Clinical Relevane of Molecular Diagnostics in Gliomas

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Agenda

1. IDH-mutant Glioma
2. Histone H3-altered Glioma
3. MAPK-altered Glioma (e.g. PXA*)
4. Glioblastoma
5. Case Examples

*PXA is mentioned exemplarily. Please be aware that the CNS WHO classification also lists further MAPK-altered gliomas and glioneuronal tumors which will not be mentioned in this presentation due to time constraints.

IDH-mutant Glioma

Astrocytoma, IDH-mutant	*IDH1, IDH2, ATRX, TP53, CDKN2A/B*

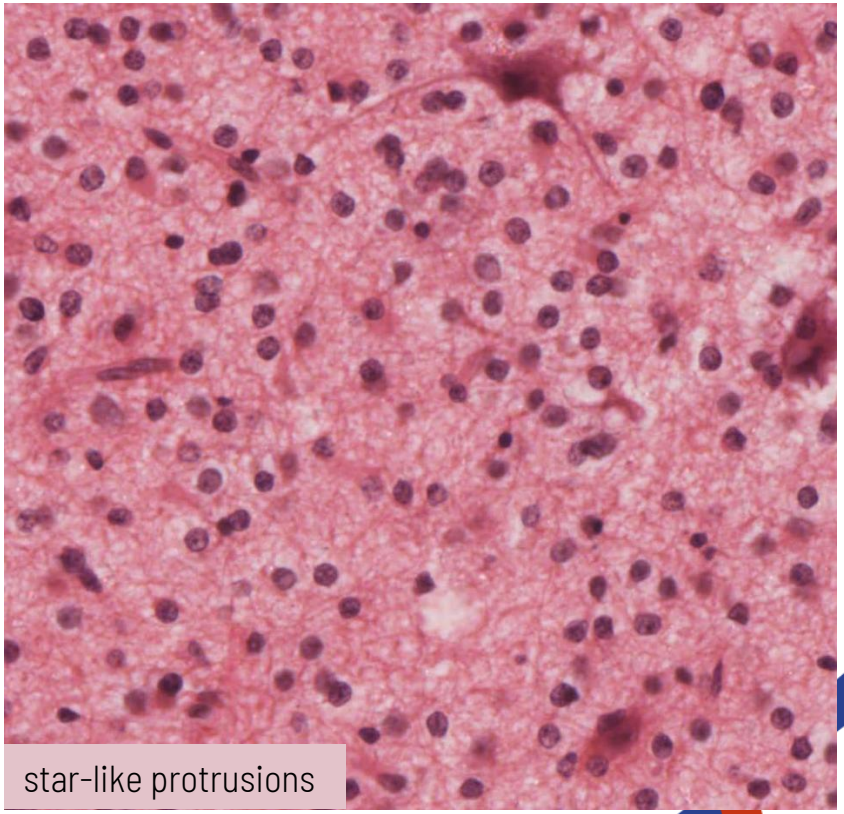
Clinical and Epidemiological Data

- mostly hemispheric location
- mostly adolescents/young adults

Diagnostic criteria

- Essential:
  diffusely infiltrating glioma
  + IDH1 R132 or IDH2 R172 mutation
  + loss of ATRX expression or ATRX mutation

- Desirable:
  astrocytic differentiation by morphology
  overexpression of p53 or TP53 mutation
  DNA methylation profile of IDH-mutant astrocytoma



star-like protrusions

IDH-mutant Glioma

Astrocytoma, IDH-mutant	*IDH1, IDH2, ATRX, TP53, CDKN2A/B*

Grading criteria

- Grade 2: no anaplasia, no/very low mitotic activity
- Grade 3: at least focal anaplasia, significant mitotic activity
- Grade 4: microvascular proliferation or necrosis or homozygous deletion of CDKN2A/B

➢ if CDKN2A/B homozygous deletion is present in an astrocytoma, this tumor is categorized as grade 4, irrespective of histology

