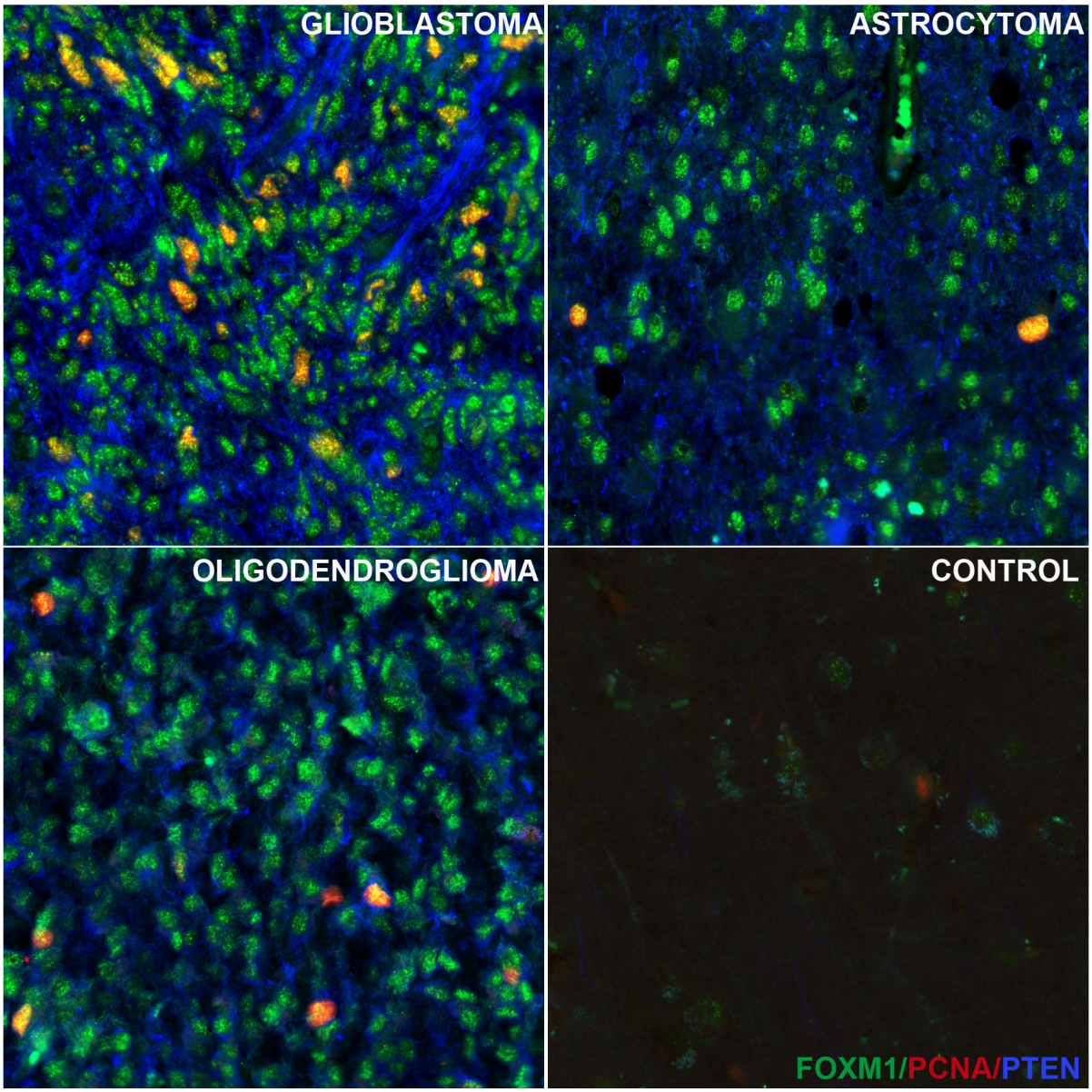

GLIOMA MARKERS

ATLAS ANTIBODIES

Gliomas are one of the most aggressive and lethal solid tumors of the central nervous system. Despite the alarming numbers, the molecular pathogenetic mechanisms of gliomas are not yet fully elucidated. Nowadays, several approaches aim to understand the biology of the disease and identify promising markers, which may provide effective novel therapies. This white paper summarizes the recent developments in glioma classification and the key molecular markers for glioma stratification. Next, it highlights the glioma proteome with a list of relevant genes with favorable and unfavorable prognostic values in glioma. It then focuses on the glioma tumor microenvironment that may provide helpful insights when developing novel therapeutic strategies.

Gliomas are solid tumors of the central nervous system (CNS). They arise from the neoplastic transformation of glial cells, namely astrocytes, oligodendrocytes, and ependymal cells.

Brain tumors comprise approximately 2% of all adult cancers but form a larger fraction within the group of childhood tumors. Gliomas account for about 80% of all malignant brain tumors and are classified according to the cell type of origin, differentiation, and malignancy grade. Gliomas show considerable variability in age of onset, grade of severity, histological features, and ability to progress, as well as to metastasize.

Survival time after diagnosis with glioma varies significantly depending on grade. According to the World Health Organization (WHO) classification, gliomas are divided into four grades. Grade 1, 2, and 3 are classified as low-grade gliomas, while grade 4 are classified as high-grade gliomas. Unfortunately, the prognosis for high-grade gliomas is poor due to limited possibilities of curative treatment.

The most common form of glioma is an astrocytoma, representing approximately 50% of all gliomas. Grade 4 astrocytoma is also known as glioblastoma or glioblastoma multiforme (GBM).

GBM is the most frequent and malignant histological type, accounting for 65% of gliomas with a very poor 5-year survival (less than 5%). GBM is typically classified into three subtypes, namely proneural, classical and mesenchymal, according to the gene expression of various biomarkers, including platelet-derived growth factor receptor (PDGFR), neurofilament light chain (NF-L), epidermal growth factor receptor (EGFR), and CD44, respectively. Pro-neural to mesenchymal transition of glioma is associated with aggressive phenotypes, unfavorable prognosis, and treatment resistance.

Gliomas are well-known tumors at the molecular level. However, these advances have not been translated into therapeutic benefits for the patients.

While our knowledge about the molecular biology of gliomas is rapidly expanding and is, to some extent, already assisting us in the design of tumor-tailored therapeutics, such as immunotherapy treatments, we are still struggling to develop efficient treatments. As a result, the overall survival in patients with gliomas has not significantly increased in the last decades.

Typical treatments for gliomas involve surgical resection of the tumor mass, radiotherapy, and chemotherapy treatments. However, relapse or even recurrence of gliomas is common and mainly due to their infiltrative growth and their high proliferative abilities.

Due to the absence of definitive surgical and medical treatments currently available, an early diagnosis coupled with an accurate tumor classification is crucial to select a personalized treatment.

Moreover, the functional heterogeneity in gliomas is defined not only by the genetic makeup of glioma cells but also through microenvironment-driven epigenetic influences that regulate glioma cell stemness.

Hence, elucidating the state transition programs and mechanisms driving cellular plasticity is essential to overcome current therapeutic limitations in GBM.

We must expand our knowledge as a step toward the design of practical and safe treatments. Therefore, the identification of molecular biomarkers is unquestionably essential and urgent for an accurate prognosis and development of critical therapeutic targets in gliomas.

