Molecular Diagnosis of Gliomas

35th Annual Park City Anatomic Pathology Update

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February 7, 2022

Sources

(Molecular Diagnostics)

- World Health Organization Classification of Tumours of the Central Nervous System. 5th ed. Lyon, International Agency for Research on Cancer; 2021. (WHO CNS5)
- cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy- Not Official WHO) updates 1-7
  - Series of papers published in leading Neuropathology journals
  - Most of the authors are editors or authors of the WHO book
  - Recognizes that the ongoing discovery of diagnostically important molecular features should be
    - broadly reported and
    - *at least considered* for incorporation into clinical practice

WHO Grading System

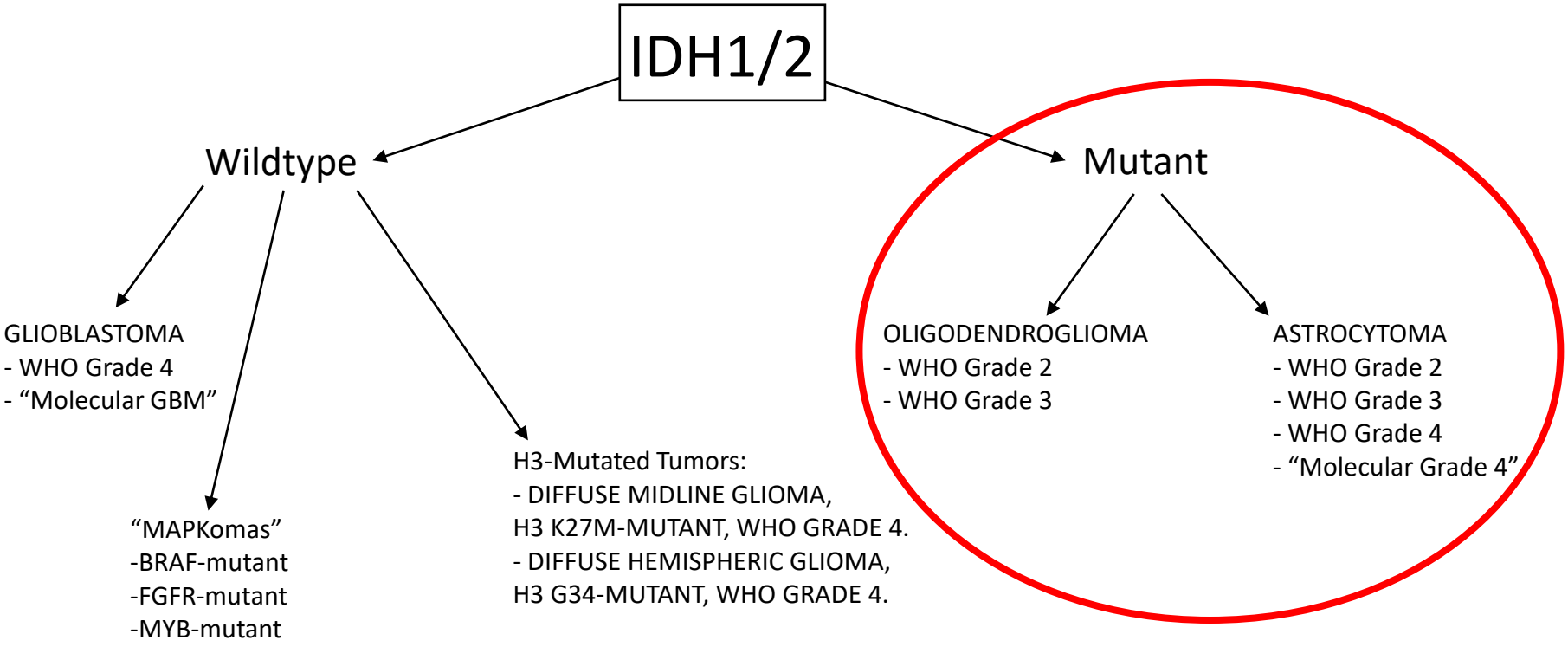
- Method of predicting clinical behavior based on
  - histopathological features **AND**
  - molecular alterations of a particular tumor

- Range is from 1 to 4 (yes, we're using Arabic numbers now)

- **WHO Grade 1**: curable with complete surgical resection
- **WHO Grade 2**: even "complete" surgical resection may not be curative (7-10 years life expectancy)
- **WHO Grade 3**: not surgically curable; may kill on its own or upon conversion to Grade IV lesion (3-7 years)
- **WHO Grade 4**: not surgically curable; chemo/rad-tx can extend life, but response is typically short-lived; kills quickly (12- 18 months)

Note: Time to death is variable and not definite.
These are approximate median survivals

My diagnostic algorithm:



IDH1/2

- Isocitrate Dehydrogenase
  - Normally converts isocitrate to α-ketoglutarate
- Mutation leads to neoenzymatic activity
  - R132 in IDH1
  - R172 in IDH2
  - α-ketoglutarate -→ 2 hydroxyglutarate
- 2-HG thought to result in changes in DNA methylation/topology favorable to tumorigenesis

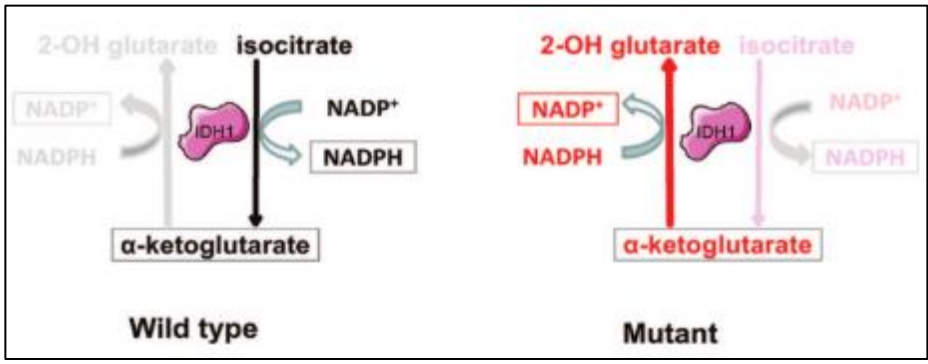


Image from researchgate.net

- IDH1 or IDH2 mutations present in vast majority of low-grade diffuse gliomas
- 95% of all mutations in these genes is the IDH1-R132H mutation (ARUP has IHC test)
- If IHC is negative, the other 5% of mutations can be demonstrated by sequencing
  - All diffuse gliomas in patients <55
  - All potential oligodendrogliomas
  - All tumors with loss of ATRX expression

Pathogenic impact of IDH mutations

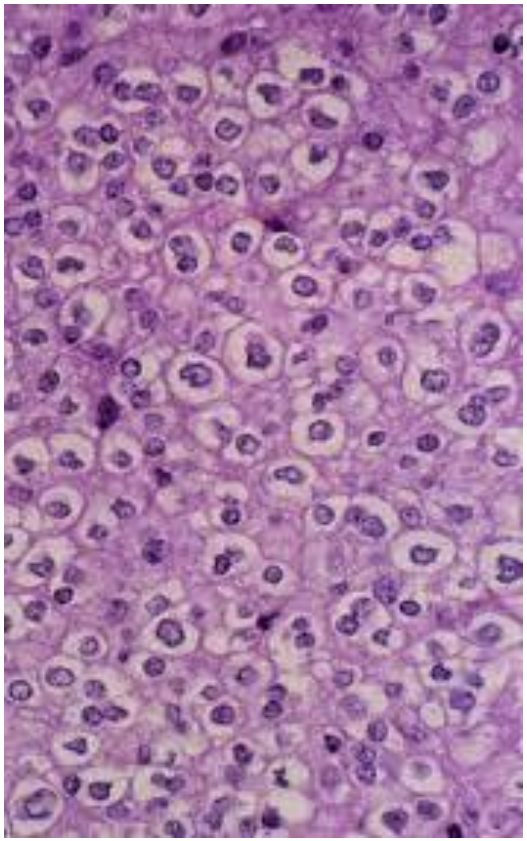
- Disruption of chromosomal topology → Allows aberrant chromosomal regulatory interactions that induce oncogene expression, e.g. PDGFRA
- Widespread gene promoter hypermethylation
  - Termed G-CIMP or "Global CpG island methylator phenotype"
- Impairment of histone demethylation

