Cancer cell heterogeneity and plasticity: A paradigm shift in glioblastoma

Yahaya A. Yabo, Simone P. Niclou, and Anna Golebiewska

NORLUX Neuro-Oncology Laboratory, Department of Oncology, Luxembourg Institute of Health, Luxembourg, Luxembourg (Y.A.Y., S.P.N., A.G.); Faculty of Science, Technology and Medicine, University of Luxembourg, Esch-sur-Alzette, Luxembourg (Y.A.Y.); Department of Biomedicine, University of Bergen, Bergen, Norway (S.P.N.)

Corresponding Author: Anna Golebiewska, PhD, Department of Oncology, Luxembourg Institute of Health, 84, Val Fleuri, L-1526 Luxembourg, Luxembourg (anna.golebiewska@lih.lu).

Abstract
Phenotypic plasticity has emerged as a major contributor to intra-tumoral heterogeneity and treatment resistance in cancer. Increasing evidence shows that glioblastoma (GBM) cells display prominent intrinsic plasticity and reversibly adapt to dynamic microenvironmental conditions. Limited genetic evolution at recurrence further suggests that resistance mechanisms also largely operate at the phenotypic level. Here we review recent literature underpinning the role of GBM plasticity in creating gradients of heterogeneous cells including those that carry cancer stem cell (CSC) properties. A historical perspective from the hierarchical to the nonhierarchical concept of CSCs towards the recent appreciation of GBM plasticity is provided. Cellular states interact dynamically with each other and with the surrounding brain to shape a flexible tumor ecosystem, which enables swift adaptation to external pressure including treatment. We present the key components regulating intra-tumoral phenotypic heterogeneity and the equilibrium of phenotypic states, including genetic, epigenetic, and microenvironmental factors. We further discuss plasticity in the context of intrinsic tumor resistance, where a variable balance between preexisting resistant cells and adaptive persisters leads to reversible adaptation upon treatment. Innovative efforts targeting regulators of plasticity and mechanisms of state transitions towards treatment-resistant states are needed to restrict the adaptive capacities of GBM.

Keywords
glioblastoma | plasticity | treatment resistance | tumor heterogeneity | tumor microenvironment

Despite aggressive treatment available for glioblastoma (GBM) patients including surgical resection, radiation, and chemotherapy, tumor recurrence is unavoidable. According to the 2021 WHO classification of CNS tumors, GBMs are classified as isocitrate dehydrogenase (IDH) wild-type (IDHwt) and represent the most aggressive form of diffuse gliomas. Based on their diverse cellular organization and histological appearance, GBMs were historically considered among the most heterogeneous tumors and were referred to as “multiforme.” GBMs commonly carry TERT promoter mutation and copy number changes at chromosomes 7 and 10 (+7/−10). Genetic alterations such as amplification of EGFR, PDGFRA, and CDK4/6, as well as deletions or inactivating mutations in TP53, PTEN, NF1, and CDKN2A/B are key determinants of inter-patient variability. GBMs corresponding to IDH1/2 mutated (IDHmut) tumors are currently a separate grade IV entity within IDHmut astrocytomas. Large-scale genetic and epigenetic profiling studies have uncovered molecular GBM subgroups characterized by distinct DNA methylation and/or expression patterns, highlighting the molecular heterogeneity of this disease. Although to date these subgroups have limited clinical relevance, it remains to be seen to what extent the different layers of inter-patient and intra-tumoral heterogeneity will inform future treatment decisions. Accumulating evidence underscores the existence of extensive intra-tumoral phenotypic heterogeneity and plasticity in GBM (Table 1). Intrinsic plasticity adds another layer of tumor complexity, allowing flexible adaptation of tumor cells during tumor initiation, progression, and treatment escape. Here we
# Table 1: A Summary of Recent Key Findings Showing Evidence of Tumor Cell Plasticity Shaping Phenotypic Heterogeneity in GBM

| Concept                                                                 | Technologies Used                                                                 | Reference |
|------------------------------------------------------------------------|-----------------------------------------------------------------------------------|-----------|
| GBM cells display variable expression of diverse programs related to oncogenesis | scRNA-seq, FACS, functional assays, Microarray                                    | Hall et al. |49 |
| Proliferating GBM cells exist on the main axis of variation between mesenchymal and proneural states, representing tumor core and invasive cells respectively | scRNA-seq, FACS, functional assays, Microarray                                    | Wang et al. |27 |
| GBM cell transition to a slow-cycling, persister-like state upon pressure from RTK inhibitors | scRNA-seq, FACS, functional assays                                                  | Eyler et al. |141 |
| GBM cells are distributed across the neurodevelopmental and metabolic axes | scRNA-seq, FACS, functional assays, Microarray                                    | Richards et al. |51 |
| GBM transcriptional states exist along a gradient centered around proliferating glial progenitor-like cells based on fetal brain signatures | scRNA-seq, FACS, functional assays, Microarray                                    | Couturer et al. |54 |
| Multiple GBM populations give rise to heterogeneous tumors with TME-specific composition | scRNA-seq, FACS, functional assays, Microarray                                    | Garofano et al. |89 |

**Abbreviations:** GBM, glioblastoma; scRNA-seq, single-cell RNA sequencing; RNA-seq, ribonucleic acid sequencing; FACS, fluorescence-activated cell sorting; PDOX, Patient-derived orthotopic xenograft; ChIP-seq, chromatin immunoprecipitation sequencing; RNA-seq, ribonucleic acid sequencing; TME, tumor microenvironment; RTK, receptor tyrosine kinase; CSC, cancer stem cell; NG2, neuron-glial antigen 2; PTPRZ1, protein tyrosine phosphatase receptor type Z1; YAP/TAZ, yes-associated protein/tafazzin; CRISPR, clustered regularly interspaced short palindromic repeats; MERSSH, multiplexed error-robust fluorescent in situ hybridization.
review the role of GBM plasticity in creating a heterogeneous and dynamic tumor ecosystem, where distinct GBM phenotypic states coexist, interacting with each other and with the evolving tumor microenvironment (TME). We consider how phenotypic heterogeneity and plasticity allow tumor cells to escape treatment and develop resistance mechanisms. Finally, we discuss the therapeutic potential of targeting molecular regulators determining GBM heterogeneity and plasticity.

Cancer Stem Cells and Intrinsic Developmental Plasticity – An Evolving Concept

Intra-tumoral heterogeneity in GBMs has been described at various molecular levels. Distinct genetic clones arise following Darwinian principles of hierarchical evolution where the selection of the fittest clones leads to a final genetic equilibrium. Models explaining the creation and maintenance of phenotypic heterogeneity, defined as diversity in epigenetic, transcriptomic, proteomic, and metabolic profiles, are more complex. The initial cancer stem cell (CSC) hypothesis, established over 20 years ago posits that so-called CSCs or Tumor Initiating Cells (TICs) are solely responsible for tumor development and establishment of intra-tumoral phenotypic heterogeneity in a hierarchical manner. CSCs were postulated to display diverse stem cell properties and to be highly tumorigenic in experimental models in vivo. Identification of CSCs in GBMs was largely based on the expression of stemness-associated cell membrane antigens such as CD133, CD15/SESA, CD44, or A2B5 or intracellular markers such as Sox2 and Nestin. Recently, Glycerol-3-phosphate dehydrogenase 1 (GPD1) was proposed as a marker of dormant GBM CSCs with a distinct metabolic profile. A hierarchical organization of GBM was also suggested based on cell clone tracing via genetic barcoding upon serial xenotransplantation and lineage tracing in mouse models. CSC phenotypes are maintained by a plethora of signaling pathways commonly active in healthy stem cells, such as WNT, Notch, TGFI, and MET pathways. In analogy to neural stem cells that terminally differentiate to neuronal and glial cells, GBM CSCs may give rise to more differentiated phenotypes with astrocytic or neuronal features (Figure 1). A number of molecules regulating the switch between CSCs and non-CSCs have been described and include nitric oxide driving activation of Notch signaling and CSC phenotypes, Bone morphogenetic protein 4 (Bmp4) driving astroglia-like differentiation and quiescence, and retinoic acid driving the aberrant neuronal differentiation process. These mechanisms are currently considered as potential therapeutic targets.

