

Divergent genomic evolution in astrocytomas and oligodendrogliomas

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Astrocytomas and oligodendrogliomas are isocitrate dehydrogenase (IDH)-mutant gliomas that most commonly arise in adults under the age of 50 and exhibit variable responses to treatment^{1–3}. To understand the genetic drivers of disease progression, here we used whole-genome and whole-exome sequencing to characterize longitudinally collected tumour specimens from 183 patients with IDH-mutant glioma. Samples were collected through the Glioma Longitudinal Analysis (GLASS) Consortium⁴. Most primary surgery samples were classified as grade 2 on the basis of histology (73% for astrocytomas and 93% for oligodendrogliomas). We validated known genomic markers of tumour progression, including hypermutation and homozygous deletion of *CDKN2A* or *CDKN2B* (hereafter, *CDKN2A/B*)⁵, and discovered genetic alterations that distinguish between the evolutionary trajectories of astrocytomas and those of oligodendrogliomas. Mutations in phosphatidylinositol 3-kinase (PI3K) and *NOTCH1* were newly acquired in recurrent oligodendrogliomas, regardless of treatment, and were associated with an increased tumour mutational burden. In recurrent astrocytomas, however, again irrespective of treatment, acquired focal oncogene amplifications and *CDKN2A/B* homozygous deletions were associated with an increase in chromosomal imbalances, which converged with markers of chromosomal instability such as chromothripsis and whole-genome doubling. In both types of IDH-mutant glioma, we observed a convergence of acquired alterations, genome-wide changes and worse patient outcomes, suggesting that treatment-refractory clones were being selected. Treatment with temozolomide was associated with the mutational signatures SBS11 and SBS119 in both tumour types, whereas the radiotherapy-associated ID8 signature increased in astrocytomas specifically. We show that the genomes of oligodendrogliomas and astrocytomas adapt to the selective pressures of tumour progression and treatment in distinct ways, suggesting that oligodendrogliomas and astrocytomas differ in their responses to treatment-induced DNA damage.

Isocitrate dehydrogenase (IDH)-mutant gliomas, named for gain-of-function mutations in the metabolic-enzyme genes *IDH1* and *IDH2*, are the most common primary malignant brain tumours in adults under the age of 50^{1,2}. The 2021 World Health Organization (WHO) classification for tumours of the central nervous system (CNS) stratifies IDH-mutant gliomas into two clinically and molecularly distinct types: (1) IDH-mutant oligodendrogliomas (WHO grades 2–3), characterized by the combined loss of chromosome arms 1p and 19q; and (2) IDH-mutant astrocytomas (WHO grades 2–4), which lack the 1p/19q codeletion⁶. Oligodendrogliomas are also characterized by mutations in the *TERT* promoter, whereas astrocytomas instead prominently feature mutations in *TP53* and *ATRX*⁷. The primary treatment for patients with an IDH-mutant glioma combines surgical debulking, alkylating chemotherapy and ionizing radiation; less aggressive tumours (WHO grade 2) might be treated with just surgical debulking⁸. Despite these therapies, virtually all IDH-mutant gliomas develop resistance to treatment, which results in tumour recurrence⁹. Because IDH-mutant gliomas are usually diagnosed in young to middle-aged individuals², the ubiquitous

emergence of treatment resistance results in a disproportionate number of life years lost³. Various molecular responses to treatment have been reported, including alkylating-agent-induced hypermutation¹⁰ and radiation-associated *CDKN2A/B* homozygous deletions¹¹, but it is unknown whether treatment-associated molecular responses exist that are specific to the individual IDH-mutant glioma types.

The Glioma Longitudinal Analysis (GLASS) Consortium⁴ seeks to advance researchers' understanding of how gliomas respond to treatment through genomic characterization of glioma tumours over time^{12,13}. Longitudinal profiling studies from the GLASS Consortium and others have shown that a subset of IDH-mutant gliomas treated with alkylating agents acquires a hypermutant genotype⁵; that gains of oncogenes such as *MYC* and *PDGFRA* are associated with shorter post-recurrence survival¹⁴; and that radiotherapy increases the frequency of the ID8 mutational signature and broad copy-number changes^{11,15,16}. These mutational footprints suggest selection of pre-existing treatment-resistant subclones or direct DNA damage associated with the radiation and alkylating agents, providing a rationale for

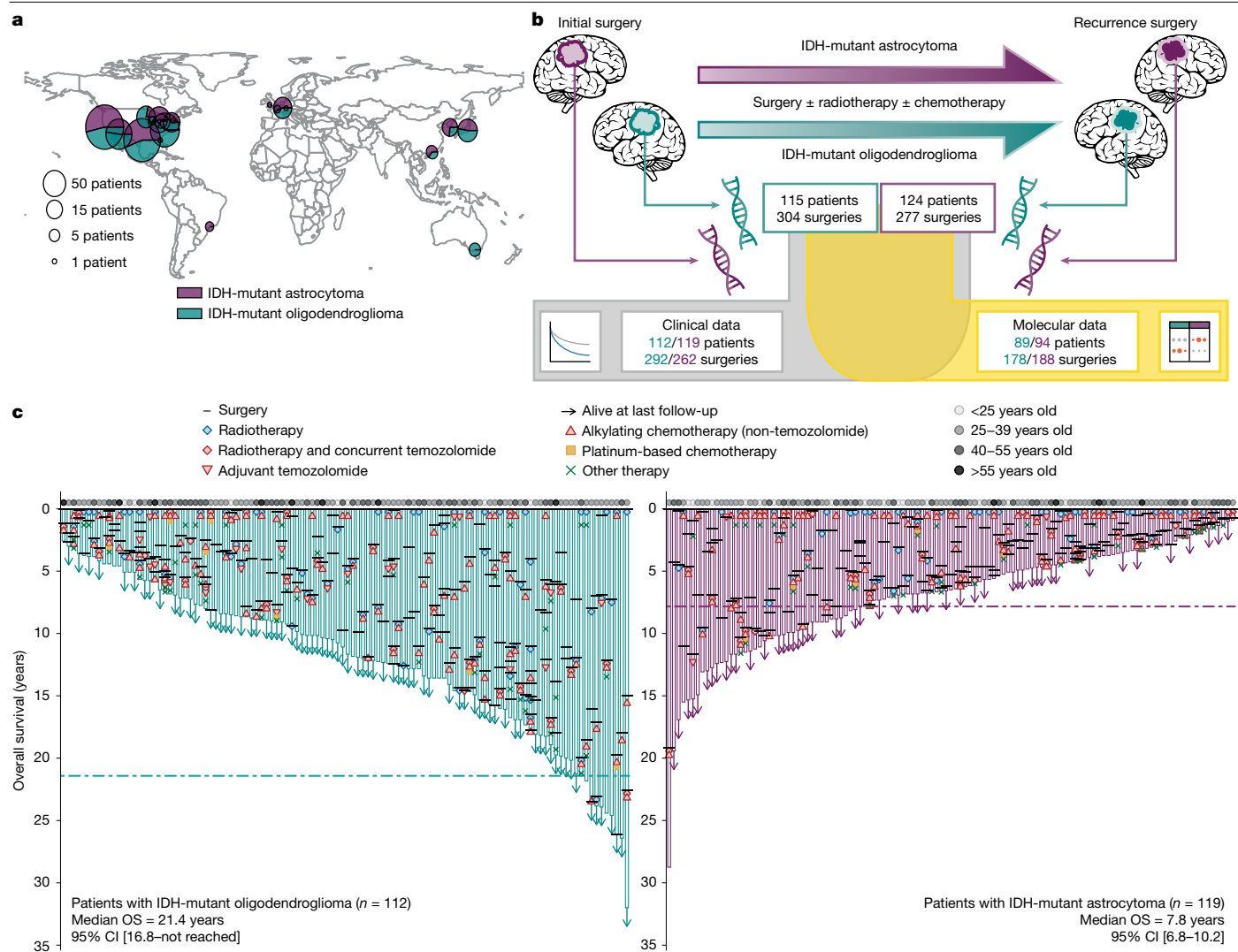


Fig. 1 | GLASS: a comprehensive global dataset on the evolution of IDH-mutant glioma. **a**, The GLASS cohort of patients with IDH-mutant glioma represents people from five continents, illustrated by the numbers of patients in the contributing cities and the proportions of the individual glioma types. **b**, The GLASS study included 239 patients with IDH-mutant glioma. Of these patients, longitudinal data were collected across at least two time-separated surgeries: clinical data ($n = 231$) and QC-passed molecular data ($n = 183$).

c, Extensive clinical data have been acquired with respect to treatment and prognostic factors; overall survival (OS) is shown for the clinical cohort ($n = 231$) of which 126 patients were still alive at last follow-up, as specified by the arrows. The dotted lines represent the median overall survival per glioma type. CI, confidence interval. Brain illustration in **b** reproduced with permission from maglyvi/Shutterstock.com (<https://www.shutterstock.com/nl/image-vector/vector-outline-illustration-human-brain-on-157155017>).

targeting the engaged biological networks¹⁷. Here, we comprehensively characterize the genomic responses to treatment of IDH-mutant gliomas by analysing whole-genome sequencing (WGS) and whole-exome sequencing (WXS) longitudinal datasets from 183 patients with highly detailed clinical annotation included in the GLASS Data Resource⁴. Our findings show how treatment shapes driver-gene alterations, mutational signatures, chromosomal instability and tumour clonality, revealing the distinct evolutionary trajectories in IDH-mutant gliomas with and without 1p/19q codeletion.

Clinical characteristics and timelines

Tumour specimens and clinical annotation from patients diagnosed with an IDH-mutant glioma were contributed by 21 institutions from Australia, Brazil, China, France, Germany, Japan, Luxembourg, South Korea, the UK and the USA (Fig. 1a). Owing to the longitudinal nature of our study, patients had undergone at least two time-separated surgical procedures to acquire tumour tissue through debulking resections (92% of surgeries) or biopsies (8%). Longitudinal datasets were generated

from 581 surgeries in 239 patients, with available clinical data for 231 patients (Fig. 1b). After applying rigorous quality filters, tumour samples from 183 patients (94 astrocytomas and 89 oligodendrogliomas) at two time points were retained for analyses, selecting the first and last available tumour sample in cases of multiples (Fig. 1b and Extended Data Fig. 1a,b). Molecular profiling was performed on 183 patients using WGS (39 astrocytomas and 56 oligodendrogliomas) and WXS (55 astrocytomas and 33 oligodendrogliomas). Across the cohort, 122 tumour pairs were obtained at diagnosis (Tx) and first recurrence (R1), and 61 tumour pairs were included with other surgery timing combinations: Tx–R2 ($n = 20$), Tx–R3 ($n = 2$), R1–R2 ($n = 19$), R1–R3 ($n = 8$), R1–R5 ($n = 3$), R2–R3 ($n = 7$), R2–R5 ($n = 1$) and R3–R4 ($n = 1$). Analyses reflecting results from all tumour pairs are referred to as initial-recurrence pairs ($n = 183$); analyses that include only primary-recurrent pairs ($n = 144$) are indicated accordingly.

The extensive clinical follow-up in the GLASS cohort was a strength of our study: a clinical annotation dataset was established by standardizing terminology across institutions, comprising 262 surgeries in 119 patients with astrocytoma and 292 surgeries in 112 patients with

oligodendroglioma (Fig. 1b,c). In addition to patient demographics, tumour pathology (2021 WHO grade and histology), surgery details (resection versus biopsy), treatment regimens (chemotherapy, radiation therapy and other treatments) and treatment outcomes (surgical interval and overall survival) were obtained.

To assess potential inclusion biases, we compared the clinical characteristics of the GLASS cohort with those of two reference datasets: The Cancer Genome Atlas (TCGA)⁷ and the Central Brain Tumor Registry of the United States (CBTRUS)². Patients from our cohort were diagnosed at a significantly younger age than were individuals with IDH-mutant glioma in TCGA (Wilcoxon test: $P = 0.006$ for astrocytomas, $P < 0.001$ for oligodendrogliomas; Extended Data Fig. 2a). This is consistent with broader epidemiological patterns showing that patients in the GLASS cohort are younger than those in CBTRUS (astrocytoma, 34 versus 37 years; oligodendroglioma, 37 versus 45 years)². Patients with oligodendroglioma in the GLASS cohort had a longer overall survival from the time of diagnosis than did those in TCGA, but overall survival from the time of birth was significantly shorter in the GLASS cohort for both patients with oligodendroglioma and those with astrocytoma (Extended Data Fig. 2b,c). CBTRUS has not yet released mature survival data for these entities². The median follow-up period for the GLASS cohort was 6.5 years for patients with astrocytoma and 11.4 years for patients with oligodendroglioma (Table 1), compared with 2.3 years and 1.9 years, respectively, in TCGA (Wilcoxon test: $P < 0.001$ for both). The differences between cohorts might have resulted from an inclusion bias towards patients who have undergone multiple surgeries; however, follow-up was significantly larger for the GLASS cohort than it was for these historical datasets.

Most of the initial tumours were classified histologically as grade 2 (astrocytomas, 73%; oligodendrogliomas, 93%). Tumour grade increased between time points in 59% of astrocytoma and 47% of oligodendrogliomas. Throughout their treatment, 74% of GLASS-cohort patients with IDH-mutant glioma were reported to have received alkylating chemotherapy, with temozolomide being the main alkylating agent used (71% for both tumour types; Fig. 1c). Platinum-based chemotherapy was the second chemotherapy of choice and was always used in addition to alkylating chemotherapy (astrocytomas, 4%; oligodendrogliomas, 6%). Radiotherapy was used to treat 68% of patients with astrocytoma and 69% of patients with oligodendroglioma. Non-standard therapies were used in 30% of patients with IDH-mutant glioma, and included anti-angiogenic therapy (15%) and retinol-based drugs such as all-trans retinoic acid (3%). A small number of patients (astrocytomas, 17%; oligodendrogliomas, 14%) only received surgical treatment during their known follow-up trajectories. A minor subset (22%) of the cohort received the combination of radiotherapy and alkylating agents as primary treatment after surgery, reflecting a post-surgical watch-and-wait approach that is an accepted strategy for low-risk grade 2 gliomas¹⁸.

All processed genomic data and clinical annotations are available via <https://www.synapse.org/glass>, and a summarized version of the processed molecular data that passed quality control (QC) per patient is available in Supplementary Table 1.

Diverging genomic driver events

We previously reported that genomic alterations such as somatic hypermutation or *CDKN2A* homozygous deletion can be detected in a subset of IDH-mutant gliomas at the time of disease progression, which were associated with alkylating chemotherapy or radiotherapy, respectively^{10,11}. We evaluated the larger GLASS cohort to identify new drivers of tumour progression that were specifically detected at the time of disease recurrence.

At initial surgery, we found a significant enrichment of mutations in canonical driver genes using dNdScv analyses (astrocytomas, *TP53* and *ATRX*; oligodendrogliomas, *CIC* and *FUBP1*), which were preserved at recurrence⁷ (Fig. 2a and Extended Data Fig. 3a–d). We did not investigate

Table 1 | Clinical characteristics of patients with IDH-mutant glioma from the GLASS cohort at baseline

	IDH-mutant oligodendroglioma (<i>n</i> = 112)	IDH-mutant astrocytoma (<i>n</i> = 119)	<i>P</i> value
Demographics			
- Age at diagnosis (years)			0.005
Median [minimum, maximum]	37 [17, 70]	34 [14, 67]	
- Sex			0.8
Female	39% (44/112)	38% (45/119)	
Male	61% (68/112)	62% (74/119)	
- Follow-up duration (years)			6.5 × 10 ⁻¹²
Median [minimum, maximum]	11.4 [2.6, 32.0]	6.5 [0.9, 28.8]	
Primary treatment			
- Surgery			0.6
Biopsy	13% (11/85)	10% (6/61)	
Resection	87% (74/85)	90% (55/61)	
Unknown	27	58	
- Radiotherapy			0.03
Non-treated	68% (60/88)	53% (56/106)	
Treated	32% (28/88)	47% (50/106)	
Unknown	24	13	
- Alkylating chemotherapy			0.2
Non-treated	64% (54/85)	54% (53/98)	
Treated	36% (31/85)	46% (45/98)	
Unknown	27	21	
- Platinum-based chemotherapy			0.5
Non-treated	94% (32/34)	100% (23/23)	
Treated	6% (2/34)	0% (0/23)	
Unknown	78	96	
- Other therapies			0.3
Non-treated	74% (25/34)	61% (14/23)	
Treated	26% (9/34)	39% (9/23)	
Unknown	78	96	
Primary pathology			
Grade 2	93% (75/81)	73% (75/103)	
Grade 3	7% (6/81)	15% (15/103)	
Grade 4		13% (13/103)	
Unknown	31	16	

Characteristics assessed included patient demographics, follow-up duration, treatment after primary surgery and tumour grade according to the 2021 WHO CNS tumour classification. These unpaired data were compared between glioma types using the two-sided Wilcoxon rank-sum test (for continuous variables), and either the two-sided Fisher's exact test or the two-sided χ^2 test (for categorical variables) where appropriate.

non-coding drivers beyond *TERT* promoter mutations, supported by previous reports in IDH-mutant gliomas¹⁹. As expected, a subset of both astrocytomas (21%) and oligodendrogliomas (18%) that received alkylating chemotherapy recurred with a significantly higher number of mutations (more than 10 mutations per Mb), of which more than 25% were attributed to the SBS11 mutational signature that has previously been associated with treatment with alkylating agents²⁰. We therefore labelled these samples as hypermutators. Among non-hypermutated recurrent oligodendrogliomas (*n* = 70), we discovered significant