Cover Image: Multiplexed IHC-IF staining of human glioblastoma, astrocytoma, olygodendrocytoma and normal cortex samples using the Anti-FOXM1 monoclonal AMAb91766 (nuclear, in green), the Anti-PCNA polyclonal HPA030522 (nuclear, in red) and the Anti-PTEN monoclonal AMAb91736 (cytoplasmic, in blue) antibodies.

Glioma classification and key molecular pathology.

Since 2008 the classification of brain tumors by the World Health Organization (WHO) has continued to evolve up to today with the latest fifth edition (WHO CNS5, 2021)(Louis 2021).

The updated classification emphasizes the need for a classification of gliomas based on histological and molecular genetic features for clinical diagnosis and outcome prediction.

With the WHO CNS5 2021 edition, several IHC diagnostic and prognostic markers have been added to the diagnostic criteria of gliomas. The new seven-layers approach for glioma classification is shown in **figure 1.**

Antibodies against ATRX, the mutated forms of IDH1 (IDH R132H), and histone H3 (H3K27M) are used to detect the respective mutations. Analysis of deletion of 1p and 19q chromosome arms is done by multiplex ligation-dependent probe amplification (MLPA) or, less commonly, by fluorescent in situ hybridization.

The distinction between the different forms of glioma is mainly based on morphological features, mutations and chromosomal aberrations (**figure 2**).

In gliomas neuropathological diagnostics, antibodies directed towards proteins such as IDH (isocitrate dehydrogenase), ATRX (alpha thalassemia/mental retardation syndrome X-linked), GFAP (glial fibrillary acidic protein), SYN (synaptophysin), EGFR (epidermal growth factor receptor), p53 (tumor suppressor protein 53), and the proliferation marker Ki-67 (MKI67) are the gold standard and routinely used.

Immunohistochemistry (IHC) plays a vital role in distinguishing between different tumor types. **Figures 3-6** show some examples of IDH1, ATRX, and GFAP multiplexed IHC-IF staining in different glioma grades.

GLIOMAS: THE 7 LAYERS CLASSIFICATION (WHO 2021)

1. Isocitrate dehydrogenase (IDH1) mutations

2. α-thalassemia/mental-retardation-syndrome-X-linked gene (ATRX) expression

3. 1p/19q co-deletion

4. CDKN2A/B homozygous deletion on 9p21

5. TERT promoter mutation/EGFR gene amplification and/or chromosomes 7 gain and 10 loss (+7/–10)

6. Histone H3 G34R/V mutations

7. Histone H3 K27M/ mutations

Figure 1. The 7 layers approach for glioma classification based on WHO CNS5 2021.

MOLECULAR PATHOLOGY IN HIGH GRADE GLIOMAS

Methylation:
MGMT, PTEN, RB1, TP53, CDKN2A, PDGFB, EMP3, SOCS1, PCDHGA11, OLIIG1/2

Chromosomal aberrations:
Gain: 1q, 19q, 20q
Loss: 6q, 9p, 13q, 14q, 22q
Concomitant: +7/-10

Mutations:
PTEN, ATRX, TP53, RB1, IDH1/2, NF1, EGFR

Amplifications:
MET, EGFR, PIK3CA, PDGFRA, CCND2, MDM2/4

Deletions:
CDKN2A, CDKN2B, CDKN2C, PTEN, RB1, NFKB1A

Figure 2. Molecular pathology in high-grade gliomas.

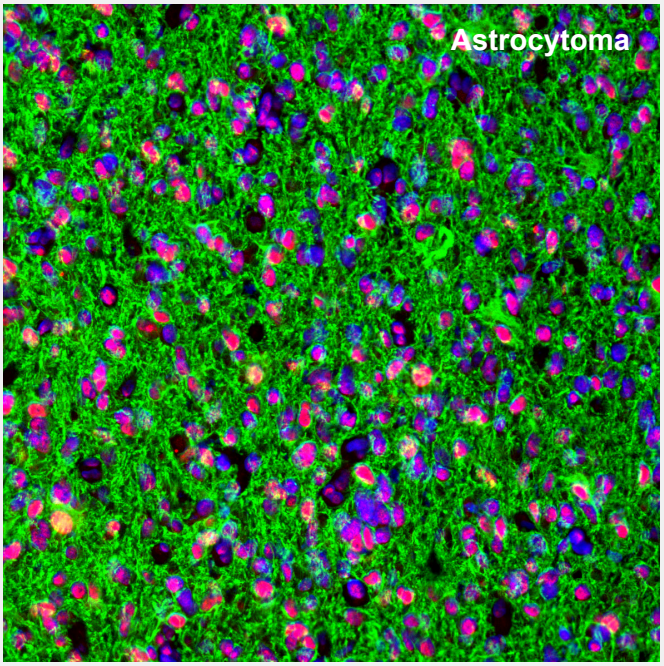

Figure 3. ATRX and GFAP in astrocytoma.
Multiplexed IHC-IF staining of astrocytoma showing ATRX (nuclear, red) and GFAP (cytoplasmic, green) immunoreactivity in tumor cells. The **Anti-ATRX AMAb90784** and **Anti-GFAP AMAb91033** monoclonal antibodies were used. Nuclei were counterstained with DAPI.

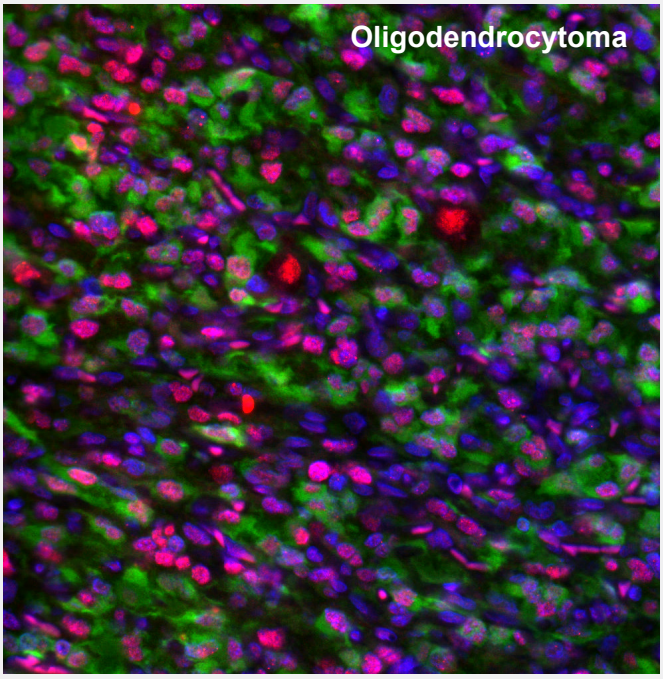

Figure 4. ATRX and IDH1 in oligodendrocytoma.
Multiplexed IHC-IF staining of anaplastic oligodendroglioma showing ATRX (nuclear, red) and IDH1 (cytoplasmic, green) immunoreactivity in tumor cells. The **Anti-ATRX AMAb90784** and **Anti-CDH1 AMAb90578** monoclonal antibodies were used, nuclei were counterstained with DAPI.