At the same time, a growing body of evidence emerged indicating that a unidirectional hierarchical CSC model is not entirely applicable to GBMs. Numerous studies showed that irrespective of CSC marker expression, cells were able to self-renew and proliferate indefinitely. Diverse GBM cells were tumorigenic in experimental models: consistently all

---

**Fig. 1** Dynamic organization of phenotypic heterogeneity in GBM. The creation of phenotypic heterogeneity in GBM differs from the hierarchical differentiation process of normal stem cells. Neural stem cells create various committed progenitors and differentiated cells in a unidirectional hierarchical process. Reversibility of the differentiation process is very limited and can occur only between closely related progenitors and stem cell populations. In contrast, GBM constitutes dynamic and diverse tumor cell populations, where high plasticity is retained in all cells and differences between CSC-like and differentiated-like states are rather small. GBM cells exist in gradients of transcriptomic states, with multiple axes of variation. Interchanges have been documented between TCGA subtypes (Proneural, Classical, Mesenchymal), single-cell states (Neural progenitor cell (NPC)-like, Oligodendrocyte progenitor cell (OPC)-like, Astrocyte (Astro)-like and Mesenchymal (Mes)-like) as well as CSC-like and differentiated-like states. The phenotypic equilibrium at the population level is dictated by the genetic background, TME cues and treatment. Created with Biorender.com.
cell populations gave rise to tumors either with equal or with different potency. 24–29 Similarly, both CSCs and non-CSCs were found to be multipotent and able to regain the initial heterogeneity, 22–24,26,29,30 incongruent with the concept of hierarchical organization. In line with this, we have shown that cellular states arise via stochastic state transitions of existing populations, evolving towards a heterogeneous equilibrium instructed by the TME. 29 Mathematical modeling confirmed the lack of a hierarchical process, yet different subpopulations may differ in the time required to establish the equilibrium. Interestingly, most plastic subpopulations (ie, fast in regenerating heterogeneity) displayed accelerated tumor growth in vivo. A similar effect was seen with NG2+ and NG2− subpopulations that displayed differential tumorigenicity in vivo following direct implantation, while this effect was lost after the recreation of initial heterogeneity in vitro prior to implantation. 27 Thus, although certain GBM subpopulations may exhibit differences in functional assays, CSCs do not appear to constitute a defined cellular entity, but rather a flexible cellular state cooperating with other states and adapting to TME cues. Importantly, unlike neural stem cell differentiation, differentiation of CSCs is not terminal, and “differentiated” GBM cells (ie, GBM cells expressing differentiation markers) can revert to CSC phenotypes (Figure 1). 19,21,29

Extensive tumor cell plasticity has also been uncovered in other malignancies, which lead to an evolving concept of the classical CSC hypothesis. 31–34 Plasticity is defined as the inherent ability to interconvert from one cellular state to another in a stochastic nonhierarchical manner. 35–37 Current data point to a very limited differentiation axis in GBMs where CSCs appear as a context-dependent phenotype (Figure 1). Both CSCs and more differentiated GBM phenotypes represent different states that can flexibly interchange while subjected to various stimuli in TME or upon treatment. While CSC-like states retain a full plasticity potential, differentiated-like phenotypes may show a more stringent potential, requiring longer times for phenotypic interchange. 37 It remains to be seen, whether the axis of bidirectional conversions between CSCs and more differentiated phenotypes can be therapeutically exploited. Whilst we refer the readers to recent seminal reviews in the CSC field, 37–42 we will focus here on novel findings highlighting GBM heterogeneity and plasticity at different molecular levels and in the context of the complex GBM ecosystem.

Transcriptomic Heterogeneity and Inferred Plasticity

Initial attempts to study cellular heterogeneity in GBMs, including CSCs, were based on a limited number of markers and isolation of cells for in vitro studies, which carry several limitations. Marker-based purification methods are not 100% efficient and do not take into account the underlying genetic heterogeneity. Many stemness markers are also expressed by nonneoplastic cells in the brain, further obscuring cell purification. 4,11,43,44 Isolated CSC and non-CSC populations were often cultured under different conditions 5 leading to a divergence of molecular profiles that reflect culture conditions rather than intrinsic cellular properties. 45 While only a limited number of markers was generally assessed at the functional level, a defining set of CSC markers could not be established for GBMs. Furthermore, it has been shown that self-renewal in vitro may not predict in vivo tumorigenic potential 29,46 and cells subjected to brain TME show distinct growth dependencies. 47 Meanwhile the assessment of intra-tumoral heterogeneity in patient samples was long hampered by capturing data from bulk populations and limited deconvolution algorithms. Bulk transcriptomic profiles of patient tumors allowed to identify inter-patient differences, which led to the initial TCGA subtyping (neural, proneural, classical, and mesenchymal) based on TME-dependent signatures. 48 More refined studies revealed that the neural subtype represents samples with limited tumor content, retaining the tumor-intrinsic signatures of proneural, classical, and mesenchymal. 4

Application of single-cell and single nuclei RNA sequencing (scRNA-seq and snRNA-seq respectively) to capture transcriptomic signatures within GBM patients revolutionized our understanding of the underlying molecular heterogeneity. The initial study by Patel et al. 49 showed that GBMs are composed of cells of different TCGA subtypes with multiple cells of intermediary signatures, suggestive of state transitions between phenotypes. Signatures linked to stemness, hypoxia, and quiescence revealed continuous gradients of expression, rather than distinct cellular subpopulations. Using scRNA-seq and cell lineage tracing combined with functional assays, Neftel et al. 50 ultimately demonstrated cellular transitions based on four single-cell transcriptomic signatures dictating the primary axis of variation: Astrocyte (AC)-like, Neural progenitor cell (NPC)-like, Oligodendrocyte progenitor cell (OPC)-like and Mesenchymal (MES)-like (Figure 1). While NPC, OPC, and AC-like expression signatures resemble neurodevelopmental programs, MES-like cells do not mirror any normal brain cells. Multiple cellular states are present in each GBM and all contain proliferating cells, incompatible with a hierarchical organization. These transcriptomic states are partially correlated with expression of cell membrane epitopes. Again, such marker-defined fractions (positive and negative) are tumorigenic in vivo and reconstitute the transcriptomic heterogeneity of the parental tumor. 50 It remains to be determined if different states reconstitute heterogeneity and in vivo tumor growth at the same speed. Recent studies revealed additional gradients based on various cellular properties including proliferation, stemness and neurodevelopmental programs, 51 proneural-to-mesenchymal axis, 52 cellular specialization, metabolism, 53 TME and injury responses (Table 1). 54 The continuous gradients of transcriptomic heterogeneity across tumor cells are in line with protein expression profiles commonly detected, for example, by flow cytometry. Though the interdependence between different gradients appears evident, the exact inter-correlations remain to be determined. Of note, proliferating cells are consistently found in multiple phenotypic states and common CSC markers are broadly expressed and patient-specific, suggesting a variety of active stemness programs across different phenotypes. 55

Single-cell transcriptomic states in IDHwt GBMs differ from those identified in lower-grade IDHmut
astrocytomas, oligodendrogliomas, and H3K27 mutant pediatric gliomas. Analyses of IDHmut and H3K27 mutant gliomas require different gene signatures and suggest a more hierarchical organization. Here proliferating cells reside mostly in stem-like states, whereas Astro-like and Oligo-like cells rarely contain cycling cells. The limited availability of patient-derived preclinical models of IDHmut lower-grade gliomas hampers the functional validation of multipotency of these states. The proportion of cycling stem-like cells increases in the most aggressive high-grade IDHmut gliomas and is high in H3K27 mutant gliomas, suggesting that stemness and plasticity correlate with tumor aggressiveness. A recent analysis revealed common pan-glioma signatures, which combine previously described entities into differentiated-like (IDHwt AC/MES-like states and IDHmut Astro/Oligo-like states), stem-like, and proliferating stem-like states (IDHwt NPC/OPC-like states and IDHmut stem-like states). The identification of cellular states and reversible plasticity between states raises the question of the underlying factors that drive these phenomena. Below we review tumor-intrinsic and TME-driven factors that contribute to the complex and dynamic organization of the GBM ecosystem (Figure 2).