Fig. 2 | sSNVs drive the progression of oligodendrogliomas, whereas astrocytomas progress through CNAs. a, Landscape of sSNVs and CNAs in patients with mutation and DNA copy-number profiles from at least two time points ($n = 183$). Individual patients (columns) are ordered by mutation frequency at recurrence. Rows from top to bottom: (1) mutation frequency (black dotted line indicates 10 mutations (mut) per Mb); (2) distribution of mutation timing; (3) driver-gene mutations in IDH-mutant glioma; (4) driver focal oncogene amplifications and homozygous deletions in IDH-mutant

glioma; and (5) clinical markers. Alkyl., alkylating. **b**, Selected examples from rows (3) and (4) in **a**. Hypermutant (HM) gliomas exhibit more than 10 mutations per Mb and have more than 25% of recurrence-specific mutations attributable to SBS11; non-hypermutant (non-HM) gliomas exhibit fewer than 10 mutations per Mb. Left parts indicate the presence of selected driver sSNV or CNA events, right parts represent the timing of driver alterations. Focal onc. amp., focal oncogene amplifications (that is, *CCND2*, *CDK4*, *CDK6*, *EGFR*, *MYC*, *MYCN*, *MET*, *PDGFRA* or *PTPRZ1*); *PI3K*, *PIK3CA* and *PIK3R1* combined.

enrichments of somatic single-nucleotide variants (sSNVs) in *NOTCH1* and the PI3K catalytic subunit *PIK3CA*, along with a trend towards enrichment of the regulatory subunit *PIK3R1* (Fig. 2b and Extended Data Fig. 3a). *NOTCH1* and PI3K mutations that were found at oligodendroglioma recurrence were not associated with specific previous treatments and showed mutual exclusivity (DISCOVER algorithm²¹; $P = 0.047$ at primary and $P = 0.01$ at non-hypermutant recurrence; Extended Data Fig. 4a). In leukaemias, *NOTCH1* has been implicated as a regulator of *PTEN* that has downstream effects on PI3K signalling, which could

explain why in oligodendrogliomas PI3K and *NOTCH1* mutations are mutually exclusive^{22,23}. *NOTCH1* mutations in oligodendrogliomas have been associated with reduced cellular differentiation and resistance to IDH inhibitors, suggesting that these mutations are biomarkers of IDH-inhibitor resistance in oligodendrogliomas²⁴. We observed the previously reported significant enrichment of *MSH6* mutations at recurrence as well as newly acquired *PIK3CA* mutations in hypermutated oligodendrogliomas (mutations at diagnosis: *MSH6* = 0/10, *PIK3CA* = 2/10; mutations at recurrence: *MSH6* = 12/16, *PIK3CA* = 13/16;

Extended Data Fig. 3b). We also noticed a non-significant increase in the mutation burden in hypermutated IDH-mutant gliomas with *MSH6* mutations, compared with *MSH6*-wild-type hypermutants, and found that this trend was consistent in both the GLASS cohort and the external validation set of the American Association for Cancer Research (AACR) Genomics Evidence Neoplasia Information Exchange (GENIE) cohort²⁵ (Extended Data Fig. 4b). Although some hypermutated astrocytomas acquired *MSH6* mutations ($n = 7/20$), this enrichment did not reach statistical significance (Extended Data Fig. 3d). In contrast to oligodendrogliomas, no gene mutations in astrocytomas were significantly associated with recurrence, regardless of hypermutation status (Extended Data Fig. 3c,d).

We used GISTIC (Genomic Identification of Significant Targets in Cancer)²⁶ to identify focal DNA copy-number gains and losses that target well-described oncogenes and tumour suppressor genes, focusing on events detected at recurrence. Focal *CDKN2A/B* homozygous deletions were significantly enriched in recurrent non-hypermutant astrocytomas (McNemar's test: $P = 0.009$) but not in non-hypermutant oligodendrogliomas (McNemar's test: $P = 0.48$; Supplementary Table 2). We found that focal high-level oncogene amplifications (chromosomal bands: *CCND2* 12p13, *CDK4* 12q14, *CDK6* 7q21, *EGFR* 7p11, *MYC* 8q24, *MYCN* 2p24, *PDGFRA* 4q12 and *MET-PTPRZ1* 7q31) were detected at significantly increased frequency in recurrent astrocytomas. In total, 38 of 94 recurrent astrocytomas contained at least one focal oncogene amplification, compared with 21 of 94 initial astrocytomas. A minority (18%) of the individual WGS-derived focal gene amplifications were classified as extrachromosomal, and all were identified in the astrocytomas. There was no significant enrichment of amplifications or homozygous deletions in recurrent oligodendrogliomas (Supplementary Table 2).

Using Sequenza-derived allele-specific copy-number profiles and PyClone-VI clustering, we classified mutations as clonal (high allelic fraction) or subclonal (reduced allelic fraction). A significant reduction in clonal mutation burden at recurrence was observed exclusively in hypermutant astrocytomas, whereas oligodendrogliomas showed no significant longitudinal change irrespective of hypermutation status (Extended Data Fig. 5a). Driver-level analysis revealed that acquired *PIK3CA*, *NOTCH1* and *PIK3R1* mutations were predominantly clonal in oligodendrogliomas. Acquired *CDKN2A* deletions were mostly clonal in astrocytomas, suggesting a strong selection effect on cells containing *CDKN2A* homozygous deletions (Extended Data Fig. 5b).

Previous studies have shown that tumours with the canonical *IDH1* p.R132H mutation differ in D-2-hydroxyglutarate levels, DNA methylation patterns and survival outcomes, compared with tumours that have non-p.R132H or *IDH2* mutations²⁷. Beyond the *IDH1* p.R132H mutation, astrocytomas favoured other *IDH1* p.R132 variants, whereas oligodendrogliomas favoured *IDH2* mutations (Supplementary Table 3a). We did not observe significant differences in the distributions of focal oncogene amplifications, *CDKN2A/B* homozygous deletions or *NOTCH1*, *PI3K* or *MSH6* mutations between *IDH1* p.R132H and other IDH-mutant groups (Supplementary Table 3b–f). Furthermore, we observed no significant associations between IDH mutation types and overall survival, mutational signatures or fraction of genome altered (FGA), potentially reflecting limited statistical power (Extended Data Fig. 6a–c).

In summary, our results show that oligodendrogliomas acquire clonal sSNVs in driver genes at recurrence, whereas astrocytomas acquire clonal focal DNA copy-number alterations (CNAs).

Prognostic relevance of driver events

Clinically applicable molecular prognostic grading markers in IDH-mutant gliomas are at present limited to homozygous deletions of *CDKN2A/B*⁶. The unfavourable prognostic effect of hypermutation at recurrence in IDH-mutant gliomas¹⁰ was confirmed in our cohort (Fig. 3a,b). *PI3K* and *NOTCH1* mutations did not further stratify survival in non-hypermutant oligodendrogliomas in GLASS (Fig. 3c). However,

PI3K- and *NOTCH1*-mutant oligodendrogliomas did associate with worse overall survival in the AACR GENIE validation set (Fig. 3d). *CDKN2A/B* homozygous deletions were associated with poor post-recurrence survival (median: 1.1 years versus 3.2 years; log-rank test: $P = 0.01$), and this association was significant in both the hypermutant and the non-hypermutant subpopulation (log-rank test: $P = 0.002$ and $P = 0.02$, respectively; Extended Data Fig. 7a and Supplementary Table 4), validating the WHO's 2021 classification of *CDKN2A/B* homozygous deletion as a grade-4 marker in recurrent astrocytomas⁶. Focal oncogene amplifications also indicated significantly shorter post-recurrence survival in patients with astrocytoma (median: 1.2 years versus 3.8 years; log-rank test: $P = 0.001$; Supplementary Table 4). Combining *CDKN2A/B* homozygous deletions and/or focal oncogene amplifications associated with inferior patient outcome in non-hypermutant astrocytomas (median post-recurrence survival: 1.5 years versus 5.3 years; log-rank test: $P < 0.001$; Fig. 3e). Moreover, tumours with only *CDKN2A/B* homozygous deletion or only focal oncogene amplifications independently associated with worse patient outcomes (multivariable Cox proportional hazards model: *CDKN2A/B* homozygous deletions, $P < 0.001$; focal oncogene amplifications, $P = 0.003$; Extended Data Fig. 7b). External validation with the AACR GENIE cohort confirmed the poor prognostic effect of these driver CNAs (Fig. 3f and Extended Data Fig. 7c). The adverse post-recurrence prognostic effect was significant in grade 2–3 GLASS tumours but not in grade 4 tumours (log-rank test: $P = 0.032$ and $P = 0.41$, respectively; Extended Data Fig. 7d). Multivariable analysis of all univariably significant clinical and molecular factors (Supplementary Tables 4–6) showed that the presence of focal oncogene amplifications was an independent poor prognostic marker in patients with astrocytoma irrespective of surgery timing (Supplementary Tables 7a–e, 8a,b and 9a,b).

We evaluated the association between enriched recurrent driver alterations (that is, treatment with radiation therapy, alkylating chemotherapy or either treatment regimen) and observed no significant differences across treatment groups after false discovery rate (FDR) correction (Supplementary Table 10a–c).

In conclusion, oligodendroglioma-enriched driver sSNVs might have prognostic relevance, but further validation is needed, whereas astrocytoma-enriched driver CNAs consistently associate with poor survival.

Mutational processes shape glioma genomes

Genome-wide somatic mutational patterns of single-base substitutions (SBSs), double-base substitutions (DBSs) and indels (ID) can indicate both tumour-inherent biology and treatment-associated damage^{28,29} and can be quantified using tumour mutational burden (TMB, comprising SBSs, DBSs and ID) and mutational signatures. The median WGS-derived TMB of primary oligodendrogliomas and astrocytomas was 1.5 mutations per Mb and 1.6 mutations per Mb, respectively, which is low relative to other tumour types^{28,29}. TMB at diagnosis increased significantly with higher tumour grade (Extended Data Fig. 8a). At recurrence, and excluding hypermutators, TMB increased significantly in both oligodendrogliomas and astrocytomas (Fig. 3g). However, in oligodendrogliomas, TMB increases were only significantly associated with tumours acquiring driver-gene alterations.

Mutations shared between time points are expected to be mostly truncal, reflecting early derivation in the evolution of a given tumour⁵. Among all mutations detected in the initial tumour, the median fraction that was also detected in the recurrent non-hypermutant tumour was 36% for oligodendrogliomas and 45% for astrocytomas (Extended Data Fig. 8b). WXS datasets from different sectors of the same tumour are available for a subset of the GLASS cohort ($n = 5$), and the median fraction of spatially shared mutations in IDH-mutant gliomas was 53%, suggesting that spatial heterogeneity partially accounts for the lack of overlap in mutations between initial and recurrent tumours (Extended

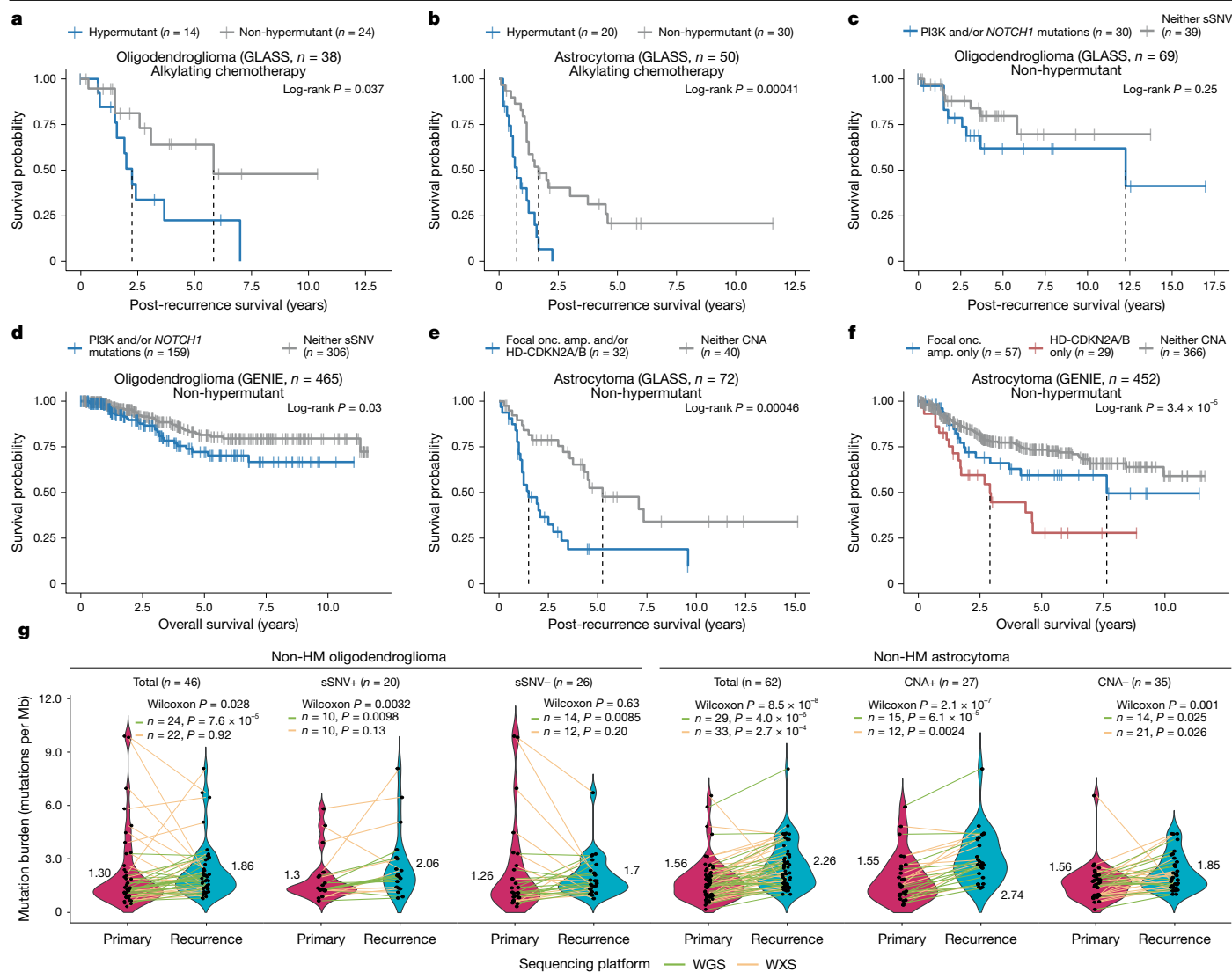


Fig. 3 | Post-recurrence survival is associated with acquired hypermutation and CNAs. **a–f**, Kaplan–Meier curves comparing the survival of different patient groups (GLASS cohort in **a–c,e**; GENIE cohort in **d,f**). Statistical significance was assessed using two-sided log-rank tests for unpaired data. **a,b**, Post-recurrence survival of patients in the GLASS cohort who received alkylating chemotherapy before recurrence to treat oligodendrogliomas (**a**) and astrocytomas (**b**), stratified for hypermutation status. Hypermutant gliomas exhibit more than 10 mutations per Mb and have more than 25% of recurrence-specific mutations attributable to SBS11; non-hypermutant gliomas exhibit fewer than 10 mutations per Mb. **c**, Post-recurrence survival of patients with non-hypermutant oligodendroglioma in the GLASS cohort, stratified by combined PI3K and *NOTCH1* mutations at recurrence. **d**, Overall survival of patients with non-hypermutant oligodendroglioma in the AACR GENIE cohort, stratified by combined PI3K and *NOTCH1* mutations in the primary tumour. **e**, Post-recurrence survival of GLASS-cohort patients with non-hypermutant astrocytoma who have a focal oncogene amplification and/or homozygous

deletion of *CDKN2A/B* (HD-CDKN2A/B), compared with patients who do not have those variants. **f**, Overall survival of AACR GENIE-cohort patients with non-hypermutant astrocytoma who have a focal oncogene amplification or HD-CDKN2A/B, compared with patients who do not have those variants. **g**, Comparison of the mutation burden (number of mutations per megabase) for non-hypermutant glioma between matched primary and recurrent samples, using two-sided Wilcoxon's signed-rank tests for paired data analysis per group and sequencing platform subgroups. Patients are grouped by glioma type and tumour-type-enriched driver-gene alterations; that is, sSNVs for non-hypermutant oligodendrogliomas (mutations of *NOTCH1*, *PIK3CA* and *PIK3R1*) and CNAs for non-hypermutant astrocytomas (focal oncogene amplifications of *CCND2*, *CDK4*, *CDK6*, *EGFR*, *MET*, *MYC*, *MYCN*, *PDGFRA* and *PTPRZ1* or homozygous deletions of *CDKN2A/B*). PI3K, *PIK3CA* and *PIK3R1* combined. Focal oncogene indicates *CCND2*, *CDK4*, *CDK6*, *EGFR*, *MYC*, *MYCN*, *MET*, *PDGFRA* or *PTPRZ1*.

Data Fig. 8c). The relatively low number of spatially and longitudinally shared mutations is consistent with previous reports^{12,14,30–32}. The length of the surgical interval was negatively correlated with the fraction of mutations shared by primary–recurrent pairs in non-hypermutant oligodendrogliomas, and positively correlated with their recurrence-only fraction—associations that were not seen in non-hypermutant astrocytomas (Extended Data Fig. 8d).