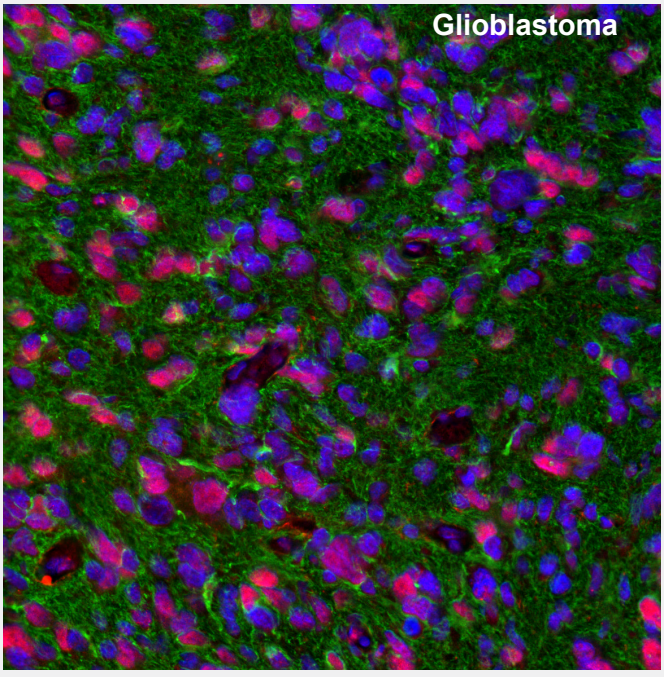

Figure 5. ATRX and GFAP in glioblastoma.
Multiplexed IHC-IF staining of glioblastoma multiforme showing ATRX (nuclear, red) and GFAP (cytoplasmic, green) immunoreactivity in tumor cells. The **Anti-ATRX AMAb90784** and **Anti-GFAP AMAb91033** monoclonal antibodies were used, nuclei were counterstained with DAPI.

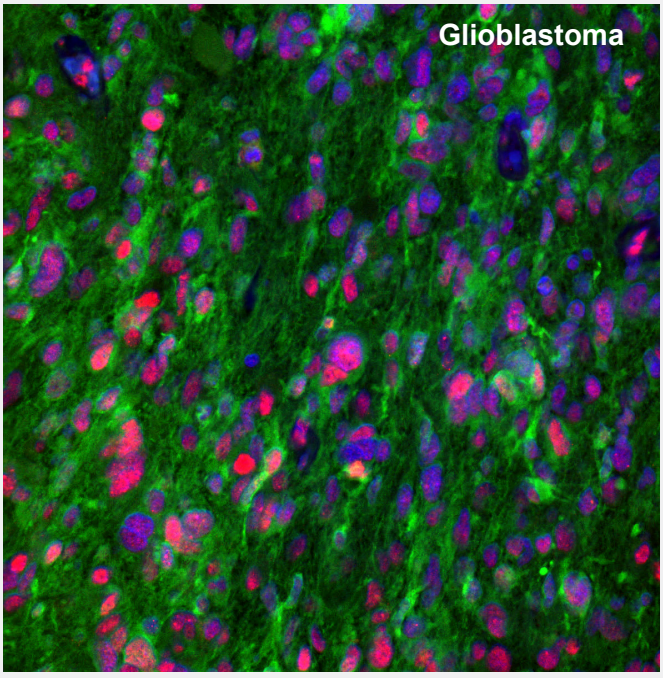

Figure 6. ATRX and IDH1 in glioblastoma.
Multiplexed IHC-IF staining of glioblastoma multiforme showing ATRX (red) and IDH1 (green) immunoreactivity in tumor cells using the **Anti-ATRX HPA001906** polyclonal and the **Anti-CDH1 AMAb90578** monoclonal antibodies. Nuclei were counterstained with DAPI.

Adult and pediatric gliomas: distinctive features.

ADULT-TYPE DIFFUSE GLIOMAS

ASTROCYTOMAS
IDH-mutant, 1p/19q non-co-deleted
Grade 2,3,4
IDH1-2, ATRX, TP53, CDKN2A/B

OLIGODENDROGLIOMAS
IDH-mutant, 1p/19q-co-deleted
Grade 2,3
IDH1-2, 1p/19q, TERT, CIC, FUBP1, NOTCH1

GLIOBLASTOMAS
IDH-wildtype
Grade 4
TERT, chromosomes +7/-10, EGFR

PEDIATRIC-TYPE DIFFUSE GLIOMAS

LOW-GRADE

DIFFUSE ASTROCYTOMA
Grade 1
MYB- or MYBL1-altered

ANGIOCENTRIC GLIOMA
Grade 1
ATRX, p53, IDH1 (R132H), BRAF V600E, H3 K27M

POLYMORPHOUS NEUROEPITHELIAL TUMOR OF THE YOUNG
Grade 1
FGFR3 amplification, BRAF V600E

DIFFUSE LOW-GRADE GLIOMA
G*
MAPK

HIGH-GRADE

DIFFUSE MIDLINE GLIOMA
G*
H3 K27-altered, PDGFR-α

DIFFUSE HEMISPHERIC GLIOMA
Grade 4
H3 G34-mutant

DIFFUSE PEDIATRIC-TYPE HIGH-GRADE GLIOMA
G*
H3-wildtype and IDH-wildtype

INFANT-TYPE HEMISPHERIC GLIOMA
G*
ALK, ROS1, NTRK1/2/3, or MET

An essential update in the WHO CNS5 2021 classification of brain tumors is the distinction of diffuse gliomas into adult-type and pediatric-type, all with distinct responses to treatment and outcomes (**figure 7**).

The distinction between different forms of gliomas is mainly based on morphological features; however, IHC analysis plays a vital role in distinguishing between different tumor types, particularly when the tumor is poorly differentiated.

Numerous reports have highlighted substantial differences in adult and pediatric gliomas mainly based on histological observations, frequency, location, and pathologic spectrum (Broniscer 2007). Pediatric high-grade gliomas, for example, often arise in brain regions that are rarely targeted in adult disease.

Pediatric diffuse gliomas are more complex and molecularly distinct from those in adults. Pediatric gliomas are generally classified as low-grade and high-grade and are characterized by circumscribed growth and frequent H3 and BRAF gene fusions or mutations (Louis 2021).

On the other hand, PTEN mutations and EGFR amplification, frequent in adult primary glioblastoma, are less common in children (Pollack 2006). Furthermore, in adults, secondary glioblastomas rarely contain EGFR amplification (Furnari 2007).

Mutations in TP53, CDKN2A, and PIK3CA are common in adult and pediatric high-grade gliomas (Gallia 2006). Furthermore, the platelet-derived growth factor receptor-α (PDGFR-α) is the predominant target of focal amplification in high-grade childhood glioma and may be a valuable target for the pediatric population (Paugh 2010).

Significant differences in copy number alterations distinguish childhood and adult glioblastoma. No IDH1 hotspot mutations were found in pediatric tumors, highlighting molecular differences with adult secondary glioblastoma. Frequent gain of chromosome 1q and lower frequency of chromosome 7 gain and 10q loss also clearly distinguished childhood from adult high-grade gliomas (Paugh 2010).

