

## Glioma through the looking GLASS: molecular evolution of diffuse gliomas and the Glioma Longitudinal Analysis Consortium

### The GLASS Consortium\*

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### Abstract

Adult diffuse gliomas are a diverse group of brain neoplasms that inflict a high emotional toll on patients and their families. The Cancer Genome Atlas and similar projects have provided a comprehensive understanding of the somatic alterations and molecular subtypes of glioma at diagnosis. However, gliomas undergo significant cellular and molecular evolution during disease progression. We review the current knowledge on the genomic and epigenetic abnormalities in primary tumors and after disease recurrence, highlight the gaps in the literature, and elaborate on the need for a new multi-institutional effort to bridge these knowledge gaps and how the Glioma Longitudinal Analysis Consortium (GLASS) aims to systemically catalog the longitudinal changes in gliomas. The GLASS initiative will provide essential insights into the evolution of glioma toward a lethal phenotype, with the potential to reveal targetable vulnerabilities and, ultimately, improved outcomes for a patient population in need.

### Keywords

characterization | evolution | glioma | sequencing | subtypes

## Introduction

Diffuse gliomas are the most frequent primary brain tumors in adults.<sup>1</sup> Almost all gliomas relapse despite intensive treatment with surgery, radiation, and chemotherapy. The most common and most aggressive gliomas, glioblastoma (GBM), are isocitrate dehydrogenase (*IDH*)-wildtype and classified as 2016 World Health Organization (WHO) grade IV. They are characterized by a median overall survival that has remained static at around 15 months for decades, even in selected clinical trial populations.<sup>2–4</sup> Patients with lower-grade (WHO grade II) *IDH*-mutated gliomas have a more favorable prognosis, but these tumors progress and recur as higher grades (III and IV) and become resistant to therapy.<sup>1</sup> The standard of care

for diffuse gliomas is maximal safe resection, followed by chemoradiation (Fig. 1).<sup>5</sup> Patients are then monitored for disease progression by imaging at regular intervals following surgery. Evaluation of disease progression is commonly guided by specific imaging criteria (eg, Response Assessment in Neuro-Oncology [RANO]),<sup>6</sup> which rely on visual evaluation of contrast enhancement and the non-enhancing hyperintense area on T2-weighted imaging. Radiologic features sometimes do not distinguish between true tumor progression and its imaging mimicker, pseudoprogression, which can result in premature withdrawal from a specific treatment or the continuation of an ineffective therapy.

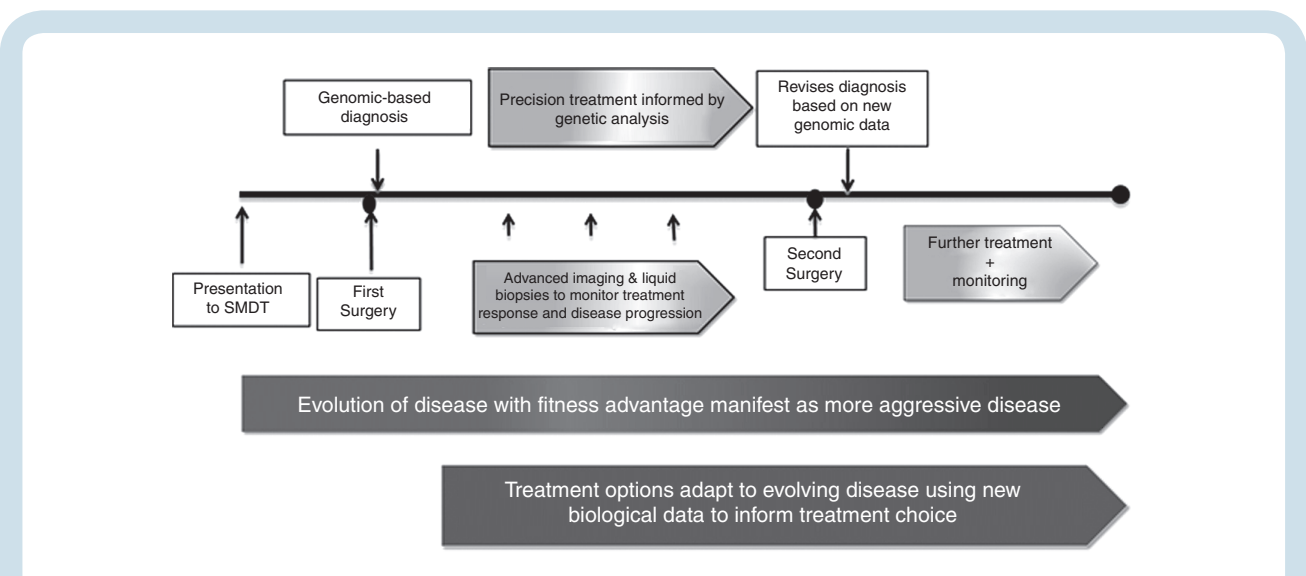
Molecular characterization of gliomas has advanced our understanding of their genesis<sup>7–18</sup> and has identified somatic alterations that allow their classification

