

Neurooncology: 2024 update

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Abstract

As in previous years, including 2023, a major focus in the neurooncological area of neuropathology was put on more precise and constantly faster diagnostic procedures, even reaching the level of ultra-fast intraoperative diagnostics based on methylation profiling. Neuropathological diagnostic precision and clinical follow-up treatment has been further increased by combining DNA methylation profiling with targeted panel sequencing. A few new, molecularly defined tumor subtypes have been proposed, among others, a glioneuronal tumor with *ATRX* alteration, kinase fusion and anaplastic features (in its abbreviated form named GTAKA) and the de novo replication repair deficient glioblastoma, IDH-wildtype both having either distinct prognostic or therapeutic implications. Regarding the understanding of brain tumor development and progression, several novel mechanisms have been presented which might also be considered as treatment targets in the future, such as a) autonomous rhythmical Ca^{2+} oscillations in interconnected glioma cell networks driving tumor growth; b) transfer of mitochondria from normal astrocytes to glioma cells enhancing proliferation and self-renewal; c) brain endothelial cell remodeling upon matrix-metalloprotease 9 secretion by tumor cells metastasizing into the CNS and d) anti-tumor activity of microglia in CNS metastasis of breast cancer. Finally, in contrast to previous years, several very promising neurooncological treatment studies have been conducted, focusing on specific targets such as H3K27M or IDH1/2 mutations for which a proper neuropathological assessment is key. The continuous translation of potential new treatment targets using faster and precise diagnostic procedures will further pave the way for better individualized clinical care of neurooncological patients.

Keywords: Neurooncology, Neuropathology, Brain tumors, Glioblastoma, Brain metastasis

Introduction

As in the previous year, the author's aim for the selection of the "top ten" paper series in the field of neurooncology was to select an equilibrated mix of high-quality manuscripts that provide new tumor biological concepts, new tumor entities, new diagnostic tools or approaches, and clinical studies with specific targeted approaches, all touching the field of neuropathology. This should not at all devalue excellent papers in the field of neurooncology with a different focus, such as neuroradiology, neurophysiology or neuropsychology, but rather focus on the central aims and scope of *Free Neuropathology*, namely morphological or molecular techniques based on biospecimens such as tissues, cells of fluids. The search strategy for this year's "neurooncology update" series was similar as previously described [Mittelbronn, 2023]. Whereas in 2022, a major focus was put on studies focusing on profiling, "omics" and new diagnostic applications, many more cutting-edge studies presenting new tumor biological mechanisms in the field of neurooncology were published in 2023. One point that negatively struck the author last year was the fact that despite the large amount of profiling data, very few studies aimed at translating those findings into clinical applications. It is a central paradigm of our field that an excellent clinical treatment necessitates precise and fast diagnosis as well as identification of specific targets, allowing for the best individualized clinical care. It is gratifying to see the development and implementation of new treatment approaches to address molecular targets that have been introduced in daily neuropathological routine over recent years. Although this year's series also presents some new tumor subtypes and further improvements in diagnostic procedures, a larger part focuses on novel, more basic tumor biological mechanisms, as well as very promising clinical studies that aim to treat brain tumors in a more specific and individualized manner to complement or even replace the classic neurooncological treatment triad of surgery, radio- and chemotherapy. With this, the "top ten" series in neurooncology for 2023 reads as follows:

1. Frequency-dependent MAPK and NFkB pathway-activation by rhythmic Ca²⁺ oscillations as potential treatment target in glioma [Hausmann et al., 2023].
2. GAP43-dependent transfer of mitochondria from astrocytes to glioma cells increasing cellular proliferation and self-renewal [Watson et al., 2023].
3. Brain endothelial cell remodeling by CNS metastatic cell-derived matrix-metalloprotease 9 (MMP9) as mechanism for extravasation [Karreman et al., 2023].
4. Microglia suppresses brain metastasis formation in breast cancer [Evans et al., 2023].
5. Proposal of a new tumor entity: Glioneuronal tumor with ATRX alteration, NTRK gene fusion and anaplastic features (GTAKA) [Bogumil et al., 2023].
6. "De novo replication repair deficient glioblastoma, IDH wildtype": a new glioblastoma subtype with implications for targeted treatment [Hadad et al., 2023].
7. Ultra-fast, machine-learning based intraoperative methylation profiling [Vermeulen et al., 2023].
8. Multiomics neuropathology improving diagnostic precision, targeted therapy and detection of cancer predisposition syndromes in pediatric neurooncology [Sturm et al., 2023].
9. Clinical vaccination study targeting H3K27M in adult diffuse midline glioma [Grassl et al., 2023].
10. Clinical study in patients with IDH mutant low-grade glioma using an oral, brain-penetrant inhibitor of mutant IDH1 and IDH2 enzymes [Mellinghoff et al., 2023].

Interdisciplinary discussions are important as the key role of neuropathological shifts from being purely diagnostic to prognostic to more and more theranostic. An increased number of neurooncological patients are currently treated based on biomarker-guided decisions jointly made in multidisciplinary molecular tumor boards [Renovanz et al., 2023]. As in many neurooncological centers the immunohistochemical, DNA/RNA in-situ, genetic

and/or epigenetic biomarker analyses are performed by neuropathologists, it is imperative that neuropathologists help to rapidly translate unequivocal findings from both basic tumor biological and clinico-oncological studies into diagnostic or theranostic tests to facilitate improved patient care.

The author strongly hopes that the selected papers will both fascinate the readers and further stimulate discussions around neurooncological topics in neuropathology.

1. Frequency-dependent MAPK and NFkB pathway-activation by rhythmic Ca²⁺ oscillations as potential treatment target in glioma [Hausmann et al., 2023].