➢ CDKN2A mutations may have equivalent prognostic significance

Prognosis

- Grade 2: 10.2 y
- Grade 3: 8.1 y
- Grade 4: 4.7 y
  - ➢ CDKN2A balanced → 5.5 y
  - ➢ CDKN2A loss → 1.8 y
- copy number variation load correlates with malignancy

IDH-mutant Glioma

Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted	*IDH1*, *IDH2*, 1p/19q, *TERT* promoter, *CIC*, *FUBP1*

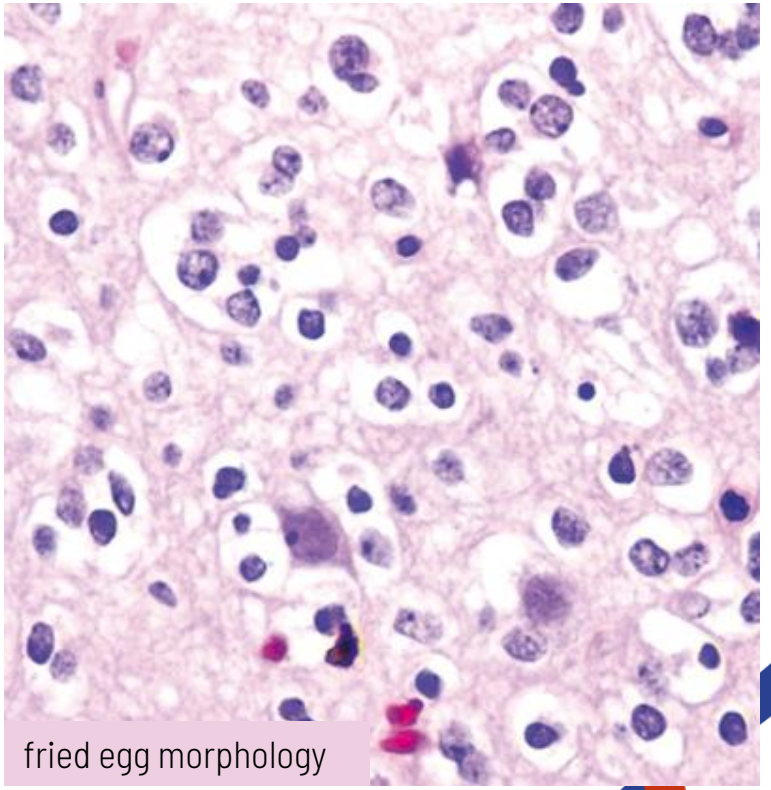
Clinical and Epidemiological Data

- mostly hemispheric location
- patients of all ages

Diagnostic criteria

- Essential:
  diffusely infiltrating glioma
  + IDH1 R132 or IDH2 R172 mutation
  + combined whole-arm deletions of 1p and 19q

- Desirable:
  TERT promoter mutation
  retained ATRX expression or ATRX wildtype
  DNA methylation profile of IDH-mutant oligodendroglioma



fried egg morphology

IDH-mutant Glioma

Oligodendroglioma, IDH-mutant, and 1p/19q-codeleted	*IDH1*, *IDH2*, 1p/19q, *TERT* promoter, *CIC*, *FUBP1*

Grading criteria

- Grade 2: no/very low mitotic activity
- Grade 3: brisk mitotic activity, microvascular proliferation, necrosis

➢ CDKN2A/B homozygous deletion is found in a subset of grade 3, but not grade 2 oligodendrogliomas

➢ CDKN2A/B homozygous deletion may serve as a molecular marker of grade 3 in oligodendrogliomas

Prognosis

- over 10 years
- CDKN2A/B homozygous deletion is linked to reduced survival in oligo-dendroglioma, independent of histological grading

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IDH-mutant Glioma

Treatment Options

Legend:

PCV = Procarbazine, Lomustine (CCNU), Vincristine
RT = Radiotherapy
TTF = Tumor Treating Fields (Optune device)
TMZ = Temozolomide