into subtypes with different biology and median survival times.<sup>19</sup> This wealth of information has provided a detailed molecular portrait of primary glioma. The Cancer Genome Atlas (TCGA), which characterized 1100 grades II–IV gliomas in detail, has by design focused on untreated tumors. The next frontier in glioma genomics is to understand recurrent disease, as patients generally die from increasingly resistant tumor regrowth after therapy. Recent pilot studies of paired tumors obtained before and after therapy show that there are many differences between the primary neoplasm at diagnosis and the recurrent tumor.<sup>20</sup> Progression of gliomas is the result of an evolutionary process that involves iterative cycles of clonal expansion, genetic diversification, and clonal selection under micro-environmental pressures, including overcoming antitumor immune responses.<sup>21</sup> The presence of multiple cell populations with an array of different somatic mutations is at least partly responsible for the rapid induction of intrinsic resistance to therapy in gliomas.<sup>22</sup> Adaptive epigenetic and phenotypic responses are equally important. The emerging understanding of this dynamic evolution of the glioma genome has major implications for cancer biology research and potential development of effective therapies. This can only be achieved through (i) profiling of sufficiently large primary/recurrent patient tumors and associated imaging to collect enough patients in order to capture low-frequency variants or subtle therapy-driving processes and (ii) standardization across biospecimen processing and data platforms. Here, we discuss the current literature on preliminary molecular longitudinal characterization of gliomas (Table 1) and introduce the Glioma Longitudinal Analysis (GLASS) Consortium, which has been initiated to establish a definitive portrait of the recurrence process and, in doing so, discover vulnerabilities that render the tumor sensitive to therapeutic intervention (Fig. 2).

## Molecular Profiling Offers New Possibilities for Diagnosis and Therapy of Gliomas

### Clinical Classification of Adult Diffuse Glioma

Historically, the diagnosis of diffuse gliomas relied purely on microscopic evaluation,<sup>23</sup> but more recently the combination of histopathology with specific molecular characteristics of gliomas has proven more objective for clinical stratification.<sup>9,11,17–19,24–29</sup> Gliomas are initially split based on the mutation status of the *IDH* 1 or 2 genes. Tumors with wild-type alleles are called *IDH*-wildtype and 95% are GBMs.<sup>12</sup> Tumors with *IDH* mutations are further subdivided based on the presence of complete 1p/19q codeletion (*IDH* mutant codeleted) or tumor suppressor protein 53 (*TP53*) mutation and alpha thalassemia/mental retardation syndrome X-linked (*ATRX*) loss (*IDH* mutant non-codeleted).<sup>9,11,17,18,24,26–29</sup> Most WHO grades II and III diffuse astrocytomas and oligodendrogliomas are *IDH* mutant and contain 1p/19q codeletion. Consensus on how this revised molecular classification should be implemented in routine clinical practice<sup>25</sup> is outlined in the latest WHO 2016 classification of CNS tumors.<sup>19</sup> For the first time, this scheme provides data for diagnosis, prognostic grading, and guiding therapeutic decisions.<sup>30,31</sup> However, this improved classification system is predicated on primary untreated disease, and it remains unclear how these molecular markers impact the biology and prognosis following diagnosis. The DNA methylation status of the O<sup>6</sup>-methylguanine-DNA methyltransferase (*MGMT*) gene promoter is predictive of response to temozolomide therapy in primary GBM, and this status appears to be largely stable between primary and recurrent disease.<sup>32</sup> The value of retesting *MGMT* status after disease progression is debatable, and



**Fig. 1** Usual course of glioma management. GLASS would improve the assessment of gliomas, particularly the prediction of malignant transformation, treatment monitoring, and assessment of tumor alterations noninvasively with imaging and/or liquid biopsies. SMDT (tumor board): specialist multidisciplinary team; RT: radiotherapy.

**Table 1** Summary of cohort based longitudinal characterization of glioma studies

#	Publication	Journal	PMID	Year	Data Types	Glioma Type at Diagnosis	Cohort Size (#patients)
1.	Phillips et al <sup>15</sup>	Cancer Cell	16530701	Mar 2006	Gene expression arrays	High grade	23
2.	Johnson et al <sup>36</sup>	Science	24336570	Dec 2013	Exome sequencing	Low grade	23
3.	Kim et al <sup>34</sup>	Genome Res	25650244	Feb 2015	Whole genome and exome sequencing, DNA copy number arrays	Glioblastoma	23 <sup>¥1</sup>
4.	Suzuki et al <sup>17</sup>	Nat Genetics	25848751	Apr 2015	Exome sequencing	Low grade	10
5.	Kim et al <sup>68</sup>	Cancer Cell	26373279	Sep 2015	Exome sequencing, array CGH, RNA sequencing	Glioblastoma	38
6.	Mazor et al <sup>43</sup>	Cancer Cell	26373278	Sep 2015	DNA methylation, RNA sequencing	Low grade	21 <sup>*1</sup>
7.	Kwon et al <sup>73</sup>	PLoS One	26466313	Oct 2015	Gene expression arrays	Glioblastoma	15
8.	Bai et al <sup>44</sup>	Nat Genetics	26618343	Nov 2015	Exome sequencing, array CGH, gene expression arrays, DNA methylation	Low grade	41
9.	Wang et al <sup>69</sup>	Nat Genetics	27270107	July 2016	Exome sequencing	Glioblastoma	39 <sup>*2</sup>
10.	DeCarvalho et al <sup>48</sup>	Biorxiv	NA	Nov 2016	Whole genome sequencing and CGH arrays	Glioblastoma	21 <sup>¥2, *3</sup>
11.	Wang et al <sup>59</sup>	Cancer Cell	28697342	June 2017	Gene expression arrays, RNA sequencing	Glioblastoma	36 <sup>¥3, *4</sup>
12.	Klughammer et al <sup>79</sup>	Biorxiv	NA	2017	DNA methylation	Glioblastoma	112
13.	Ferreira de Souza et al <sup>78</sup>	Biorxiv	NA	2017	DNA methylation	Low grade	32 <sup>¥4, *4</sup>

\*1 Additional characterization on cohort from #2.

