T.E. Bates, K. Muir, Tricyclic antidepressants and the incidence of certain cancers: a study using the GPRD, *Br. J. Cancer* 104 (2011) 193–197, <https://doi.org/10.1038/sj.bjc.6605996>.
- [141] S. Dolma, H.J. Selvadurai, X. Lan, L. Lee, M. Kushida, V. Voisin, H. Whetstone, M. So, T. Aviv, N. Park, X. Zhu, C. Xu, R. Head, K.J. Rowland, M. Bernstein, I. D. Clarke, G. Bader, L. Harrington, J.H. Brumell, M. Tyers, P.B. Dirks, Inhibition of dopamine receptor D4 impedes autophagic flux, proliferation, and survival of glioblastoma stem cells, *Cancer Cell* 29 (2016) 859–873, <https://doi.org/10.1016/j.ccr.2016.05.002>.
- [142] J. Li, S. Zhu, D. Kozono, K. Ng, D. Futalan, Y. Shen, J.C. Akers, T. Steed, D. Kushwaha, M. Schlabach, B.S. Carter, C.-H. Kwon, F. Furnari, W. Cavenee, S. Elledge, C.C. Chen, Genome-wide shRNA screen revealed integrated mitogenic signaling between dopamine receptor D2 (DRD2) and epidermal growth factor receptor (EGFR) in glioblastoma, *Oncotarget* 5 (2014) 882–893, <https://doi.org/10.18632/oncotarget.1801>.
- [143] C. Mahé, M. Bernhard, I. Bobirnac, C. Keser, E. Loetscher, D. Feuerbach, K.K. Dev, P. Schoeffter, Functional expression of the serotonin 5-HT7 receptor in human glioblastoma cell lines, *Br. J. Pharmacol.* 143 (2004) 404–410, <https://doi.org/10.1038/sj.bjp.0705936>.

- [144] C. Labrakakis, S. Patt, J. Hartmann, H. Kettenmann, Functional GABA(A) receptors on human glioma cells, *Eur. J. Neurosci.* 10 (1998) 231–238, <https://doi.org/10.1046/j.1460-9568.1998.00036.x>.
- [145] V. Gallo, D.K. Patneau, M.L. Mayer, F.M. Vaccarino, Excitatory amino acid receptors in glial progenitor cells: molecular and functional properties, *Glia* 11 (1994) 94–101, <https://doi.org/10.1002/glia.440110204>.
- [146] V. Venkataramani, D.I. Tanev, C. Strahle, A. Studier-Fischer, L. Fankhauser, T. Kessler, C. Körber, M. Kardorff, M. Ratliff, R. Xie, H. Horstmann, M. Messer, S. P. Paik, J. Knabbe, F. Sahn, F.T. Kurz, A.A. Acikgöz, F. Herrmannsdörfer, A. Agarwal, D.E. Bergles, A. Chalmers, H. Miletic, S. Turcan, C. Mawrin, D. Hänggi, H.-K. Liu, W. Wick, F. Winkler, T. Kuner, Glutamatergic synaptic input to glioma cells drives brain tumour progression, *Nature* 573 (2019) 532–538, <https://doi.org/10.1038/s41586-019-1564-x>.
- [147] H.S. Venkatesh, W. Morishita, A.C. Geraghty, D. Silverbush, S.M. Gillespie, M. Arzt, L.T. Tam, C. Espenal, A. Ponnuswami, L. Ni, P.J. Woo, K.R. Taylor, A. Agarwal, A. Regev, D. Brang, H. Vogel, S. Hervey-Jumper, D.E. Bergles, M. L. Suvà, R.C. Malenka, M. Monje, Electrical and synaptic integration of glioma into neural circuits, *Nature* 573 (2019) 539–545, <https://doi.org/10.1038/s41586-019-1563-y>.
- [148] Q. Zeng, I.P. Michael, P. Zhang, S. Saghafinia, G. Knott, W. Jiao, B.D. McCabe, J. A. Galván, H.P.C. Robinson, I. Zlobec, G. Ciriello, D. Hanahan, Synaptic proximity enables NMDAR signalling to promote brain metastasis, *Nature* 573 (2019) 526–531, <https://doi.org/10.1038/s41586-019-1576-6>.