Astrocytoma, IDH mutant	Grade 2	observation possible (if low-risk) RT + PCV or TMZ (if high-risk) vorasidenib (FDA)
	Grade 3	RT + TMZ maintenance
	Grade 4	RT (+ concurrent TMZ) + TMZ maintenance TTF (off-label)
Oligodendroglioma, IDH mutant and 1p/19q co-deleted	Grade 2	observation possible (if low-risk) RT + PCV (if high-risk) vorasidenib (FDA)
	Grade 3	RT + PCV or PCV + RT

Histone H3-altered Glioma

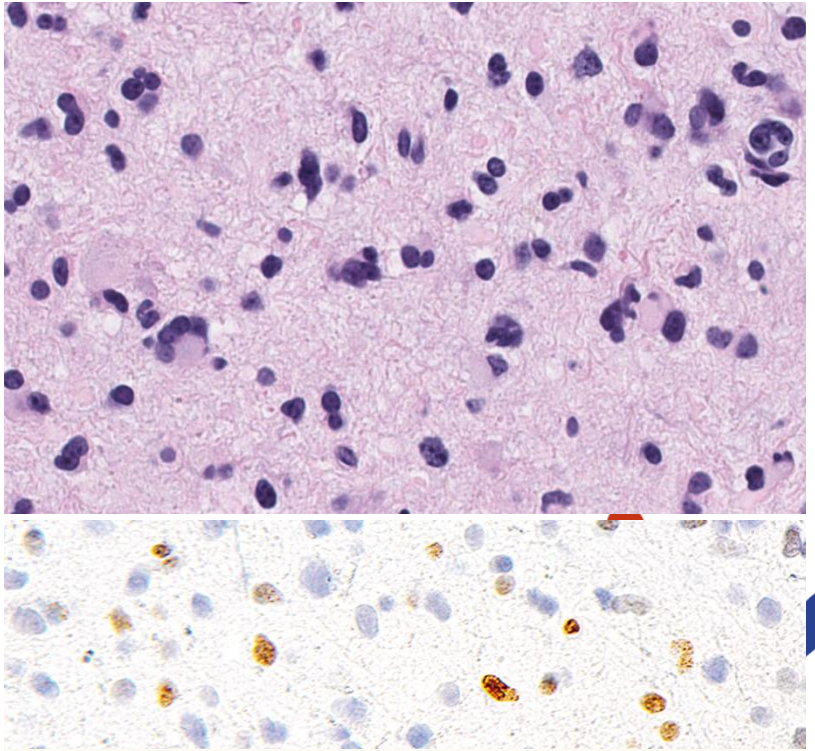
Diffuse midline glioma, H3 K27-altered	H3 K27, *TP53*, *ACVR1*, *PDGFRA*, *EGFR*, *EZHIP*

Diagnostic criteria

- Essential:
  diffuse glioma located in the midline
  + loss of nuclear expression of H3 K27me3 (IHC)
  + H3 p.K27M or p.K27I mutation
    OR EGFR amplification
    OR EZHIP overexpression (RNA/IHC)
    OR DNA methylation profile of diffuse midline glioma

- Desirable:
  molecular analyses that enable discrimination of K27 alterations in the histone family members H3.3, H3.2 and H3.1

- some cases may have concurrent ATRX or TP53 alterations



loss of H3K27me3 in the tumor cell nuclei

Histone H3-altered Glioma

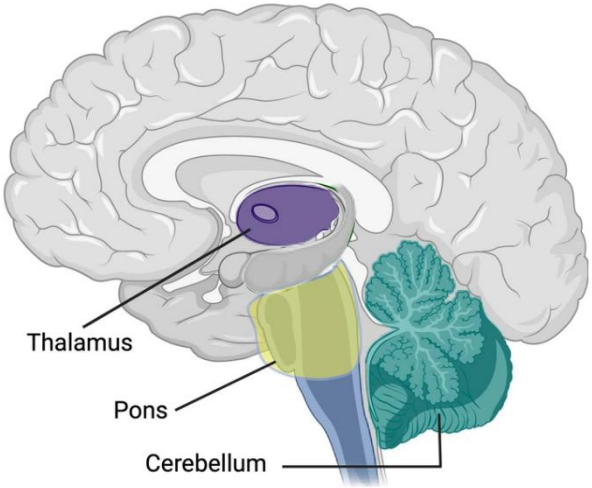
Diffuse midline glioma, H3 K27-altered	H3 K27, *TP53*, *ACVR1*, *PDGFRA*, *EGFR*, *EZHIP*