} Silencing of cellular differentiation factors induces stem cell-like physiological state in glioma

Josh Coleman

Oligodendroglioma

- 3rd-4th decade of life
- Diffuse glioma with oligodendroglial differentiation
- WHO Grading:
  - Grade 2 (no/minimal mitotic activity, no necrosis/MVP)
  - Grade 3 (conspicuous mitotic activity, and/or necrosis/MVP)
- For a DEFINITIVE diagnosis of Oligodendroglioma:
  - IDH1 or IDH2 mutation (IHC or seq); AND
  - Co-deletion of chromosome arms 1p and 19q (currently performed with FISH)



"Fried-eggs"

WHO CNS5; cIMPACT-NOW Update 2

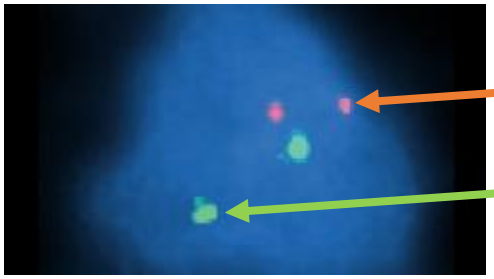
OLIGODENDROGLIOMA, IDH1-MUTANT, 1p/19q-CODELETED, WHO GRADE 2 or 3.

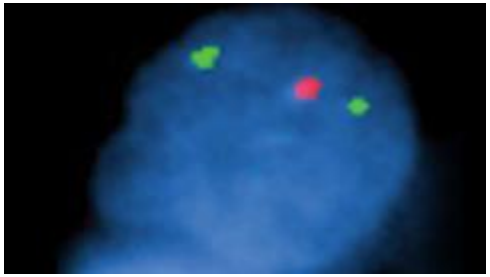
<u>Common Molecular Features:</u>

- IDH1 or IDH2 mutations
  - Oligo more likely than Astro to harbor an IDH2 mutation
  - If IDH1-R132H IHC is negative, *AND*….
    - patient <55 *AND/OR*
    - histology is suspicious for oligo….
  - …..Reflex to IDH1/2 sequencing
- 1p/19q codeletion
  - FISH
- p53 WT
- ATRX WT
  - Assessed by ATRX IHC: retained expression
- pTERT mutation (leads to overexpression and maintenance of telomeres)

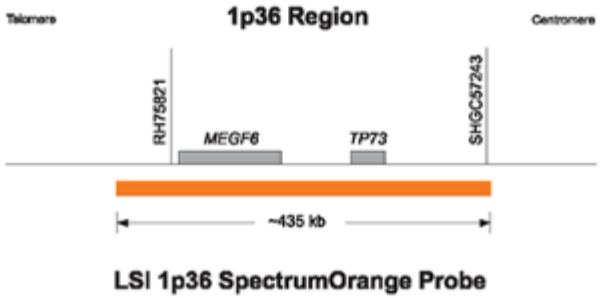
WHO CNS5; cIMPACT-NOW Update 2

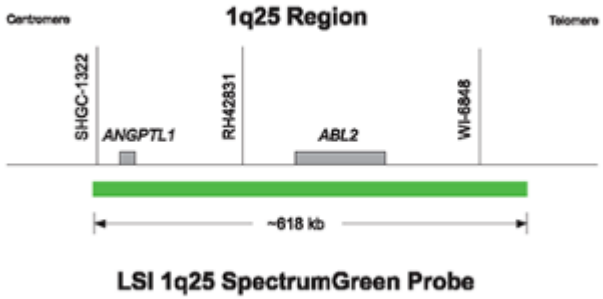
1p FISH





Loss of 1p relative to 1q





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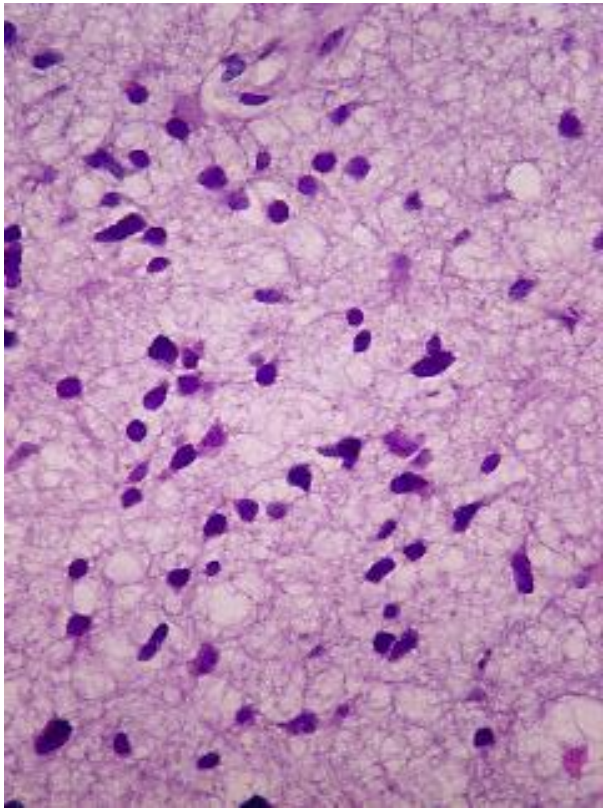
Astrocytoma, IDH-mutant, WHO Grades 2-4

- 3rd-4th decades of life
- Diffuse glioma with astrocytic differentiation
- WHO Grading:
  - Grade 2 (no mitotic activity, no nec./MVP)
  - Grade 3 (anaplasia, mitotic activity, no nec./MVP)
  - Grade 4 (anaplasia, mitotic activity, necrosis AND/OR MVP)
- Molecular demonstration of this entity:
  - IDH1 or IDH2 mutation (IHC or seq); AND
  - NO 1p/19q CODELETION, i.e. RETENTION of chromosome arms 1p and/or 19q (currently performed with FISH)



WHO CNS5; cIMPACT-NOW Update 2

“Molecular Grade 4 Astrocytoma”

- Homozygous deletion of CDKN2A/B has long been recognized as a negative prognostic factor in IDH-mutant gliomas
- Loss of CDKN2A/B is now known to confer malignant behavior
- When classifying a histologic Grade 2/3 IDH-mutant glioma, assessment of CDKN2A/B status is **necessary**
  - Useful adjunct is p16 IHC
  - FISH is the gold standard
- Homozygous deletion of CDKN2A/B = “ASTROCYTOMA, IDH1-MUTANT, WHO GRADE 4”
  - *Regardless* of histologic grade

WHO CNS5; cIMPACT-NOW Update 5

Updated IDH-mutant astrocytoma diagnoses:

- "Diffuse Astrocytoma, IDH1-mutant, WHO Grade II" no longer exists.
  - Now is *"Astrocytoma, IDH1-mutant, WHO Grade 2"*

- "Anaplastic Astrocytoma, IDH1-mutant, WHO Grade III" no longer exists.
  - Now is *"Astrocytoma, IDH1-mutant, WHO Grade 3"*

- "Glioblastoma, IDH1-mutant, WHO Grade IV" no longer exists.
  - Now is *"Astrocytoma, IDH1-mutant, WHO Grade 4"*

WHO CNS5

ASTROCYTOMA, IDH1-MUTANT, WHO GRADE 2/3/4.