\*2 Analysis additionally includes data from cohorts in #2, #3, #4, #5.

\*3 Analysis additionally includes data from cohorts in #3, #5.

\*4 Additional characterization on cohort from #3, includes re-analysis of cohorts from #1, #6, #7.

\*5 Analysis additionally includes data from cohorts in #6, #8.

¥1 Including 13 glioma pairs from TCGA.

¥2 Including 14 glioma pairs from TCGA.

¥3 Additional characterization on 27 glioma pairs from TCGA, overlapping with ¥1 and ¥2

¥4 Including 27 glioma pairs from TCGA, overlapping with ¥1 and ¥2

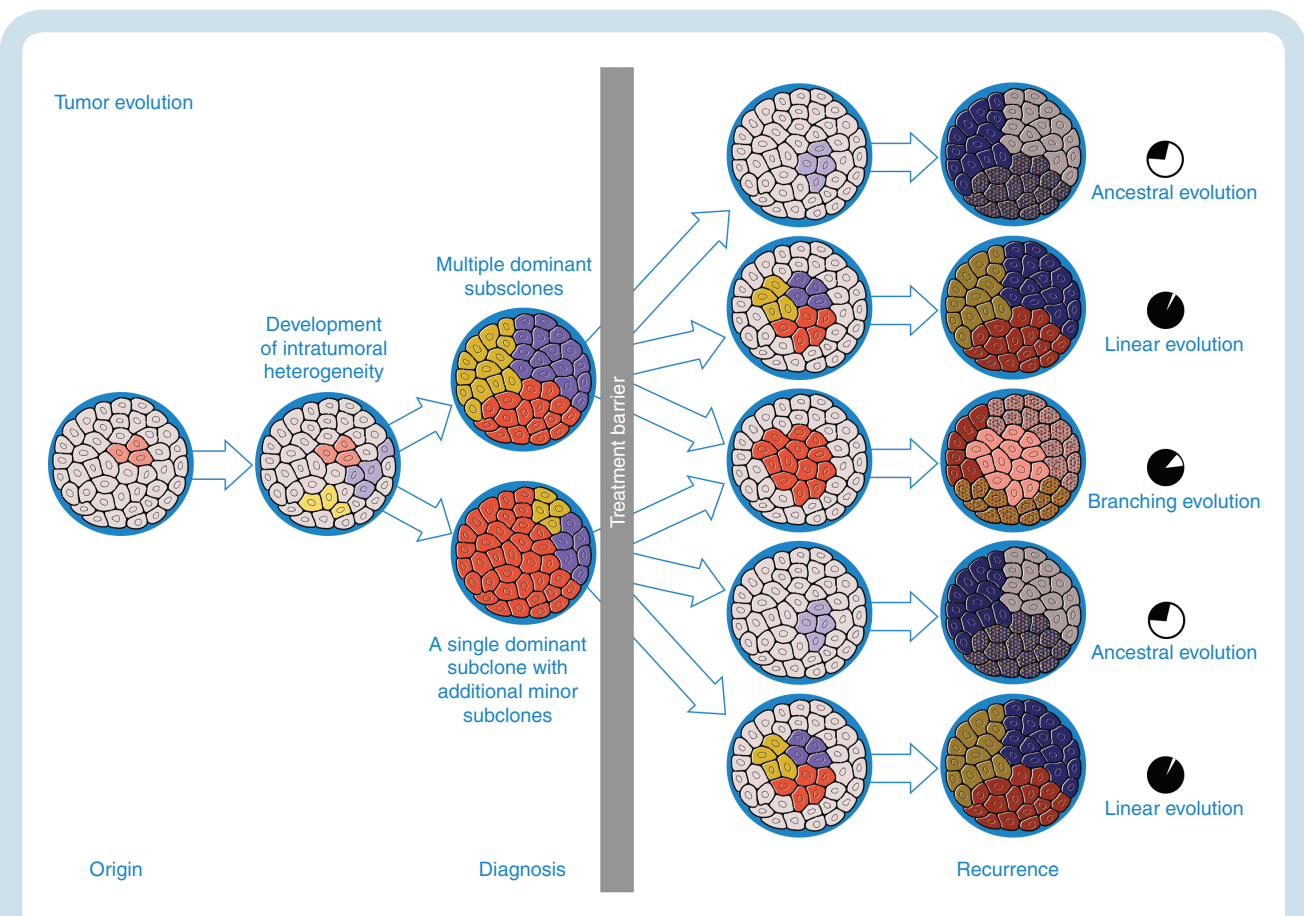
a methylated *MGMT* promoter continues to predict treatment response at this stage.

### Intratumoral Heterogeneity in Primary Gliomas

Cancer results from a single normal cell that has acquired molecular alterations providing it with a growth advantage. In glioma, the most frequent somatic abnormalities are thought to be founding events.<sup>33</sup> This includes somatic mutations in the *IDH* genes and in the promoter of the telomerase reverse transcriptase gene, which is characteristic of *IDH*-wildtype GBM as well as *IDH*-mutant codeleted gliomas.<sup>24</sup> Major aneuploidy, such as 1p/19q codeletion, whole chromosome 7 gain, and chromosome 10 loss (*IDH*-wildtype gliomas), are also thought to be glioma-initiating alterations.<sup>34–36</sup> The 3 major glioma subtypes reflect different patient age at diagnosis distributions, which further suggests that the 3 groups represent distinct gliomagenic biologies.

