Molecular Profiling in Glioma

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- Gliomas
  - Diffuse astrocytic and oligodendroglial tumors
    - Diffuse astrocytoma, IDH mutant
      - Gemistocytic astrocytoma, IDH mutant
    - Diffuse astrocytoma, IDH wild-type
    - Diffuse astrocytoma, NOS
    - Anaplastic astrocytoma, IDH mutant
    - Anaplastic astrocytoma, IDH wild-type
    - Anaplastic astrocytoma, NOS
    - Glioblastoma, IDH wild-type
      - Giant cell glioblastoma
      - Gliosarcoma
      - Epithelioid glioblastoma
    - Glioblastoma, IDH mutant
    - Glioblastoma, NOS
    - Diffuse midline glioma, H3-K27M mutant
    - Oligodendroglioma, IDH mutant and 1p/19q co-deleted
    - Oligodendroglioma, NOS
    - Anaplastic oligodendroglioma, IDH mutant and 1p/19q co-deleted
    - Anaplastic oligodendroglioma, NOS
    - Oligoastrocytoma, NOS
    - Anaplastic oligoastrocytoma, NOS

- Gliomas
  - Other Astrocytic Tumors
    - Pilocytic astrocytomas
      - Pilomyxoid astrocytoma
    - Subependymal giant cell astrocytoma
    - Pleomorphic xanthoastrocytoma
    - Anaplastic pleomorphic xanthoastrocytoma
  - Other Gliomas
    - Chordoid glioma of the third ventricle
    - Angiocentric glioma
    - Astroblastoma

- Ependymal Tumors
  - Subependymomas
  - Ependymoma
  - Anaplastic Ependymoma
  - Myxopapillary Ependymoma
  - Ependymoma. RELA fusion-positive
- Glio-neuronal Tumors
  - DNET
  - Gangliocytoma
  - Ganglioglioma and Anaplastic Ganglioglioma
  - Dysplastic Cerbellar Gangliocytoma
  - Desmoplastic Infantile Astrocytoma & Ganglioglioma
  - Central Neurocytoma
  - Extraventrticular Neurocytoma
  - Cerebellar Liponeurocytoma
  - Paraganglioma
  - Papillary glioneuronal tumor
  - Rosette-forming glioneuronal tumor
  - Diffuse leptomeningeal glioneuronal tumor

- WHO classification of CNS tumors of 2007/Histologic grading
  - Interobserver variability
  - Variability within each morphologically defined glioma entity
  - Histologically ambiguous gliomas cannot be classified

- Pathogenesis
- Response to therapy and prognosis
- Diagnostic markers for more accurate classification

- Haarlem consensus guidelines in 2014/revised WHO classification of CNS tumors in 2016
  - Layer 1: Integrated diagnosis
  - Layer 2: Histology
  - Layer 3: Grading
  - Layer 4: Molecular Information
- NOS

Adult Tumors

Diffuse Gliomas of WHO Grade II and III

- Astrocytic and oligodendroglial
- The hallmark genetic aberration in these tumors is mutation of IDH1 or, less commonly, IDH2.
  - Increases DNA and histone methylation, eventually predisposing neural stem or progenitor cells to neoplastic transformation.
  - IDH mutation leads to reduced levels of hypoxia-inducible factor and enhanced proliferation of human astrocytes.
  - Increased oxidative stress which leads to tumorigenesis.

- IDH mutation likely represents the ***initiating somatic aberration*** in the vast majority of diffuse and anaplastic gliomas of WHO grade II and III; however, it ***does not appear to be sufficient*** by itself for induction of tumor growth.

- Next participating genes are:
  - TP53 and ATRX genes in diffuse and anaplastic astrocytomas
  - 1p/19q co-deletion and telomerase reverse transcriptase (TERT) promoter mutation in oligodendroglial tumors
- These two genetic profiles, i.e., IDH, TP53, and ATRX mutation versus IDH mutation, 1p/19q co-deletion, and TERT promoter mutation, ***are virtually exclusive*** and considered as the ***defining molecular characteristics*** of astrocytic versus oligodendroglial lineage tumors.