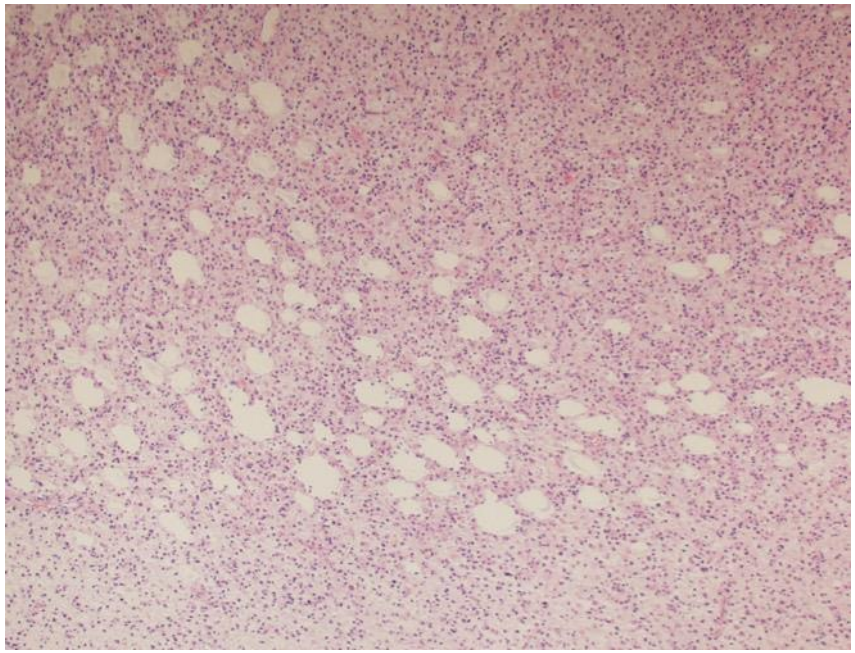
Common Molecular Features:

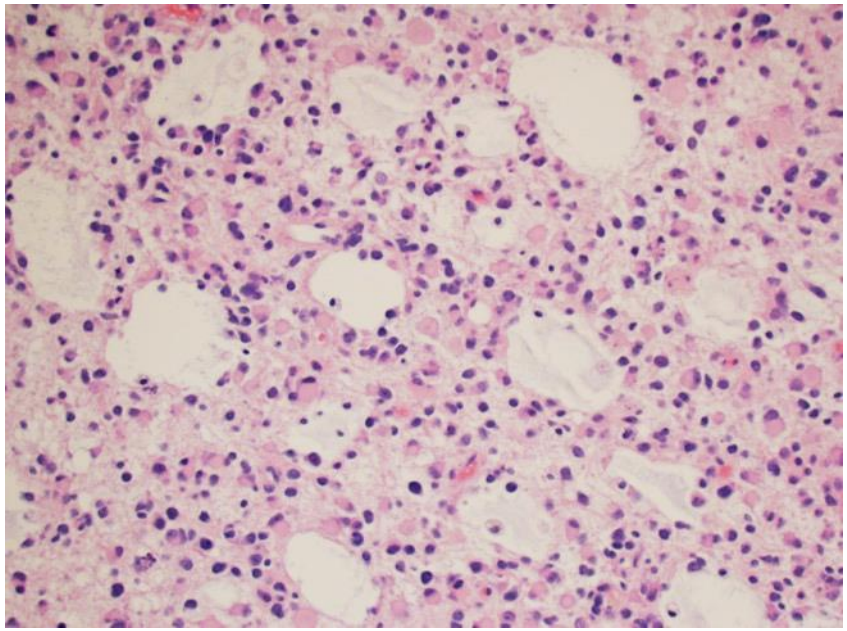
- IDH1 or IDH2 mutations
  - Oligo more likely than Astro to show IDH2 mutation
- Retained 1p and/or 19q (i.e. ABSENCE of 1p/19q codeletion)
- p53 mutation
- ATRX mutation
  - Assessed by ATRX IHC: Loss of expression
- pTERT WT

IDH1/2 analysis in astrocytomas

- IDH1-R132H IHC on <u>ALL</u> gliomas

- When to sequencing IDH1/2?
- If…
  - The patient is <55, *AND/OR*
  - There is loss of ATRX expression, *AND/OR*
  - Histology (myxoid/mucinous cysts) could suggest IDH1/2 mutation





Genetic alterations of astrocytomas

- ATRX mutations
  - Essential chromatin-binding and remodeling protein that may play a role in repair of DNA double-stranded breaks
  - Deficiency has been associated with
    - Epigenomic dysregulation
    - Alternative lengthening of telomeres (ALT)
    - TEST: ATRX IHC is sensitive for loss of expression
- TP53 mutations
  - Loss of tumor suppressor function (Gain-of-function as well)
  - May enable tumor survival in the face of cellular dysregulation caused by ATRX loss
  - May permit genomic instability, e.g. copy number gain of MYC, CCND2, other oncogenes

Josh Coleman

Oligo vs. Astro

OLIGODENDROGLIOMA

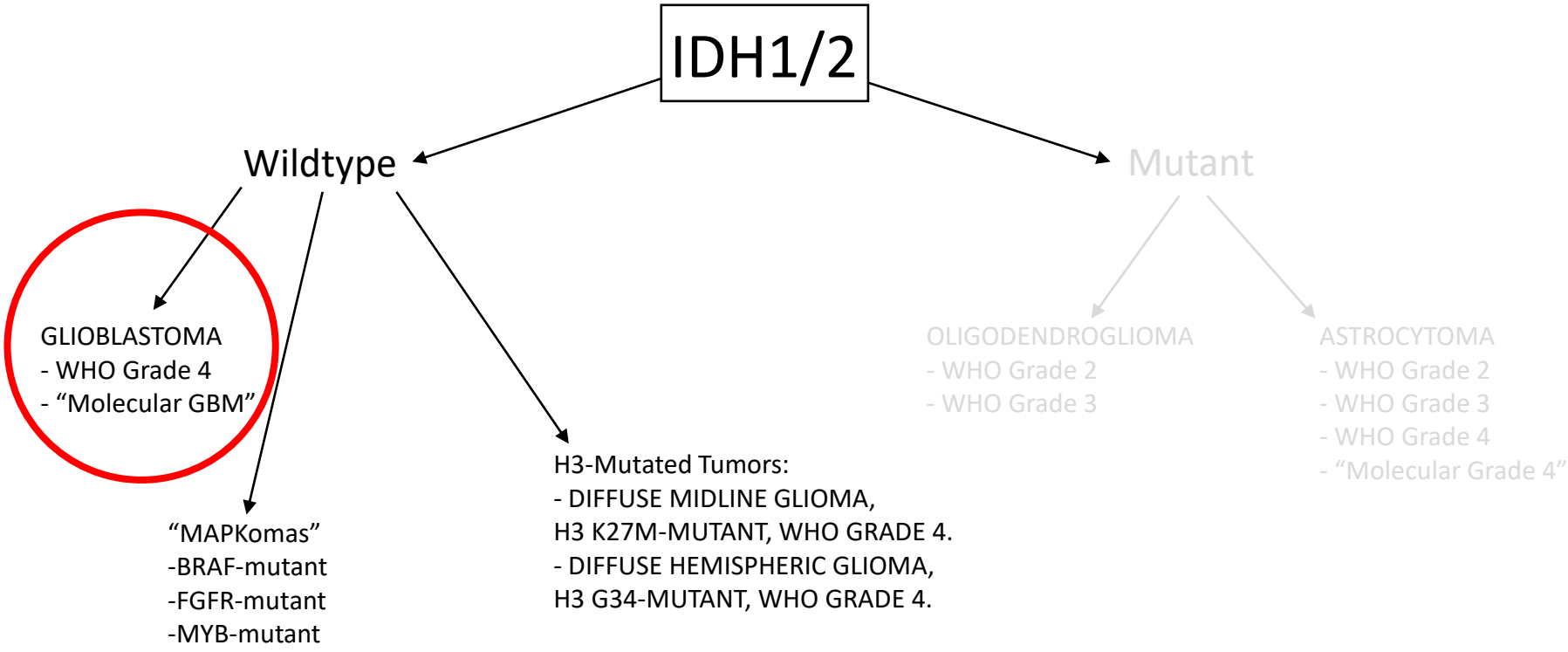
- IDH1 or IDH2 mutations
- 1p/19q codeletion
- p53 WT
- ATRX WT
- pTERT mutation