Cancer cell descendants of the same cell of origin may contain a wide range of genetic and epigenetic states.<sup>37,38</sup> This intratumoral heterogeneity confounds diagnosis, challenges the design of effective therapies, and is a determinant of tumor resistance.<sup>39</sup> Molecular heterogeneity in GBM has been characterized using multiple approaches. For

example, fluorescent in situ hybridization analysis of the most commonly amplified receptor tyrosine kinases (RTKs) in GBM (epidermal growth factor receptor [*EGFR*], platelet derived growth factor receptor alpha [*PDGFRA*], and *MET*) revealed a mosaic of tumor subclones marked by different RTK amplifications in 2%–3% of GBM,<sup>40,41</sup> possibly indicating cooperation between cell populations. Single-cell sequencing demonstrated comparable non-overlapping subclonal GBM cell populations marked by different *EGFR* truncation variants, suggesting convergent evolution of *EGFR* mutations.<sup>42</sup> Genomic profiling of spatially distinct tumor sectors has revealed partial overlap in the mutation content in multiple samples from *IDH*-mutant lower-grade glioma<sup>17,36,43,44</sup> and *IDH*-wildtype GBMs.<sup>34,35,45–47</sup> Somatic mutations/DNA copy number alterations in important glioma driver genes such as *TP53* and phosphatase and tensin homolog (*PTEN*) have been found to be subclonal, suggesting they were acquired after tumor initiation. These unexpected discoveries show the many genetic routes tumor cells can take to overcome anti-tumorigenic barriers such as senescence and genomic instability. The possibility of extrachromosomal oncogene amplification adds an additional layer of complexity, allowing tumors to rapidly increase intratumoral heterogeneity in response to a microenvironment sparse in resources.<sup>48–53</sup>



**Fig. 2** Simplified glioma evolution models. The glioma-initiating cell evolves into the tumor at diagnosis with selective pressures resulting in intratumoral heterogeneity. Recurrent tumors share few or the majority of the somatic alterations seen in the diagnostic tumors depending on the evolutionary pattern (linear, branching, or ancestral evolutions). Subclones may be marked by mutations or extrachromosomal DNA elements.

Intratumoral mutation retention rates may be correlated with the geographical distance between samples in the tumor,<sup>47</sup> and by extension, the level of heterogeneity between different lesions of multifocal GBM is greater than between different areas in the same GBM.<sup>47,54,55</sup> Spatial heterogeneity determined by genetic alterations is reflected in the epigenetic patterns of different tumor sections examined by combined analysis of DNA methylation and genetic abnormalities.<sup>43,46</sup> These accumulating data suggest that intratumoral heterogeneity is encoded through a genomic–epigenomic codependent relationship,<sup>43</sup> in which epigenetic changes may modulate mutational susceptibility in proximal cells, and specific mutations dictate aberrant epigenetic patterns.<sup>43,56,57</sup> Although gene expression signatures can be used to subclassify GBMs, the predominant subtype often varies from region to region within a given tumor.<sup>35,46</sup> This relative instability may be in part due to the variable levels of tumor-associated non-neoplastic cells that can be found in different parts of the tumor.<sup>58,59</sup> Single-cell RNA sequencing of GBM cells has shown that glioma cells from the same tumor can correspond to different glioma subtypes, often with one dominating the others.<sup>47,59–61</sup> Single-cell transcriptomics extend previous observations of mosaic RTK amplification in a small subset of GBM to be

a more common disease characteristic.<sup>60,61</sup> Single-cell RNA sequencing further has shown cellular hierarchies along an axis of undifferentiated progenitors to more differentiated cell populations, reminiscent of the hematopoietic stem cell hierarchy. The balance shifts toward proliferating progenitors in *IDH*-wildtype glioma, reflecting the clinically more aggressive disease course.<sup>62,63</sup> These developmental and functional hierarchies are associated with dynamic neural stem cell expression patterns in which stem or progenitor cells may function as units of evolutionary selection (Fig. 2).

### Longitudinal DNA Profiling in Pretreatment and Posttreatment Tumors

One of the earliest reports on the effects of therapy on the tumor genomic landscape analyzed a 23-patient cohort of *IDH*-mutant lower-grade gliomas treated with temozolomide chemo.<sup>64</sup> A subset of the recurrent tumors acquired hundreds of new mutations that bore a characteristic signature of temozolomide-induced mutagenesis, suggesting that treatment pressure from an alkylating agent induced the growth of tumor cells with new mutations.<sup>36</sup> These