Clinical and Epidemiological Data

- midline location (thalamus, cerebellum, brainstem, pons, spine)
- children and young adults frequently affected
- former synonym of this entity: diffuse intrinsic pontine glioma (DIPG)

Grading and Prognosis

- poor outcome with OS of 11-16 months
- Grade 4



https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-022-02630-8

Histone H3-altered Glioma

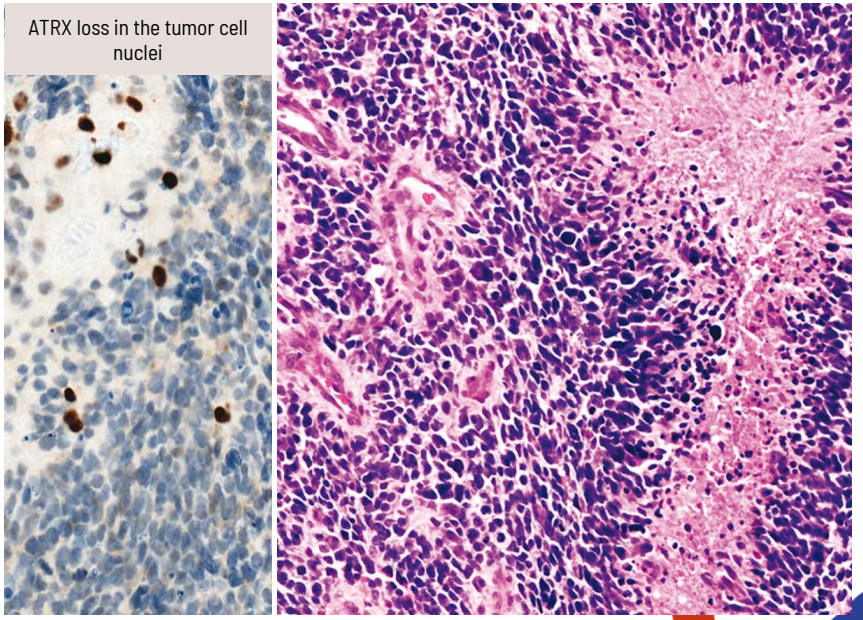
Diffuse hemispheric glioma, H3 G34-mutant	H3 G34, *TP53*, *ATRX*

Diagnostic criteria

- Essential:
  cellular, infiltrative glioma with mitotic activity
  + H3.3 p.G34R or p.G34V mutation
  + hemispheric location
  in unresolved cases DNA methylation profile of H3 G34–mutant diffuse hemispheric glioma

- Desirable:
  Olig2 positivity
  loss of ATRX expression or ATRX mutation
  overexpression of p53 or TP53 mutation

ATRX loss in the tumor cell nuclei



Histone H3-altered Glioma

Diffuse hemispheric glioma, H3 G34-mutant	H3 G34, *TP53*, *ATRX*

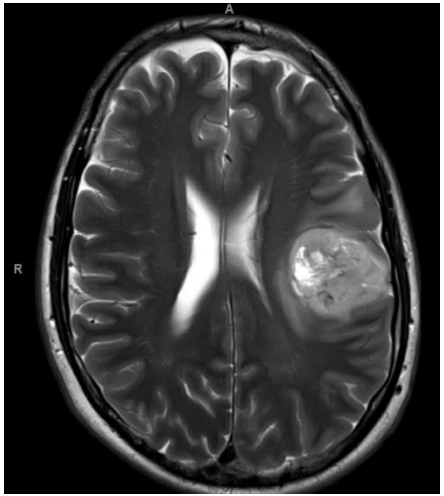
Clinical and Epidemiological Data

- located in the cerebral hemispheres
- adolescents and young adults frequently affected (rare in elderly patients)
- former synonym of this entity: glioblastoma, IDH-wildtype, H3 G34 mutant

Due to the use of different reference transcripts for sequencing ananysis, H3 K27 alterations are sometimes referred to as K28 and H3 G34 alterations as G35.