Mutational signatures reflect the underlying mutational processes in a tumour and can be associated with external environmental exposures

or internal biological processes such as defective DNA repair. Other than ionizing radiation, no carcinogenic exposures have been causally linked to incidence risk in IDH-mutant glioma³³. We examined the developmental history of IDH-mutant gliomas by analysing mutational signatures using sSNVs extracted from all WGS datasets, excluding four non-hypermutant oligodendrogliomas that had a TMB higher than 10 mutations per Mb only at the initial surgery (78 genomes from 39 astrocytomas and 106 genomes from 52 oligodendrogliomas; Supplementary Tables 11a–d and 12a–d). This analysis revealed 28 SBS

or DBS signatures and 12 ID signatures similar to those previously reported in the combined databases of the Catalogue Of Somatic Mutations In Cancer (COSMIC)²⁸ and Signal²⁹. Amongst mutations that were detected in untreated primary tumours and mutations that were shared between primary and matching recurrent tumours, six SBS and seven ID signatures were found in at least five samples. These include the age-related clock-like SBS1, ID1 and ID2 signatures (Extended Data Fig. 9). We observed the presence of SBS120—a signature of unknown origin that is unique to tumours in the CNS²⁹—in both types of IDH-mutant glioma. The SBS5, SBS8 and SBS40 signatures are associated with late replication regions in different genomic contexts and were differentially observed between oligodendrogliomas and astrocytomas (SBS5 and SBS40 versus SBS8, respectively; Fig. 4a). The C>T SBS97 signature, which has previously been associated with deficiencies in mismatch repair, was found only in astrocytomas, as were SBS37 and SBS101, which are signatures of unknown aetiology. By contrast, C>A transversions dominated the SBS107 signature, which was detected in oligodendrogliomas but not in astrocytomas. This signature shows similarity to the SBS4 tobacco smoking signature²⁹, and previous SBS107 associations have been found in bladder, renal and liver carcinomas^{29,34}. Signatures ID3, also associated with tobacco smoking, and ID23, related to aristolochic acid exposure³⁵, were exclusively detected in subsets of astrocytomas. Smoking is not associated with an appreciably increased risk of IDH-mutant glioma, and exposure to aristolochic acid also results in increased fractions of SBS22a and SBS22b, which were not detected, suggesting that alternative mechanisms are able to generate SBS107, ID3 and ID23. ID5, reported to be a clock-like signature, was found in oligodendrogliomas but not in astrocytomas²⁸.

In summary, we identified significant differences in mutational signatures between oligodendrogliomas and astrocytomas, suggesting that a variety of mechanisms underlies the initiation, DNA damage-repair response and clonal selection processes of both glioma types.

Therapy-associated mutational signatures

Therapeutic bottlenecks drive the clonal selection of tumour cells that can repair treatment-induced DNA damage effectively, and their mutational signatures might represent the footprint of the associated DNA damage-repair mechanisms. We evaluated the enrichment of mutational signatures after therapy relative to matched pre-treatment WGS samples. The temozolomide-associated hypermutation signature SBS11 was detected in 7 out of 18 astrocytomas (39%) and in 12 out of 23 oligodendrogliomas (52%) that were treated with temozolomide before tumour recurrence (Fig. 4b). SBS119 was detected in 8 out of 13 oligodendrogliomas and in 3 out of 12 astrocytomas that were temozolomide-treated and non-hypermuted (Fig. 4c). SBS119 was previously observed in three patients with CNS tumours, and resembles a signature that was identified when induced pluripotent stem cells were treated with temozolomide *in vitro*^{29,36}. SBS11 is dominated by C>T transversions, whereas SBS119 is enriched for T>C substitutions and shares similarities with microsatellite-instability-associated signatures, such as SBS26 (Supplementary Table 12a,b). SBS119 was not associated with artefact mutational patterns of formalin-fixed paraffin-embedded (FFPE) tissue or guanine oxidation. Spearman's correlation analyses revealed that there was a positive association between the number of temozolomide cycles and the proportion of the recurrence-specific SBS119 signature in non-hypermuted gliomas ($R = 0.58$, $P = 0.0037$; Extended Data Fig. 10a). No correlation was observed in hypermutant gliomas between cumulative temozolomide exposure and the proportion of recurrence-specific SBS11 mutations in either tumour type (Extended Data Fig. 10b). We also tested whether the baseline mutational signature composition of the initial tumours predicted the subsequent acquisition of alkylating-agent-associated signatures, and did not find any significant associations with acquired SBS11 hypermutation after FDR correction.

Radiation-treated astrocytomas that were not hypermutated showed a significant enrichment of signature ID8 (Fig. 4b,c). Although this signature was also detected in non-irradiated astrocytomas, it was highest in radiation-treated astrocytomas, suggesting that radiation increases selection for ID8 mutations, but does not necessarily increase their formation. Radiation-treated oligodendrogliomas also showed a difference in ID8 mutations between the signature timing fractions, but this trend did not reach statistical significance. High-recurrence fractions of the ID8 signature were significantly associated with inferior post-recurrence outcomes in both glioma types (Fig. 4d,e).

Altogether, the results from these mutational signatures further delineate the differences in evolutionary trajectories and responses to treatment between the two types of IDH-mutant glioma.

Increasing chromosomal instability in astrocytoma

High levels of aneuploidy have been reported to be associated with worse patient outcomes in astrocytoma but not in oligodendroglioma³⁷. We evaluated broad patterns of DNA CNAs in association with treatment status and acquired genomic drivers (Fig. 5a). Newly diagnosed oligodendrogliomas exhibited few changes in chromosomal arms other than the 1p/19q codeletion, whereas astrocytomas had higher levels of gains and losses at the chromosomal arm level (Extended Data Fig. 11a,b). Arm-level events were predominantly shared between time points in oligodendrogliomas but were mostly specific to either primary or recurrent tumours in astrocytomas. Our analysis also revealed a frequent chromosome-arm-level loss of 4p and 4q in oligodendrogliomas, further enriched at recurrence (23% to 33%), whereas gain of 7q and loss of 10q were frequently detected in astrocytomas (32%; Extended Data Fig. 11a,b). The overall level of aneuploidy, defined as FGA, increased with grade in both tumour types (Extended Data Fig. 11c).

At recurrence, FGA increased significantly in astrocytomas and in hypermutant but not in non-hypermuted oligodendrogliomas (Fig. 5b). The increased FGA at recurrence persisted after adjusting for Sequenza-estimated purity and ploidy and after excluding low-purity samples (purity < 0.4). In non-hypermuted astrocytomas, the increase in FGA was significantly higher in tumours with *CDKN2A/B* homozygous deletions and focal oncogene amplifications than it was in tumours that did not acquire such events, suggesting that an increased sensitivity to acquired broad CNAs predisposes to focal DNA lesions (Extended Data Fig. 12a). This effect was most evident in tumours that were originally diagnosed as grade 2; limited sample sizes precluded definitive analysis in grade 3 and 4 tumours (Extended Data Fig. 12b).

To gain insight into the mechanisms that underlie changes in FGA at recurrence, we investigated features of chromosomal instability, including through copy-number (CN) signatures³⁸. Whereas whole-genome doubling (WGD) rates were comparable between initial astrocytomas and oligodendrogliomas, WGD frequency increased significantly in recurrent astrocytomas (20% versus 7%; McNemar's test: $P = 0.006$) but not in recurrent oligodendrogliomas (10% versus 7%; McNemar's test: $P = 0.55$; Fig. 5c). Samples that had undergone WGD showed enrichment for the WGD-associated signatures CN2 and CN20 (Extended Data Fig. 13a), independent of glioma type. The number of chromothripsis events was higher in initial astrocytomas relative to initial oligodendrogliomas (Fisher's exact test: $P = 2.1 \times 10^{-6}$; Fig. 5d). At recurrence, the frequency of chromothripsis increased in both tumour types, and continued to be significantly more frequent in astrocytomas (Fisher's exact test: $P = 6.6 \times 10^{-6}$). The presence of chromothripsis was significantly associated with previous radiotherapy in astrocytomas but not in oligodendrogliomas (Fisher's exact test: $P = 0.046$ versus $P = 0.24$), linking treatment exposure to tumour-type-specific patterns of acquired chromosomal instability (Fig. 5e). Chromothripsis cases were enriched for chromothripsis-associated signatures CN4–CN8 (ref. 38), with the chromothripsis-associated amplification signatures CN7 and CN8 showing the largest contributions (Extended Data Fig. 13b). The rates of

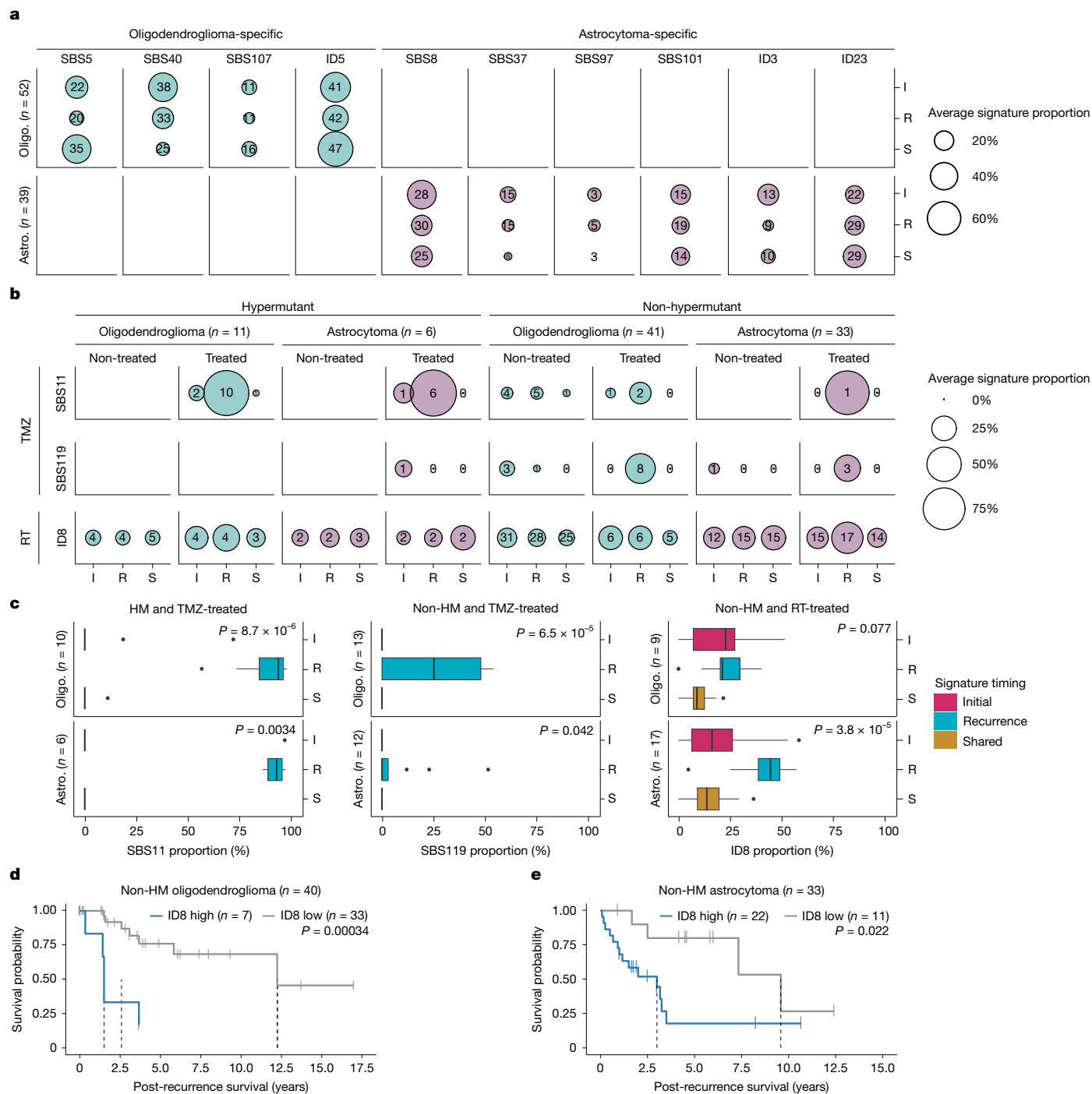


Fig. 4 | Mutational signatures SBS11 and SBS119 are associated with temozolomide exposure, and ID8 is linked to radiotherapy. **a**, SBS and ID mutational signatures were compared using the average percentage of alterations attributable to these signatures (circle size) and the number of patients with any alteration attributable to these signatures (number in circle) per glioma type and timing of the signatures. In oligodendrogliomas, four treatment-agnostic SBS and ID signatures were detected that were not seen in astrocytomas (oligodendroglioma-specific), and conversely, six other signatures were exclusively detected in astrocytomas (astrocytoma-specific). Astro., astrocytoma; Oligo., oligodendroglioma; ID, small insertions and deletions signature; I, initial; R, recurrence; S, shared. **b**, Treatment-associated SBS and ID signatures were compared using the average percentage of alterations attributable to these signatures (circle size) and the number of patients with

any alteration attributable to these signatures (number in circle) per glioma type, hypermutation status, treatment regimen and timing of the signatures. RT, radiotherapy; TMZ, temozolomide. **c**, Box plots from **b** assessing significant differences between timing fractions per glioma type, using the two-sided Kruskal–Wallis test for unpaired data. Sample sizes represent the number of patients for whom all three timing fractions were assessed for each mutational signature and tumour type. Box plots show median (centre) with interquartile range (box bounds), 1.5× the interquartile range (whiskers) and outliers (points). **d**, **e**, Post-recurrence survival of patients with non-hypermutant oligodendroglioma (**d**) or astrocytoma (**e**) by ID8 mutation frequency (cohort split by mean ID8 for all recurrent gliomas), compared using two-sided log-rank tests for unpaired data.

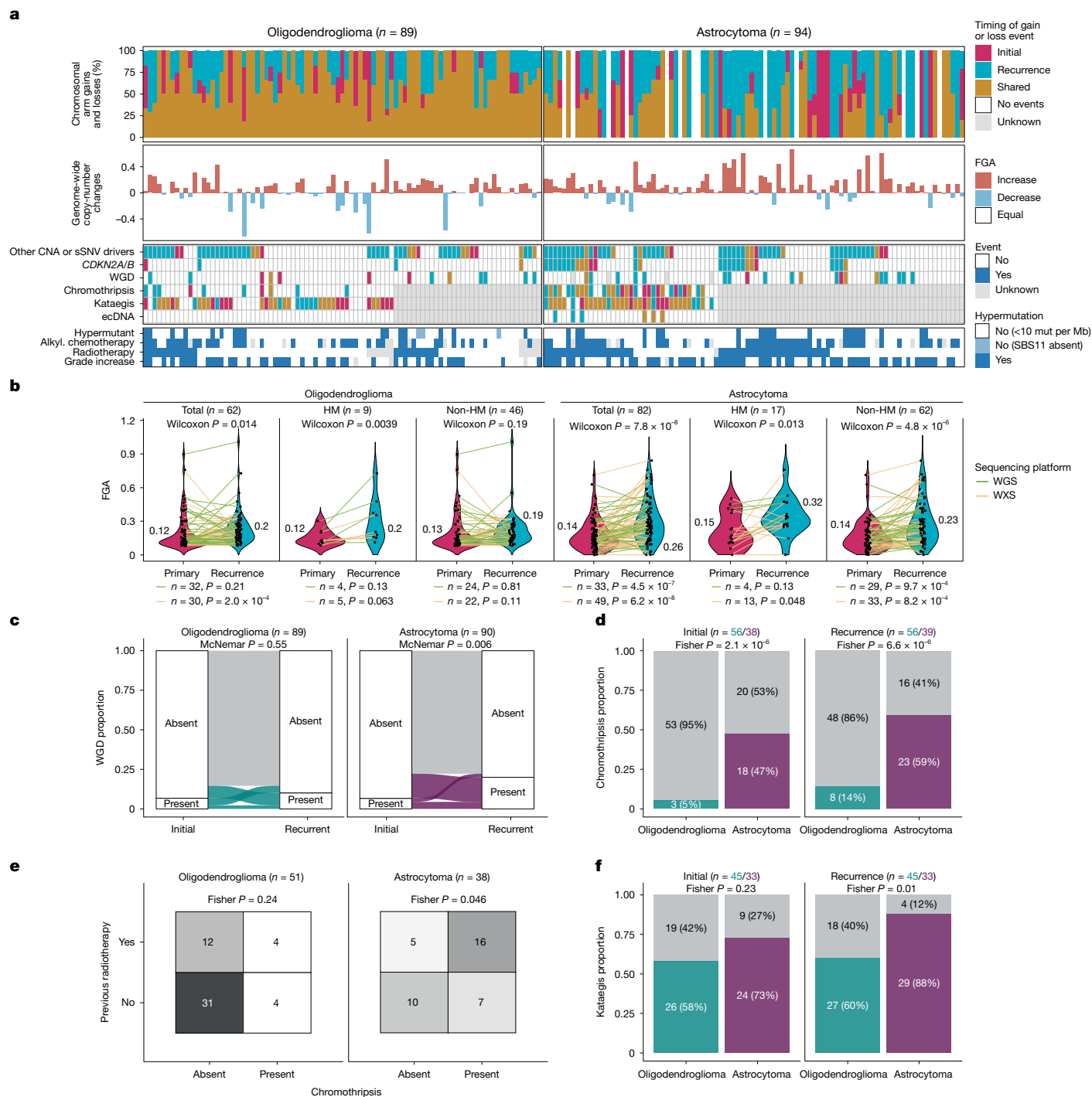


Fig. 5 | Aneuploidy increases and markers of chromosomal instability are more prevalent in astrocytomas. a, Landscape of aneuploidy in patients with mutation and DNA copy-number profiles from at least two time points ($n = 183$). Individual patients (columns) are ordered by sequencing platform (left, WGS; right, WXS; samples lacking chromothripsis, kataegis or extrachromosomal DNA (ecDNA) annotation) and enriched sSNV or CNA driver status. Rows from top to bottom: (1) shared and private copy-number segment fractions by tumour; (2) change in FGA between initial and recurrent tumour (higher values indicate a higher FGA in the recurrent tumour); (3) molecular markers; and (4) clinical markers. **b**, Comparison of genome-wide aneuploidy (measured as FGA) between matching primary and recurrent gliomas using two-sided Wilcoxon's signed-rank test for paired data analysis per group and sequencing platform subgroups. Patients are grouped by glioma type and the presence of

hypermutation, defined as more than 10 mutations per Mb and more than 25% of recurrence-specific mutations attributable to SBS11. **c**, Prevalence of WGD separated by tumour type and comparing time points using two-sided McNemar's test for paired data. **d**, Prevalence of chromothripsis comparing tumour types separated by time point; significance was determined using two-sided Fisher's exact test for unpaired data. **e**, Tile plots, separated by tumour type, depicting the prevalence of chromothripsis events at recurrence in relation to previous radiotherapy treatment; significance was determined using two-sided Fisher's exact test for unpaired data. **f**, Prevalence of kataegis in non-hypermutant samples comparing tumour types separated by time point; significance was determined using two-sided Fisher's exact test for unpaired data.

kataegis events, which are focal hypermutation bursts due to APOBEC mutagenesis³⁹, were comparable between initial astrocytomas and oligodendrogliomas (Fisher's exact test: $P = 0.23$) and, like chromothripsis, were significantly higher in recurrent astrocytomas than they were in recurrent oligodendrogliomas (Fisher's exact test: $P = 0.01$), further supporting the idea that chromosomal instability processes are specific to tumour type (Fig. 5f). Kataegis was significantly associated with increased chromothripsis rates (Fisher's exact test: $P = 9.1 \times 10^{-4}$) at recurrence (Extended Data Fig. 13c). Finally, treatment-associated hypermutation showed a nominal association with the chromothripsis signature CN7, potentially linked to the longitudinal increase in FGA in hypermutators (Extended Data Fig. 13d). WGD, chromothripsis and hypermutation were each associated with a significantly higher FGA (Extended Data Fig. 13e–g).