- Co-deletion of 1p and 19q in oligodendroglial tumors is frequently associated with mutations in the CIC gene located on 19q13.2.
- A subset of 1p/19q co-deleted tumors carries mutations in the far upstream element-binding protein 1 gene (FUBP1) at 1p31.1.
- Partial deletions on either chromosome are not diagnostic but in fact may be detected in a subset of high-grade astrocytomas, including glioblastomas.

IS WHO GRADING STILL IMPORTANT FOR IDH MUTANT DIFFUSE GLIOMAS?

- Histologic grading of diffuse gliomas has traditionally been regarded as an independent prognostic factor alongside certain clinical parameters like patient age, clinical performance score, and extent of surgical resection.
- The histologic grade is often used to guide postoperative therapy, with WHO grade III gliomas being invariably treated with adjuvant radio- and/or chemotherapy, while treatment of clinically asymptomatic patients with WHO grade II lesions may be deferred until progression.
- The prognostic value of WHO grading by demonstrating similar overall survival (OS) of patients with IDH mutant WHO grade II and WHO grade III astrocytic gliomas, has been challenged.

Glioblastoma WHO Grade IV

- IDH wild-type: 90%
  - Copy number gains on chromosome 7
  - Monosomy of chromosome 10
  - Mutations in the phosphatase and tensin homolog on chromosome 10 (PTEN) tumor suppressor gene
  - Recurrent mutations in the TP53: 10%
  - Phosphatidylinositol 3-kinase regulatory subunit 1 (PIK3R1): 10%
  - Phosphatidylinositol 3-kinase, catalytic, alpha (PIK3CA): 10%
  - Neurofibromatosis type 1 (NF1): 10%
  - Homozygous deletion of the cyclin-dependent kinase inhibitor 2A and 2B (CDKN2A/p14ARF and CDKN2B) loci on 9p21
  - BRAF V600E mutations: 5%
  - Oncogenic fusions between fibroblast growth factor receptor (FGFR) genes and transforming, acidic, coiled coilcontaining protein (TACC) genes: rare
  - EGFR amplification: 40%
    - About half of EGFR amplified glioblastomas additionally carry a constitutively active deletion mutation lacking parts of the external domain encoded by exons 2–7.
  - TERT promoter mutations: 70%
    - TERT promoter mutations leading to increased expression of TERT in IDH wild-type GBMs are mutually exclusive to ATRX mutations that are common in IDH mutant astrocytic gliomas.
    - These are often associated with epidermal growth factor receptor (EGFR) amplification.

- IDH mutant: 10%
  - TERT promoter mutation is preferentially detected in oligodendroglial tumors.

- Amplification of proto-oncogenes is typical for adult glioblastomas, in particular affecting the EGFR, platelet-derived growth factor receptor A (PDGFRA), and hepatocyte growth factor receptor (MET) genes, the cyclin-dependent kinase genes CDK4 and CDK6, as well as the murine double minute genes MDM2 and MDM4.
- BRAF mutations are found in up to 50% of epithelioid glioblastoma.

Large-Scale Molecular Profiling for Sub-categorization

- Four ***gene expression-based molecular subgroups*** of glioblastoma, termed ***proneural, neural, classic, and mesenchymal subgroups***, have been distinguished.
- Gene expression classes may be unstable, i.e., glioma stem cells with proneural expression profile may switch to the mesenchymal profile upon nuclear factor kappa B (NF-kB) activation.
- Regional heterogeneity of expression classes has been reported in individual glioblastomas.

- ***Molecular classification based on DNA methylation profiles*** appears to be more robust as it likely reflects the cell of origin and remains stable over tumor evolution.