Intrinsic Tumor Characteristics
Defining Intra-tumoral Phenotypic Heterogeneity and Plasticity

Genetic Background

The underlying genetic background of the tumor directly contributes to plasticity and phenotypic heterogeneity. GBMs rarely contain equal proportions of single-cell transcriptomic states and the ratio is skewed by patient-specific genetic associations (e.g., PDGFRα, EGFR, and CDK4 amplification, NF1 loss), where the most abundant phenotypic state defines the molecular subtype at the bulk level. PDGFRα amplified tumors are generally enriched for OPC-like states, CDK4 amplified tumors for NPC-like states, and EGFR amplified tumors for AC-like states, while GBMs with NF1 loss contain higher proportions of MES-like states. The molecular mechanisms behind these differences are still unknown. Regardless of this genetic bias, scRNA-seq showed that genetic clones defined, for example, by different chromosomal aberrations present within the same tumor clearly recapitulate phenotypic heterogeneity and display multiple transcriptomic states. At the functional level it was shown that genetic clones defined by different ploidy (i.e., number of sets of chromosomes) display heterogeneous cell membrane marker expression and recreate phenotypic heterogeneity at the population level, suggesting that phenotypic heterogeneity provides a survival advantage to the tumor. These heterogeneous profiles can adapt upon clonal selection, most likely in response to TME niches. Genetic clones are also spatially distributed across different niches, further impacting phenotypic heterogeneity in the spatial context. This implies that phenotypic heterogeneity and plasticity of GBM cells represent a general phenomenon also in the framework of underlying genetic heterogeneity.

In contrast to the hierarchical process of conventional genetic evolution, extrachromosomal DNA (ecDNA) carrying oncogenes shows a more plastic behavior. As ecDNA structures do not contain centromeres, they are randomly distributed to daughter cells, creating an additional level of heterogeneity. Although the mechanisms regulating ecDNA maintenance are not fully understood, it was found that the generation of ecDNA can be flexibly regulated following therapeutic stress. EGFRvIII-containing ecDNAs are lost upon treatment with erlotinib and reemerge rapidly in surviving GBM cells. Genetic heterogeneity based on ecDNA carrying EGFRvIII is also rapidly reconstituted from purified cells with or without EGFRvIII amplification, further demonstrating the plastic behavior of ecDNA emergence. Whether other sub-clonal ecDNA events such as EGFR or PDGFRα amplifications display similar plasticity remains to be seen. Gene amplifications carried on ecDNAs are often dynamic during glioma treatment, and it is currently unclear if this is due to clonal selection, plasticity of ecDNA production, or both.

Permissive Epigenome

Epigenetic mechanisms such as DNA methylation, histone modification, and chromatin remodeling are essential in shaping dynamic gene expression. The phenotypic equilibrium in GBM is dictated in part by the cell of origin, implicating a key role of the subjacent epigenome in shaping phenotypic heterogeneity. DNA methylation further magnifies the outcome of chromosomal aberrations, as amplified genomic regions (e.g., chromosome 7) show low DNA methylation, whereas regions of copy number loss appear highly methylated. Copy number alterations also associate with DNA methylation disorder, that is, discordant DNA methylation status comprising methylated and unmethylated CpGs in regulatory sequences, which mark epigenetically dynamic regions. DNA methylation profiles enable the stratification of GBMs into several subclasses that largely correlate with transcriptomic subtypes. Similar to transcriptomic states, disparate DNA methylation and chromatin accessibility profiles are found not only in spatially separated tumor zones but also at the single-cell level in different phenotypic states. The recent analysis by Chaligine et al. showed that IDHmut gliomas contain cells with Lgm1-Lgm3 DNA methylation subtypes, while IDHwt GBMs show gradients of Lgm4-Lgm5 subtypes. Lgm4 cells represent AC-like and MES-like states and Lgm5 cells are mostly NPC-like and OPC-like, highlighting a closer resemblance of these state pairs at the DNA methylation level.

Still, the epigenetic regulation of flexible GBM cellular states remains poorly understood. Globally, DNA of GBM cells is hypomethylated, creating open and active chromatic areas similar to pluripotent states. Analysis of phylogenetic trees based on DNA methylation revealed that most differences between phenotypic states arise from stochastic passenger changes, rather than encoded cell state differentiation events. Promoter regions regulated by Polycomb repressive complex 2 (PRC2, responsible
for depositing H3K27me3 repressive mark) linked to stemness pathways show strong hypomethylation and low DNA methylation disorder (HOX and Homeobox genes, lineage-specific transcription and growth factors).\(^{59,66}\) Interestingly, NPC/OPC-like states show modestly higher DNA hypomethylation of PRC2 targets than AC/MES states, which correlates with enhanced chromatin accessibility and increased active histone marks such as H3K4me3 and H3K27ac.\(^{66}\) On the other hand, regulatory elements of AC/MES-like signature genes are highly hypomethylated and accessible in these states, suggesting a combined role of DNA and histone methylation with DNA accessibility in state transitions. Intermediate states at the proneural–mesenchymal axis show heterogeneous profiles with partial overlap of proneural (NPC/OPC-like) and mesenchymal accessibility features, and appear largely associated with AC-like states.\(^{52,69}\) Again, stemness-associated chromatin profiles were present across different phenotypic states and heterogeneous CSC-like states. The relative difference in DNA methylation levels between phenotypic states are minor and certain gene promoters (e.g., Prominin-1/CD133 (PROM1), MutL Homolog 1 (MLH1)) show heterogeneous accessibility profiles without detectable DNA methylation. Promoters of many developmental and signature-specific genes were also identified as bivalent in GBM, defined by the presence of active H3K4me3 and repressive H3K27me3 histone marks combined with low DNA methylation levels.\(^{70}\) Bivalent domains, initially described in pluripotent
stem cells, are indicative of plasticity as they allow for temporal suppression of transcription while protecting the genes from irreversible silencing by DNA methylation and keeping them “poised” for action.\textsuperscript{71} Such domains were reported on PRC2 target gene promoters and were enriched in NPC/OPC-like states.\textsuperscript{66} Although the simultaneous presence of active and repressive histone marks remains unproven at the single-cell level, current data suggest a “primed” status of nonexpressed signatures and epigenetically-encoded plasticity between transcriptomic states. On the other hand, higher DNA methylation levels and DNA methylation disorder are present at the promoters of genes associated with cell differentiation processes, leading to reduced gene expression. High DNA methylation disorder is also present at DNA elements regulated typically by transcription factors associated with extracellular stress stimuli such as hypoxia (eg, \textit{HIF1}\textalpha), most probably facilitating plasticity during stress.\textsuperscript{59} Continuous pressure via hypoxia or radiotherapy leads to accumulation of additional DNA methylation disorder, further enhancing GBM plasticity.

**Stemness Transcriptional Network**

GBMs highjack core transcriptional networks of reprogramming reminiscent of pluripotent stem cells. The pluripotency reprogramming transcription factors Sox2 and c-Myc are widely active in GBM cells; Oct3/4, Nanog, and Klf4 were also reported though at lower levels.\textsuperscript{72} Genetic activation of pluripotency or of neural-specific transcription factors (Brm2, Sox2, Sall2, Olig2) induces tumorigenic CSC-like states in GBM via modulation of epigenetic regulators (eg, HOTAIR, MALAT-1) and noncoding RNAs (eg, HOTAIR, MALAT-1).\textsuperscript{73–76} Such reprogramming can be triggered by oncogenic pathways, such as HGF/cMET signaling,\textsuperscript{77} or TME cues, such as hypoxia.\textsuperscript{78} Of note, Sox2 expression appears rather ubiquitous in GBM cells, regulating distinct downstream gene networks in stem-like and differentiated-like cells.\textsuperscript{79} A set of active enhancers and transcription factors were also found to be subtype-specific, where Sox10 repression led to chromatin remodeling and transition towards the mesenchymal state. Other factors, such as Ascl1 can activate a switch towards more differentiated states.\textsuperscript{80}