Collectively, these findings indicate that chromosomal instability—as reflected by FGA, WGD and chromothripsis—and mutational phenomena, such as kataegis and hypermutation, drive tumour-type-specific and treatment-associated increases in chromosomal instability over time.

Discussion

IDH-mutant oligodendroglioma and astrocytoma account for a disproportionate number of life years lost, emphasizing the need for new therapeutic modalities³. The US Food and Drug Administration (FDA) approval of the IDH inhibitor vorasidenib for the treatment of progressive WHO grade 2 IDH-mutant gliomas in 2024 was an important breakthrough. Although both IDH-inhibitor treatment and chemoradiation improve survival outcomes, the tumours eventually progress to higher grade and become resistant to treatment⁴⁰. The GLASS Consortium was established to characterize adaptive changes that occur in response to therapeutic treatments, by comparing pre- and post-treatment gliomas, and to discover therapeutic vulnerabilities⁴. The current GLASS IDH study integrated WGS and WXS data from a global, multi-institutional cohort spanning ten countries. Detailed clinical annotation reveals the range of common treatment modalities over three decades, with the preferential treatment comprising both radiotherapy and alkylating chemotherapy in the form of temozolomide for patients with oligodendroglioma or astrocytoma.

Our analysis focused on type-stratified evolution, which showed distinct evolutionary trajectories. This included the identification of recurrence-enriched driver-gene alterations, some of which have previously been implicated in the prognosis and evolution of IDH-mutant glioma, including temozolomide-induced hypermutation; PI3K, *MSH6* and *NOTCH1* sSNVs; and cell-cycle CNAs^{7,14,41,42}. The concordance of our findings with previous reports in IDH-mutant gliomas supports their translational relevance and increases the likelihood of reproducibility in independent datasets. We further revealed increases in mutational and aneuploidy burden and in treatment-associated mutational signatures, including SBS11, SBS119 and ID8. Considered together, these results reveal a dichotomous pattern. Oligodendrogliomas progress through new sSNVs in driver genes, whereas astrocytomas activate oncogenes and deactivate tumour suppressor genes through focal CNAs, which also coincide with significant increases in aneuploidy burden, as induced by multiple chromosomal instability aetiologies (Extended Data Fig. 14). Notably, acquired focal amplification of at least one well-described oncogene in non-hypermutated astrocytomas seemed to indicate an unfavourable patient outcome, pointing to additional prognostic biomarkers to guide clinical management and extending previous observations⁴³. The divergent resistance mechanisms of IDH-mutant gliomas could reflect early, type-specific evolutionary trajectories, with the enrichment of sSNVs and focal CNAs occurring independently of treatment as well. The near-ubiquitous presence of *TP53* mutations in astrocytoma might contribute to progression mechanisms such as mitotic errors, resulting in chromosomal

mis-segregation events⁴⁴, and ATRX deficiency has been repeatedly associated with replication stress, DNA damage and abnormalities in chromosomal cohesion and congression⁴⁵. Together, these alterations could create a genome that is prone to acquiring new CNAs that enable disease progression. By contrast, oligodendrogliomas arise from a genomic background defined by 1p/19q codeletion, and are further characterized by intact p53 and ATRX pathways^{6,7}. We recently showed that acquired genetic alterations such as those described here coincide with malignant cell-state changes that are shared across oligodendrogliomas and astrocytomas, suggesting that distinct genomic adaptations can converge on common transcriptional programs⁴².

A limitation of this study is the inherent selection bias of longitudinal, multi-resection cohorts, because inclusion requires survival to at least a second surgery. Although we partially addressed this bias through comparison with the TCGA IDH-mutant glioma cohort, these factors should be considered when interpreting survival outcomes and biomarker frequencies derived from the GLASS cohort.

In summary, astrocytomas and oligodendrogliomas follow fundamentally distinct evolutionary trajectories in response to therapy: astrocytomas accumulate CNAs, such as focal oncogene amplifications, and exhibit widespread aneuploidy, whereas oligodendrogliomas acquire new driver mutations. Our results suggest that further research could enable the development of type-stratified and evolution-informed treatment strategies in IDH-mutant glioma. In addition, this work provides a key framework for future work on the molecular changes that take place after gliomas become resistant to treatment with IDH inhibitors.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-026-11150-x>.

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Methods

DNA sequencing

WGS and WXS data were newly generated for the MD Anderson Cancer Center, Duke University Hospital, Henry Ford Hospital, Case Western Reserve University, Chinese University of Hong Kong, Mayo Clinic, Northwestern Medicine, Olivia Newton-John Cancer Research Institute, University of California, San Francisco, Saint Joseph's Hospital and Medical Center, Seoul National University and Luxembourg Institute of Health at the Jackson Laboratory for Genome Medicine sequencing core. All were subjected to 150 base pair (bp) paired-end sequencing using Novaseq (Illumina) according to the manufacturer's protocol. Previously published longitudinal data from GLASS^{5,12} and TCGA⁷ were also used and the sequencing information for those can be found in previous publications. In total, sequencing data were contributed by 21 institutions: Columbia University, University of Florida, German Cancer Research Center, Pitié-Salpêtrière, Samsung Medical Center, Biobank Japan, University of South Wales, University of Sao Paulo and the aforementioned 13 institutions including the Jackson Laboratory. For the Seoul National University cohort, both DNA and RNA were concurrently extracted from each tumour sample at the Jackson Laboratory for Genomic Medicine using QIAGEN's AllPrep DNA/RNA Mini Kit (80204). For blood samples, DNA extraction was done using QIAGEN's QIAamp DNA Mini and Blood Mini Kit (51104). A total of 200 ng of DNA was fragmented to 400 bp using an LE220 focused-ultrasonicator (Covaris) and then size-selected with Ampure XP beads (Beckman Coulter). The DNA fragments underwent end repair, A-tailing and ligation of Illumina unique adapters (Illumina) using the KAPA Hyper Prep Kit from Roche (7962363001). The resulting whole-genome libraries were sequenced with 150-bp paired-end reads on the Illumina NovaSeq 6000. Sequencing coverage on average was 27× and more than 100×, respectively, across WGS and WXS longitudinal samples, and driver mutations were consistently supported by sufficient depth (ranging from 30×–80× for WGS and 80×–300× for WXS) in both primary and recurrent tumours, minimizing the effect of coverage variability on mutation detection.

Reference cohorts

GLASS data were compared with data from two reference cohorts: TCGA⁷ and AACR GENIE (v.18.5)²⁵. Clinical data for patients in the GLASS cohort at baseline were compared with data from TCGA patients with primary IDH-mutant gliomas with respect to age of onset and overall survival^{15,7,12}. Survival data for patients with oligodendrogliomas and astrocytomas were compared between the GLASS and GENIE cohorts. The GENIE cohort includes mainly panel sequencing data, and in most cases lacks copy-number data or coverage of *TERT* promoter hotspots. Oligodendrogliomas in the GENIE cohort were therefore defined as IDH-mutant gliomas without alterations in *TP53* or *ATRX*, as determined by high-coverage panel sequencing, whereas IDH-mutant gliomas with these alterations were called astrocytomas.

GLASS identifiers

A GLASS barcode system based on the TCGA barcode design was used to identify all samples and their corresponding patients. In brief, the 24-character barcode includes information about the project, collection centre, patient identification, sample type, analyte type and sequencing platform. Detailed information on the barcode breakdown is available in a previous GLASS publication¹².

Computational pipelines

GLASS pipelines for variant and copy-number calling were developed using Snakemake (v.5.2.2)⁴⁶. The Genome Analysis Toolkit 4 (GATK4, v.4.1.0.0)⁴⁷ suite was used unless otherwise specified. All data were analysed using homogeneous pipelines capable of processing raw FastQ files.

Alignment and variant calling

In brief, in alignment and preprocessing, the study adhered to GATK Best Practices, using various tools to manage and sanitize binary alignment map files, assign and manage read groups, perform QC, mark sequencing adapters, align sequences to the b37/hg19 genome (human_g1k_v37_decoy), merge and mark duplicates and perform base recalibration. For variant detection, GATK4 was used, with germline variants called from control samples, and somatic variants identified in tumour samples and patient cohorts. QC measures included evaluating cross-sample contamination and read orientation artefacts. In variant post-processing, BCFTools (v.1.9)⁴⁸ normalized, sorted and indexed variants to generate a consensus variant call format (VCF) file, which was then annotated and used to compare allele frequencies between related samples, with single-sample variants overlaid on multi-sample calls to infer individual sample mutations. Further details of these steps can be found in the previously published GLASS paper⁵.

Copy-number calling

Copy-number identification was performed according to GATK Best Practices, with different methods for WGS and WXS data. For WGS data, the genome was segmented into 10-kb bins, whereas overlapping regions from various capture kits were merged for WXS data. Both strategies included counting reads in these bins, subsetting germline variants and separating the cohort by sequencing centre to create a panel of normals. This involved denoising read counts, annotating intervals for GC content and inspecting results for quality. Copy-number calling used a method based on GATK CallCopyRatioSegments and GISTIC (v.2.0.23)²⁶, identifying neutral segments and classifying amplifications or deletions on the basis of weighted means and standard deviations. Chromosome arms were analysed to set high-level thresholds, and gene-level copy numbers were called by intersecting gene boundaries with segment intervals and comparing the weighted non-log₂ copy ratio to these thresholds. These steps have also been described in detail previously⁵.

Molecular data cohort selection

Stringent QC measures were put in place to retain time-separated surgeries for molecular analyses (Extended Data Fig. 1b). QC criteria required (QC1) that a tumour sample was available and WGS and/or WXS data were generated from the sample; (QC2) that fingerprinting confirmed sample identity using the Picard CrosscheckFingerprints tool⁴⁹; (QC3) that sequencing coverage was at least 8× for WGS samples and at least 28× for WXS samples, and that the mutation frequency was not zero and not abnormally elevated (indicative of FFPE artefacts); (QC4) a minimum interval of 3 months between the initial and recurrent surgery; (QC5) the exclusion of patients with only one surgery remaining and a restriction to two distinct surgeries per patient, choosing the first and last surgery available; and (QC6) that copy-number profiles met quality thresholds without excessive noise, unexpected genomic stability or insufficient signal, as independently assessed and jointly reviewed by three of the authors (T.C., K.C.J. and F.S.V.).

sSNV calling

dN/dS ratios were estimated using dNdScv (v.0.1.0)⁵⁰ with the default and recommended parameters applied to all mutations in initial and recurrent tumour samples, as well as for each mutational fraction (unique to initial, unique to recurrent, and shared). These analyses were performed separately for each of the two types of IDH-mutant glioma. Adequate sequencing depth and reciprocal mutation calling between paired samples minimized coverage-related false negatives; because our analysis compares shared mutations with sample-specific ones, coverage variation is unlikely to affect the qualitative patterns observed.

Clonality analysis

We used PyClone-VI⁵¹ to assess the clonal status of all sSNVs in our dataset. In brief, variants with a minimum coverage of 10× in at least one sample from each paired set were included in the analysis. PyClone-VI was run in beta-binomial mode using default parameters. Mutations with a cancer-cell fraction higher than 0.9 were classified as clonal, and those below this threshold were considered subclonal.

GISTIC

GISTIC²⁶ was run on the public version of the GenePattern server, on the segmentation data of primary and recurrent tumours for each type of IDH-mutant glioma individually to identify broad and focal copy-number changes of significance in each group. The analysis was done using the GeneGISTIC method, with broad event detection and gene collapse values set to extreme. Additional parameters included a focal length cut-off of 0.5 or higher, with a confidence level of 99% and a chromosome-arm adjustment capped at 1.0. Copy-number driver genes were identified from the GeneGISTIC results.

Aneuploidy calculation

Aneuploidy was calculated at three different levels for all samples: arm level, segment level and genome-wide level. For the arm-level aneuploidy calculation, a previously described method was used⁵². In brief, adjacent segments with identical arm-level calls (−1, 0 or 1) were merged, resulting in a single call for each merged segment. The proportion of the chromosome arm spanned by each merged segment was calculated. Segments covering over 80% of the arm were called −1 (loss), 0 (neutral), +1 (gain) or NA, if no segment covered at least 80%. For each sample, the number of arms with non-neutral events was then counted. As a secondary method, average $\log_2$ (copy-number ratios) were calculated to compare arm-level aneuploidies between groups. Lower thresholds were set at ± 0.1 for significant arm-level gain or loss at any time point and ± 0.05 for significant arm-level changes (Δ gains or losses).

For segment-level calculation, each segment was assigned a status of gain, loss or neutral on the basis of the $\log_2$ copy ratio threshold of ± 0.2 . Segments with an absolute $\log_2$ ratio greater than 0.2 were considered altered. In each sample, the total number of altered segments (gains and losses) was counted.

High-level amplification and homozygous deletion thresholds were determined per sample from segmented copy-number data by calculating the weighted mean and s.d. of non- $\log_2$ copy ratios among neutral segments (0.9–1.1), with segment length used as weights and iterative filtering performed twice to remove outliers. Thresholds for amplification and homozygous deletion were then defined as the twice-filtered weighted mean ± 2 s.d.

For genome-wide level calculation, FGA was calculated with the following formula: $FGA = 1 - (\text{total base pairs in neutral segments} / \text{total callable base pairs})$. For WGS samples, the callable genome size was assumed to be 3×10^9 bp. For WXS samples, callable size was set to 5×10^7 bp on the basis of the exome capture target region. FGA values were calculated per sample using platform-specific copy-number profiles and normalized to a 0–1 scale; WGS and WXS represent mostly non-overlapping tumour sets.

Structural variant calling

Structural variants (SVs) were called on WGS data only using SvABA (v.1.2.0)⁵³, against the hs37d5 reference genome, incorporating dbSNP build 138 (b37) and excluding ENCODE hg19 blacklist regions. Somatic SVs were extracted from SvABA VCFs and parsed in R, with break-end records resolved to obtain partner coordinates and strand orientation. Only autosomes and chromosome X were retained, and events on decoy, random or alternative contigs were excluded. Reciprocal break ends were paired to reconstruct complete events and classified

as deletions, duplications, head-to-head inversions, tail-to-tail inversions or translocations, with duplicate mates collapsed to a single representative event per sample.

Chromothripsis

Chromothripsis events were identified using ShatterSeek (v.1.1)⁵⁴. Copy-number segments were derived from GATK segmentation output for WGS aliquots, and somatic SVs were called using SvABA. Only samples with both SV and copy-number data were retained for downstream chromothripsis analyses. Of the copy-number segments, $\log_2$ copy ratios were converted to integer total copy number and capped at 20, and adjacent segments with an identical copy number were merged. Chromothripsis was assessed on the basis of break-point clustering, oscillating two-state total copy-number patterns and fragment-join randomness, and chromosome-level summaries were used to define confidence categories. High-confidence events required more than five intrachromosomal SVs, more than six oscillating segments, significant break-point enrichment or clustering ($P < 0.05$) and non-random fragment joins ($P > 0.05$); low-confidence events met similar criteria, with four to six oscillating segments. Alternatively, high-confidence events required more than two intrachromosomal SVs, with more than three interchromosomal events and more than six oscillating segments.

Kataegis

Kataegis events were called using SigProfilerSimulator and SigProfilerClusters (v.1.2.2)⁵⁵ as previously described. For each sample, 100 simulated genomes were generated under a pentanucleotide context model, preserving chromosome-level mutation burdens and transcriptional strand bias, to derive empirical intramutational distance thresholds. Cluster detection incorporated genome-wide correction for regional mutation density using 1-Mb windows, and variant allele frequencies were considered during subclassification. From the outputs, kataegis event results were selected and mutations sharing a cluster group identifier were aggregated and defined as a single kataegis event. Finally, the number of events per sample was counted to establish the kataegis burden ($\log_2(\text{kataegis count} + 1)$) per sample. Kataegis analyses were restricted to non-hypermutator samples to avoid violation of the genome-wide background mutation rates.

WGD

ASCAT was used in multi-sample mode to derive patient- and sample-level WGD status⁵⁶. Denoised copy ratio and tumour and normal allelic count files from the GATK CNV caller were transformed into tumour and normal B-allele frequency as well as tumour and normal logR files as input for ASCAT. ASCAT was run in case-based multi-sample mode. WGD was called from the ASCAT solution as part of the reported summary metrics. To avoid overestimating WGD, we applied a second filter and used a cut-off of major copy-number fraction ≥ 0.5 assessed by Sequenza in samples that were predicted to have undergone WGD by ASCAT.