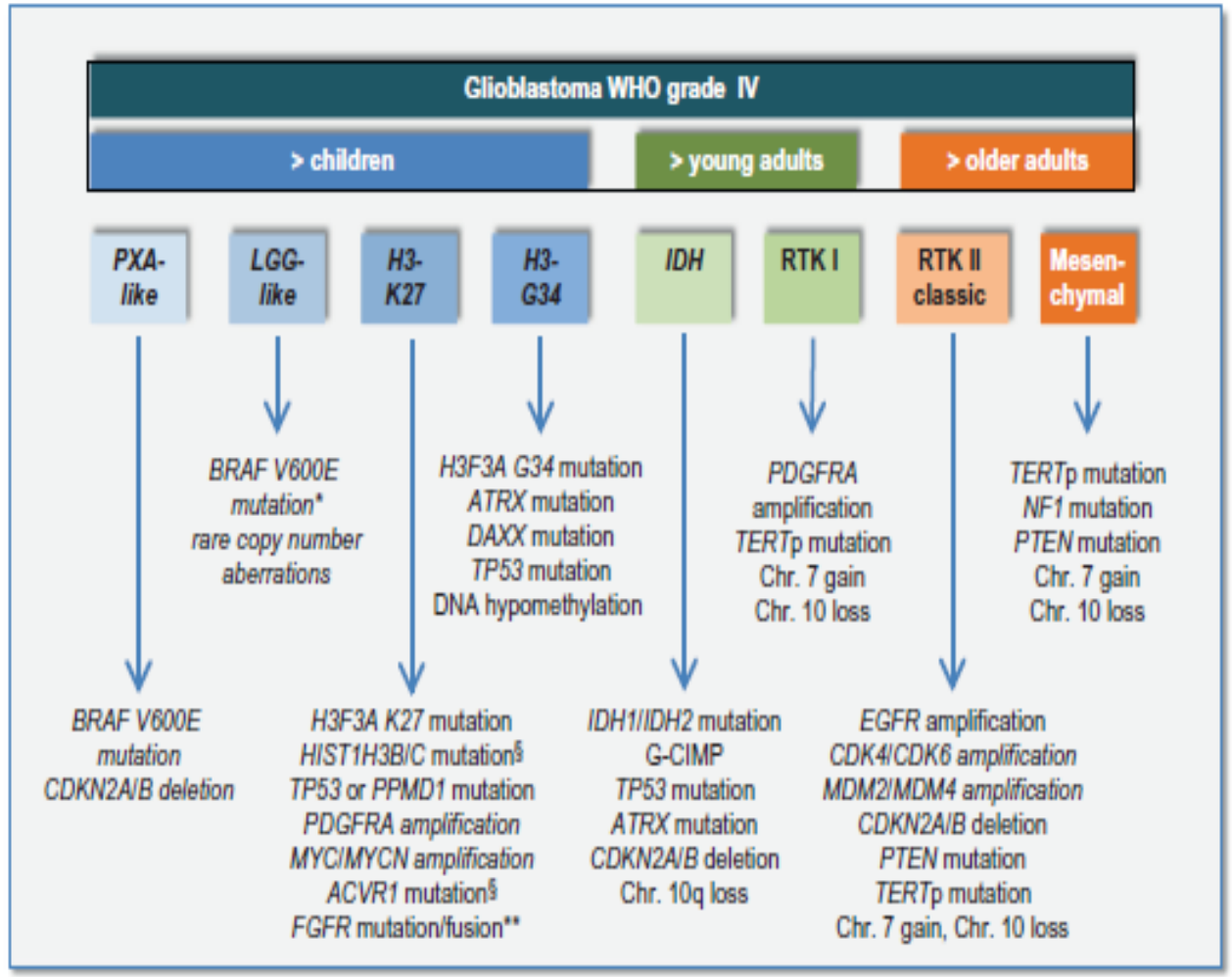


Fig. 6.2. Glioblastoma subgroups in children and adults as defined by distinct DNA methylation profiles and most commonly associated genetic alterations (Sturm et al., 2012; Korshunov et al., 2015). The four subgroups on the left side are predominant in children, among which "PXA-like" and "LGG-like" glioblastoma subgroups are associated with more favorable outcome, while the "H3-K27" subgroup associates with poor outcome. The "IDH" and "RTK I" subgroups typically manifest in young adults, while the "RTK II (classic)" and "mesenchymal subgroups" mostly develop in elderly patients (>50 years of age). The "H3-K27" subgroup includes diffuse midline gliomas in the thalamus, pons, and spinal cord. §*HIST1H3B* or *HIST1H3C* K27 mutations are specifically found in subsets of diffuse intrinsic pontine gliomas without *H3F3A*-K27 mutation; **BRAF*-V600E mutation detectable in a minor