Figure 7. Adult- and pediatric-type of diffuse gliomas: classification, grade and key diagnostic genes.
Gliomas are classified according to cell type of origin, differentiation and malignancy grade. Type, grade, and molecular signatures of glioma determine diagnosis and treatment options. Based on an integrative molecular profiling, diffusely infiltrating gliomas in adults are separated into three overarching groups depending on origins, mutations in isocitrate dehydrogenase (IDH), ATRX and 1p/19q expression. In the pediatric population diffuse gliomas are classified as low-grade gliomas and high-grade gliomas. G* (attribution of a precise grade depends on the specific characterization following the integration of histo-pathological and molecular information).

The glioma proteome

The glioma proteome is revealed and accessible on the Human Protein Atlas (HPA) website (proteinatlas.org). It is built on the GBM data available from The Cancer Genome Atlas program (TCGA) and combines transcriptomic data with antibody-based protein data.

The transcriptome analysis shows that 72% (n=14370) of all human genes (n=20090) are expressed in glioma. **Figure 8** shows the number of elevated and prognostic (unfavorable and favorable) genes in glioma.

The top 20 significant genes related to unfavorable and favorable prognosis in GBM are listed in **Table 1 and Table 2**, respectively. For *unfavorable genes*, higher relative expression levels at diagnosis give significantly lower overall survival for the patients. For *favorable genes*, higher relative expression levels at diagnosis give significantly higher overall survival for the patients.

Figure 9 shows the different composition of mutated genes across high-grade (GBM) and low-grade gliomas.

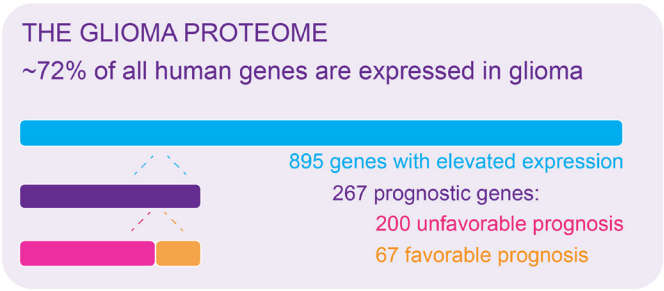

Figure 8. Elevated and prognostic genes in glioma.
The glioma proteome is built using TCGA transcriptomics data and antibody-based protein data. It shows that 895 genes have elevated expression in glioma, 267 of which are suggested as prognostic. Among the prognostic genes, 200 are associated with unfavorable prognosis and 67 are associated with favorable prognosis.

Table 1 and Table 2 list the top 20 most significant genes related to an unfavorable and favorable prognosis in GBM.

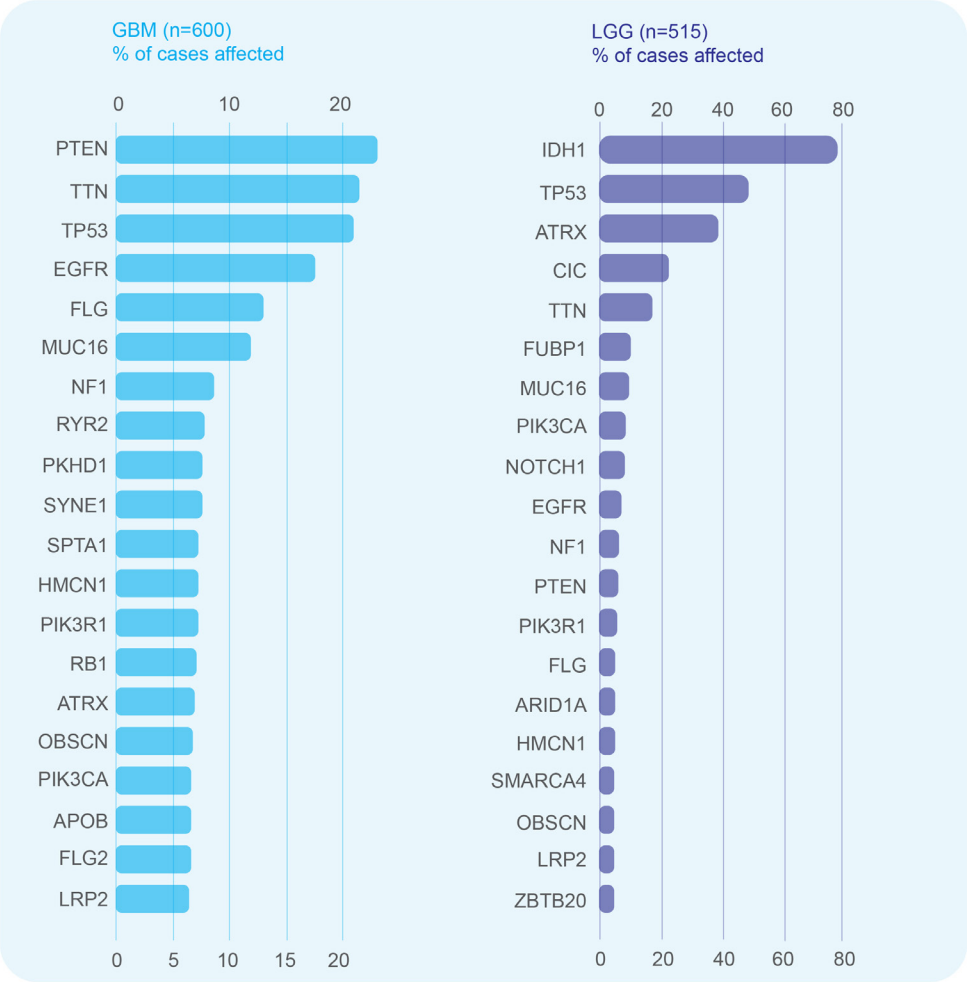

Figure 9. High-grade and low-grade gliomas differ in the composition of mutated genes.
Distribution of most frequently mutated genes in high-grade (GBM) and low-grade gliomas expressed as % of total cases (n). Source TCGA.

Table 1.
Top 20 genes with highest significance associated with **unfavorable prognosis** in GBM.

Product Name	Predicted Location	Prognostic p-Value	Product Number
Anti-ADAM15	Intracellular, Membrane	5.83e-05	HPA011633
Anti-ARMC10	Intracellular, Membrane	8.9e-06	HPA011036, HPA011057
Anti-CEND1	Membrane	4.38e-05	HPA042527
Anti-DBNL	Intracellular	2.12e-05	HPA020265, HPA027735
Anti-EN2	Intracellular	1.74e-06	HPA045646, HPA069809
Anti-FAM174A	Membrane	2.57e-06	HPA019539
Anti-KDELR2	Membrane	5.4e-05	HPA016459
Anti-LRRC61	Intracellular	1.11e-05	HPA019355
Anti-MED10	Intracellular	2.65e-05	HPA042795, HPA054188
Anti-MGAT4B	Intracellular	6.15e-05	HPA012804, HPA052134
Anti-PODNL1	Intracellular, Secreted	5.61e-08	HPA042807
Anti-PTPRN	Intracellular, Membrane	1.45e-07	HPA007152, HPA007179
Anti-RPL39L	Intracellular	1.3e-06	HPA047105
Anti-RPP25	Intracellular	2.24e-05	HPA046900
Anti-SLC6A6	Membrane	5.75e-08	HPA016488
Anti-SPAG4	Intracellular, Membrane	6.31e-05	HPA048393, HPA061789
Anti-STC1	Intracellular, Secreted	6.44e-05	HPA023918
Anti-TSPAN13	Membrane	3.56e-05	HPA007426
Anti-WFDC2	Intracellular, Secreted	3.04e-05	HPA042302
Anti-ZBED6CL	Intracellular	3.12e-05	HPA019724, HPA055805

Table 2.
Top 20 genes with highest significance associated with **favorable prognosis** in GBM.