**Molecular Crosstalk and Tumor Networks**

Paracrine crosstalk between phenotypic states plays a key role in shaping the overall GBM ecosystem. Wang et al. showed that reciprocal crosstalk between tumor cells of different phenotypes creates supportive growth stimuli via BDNF-NTRK2-VGF paracrine signaling.\textsuperscript{83} Cells with more differentiated phenotypes stimulate stem-like states, promoting tumor initiation and growth.\textsuperscript{82,83} Such paracrine mechanisms could explain the increased tumor growth capacity of those GBM subpopulations, that are more efficient in recreating heterogeneity.\textsuperscript{84} Paracrine crosstalk via soluble CD109 was reported between cells of the tumor core and invasive edge.\textsuperscript{83} Apoptotic GBM cells in the necrotic zone release extracellular vesicles that transport components of spliceosomes to neighboring viable cells, which subsequently modulate RNA splicing and promote survival in the recipient cells.\textsuperscript{85} Phenotypic crosstalk also exists between different genetic clones, for example, \textit{EGFRvIII}-amplified cells release cytokines such as IL-6 and LIF, which directly activate gp130 and \textit{EGFR} in surrounding \textit{EGFRwt}-amplified cells, leading to sustained tumor growth.\textsuperscript{86}

In addition to paracrine signaling, GBM cells communicate with each other via direct cell-cell contacts, via exosomes or microtubes. IDHwt GBM and IDHmut high-grade astrocytoma cells interconnect via ultra-long tumor microtubes protruding from the cell membrane, which enhances survival and resistance to radio-
Yabo et al. Glioblastoma plasticity

676

Crosstalk with TME Subpopulations

Direct interactions between tumor cells and nonneoplastic cells play a vital role in the maintenance of cellular plasticity in GBM. Recent data demonstrate a critical role for physical contacts between tumor cells and neurons, where crosstalk occurs via molecular and electrochemical signaling through a neuron-to-glioma cell synapse. Some glioma cells (10–30%) can thus hijack the neuronal network to receive electrochemical and paracrine signals promoting growth and invasion. Membrane depolarization further enhances cellular communication, where depolarization-induced nonsynaptic calcium currents are amplified via gap junctions of the tumor network itself. Tumor cells are also impacted by the bidirectional crosstalk with nonneuronal cells, including glial cells, endothelial cells, pericytes, resident microglia and infiltrating immune cells. These interactions involve cell-cell contact and paracrine mechanisms, leading to phenotypic adaptation of both tumor and TME subpopulations in different tumor niches. This complex reciprocal interplay has been thoroughly reviewed elsewhere.

The genetic and phenotypic status of tumor cells is important in the bidirectional crosstalk and in shaping the TME, although the “what comes first” question remains unresolved. For example, the immune component is influenced by the IDH status in gliomas and differs significantly from brain metastases. IDHmut gliomas display an increased proportion of microglia-derived macrophages, whereas IDHwt GBMs show enhanced infiltration of monocyte-derived macrophages and lymphocytes. The TME varies across different transcriptional GBM subtypes: while mesenchymal tumors contain lower tumor content and a higher proportion of macrophages, neutrophils, and neuroglial cells, classical tumors have increased dendritic cell signatures. Transitions towards mesenchymal/injury response-like GBM states may occur via inflammatory cytokines released by mesenchymal-specific macrophages, such as TNFα, CCL5, CCL12, and G-CSF, further underlining the reciprocal crosstalk between the TME and tumor cell phenotypic states. A recent study by Hara et al. shows that Oncostatin M (OSM) released by macrophages induces GBM transitions towards the mesenchymal state through activation of OSMR/LIFR-GP130 receptors and STAT3 signaling in GBM cells.

Tumor Plasticity as a Mechanism of Resistance

Plasticity allows for the creation of a plethora of cellular states with different sensitivity to the treatment. Treatment-related phenotypic changes can generally be attributed to two scenarios: (1) increased proliferation and selection of preexisting resistant cellular states over time or (2) adaptation of tumor cells towards resistant phenotypes (Figure 3). Such plastic tumor cells, so-called drug-tolerant persisters, can survive therapeutic pressure by adapting towards treatment-resistant states with a faster response than Darwinian selection. Although quiescence was proposed as a main feature of adaptation, proliferating persisters have also been reported. Preexisting resistance may involve different genetic clones, different cellular states or both. While the selected treatment-resistant genetic clones and/or preexisting resistant phenotypic states retain their genotype and phenotype over time,
drug persisters can revert back to the initial states upon a drug holiday period. Recent reports from breast cancer, lung cancer, and melanoma point to multifactorial resistance, indicating that heterogeneous subpopulations can undergo diverse state transitions and activate concurrent genetic and nongenetic resistance mechanisms upon treatment. By following so-called Lamarckian adaptation, that is, inheritance of acquired characteristics, cells surviving drug treatment first undergo plastic and reversible changes at the phenotypic level, some of which may become permanent over time.

To what extent GBM cells with different sensitivity to treatment are reflected by various phenotypic states remains to be determined. The initial CSC studies describe preexisting GBM CSCs to be highly resistant to radio- and chemotherapy through, for example, enhanced activation of DNA repair mechanisms (eg, via MGMT, Chk1, and Chk2), and inhibitors of apoptosis (eg, FLIP, BCL-2, and BCL-XL) Mouse models show that GBM cells can also escape chemotherapy via ABCG2-driven drug efflux, which is however not reflected in human GBM.

On the other hand, a preexisting proneural-to-mesenchymal gradient was shown to correlate with resistance to radiation and multi-drug treatment, without a direct link to CSC-like phenotypes. Other studies also do not find convincing evidence for CSC selection and describe resistance in non-CSCs. This controversy may in part be explained by diverse definitions of CSCs (eg, quiescent vs proliferative) and variable proliferative properties of the studied populations across patient tumors and preclinical models. Tumors containing quiescent CSC-like cells may show increased stemness upon treatment due to their lower susceptibility to radio- and chemotherapy, whereas tumors driven by proliferative CSC-like cells may not show such selection. The recent insight into GBM plasticity proposes additional scenarios. While some GBM cells may preexist in highly treatment-resistant states, persister cells can activate various adaptive mechanisms upon treatment, such as quiescence, induction of regulatory loops of mRNAs, small and long noncoding RNAs, and transition to stem-like states. Stemness pathways can then act as protectors against treatment, for example, Notch signaling attenuates resistance to radiotherapy via upregulation of PI3K/Akt and Bcl-2 survival pathways. Ionizing radiation activates a switch from CD133+ to CD109+ stem-like phenotypes in invasive cells, concomitant with CCAAT/enhancer binding protein β (C/EBPβ)-mediated transition from proneural to YAP/TAZ-dependent mesenchymal signatures. Similar plasticity has been described in the
context of anti-angiogenic treatment, where tumor cells adapt to TME changes by upregulating glycolysis, invasion, and mesenchymal features via ZEB1-regulated mechanisms. 137,138

Cellular plasticity is also involved in the resistance to targeted drugs. Receptor tyrosine kinase inhibitors, a major class of targeted therapeutics, generally lead to plastic escape mechanisms via activation of alternative signaling pathways. 139 Dasatinib, a PDGFRα inhibitor, was shown to activate reversible GBM transitions towards quiescent Notch and KDM6-dependent persister states via remodeling of H3K27 modifications from H3K27me3 to H3K27ac and activation of neurodevelopmental programs. 140 These states can also preexist in treatment-naïve GBM and are high at baseline in certain stem-like cultures, suggesting a variable balance between preexisting and adaptive resistance in different tumors. Moreover, scRNA-seq combined with lineage tracing showed that this adaptive resistance coexists with irreversible genetic evolution towards novel resistant clones. 141 Adaptive resistance was also observed via single-cell phosphoproteomic analysis upon mTOR inhibition, where GBM cells shift from mTORC1/C2 to ERK and Src signaling. 142 Further studies are needed to reveal the molecular mechanisms and epigenetic regulators underlying treatment-induced GBM plasticity in the context of standard-of-care and targeted therapies. We speculate that GBMs may differ with regards to the ratio of preexisting resistant cells versus adaptive persister. Based on the vast plasticity described in GBM, resistance most likely originates in large part from adaptive changes of drug-tolerant persister states. Moreover, the signatures of resistance are likely to be treatment-specific rather than universal.