hypermutated tumors may be sensitive to immune checkpoint inhibitors,<sup>22</sup> including programmed death 1 (PD-1) inhibitors<sup>65</sup> and poly-adenosine diphosphate ribose polymerase inhibitors.<sup>66</sup> However, clinical trial data supporting these hypotheses have yet to emerge. Another study used whole-genome and multisector exome sequencing of 23 predominantly *IDH*-wildtype GBM and matched recurrent tumors.<sup>34</sup> This study showed that some GBM recurrences carried ancestral p53 driver mutations detectable in the primary GBM counterparts, suggesting an intrinsic resistance mechanism. Other recurrences were driven by branched subclonal mutations not present in the parental primary GBM. This may imply secondary or extrinsic resistance, reflecting treatment-induced resistance through DNA mutagenesis and a distinct evolutionary process (Fig. 2).<sup>34</sup> As in the study of *IDH*-mutant lower-grade gliomas, a subset of the disease recurrences was characterized by an accumulation of mutations in association with temozolomide treatment. Notably, this effect was limited to cases with *MGMT* promoter methylation. *MGMT* is a gene in the DNA repair pathway, and somatic mutations of other pathway members, such as mutS homolog 2 (*MSH2*) and *MSH6*, have been identified as drivers of the hypermutation process.<sup>67</sup> The spatiotemporal evolutionary trajectory in paired gliomas between initial diagnosis and relapse was further portrayed via integrative genomic and radiologic analyses through whole-exome sequencing (WES) of 38 primary and corresponding recurrent tumors.<sup>68</sup> Linear evolution, reminiscent of intrinsic resistance in which a recurrent tumor is genetically similar to the initial tumor, was predominantly observed in recurrent tumors that relapsed adjacent to the primary site. Branched evolution, associated with secondary or extrinsic resistance, was more common in recurrences at distant sites, which were marked by a substantial genetic divergence in their mutational profile from the initial tumor, with key driver alterations differing in more than 30% of cases. Geographically separated multifocal tumors and/or long-term recurrent tumors were seeded by distinct clones, as predicted by an evolution model defined as multiverse, ie, driven by multiple subclonal cell populations.<sup>47</sup> In an effort to elucidate the diverse evolutionary dynamics by which gliomas are initiated and recur, the clonal evolution of GBM under therapy was assessed from an aggregated analysis of datasets generated by multiple institutions.<sup>69</sup> Systematic review of the exome sequences from 93 patients revealed highly branched evolutionary patterns involving a Darwinian process of clonal replacement in which a subset of clones with a selective advantage during a standard treatment regimen renders the tumor susceptible to disease progression (Fig. 2). Mathematical modeling delineated the sequential order of somatic mutational events that constitute GBM genome architecture, identifying somatic mutations in *IDH1*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), and *ATRX* as early events of tumor progression, whereas *PTEN*, neurofibromatosis type 1 (*NF1*), and *EGFR* alterations were predicted to occur at a later stage of the evolution.<sup>47</sup> Similar observations have been reported from studies of low-grade gliomas, demonstrating that the somatic mutations in *IDH1*, *TP53*, and *ATRX* were frequently early and retained throughout tumor progression from primary to relapse.<sup>17,44</sup>

Longitudinal profiling of paired samples continues to provide deeper insights into the genomic background of treatment-induced hypermutagenesis. The latter has potential to increase aggressive clinical behavior and relevance in targeted and immunotherapy.<sup>17,44,70,71</sup> The implications of these pilot data and how these insights can be integrated into clinical practice require further evaluation. Collectively, longitudinal genomic profiling will be essential in implementing clinical application toward patient-tailored treatment regimens.

### Transcriptional Changes During Glioma Progression

Unsupervised transcriptome analysis of GBM converged on 4 expression subtypes, referred to as classical, mesenchymal, neural, and proneural, which are associated with specific genomic abnormalities.<sup>12,14,15,72</sup>

Transcriptional subtypes of the relatively homogeneous *IDH*-mutant and *IDH*-mutant 1p/19q-codeleted groups have been less emphasized in the literature, as these cases usually carry a proneural signature.<sup>10,12</sup> While expression subtype classification is a widely used research tool, it has not been shown to correlate with clinical outcome, and has not been incorporated in the recent 2016 WHO CNS tumor classification update. Much is still unknown about how transcriptional subclasses evolve under therapy. A switch from proneural to mesenchymal expression has been observed upon disease recurrence and was proposed to be a source of treatment resistance in GBM relapse,<sup>15,73,74</sup> but the relevance of this phenomenon in glioma progression remains ambiguous, particularly considering (i) the increased fraction of microglial/macrophage cells in mesenchymal GBM that confound subtype characterization<sup>58,59</sup> and (ii) glioma neurospheres derived from mesenchymal GBM that are frequently classified as proneural.<sup>74</sup> Deriving an expression subtype classification on the basis of glioma-intrinsic genes has maintained the proneural, classical, and mesenchymal classes.<sup>59</sup> Determining subtypes in a cohort of 91 *IDH*-wildtype GBM showed subtype switching following therapy and disease relapse in 45% of the cohort.<sup>59</sup> These patterns converged with changes in the microenvironment but also revealed that *NF1* loss results in macrophage/microglia recruitment. The ability of genomic abnormalities to regulate the tumor microenvironment shows how tumors act as a system, rather than an aggregation of individual cells.