It has been previously reported that glioma cells are not only interconnected between each other but also to neurons via microtubes enabling

intercellular communication via calcium signaling [Venkataramani et al., 2019]. Calcium signaling induced by neuronal activity increased the formation of glioma microtubes and cell invasion [Venkataramani et al., 2022]. However, the exact mechanisms how the intercellular communication between neurons and glioma cells or uniquely amongst glioma cells were still not fully deciphered. In the present study, Hausmann et al. showed that a minor glioma cell fraction, accounting for only 1–5 % of all tumor cells is able to rhythmically trigger Ca²⁺ oscillations [Hausmann et al., 2023]. Those cells belong to the cellular fraction showing a considerably higher number of tumor microtube connections to other glioma cells. As the Ca²⁺ oscillations in glioma cells were not only observed *in vivo* in the presence of neurons but also in neuron-free cell culture conditions, one can assume that this periodic activity is a glioma-cell autonomous mechanism. This hypothesis was further corroborated by the finding that blocking gap junction and/or tumor

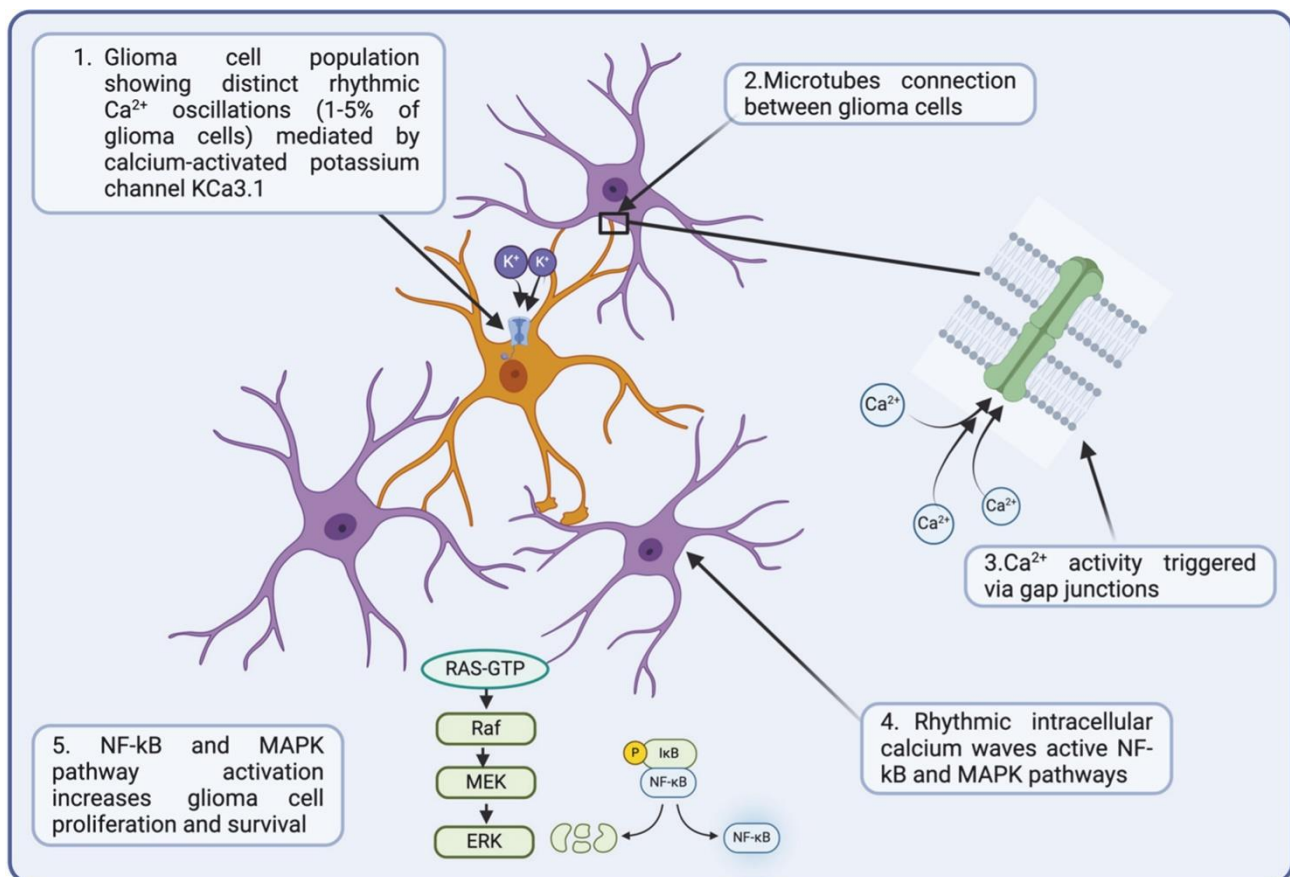


Figure 1: Rhythmic Ca²⁺ oscillation mechanism in glioma cell networks leading to enhanced malignancy via NF-κB and MAPK pathway activation.

microtubule formation did not reduce the periodic Ca^{2+} activity in this small glioma subpopulation, but considerably reduced the related Ca^{2+} activity in the intertumoral network. A pharmacological screen revealed that specific inhibition of the calcium-activated potassium channel KCa3.1 reduced Ca^{2+} activity also in those glioma cells. The respective potassium channel appears to be of interest in glioma biology as implications for enhanced malignant behavior, in particular enhanced glioma cell invasion in the presence of KCa3.1 , have been reported [D'Alessandro et al., 2013; Turner et al., 2014]. KCa3.1 -positive cells rhythmically stimulated Ca^{2+} signaling at a mean frequency of 12.5–12.7 mHz (*in vivo*) and 9.7–11.3 mHz (*in vitro*) within the glioma network, apparently a range in which also the MAPK and NF κ B pathways increase cell proliferation and survival. Along this line, the inhibition or knockout of KCa3.1 led to reduced MAPK and NF κ B pathway activity while its stimulation enhanced the activity of both pathways. The knockdown of KCa3.1 was associated with significantly reduced glioma growth in animal models and survival analyses of an openly accessible patient cohort (The Cancer Genome Atlas - TCGA) revealed that glioblastoma patients with high KCa3.1 expression showed significantly worse survival as compared to their counterpart with low KCa3.1 expression. In summary, those findings point to a new treatment option for glioblastoma patients by targeting more specifically KCa3.1 thereby focusing on the cell population that seems to negatively orchestrate the glioma cell network. Although it was demonstrated that the cell state of rhythmically performing Ca^{2+} signaling is hierarchical, but highly plastic, it still remains to be determined why only a very small subpopulation of glioma cells acquire this state at any given time (**summary of the proposed mechanism in Figure 1**).

2. GAP43 -dependent transfer of mitochondria from astrocytes to glioma cells increases cellular proliferation and self-renewal [Watson et al., 2023].