Grading and Prognosis

- poor outcome with OS of 18-22 months
- Grade 4



https://www.pathologyoutlines.com/topic/cnstumordiffuseH3G34mutant.html

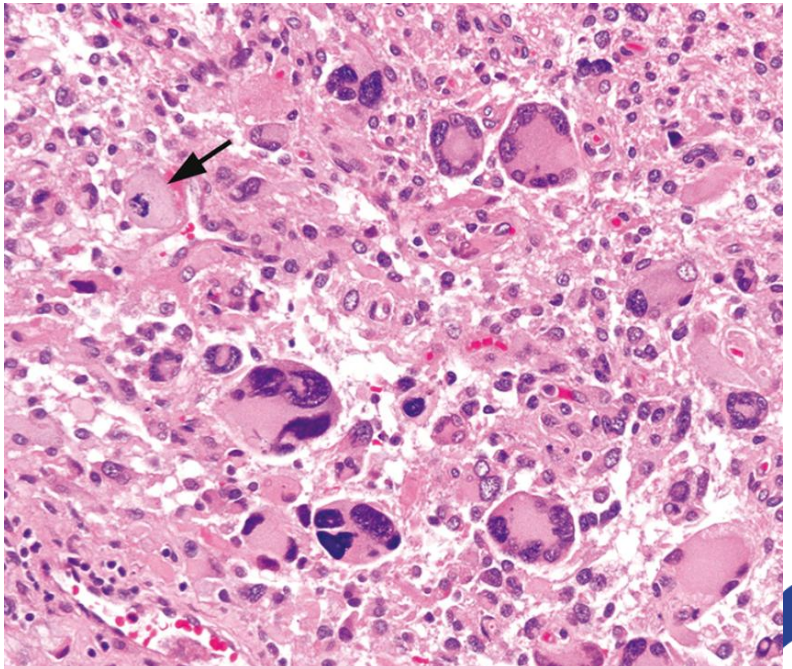
MAPK-altered Glioma (e.g. PXA)

Zentrum für Humangenetik Tübingen

Pleomorphic xanthoastrocytoma (PXA) *BRAF, CDKN2A/B*

Diagnostic criteria

- Essential:
  astrocytoma with pleomorphic tumor cells and eosinophilic granular bodies
- Desirable:
  reticuline deposition
  BRAF mutation (mostly V600E) or other MAPK alteration combined with homozygous deletion of CDKN2A/B
  DNA methylation profile of pleomorphic xanthoastrocytoma
- alterations typical for glioblastoma should be absent



highly pleomorphic and xanthomatous tumor cells

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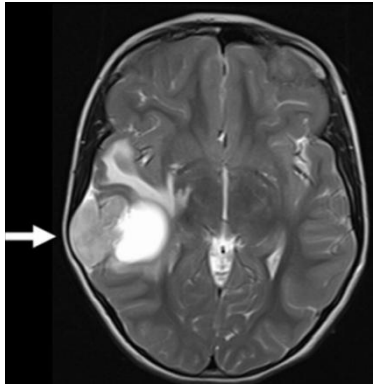
MAPK-altered Glioma (e.g. PXA)

Zentrum für Humangenetik Tübingen

Pleomorphic xanthoastrocytoma (PXA) *BRAF, CDKN2A/B*

Clinical and Epidemiological Data

- located in the cerebral hemispheres, often superficially involving the leptomeninges
- often occurs in children and young adults, rarely observed in elderly patients



doi: 10.21037/tp-24-306

Grading and Prognosis

- grading according to mitotic count
- Grade 2: 5-year OS 90%
- Grade 3: 5-year OS 57%
- rare cases may have a TERT promoter mutation or TERT copy number gain (may predict worse outcome)

Despite PXA is mainly defined by histology in the current CNS WHO classification, molecular studies focusing on DNA methylation-based classification suggest a wider morphological spectrum of this entity. Therefore, comprehensive molecular testing is recommended.