### Epigenetic Changes During Glioma Progression

DNA methylation profiling of gliomas has prognostic value independent of patient age and the pathologic grade of the tumor.<sup>9</sup> Evidence suggests that evolutionary selection can also act on the epigenome, affording cells plasticity to resist therapy.<sup>9,43</sup> For example, recurrent *IDH*-mutant gliomas profiled for mutations and DNA methylation independently evolved deregulation of their cell cycle programs through genetic mutations or epigenetic mechanisms.<sup>43</sup>

Nearly all *IDH*-mutant gliomas exhibit a characteristic cytosine-phosphate-guanine island hypermethylator phenotype (G-CIMP), which (i) induces silencing of key

extracellular matrix and cell migration gene promoters.<sup>10</sup> (ii) mediates alteration of chromosome topography, leading to oncogene upregulation,<sup>75,76</sup> (iii) mediates histone methylation-related changes in gene expression, and (iv) may play a role in creating an immunosuppressed micro-environment.<sup>77</sup> While almost all *IDH*-mutant tumors are G-CIMP at diagnosis, a longitudinal analysis showed that 34% of cases exhibited demethylation toward G-CIMP–intermediate or G-CIMP–low DNA methylation at recurrence.<sup>78</sup> Substantial epigenetic heterogeneity between tumor samples from the same patient collected at subsequent surgeries was also observed in a cohort of 112 primary mostly non-G-CIMP GBM patients.<sup>79</sup> Characteristic trends in DNA methylation between primary and relapsed GBM included a prominent demethylation of gene promoters related to Wnt signaling, which was associated with worse patient outcome. Moreover, patients whose primary tumors harbored higher levels of DNA methylation heterogeneity showed longer progression-free survival and a trend toward longer overall survival.<sup>79</sup>

### Imaging and (Epi)genomics

MRI is noninvasive, with no risk of radiation exposure. Standard MRI includes precontrast and postcontrast T1-weighted (T1w) and T2-weighted (T2w)/T2w fluid-attenuated inversion recovery (T2-FLAIR) imaging assessing tumor location, size, and other features.<sup>80</sup> Newer techniques such as perfusion imaging provide a measure of tumor vascularization in terms of relative cerebral blood volume, which correlates with tumor grade.<sup>81,82</sup> There is interest in exploring the relationships between MR findings such as cerebral blood volume with the biological behavior of tumors—for example, to determine risk prior to surgery.

In the rapidly growing field called radiogenomics,<sup>83</sup> quantitative imaging features can be linked with genomic profiles, with recent applications in high-grade glioma.<sup>83,84</sup> A priority of radiogenomics is to identify MRI-based biomarkers for glioma subtypes such as *IDH*-mutant versus wildtype and 1p/19q codeleted versus non-codeleted. Noninvasive phenotypic assessment provides an early test to stratify *IDH*-mutant non-codeleted gliomas and may offer prognostic information through MRI with the potential to influence patient outcomes and determine risk prior to surgery.<sup>85</sup> It may also help in selecting personalized treatments in clinical trials.<sup>86</sup> A detailed global assessment of the spatial and longitudinal heterogeneity of gliomas is potentially feasible.<sup>87</sup>

### Barriers to Progress

The major obstacle for glioma patients is a lack of effective treatments, which may result from cell-intrinsic resistance or treatment-resistant glioma cells being favored over treatment-sensitive cells, augmented or attenuated by micro-environmental influences, including hypoxia and stromal elements. That therapy has profound effects on tumor composition is reflected by the temozolomide-induced hypermutator phenotype.<sup>64</sup> As a result, the molecular characteristics of the recurrent tumor differ in significant ways

from those found in the primary tumor.<sup>34,36</sup> TCGA and similar initiatives elsewhere have established comprehensive portraits of the interpatient variability of untreated glioma genomes. Single-cell sequencing and barcoding experiments have demonstrated functional hierarchies providing important insights into characteristics of the most relevant cells to target.<sup>62,63</sup> We are increasingly able to infer the life history of glioma,<sup>33</sup> from germline predispositions<sup>88,89</sup> and tumor-initiating events such as *IDH1* mutation to tumor-promoting events such as RTK alterations. To improve the outcomes of patients with gliomas, we need to establish a thorough understanding of the treatment-induced molecular and genetic diversity that leads to resistance.

A detailed understanding of the biological diversity within every tumor following clinical presentation and disease progression is needed if we are to successfully understand how treatment affects glioma progression. This is an essential step toward the integration of precision therapeutics into clinical decision making, highlighting the danger in considering treatment options for patients with recurrent tumors solely on the basis of the molecular analysis of their treatment-naïve tumors. This is particularly important in the setting of clinical research, which often recruits patients with recurrent GBM to evaluate drugs developed on the basis of mechanistic data obtained on treatment-naïve tumors.

Studying the heterogeneity and spatiotemporal evolution of cancer in general, and particularly in brain cancer, is challenging. Many tumor samples—and therefore large-scale collaboration—are needed to achieve meaningful comprehensive results and to capture low-frequency alterations or subtle therapy-driving processes. Individual research groups typically do not have the resources to use a multiplatform analysis of their samples, owing to cost or the availability of expertise. Published longitudinal datasets consist of a mixture of different modalities, ranging from only exomes<sup>36</sup> or DNA methylation profiles<sup>43,79</sup> to a combination of exome sequencing, RNA sequencing, and DNA copy number profiling,<sup>34,59</sup> thwarting meta-analyses based on cross-publication comparisons. The value of establishing a comprehensive multiplatform reference dataset quickly has been demonstrated by the success of TCGA, the International Cancer Genomics Consortium (ICGC), the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) Consortium, and other glioma projects, which have led to a fundamental reclassification of gliomas by the WHO<sup>19</sup> and are highly cited.<sup>8,10–12,90,91</sup> Similarly, a consortium would be the most effective approach to assemble the large cohorts of primary and recurrent tumor pairs needed to identify somatic alterations enriched after disease progression. Systematizing and standardizing what we do and how we do it will be essential for change to clinical practice in neuro-oncology. This philosophy is at the core of the international GLASS Consortium.