ASTROCYTOMA

- IDH1 or IDH2 mutations
- Retained 1p and/or 19q
- p53 mutation
- ATRX mutation
- pTERT WT

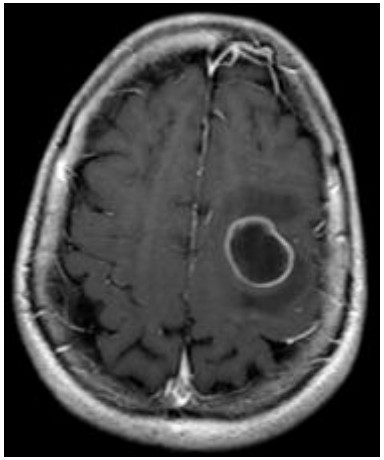
RED = Mutations that enhance telomere maintenance/lengthening

My diagnostic algorithm:

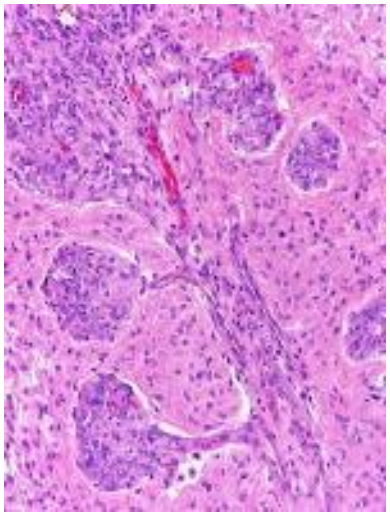


Glioblastoma, IDH-WT, WHO Grade 4.

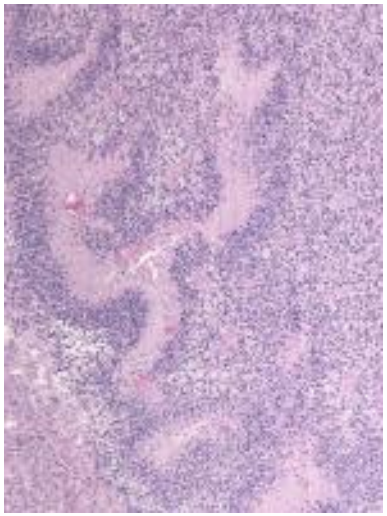
- Most common in 6th-8th decades of life
- Most common primary brain tumor in humans
- Diffusely infiltrative astrocytic neoplasm with
  - Conspicuous mitotic activity
  - Necrosis (palisading) and/or microvascular proliferation



Rim(or ring)-enhancing lesion



Microvascular proliferation



Palisading necrosis

WHO CNS5; cIMPACT-NOW Update 3

Glioblastoma

Common Molecular Features:

- IDH1/2 wildtype
- RTK amplification
  - EGFR
  - MET
  - PDGFRA
- pTERT mutation
- ATRX WT
- Trisomy 7/monosomy 10 (+7/-10)

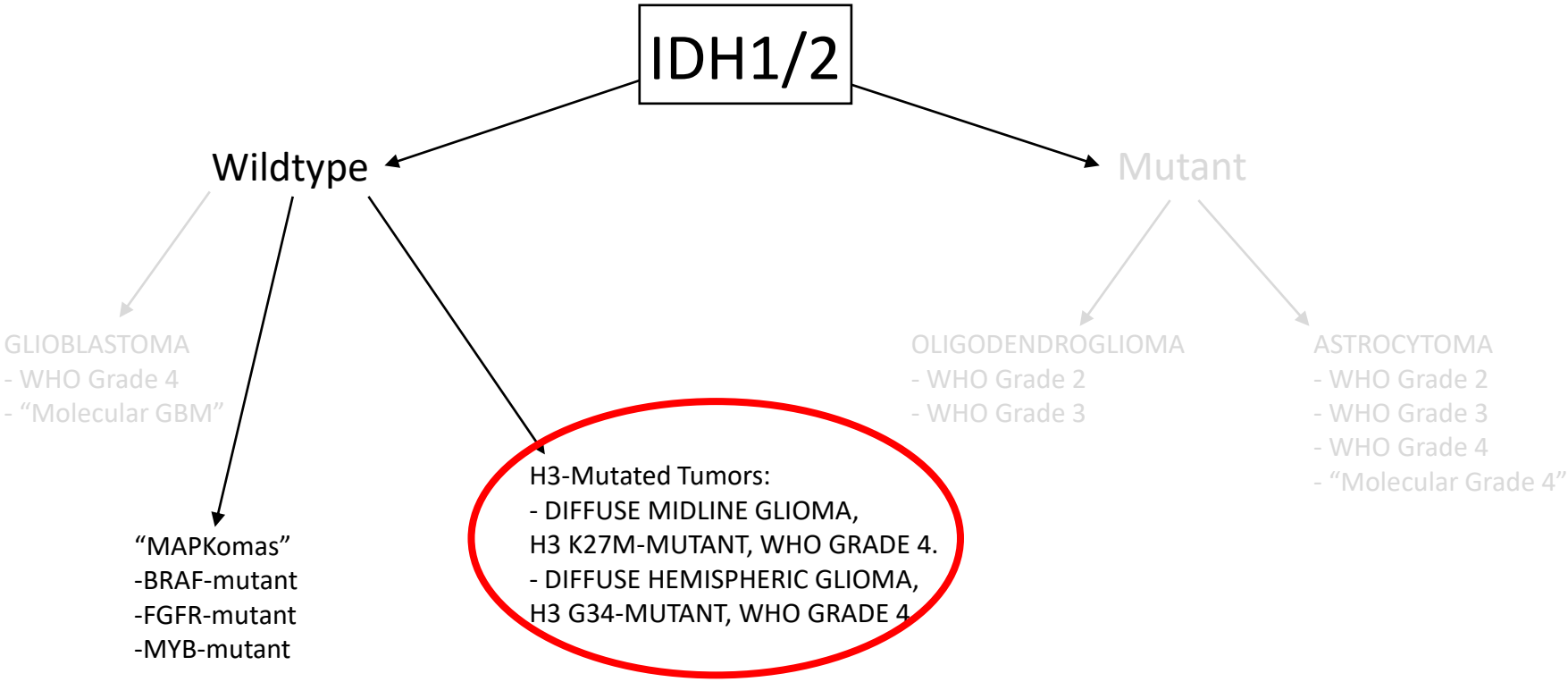
WHO CNS5; cIMPACT-NOW Update 3

“Molecular GBM”