fraction of tumors; **preferentially in thalamic tumors; G-CIMP, glioma-associated CpG island methylator phenotype; LGG-like, low-grade glioma-like molecular profile; PXA-like, pleomorphic xanthoastrocytoma-like molecular profile; RTK, receptor tyrosine kinase; TERTp, TERT promoter.

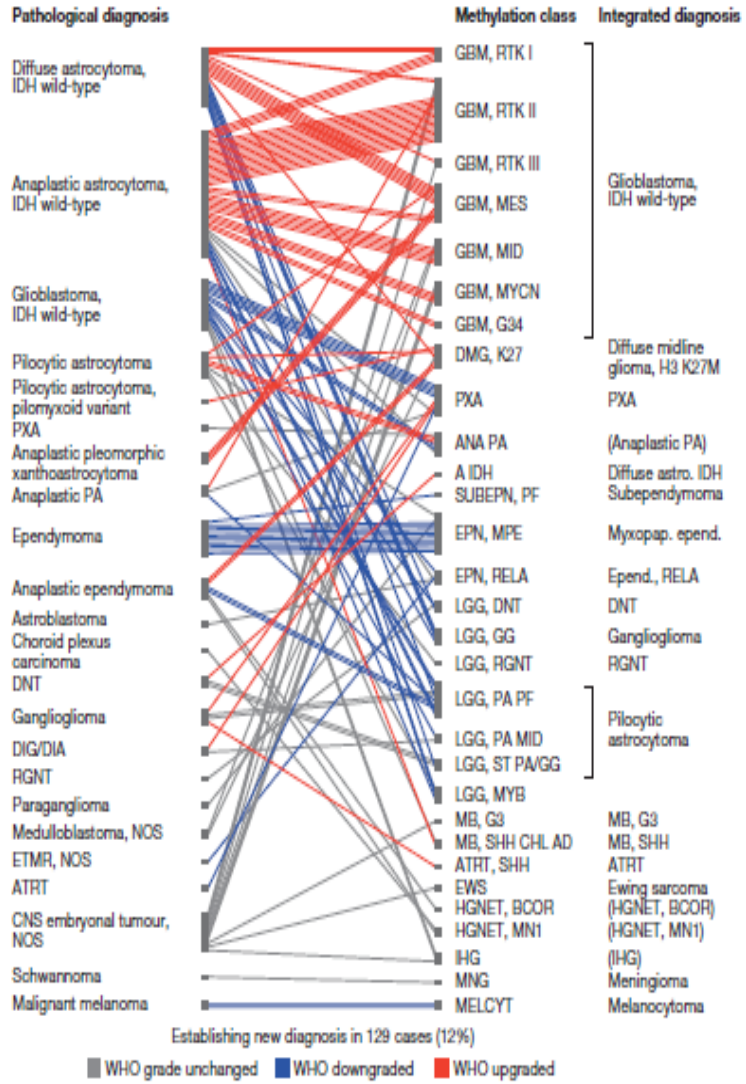


Figure 4 | Reassessment of discrepant cases and establishment of new diagnosis. Discrepancy between pathological diagnosis (left) and methylation profiling (middle) was observed for 139 cases. For 129 cases, histological and molecular reassessment (Supplementary Table 5) resulted in a change in the initial diagnosis with formulation of a new integrated diagnosis (right). For 92 cases, this involved a change in the WHO grading, with both down- (blue) and upgrading (red). Integrated diagnoses in brackets are not recognized as a WHO entity. For methylation class abbreviations see Supplementary Table 1.