OncoEdu Research | Education | Treatment

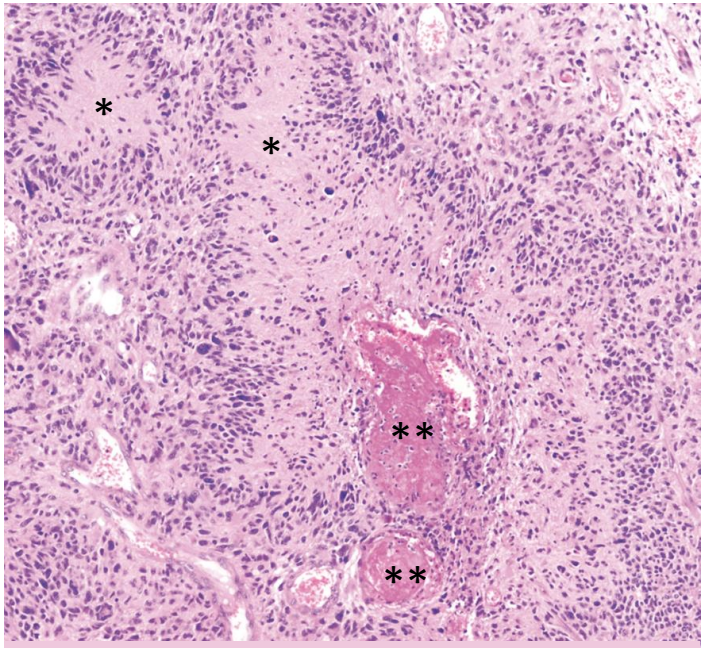
Glioblastoma, IDH-wildtype (GBM)

Glioblastoma, IDH-wildtype	IDH-wildtype, *TERT* promoter, chromosomes 7/10, *EGFR*

Diagnostic criteria

- Essential:
  IDH-wildtype, H3-wildtype, diffuse astrocytic glioma
  + one or more of the following
    - microvascular proliferation
    - necrosis
    - TERT promoter mutation
    - EGFR amplification
    - chromosome 7 gain combined with chromosome 10 loss

- Desirable:
  DNA methylation profile of glioblastoma, IDH-wildtype



glial tumor with palisading necrosis* and thrombosed vessels**

Glioblastoma, IDH-wildtype (GBM)

Glioblastoma, IDH-wildtype	IDH-wildtype, *TERT* promoter, chromosomes 7/10, *EGFR*

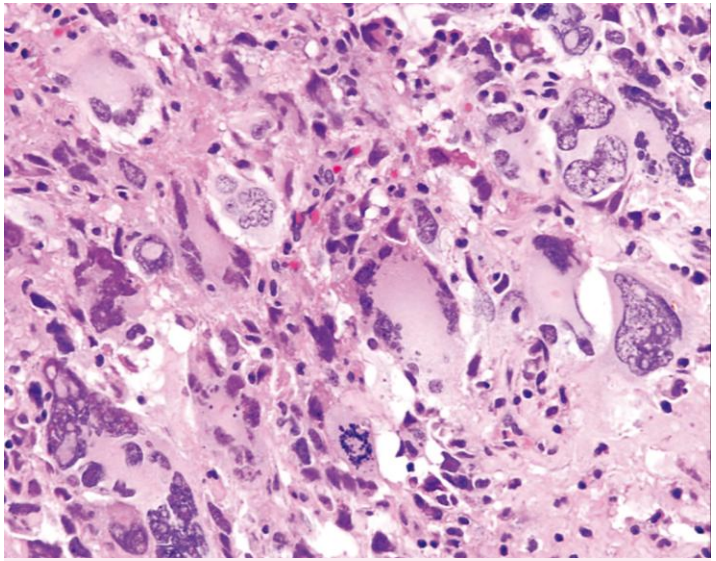
Clinical and Epidemiological Data

- mostly located in the cerebral hemispheres
- rarely located in the cerebellum or spinal cord (<10%)

Rarely, glioblastomas may have a BRAF mutation and may also histologically look like PXA. In order to enable distinction from PXA, diagnostic glioblastoma criteria must apply regardless of the BRAF mutation.