It has previously been shown that brain cells interact via tunneling nanotubes (TNT), in neoplastic conditions also known as tumor microtubules (MTs), by which larger cellular organelles such as mitochondria can be transferred. In particular, glioblastoma

cell-derived mitochondria can be shuttled into non-neoplastic astrocytes that subsequently adapted to tumor-like metabolism and hypoxia conditions [Valdebenito et al., 2021]. The study of Watson et al. analyzed the reverse, namely how mitochondria are transported from residual non-neoplastic astrocytes to glioma cells and how this impacted glioma cell function [Watson et al., 2023]. To elucidate this question, the authors used different mouse and rat glioma models with mitochondria and glioma cells fluorescing in different colors. By doing so, they could demonstrate that between 15 to 60% of glioma cells incorporated mitochondria of non-neoplastic astrocytic, and to a lesser extent also microglial origin. It could further be shown that physical contact between non-neoplastic astrocytes and glioma cells is necessary for mitochondrial transfer. Inhibition of actin polymerization by cytochalasin B reduced the mitochondrial transfer rate, indicating an important role of actin cytoskeleton remodeling as an underlying mechanism. Hypothesizing that the actin-associated protein GAP43 that is involved in neurite outgrowth could be implicated in this mechanism, the authors knocked down GAP43 and observed significantly reduced mitochondrial transfer. Importantly, RNA sequencing showed an increase of gene activity related to electron transport and mitochondrial organization therefore suggesting a functional implication of mitochondrial transfer. This was corroborated by metabolic analyses showing a higher basal and maximal respiration rate in glioma cells having incorporated a higher number of astrocyte-derived mitochondria. This glioma fraction was also more aerobic and energetic as compared to their counterpart with lower mitochondria incorporation rate. In addition, amino acid and nucleotide metabolism were significantly altered in glioma cells with a higher transfer rate of mitochondria indicating a metabolic reprogramming that might be important for pro-proliferative, antioxidative and various cell signaling processes. The first evidence that this mechanism might play an important clinical role in neurooncology was observed in a mouse glioma model in which animals with glioma cells that incorporated astrocyte-derived mitochondria showed significantly shorter survival rates as compared to glioma cells without mitochondria transfer (**summary of the proposed mechanism in Figure 2**).

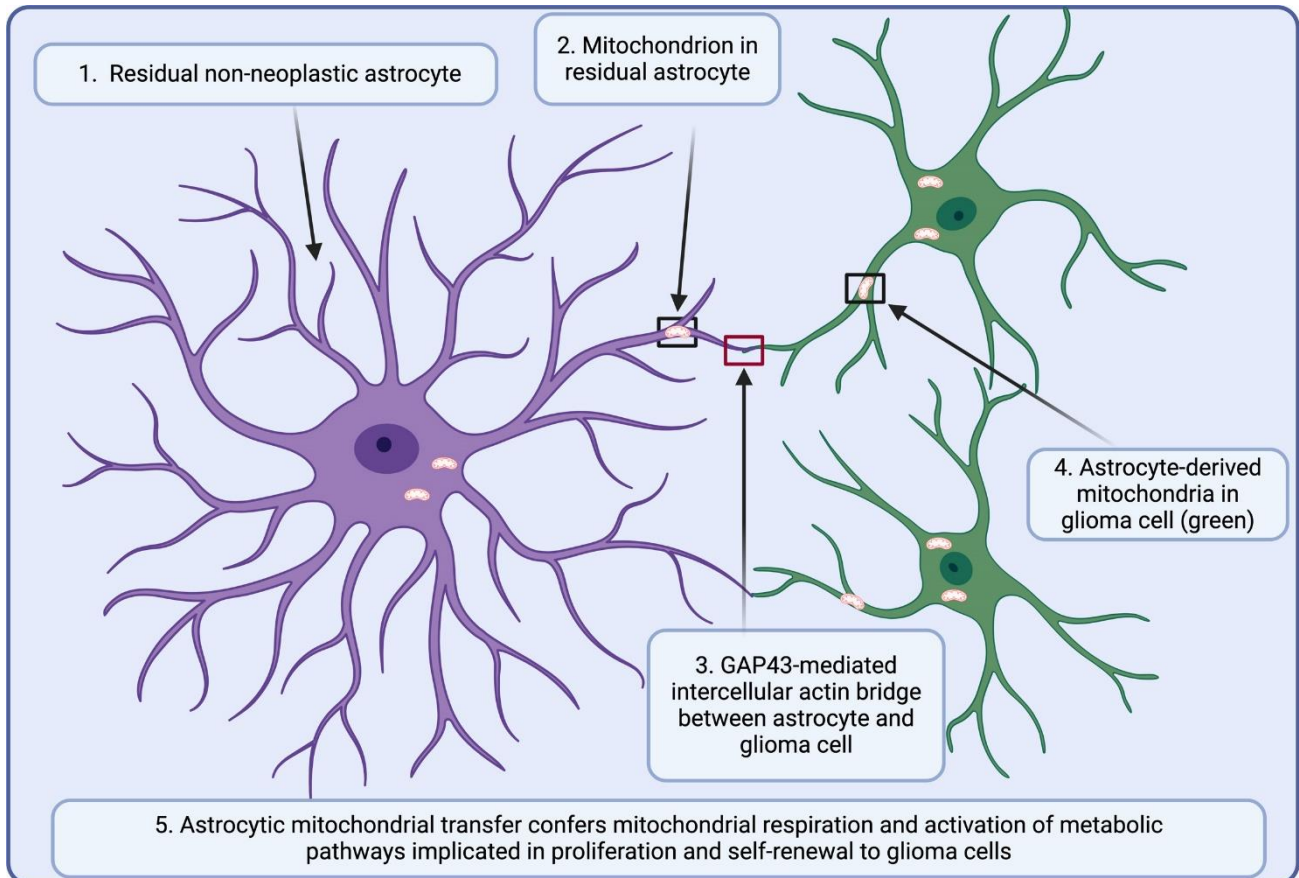


Figure 2: Mitochondrial transfer from residual non-neoplastic astrocytes to glioma cells thereby conferring respiratory and metabolic functions linked to glioma cell proliferation and tumorigenicity.

3. Brain endothelial cell remodeling by CNS metastatic cell-derived matrix-metalloprotease 9 (MMP9) as a mechanism for extravasation [Karreman et al., 2023].