Pediatric Tumors

Gliomas with Circumscribed Growth

- Pilocytic astrocytoma WHO grade I:
  - Frequent truncating duplications and subsequent oncogenic fusions of the serine/threonine-protein kinases BRAF or v-raf-1 murine leukemia viral oncogene homolog (RAF1), with the KIAA1549-BRAF fusion being by far most commonly observed.
  - Alternative aberrations occurring in smaller fractions of tumors include activating mutations in BRAF, v-ki-ras 2 Kirsten rat sarcoma viral oncogene homolog (KRAS), FGFR1, and protein tyrosine phosphatase non-receptor type 11 (PTPN11), neurotrophic tyrosine kinase receptor type 2 (NTRK2) gene fusions, and NF1 gene mutations.
  - Alterations in any of these genes result in aberrant signaling of the ***mitogen-activated protein kinase (MAPK) pathway***.
- The absence of significant mutations in other pathway genes indicates that pilocytic astrocytoma represents a ***"single-pathway disease"***, with corresponding implications for both molecular diagnostics and targeted therapy.

- Pleomorphic xanthoastrocytoma (WHO grade II) is genetically characterized by frequent BRAFV600E mutation that is detectable in up to 70% of all cases and more than 90% of temporal-lobe tumors.
- Homozygous deletions of the CDKN2A locus and loss of p16INK4a expression are detectable in approximately 60% of PXAs.
- Anaplastic tumors have possible overlap with BRAF mutant glioblastomas, in particular of the epithelioid variant.
- Genetic alterations underlying malignant progression of PXA remain to be determined.

- Subependymal giant cell astrocytomas demonstrate mutations either in the hamartin gene (TSC1) or the tuberin gene (TSC2), usually accompanied by loss of heterozygosity at the respective gene loci.
- Inactivation of TSC1 or TSC2 causes aberrant activation of mammalian target of rapamycin (mTOR) signaling that drives tumorigenesis.

Diffuse Gliomas

- Pediatric diffuse astrocytomas and oligodendrogliomas
  - Absence of IDH mutation and the lack of 1p/19q co-deletion
  - FGFR1 intragenic duplications and rearrangements of v-myb avian myeloblastosis viral oncogene homolog (MYB) or MYB-like (MYBL) genes: 50%

Diffuse Midline Gliomas

- Diffuse midline glioma, H3-K27M mutant, is a newly defined entity that is characterized by midline tumor location, including thalamus, brainstem, and spinal cord, K27M mutation in either H3F3A (encoding histone H3.3) or HIST1H3B/C (encoding histone H3.1).
- Most H3-K27M mutant gliomas correspond to high-grade astrocytic glioma, i.e., anaplastic astrocytoma or glioblastoma.
- These tumors often:
  - Carry mutations in TP53 or the magnesium/manganese dependent protein phosphatase gene 1 (PPMD1)
  - Show frequent amplification of proto-oncogenes:
    - PDGFRA
    - v-myc avian myeloblastosis viral oncogene homolog (MYC) or v-myc avian myeloblastosis viral-related oncogene
    - neuroblastoma-derived (MYCN)
    - cyclin dependent kinase 4 or 6 (CDK4 or CDK6) or cyclin D (CCND1-3) genes
    - inhibitor of DNA-binding 2 (ID2)
    - MET

- Mutations in the ATRX and death-associated protein 6 (DAXX) genes are rare in H3-K27M mutant gliomas but common in H3F3A-G34 mutant cases.
- Mutations in the activing receptor 1 gene (ACVR1) have been detected in about 20% of diffuse intrinsic pontine gliomas while activating FGFR1 mutations or FGFR1 fusions are seen in subsets of thalamic tumors.

Pediatric Glioblastomas

- H3F3A mutations: 58%
  - K27 mutations: 43%
  - G34 mutations: 15%
- IDH mutations: 6%
- H3F3A and IDH wild-type: 36%

- A total of 20% of histologically typical glioblastomas (most in secondary ones) in children demonstrated DNA methylation profiles of low-grade gliomas, including a PXA-like signature associated with BRAF mutation and homozygous CDKN2A deletion.