- [149] H. Zhang, Y. Zhou, B. Cui, Z. Liu, H. Shen, Novel insights into astrocyte-mediated signaling of proliferation, invasion and tumor immune microenvironment in glioblastoma, *Biomed. Pharmacother.* 126 (2020) 110086, <https://doi.org/10.1016/j.biopha.2020.110086>.
- [150] D. Henrik Heiland, V.M. Ravi, S.P. Behringer, J.H. Frenking, J. Wurm, K. Joseph, N.W.C. Garrelfs, J. Strähle, S. Heynckes, J. Grauvogel, P. Franco, I. Mader, M. Schneider, A.-L. Potthoff, D. Delev, U.G. Hofmann, C. Fung, J. Beck, R. Sankowski, M. Prinz, O. Schnell, Tumor-associated reactive astrocytes aid the evolution of immunosuppressive environment in glioblastoma, *Nat. Commun.* 10 (2019) 2541, <https://doi.org/10.1038/s41467-019-10493-6>.
- [151] L.A. Edwards, K. Woolard, M.J. Son, A. Li, J. Lee, C. Ene, S.A. Mantey, D. Maric, H. Song, G. Belova, R.T. Jensen, W. Zhang, H.A. Fine, Effect of Brain- and Tumor-Derived Connective Tissue Growth Factor on Glioma Invasion, *J. Natl. Cancer Inst.* 103 (2011) 1162–1178, <https://doi.org/10.1093/jnci/djr224>.
- [152] A. Louveau, I. Smirnov, T.J. Keyes, J.D. Eccles, S.J. Rouhani, J.D. Peske, N. C. Derecki, D. Castle, J.W. Mandell, K.S. Lee, T.H. Harris, J. Kipnis, Structural and functional features of central nervous system lymphatic vessels, *Nature* 523 (2015) 337–341, <https://doi.org/10.1038/nature14432>.
- [153] F.S. Hodi, S.J. O'Day, D.F. McDermott, R.W. Weber, J.A. Sosman, J.B. Haanen, R. Gonzalez, C. Robert, D. Schadendorf, J.C. Hassel, W. Akerley, A.J.M. van den Eertwegh, J. Lutzky, P. Lorigan, J.M. Vaubel, G.P. Linette, D. Hogg, C. H. Ottensmeier, C. Lebbé, C. Peschel, I. Quirt, J.J. Clark, J.D. Wolchok, J.S. Weber, J. Tian, M.J. Yellin, G.M. Nichol, A. Hoos, W.J. Urba, Improved survival with ipilimumab in patients with metastatic melanoma, *N. Engl. J. Med.* 363 (2010) 711–723, <https://doi.org/10.1056/NEJMoa1003466>.
- [154] M. Reck, D. Rodríguez-Abreu, A.G. Robinson, R. Hui, T. Csőszi, A. Fülöp, M. Gottfried, N. Peled, A. Tafreshi, S. Cuffe, M. O'Brien, S. Rao, K. Hotta, M. A. Leiby, G.M. Lubiniecki, Y. Shentu, R. Rangwala, J.R. Brahmer, KEYNOTE-024 Investigators, Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer, *N. Engl. J. Med.* 375 (2016) 1823–1833, <https://doi.org/10.1056/NEJMoa1606774>.
- [155] D.A. Reardon, A.A. Brandes, A. Omuro, P. Mulholland, M. Lim, A. Wick, J. Baehring, M.S. Ahluwalia, P. Roth, O. Bähr, S. Phuphanich, J.M. Sepulveda, P. De Souza, S. Sahebjam, M. Carleton, K. Tatsuoka, C. Taitt, R. Zwirter, J. Sampson, M. Weller, Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: the CheckMate 143 phase 3 randomized clinical trial, *JAMA Oncol.* 6 (2020) 1003–1010, <https://doi.org/10.1001/jamaoncol.2020.1024>.
- [156] A.M. Newman, C.L. Liu, M.R. Green, A.J. Gentles, W. Feng, Y. Xu, C.D. Hoang, M. Diehn, A.A. Alizadeh, Robust enumeration of cell subsets from tissue expression profiles, *Nat. Methods* 12 (2015) 453–457, <https://doi.org/10.1038/nmeth.3337>.
- [157] M. Martínez-Lage, T.M. Lynch, Y. Bi, C. Cocito, G.P. Way, S. Pal, J. Haller, R. E. Yan, A. Ziober, A. Nguyen, M. Kandpal, D.M. O'Rourke, J.P. Greenfield, C. S. Greene, R.V. Davuluri, N. Dahmane, Immune landscapes associated with different glioblastoma molecular subtypes, *Acta Neuropathol. Commun.* 7 (2019), <https://doi.org/10.1186/s40478-019-0803-6>.