Brain metastases still constitute the most frequent malignant CNS tumors, being much more frequent than their primary CNS tumor counterparts. There is a broad agreement on the key steps of cancer cell extravasation from the bloodstream to the CNS similarly to leukocyte extravasation including a) rolling/adhesion of cancer cells to brain endothelia, b) alteration of the blood-brain barrier (BBB) permeability and c) trans-endothelial migration, however, the exact underlying mechanisms are still not fully understood [Alsabbagh et al., 2023]. Previous observations, that primary cancer cells are able to considerably change the structure of brain vessels [Haskó et al., 2019], prompted the authors to deci-

pher more precisely the potential underlying mechanism. By combining fluorescent and electron microscopy on murine brain metastasis models, the authors were able to trace the moment of interaction between cancer and endothelial cells and study those events at high resolution. The observed vascular arrest of cancer cells was mainly seen at vessel branch points where cancer cells acquired an elongated shape and surrounding vessels a significantly dilated state with disorganized basement membranes. Furthermore, the moment of extravasation could also be captured by showing tumor cell nuclei both partly inside and outside of brain capillaries with a nuclear diameter of less than 1 μm . The most prominent disruption of the basement membranes was observed during the extravasation process of cancer cells, without surpassing the astrocytic or microglial cell layers. Brain capillaries affected by cancer cell extravasation showed three major, reproducible findings, namely a) the formation of neo-lumina parallel to the pre-existing ones,

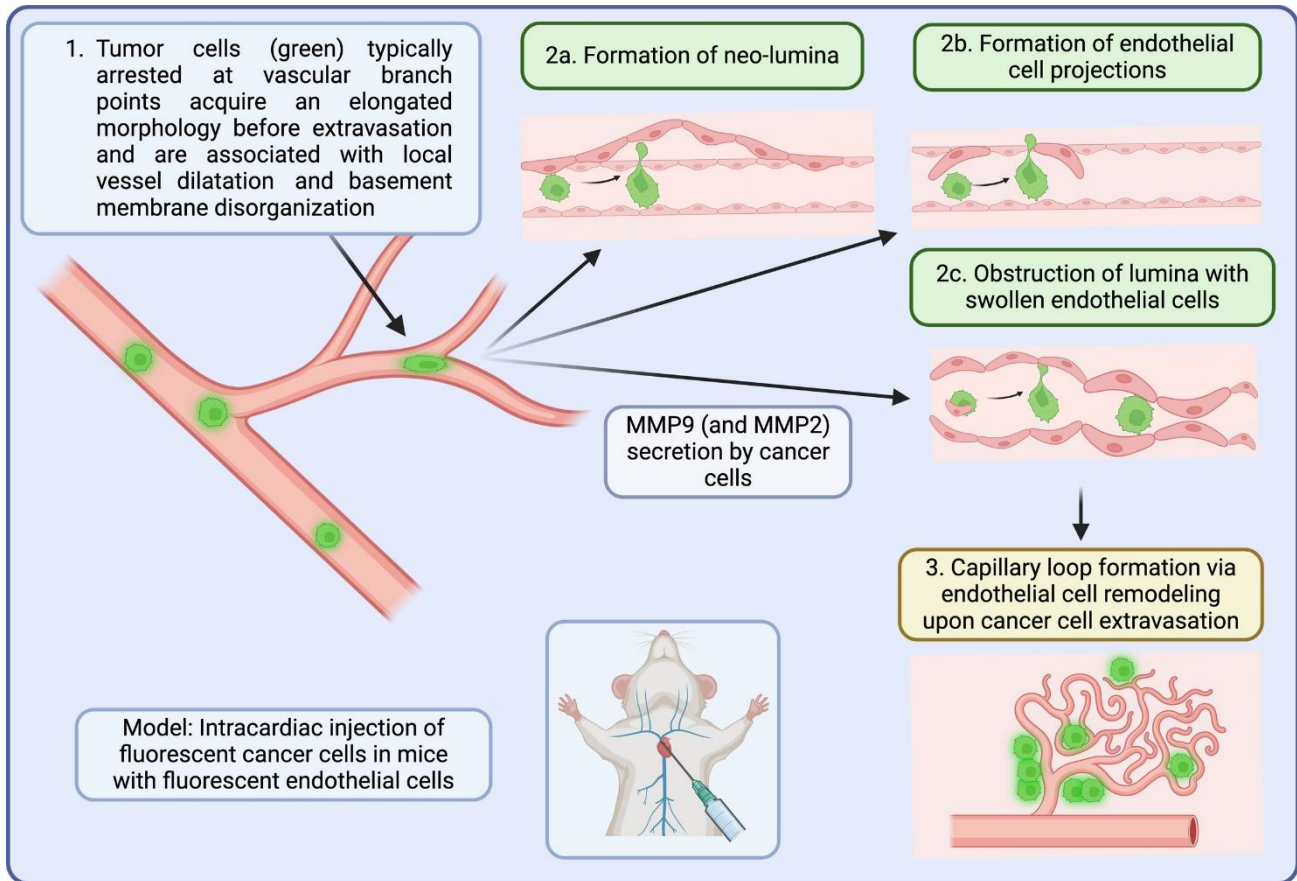


Figure 3: Alteration of brain endothelium upon interaction with MMP9 producing cancer cells.

b) formation of endothelial cell projections and c) obstruction of brain capillary lumina by swollen endothelial cells (**Figure 3**). Of note, those changes were reversible within 4 days if cancer cells left the respective blood vessel without performing extravasation. Furthermore, the faster cancer cells were able to extravasate, the more successful the survival rate of those cells was in the perivascular niche. Once cancer cells successfully extravasated and started perivascular metastatic growth, prominent endothelial cell remodeling led to the formation of capillary loops that followed the localization of tumor cells. The formation of capillary loops was even a prerequisite for the formation of macrometastases. This mechanism was mediated by cancer cell derived matrix metalloprotease 9 (MMP9). Pharmacological or genetic inhibition of MMP9 expression led to significantly reduced endothelial cell remodeling, extravasation of cancer cells, and the formation of metastases. These findings contribute to a better understanding of brain metastasis formation and may pave the way for new prevention or treatment strategies.

4. Microglia suppress breast cancer brain metastasis formation [Evans et al., 2023].

Multiple studies have suggested a detrimental role of microglia in the development and/or progression of brain metastases [Izraely S et al., 2023; Pukrop et al., 2010]. To overcome the issue of distinguishing the contribution of primary cerebral microglia from secondarily invaded cells of the myeloid lineage (most importantly macrophages), the authors chose a complex study design including single cell RNA sequencing (scRNAseq) as well as genetic and humanized mouse models approaches [Evans et al., 2023]. In a murine breast carcinoma brain metastasis model in which intracardiac injection of tumor cells led to brain metastasis formation, scRNAseq revealed stable pro-inflammatory expression signatures related to cytokine production, antigen processing and presentation, and interferon-beta responses. As this signature was microglia-specific and only encountered upon brain