Ependymal Tumors

- DNA methylation profiling revealed nine distinct biologic subgroups:
  - Supratentorial:
    - Subependymoma (I)
    - Ependymoma (II/III): RELA fusion, 11q aberrations
    - Ependymoma (II/III): YAP1 fusion, 11q aberrations
  - Infratentorial:
    - Subependymoma (I)
    - Ependymoma of posterior fossa type A (II/III)
    - Ependymoma of posterior fossa type B (II/III): Chromosomal instability
  - Spinal cord:
    - Subependymoma (I): 6q deletion
    - Myxopapillary Ependymoma (I): Chromosomal instability
    - Ependymoma (II/III): 22q deletion, chromosomal instability, NF2 mutation

Predictive Markers for High-grade Gliomas

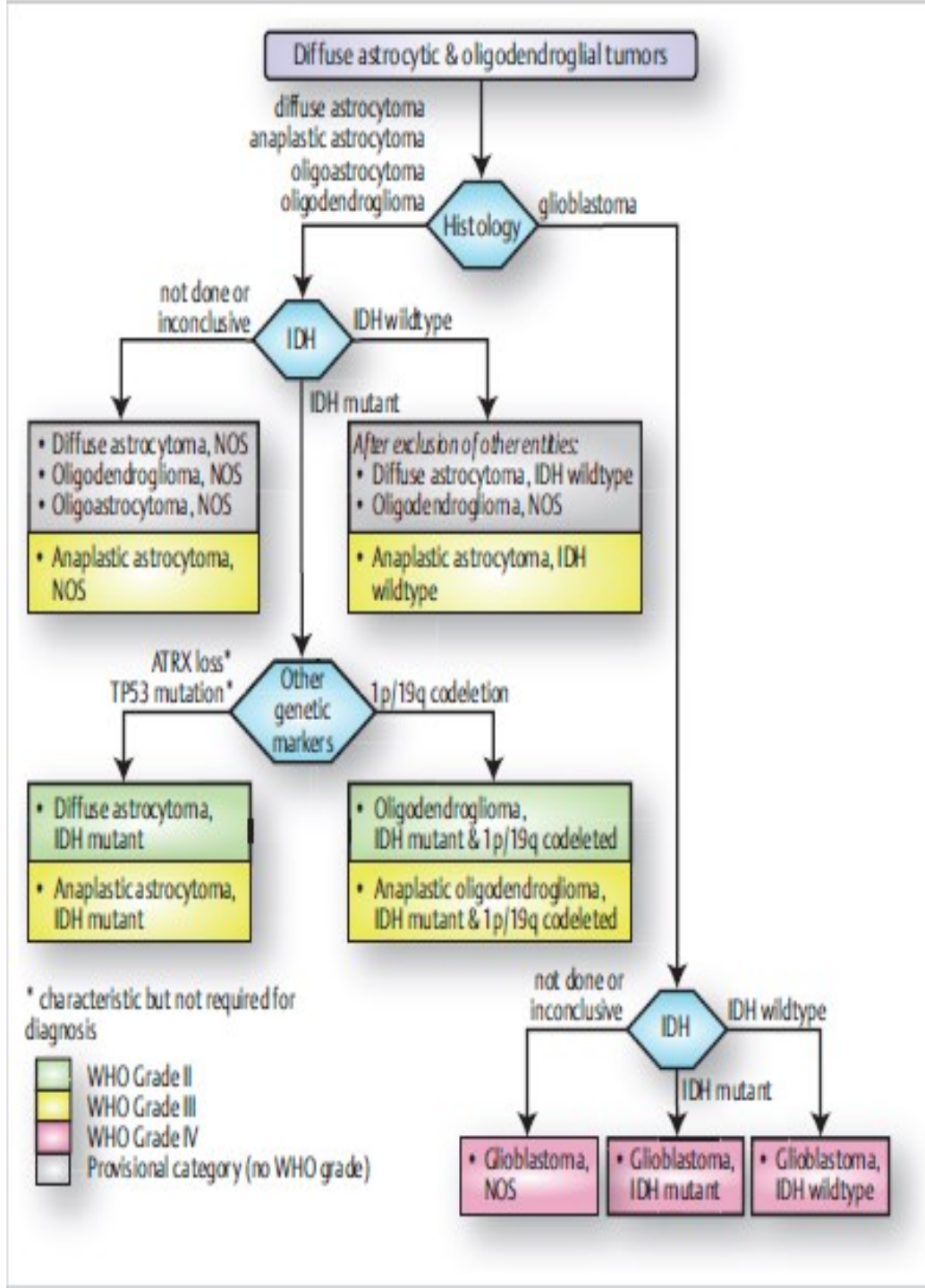
O6-methylguanine-DNA Methyltransferase (MGMT) Promoter Methylation