### Perspectives

The concept of CSCs at the apex of a hierarchical organization in GBM brought major hopes for straightforward therapies that could eradicate the entire tumor by specifically targeting CSCs at their roots. Over the years numerous promising targets have been proposed including cell membrane markers and stemness signaling pathways. 18,40 The evidence of powerful intrinsic cellular plasticity dampens these expectations as at the therapeutic level, tumor plasticity represents a conceptual departure from the classical CSC hypothesis. Indeed, so far none of the identified targets passed the preclinical efficacy. For example, CD133+ CSCs with anti-CD133 antibodies or CD133-specific CAR-T cells did not result in complete elimination of GBM in preclinical models, only temporary effects are observed and tumors regrow as soon as the treatment is halted. 142 Similarly, cell differentiation protocols are largely unsuccessful in eliminating proliferating GBM cells. Thus, GBM eradication will require targeting the dynamic states rather than single entities. To achieve this, further studies are needed to reveal the drivers of plasticity and treatment escape. The molecular signatures of preexisting treatment-resistant and plastic persister GBM cells in the context of standard-of-care and targeted therapies remain largely unknown. Future studies should address which of the phenotypic changes are fast and reversible, and which are retained in tumors long after treatment. The assessment of the ratio between preexisting treatment-resistant and persister cells may allow patient stratification according to different treatments. Initiatives such as the GLASS consortium 112 will reveal long-term changes in longitudinal patient samples prior and after treatment. While scRNA-seq is still limited to fresh samples, adaptation of the technology to single nuclei extracted from frozen or fixed tissue samples opens new opportunities. Tumor multisampling, spatial-omics and emerging technologies permitting simultaneous assessment of genetic, epigenetic, and transcriptomic information will foster an integrative analysis of dynamic states in a spatio-temporal context.

On the other hand, identifying short-term reversible drug-induced adaptations will require experimental models. These changes may be masked in recurrent patient samples because of the drug holiday phenomenon and/or long-term evolution of the tumor post-treatment. Combining drug exposures directly with single-cell multi-omics 144 and functional analyses in clinically-relevant models will accelerate the functional characterization of preexisting and adaptive resistant states. In this context, patient-derived organoids 145–147 and orthotopic xenografts (PDOXs) 34,45,148,149 which recapitulate tumor heterogeneity and TME niches, should be preferred over in vitro cell lines. Barcoding lineage tracing strategies 150,121 will allow the tracking of single cells in a spatio-temporal manner. This may overcome the limitation of (sc)RNA-seq that captures gene expression at a specific snapshot in time and does not reveal the relationship between treatment-naïve cells and their resistant progeny. Inclusion of (sc)RNA-seq analysis of tumor dynamics as part of clinical trials may be key to investigating resistance mechanisms towards targeted treatment and discriminate responders from nonresponders.

The pressing question remains on how to design therapies against a dynamic target. Gene regulatory networks, master regulators, and epigenetic modifiers dictating tumor plasticity may represent more powerful targets than signature molecules of resistant subpopulations per se. 88,150 Noncoding regulators, such as miRNAs or long noncoding RNAs are additional emerging therapeutic targets. 76 Reversible feedback loops in signaling pathways and selective translation of mRNAs marked by N6-methyladenosine (m^5^A) modification are emerging examples of other molecular layers of plastic regulation of state transitions. Interestingly, Shen et al. showed that mRNAs selected for translation in melanoma persister cells largely comprise chromatin regulators and stress-response kinases. 151 Blocking cellular state transitions in melanoma 124 and other cancers 152,153 effectively decreased heterogeneity and delayed the onset of resistance. Targeting of Retinoid X receptor-γ (RXRG), a master regulator responsible for the reversible shift towards treatment-resistant melanoma, successfully inhibited transitions towards drug-resistant states. 154 Regulators of mesenchymal states, such as NF-κB, STAT3, YAP/TAZ, or C/EBPβ might represent therapeutic targets for GBM resisting standard-of-care therapy. On the other hand, mesenchymal states were recently linked to a higher abundance of cytotoxic T cells, 39 creating novel opportunities for immunotherapies. Lastly, the synergistic effects of genetic evolution and nongenetic state transitions upon treatment will have to be considered, 36 as new genetic modifications may influence the capacity of state transitions and the
population equilibrium of phenotypic states. Relying solely on hierarchical Darwinian selection (genetic or nongenetic) or tumor plasticity may not be sufficient. Models developed by evolutionary ecology, which simultaneously take into account selective and adaptive factors, may bring novel understanding of the dynamic processes in tumors. Novel modalities such as the use of nonlethal doses to control state transitions and retain sufficiently less aggressive drug-sensitive/permmissive states in the tumor ecosystem merits experimental validation. In conclusion, major research efforts are needed to unravel the molecular mechanisms and regulators of GBM plasticity and generate effective drugs against a moving target.

Funding
This work was supported by the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie ‘GLIOTRAIN’ ITN initiative (agreement No 766069).

Conflict of interest statement. Authors declare no conflict of interest.