Grading and Prognosis

- poor outcome with OS of 1.6 years
- Grade 4



histological variant with bizarre and multi-nucleated tumor cells

IDH-wildtype Glioma

Treatment Options

Legend:

CCNU = Lomustine
RT = Radiotherapy
TTF = Tumor Treating Fields (Optune Device)
TMZ = Temozolomide
CAR-T = chimeric antigen receptor T-cell

H3-altered Glioma	diffuse midline glioma, H3 K27-altered	RT only, clinical trials (ONC201, ONC206, GD2-CAR-T, H3.3 K27M peptide vaccine +/- immune checkpoint inhibitor)
	diffuse hemispheric glioma, H3 G34-mutant	RT + TMZ (glioblastoma-like protocols) clinical trials (neoantigen-targeted peptide-pulsed dendritic cell vaccine)
Pleomorphic Xanthoastrocytoma (Grade 2 or 3)		RT (+ adjuvant TMZ or CCNU) dabrafenib/trametinib clinical trials (BRAF/MEK inhibitors)
Glioblastoma, IDH wildtype		RT (+ concurrent TMZ) + TMZ maintenance ± TTF in patients up to 70 years of age MGMT+ → TMZ only, MGMT- → RT only in patients over 70 years of age in case of progression TMZ, bevacizumab, regorafenib, CCNU, clinical trial

Case Examples

Case 1

External Diagnosis: Glioblastoma, IDH-wildtype, CNS WHO Grade 4

Histological Diagnosis: Diffuse glioma with singular mitoses
Molecular Findings: BRAF V600E, homozygous CDKN2A/B deletion, lack of molecular GBM markres

Revised Integrated Diagnosis: Pleomorphic Xanthoastrocytoma, CNS WHO Grade 2

- BRAF V600E as therapeutic option in case of progression/recurrence

Case 2

External Diagnosis: Suggestive of low-grade glioma

Histological Diagnosis: CNS tissue with diffusely elevated cell denstity
Molecular Findings: EGFR amplification, TERT promoter mutation

Revised Integrated Diagnosis: Infiltration Zone of Glioblastoma, IDH-wildtype, CNS WHO Grade 4

Further Molecular Alterations With Potential Relevance in Gliomas

Options that can be discussed in a molecular tumor board

Altered Gene	Inhibitor
EGFR (activating varinats, e.g. vIII)	Osimertinib, Afatinib
PTEN, PIK3CA	Temsirolimus, Everolimus
NF1	Selumetinib, Trametinib
FGFR	Erdafitinib
MET	Capmatinib, Crizotinib
NTRK	Entrectinib, Larotrectinib
CDK4, CDK6, CDKN2A/B	Abemaciclib
PDGFRA	Regorafenib, Dasatinib
VEGFA (overexpression)	Bevacizumab

https://staging.asiabiotech.com/collective-wisdom-in-action/

Literature

- WHO Classification of Tumours Editorial Board. Central nervous system tumours. Lyon (France): International Agency for Research on Cancer; 2021 (WHO classification of tumours series, 5th ed.; vol. 6). Available from: https://tumourclassification.iarc.who.int/chapters/45.
- The 2021 WHO Classification of Tumors of the Central Nervous System: a summary (PMID: 34185076)
- Improved prognostic stratification of patients with isocitrate dehydrogenase-mutant astrocytoma (PMID: 38183430)
- Novel, improved grading system(s) for IDH-mutant astrocytic gliomas (PMID: 29687258)
- CDKN2A mutations have equivalent prognostic significance to homozygous deletion in IDH-mutant astrocytoma (PMID: 37550258)
- Pleomorphic xanthoastrocytoma is a heterogeneous entity with pTERT mutations prognosticating shorter survival (PMID: 35012690)
- Wick W. et al, Glioma, S2k guideline, 2021, in: German Society of Neurology, Guidelines for Diagnosis and Therapy in Neurology. Online: www.dgn.org/leitlinien (accessed on 03.07.2025
- https://clinicaltrials.gov/

Thank You!

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