Product Name	Predicted Location	Prognostic p-Value	Product Number
Anti-ARHGAP12	Intracellular	0.000136	HPA000412
Anti-CDYL	Intracellular	0.000153	HPA035578
Anti-ETNPPL	Intracellular	9.52e-05	HPA044546, HPA072938
Anti-MARS2	Intracellular	0.000112	HPA035589, HPA035590
Anti-MIER1	Intracellular	3.44e-08	HPA019589, HPA050306
Anti-MTHFD2	Intracellular	0.000101	HPA049657
Anti-NEUROD1	Intracellular	0.000134	HPA003278
Anti-PATZ1	Intracellular	0.000106	HPA047893
Anti-RCOR3	Intracellular	1.35e-05	HPA007413, HPA007621, HPA071997
Anti-SAMD13	Intracellular	1.14e-08	HPA058929
Anti-SLC39A10	Intracellular, Membrane	1.46e-05	HPA036512, HPA036513, HPA066087
Anti-SOX21	Intracellular	8.79e-05	HPA048337, HPA064084, AMAb91309 AMAb91311
Anti-STARD7	Intracellular	3.89e-06	HPA064958, HPA064978
Anti-TBL1XR1	Intracellular	5.16e-05	HPA019182
Anti-ZBTB6	Intracellular	2.06e-05	HPA054111, HPA076894
Anti-ZFP1	Intracellular	4.24e-05	HPA044916, HPA062910
Anti-ZNF322	Intracellular	0.000128	HPA043161, HPA046692
Anti-ZNF420	Intracellular	3.57e-05	HPA059675
Anti-ZNF639	Intracellular	5.45e-05	HPA049023, HPA052163
Anti-ZNF821	Intracellular	0.00012	HPA036372, HPA042742

The glioma tumor microenvironment.

The dynamic communication between tumor cells and the surrounding tumor microenvironment (TME) plays a crucial role in the sustained growth, proliferation, and invasion of gliomas, survival, evasion of cell death, metabolism, migration, and metastasis.

The glioma TME is highly heterogeneous, consisting of a multiplex of both cancerous and non-cancerous cells, including endothelial cells (ECs), immune cells, glioma stem-like cells (GSCs), and astrocytes, as well as non-cellular components such as the extracellular matrix (ECM) (Boyd 2021; Yekula 2020).

Several means of intercellular communication between the glioma tumoral cells and the TME have been documented. In the glioma TME, cells communicate through endothelial cells, growth factors, cytokines, chemokines, monocytes, macrophages, mast cells, microglia, T-cells, astrocytes, oligodendrocytes, and cancer stem cells (Cole 2020; Radin 2020).

All the different means of communication show a multifaceted role in supporting several hallmarks of cancer (**figure 10**).

Figures 11-16 show examples of immunostainings using PrecisA Monoclonals antibodies against TME markers in different grades of gliomas.

Table 3 lists the newly released PrecisA Monoclonals markers from Atlas Antibodies.

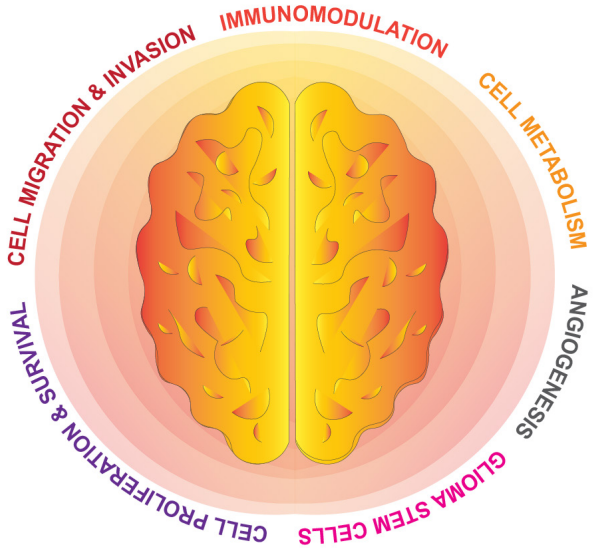

Figure 10. Understanding the dynamic glioma tumor microenvironment is necessary for the development of new immunotherapeutic strategies.

Glioma cells coexist with normal non-neoplastic cells. Glioma cells proliferate in an hypoxic immunosuppressive TME, with aberrant vasculature, abundant and distinct extracellular matrix and impaired blood-brain tumor barrier.

Four mechanisms in the TME have emerged as paramount for our understanding of gliomas and crucial for the development of new treatment strategies such as immunomodulation, angiogenesis, cancer stem cells, and drug resistance. Many of these mechansims are interdependent and carry specific molecular signatures.

Immunomodulation

In recent years, immunotherapy has become one of the major options for anti-cancer therapy. However, the complex mechanisms of glioma immunogenicity and microenvironment interactions have only partially been elucidated (Desland 2020). The ability of tumor cells to suppress both local and systemic immune responses and to hijack the communication with the TME, severely restrict treatment efficacy.

Immune cells constitute an important component of the glioma TME as they can reach up to 50% of the tumor mass content. Immune cells in glioma switch on the inflammatory process in the TME thus enhancing tumor development (Gieryng 2017; Strepkos 2020). Consistently, high-grade gliomas have a more immunosuppressive profile than low-grade gliomas.

Among the immune-related genes ADORA2A, CD160, CD276, NRP1 and VTCN1 are significantly overexpressed in low-grade gliomas. In high-grade gliomas, VTCN1, BTNL2 and METTL21B are overexpressed, while the expression of CD86, HAVCR2, LAIR1 and VSIR is significantly decreased.

Cytokines and chemokines secreted by glioma cells induce infiltration of immunosuppressive cells (MDSCs, Tregs and TAMs) and the acquisition of pro-tumoral phenotypes by myeloid cells (M2 phenotype differentiation and PDL-1 and B7 expression).

Angiogenesis.

The poor prognosis for glioblastoma is also partly due to the lack of successful drug delivery across the blood-brain tumor barrier.

Glioblastomas are highly vascularized tumors, and glioma growth depends on the formation of new blood vessels. Angiogenesis is a complex process involving proliferation, migration, and differentiation of vascular endothelial cells under the stimulation of specific signals.

The high metabolic demands of high-grade glioma, for instance, create hypoxic areas, that trigger the expression of PLVAP (a vascular marker of disrupted blood-brain barrier) and angiogenesis, leading to the formation of abnormal vessels and a dysfunctional blood-brain tumor barrier. Endoglin (CD105), a marker for immature blood vessels, is significantly higher in glioblastoma compared with peritumoral normal tissue (Burghardt 2021).

Glioma cancer stem cells and drug resistance.