Copy-number signatures

Allele-specific copy-number profiles from ASCAT were used as input for copy-number signature calling using the SigProfilerToolkit⁵⁵. Sample-level 48-feature copy-number matrices were generated using SigProfilerMatrixGenerator. Assignment of copy-number signatures was performed using SigProfilerAssignment⁵⁵ with the COSMIC v.3.5 reference copy-number signature set³⁸. Per-sample absolute and relative signature activities were exported for downstream analyses.

Extrachromosomal DNA

Complex focal amplifications and extrachromosomal DNA (ecDNA) were detected using AmpliconSuite⁵⁷, which integrates

AmpliconArchitect and AmpliconClassifier. Case-based multi-sample mode was used with default AmpliconSuite parameters. From the output that categorizes amplicons into predefined classes (ecDNA, breakage–fusion–bridge cycles and linear amplification), we classified samples into samples lacking or containing ecDNA.

Mutational signatures

Mutational signature analysis was done in three steps using previously published methods^{28,58}. First, WGS data for all the samples were used to create a mutational matrix and extract de novo signatures using SigProfilerExtractor (v1.2.1)⁵⁸. We used the b37/hg19 reference genome, maximum signatures of 10 and non-negative matrix factorization replicates of 100 for the SigProfiler run. Seven SBS and seven ID de novo signatures were extracted from the oligodendroglioma cohort and ten SBS and six ID de novo signatures from the astrocytoma cohort, with an average stability higher than 0.8. Extracted signatures with a cosine similarity to a reference signature of 0.8 or higher were deconvoluted, whereas those with a similarity lower than 0.8 were retained as novel signatures (Supplementary Table 12). Then, de novo signatures were deconvoluted to a curated list of reference signatures for SBS, DBS and ID. To curate the reference signature list, we combined two public mutational signature databases: the COSMIC database (<https://cancer.sanger.ac.uk/signatures>)²⁸ for SBS signatures 1–96 and all ID signatures, and the Signal database (<https://signal.mutationalsignatures.com/explore/main/cancer/signatures>)²⁹ for SBS signatures 97 and higher. To ensure confidence in signature assignment probability, we used only WGS data from our cohort, aligning with the WGS-based curated reference. Finally, we used the deconvoluted reference signatures identified in step 2 to estimate the exposure of mutational signatures in each sample. The R package Palimpsest (v.2.0.0)⁵⁹ was then used to assign the most likely signature to each mutation in a sample. We excluded four cases from downstream mutational signature analyses in which the initial, but not the recurrent sample, had a mutation burden exceeding 10 mutations per Mb.

Mutational burden

Mutational burden was determined by the number of mutations per Mb sequenced, requiring a minimum base coverage of 15×. Mutational burden was calculated per sample and shown on a common scale (0–10 for non-hypermutated tumours); WGS and WXS represent distinct tumour sets rather than matched samples sequenced on both platforms. DNA hypermutation was defined for recurrent tumours with a combination of more than 10 mutations per Mb and more than 25% of recurrence-specific mutations attributable to the COSMIC mutational signature SBS11. Tumours with fewer than 10 mutations per Mb were classified as non-hypermutant, whereas hypermutation status was left unassigned for tumours with more than 10 mutations per Mb but fewer than 25% of recurrence-specific mutations attributable to SBS11.

Mutual exclusivity analysis

Mutual exclusivity of genomic alterations was assessed using the DISCOVER (discrete independence statistic controlling for observations with varying event rates) algorithm (v.3.1.7)²¹, which computes tumour-specific probabilities of gene alterations while preserving the observed marginal totals per gene and per tumour. Expected co-occurrence rates between gene pairs were derived under a Poisson binomial model, accounting for these probabilities. Significantly fewer co-alterations than expected under this null model were interpreted as evidence of mutual exclusivity.

Assessment of batch effects

To assess potential sequencing-centre-related batch effects, we tested for associations between treatment centres and recurrent sSNVs, CNAs and mutational signatures using tumour-type- and

hypermutation-status-stratified logistic regression models, which showed no significant centre-specific effects.

Survival analysis

Survival end points included overall survival from diagnosis and time to recurrence. Overall survival from diagnosis was defined as the interval between the date of primary surgical resection and the date of death or the date of last follow-up if censored. Time to recurrence was defined as the interval between the date of primary surgical resection and the date of first recurrence surgery. No censoring was required for time to recurrence because all patients had undergone a secondary surgery by study design. Overall survival from birth was calculated by adding age at diagnosis to OS from diagnosis. Post-recurrence survival was defined as the interval from the date of the QC-passed last recurrence surgery to death or censoring at last follow-up. Kaplan–Meier survival curves were generated and compared using the log-rank test with the survival and survminer R packages. Cox proportional hazards regression was used to estimate hazard ratios; variables with likelihood ratio test $P \leq 0.10$ were considered for inclusion in multivariable models. Schoenfeld residuals were examined to verify the proportional hazards assumption; variables violating this assumption were excluded. Prognostic models were refined through stepwise backward elimination, and model performance was quantified using Harrell's concordance index with 1,000 bootstrap resamples.

Other statistical methods

All statistical analyses were performed using R (v.4.3.2) and Python (v.3.10.13). For cohort-level comparisons that did not account for patient-level pairing, unpaired categorical variables were assessed using Fisher's exact test or the χ^2 test, and unpaired continuous variables using the Wilcoxon's rank-sum or Kruskal–Wallis' test, as appropriate. For cohort-level analyses specifically evaluating patient-level discordance over time, paired categorical variables were compared using McNemar's test and paired continuous variables using the Wilcoxon's signed-rank test. Statistical significance was defined as two-tailed $P < 0.05$ unless specified otherwise.

Ethics statement

The GLASS Consortium is an international observational study aimed at understanding the molecular response to treatment in adult glioma, in which data is pooled from different sources. The protocols used to collect and sequence specimens, or to collect and analyse data from patients, were approved by the institutional review board of Yale University (2000036542, 2000041140, 2000039350, 2000036441, 2000035189, 2000034831, 2000035190, 2000035426 and 2000035453) and as previously reported^{5,12}. Anonymized patient and sample identifiers were generated as previously reported⁵. All participants provided written informed consent before enrolment, and the study was performed in accordance with the Declaration of Helsinki and applicable institutional and national ethical guidelines.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Deidentified somatic variant and clinical datasets are publicly available through Synapse (<https://www.synapse.org>) with Project SynID syn17038081. A summarized version of the processed data per patient is available in Supplementary Table 1. For the reference cohorts, data are available through the GDC Data Portal (<https://gdc.cancer.gov>) for the TCGA-LGG cohort, the GENIE Synapse (<https://www.synapse.org/genie>) and the GENIE cBioPortal (<https://genie.cbioportal.org>).

Code availability

Custom computational pipeline scripts for sequencing data preprocessing and downstream data analysis scripts for this study are accessible through the project's GitHub repository (<https://github.com/verhaaklab/glass-idh>).

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done by T.C., C.M.S.T. and F.S.V. with additional support from C. Heo, G.Y.Y., T.E.W., S.B.A., E.S., K.J.A. and H.-E.M. K.C.J., E.K., K.J.A., F.P.B., F.S.V., P.S.G., E.S., D.M.A., E.B.C. and R.G.W.V. provided further insights into data analysis and interpretations. A.L., A.E.S., A.R.B., A.G., A.I., B.H., C.E., C. Hong, C. Horbinski, D.R.O., E.G.V.M., H.K.N., H.-E.M., J.T.H., J.M.C., J.S.B.-S., J.F.C., K.M., K.L., K.W., L.M.P., M.P.N., M.P., M.C.M.K., M.K., N.S., P.W., P.S.L., P.J.F., R.V., S.H.P., S.M., W.K.A.Y., W.R.V. and Y.H. collected samples and/or clinical data. P.L.G. helped with sample sequencing. T.C., C.M.S.T., E.K., F.S.V. and R.G.W.V. led manuscript writing, assisted by editing and revisions from K.C.J., A.E.S., A.G., A.I., A.L., A.M.E.W., A.R.B., A.V.C., B.H., B.K., B.A.W., C.E., C. Heo, C. Hong, C. Horbinski, C.K., C.W., D.J.B., D.M.A., D.R.O., E.B.C., E.G.V.M., E.S., F.P.B., G.F., G.Y.Y., H.K.G., H.K.N., H.-E.M., I.D., J.F.C., J.H.L., J.M.C., J.S.B.-S., J.T.H., K.A., K.J.A., K.L., K.M., K.W., L.G., L.M.P., M.C.M.K., M.K., M.P., M.P.N., M.W., N.S., P.J.F., P.L.G., P.S.G., P.S.L., P.W., R.V., S.B.A., S.H.P., S.M., S.P., T.E.W., T.W., W.K.A.Y., W.R.V., Y.H. and Z.E.-O. T.C., C.M.S.T., E.K., K.C.J., J.H.L., Z.E.-O., C. Heo, K.J.A., D.M.A., E.B.C., A.I., D.R.O., D.J.B., E.G.V.M., F.P.B., G.F., H.K.G., I.D., K.A., L.G., M.P.N., M.K., N.S., P.S.L., P.J.F., S.H.P., S.M., S.P., F.S.V. and R.G.W.V. participated in internal discussions about data interpretation through offline meetings and/or teleconferences. J.H.L., Z.E.-O., C. Hong, C. Horbinski, S.B.A., E.S., K.J.A., A.V.C., A.E.S., A.R.B., A.G., A.M.E.W., A.I., B.A.W., C.W., D.J.B., D.M.A., E.B.C., E.G.V.M., G.F., H.K.G., I.D., J.T.H., J.M.C., J.S.B.-S., J.F.C., K.A., L.M.P., L.G., M.P.N., M.C.M.K., M.W., M.K., N.S., P.S.L., P.J.F., S.H.P., S.M., S.P., T.E.W., T.W., W.K.A.Y., F.S.V. and R.G.W.V. are GLASS consortium members. R.G.W.V. led the GLASS consortium and this project.

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Competing interests R.G.W.V. is a co-founder of and holds equity in Boundless Bio. E.B.C. is a member of the research advisory committee of Servier Pharmaceuticals. E.G.V.M. is a co-founder and chief scientific officer of, and equity holder in, OncoSpherix. M.W. has received research grants from Novartis, Quercis and Versameb, and honoraria for lectures, advisory board participation or consulting from Anheart, Bayer, Curevac, Medac, Neurosense, Novartis, Novocure, Orbus, Pfizer, Philogen, Roche and Servier. P.J.F. has received research support from Servier. T.W. has received research grants from Cellis and honoraria for advisory board from Philogen. The remaining authors declare no competing interests.

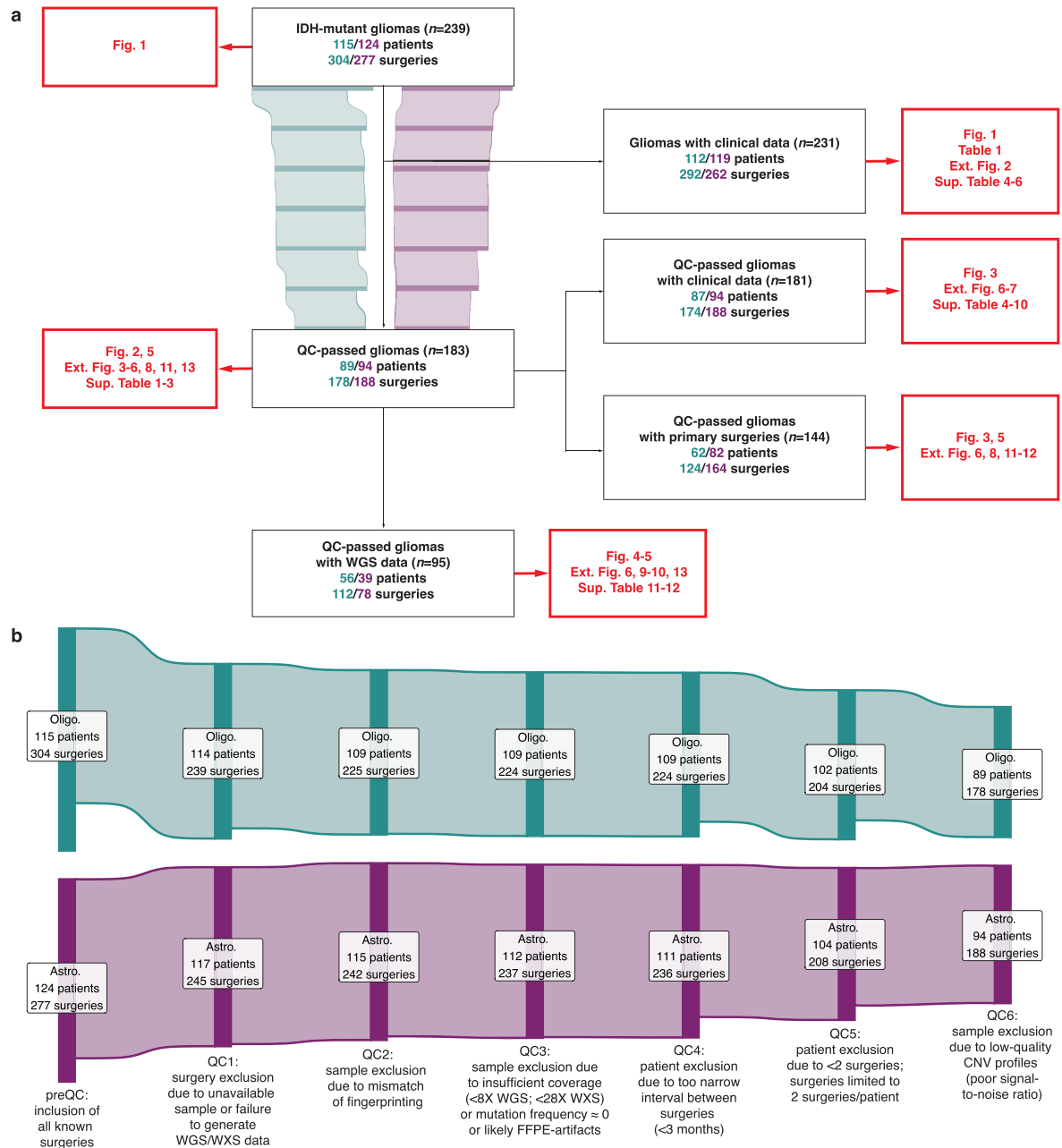
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-026-11150-x>.

Correspondence and requests for materials should be addressed to Frederick S. Varn or Roel G. W. Verhaak.

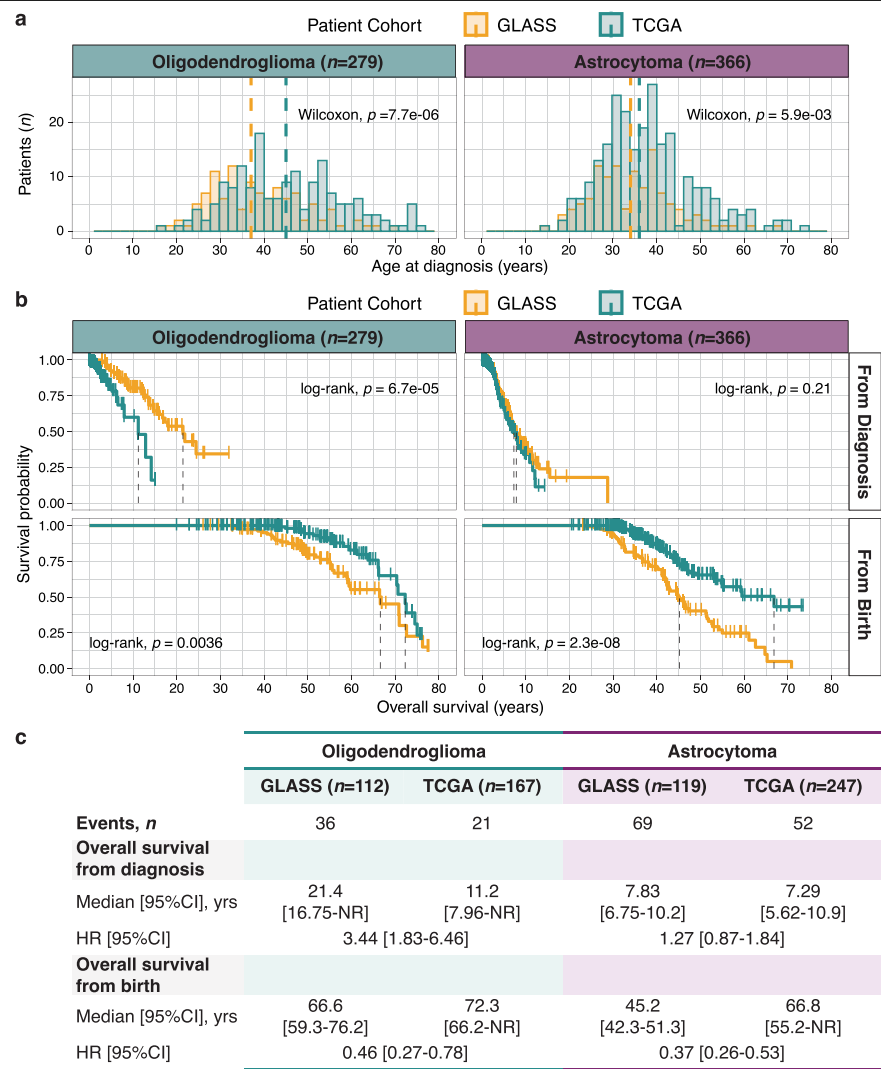
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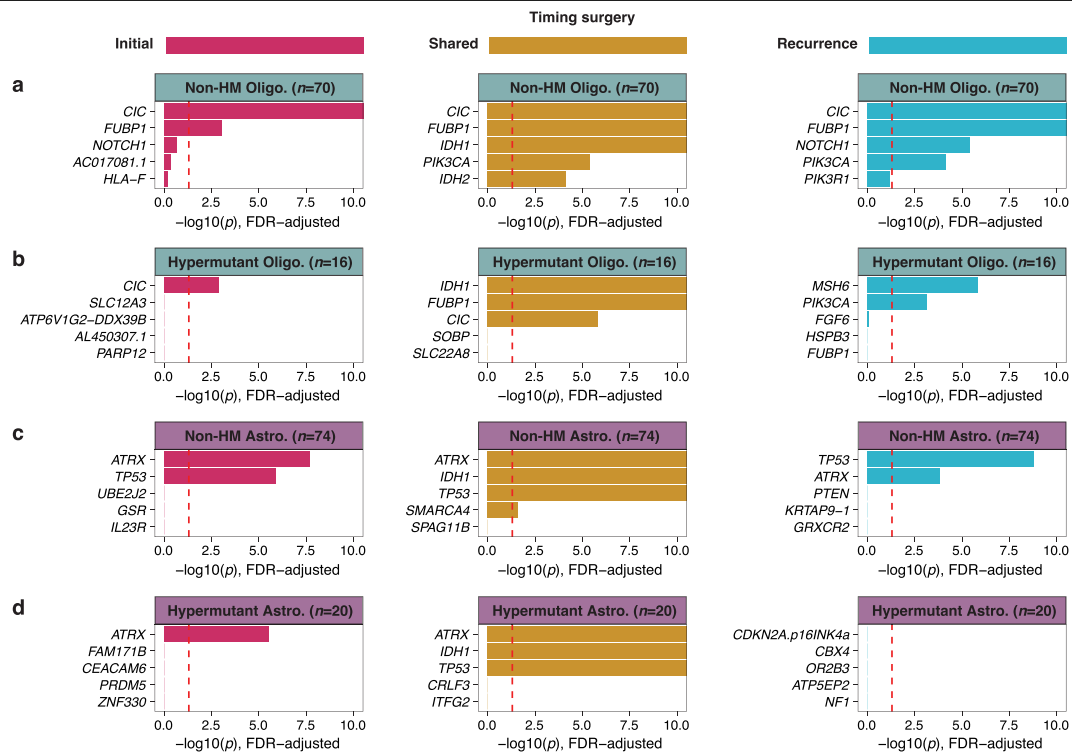
Extended Data Fig. 1 | Comprehensive overview of GLASS cohort selection, quality control and sample distribution across analyses. a, Flow chart summarizing the included patients and surgeries, annotated with the specific Figures and Tables in which each subset was used. In total, clinical and/or molecular data were available for 581 surgeries from 239 patients with IDH-mutant glioma. Clinical data were available for 231 patients, and molecular data were available for gliomas for which sequencing data across at least two time points passed all QC steps (see **b**) for 183 patients. 181 patients with QC-passed sequencing data across at least two time points had accompanying clinical data suitable for survival analyses, and 144 included data from primary surgeries rather than non-primary initial surgeries. A total of 95 patients with QC-passed gliomas had WGS data enabling mutational signature and chromosomal instability analyses. **b,** Sankey diagram illustrating the