### The Glioma Longitudinal Analysis (GLASS) Consortium

Large-scale collaborations are needed to help us understand the impact of treatment on evolutionary dynamics

and thereby develop novel treatments to prevent and overcome resistance to treatment. GLASS aims to perform comprehensive molecular profiling of matched primary and recurrent glioma specimens from 1500 patients, 500 in each of the 3 major glioma molecular subtypes. At the time of writing, the consortium includes investigators from 34 academic hospitals, universities, and research institutes from 12 countries (see list of participants on the GLASS website, <http://www.glass-consortium.org>). By analogy with the ICGC,<sup>90</sup> GLASS is structured into country-specific franchises (GLASS-NL, GLASS-AT, GLASS-AU, GLASS-Korea, etc) led by local investigators who are invested in the team's overall goal, while taking advantage of country-specific opportunities. This enables each GLASS branch to have unique features that allow a deeper analysis of subcohorts, that is, with additional imaging annotation, parallel characterization of drug response through xenografting of tumor samples, autopsies, a specific focus on a glioma subtype, etc, thereby making them competitive and enabling them to address non-overlapping aspects of the phenotypic diversity seen in the clinic. Country-specific branches will be coordinated to connect with the larger analyses and to drive specific research topics for both. There are no explicit restrictions on publishing, and each group is invited to publish their sub-studies independently. The overall goal is to establish a reference dataset by pooling samples and aggregate data from all multiplatform analyses, countries, and substudies, and to make datasets comparable through coordinated sample and data processing guidelines. Country franchises are centrally connected through a number of committees, each overseeing different aspects of the analysis.

### Biospecimen Acquisition and Characterization Platforms

Biospecimens from gliomas are often snap-frozen or conserved as formalin-fixed, paraffin-embedded (FFPE) samples. For genomic and transcriptomic analyses, snap-frozen material is preferred, while historically FFPE is the common approach to tissue preservation. Methods for generating sequencing data from FFPE material are increasingly improving, with 5%–20% of samples failing quality controls. Given that samples from multiple timepoints are required for inclusion into GLASS, patients for whom only FFPE material is available are twice as likely to not yield sufficient high-quality DNA. While the increased failure rate means we will have to include a higher number of samples, we do not see this as prohibitive and are actively pursuing the use of FFPE material. RNA extracted from glioma tissue is often highly degraded, resulting in higher attrition rates,<sup>92</sup> but high-quality RNA sequencing data from FFPE samples have been reported.<sup>93</sup> For DNA methylation profiling of FFPE material, a recent study focusing on primary glioblastoma reported a high success rate using the reduced representation bisulfite sequencing assay.<sup>79</sup>

While we require the availability of a matching germline sample (blood or other) for inclusion of DNA sequencing data into GLASS, cases without a germline match may be candidates for transcriptome and DNA methylation analysis. Ideally, we aim to generate DNA, RNA, and epigenomic sequencing data from every tumor. Single-cell analysis

methods require fresh tissue from which individual cells can be dissociated; this may be considered in the future as the project evolves or as part of specific subprojects. Similarly, subsets of the GLASS cohort will be compared longitudinally by spatial correlation using multisector analysis (3–6 samples per tumor) to understand whether any differences between paired tumor samples are the result of intratumoral heterogeneity or longitudinal heterogeneity. Where available, these will be correlated with conventional and novel MR imaging to explore spatiotemporal heterogeneity noninvasively. We aim to take current radiogenomic approaches further, not only to establish the features of genetic characteristics at first diagnosis, but also in relation to molecular alterations over time and under the pressure of standard therapy. Comprehensive genomic sequencing is needed to identify patterns of disease evolution as well as the key mutations and chromosomal alterations that confer resistance to standard radiation, temozolomide, and novel clinical trial therapies. Sequencing paradigms and their costs are rapidly evolving, and each method provides different but complementary information. There is no consensus on optimal methods. With the accessibility of 30x coverage whole-genome sequencing (WGS) at \$1100 per biospecimen, the costs of WGS and WES have become comparable. The coverage of a typical WES is between 60x and 100x, which enables greater sensitivity in detecting mutations in coding regions, but WES does not interrogate noncoding regions of the genome and is not able to detect structural variants or noncoding copy number variants. The comprehensive nature of WGS enables analysis of evolution and clonality at higher resolution. WGS and WES combined may provide the optimal window on the breadth, depth, and allelic fraction of somatic events. However, where limitations in tissue or resources mandate a choice of one or the other, the decision will depend on the purpose of the (sub)project. GLASS franchises with a focus on clinical relevance may lean toward WES, while projects aiming to define clonal relationships may opt to perform WGS.

Targeted sequencing data analysis in the absence of a matching germline sample is frequently performed in the clinical setting, and such datasets, which are typically able to provide mutation calls on 20–400 genes, may therefore be easily accessible. While GLASS does not intend to pursue generating such datasets, aggregating information from existing resources may be a viable option to learn or validate mutations enriched at diagnosis or recurrence.