Chemoresistance and reoccurence are major issues in the management of patients with glioma and the main cause of mortality. Reoccurence of gliomas is largely associated with the ability of tumor cells to regenerate from treatment-resistant cancer stem cells within and surrounding the primary tumor after initial treatment. Glioma cancer stem cells (GSCs) are the main drivers of uncontrolled cells growth in high-grade gliomas, but also the resistance to therapy (Boyd 2021; Ma 2018).

GSCs, acts through a core set of neurodevelopmental transcription factors and oncogenes. High-grade gliomas show elevated transcription of stem cells such as ALDH1A1, EZH2, GFAP, SALL4, NANOG, and POSTN. Molecular surface markers such as CD133, CD44, A2B5, CD15, CD171 have also been implicated in the reoccurrence of gliomas and increased aggressiveness of glioblastoma.

Chi3l1 as modulator of stem cells cellular states.

Phenotypic plasticity in human tumors can be driven by activation of epithelial-to-mesenchymal transition (EMT) process by which cells acquire plasticity and gain the properties of stem cells.

CHI3L1 is a secreted protein acting as modulator of stem cell states and highly expressed in gliomas. CHI3L1 is used as a marker for invasion, migration and angiogenesis in glioblastoma (Guetta-Terrier 2021).

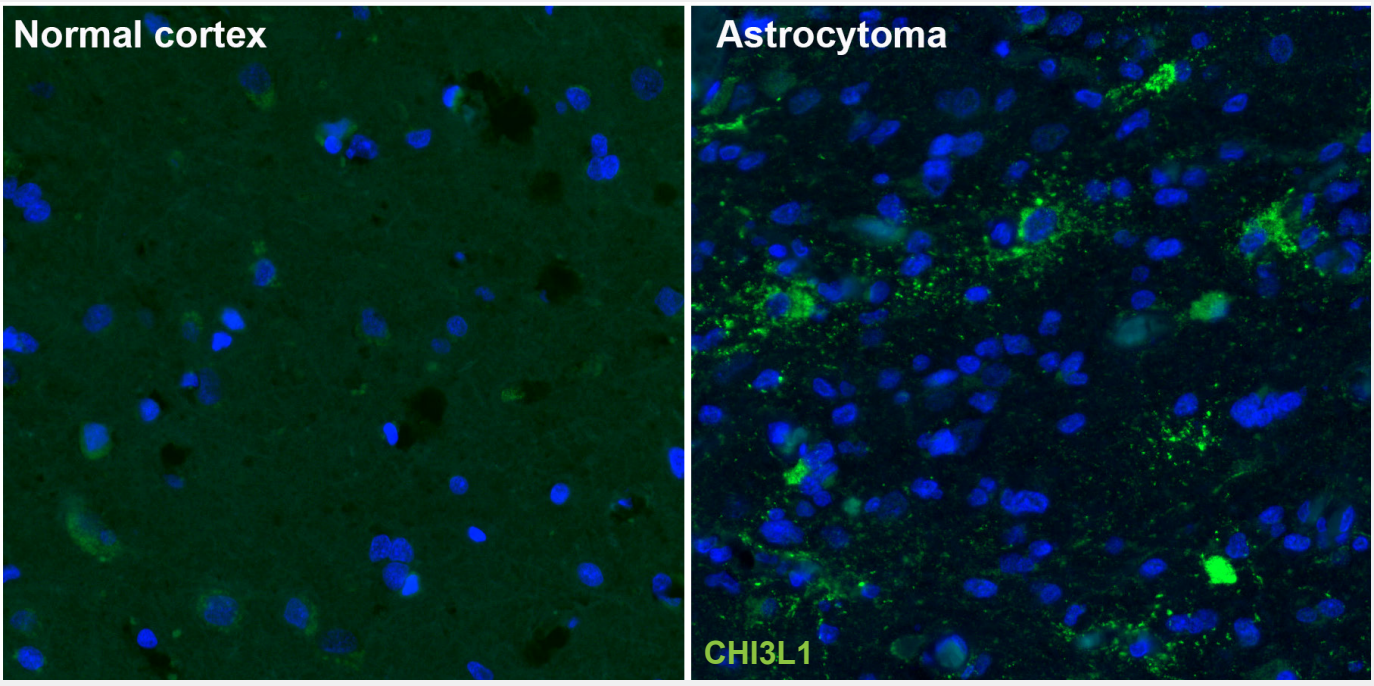

Figure 11. Fluorescence immunohistochemistry image of human normal cortex (left) and astrocytoma (right) samples stained with the monoclonal **Anti-CHI3L1 antibody (AMAb91777)** showing strong protein expression in astrocytoma (in green). Nuclei are counterstained by DAPI (in blue).

TME, including infiltration of myeloid cells such as microglia and macrophages, plays a significant role in glioma progression (Brandenburg 2020).

Glioma accumulated microglia/macrophages are a mixed cell population with pro- and anti-tumoral properties, although tumor-supporting effects generally predominate.

Microglial cells which present the antigens to the CD4-positive T-cells, express HLA-DRA protein which belongs to the HLA class II proteins (Neefjes 2011).

HLA expression is increased in glioblastoma patients, and there is some evidence suggesting that HLA-family can be used a specific molecular target in treatment of glioblastoma (Wang 2015).

METTL21B is involved in cell adhesion, angiogenesis and cell proliferation. Its expression is positively associated with glioma grade at the protein level. METTL21B facilitates immune evasion of tumor and affect prognosis by mediating macrophage polarization from M1 to M2 and regulating expression of immune checkpoints.

Nevertheless, patients with high METTL21B level are likely to have better response to immune checkpoints blockage therapy. Because of its substrate specificity, METTL21B is a promising target for the treatment of glioma (Shu 2021).

Figure 12 shows the high METTL21B expression in glioblastoma tumor cells as well as increased number of activated microglial cells (HLA-DRA) compared to controls.