sequential QC process, which reduced 581 surgeries from 239 patients (pre-QC) to 366 surgeries from 183 patients. QC1 selected surgeries for which a tumour sample was available and WGS and/or WXS data could be generated. QC2 excluded samples with fingerprinting mismatches. QC3 retained samples with sufficient coverage (≥8X for WGS; ≥28X for WXS), a non-zero mutation frequency, and no abnormally high mutation counts suggestive of FFPE artefacts. QC4 excluded one patient because the time between the available surgeries was less than 3 months. QC5 excluded patients with only one remaining surgery and retained only the first and last available surgery per patient. Lastly, QC6 excluded patients with CNV plots exhibiting a poor signal-to-noise ratio. Abbreviations: Ext., Extended Data; Sup., Supplementary; Oligo., oligodendroglioma; Astro., astrocytoma; CNV, copy-number variations.



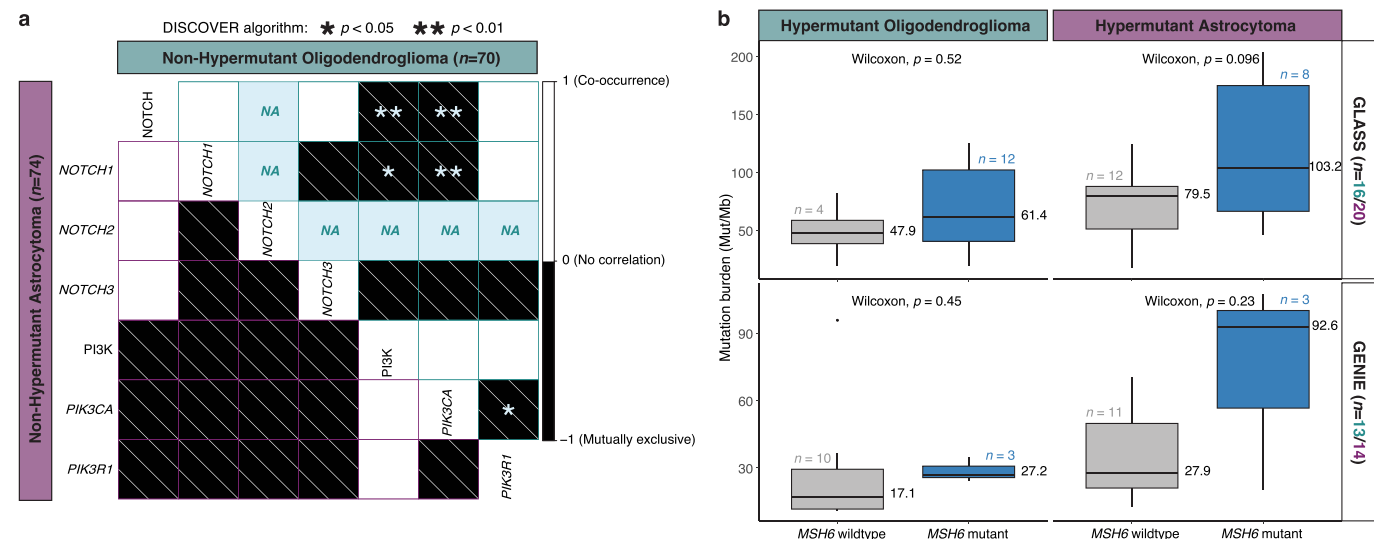
Extended Data Fig. 2 | Early disease onset in patients in the GLASS cohort is associated with reduced life expectancy, compared with those in the TCGA cohort. a, Age distribution at time of diagnosis of GLASS and TCGA cohorts compared using two-sided Wilcoxon rank-sum tests for unpaired data. Dotted lines represent median age across the respective cohorts. **b**, Kaplan–Meier estimates comparing overall survival with two-sided log-rank tests of GLASS and TCGA cohort patients measured from the time of diagnosis,

(top Kaplan–Meier panels) and from birth (bottom Kaplan–Meier panels). Projected overall survival from birth was calculated by adding individually reported overall survival years from diagnosis to age of disease onset. **c**, Tabular summary of **b** to emphasize the confidence intervals per group and hazard ratios between groups. Abbreviations: CI, confidence interval; yrs, years; HR, hazard ratio; NR, not reached.



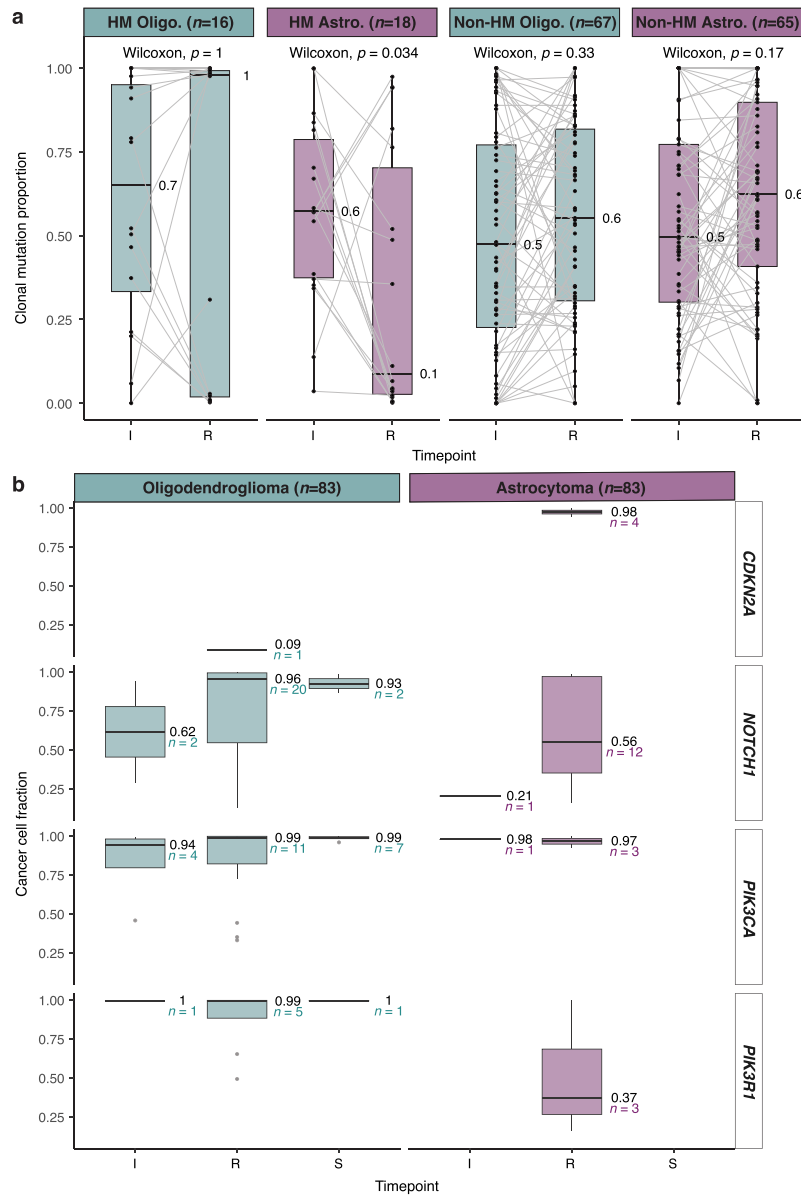
Extended Data Fig. 3 | Enrichment of driver sSNVs in PI3K, NOTCH1 and MSH6 in recurrent oligodendrogliomas. a–d, Enrichment dN/dS-based analyses with two-sided likelihood ratio tests were stratified by timing of surgery (left: initial, middle: shared between initial and recurrence, right: recurrence) and further divided by hypermutation status and tumour type:

non-hypermutant oligodendroglioma (a), hypermutant oligodendroglioma (b), non-hypermutant astrocytoma (c) and hypermutant astrocytoma (d). The red dotted line denotes FDR-adjusted $P = 0.05$. Abbreviations: Non-HM, non-hypermutant; Oligo., oligodendroglioma; FDR, false discovery rate; Astro., astrocytoma.



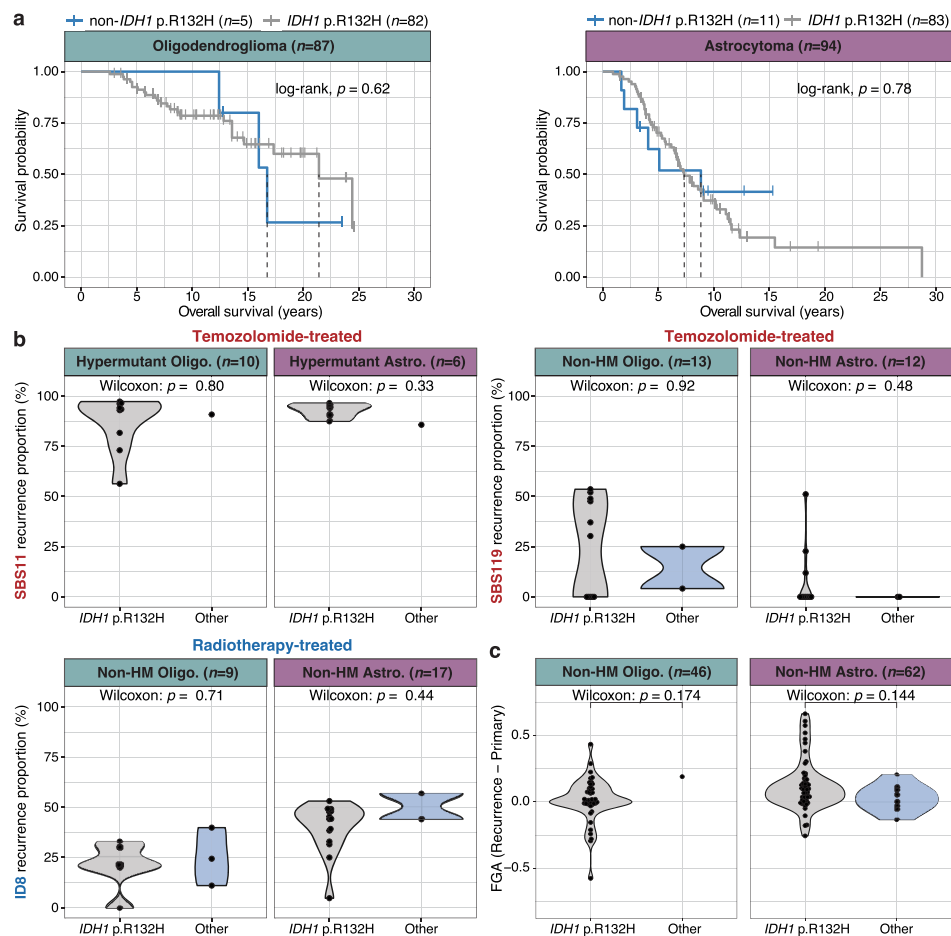
Extended Data Fig. 4 | Mutual exclusivity of PI3K and NOTCH1 sSNVs and high TMB in MSH6-mutant gliomas. a, Heat map of two-sided mutual exclusivity DISCOVER test²¹ P values of sSNVs detected in PI3K and NOTCH genes in non-hypermutant gliomas at recurrence. **b**, Comparison of mutational burden between MSH6-mutant and MSH6-wildtype hypermutant oligodendrogliomas and astrocytomas in the GLASS and AACR GENIE cohorts using two-sided

Wilcoxon rank-sum tests for unpaired data. Box plots depict median (centre) with interquartile range (box bounds), 1.5× the interquartile range (whiskers) and outliers (points). Abbreviations: DISCOVER, discrete independence statistic controlling for observations with varying event rates; NOTCH, NOTCH1, NOTCH2 and NOTCH3 combined; NA, not applicable; PI3K, PIK3CA and PIK3R1 combined; Mut, mutations; Mb, megabase.



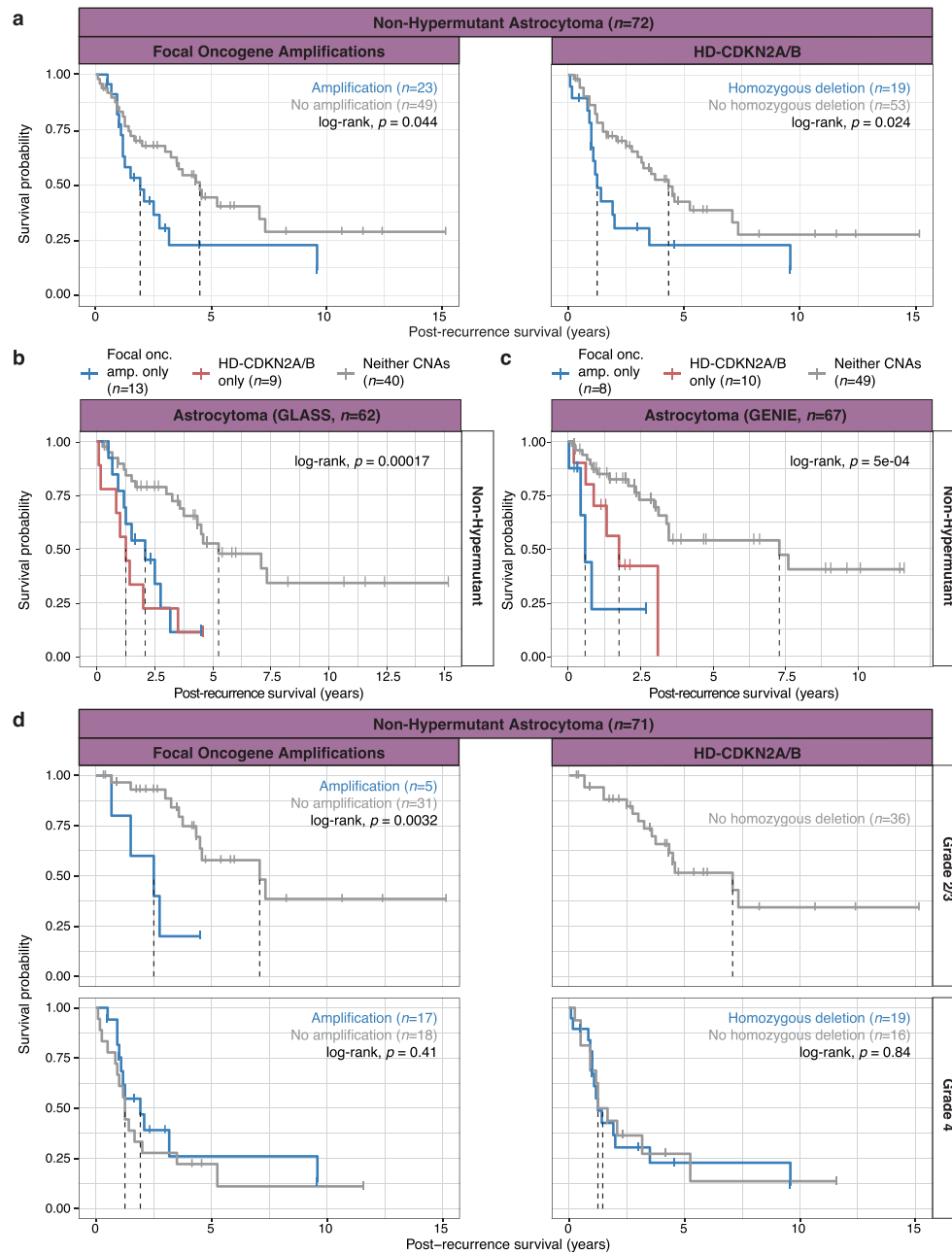
Extended Data Fig. 5 | Clonal mutation patterns and driver-gene clonality across IDH-mutant tumour types and time points. a, Clonal mutation proportion across IDH-mutant tumour types and by hypermutation status; significance determined by paired two-sided Wilcoxon's signed-rank test and sample sizes represent patients with matched initial-recurrence data per category. Box plots depict median (centre) with interquartile range

(box bounds), 1.5× the interquartile range (whiskers), and individual data points (points). **b,** Cancer-cell fraction values of enriched driver genes reflecting their clonal status by time point. Box plots depict median (centre) with interquartile range (box bounds), 1.5× the interquartile range (whiskers) and outliers (points). Abbreviations: HM, hypermutant; Oligo., oligodendroglioma; Astro., astrocytoma; Non-HM, non-hypermutant; I, initial; R, recurrence; S, shared.