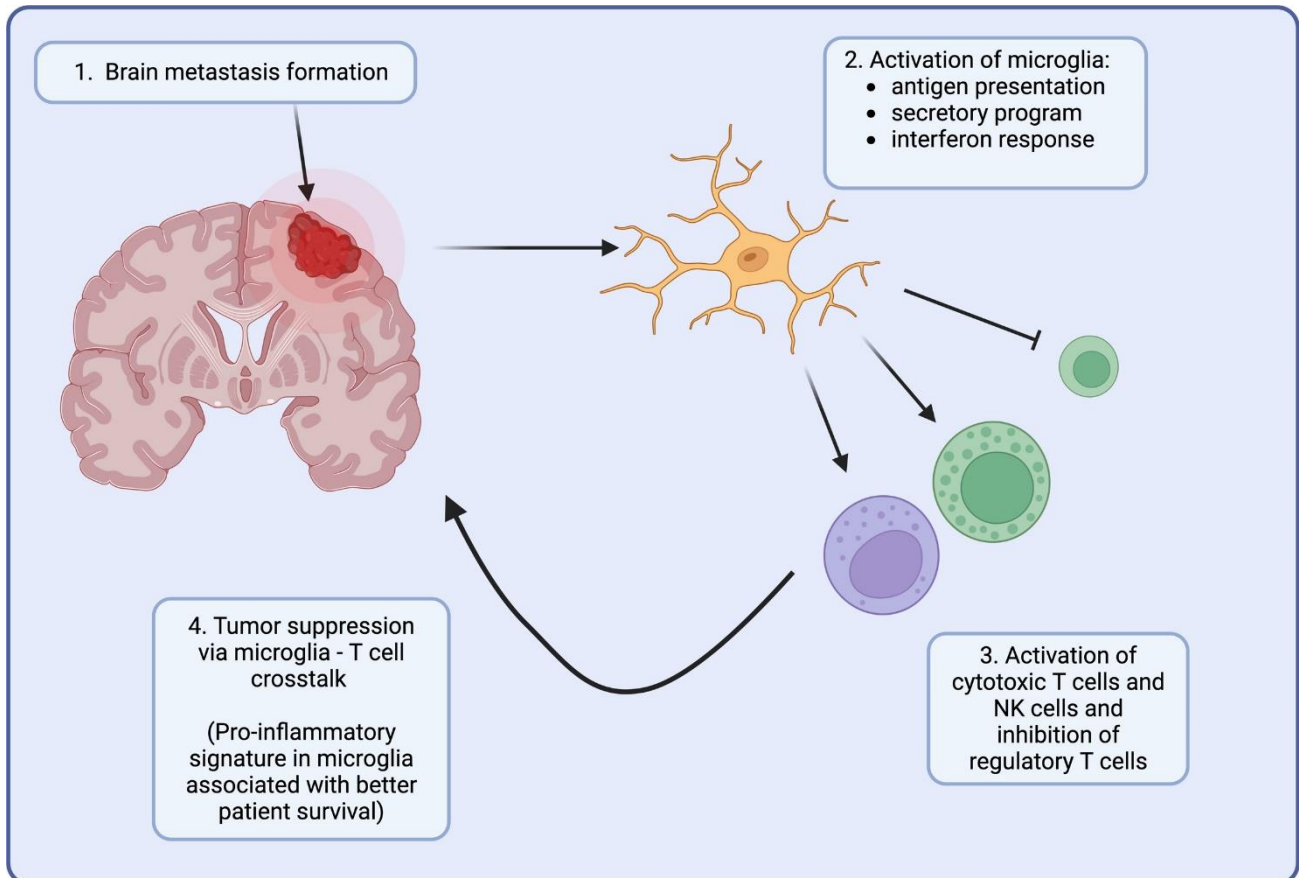


Figure 4: Proinflammatory microglial signature associated with activation of cytotoxic T and NK cells and inhibition of regulatory T cells inhibiting brain metastasis growth.

metastasis formation, the authors hypothesized that these transcriptional changes were related to tumor cell sensing and pathological tissue alterations. An experimental depletion of microglial cells using a genetic model with deletion of a key super-enhancer in the *Csf1r* locus was associated with enhanced metastasis formation and reduced survival rates. Mechanistically, the authors showed that in the context of brain metastasis formation, microglial cells activate NK/NKT and $CD8^+$ T cells, while attracting relatively less immunosuppressive T regulatory cells. Of note, experimental depletion of effector T cells led to reduced antigen presentation and IFN response in microglial cells, suggesting that only the orchestrated interaction between both cell types enables their full antitumoral capacity (**Figure 4**). The antitumoral responses were conserved in an additional experimental mouse model with human microglial cells. The authors reanalyzed data from an RNA sequencing study from human breast cancer brain metastasis patients [**Varešlija et al., 2019**]. In line with their findings and hypotheses,

this patient cohort demonstrated significantly longer survival rates if breast cancer brain metastases were associated with a high expression of canonical microglial markers indicating a higher tumor infiltration by microglial cells. Although still many scientific papers stress a pro-tumorigenic role of microglial cells in primary and secondary brain tumors, the findings of the present study are also in line with some human tissue-based studies describing a beneficial contribution of microglial cells to prolonged patient survival in glioblastoma [**Zeiner et al., 2019**].

5. Proposal of a new tumor entity: Glioneuronal tumor with ATRX alteration, NTRK gene fusion and anaplastic features (GTAKA) [Bogumil et al., 2023].

Although glioneuronal neoplasms constitute a fairly rare group of brain tumors, new entities belonging to this group are constantly emerging,

mainly due to the increasing use of molecular methods and respective analyses in larger, centralized databases that allow for a faster collection of isolated rare cases. DNA methylation profiling revealed a distinct new group of 20 cases (median age: 19 years) of mainly supratentorial (84 %) location with histologically glioneuronal features as compared to a reference cohort of more than 100,000 patients. In a more fine-tuned approach comparing those cases with a reference cohort of 718 neuroepithelial tumors, unsupervised, non-linear t-distributed stochastic neighbor embedding (t-SNE) projection, this distinct subgroup could be confirmed. Further detailed neuropathological and molecular pathological analyses could be performed in 16 out of those 20 tumors. All tumors showed alterations of *ATRX*, assessed by DNA sequencing and/or immunohistochemistry. In addition, all cases showed a potentially

druggable gene fusion involving members of the receptor tyrosine-kinase family, mainly *NTRK1-3*. In slightly more than half of the patients, homozygous deletion of *CDKN2A/B* was observed. The neuropathological assessment of those tumors showed isomorphic, round, often condensed nuclei with perinuclear clearing, prominent mitotic indices and vascular proliferations. For this new entity, the proposed acronym “GTAKA” is composed of its central neuropathological and molecular features, namely **G**lioneuronal Tumor with **A***TRX* alteration, **K**inase fusion and **A**naplastic features (**Table 1**). At the current stage, it is too early to provide precise prognostic estimations, however this new subgroup of glioneuronal tumors with a median progression-free survival of only 12.5 months seems to be clearly more aggressive than many other, already established glioneuronal tumor entities.