- A DNA repair enzyme that can counteract the efficacy of chemotherapy with temozolomide.
- The MGMT gene at 10q26 is transcriptionally silenced by aberrant DNA methylation of its 5’-associated CpG-island, in approximately 40% of IDH wild-type glioblastomas as well as the vast majority of IDH mutant and G-CIMP-positive gliomas.
- Patients with MGMT promoter methylated glioblastomas respond better to treatment with DNA alkylating agents like TMZ and carmustine and survive longer when treated as compared to glioblastoma patients with MGMT promoter unmethylated tumors.
- MGMT promoter methylation is an important prognostic marker in glioblastoma patients treated according to the current standard of therapy.

- MGMT promoter methylation in these patients is clinically important as a predictive biomarker for guiding the decision between either radiotherapy alone (when the tumor shows an unmethylated MGMT promoter) or TMZ alone (when the tumor shows a methylated MGMT promoter).
- MGMT promoter methylation is detectable in the vast majority of IDH mutant gliomas, and associated with longer survival but independent of therapy, i.e., radio- or chemotherapy.
- The prognostic versus predictive role of MGMT promoter methylation in malignant gliomas depends on the IDH mutation status.

1p/19q Co-deletion

- Its presence or absence has a role in predicting long-term survival following aggressive multimodal treatment comprising surgical resection followed by upfront combined radiotherapy and chemotherapy with procarbazine, CCNU, and vincristine (PCV).
  - Anaplastic oligodendrogliomas, IDH mutant and 1p/19q co-deleted, had significantly longer median OS times when treated upfront with radiotherapy plus PCV as compared to upfront treatment with radiotherapy alone.
  - Survival of patients with 1p/19q intact anaplastic gliomas was significantly shorter and did not differ between radiochemotherapy and radiotherapy arms.


Diffuse astrocytic & oligodendroglial tumors
diffuse astrocytoma
anaplastic astrocytoma
oligoastrocytoma
oligodendroglioma
Histology
glioblastoma
not done or inconclusive
IDH
IDH wildtype
IDH mutant
• Diffuse astrocytoma, NOS
• Oligodendroglioma, NOS
• Oligoastrocytoma, NOS
• Anaplastic astrocytoma, NOS
After exclusion of other entities:
• Diffuse astrocytoma, IDH wildtype
• Oligodendroglioma, NOS
• Anaplastic astrocytoma, IDH wildtype
ATRX loss*
TP53 mutation*
Other genetic markers
1p/19q codeletion
• Diffuse astrocytoma, IDH mutant
• Anaplastic astrocytoma, IDH mutant
• Oligodendroglioma, IDH mutant & 1p/19q codeleted
• Anaplastic oligodendroglioma, IDH mutant & 1p/19q codeleted
* characteristic but not required for diagnosis
WHO Grade II
WHO Grade III
WHO Grade IV
Provisional category (no WHO grade)
not done or inconclusive
IDH
IDH wildtype
IDH mutant
• Glioblastoma, NOS
• Glioblastoma, IDH mutant
• Glioblastoma, IDH wildtype

Thanks for Your Attention!