- [158] H.J. Close, L.F. Stead, J. Nsengimana, K.A. Reilly, A. Droop, H. Wurdak, R. K. Mathew, R. Corns, J. Newton-Bishop, A.A. Melcher, S.C. Short, G.P. Cook, E. B. Wilson, Expression profiling of single cells and patient cohorts identifies multiple immunosuppressive pathways and an altered NK cell phenotype in glioblastoma, *Clin. Exp. Immunol.* 200 (2020) 33–44, <https://doi.org/10.1111/cei.13403>.
- [159] M. Mohme, S. Schliffke, C.L. Maire, A. Rüntger, L. Glau, K.C. Mende, J. Matschke, C. Gehbauer, N. Akyüz, S. Zapf, M. Holz, M. Schaper, T. Martens, N.O. Schmidt, S. Peine, M. Westphal, M. Binder, E. Tolosa, K. Lamszus, Immunophenotyping of newly diagnosed and recurrent glioblastoma defines distinct immune exhaustion profiles in peripheral and tumor-infiltrating lymphocytes, *Clin. Cancer Res.* 24 (2018) 4187–4200, <https://doi.org/10.1158/1078-0432.CCR-17-2617>.
- [160] B. Otvos, D.J. Silver, E.E. Mulkearns-Hubert, A.G. Alvarado, S.M. Turaga, M. D. Sorensen, P. Rayman, W.A. Flavahan, J.S. Hale, K. Stoltz, M. Sinyuk, Q. Wu, A. Jarrar, S.-H. Kim, P.L. Fox, I. Nakano, J.N. Rich, R.M. Ransohoff, J. Finke, B. W. Kristensen, M.A. Vogelbaum, J.D. Lathia, Cancer stem cell-secreted macrophage migration inhibitory factor stimulates myeloid derived suppressor cell function and facilitates glioblastoma immune evasion, *Stem Cells* 34 (2016) 2026–2039, <https://doi.org/10.1002/stem.2393>.
- [161] M. Couto, V. Coelho-Santos, L. Santos, C. Fontes-Ribeiro, A.P. Silva, C.M. F. Gomes, The interplay between glioblastoma and microglia cells leads to endothelial cell monolayer dysfunction via the interleukin-6-induced JAK2/STAT3 pathway, *J. Cell. Physiol.* 234 (2019) 19750–19760, <https://doi.org/10.1002/jcp.28575>.
- [162] M.C. Kuppper, M.F. Hamou, Y. Sawamura, S. Bodmer, N. de Tribolet, Inhibition of lymphocyte function by glioblastoma-derived transforming growth factor beta 2, *J. Neurosurg.* 71 (1989) 211–217, <https://doi.org/10.3171/jns.1989.71.2.0211>.
- [163] O. Bloch, C.A. Crane, R. Kaur, M. Safaee, M.J. Rutkowski, A.T. Parsa, Gliomas promote immunosuppression through induction of B7-H1 expression in tumor-associated macrophages, *Clin. Cancer Res.* 19 (2013) 3165–3175, <https://doi.org/10.1158/1078-0432.CCR-12-3314>.
- [164] A.T. Parsa, J.S. Waldron, A. Panner, C.A. Crane, I.F. Parney, J.J. Barry, K. E. Cachola, J.C. Murray, T. Tihan, M.C. Jensen, P.S. Mischel, D. Stokoe, R. O. Pieper, Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma, *Nat. Med.* 13 (2007) 84–88, <https://doi.org/10.1038/nm1517>.
- [165] J. Wischhusen, M.A. Friese, M. Mittelbronn, R. Meyermann, M. Weller, HLA-E positive glioma cells from NKG2D-mediated immune responses in vitro: implications for immune escape in vivo, *J. Neuroimmunol. Exp. Neurol.* 64 (2005) 523–528, <https://doi.org/10.1093/jnen/64.6.523>.
- [166] V.V. Didenko, H.N. Ngo, C. Minchew, D.S. Baskin, Apoptosis of T lymphocytes invading glioblastomas multiforme: a possible tumor defense mechanism, *J. Neurosurg.* 96 (2002) 580–584, <https://doi.org/10.3171/jns.2002.96.3.0580>.