- Diffuse, astrocytic, IDH1/2 WT glioma without WHO Grade 4 histologic features but showing:
  - EGFR amplification, AND/OR
  - pTERT mutation, AND/OR
  - Trisomy 7 AND Monosomy 10
- cIMPACT-NOW 3:
  - “Diffuse Astrocytic Glioma, IDH-WT, with Molecular Features of Glioblastoma, WHO Grade 4.”
  - Cumbersome much?
- cIMPACT-NOW 6:
  - “Glioblastoma, IDH-WT, WHO Grade 4”

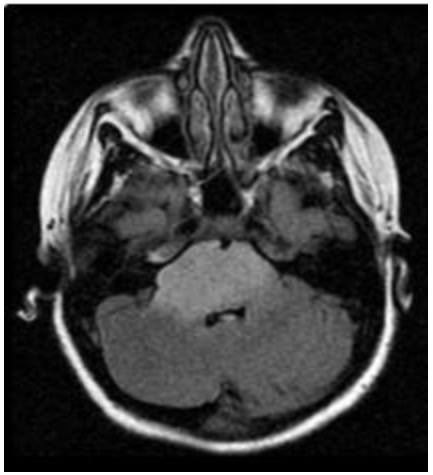
WHO CNS5; cIMPACT-NOW Updates 3 and 6

My diagnostic algorithm:

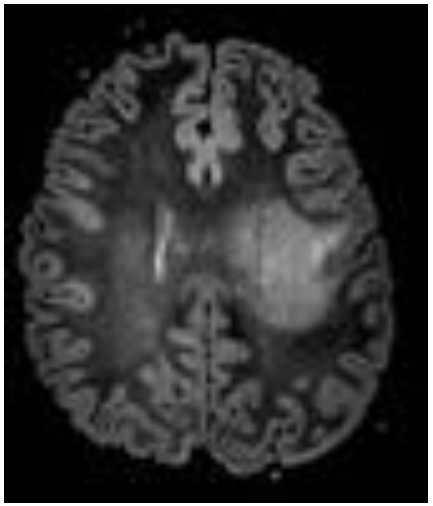


Histone 3-Mutant Gliomas

- Rare, diffusely infiltrating gliomas of young patients (2nd-3rd decades of life)
- Diffusely infiltrating glioma of midline structures:
  - Diffuse midline glioma, H3 K27M mutant, WHO Grade 4
- Diffusely infiltrating glioma of cerebral hemispheres:
  - Diffuse Hemispheric Glioma, H3 G34 mutant, WHO Grade 4
- Both are very aggressive
- *Regardless of histologic grade*, these are WHO Grade 4 neoplasms



Midline



Hemispheric

WHO CNS5; cIMPACT-NOW Update 2

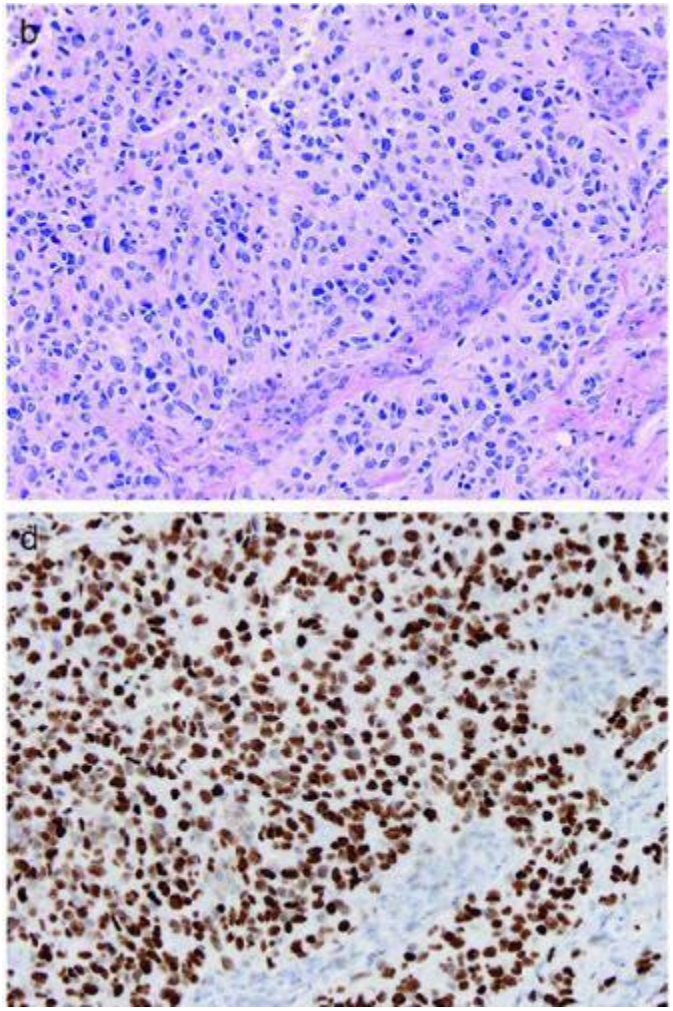
H3-mutant gliomas: Diagnosis

- Diffuse glioma (usually astrocytic in appearance) in a young person showing….
  - No IDH1/2 mutations
  - No EGFR Amplification
  - No combined tri7/mono10
  - No pTERT mutation
- Work-up: H3 K27M (midline) and/or H3 G34 IHC (hemispheric)

WHO CNS5; cIMPACT-NOW Update 2

H3 K27M Antibody

- Highly specific antibody for the K27M mutation
- Regardless of histologic grade, a diffuse glioma
  - affecting midline structures, *AND*
  - H3 K27M mutant
- ….is a WHO Grade 4 neoplasm.