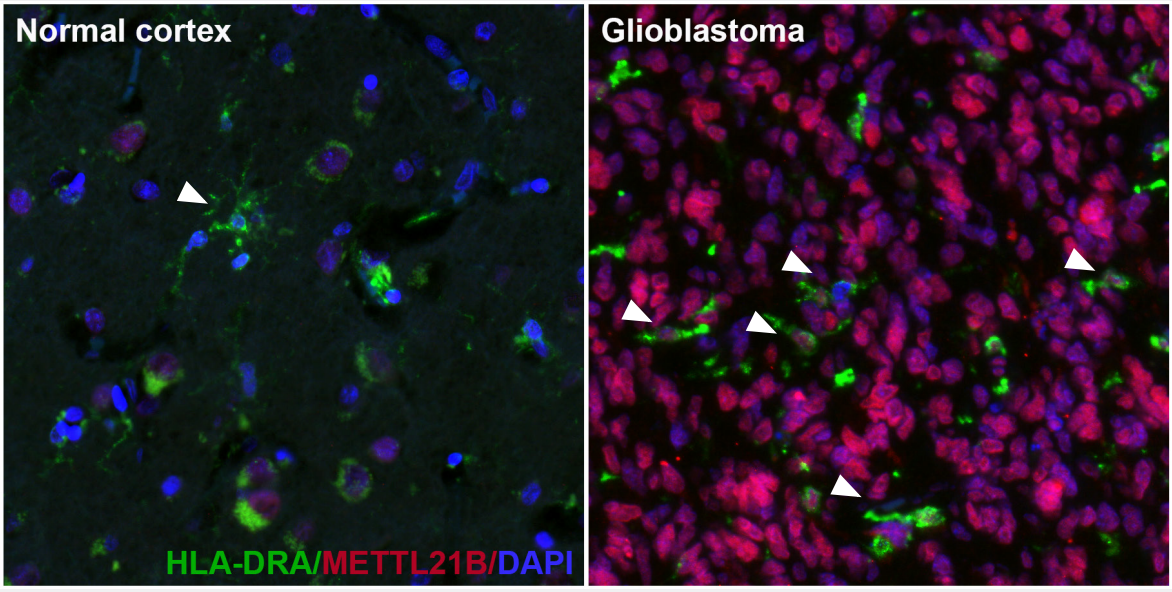

Figure 12 . Multiplexed IHC-IF staining of human normal cortex (left) and glioblastoma (right) samples using the **Anti-HLA-DRA monoclonal AMAb91674** (cytoplasmic, in green) and the **Anti-METTL21B polyclonal HAP043020** (nuclear, in red) antibodies. Arrows indicate activated microglia (HLA-DRA positive staining).

Targeting GSCs is an extremely important aspect of the clinical treatment of gliomas. A better comprehension of glioma GSCs provide functional insights into the dynamic of cellular communication during gliomagenesis, creating new opportunities for diagnostics and therapeutics.

The transcription factor SALL4 participates in cell proliferation, apoptosis, cycle, invasion, evolution and drug resistance. SALL4 is overexpressed in glioma patients and correlated with poor outcome (Zhang L. 2015).

SALL4 acts by strengthening the PI3K/AKT signaling pathway (which is a well-known pathway in the regulation of tumorigenesis, significantly activated in glioma) thus reducing the expression of the tumor suppressor PTEN (Liu 2017).

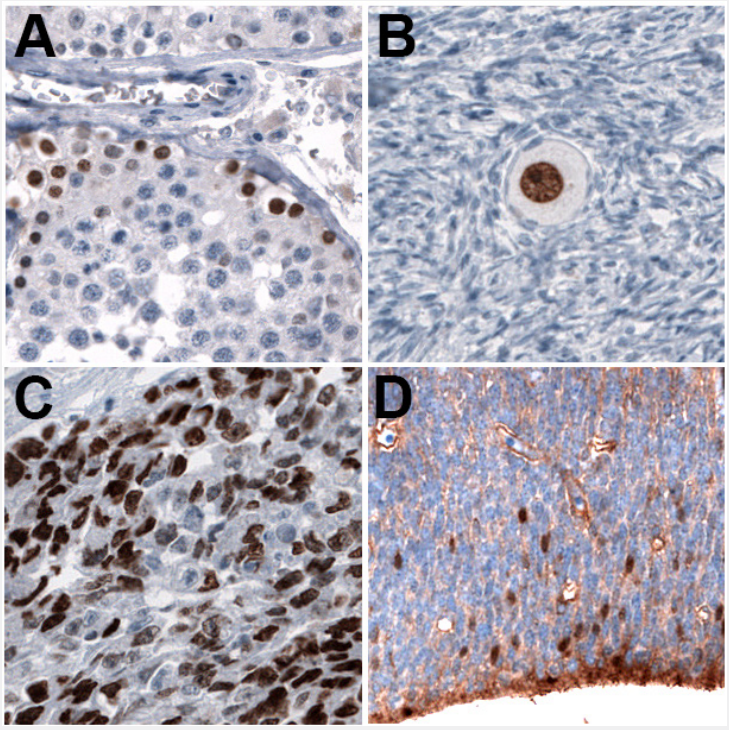

Figure 13. Characterization of SALL4 in human and mouse tissues.
Immunohistochemical staining using the **Anti-SALL4 AMAb91769** monoclonal antibody showing nuclear positivity in germinal cells in testis (**A**), oocyte (**B**) and embryonal testis carcinoma (**C**) human tissues, as well as nuclear positivity in a subset of cells in the developing brain in mouse embryo E11 (**D**).

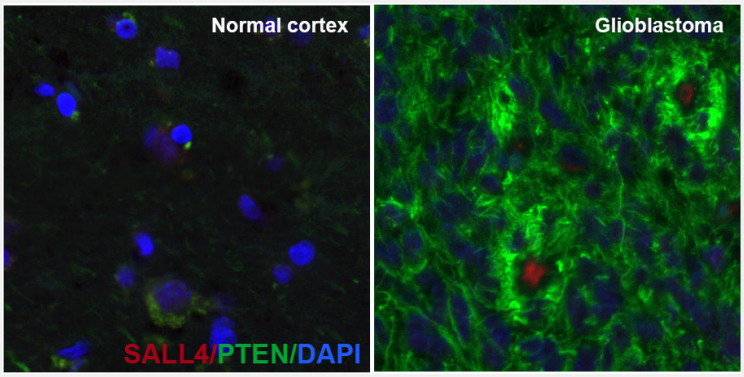

Figure 14. SALL4 is up-regulated in glioma samples. Multiplexed IHC-IF staining of human normal cortex and glioblastoma samples using the **Anti-SALL4 AMAb91769** monoclonal (nuclear, in red) and the Anti-PTEN monoclonal AMAb91736 (cytoplasmic, in green) antibodies. Nuclei are counterstained with DAPI (in blue).

The transcription factor EZH2 is highly expressed in gliomas and a potential target for immune therapy.

EZH2 is a histone H3 lysine methyltransferase that promotes tumorigenesis in a variety of human malignancies including gliomas by altering the expression of tumor suppressor genes. EZH2 overexpression in gliomas is associated with several immune checkpoints, cell cycle, DNA replication, mismatch repair, p53 signaling and tumor-infiltrating lymphocytes (Chen 2021).

The aldehyde dehydrogenases ALDH1A3 is associated with cell adhesion and tumor invasion and a marker of Mes-subtype of gliomas.

ALDH is a marker of cancer stem cells associated with the malignant phenotype in gliomas.

Among the ALDH isoforms, ALDH1A3 is overexpressed in high-grade than in low-grade gliomas, while ALDH1A1 is overexpressed in low-grade compared to high-grade gliomas.

Most of the Mes-subtype patients have high ALDH1A3 mRNA expression, indicating ALDH1A3 as a useful marker for Mes-subtype of gliomas (Zhang W. 2015).

Shugoshin 2 is critical in cell division and cell cycle progression.

Shugoshin 2 (SGO2) plays a crucial role in glioma cells proliferation. Data show that SGO2 expression positively correlates with WHO grading of human gliomas (Kao 2021).