- [167] S.A. Dusoswa, J. Verhoeff, E. Abels, S.P. Méndez-Huergo, D.O. Croci, L.H. Kuijper, E. de Miguel, V.M.C.J. Wouters, M.G. Best, E. Rodriguez, L.A.M. Cornelissen, S. J. van Vliet, P. Wesseling, X.O. Breakefield, D.P. Noske, T. Würding, M.L. D. Broekman, G.A. Rabinovich, Y. van Kooyk, J.J. Garcia-Vallejo, Glioblastomas exploit truncated O-linked glycans for local and distant immune modulation via the macrophage galactose-type lectin, *Proc Natl Acad Sci U S A.* 117 (2020) 3693–3703, <https://doi.org/10.1073/pnas.1907921117>.
- [168] T.J. Alban, D. Bayik, B. Otvos, A. Rabljenovic, L. Leng, L. Jia-Shiun, G. Roversi, A. Lauko, A.A. Momin, A.M. Mohammadi, D.M. Peereboom, M.S. Ahluwalia, K. Matsuda, K. Yun, R. Bucala, M.A. Vogelbaum, J.D. Lathia, Glioblastoma myeloid-derived suppressor cell subsets express differential macrophage migration inhibitory factor receptor profiles that can be targeted to reduce immune suppression, *Front. Immunol.* 11 (2020), <https://doi.org/10.3389/fimmu.2020.01191>.
- [169] D.M. Peereboom, T.J. Alban, M.M. Grabowski, A.G. Alvarado, B. Otvos, D. Bayik, G. Roversi, M. McGraw, P. Huang, A.M. Mohammadi, H.I. Kornblum, T. Radivoyevitch, M.S. Ahluwalia, M.A. Vogelbaum, J.D. Lathia, Metronomic capecitabine as an immune modulator in glioblastoma patients reduces myeloid-derived suppressor cells, *JCI Insight* 4 (2019), <https://doi.org/10.1172/jci.insight.130748>.
- [170] D. Bayik, Y. Zhou, C. Park, C. Hong, D. Vail, D.J. Silver, A. Lauko, G. Roversi, D. C. Watson, A. Lo, T.J. Alban, M. McGraw, M. Sorensen, M.M. Grabowski, B. Otvos, M.A. Vogelbaum, C. Horbinski, B.W. Kristensen, A.M. Khalil, T. H. Hwang, M.S. Ahluwalia, F. Cheng, J.D. Lathia, Myeloid-derived suppressor cell subsets drive glioblastoma growth in a sex-specific manner, *Cancer Discov.* (2020), <https://doi.org/10.1158/2159-8290.CD-19-1355>.
- [171] T. Kees, J. Lohr, J. Noack, R. Mora, G. Gdynia, G. Tödt, A. Ernst, B. Radlwimmer, C.S. Falk, C. Herold-Mende, A. Régnier-Vigouroux, Microglia isolated from patients with glioma gain antitumor activities on poly (I:C) stimulation, *Neuro Oncol.* 14 (2012) 64–78, <https://doi.org/10.1093/neuonc/nor182>.
- [172] W. Li, M.B. Graeber, The molecular profile of microglia under the influence of glioma, *Neuro Oncol.* 14 (2012) 958–978, <https://doi.org/10.1093/neuonc/nos116>.
- [173] S.J. Coniglio, E. Eugenin, K. Dobrenis, E.R. Stanley, B.L. West, M.H. Symons, J. E. Segall, Microglial stimulation of glioblastoma invasion involves epidermal growth factor receptor (EGFR) and colony stimulating factor 1 receptor (CSF-1R) signaling, *Mol. Med.* 18 (2012) 519–527, <https://doi.org/10.2119/molmed.2011.00217>.
- [174] D.S. Markovic, K. Vinnakota, S. Chirasani, M. Synowitz, H. Raguete, K. Stock, M. Sliwa, S. Lehmann, R. Kälin, N. van Rooijen, K. Holmbeck, F.L. Heppner, J. Kiwit, V. Matyash, S. Lehnardt, B. Kaminska, R. Glass, H. Kettenmann, Gliomas induce and exploit microglial MT1-MMP expression for tumor expansion, *Proc. Natl. Acad. Sci. U. S. A.* 106 (2009) 12530–12535, <https://doi.org/10.1073/pnas.0804273106>.