Essential
Glioneuronal tumor often mimicking oligodendroglioma or neurocytoma (often with isomorphic nuclei with perinuclear clearing, vascular proliferations, increased mitotic activity (median: 4 mitoses/mm ²) and/or necrosis)
and
Alteration of <i>ATRX</i> (either by immunohistochemistry (loss of expression) or DNA sequencing (mutation))
and
Gene fusion of members of receptor tyrosine kinase family (mainly <i>NTRK2</i>)
desirable if present
Homozygous loss of <i>CDKN2A/B</i>
or
DNA methylation profiling clustering with GTAKA cases

Table 1: Proposition of diagnostic criteria for Glioneuronal Tumor with *ATRX* alterations, Kinase fusion and Anaplastic features (“GTAKA”).

6. “De novo replication repair deficient glioblastoma, IDH wildtype”: a new glioblastoma subtype with implications for targeted treatment [Hadad et al., 2023].

Over decades, the treatment of glioblastoma patients consisted of neurosurgical resection followed by radio-chemotherapy and is, however, still associated with a detrimental prognosis. Despite a multitude of clinical studies, valid biomarkers for potential targeted therapies are largely lacking [Rodgers et al., 2024]. Therefore, it is highly important to define glioblastoma subtypes, even if they only occur at low percentages, that might benefit from new treatment approaches. In a recent larger series of 459 consecutive glioblastoma patients, 9 patients (2 %) with a biallelic inactivation of a canonical mismatch repair gene (MSH2, MSH6 or MLH1) were identified, partly with a heterozygous germline mutation (indicating an underlying Lynch syndrome) in a part of the glioblastoma patients [Hadad et al., 2023]. While overall mutation rate was significantly increased in this tumor group, they showed lower rates of classic genetic glioblastoma alterations such as trisomy of chromosome 7, monosomy of chromosome 10, TERT promoter mutation, EGFR amplification or homozygous

CDKN2A deletion. In 3 out of those 9 patients, additional mutations in the proofreading domain of the DNA polymerase *POLE* were detected being associated with an ultrahypermutational state of more than 100 mutations per megabase. Of note, the mismatch repair deficient (MRD) glioblastomas presented as a giant cell variant of glioblastoma and DNA methylation profile either matched with “diffuse pediatric-type HGG, RTK1 subtype, subclass A”, “adult-type diffuse HGG, IDH-wt, subtype E” or did not match at all. Clinically, the median age at tumor occurrence was significantly lower (50 years) in the MRD group as compared to non-MRD glioblastoma patients (63 years). In addition, median overall survival was more than twice as long (36.8 months) in MRD glioblastoma as compared to non-MRD patients (15.5 months). Microscopically, MRD glioblastoma were associated with significantly more CD8⁺ cytotoxic T cells and microglia cells indicating a stronger immune activation in the context of a hypermutational state. As smaller studies demonstrated that some MRD glioblastoma patients radiologically and clinically profited from immune checkpoint inhibition treatment, the identification of such patients (Table 2) is important [Bouffet et al., 2016]. With this, a better clinical patient stratification regarding improved survival times and suitability for treatments with immune checkpoint inhibitors is possible.

Recommendation for mismatch repair (MMR) gene and tumor mutational burden (TMB) testing in glioblastoma
Clinical parameter: patient age at first tumor occurrence around 50 years
Microscopic parameter: giant cell variant of glioblastoma
Methylation profiling: "diffuse pediatric-type HGG, RTK1 subtype, subclass A" or "adult-type diffuse HGG, IDH-wt, subtype E" or "no match" (MNP v12.7)
Mutational analysis: lack of a) trisomy of chromosome 7, b) monosomy of chromosome 10, c) EGFR amplification, d) homozygous deletion of CDKN2A or e) TERT promoter mutation

Table 2: Clinical and neuropathological parameters that should trigger MMR and TMB testing in patients with high-grade gliomas.

7. Ultra-fast, machine-learning based intraoperative methylation profiling [Vermeulen et al., 2023].

Methylation profiling considerably improved the unbiased and precise classification of brain tumors and was rapidly acknowledged as an essential criterium for diagnostics of most brain tumor entities by the WHO guidelines [Capper et al., 2018; WHO classification of brain tumors, 2021]. Most institutions performing methylation profiling still use an array-based approach for which the entire laboratory process before bioinformatic analyses takes approximately one week. However, for the intraoperative surgical guidance and potentially upcoming new treatment approaches with application of substances or cells directly in the tumor cavity, it might be important to have a precise, rapid on-site diagnosis. As brain tumors are more and more stratified according to molecular classes, an intraoperative histological assessment for primary cerebral neoplasms is of limited interest in many such cases. To overcome those obstacles, a fast genomic and epigenomic assessment using nanopore sequencing had been previously developed [Euskirchen et al., 2017]. The authors of the current study applied nanopore sequencing in combination with a neural network analysis (named “Sturgeon”) to molecularly classify CNS tumors intraoperatively [Vermeulen et al., 2023]. In 45 out of 50 samples, an accurate diagnosis was already obtained 40 min after initiation of the sequencing procedure in a retrospective cohort. After this highly promising proof of concept results, the authors were able to achieve intraoperative molecular diagnoses within a turnaround time of 90 min. A major advantage of the Sturgeon model is that it can be universally used without retraining (see <https://github.com/marcpaga/sturgeon>) on several different flow cell types (e.g. MinION or PromethION using R9 and R10 chemistry). However, the method is still strongly dependent on the tumor cell content. Therefore, a concomitant mirroring histological assessment of the tissue assessed by the Sturgeon model could be beneficial.

8. Multiomics neuropathology improving diagnostic precision, targeted therapy and detection of cancer predisposition syndromes in pediatric neurooncology [Sturm et al., 2023].