References

1. Louis DN, Perry A, Wesseling P, et al. The 2021 WHO classification of tumors of the central nervous system: a summary. Neuro Oncol. 2021; 23(8):1231–1251.
2. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018; 555(7687):469–474.
3. Ceccarelli M, Barthel FP, Malta TM, et al.; TCGA Research Network. Molecular profiling reveals biologically discrete subsets and pathways of progression in diffuse glioma. Cell. 2016; 164(3):550–563.
4. Wang Q, Hu B, Hu X, et al. Tumor evolution of glioma-intrinsic gene expression subtypes associates with immunological changes in the microenvironment. Cancer Cell. 2017; 32(1):42–56.e6.
5. Turajic S, Sottrivia A, Graham T, Swanton C. Resolving genetic heterogeneity in cancer. Nat Rev Genet. 2019; 20(7):404–416.
6. Latthia JD, Mack SC, Mulkearns-Hubert EE, Valentim CI, Rich JN. Cancer stem cells in glioblastoma. Genes Dev. 2015; 29(12):1203–1217.
7. Singh SK, Hawkins C, Clarke ID, et al. Identification of human brain tumour initiating cells. Nature. 2004; 432(7015):396–401.
8. Galli R, Binda E, Orfanelli U, et al. Isolation and characterization of tumorigenic, stem-like neural precursors from human glioblastoma. Cancer Res. 2004; 64(19):7011–7021.
9. Bao S, Wu Q, McLendon RE, et al. Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature. 2006; 444(7120):756–760.
10. Son MJ, Woolard K, Nam DH, Lee J, Fine HA. SSEA-1 is an enrichment marker for tumor-initiating cells in human glioblastoma. Cell Stem Cell. 2009; 4(5):440–452.
11. Ogden AT, Waziri AE, Lochhead RA, et al. Identification of A2B5+CD133+ tumor-initiating cells in adult human gliomas. Neurosurgery. 2008; 62(2):505–514; discussion 514.
12. Anido J, Sáez-Borderías A, González-Juncà A, et al. TGF-β receptor inhibitors target the CD44(high)/Id1(high) glioma-initiating cell population in human glioblastoma. Cancer Cell. 2010; 18(6):655–668.
13. Rusu P, Shao C, Neuerburg A, et al. GD1 difficult markers dormant glioma stem cells with a distinct metabolic profile. Cell Stem Cell. 2019; 25(2):241–257.e8.
14. Lan X, Jörg DJ, Cavalli FMM, et al. Fate mapping of human glioblastoma reveals an invariant stem cell hierarchy. Nature. 2017; 549(7671):227–232.
15. Chen J, Li Y, Yu TS, et al. A restricted cell population propagates glioblastoma growth after chemotherapy. Nature. 2012; 488(7412):522–526.
16. Charles N, Ozawa T, Squatrito M, et al. Perivascular nitric oxide activates notch signaling and promotes stem-like character in PDGF-induced glioma cells. Cell Stem Cell. 2010; 6(2):141–152.
17. Joo KM, Jin J, Kim E, et al. MET signaling regulates glioblastoma stem cells. Cancer Res. 2012; 72(15):3828–3838.
18. Peñuelas S, Anido J, Prieto-Sánchez RM, et al. TGF-beta increases glioma-initiating cell self-renewal through the induction of LIF in human glioblastoma. Cancer Cell. 2009; 15(4):315–327.
19. Sachdeva R, Wu M, Johnson K, et al. BMP signaling mediates glioma stem cell quiescence and confers treatment resistance in glioblastoma. Sci Rep. 2019; 9(1):14569.
20. Piccirillo SG, Reynolds BA, Zanetti N, et al. Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. Nature. 2006; 444(7120):761–765.
21. Campos B, Centner FS, Bermejo JL, et al. Aberrant expression of retinoic acid signaling molecules influences patient survival in astrocytic gliomas. Am J Pathol. 2011; 178(5):1953–1964.
22. Kenney-Herbert E, Al-Mayhani T, Piccirillo SG, et al. CD15 expression does not identify a phenotypically or genetically distinct glioblastoma population. Stem Cells Transl Med. 2015; 4(7):822–831.
23. Wang J, Sakariassen PØ, Tsikalovskiy O, et al. CD133 negative glioma cells form tumors in nude rats and give rise to CD133 positive cells. Int J Cancer. 2008; 122(4):761–768.
24. Chen R, Nishimura MC, Bumbaca SM, et al. A hierarchy of self-renewing tumor-initiating cell types in glioblastoma. Cancer Cell. 2010; 17(4):362–375.
25. Auffinger B, Tobias AL, Han Y, et al. Conversion of differentiated cancer cells into cancer stem-like cells in a glioblastoma model after primary chemotherapy. Cell Death Differ. 2014; 21(7):1119–1131.
26. Brescia P, Ortonsi B, Fornasiari L, Levi D, Broggi G, Pelicci G. CD133 is essential for glioblastoma stem cell maintenance. Stem Cells. 2013; 31(5):857–869.
27. Al-Mayhani TF, Heywood RM, Venireddy V, Latthia JD, Piccirillo SGM, Watts C. A non-hierarchical organization of tumorigenic NG2 cells in glioblastoma promoted by EGFR. Neuro Oncol. 2019; 21(6):719–729.
28. Couturier CP, Ayyadhury S, Le PU, et al. Single-cell RNA-seq reveals that glioblastoma recapitulates a normal neurodevelopmental hierarchy. Nat Commun. 2020; 11(1):3406.
29. Dikre A, Golebiewska A, Buder T, et al. Stem cell-associated heterogeneity in Glioblastoma results from intrinsic tumor plasticity shaped by the microenvironment. Nat Commun. 2019; 10(1):1787.
30. Brown DV, Filiz G, Daniel PM, et al. Expression of CD133 and CD44 in glioblastoma stem cells correlates with cell proliferation, phenotype stability and intra-tumor heterogeneity. PloS One. 2017; 12(2):e0172791.
31. Meacham CE, Morrison SJ. Tumour heterogeneity and cancer cell plasticity. Nature. 2013; 501(7467):328–337.
32. Bjerkvig R, Johansson M, Miletic H, Niclou SP. Cancer stem cells and angiogenesis. Semin Cancer Biol. 2009; 19(5):279–284.

33. Li Y, Laterra J. Cancer stem cells: distinct entities or dynamically regulated phenotypes? Cancer Res. 2012; 72(3):576–580.

34. Nguyen LV, Vannier R, Dirks P, Eaves CJ. Cancer stem cells: an evolving concept. Nat Cancer. 2012; 12(12):133–143.

35. Huang S. Reconciling non-genetic plasticity with somatic evolution in cancer. Trends Cancer. 2021; 7(4):309–322.

36. Gupta PB, Pastushenko I, Skibinski A, Blanpain C, Kuperwasser C. Phenotypic plasticity: driver of cancer initiation, progression, and therapy resistance. Cell Stem Cell. 2019; 24(11):65–78.

37. Prager BC, Bhargava S, Mahadev V, Hubert CG, Rich JN. Glioblastoma stem cells: driving resilience through Chaos. Trends Cancer. 2020; 6(3):223–235.

38. Saygin C, Matei D, Majeti R, Reizes O, Lathia JD. Targeting cancer stemness in the clinic: from hype to hope. Cell Stem Cell. 2019; 24(1):25–40.

39. Bayik D, Lathia JD. Cancer stem cell-immune crosstalk in tumour progression. Nat Rev Cancer. 2021; 21(8):526–536.

40. Ramos EK, Hoffmann AD, Gerson SL, Liu H. New opportunities and challenges to defeat cancer stem cells. Trends Cancer. 2017; 3(11):780–796.

41. Mitchell K, Troike K, Silver DJ, Lathia JD. The evolution of the cancer stem cell state in glioblastoma: emerging insights into the next generation of functional interactions. Neuro Oncol. 2021; 23(2):198–213.

42. Wainwright EN, Scaffidi P. Epigenetics and cancer stem cells: unleashing, hijacking, and restricting plasticity. Trends Cancer. 2017; 3(5):372–386.

43. Golebiewska A, Hau AC, Oudin A, et al. Patient-derived organoids and orthotopic xenografts of primary and recurrent gliomas represent relevant patient avatars for precision oncology. Acta Neuropathol. 2020; 140(6):919–949.

44. Golebiewska A, Bougnad S, Stieber D, et al. Side population in human glioblastoma is non-tumorigenic and characterizes brain endothelial cells. Brain. 2013; 136(Pt 5):1462–1475.

45. Pine AR, Cirigiano SM, Nicholson JG, et al. Tumor microenvironment is critical for the maintenance of cellular states found in primary glioblastomas. Cancer Discov. 2020; 10(7):964–979.

46. Barrett LE, Granot Z, Coker C, et al. Self-renewal does not predict tumor growth potential in mouse models of high-grade glioma. Cancer Cell. 2020; 21(11):11–14.

47. Miller TE, Liau BB, Wallace LC, et al. Transcription elongation factors relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer Discov. 2018; 355(6332):eaai8478.

48. Richards LM, Whitely OKNN, MacLeod G, et al. Gradient of developmental and injury response transcriptional states defines functional vulnerabilities underpinning glioblastoma heterogeneity. Nat Cancer. 2021; 2(2):157–173.

49. Bhaduri A, Di Lullo E, Jung D, et al. Outer radial glia-like cancer stem cells contribute to heterogeneity of glioblastomas. Cell Stem Cell. 2020; 26(1):48–63.e6.

50. Venteicher AS, Tirosch I, Hebert C, et al. Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. Science. 2017; 355(6332):eaai8478.

51. Tirosch I, Venteicher AS, Hebert C, et al. Single-cell RNA-seq supports a developmental hierarchy in human oligodendroglioma. Nature. 2016; 539(7628):309–313.

52. Filbin MG, Tirosch I, Honestadt V, et al. Developmental and oncogenic programs in H3K27M gliomas dissected by single-cell RNA-seq. Science. 2018; 360(6386):331–335.

53. Johnson KC, Anderson JK, Courtois ET, et al. Single-cell multimodal glioma analyses identify epigenetic regulators of cellular plasticity and environmental stress response. Nat Genet. 2021; 53(10):1456–1468.

54. Stieber D, Golebiewska A, Evers L, et al. Glioblastomas are composed of genetically divergent clones with distinct tumorigenic potential and variable stem cell-associated phenotypes. Acta Neuropathol. 2014; 127(2):203–219.

55. Sotorrova A, Spiteri I, Piccirillo SG, et al. Intratumor heterogeneity in human glioblastoma reflects cancer evolutionary dynamics. Proc Natl Acad Sci U S A. 2013; 110(10):4009–4014.

56. Verhaak RGW, Bafna V, Mischel PS. Extrachromosomal oncogene amplification in tumour pathogenesis and evolution. Nat Rev Cancer. 2019; 19(5):283–288.

57. deCarvalho AC, Kim H, Poisson LM, et al. Discordant inheritance of chromosomal and extrachromosomal DNA elements contributes to dynamic disease evolution in glioblastoma. Nat Genet. 2018; 50(5):708–717.