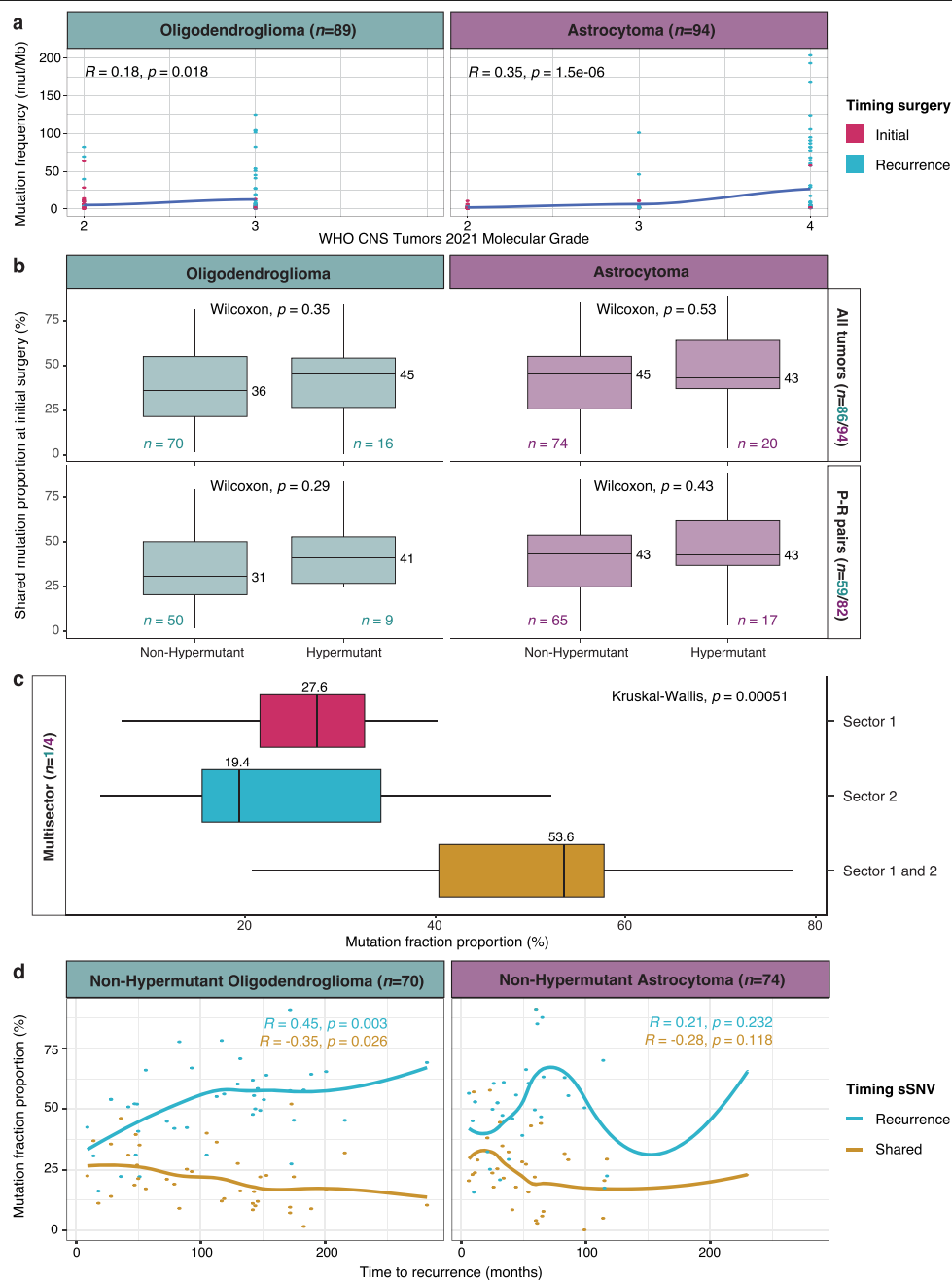
Extended Data Fig. 6 | Comparable overall survival, mutational signatures and FGA between *IDH1* p.R132H and other IDH mutations in both tumour types. a–c. Analyses comparing gliomas (left: oligodendroglioma; right: astrocytoma) with the most common IDH mutation, i.e. *IDH1* p.R132H, to gliomas with other canonical *IDH1* and *IDH2* mutations. **a**, Kaplan–Meier curves of overall survival with two-sided log-rank tests. **b**, Recurrent-specific proportions of mutational signatures for SBS11 in hypermutant gliomas treated with

temozolomide (top left), SBS119 in non-hypermutant gliomas treated with temozolomide (top right), and ID8 in non-hypermutant gliomas treated with radiotherapy (bottom left) with unpaired two-sided Wilcoxon rank-sum tests. **c**, Difference in FGA over time in matched primary and recurrence samples with the unpaired two-sided Wilcoxon rank-sum test. Abbreviations: Oligo., oligodendroglioma; Astro., astrocytoma; Non-HM, non-hypermutant; SBS, single-base substitutions signature; ID, small insertions and deletions signature.



Extended Data Fig. 7 | Post-recurrence survival in non-hypermutant astrocytomas is influenced by enriched driver CNAs. a–d. Post-recurrence survival Kaplan–Meier estimates for patients with non-hypermutant astrocytoma, with two-sided log-rank tests to determine significance. **a**, GLASS cohort stratified for focal oncogene amplifications and HD-CDKN2A/B. **b**, GLASS cohort stratified for focal oncogene amplifications and HD-CDKN2A/B

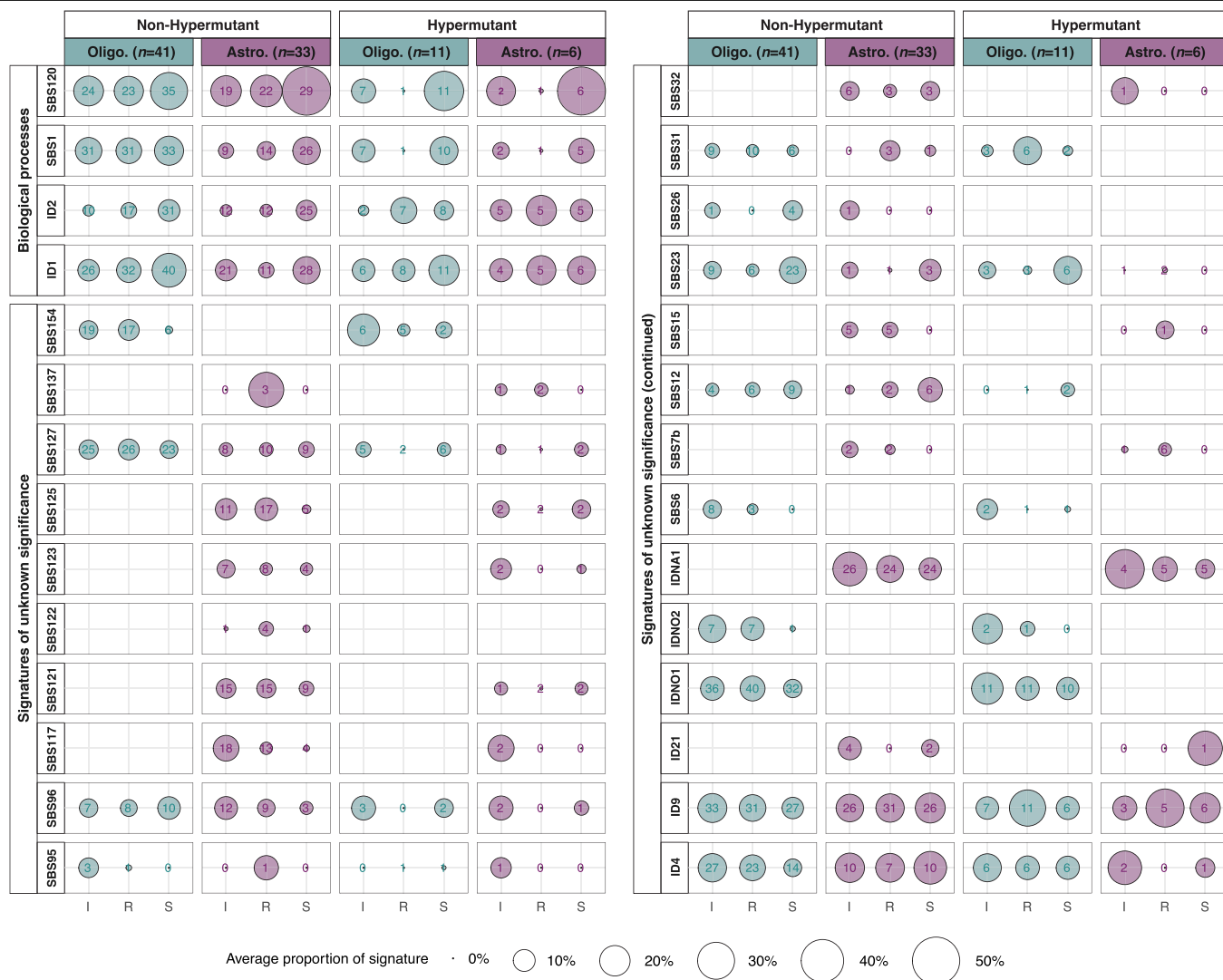
excluding those with both CNAs. **c**, GENIE cohort stratified for focal oncogene amplifications and HD-CDKN2A/B excluding those with both CNAs. **d**, GLASS cohort stratified for focal oncogene amplifications and HD-CDKN2A/B as well as grade. Abbreviations: Focal onc. amp., focal oncogene amplifications i.e. *CCND2*, *CDK4*, *CDK6*, *EGFR*, *MYC*, *MYCN*, *MET*, *PDGFRA*, or *PTPRZ1*; HD-CDKN2A/B, homozygous deletions of *CDKN2A/B*.



Extended Data Fig. 8 | Intratumoral heterogeneity is suggested by variation in mutation distributions across different sectors of the tumour.

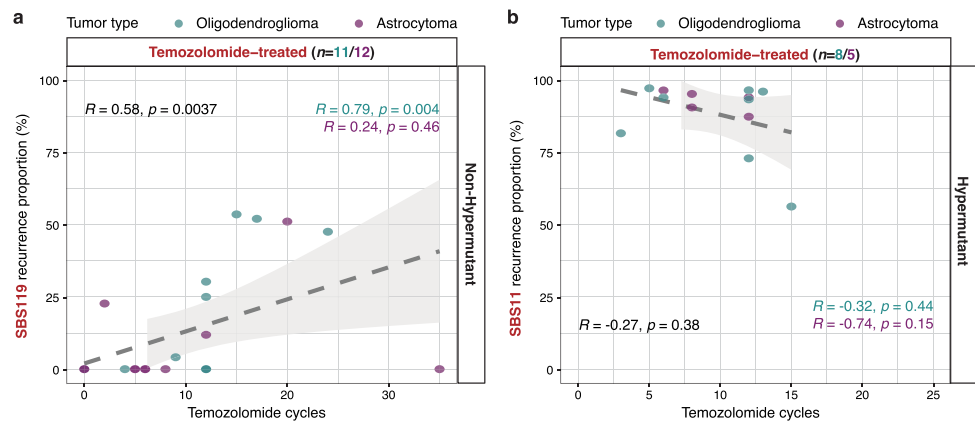
a, Two-sided Spearman's correlations of the mutation frequency stratified by WHO molecular grade. **b, c**, Box plots depict median (centre) with interquartile range (box bounds) and 1.5× the interquartile range (whiskers). **b**, Shared mutation proportion which were present at initial surgery in oligodendrogliomas and astrocytomas compared between hypermutant and non-hypermutant tumours using two-sided unpaired Wilcoxon rank-sum tests. Separate analyses were performed for all available tumours (top) and only those with matched

primary and recurrence surgeries (bottom). **c**, Multisector sampling during the same surgery, comparing mutation distributions across different sectors in 1 oligodendroglioma and 4 astrocytomas using the two-sided Kruskal-Wallis test for unpaired data. Median fraction of mutations detected uniquely in sector 1 (top), uniquely detected in sector 2 (middle) or shared between sectors 1 and 2 (bottom). **d**, Two-sided Spearman's correlations of the recurrence-only and the shared mutation burden fractions non-hypermutant gliomas (left: oligodendrogliomas; right: astrocytomas) relative to time between surgeries. Abbreviations: Mut, mutations; Mb, megabase; P-R, primary-recurrence.



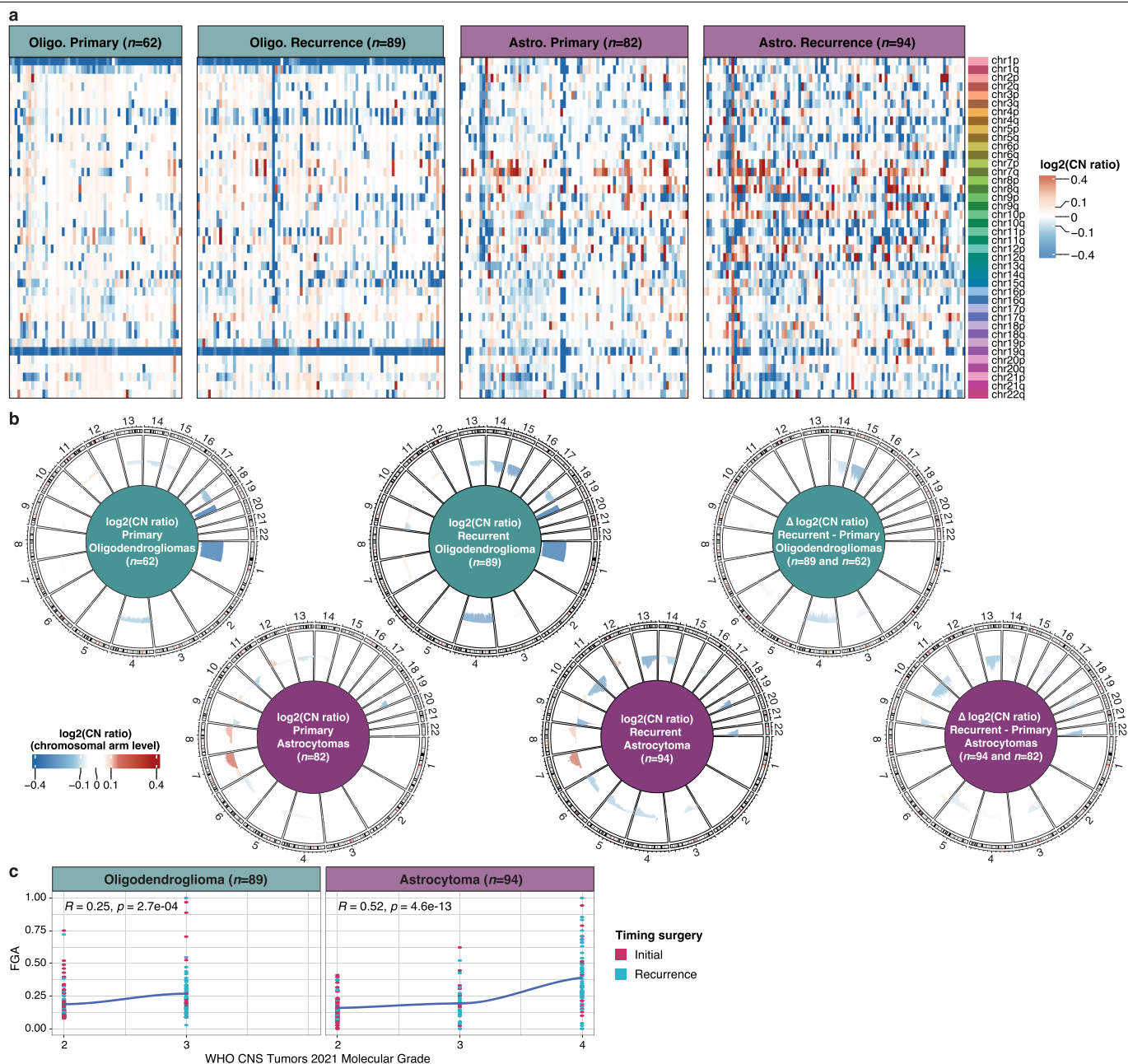
Extended Data Fig. 9 | Distinct mutational signatures provide insights into the aetiology and evolution of gliomas. Comparison of SBS and ID mutational signatures using the average percentage of alterations attributed to these signatures (circle size) and the number of patients with any alteration attributable to these signatures (number in circle) per glioma type, hypermutation status, treatment regimen, and timing of the signatures. De novo extracted signatures without >0.8 similarity to known reference