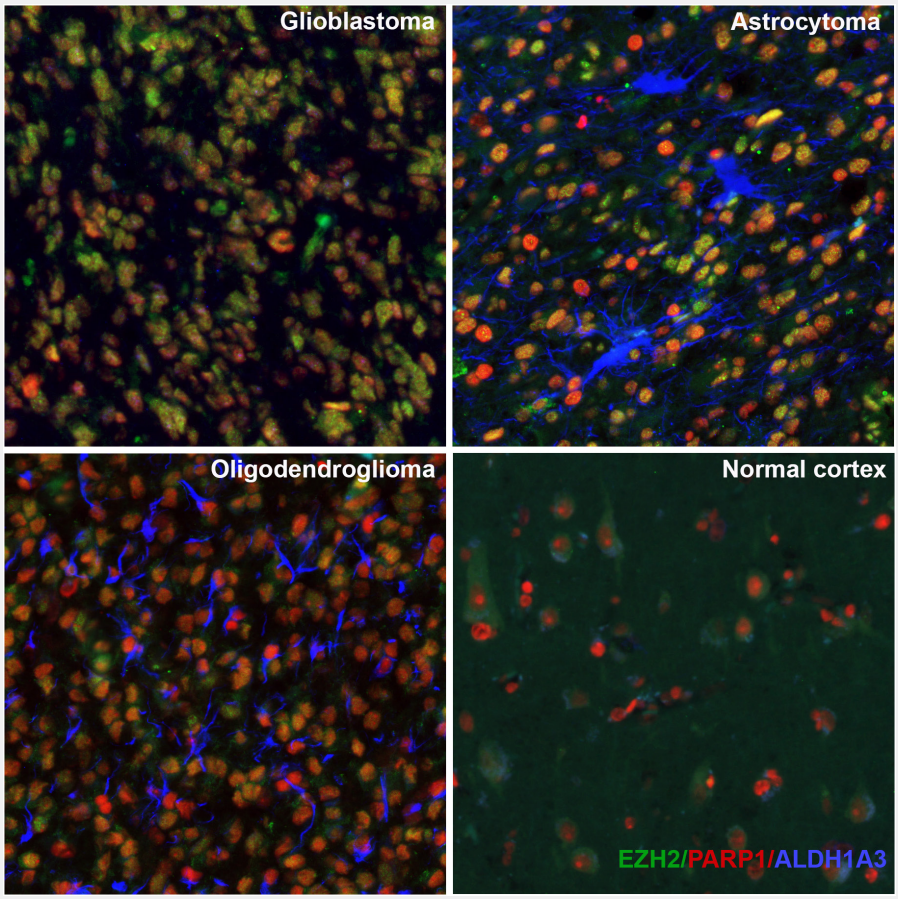

Figure 15. Multiplexed IHC-IF staining of human glioblastoma, astrocytoma, olygodendrocytoma and normal cortex samples using the **Anti-EZH2 AMAb91752** (green, nuclear), the **Anti-PARP1 AMAb90959** (red, nuclear) and the **Anti-ALDH1A3 AMAb91754** (blue, cytoplasmic) monoclonal antibodies.

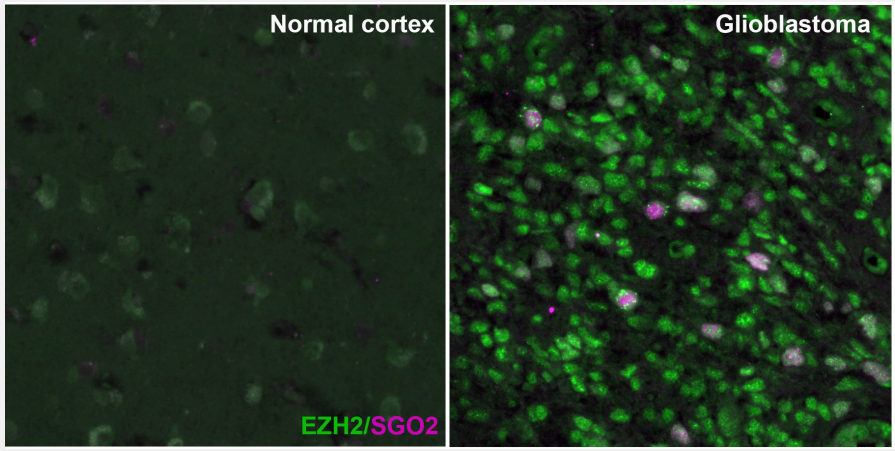

Figure 16. Multiplexed IHC-IF staining of human normal cortex and glioblastoma samples using the **Anti-EZH2 AMAb91750** (green, nuclear) monoclonal and the **Anti-SGO2 HPA035163** (magenta, nuclear) polyclonal antibodies.

Table 3.
Newly released PrecisA Monoclonals targeting glioma TME markers.

Biological role in gliomas	Product Name	Protein Name	Product Number	Validated Applications	Isotype
Marker for mesenchymal glioma phenotype.	Anti-ALDH1A3	Aldehyde dehydrogenase family 1 member A3	AMAb91754	IHC*, WB, ICC-IF*	IgG2a
Carbohydrate-binding protein. Marker for invasion, migration and angiogenesis in glioblastoma.	Anti-CHI3L1	Chitinase-3-like protein 1	AMAb91777	IHC, WB	IgG2b
			AMAb91778	IHC, WB	IgG1
Transcription factor. Marker for glioma stem cells.	Anti-EZH2	Enhancer of zeste 2 polycomb repressive complex 2 subunit	AMAb91749	IHC*, WB, ICC-IF	IgG2b
			AMAb91750	IHC*, ICC-IF	IgG2a
			AMAb91752	IHC*, WB, ICC-IF	IgG2b
Transcriptional activator involved in cell proliferation. Marker for glioma progression.	Anti-FOXM1	Forkhead box M1	AMAb91766	IHC, ICC-IF	IgG1
Cancer cell migration.	Anti-GLI1	GLI family zinc finger 1	AMAb91771	WB, ICC-IF	IgG1
			AMAb91772	IHC	IgG1
			AMAb91773	IHC, ICC-IF	IgG2b
Transcriptional regulator. Critical for glioblastoma initiation and chemoresistance.	Anti-ID1	Inhibitor of dna binding 1, hlh protein	AMAb91756	IHC, ICC-IF	IgG1
			AMAb91757	IHC*, ICC-IF	IgG2a
Immunomodulation.	Anti-NF1	Neurofibromin 1	AMAb91741	ICC-IF	IgG2b
			AMAb91745	ICC-IF	IgG1
Cell cycle progression and proliferation. Marker for mesenchymal phenotype.	Anti-PTEN	Phosphatase and tensin homolog	AMAb91735	IHC*, WB	IgG1
			AMAb91736	IHC*, WB	IgG2a
Extracellular matrix protein. Cancer stem cell maintenance and metastasis.	Anti-POSTN	Periostin	AMAb91763	IHC*, ICC-IF	IgG2a
			AMAb91764	IHC*, ICC-IF	IgG2a
Transcription Factor. Marker for neural glyoma phenotype.	Anti-RBFOX3	RNA binding protein fox-1 homolog 3	AMAb91746	IHC*	IgG2b
			AMAb91748	IHC*	IgG2b
Transcription factor. Maintenance and renewal of stem cells.	Anti-SALL4	Spalt like transcription factor 4	AMAb91768	IHC*, WB, ICC-IF	IgG1
			AMAb91769	IHC*, WB, ICC-IF	IgG1
			AMAb91770	ICC-IF	IgG2a

* Products with enhanced validation for indicated applications.

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