Over the past decade, diagnostic improvement in neuropathology was strongly linked to constantly improved methylome profiling [<https://www.molecularneuropathology.org/mnp> based on Capper et al. 2018]. However, this approach has not yet contributed significantly to improved treatment approaches, partially due to a lack of related prognostic data or information about underlying genetic predisposition for tumor development that might be additionally important for further clinical follow-up [Farouk et al., 2021]. In their study, Sturm et al. combined methylation profiling with targeted panel sequencing in a cohort of more than 1,200 newly diagnosed pediatric brain tumors [Sturm et al., 2023]. With this approach, they were able to achieve a refined diagnosis in almost every second case as compared to current WHO classification alone. Moreover, in almost every second case, a potential therapeutically relevant alteration was detected and 15 % of all tumors revealed a directly druggable genetic alteration with BRAF-V600E being the most frequent (7.4 %), followed by FGFR1/3 (4.0 %), ALK (0.8 %), NTRK2/3 (0.4 %), MET (0.1 %) and RET (0.1 %). For 10 % of all cases an underlying cancer predisposition syndrome was found, amongst those, most frequently neurofibromatosis type 1 (1.5 %), followed by TP53-related Li-Fraumeni syndrome (1.2 %), constitutional mismatch repair gene deficiency or Lynch syndrome including MLH1, MSH2 and MSH6 genes (1.1 %), ataxia-telangiectasia and ATM heterozygous carriers (0.9 %), neurofibromatosis type 2 (0.8 %), DICER syndrome (0.6 %) and rhabdoid tumor predisposition syndrome 1 related to SMARCB1 (0.4 %) were observed. Compared to the initial cohort [Capper et al., 2018], 3 % of pediatric neurooncological cases in the present study belong to potentially new tumor classes. Of note, in up to one third of this pediatric patient cohort, discordant results were obtained when comparing standard WHO- and methylation-based analyses, particularly for the children histologically displaying tumors with characteristics of a

Molecular classes of high-grade glioma (CNS WHO grade 3-4)		
	DNA methylation class	Mutation-based
Molecular high risk	High-grade glioma (HGG), G34 Diffuse midline glioma (DMG), K27 High-grade glioma (HGG), MYCN High-grade glioma (HGG), midline High-grade glioma (HGG), RTK High-grade glioma (HGG), posterior fossa	H3 K27M H3 G34R/V
Molecular intermediate risk	Astrocytoma, IDH High-grade glioma (HGG), IDH Oligodendroglioma, IDH Anaplastic pilocytic astrocytoma Pleomorphic xanthoastrocytoma Infantile hemispheric glioma (IHG) CNS neuroblastoma, FOXR2 Diffuse glioneuronal tumour with oligodendroglial features and nuclear clusters (DGONC)	IDH1/2 (R132H) ALK fusion NTRK fusion ROS1 fusion MET fusion Co-occurrence of BRAF-V600E and homozygous deletion of CDKN2A/B
Molecular low risk	Pilocytic astrocytoma, posterior fossa Pilocytic astrocytoma, midline Pilocytic astrocytoma, ganglioglioma, hemispheric Low-grade glioma (LGG), MYB/MYBL1 Ganglioglioma Diffuse leptomeningeal glioneuronal tumor (DLGNT)	BRAF fusion BRAF-V600E in absence of CDKN2A/B deletion Low-grade glioma (LGG), not otherwise specified (NOS)

Table 3: Molecular risk stratification classes for of pediatric high-grade gliomas otherwise classified according to CNS WHO grade 3–4. The molecular risk remains unknown for cases revealing high score for non-neoplastic control tissue or being unclassifiable in the DNA methylation classifier. As the biological behavior is still unclear for pediatric brain tumor with PATZ1 or PLAGL1 fusion, those tumors were excluded from the risk stratification.

high-grade glioma. Taken into account that clinical follow-up revealed that molecular profiling provides a better clinical prediction for patient prognosis than morphological assessment, it was highly recommended to implement multiomics neuropathological assessment in the diagnostic routine. The present study still highlights the importance of methylation profiling, as methylation data being discordant to the initial neuropathological diagnosis was considered as relevant in up to 70 % of the cases discussed in interdisciplinary tumor boards. A very useful tool of the study is the proposed molecular risk stratification of pediatric high-grade gliomas that have been classified according to CNS WHO grade 3–4 (**Table 3**). Although the WHO classification would put such tumors in a very similar biological class, it has to be taken into account that their clinical behavior might be very different and that

multiomics, in particular methylation profiling, provides a much more precise prediction in those cases. Of note, the highly successful results of the present study further had a strong impact on health politics. German national health insurances now provide financing for DNA methylation profiling and gene panel sequencing (for the latter in both tumor and blood leukocyte samples) in parallel for every newly diagnosed brain tumor case in children and adolescents, thereby changing the standard-of-care.

9. Clinical vaccination study targeting H3K27M in adult diffuse midline glioma [Grassl et al., 2023].

While mutations in histone H3 have been detected in several human tumors, the H3K27M mutation in diffuse gliomas of mainly younger patients

constitutes a specific brain tumor entity usually showing a detrimental clinical prognosis [WHO classification of brain tumors, 2021]. Although, H3K27M mutant diffuse midline gliomas are incurable, the first promising results were obtained from MHC-humanized mice showing a mutation specific immune response to a H3K27M long peptide vaccine [Ochs et al., 2017]. This prompted the authors to start a phase I clinical trial with 8 patients suffering from a H3K27M-positive diffuse midline glioma [Grassl et al., 2023]. All patients received a 27-mer peptide (p14-40) including the mutant H3K27M via bi-weekly subcutaneous injection over 6 weeks followed by monthly administration for 4 months and quarterly afterwards until disease progression. The vaccine was well tolerated and without any relevant higher-grade side effects. After 2 injections, H3K27M-vaccine related neopeptide-specific immune responses (in blood and CSF) were detected in 5 out of 8 patients and were associated with promising neuroradiological findings showing reduced axial contrast enhancement. For patients from which previous tumor material was accessible, a colocalization of mutant H3K27M and HLA class-II-DR was detected, indicating that the neopeptide is probably presented by antigen-presenting cells which might lead to a stimulation of H3K27M-specific T cells. Of note, the two patients of this phase I study with the best clinical outcome after detection of peripheral immune response also showed highest numbers of HLA class-II-DR-positive cells associated with mutant H3K27M which may indicate that enhanced immune cell responses associated with mutant neopeptide presentation are responsible for better patient prognosis. With this first human vaccination trial for H3K27M mutant diffuse midline glioma, the authors demonstrated patient safety and neopeptide immune responses. As a most promising finding, complete remission for more than 31 months was achieved in one patient. However, all other patients died within the study period. To further improve the H3K27M vaccine-based effects, the authors propose to administer the vaccine concomitantly with first line treatment to increase the time for T cell mediated antitumoral effects.

10. Clinical study in patients with IDH mutant low-grade glioma using an oral, brain-penetrant inhibitor of mutant IDH1 and IDH2 enzymes [Mellinghoff et al., 2023].