- [175] S.B. Willingham, J.-P. Volkmer, A.J. Gentles, D. Sahoo, P. Dalerba, S.S. Mitra, J. Wang, H. Contreras-Trujillo, R. Martin, J.D. Cohen, P. Lovelace, F.A. Scheeren, M.P. Chao, K. Weiskopf, C. Tang, A.K. Volkmer, T.J. Naik, T.A. Storm, A. R. Mosley, B. Edris, S.M. Schmid, C.K. Sun, M.-S. Chua, O. Murillo, P. Rajendran, A.C. Cha, R.K. Chin, D. Kim, M. Adorno, T. Raveh, D. Tseng, S. Jaiswal, P. Ø. Enger, G.K. Steinberg, G. Li, S.K. So, R. Majeti, G.R. Harsh, M. van de Rijn, N.N. H. Teng, J.B. Sunwoo, A.A. Alizadeh, M.F. Clarke, I.L. Weissman, The CD47-signal regulatory protein alpha (SIRPα) interaction is a therapeutic target for human solid tumors, *Proc. Natl. Acad. Sci. U. S. A.* 109 (2012) 6662–6667, <https://doi.org/10.1073/pnas.1121623109>.
- [176] S.M. Turaga, D.J. Silver, D. Bayik, E. Paouri, S. Peng, A. Lauko, T.J. Alban, N. Borjini, S. Stanko, U. Naik, R.A. Kerl, J.R. Connor, J.S. Barnholtz-Sloan, J.B. Rubin, M. Berens, D. Davalos, J.D. Lathia, JAM-A functions as a female microglial tumor suppressor in glioblastoma, *Neuro Oncol.* (n.d.), <https://doi.org/10.1093/neuonc/noaa148>.

- [177] W. Yang, N.M. Warrington, S.J. Taylor, P. Whitmire, E. Carrasco, K.W. Singleton, N. Wu, J.D. Lathia, M.E. Berens, A.H. Kim, J.S. Barnholtz-Sloan, K.R. Swanson, J. Luo, J.B. Rubin, Sex differences in GBM revealed by analysis of patient imaging, transcriptome, and survival data, *Sci. Transl. Med.* 11 (2019), <https://doi.org/10.1126/scitranslmed.aao5253>.
- [178] E. Song, T. Mao, H. Dong, L.S.B. Boisserand, S. Antila, M. Bosenberg, K. Alitalo, J.-L. Thomas, A. Iwasaki, VEGF-C-driven lymphatic drainage enables immunosurveillance of brain tumours, *Nature* 577 (2020) 689–694, <https://doi.org/10.1038/s41586-019-1912-x>.
- [179] C. Lee-Chang, J. Miska, D. Hou, A. Rashidi, P. Zhang, R.A. Burga, I. Jusué-Torres, T. Xiao, V.A. Arrieta, D.Y. Zhang, A. Lopez-Rosas, Y. Han, A.M. Sonabend, C. M. Horbinski, R. Stupp, I.V. Balyasnikova, M.S. Lesniak, Activation of 4-1BBL+ B cells with CD40 agonism and IFN $\gamma$  elicits potent immunity against glioblastoma, *J. Exp. Med.* 218 (2021), <https://doi.org/10.1084/jem.20200913>.
- [180] T. Daubon, A. Hemadou, I. Romero Garmendia, M. Saleh, Glioblastoma immune landscape and the potential of new immunotherapies, *Front. Immunol.* 11 (2020) 585616, <https://doi.org/10.3389/fimmu.2020.585616>.
- [181] G.V. Long, V. Atkinson, A.M. Menzies, S. Lo, A.D. Guminski, M.P. Brown, M. Gonzalez, K. Diamante, S.K. Sandhu, R.A. Scolyer, L. Emmett, G.A. McArthur, A randomized phase II study of nivolumab or nivolumab combined with ipilimumab in patients (pts) with melanoma brain metastases (mets): the Anti-PD1 Brain Collaboration (ABC), *JCO* 35 (2017), [https://doi.org/10.1200/JCO.2017.35.15\\_suppl.9508](https://doi.org/10.1200/JCO.2017.35.15_suppl.9508), 9508–9508.