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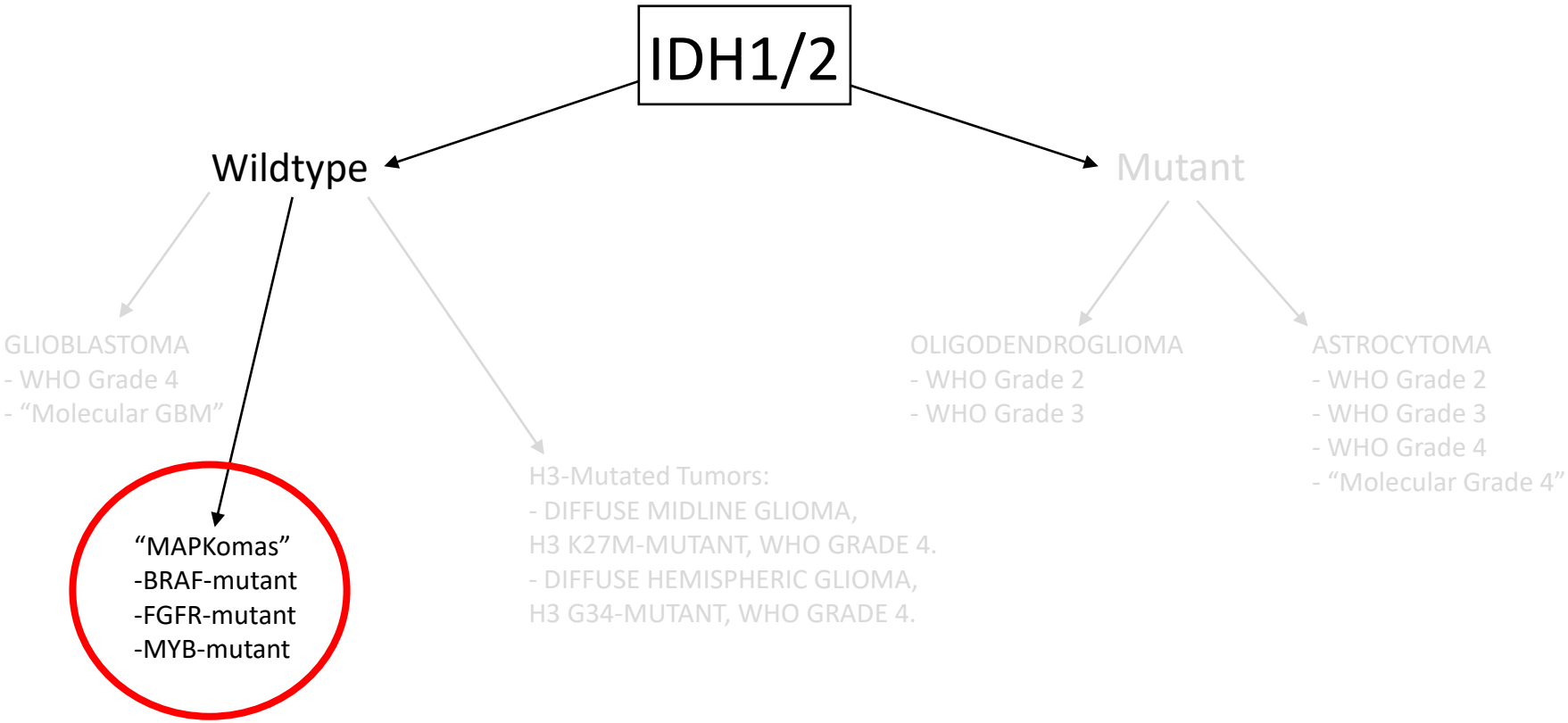


"MAPKomas"

- Over the last several years, numerous studies have demonstrated the mutation-driven activation of MAPK in numerous gliomas, mostly in children/young patients (1^{st}-3^{rd} decades)
- OFTEN but not always in the temporal lobe of young people being worked up for epilepsy
- Suspicion for a MAPKoma:
  - Certain histologies suggest this class
  - Glioma in a young patient that is H3 and IDH1/2 WT

WHO CNS5; cIMPACT-NOW Update 4

IDH and H3 WT Histologies/Genetics: BRAFomas

- Pilocytic Astrocytoma, WHO Grade 1……..BRAF-KIAA1549 fusion
- Gangliocytoma/Ganglioglioma, WHO Grade 1
- Dysembryoplastic Neuroepithelial Tumor (DNET), WHO Grade 1
- Angiocentric Glioma, WHO Grade 1
- Polymorphous Neuroepithelial Tumor of the Young (PLNTY)
- Pleomorphic Xanthoastrocytoma, WHO Grade 2
- Anaplastic Pleomorphic Xanthoastrocytoma, WHO Grade 3
- Diffuse glioma, BRAF V600E-mutant

BRAF-V600E

WHO CNS5; cIMPACT-NOW Update 4

IDH and H3 WT Histologies/Genetics: MYBomas

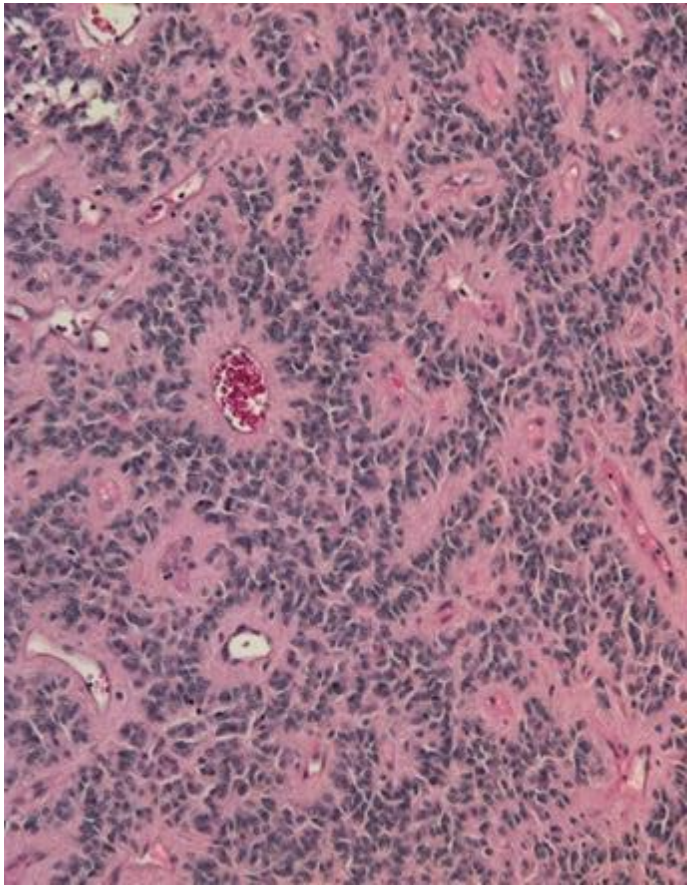
- Diffuse glioma, MYB-altered
- Diffuse glioma, MYBL-altered
- Angiocentric glioma, WHO Grade 1 (if not BRAF, MYB alteration is common)

IDH and H3 WT Glioma Genetics: FGFRomas

- Diffuse glioma, FGFR1-mutant
- Diffuse glioma, FGFR1 TKD-duplicated
- PLNTY (if not BRAF, FGFR2/3 fusions are common)

Ependymomas

- Tumors with ependymal differentiation
- Perivascular pseudorosettes
- Occur anywhere there is an ependymal surface
- Lateral Ventricles (supratentorial; ST): young adults
- Fourth Ventricle (posterior fossa; PF): young kids
- ST and PF Histologic Grading:
  - Prior to cIN-7: 2 or 3 (anaplastic)
  - After cIN-7: No grading currently endorsed
    - Sufficient data does not exist to grade molecularly defined ependymomas
- Spinal cord (SC): Adults
  - Cervical/thoracic (NF2), WHO Grade 2
  - Lumbar/cauda equina: Myxopapillary Ependymoma, WHO Grade 2



WHO CNS5; cIMPACT-NOW Update 7

Subclasses of Ependymoma

- ST-A: c19orf95 fusion (previously RELA fusion-positive)
  - Poor prognosis
- ST-B: YAP1 fusion
  - Good prognosis

- PF-A: *Hypo*methylation of H3 K27: poor outcome (testable with H3 K27me3 IHC)
- PF-B: "Normomethylation" of H3 K27
  - Assessed with H3 K27me3 IHC
  - Previously a Mayo sendout
  - In-house validation is finished, and should be orderable in Millennium soon
  - *cIN-7 recommends at least consideration of methylation profiling in the PF subgroup as frontline diagnostic modality*

- SC:
  - Predominantly WHO Grade 2.
  - MYCN amplification define new subset of aggressive spinal ependymoma (*in-house already*)
  - Myxopapillary (now considered WHO Grade 2)

NOS/NEC

- NOS
- Not Otherwise Specified
- Used when light microscopic diagnosis is made, but further molecular classification is
  - 1) unavailable, or
  - 2) technically failed
- E.g.: A histologically classic oligodendroglioma diagnosed at an institution without FISH capabilities:
  - Oligodendroglioma, NOS.