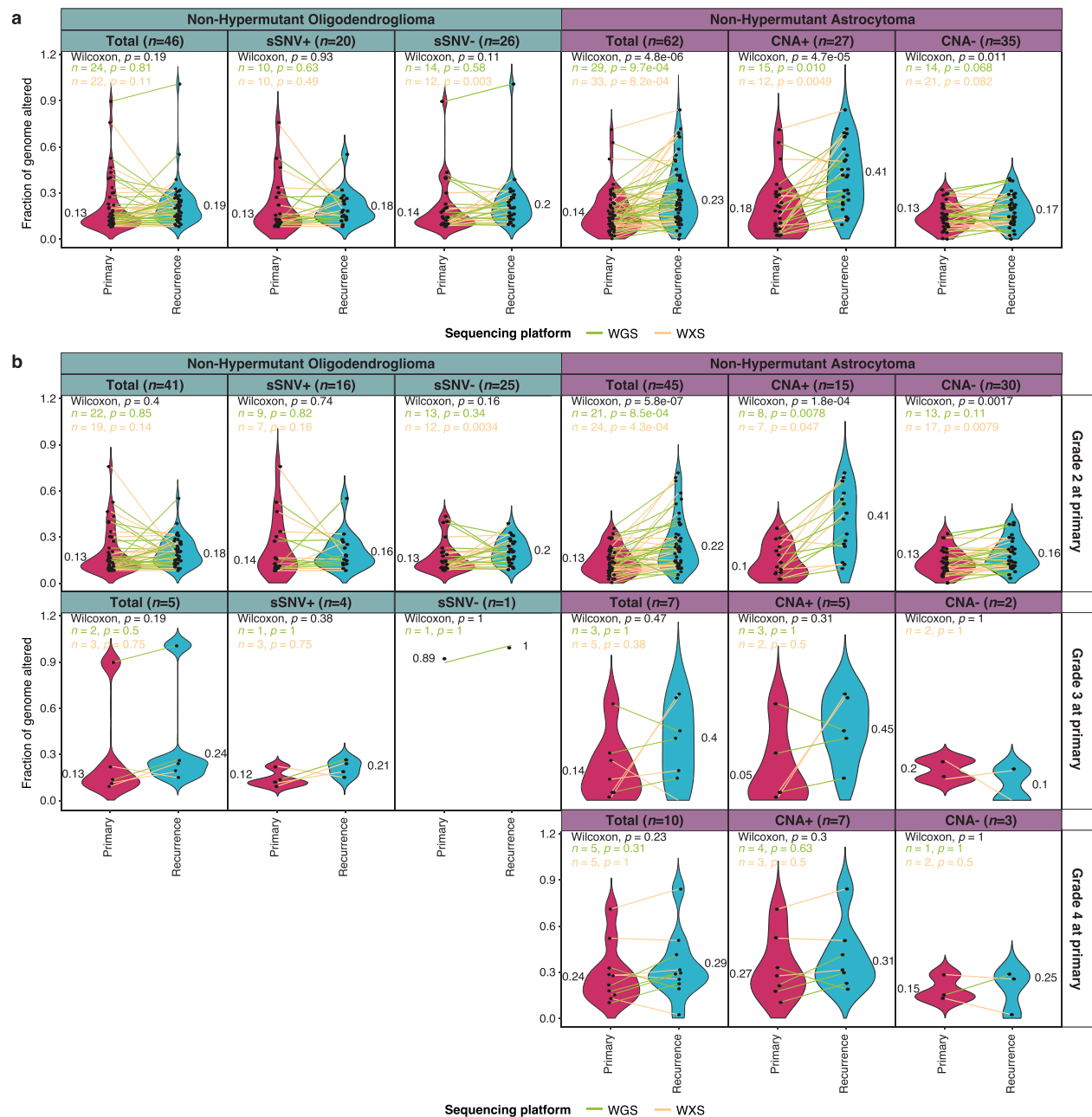
signatures were classified as novel signatures; this included two ID signatures for oligodendrogliomas and one ID signature for astrocytomas. Abbreviations: Oligo., oligodendroglioma; Astro., astrocytoma; SBS, single-base substitutions signature; ID, small insertions and deletions signature; IDNA, not previously reported ID mutational signature detected in the astrocytoma cohort; IDNO, not previously reported ID mutational signature detected in the oligodendroglioma cohort; I, initial; R, recurrence; S, shared.



Extended Data Fig. 10 | SBS119 but not SBS11 is correlated with the number of temozolomide cycles administered. a, b. Correlation between the number of received temozolomide cycles and the recurrence-specific proportion of the SBS119 signature in non-hypermutant gliomas (a) and the SBS11 signature in hypermutant gliomas (b) (purple: astrocytoma; teal: oligodendroglioma). Correlation strength and significance were evaluated using Spearman's

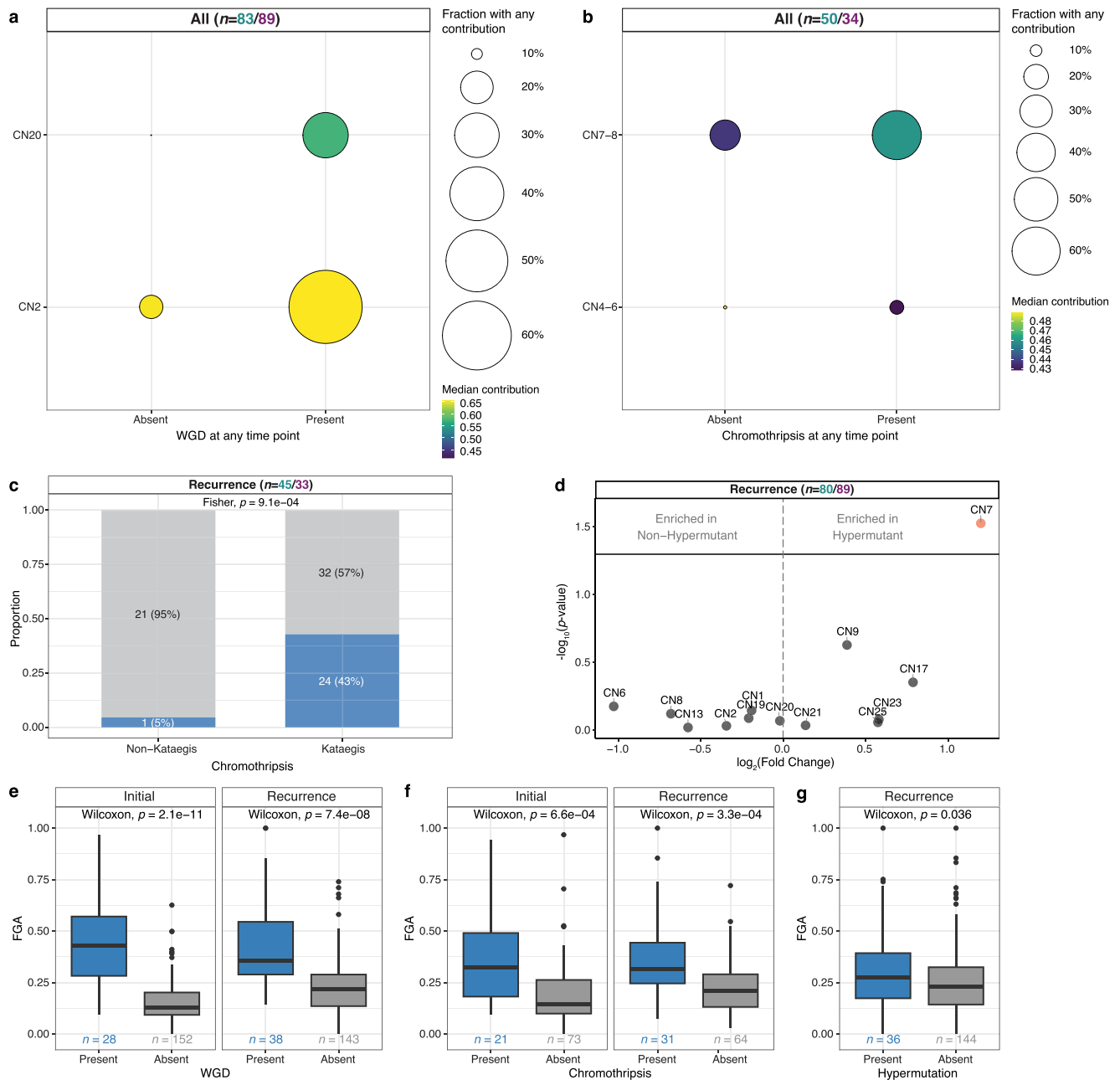
correlation coefficient (R) and corresponding two-sided P values (purple: astrocytoma; teal: oligodendroglioma; black: both tumour types). Dotted lines depict fitted linear regression models, with the shaded grey areas indicating their 95% confidence intervals. Abbreviations: SBS, single-base substitution signature.





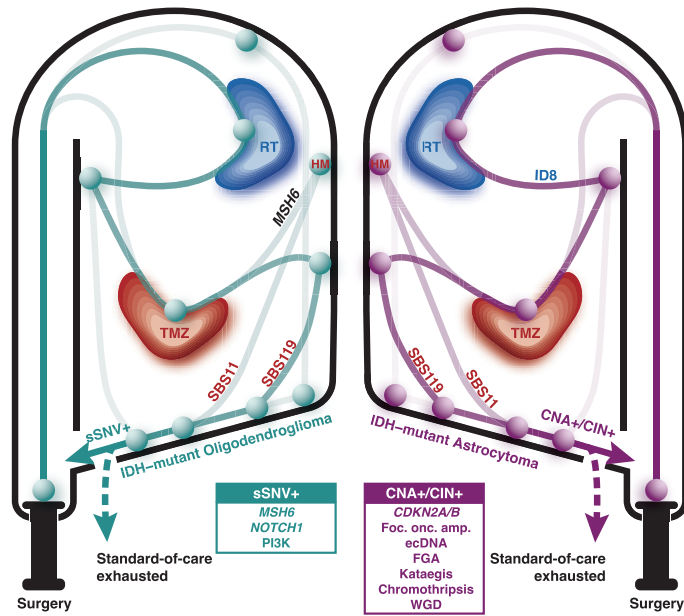
Extended Data Fig. 12 | Stable FGA in non-hypermutant oligodendrogliomas only, independent of driver status and primary grade. a. Comparison of non-hypermutant genome-wide aneuploidy (measured as FGA) between matching primary and recurrent samples using two-sided Wilcoxon's signed-rank test for paired data analysis per group and sequencing platform subgroups. Patients are grouped by glioma type and tumour-type-enriched driver-gene alterations; that

is, the sSNVs for non-hypermutant oligodendrogliomas (mutations of *NOTCH1*, *PIK3CA*, and *PIK3R1*) and the CNAs for non-hypermutant astrocytomas (focal oncogene amplifications of *CCND2*, *CDK4*, *CDK6*, *EGFR*, *MET*, *MYC*, *MYCN*, *PDGFRA*, and *PTPRZ1* or homozygous deletions of *CDKN2A/B*). **b.** Grade-related separation of analyses of **a**, where analyses were stratified for grade at primary surgery.



Extended Data Fig. 13 | Copy-number signatures associated with WGD or chromothripsis and FGA associated with genomic features. **a, b**, Bubble plots depicting copy-number signature prevalence (size corresponding to fraction of samples with any contribution of the signature) and contribution (colour intensity corresponding to median contribution of samples) for samples for which copy-number signature data were available. **a**, Comparison of samples separated based on WGD status. **b**, Comparing WGS samples for which chromothripsis annotation and copy-number signature data were available. Samples separated based on chromothripsis status and grouped into two different chromothripsis signature groups CN4-8 and CN7-8, respectively. **c**, Prevalence of chromothripsis in non-hypermutant recurrent samples separated by presence and absence of kataegis; data for tumour types are combined and significance determined using two-sided Fisher's exact test for unpaired data. **d**, Association between hypermutation status and relative

copy-number signature contribution of all gliomas at recurrence depicting the effect size as $\log_2$ (fold change) between mean relative contribution in each group and $-\log_{10}(P)$. Orange colour indicates nominal copy-number signature association (unadjusted $P < 0.05$) determined using two-sided unpaired Wilcoxon rank-sum tests. **e–g**, Box plots depict median (centre) with interquartile range (box bounds), $1.5 \times$ the interquartile range (whiskers) and outliers (points). **e**, Box plots comparing FGA in samples containing or lacking WGD separated by time point (n = samples at time point) using two-sided unpaired Wilcoxon rank-sum tests. **f**, Box plots comparing FGA in WGS samples containing or lacking chromothripsis separated by time point (n = samples at time point) using two-sided unpaired Wilcoxon rank-sum tests. **g**, Box plots comparing FGA in number of samples at recurrence containing or lacking hypermutation using two-sided unpaired Wilcoxon rank-sum tests. Abbreviations: CN, copy-number signature.



Extended Data Fig. 14 | IDH-mutant gliomas evolve under shared pressures, but the resulting alterations differ in their type or extent. Throughout their clinical trajectories, most patients with IDH-mutant glioma are treated with radiotherapy and/or temozolomide. Although shaped by the same treatment-based selection pressures, changes in the genomes of astrocytomas and oligodendrogliomas suggest that different DNA damage response mechanisms are activated in response to therapy, resulting in acquiring of distinct sets of somatic alterations. At progression, astrocytomas are enriched for focal DNA CNAs, the ID8 signature, aneuploidies and chromosomal instability markers, whereas recurrent oligodendrogliomas evolve with new mutations in PI3K, *MSH6* and *NOTCH1* genes. The inevitable recurrence of disease either leads to a recurrence surgery and possible (re-)treatment with chemoradiation or a palliative setting when all standard-of-care options have been exhausted. Abbreviations: RT, radiotherapy; TMZ, temozolomide; HM, hypermutation; SBS, single-base substitutions signature; ID, small insertions and deletions signature; CIN, chromosomal instability; PI3K, *PIK3CA* and *PIK3RI* combined; Foc. onc. amp., Focal oncogene amplifications i.e. *CCND2*, *CDK4*, *CDK6*, *EGFR*, *MYC*, *MYCN*, *MET*, *PDGFRA*, or *PTPRZ1*; ecDNA, extrachromosomal DNA.

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☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	No software was used for data collection.
Data analysis	Data analysis was conducted and visualized using R (v4.3.2), Python (v3.10.13), BCFTools (v1.9), Circlize (v0.4.16), ComplexHeatmap (v1.10.2), Corrplot (v0.95), DBI (v1.2.2), DISCOVER (v3.1.7), dNdScv (v0.1.0), Duckplyr (v1.1.1), GATK4 (v4.1.0.0), GenomicRanges (v1.24.1), GeoPandas (v1.1.1), Ggh4x (v0.3.0), Ggplot2 (v3.5.1), Ggpubr (v0.6.0), GISTIC (v2.0.23), Grid (v3.6.2), GridExtra (v2.3), Gtsummary (v1.6.3), Matplotlib (v3.10.5), MutSigCV (v1.3.5), Numpy (v2.3.2), Palimpsest (v2.0.0), Pandas (v2.3.1), Patchwork (v1.3.1), RPostgres (v1.4.4), Scales (v1.4.0), Shapely (v2.1.1), SigProfilerExtractor (v1.2.1), Snakemake (v5.2.2), Survival (v3.8-3), Survminer (v0.5.0), and Tidyverse (v2.0.0). Custom computational pipeline scripts for sequencing data pre-processing and downstream data analyses are accessible via the project's GitHub repository (https://github.com/verhaaklab/glass-idh).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Deidentified somatic variant and clinical GLASS datasets are publicly available via Synapse (<https://synapse.org/glass>) with Project SynID syn17038081. A summarized version of the processed data per patient is available in Supplementary Table 1.

For the reference cohorts data is available at CGHub (<https://cghub.ucsc.edu>), the Cancer Genome Atlas Data Coordinating Center (<http://cancergenome.nih.gov>), the Cancer Genome Atlas lower-grade glioma publication page (https://tcga-data.nci.nih.gov/docs/publications/lgg_2015), The GENIE Synapse (<https://www.synapse.org/genie>) and the GENIE cBioPortal (<https://genie.cbioportal.org>).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Self-reported patient sex was accounted for in survival analyses because this is historically known to be a significant prognostic factor for patients with IDH-mutant gliomas. Gender-based analyses have not been performed nor has data on patient gender been collected.
Reporting on race, ethnicity, or other socially relevant groupings	Data on patient race or ethnicity have not been collected.
Population characteristics	Human research participants consisted of patients at Yale University with genomically confirmed IDH-mutant gliomas. No participants were preselected based on demographic or clinical characteristics. Relevant population and clinical characteristics considered in downstream analyses included patient age, sex, treatment regimen, surgical details, and tumor pathology.
Recruitment	Individual tissue source centers retrospectively identified patients who had undergone at least two surgeries at different time points.
Ethics oversight	Each tissue source center obtained approval from its respective Institutional Review Board (IRB) and informed consent from all patients included in the cohort. Data aggregation at The Jackson Laboratory and Yale University was conducted under the oversight of their own IRBs. The protocol used to collect and sequence specimens, or collect and analyze data from patients, was approved by the institutional review board (IRB) of Yale University (2000036542, 2000041140, 2000039350, 2000036441, 2000035189, 2000034831, 2000035190, 2000035426, 2000035453) and as previously reported in Barthel FP et al. Nature 2019 and Varn FS et al. Cell 2022

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was determined by the availability of patient-derived tumor specimens collected at two distinct time points from the GLASS cohort.
Data exclusions	Patients with IDH-wildtype gliomas were excluded from analysis for this manuscript, because this is a biologically different entity.
Replication	Data from the GLASS cohort was compared to two reference cohorts: the LGG TCGA database, and the AACR GENIE database. This established that clinical characteristics were similar among cohorts.
Randomization	The current study was performed through retrospective analysis, and randomization was therefore not applicable.

Blinding

The current study was performed through retrospective analysis, and blinding was therefore not applicable.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A