58. Wang Z, Sun D, Chen YJ, et al. Cell lineage-based stratification for glioblastoma. Cancer Cell. 2020; 38(3):367–379.e8.

59. Chaligne R, Gaiti F, Silverbush D, et al. Epigenetic encoding, heritability and plasticity of glioma transcriptional cell states. Nat Genet. 2021; 53(10):1469–1479.

60. Wengr A, Ferreya Vega S, Kling T, Bontell TO, Jakola AS, Carén H. Intratumor DNA methylation heterogeneity in glioblastoma: implications for DNA methylation-based classification. Neuro Oncol. 2019; 21(5):616–627.

61. Nabielski NH, Deleyrolle LP, Darst RP, Riva A, Reynolds BA, Kladde MP. Multiplex mapping of chromatin accessibility and DNA methylation within targeted single molecules identifies epigenetic heterogeneity in neural stem cells and glioblastoma. Genome Res. 2014; 24(2):329–339.

62. Guilhamon P, Chesnelong C, Kushida MM, et al. Single-cell chromatin accessibility profiling of glioblastoma identifies an invasive cancer stem cell population associated with lower survival. Elife. 2021; 10:e64090.

63. Hall AW, Battenhouse AM, Shirvam H, et al. Bivalent chromatin domains in glioblastoma reveal a subtype-specific signature of glioma stem cells. Cancer Res. 2018; 78(10):2463–2474.

64. Golesbiewska A, Atkinson SP, Lako M, Armstrong L. Epigenetic landscaping during hESC differentiation to neural cells. Stem Cells. 2009; 27(6):1298–1308.

65. Rheimbay E, Suvá ML, Gillespie SM, et al. An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. Cell Rep. 2013; 35(5):1567–1579.
73. Suvà ML, Rheinbay E, Gillespie SM, et al. Reconstructing and reprogramming the tumor-propagating potential of glioblastoma stem-like cells. Cell. 2014; 157(3):580–594.

74. Lopez-Bertoni H, Lal B, Li A, et al. DNMT-dependent suppression of microRNA regulates the induction of GBM tumor-propagating phenotype by Oct4 and Sox2. Oncogene. 2015; 34(30):3994–4004.

75. Acanda De La Rocha AM, Lopez-Bertoni H, Guruceaga E, et al. Analysis of SOX2-regulated transcriptome in glioma stem cells. PLoS One. 2016; 11(9):e0163155.

76. Schwerdtfeger M, Desiderio V, Kobold S, Regad T, Zappavigna S, Caraglia M. Long non-coding RNAs in cancer stem cells. Transl Oncol. 2021; 14(6):11134.

77. Li Y, Li A, Glas M, et al. c-Met signaling induces a reprogramming network and supports the glioblastoma stem-like phenotype. Proc Natl Acad Sci U S A. 2011; 108(24):9951–9956.

78. Heddleston JM, Li Z, McLendon RE, Hjelmeland AB, Rich JN. The hypoxic microenvironment maintains glioblastoma stem cells and promotes reprogramming towards a cancer stem cell phenotype. Cell Cycle. 2009; 8(20):3274–3284.

79. Berezovsky AD, Poisson LM, Cherba D, et al. Sox2 promotes malignancy in glioblastoma by regulating plasticity and astrocytic differentiation. Neoplasia. 2016; 18(3):193–206, 206.e19.

80. Wu Y, Fletcher M, Gu Z, et al. Glioblastoma epigenome profiling identifies SOX10 as a master regulator of molecular tumour subtype. Nat Commun. 2020; 11(1):6434.

81. Park NJ, Guilhamon P, Desai K, et al. ASCL1 reorganizes chromatin to direct neuronal fate and suppress tumorigenicity of glioblastoma stem cells. Cell Stem Cell. 2017; 21(3):411.

82. Damans S, Sloan SA, Croote D, et al. Single-cell RNA-Seq analysis of infiltrating necastic cells at the migrating front of human glioblastoma. Cell Rep. 2017; 21(5):1399–1410.

83. Bastola S, Pavlyukov MS, Yamashita D, et al. Glioma-initiating cells at tumor edge gain signals from tumor core cells to promote their malignancy. Nat Commun. 2020; 11(1):4660.

84. Joseph JV, Conroy S, Tomar T, et al. TGF-β is an inducer of ZEB1-dependent mesenchymal transdifferentiation in glioblastoma that is associated with tumor invasion. Cell Death Dis. 2014; 5:e1443.

85. Flavahan WA, Wu Q, Hitomi M, et al. Brain tumor initiating cells adapt to restricted nutrition through preferential glucose uptake. Nat Neurosci. 2013; 16(10):1373–1382.

86. Hjelmeland AB, Wu Q, Heddleston JM, et al. Acidic stress promotes a glioma stem cell phenotype. Cell Death Differ. 2011; 18(5):829–840.

87. Abdul Rahim SA, Dirkse A, Oudin A, et al. Regulation of hypoxia-induced autophagy in glioblastoma involves ATG9A. Br J Cancer. 2017; 117(6):813–825.

88. Jin X, Kim LJY, Wu Q, et al. Targeting glioma stem cells through combined BM11 and EZH2 inhibition. Nat Med. 2017; 23(11):1352–1361.

89. Hara T, Chanoch-Myers R, Mathewson ND, et al. Interactions between cancer cells and immune cells drive transitions to mesenchymal-like states in glioblastoma. Cancer Cell. 2021; 39(6):779–782.e11.

90. Griguer CE, Oliva CR, Gobin E, et al. CD313 is a maker of bioenergetic stress in human glioma. PLoS One. 2008; 3(11):e3655.

91. Bar EE, Lin A, Mahairaki V, Matsui W, Eberhart CG. Hypoxia increases the expression of stem-cell markers and promotes clonogenicity in glioblastoma neurospheres. Am J Pathol. 2010; 177(3):1491–1502.

92. Jung J, Kim LJ, Wang X, et al. Nicotinamide metabolism regulates glioblastoma stem cell maintenance. JCI Insight. 2017; 2(10):e90019.

93. Wang X, Prager BC, Wu Q, et al. Reciprocal signaling between glioblastoma stem cells and differentiated tumor cells promotes malignant progression. Cell Stem Cell. 2018; 22(4):514–528.e5.

94. Yan K, Wu Q, Yan DH, et al. Glioma cancer stem cells secrete Gremlin1 to promote their maintenance within the tumor hierarchy. Genes Dev. 2014; 28(10):1089–1100.

95. Pavlyukov MS, Yu H, Bastola S, et al. Apoptotic cell-derived extracellular vesicles promote malignancy of glioblastoma via intercellular transfer of splicing factors. Cancer Cell. 2018; 34(1):119–135.e10.

96. Inda MM, Bonavia R, Mukasa A, et al. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev. 2010; 24(16):1731–1745.

97. Owsswald M, Jung E, Sahm F, et al. Brain tumour cells interconnect to a functional and resistant network. Nature. 2015; 528(7580):93–98.

98. Weil S, Owsswald M, Solecki G, et al. Tumor microtubes convey resistance to surgical lesions and chemotherapy in gliomas. Neuronal Cell. 2019; 17(10):1316–1326.

99. Xie R, Kessler T, Grosch J, et al. Tumor cell network integration in glioma represents a stemness feature. Neuronal Cell. 2021; 23(5):757–769.

100. Jung E, Owsswald M, Ratliff M, et al. Tumor cell plasticity, heterogeneity, and resistance in crucial microenvironmental niches in glioma. Nat Commun. 2021; 12(1):1014.

101. Venkatesh HS, Morishita W, Geraghty AC, et al. Electrical and synaptic integration of glioma into neural circuits. Nature. 2019; 573(7775):539–545.

102. Venkataramani V, Tanev DI, Strahle C, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. Nature. 2019; 573(7775):532–538.

103. Ouart DF, Joyce JA. The microenvironmental landscape of brain tumors. Cancer Cell. 2017; 31(3):326–341.

104. Fries-Afonso Y, Niclou SP, Michelucci A. Revealing and harnessing tumor-associated microglia/macrophage heterogeneity in glioblastoma. Int J Mol Sci. 2020; 21(3):689.