- NEC
- Not Elsewhere Classified
- Used when adequate workup is performed, and the tumor does not fit into an established classification system
  - E.g., a mismatch between microscopic and genetic features
- Sometime termed a “descriptive diagnosis”

WHO CNS5; Louis et. al.

Methylation Profiling

- DNA methylation is the most extensively studied epigenetic mechanism
- Plays a key role in gene expression and development
- Aberrations of methylation present in various disease, including cancer
- Methylation pattern can be used to identify tumor class
- But how helpful? (*How accurate?)*

Why utilize methylation-based classification?

- Interobserver variability in the histopathologic diagnosis of gliomas, embryonal tumors, and ependymomas
- Unusual histology or molecular profile
- Patient-specific retrospective analysis (e.g., a patient diagnosed with GBM in 2008 who is still alive)
- Overall, diagnostic discordance can confound
  - Clinical practice
  - Clinical trials

Methylation profiling in gliomas

- 166 low-grade gliomas
- WHO 2016 workup (microscopy, IHC, and molecular) compared to methylation profiles

Ferreyra Vega *et al. Clin Epigenet* (2021) 13:102
https://doi.org/10.1186/s13148-021-01085-7

Clinical Epigenetics

RESEARCH Open Access

DNA methylation profiling for molecular classification of adult diffuse lower-grade gliomas

Sandra Ferreyra Vega[1,2], Thomas Olsson Bontell[3,4], Alba Corell[1,5], Anja Smits[1,6], Asgeir Store Jakola[1,5,7*†] and Helena Carén[2*†]

Vega et. al.

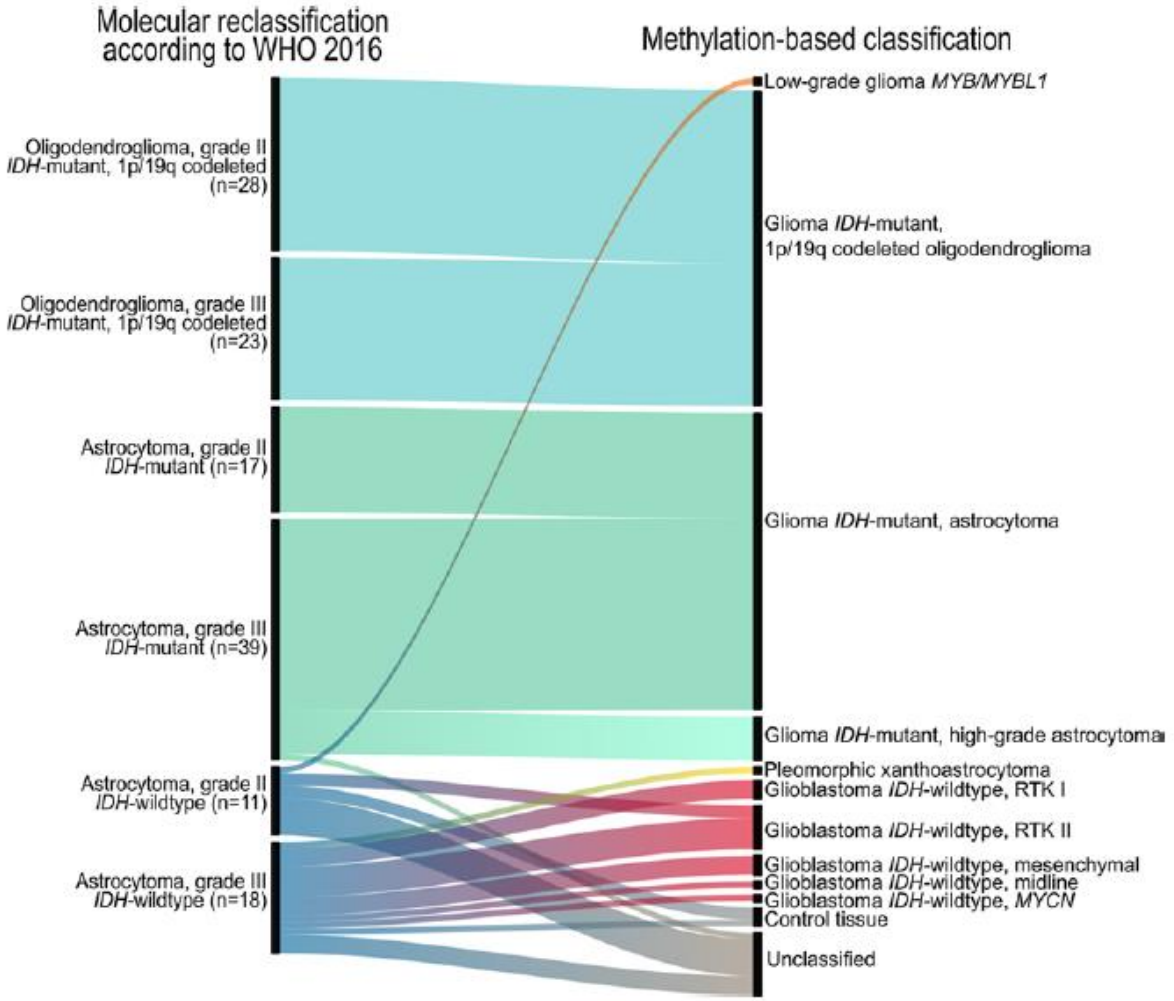


Fig. 3 Molecular reclassification of the adult diffuse lower-grade glioma cohort according to WHO 2016 classification system. Associations of the molecular reclassification including WHO grading and molecular data (*IDH* mutation status and 1p/19q codeletions) generated at time of diagnosis and retrospectively in the study (left) with the outcome of methylation-based classification (right) with the MNP classifier [13]

Vega et. al.

ARTICLE

doi:10.1038/nature26000

DNA methylation–based classification of central nervous system tumours

A list of authors and their affiliations appears in the online version of the paper.

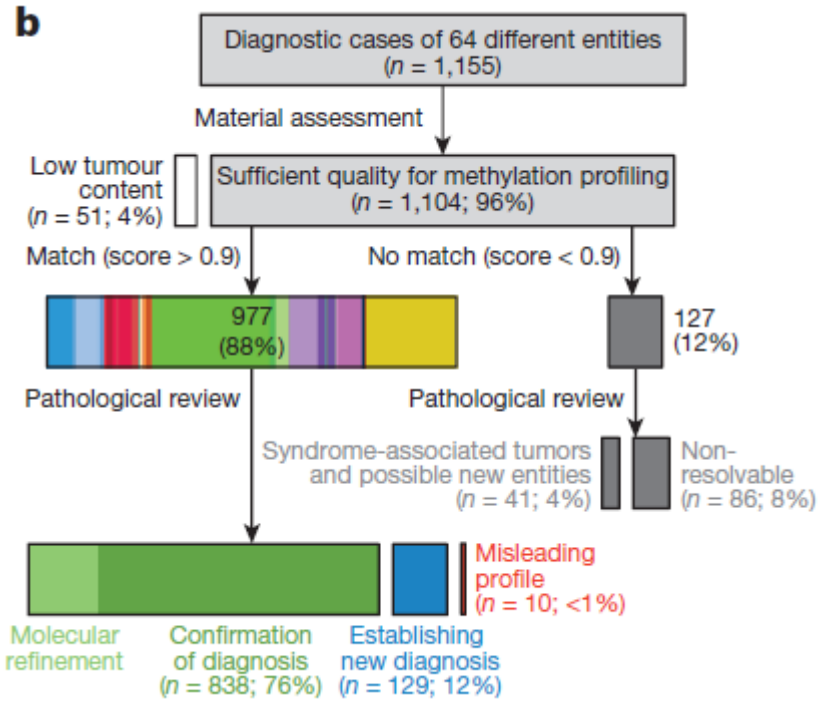
Accurate pathological diagnosis is crucial for optimal management of patients with cancer. For the approximately 100 known tumour types of the central nervous system, standardization of the diagnostic process has been shown to be particularly challenging—with substantial inter-observer variability in the histopathological diagnosis of many tumour types. Here we present a comprehensive approach for the DNA methylation-based classification of central nervous system tumours across all entities and age groups, and demonstrate its application in a routine diagnostic setting. We show that the availability of this method may have a substantial impact on diagnostic precision compared to standard methods, resulting in a change of diagnosis in up to 12% of prospective cases. For broader accessibility, we have designed a free online classifier tool, the use of which does not require any additional onsite data processing. Our results provide a blueprint for the generation of machine-learning-based tumour classifiers across other cancer entities, with the potential to fundamentally transform tumour pathology.