### Clinical Annotation in GLASS

Aggregating clinical annotation across the consortium will help enable linkage of genotype with clinical and morphological phenotype in primary and recurrent settings. The number of clinical annotation elements will be different in each country with minimal requirements (Box 1). The GLASS clinical annotation committee will standardize clinical and imaging data collection for prospective studies and oversee collection of the clinical and imaging data from patients whose profiles are already included in the composite dataset. Each individual franchise will make data accessible in a comprehensible way by integrating

**Box 1.** GLASS aims to collect genome-wide DNA, RNA, and epigenomic sequencing data on 1500 glioma tissues and matched recurrent tissues. To be included in this core set of cases, tissues and germline reference are required, with a minimal clinical dataset. Submission of standard cases without a germline source or without complete molecular profiling is encouraged. To generate a comprehensive data resource for the molecular study of glioma recurrence, cases with molecular data on matched primary/recurrent specimens will be collected into an archive.

\* All data should be provided in compliance with HIPAA regulations, ie, dates as intervals.

#### CORE CASE REQUIREMENTS

- Primary diagnosis of glioma (WHO Grade II-IV) with frozen/FFPE tumor specimen
- Matched recurrent diagnosis of glioma (WHO Grade II-IV) with frozen/FFPE tumor specimen
- Matched germline reference specimen

#### OR

- Global DNA sequencing (WES or WGS) on matched glioma pairs (Grade II-IV primary) and germline reference specimen **and** RNA sequence **and** DNA methylation on matched glioma pairs (Grade II-IV primary)

#### WITH

- Clinical data: age at diagnosis, year of diagnosis, time from diagnosis to recurrence, treatment history between diagnoses\*

#### STANDARD CASE REQUIREMENTS

- Primary diagnosis of glioma (WHO Grade II-IV) with frozen/FFPE tumor specimen
- Matched recurrent diagnosis of glioma (WHO Grade II-IV) with frozen/FFPE tumor specimen

#### OR

- Global DNA sequencing (WES or WGS) on matched glioma pairs (Grade II-IV primary) and germline reference specimen **and/or** RNA sequence **and/or** DNA methylation on matched glioma pairs (Grade II-IV primary)

#### WITH

- Clinical data: age at diagnosis, year of diagnosis, time from diagnosis to recurrence, treatment history between diagnoses\*
- IDH mutation and 1p/19q co-deletion status if DNA sequence is not available

#### ARCHIVE CASE REQUIREMENTS

- Molecular data on primary/recurrent matched glioma specimens

#### WITH

- Clinical data: age at diagnosis, year of diagnosis, time from diagnosis to recurrence\*

clinical, imaging, and molecular parameters to explore correlation with relapse data. Currently, radiology and imaging are part of the clinical annotation committee. By mapping imaging features in a voxel-wise manner and correlating these spatially with molecular alterations obtained from different parts of the tumor, we aim to assess the entire tumor and to determine intratumoral heterogeneity.

## Data Infrastructure

A designated committee will maintain standardized data processing, data management, and data sharing. A characteristic of the GLASS Consortium is that data will be generated at multiple institutions distributed over multiple countries. As the regulations pertaining to ethical use of sequencing datasets are continuously evolving, GLASS will follow the example set by ICGC to perform decentralized data analysis to avoid cross-border exchange of patient-sensitive raw sequencing data. Batch effects may arise from varying library preparations, analyzing fresh-frozen versus FFPE tissue, sequencing platforms, laboratories, etc. Batch effects are most perturbing when performing unsupervised

analysis, such as unsupervised clustering from expression or DNA methylation profiles. Adequately correcting for these items will be necessary to obtain usable data.

The GLASS data infrastructure committee has developed Docker software images that are shared among participating institutions and that enable analysis uniformity. Like a shipping container, a Docker image packages one or more software tools to establish a workflow resembling an executable application. Comparable to platform-independent Java software, the ready-to-run Docker images are independent of the local computational environment. Along the same lines, comparable Singularity images have been prepared. The GLASS participants run these images locally, which initializes a per-sample-per-analysis Docker/Singularity container, resulting in data analysis using an identical software environment and run parameters. Docker/Singularity images and documentation are available for download through <http://docker.glass-consortium.org>.

The data infrastructure committee will also coordinate mechanisms for dissemination of results, so as to widely share datasets with the community. We may explore mechanisms such as the Genomic Data Commons, or similar, to align our efforts with other molecular profiling studies.



## Final Remarks and Perspectives

Survival and quality of life for patients with diffuse gliomas remain dismal with standard treatments. Diffuse glioma is a fatal disease with an enormous societal burden as a result of the short survival following high-grade disease and the young age at diagnosis of lower-grade disease. This not only affects patients in the prime of their lives, but also puts enormous burden on their immediate entourage, as they need extensive supportive care and navigation through a complicated medical landscape, and experience difficulties with medical costs and insurance. While cures of diffuse gliomas remain elusive, our patients demand better therapies. With no substantive impact of molecular medicine to date, in practice treatments remain “one size fits all.” The GLASS Consortium aims to improve clinical outcomes by establishing a broadly useful dataset that will provide pivotal new insights into the mechanisms used by gliomas to defy therapeutic challenges.

Importantly, GLASS is also an opportunity for the exchange of knowledge among an international group of collaborators to ultimately build smarter clinical trials and develop therapies that will extend survival and improve the quality of life of people with diffuse gliomas. GLASS is well positioned to demonstrate the value of well-coordinated collaborative efforts. To that end, new investigators are invited to join the consortium, where the major criteria for participation are the ability to offer datasets of longitudinally profiled glioma patients or the availability of suitable tissue samples.

In summary, through the GLASS Consortium, we aspire to continue the immeasurable success of TCGA while increasing the focus on making a difference to patients and their families.

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