In 2009, the detection of specific point mutations in *IDH1* and *IDH2* genes in distinct brain tumor entities, in particular diffuse astrocytoma and oligodendroglioma, considerably changed the neuropathological diagnostic approach paving the way from a mainly histological to a more and more molecular classification [Yan et al., 2009]. The most frequently altered amino acid is 132 in *IDH1* and 172 in *IDH2*. Thus, those alterations make them a promising treatment target. The application of the dual IDH1 and IDH2 inhibitor Vorasidenib in an orthotopic murine glioma model showed considerably reduced 2-hydroxyglutarate levels that acts as an oncometabolite [Kontekis et al., 2020]. In addition, it was demonstrated that Vorasidenib also penetrated the brain of several non-human species therefore showing its CNS bioavailability in preclinical models. This prompted several neurooncological teams to test IDH1/IDH2 inhibitors in the clinical context of which the double-blind, phase 3 trial of Mellinghoff and colleagues is one of the most advanced studies for residual or recurrent CNS WHO grade 2 glioma comprising 331 patients in total [Mellinghoff et al., 2023]. The patients orally received Vorasidenib or placebo daily until disease progression or toxic treatment side effects occurred. Vorasidenib treatment was mainly associated with low-grade toxic side effects; however grade 3 or higher side effects were observed in 22.8 % of the patients in the Vorasidenib as compared to 13.5 % in the placebo control group. Of note, only 1.8 % of the patients treated with Vorasidenib showed serious side effects, therefore showing a clinically acceptable safety profile. The median progression-free survival was significantly improved upon Vorasidenib treatment, reaching 27.7 months compared to only 11.1 months in the placebo control group. Furthermore, time to next follow-up treatment was significantly longer in the Vorasidenib treated patients for which the median had not been reached by the time of publication of the study while the placebo group reached the

median at 17.8 months. This study is another milestone on the way to more specific, efficacious and safe brain tumor treatments. The US Food and Drug Administration has now approved Vorasidenib as a systemic therapy for patients with Grade 2 astrocytoma or oligodendroglioma with a susceptible IDH1 or IDH2 mutation, based largely on the result of this study (see <https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-vorasid-enib-grade-2-astrocytoma-or-oligodendroglioma-susceptible-idh1-or-idh2-mutation>).

Discussion

In 2023, several novel pathogenic mechanisms were published that might serve as potential targets for oncological treatment approaches in the future. However, it still remains to be determined if those mechanisms can be targeted with high specificity without having negative side effects. For example, spontaneous Ca^{2+} oscillations are key in the modulation of glioneuronal synaptic information [Goenaga et al., 2023]. Therefore, it is unclear if rhythmic Ca^{2+} oscillations that activate frequency-dependent MAPK and NF κ B pathway in the context of brain tumors may be selectively targeted without negatively impacting the normal neurophysiological circuits [Hausmann et al., 2023]. To which degree a potential inhibition of GAP43-dependent transfer of mitochondria from normal astrocytes to glioma cells may slow down tumor growth also in the human context has to be clarified [Watson et al., 2023]. Although it seems obvious that fresh and healthy mitochondria transferred to potentially exhausted glioma cells may serve as a powerhouse, a high metabolic plasticity has been reported for glioma cells allowing for sustainable tumor propagation via glycolytic energy production if mitochondrial-type energy supply is impaired [Shibao et al., 2018]. This glioma cell feature may limit a treatment approach targeting the intercellular transfer of mitochondria between normal and neoplastic glial cells. The findings regarding the involvement of matrix-metalloprotease (MMP) in the process of brain metastasis formation may serve as another promising therapeutic target in the prevention and treatment of brain metastasis, especially as several synthetic MMP inhibitors have been produced [Karreman et al., 2023]. Unfortunately, to date,

many oncological trials applying MMP inhibitors failed because of either lack of efficacy or severe adverse side effects [Lopez-Navarro and Gutierrez, 2022]. Finally, the tumor-suppressive effects of microglia cells demonstrated in the context of brain metastasis formation need a careful reassessment in humans as to date still many studies claim a promoting microglial effect for brain tumor formation [Evans et al., 2023]. As previous studies in glioma have shown, the role of microglial cells in brain tumors is probably less black and white and rather reflects a high cellular plasticity that is related to intratumoral microenvironmental changes resulting in both classic pro- and anti-tumoral features within the same tumor [Zeiner et al., 2019]. In summary, although the aforementioned studies provide fascinating findings about brain tumor pathomechanisms, it seems to be too early to concretely judge the related diagnostic and, most importantly, therapeutic potential. In contrast, the diagnostic and prognostic potential for the proposed new tumor entities seem to be clear. For the glioneuronal tumor with ATRX alteration, NTRK gene fusion and anaplastic features (GTAKA), the detrimental clinical course that is rather unusual for many glioneuronal tumors has to be stressed [Bogumil et al., 2023]. As GTAKA could be unequivocally defined as a new class using methylation classification, its diagnosis also seems to be straightforward. The proposed new entity of “De novo replication repair deficient glioblastoma, IDH wildtype” harbors a better prognosis as compared to other glioblastoma variants [Hadad et al., 2023], and may also profit from checkpoint inhibitors. Both diagnosis and treatment rely on a neuropathological diagnostic approach requiring MMR and TMB testing in diffuse high-grade gliomas: a) presenting in patients with a younger median age (50 years) as compared to conventional glioblastoma, b) showing a giant cell morphology, and c) lacking classic methylation or mutational features usually observed in conventional glioblastoma (Table 2). The diagnostics of such new entities should also be taken into account when developing new diagnostic tools. The ultra-fast, machine-learning based intraoperative methylation profiling can be applied without retraining but it is still based on methylation data that has been published a few years ago [Vermeulen et al., 2023]. It is therefore essential

that the neuropathological community aligns on joint diagnostic standards, techniques and platforms so that molecular profiling can achieve similar results world-wide. This is even more important if different layers of omics approaches are combined [Sturm et al., 2023]. Over the recent years, neuropathology was always at the forefront regarding technical development (e.g. methylation profiling) and diagnostic precision in oncology (e.g. entity specific mutations such as IDH1/IDH2, H3K27M, ATRX only to name a few), subsequently also impacting other oncological domains. These developments prompted an extremely rapid clinical translation focusing on specific drug targets such as H3K27M, IDH1/IDH2 or MRD tumors [Hadad et al., 2023; Grassl et al., 2023; Mellinghoff et al., 2023]. In conclusion, it is fascinating to see how fast the improvements in diagnostic neuropathology with the definition of specific treatment targets reached the bedside. After having shown drug safety and initial clinical efficacy, larger clinical trials are now necessary to define best treatment schemes.

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Conflicts of Interest Statement

The author does not have any conflict of interest to declare.

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