105. Klemm F, Maas RR, Bowman RL, et al. Interrogation of the microenvironmental landscape in brain tumors reveals disease-specific alterations of immune cells. Cell. 2020; 181(7):1643–1666.e17.

106. Bhat KPL, Balasubramaniyan V, Vaillant B, et al. Mesenchymal differentiation mediated by NF-κB promotes radiation resistance in glioblastoma. Cancer Cell. 2013; 24(3):331–346.

107. Sa JK, Chang N, Lee HW, et al. Transcriptional regulatory networks of tumor-associated macrophages that drive malignancy in mesenchymal glioblastoma. Genome Biol. 2020; 21(11):216.

108. Erasimus H, Gobin M, Niclou S, Van Dyck E. DNA repair mechanisms and their clinical impact in glioblastoma. Mutat Res Rev Mutat Res. 2016; 769:19–35.

109. Wellmer M, van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat Rev Clin Oncol. 2021; 18(3):170–186.

110. Meyer M, Reimand J, Lan X, et al. Single cell-derived clonal analysis of human glioblastoma links functional and genomic heterogeneity. Proc Natl Acad Sci U S A. 2015; 112(3):851–856.

111. Reintz R, Wang S, Kebir S, et al. Functional subclone profiling for prediction of treatment-induced intratumor population shifts and discovery of rational drug combinations in human glioblastoma. Clin Cancer Res. 2017; 23(2):562–574.

112. Aldape K, Amin SB, Ashley DM, et al. Glioma through the looking GLASS: molecular evolution of diffuse gliomas and the Glioma Longitudinal Analysis Consortium. Neuro Oncol. 2018; 20(7):837–884.

113. Barthel FP, Johnson KC, Varn FS, et al.; GLASS Consortium. Longitudinal molecular trajectories of diffuse glioma in adults. Nature. 2019; 576(7785):112–120.

114. Köhrer V, Yang J, Barah P, et al. Evolutionary trajectories of IDHWT glioblastomas reveal a common path of early tumorigenesis instigated years ahead of initial diagnosis. Cancer Cell. 2019; 35(4):692–704.e12.
115. Spiteri I, Caravagna G, Cresswell GD, et al. Evolutionary dynamics of residual disease in human glioblastoma. Ann Oncol. 2019; 30(3):456–463.

116. Kim J, Lee IH, Cho HJ, et al. Spatiotemporal evolution of the primary glioblastoma genome. Cancer Cell. 2015; 28(3):318–328.

117. de Souza CF, Sabedot TS, Malta TM, et al. A distinct DNA methylation shift in a subset of glioma CpG island methylator phenotypes during tumor recurrence. Cell Rep. 2018; 23(2):637–651.

118. Pombo Antunes AR, Scheyltjens I, Lodi F, et al. Single-cell profiling of myeloid cells in glioblastoma across species and disease stage reveals macrophage competition and specialization. Nat Neurosci. 2021; 24(4):595–610.

119. Akkari L, Bowman RL, Tessier J, et al. Dynamic changes in glioma macrophage populations after radiotherapy reveal CSF-1R inhibition as a strategy to overcome resistance. Sci Transl Med. 2020; 12(552):eaaw7843.

120. Shen S, Vagner S, Robert C. Persistent cancer cells: the deadly survivors. Cell. 2020; 183(4):860–874.

121. Oren Y, Tsabar M, Cuoco MS, et al. Cycling cancer persister cells arise from lineages with distinct programs. Nature. 2021; 596(7873):576–582.

122. Risom T, Langer EM, Chapman MP, et al. Differentiation-state plasticity of cycling cancer persister cells is necessary or sufficient for a cancer stem cell phenotype in glioblastoma. Mol Cancer. 2020; 18(1):19.

123. Stewart CA, Gay CM, Xi Y, et al. Single-cell analyses reveal increased intratumoral heterogeneity after the onset of therapy resistance in small-cell lung cancer. Nat Cancer. 2020; 1:423–436.

124. Rambow F, Rogiers A, Marin-Bejar O, et al. Toward minimal residual disease-directed therapy in melanoma. Cell. 2018; 174(4):843–855.e19.

125. Liu G, Yuan X, Zeng Z, et al. Analysis of gene expression and intratumoral heterogeneity after the onset of therapy resistance in small-cell lung cancer. Cell Stem Cell. 2019; 18(3):322–336.

126. Bleau AM, Hambardzumyan D, Ozawa T, et al. PTEN/PI3K/Akt pathway regulates the side population phenotype and ABCG2 activity in glioma stem-like cells. Cell Stem Cell. 2009; 4(3):226–235.

127. Broadley KW, Hunn MK, Farrand KJ, et al. Side population is not necessary or sufficient for a cancer stem cell phenotype in glioblastoma multiforme. Stem Cells. 2011; 29(3):452–461.

128. Golebiewska A, Bons NH, Bjerkvig R, et al. Clonal ZEB1-driven mesenchymal transition promotes targetable oncologic antiangiogenic therapy resistance. Cancer Res. 2020; 80(7):1498–1511.

129. Chandra A, Jahangiri A, Chen W, et al. Clonal ZEB1-driven mesenchymal transition promotes targetable oncologic antiangiogenic therapy resistance. Cancer Res. 2020; 80(7):1498–1511.

130. Sreenivasan S, Snider J, Lyakiskeva A, Wong V, Stagljar I. Rho receptor tyrosine kinases and cancer: oncogenic mechanisms and therapeutic approaches. Oncogene. 2021; 40(24):4079–4093.

131. Liu BB, Sievers C, Donohue LK, et al. Adaptive chromatin remodeling drives glioblastoma stem cell plasticity and drug tolerance. Cell Stem Cell. 2017; 20(2):233–246.e7.

132. Eyler CE, Matsunaga H, Vantine SJ, van Galen P, Bernstein BE. Single-cell lineage analysis reveals genetic and epigenetic interplay in glioblastoma drug resistance. Genoma Biol. 2020; 21(1):174.

133. Wei W, Shin YS, Xue M, et al. Single-cell phosphoproteomics resolves adaptive signaling dynamics and informs targeted combination therapy in glioblastoma. Cancer Cell. 2016; 29(4):563–573.

134. Vora P, Venugopal C, Salim SK, et al. The rational development of CD133-targeting immunotherapies for glioblastoma. Cell Stem Cell. 2020; 26(6):832–844.e6.

135. Ye C, Ho DJ, Neri M, et al. DRUG-seq for miniaturized high-throughput transcriptome profiling in drug discovery. Nat Commun. 2018; 9(1):4307.

136. Jacobs F, Sinanis RD, Zhang DY, et al. A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. Cell. 2020; 180(1):188–204.e22.

137. Klein E, Hau AC, Oudin A, Golubiewska A, Niclou SP. Glioblastoma organoids: pre-clinical applications and challenges in the context of immunotherapy. Front Oncol. 2020; 10:604121.

138. Oudin A, Baus V, Barthelemy V, et al. Protocol for derivation of organoids and patient-derived orthotopic xenografts from glioma patient tumors. STAR Protoc. 2021; 2(2):100534.

139. Voaal RA, Tian S, Remonde D, et al. Genomic and phenotypic characterization of a broad panel of patient-derived xenografts reflects the diversity of glioblastoma. Clin Cancer Res. 2020; 26(5):1094–1104.

140. Aibar S, González-Blas CB, Moerman T, et al. SCENIC: single-cell regulatory network inference and clustering. Nat Methods. 2014; 11(10):1033–1038.

141. Serresi M, Kertalli S, Li L, et al. Functional antagonism of chromatin modulators regulates epithelial-mesenchymal transition. Sci Adv. 2021; 7(9):eabd7974.

142. Marusyk A, Janiszewska M, Polvak K. Intratumor heterogeneity: the Rosetta stone of therapy resistance. Cancer Cell. 2020; 37(4):471–484.

143. West J, You L, Zhang J, et al. Towards multidrug adaptive therapy. Cancer Res. 2020; 80(7):1578–1589.