- 76 histopathologic entities (at least 8 per entity) were subjected to methylation profiling Methylation 450 BeadChip Array (Illumina)

Capper et. al.

Prospective use of methylation profiling

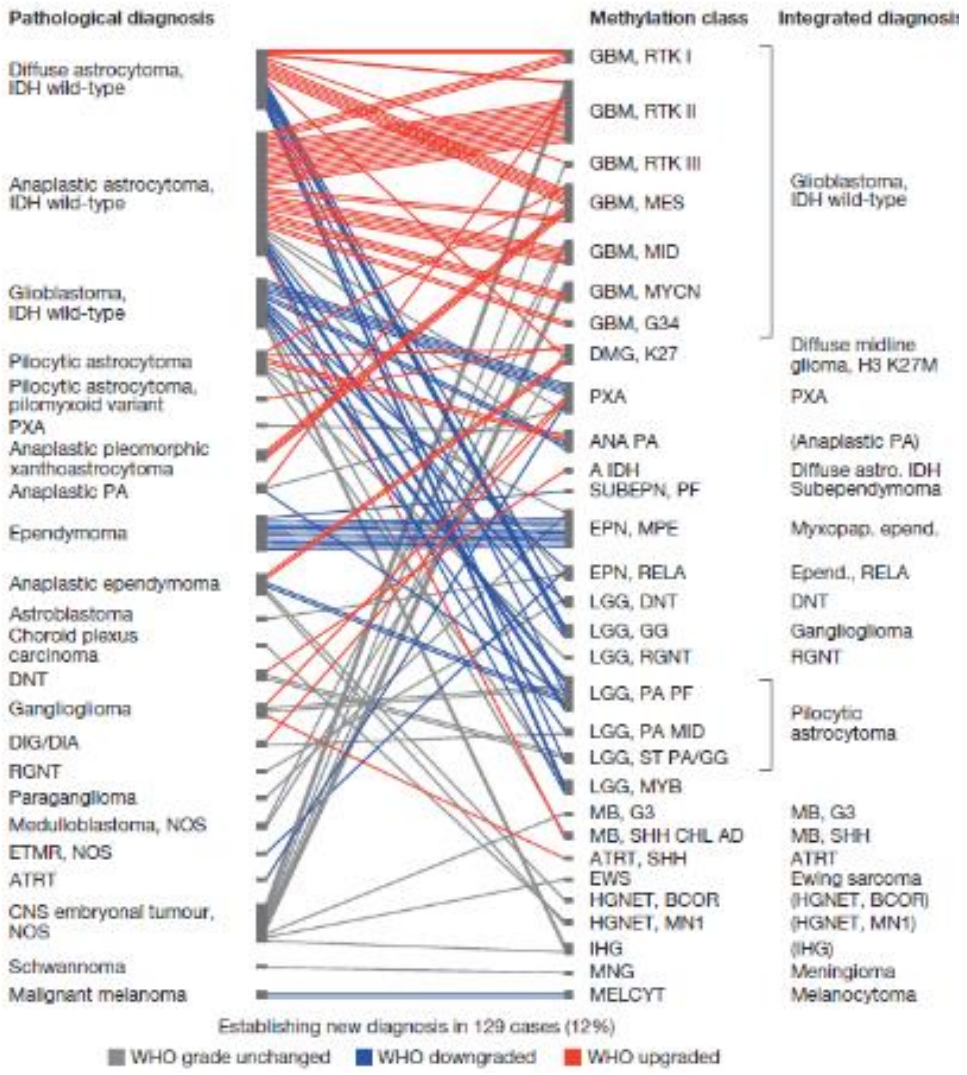
- Frequent confirmation of histologic dx, but only 76%
  - Small fraction was similar but molecularly refined
- Methylation suggested alternate diagnosis in 139 of 1155 cases
- Additional molecular testing confirmed new diagnosis in 129 cases (***12%*** total cohort)
- Poll of 5 external centers who implement methylation profiling in tumor dx:
  - In 50/401 (***12%***) cases, methylation established a new diagnosis



Capper et. al.

The changed diagnoses

- Profound clinical impact
- Change in WHO Grading seen in ***71%*** of cases
  - Upgrade: 41%
  - Downgrade: 30%



Capper et. al.

CNS tumor Methylation Profiling

- Microscopy-Methylation correlation varies from 49-95% (refs. 11-15)….why?
- Different cohorts
- Enrichment for diagnostically challenging cases results in lower correlation
- Whereas sampling tumors that are more representative of routine neuropathology practice results in higher correlation
- Highest correlation seen in IDH-mutant tumors

Methylation is not just for CNS neoplasia….

Published: 01 May 2017

Genome-wide DNA methylation profiling reveals cancer-associated changes within early colonic neoplasia

M P Hanley, M A Hahn, A X Li, X Wu, J Lin, J Wang, A H Choi, Z Ouyang, Y Fong, G P Pfeifer, T J Devers & D W Rosenberg

Oncogene **36**, 5035–5044 (2017) | Cite this article

PLOS ONE

OPEN ACCESS PEER-REVIEWED

RESEARCH ARTICLE

Genome wide DNA methylation profiling identifies specific epigenetic features in high-risk cutaneous squamous cell carcinoma

David Hervás-Marín, Fealiemah Higgins, Onofre Sanmartín, Jose Antonio López-Guerrero, M. Carmen Bañó, J. Carlos Igual, Inma Quilis, Juan Sandoval

Published: December 20, 2019 • https://doi.org/10.1371/journal.pone.0223341

LYMPHOID NEOPLASIA | MARCH 19, 2015

DNA methylation profiling identifies two splenic marginal zone lymphoma subgroups with different clinical and genetic features

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Sarcoma classification by DNA methylation profiling

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Conclusions

- Accurate glioma diagnosis (and therefore, accurate patient education and appropriate therapy) relies heavily on the molecular alterations present in these tumors.
- Integration of histologic and molecular characteristics is the new norm in our field
- Methylation is the next step in further refinement of glioma diagnosis (as well as neoplasia in diverse organ systems)
  - That said, many tumors commonly seen in neuropathology practice do not require methylation for appropriate classification
  - Methodologies and regulatory aspects of methylomics not yet resolved
  - *So for now*, prudent selection of cases for methylation profiling is the key

Thanks!

Questions?

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