- [182] L. Garofano, S. Migliozi, Y.T. Oh, F. D'Angelo, R.D. Najac, A. Ko, B. Frangaj, F. P. Caruso, K. Yu, J. Yuan, W. Zhao, A. Luisa Di Stefano, F. Bielle, T. Jiang, P. Sims, M.L. Suvà, F. Tang, X.-D. Su, M. Ceccarelli, M. Sanson, A. Lasorella, A. Iavarone, Pathway-based classification of glioblastoma uncovers a mitochondrial subtype with therapeutic vulnerabilities, *Nature Cancer* 2 (2021) 141–156, <https://doi.org/10.1038/s43018-020-00159-4>.
- [183] M. Castellán, A. Guarnieri, A. Fujimura, F. Zanconato, G. Battilana, T. Panciera, H.L. Sladitschek, P. Contessotto, A. Citron, A. Grilli, O. Romano, S. Bicchato, M. Fassan, E. Porcù, A. Rosato, M. Cordenonsi, S. Piccolo, Single-cell analyses reveal YAP/TAZ as regulators of stemness and cell plasticity in glioblastoma, *Nature Cancer* 2 (2021) 174–188, <https://doi.org/10.1038/s43018-020-00150-z>.
- [184] L.M. Richards, O.K.N. Whitley, G. MacLeod, F.M.G. Cavalli, F.J. Coutinho, J. E. Jaramillo, N. Svergun, M. Riverin, D.C. Croucher, M. Kushida, K. Yu, P. Guilhamon, N. Rastegar, M. Ahmadi, J.K. Bhatti, D.A. Bozek, N. Li, L. Lee, C. Che, E. Luis, N.I. Park, Z. Xu, T. Ketela, R.A. Moore, M.A. Marra, J. Spears, M. D. Cusimano, S. Das, M. Bernstein, B. Haibe-Kains, M. Lupien, H.A. Luchman, S. Weiss, S. Angers, P.B. Dirks, G.D. Bader, T.J. Pugh, Gradient of Developmental and Injury Response transcriptional states defines functional vulnerabilities underpinning glioblastoma heterogeneity, *Nature Cancer* 2 (2021) 157–173, <https://doi.org/10.1038/s43018-020-00154-9>.
- [185] K. Mitchell, K. Troike, D.J. Silver, J.D. Lathia, The evolution of the cancer stem cell state in glioblastoma: emerging insights into the next generation of functional interactions, *Neuro-Oncology* 23 (2021) 199–213, <https://doi.org/10.1093/neuonc/noaa259>.
- [186] D. Wang, R. Starr, W.-C. Chang, B. Aguilar, D. Alizadeh, S.L. Wright, X. Yang, A. Brito, A. Sarkissian, J.R. Ostberg, L. Li, Y. Shi, M. Gutova, K. Aboody, B. Badie, S.J. Forman, M.E. Barish, C.E. Brown, Chlorotoxin-directed CAR T cells for specific and effective targeting of glioblastoma, *Sci Transl Med* 12 (2020), <https://doi.org/10.1126/scitranslmed.aaw2672>.
- [187] D.M. O'Rourke, M.P. Nasrallah, A. Desai, J.J. Melenhorst, K. Mansfield, J.J. D. Morrisette, M. Martinez-Lage, S. Brem, E. Maloney, A. Shen, R. Isaacs, S. Mohan, G. Plesa, S.F. Lacey, J.-M. Navenot, Z. Zheng, B.L. Levine, H. Okada, C. H. June, J.L. Brogdon, M.V. Maus, A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma, *Sci Transl Med* 9 (2017), <https://doi.org/10.1126/scitranslmed.aaa0984>.
- [188] D.N. Louis, P. Wesseling, K. Aldape, D.J. Brat, D. Capper, I.A. Cree, C. Eberhart, D. Figarella-Branger, M. Fouladi, G.N. Fuller, C. Giannini, C. Haberler, C. Hawkins, T. Komori, J.M. Kros, H.K. Ng, B.A. Orr, S.-H. Park, W. Paulus, A. Perry, T. Pietsch, G. Reifenberger, M. Rosenblum, B. Rous, F. Sahm, C. Sarkar, D.A. Solomon, U. Tabori, M.J.A. van den Bent, von Deimling, M. Weller, V. A. White, D.W. Ellison, cIMPACT-NOW update 6: new entity and diagnostic principle recommendations of the cIMPACT-Utrecht meeting on future CNS tumor classification and grading, *Brain Pathology* 30 (2020) 844–856, <https://doi.org/10.1111/bpa.12832>.