DIFFUSE GLIOMAS – DIAGNOSTIC APPROACH AND ANCILLARY TESTS FOR CLASSIFICATION

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University of California San Francisco

AMERICAN ASSOCIATION
OF NEUROPATHOLOGISTS

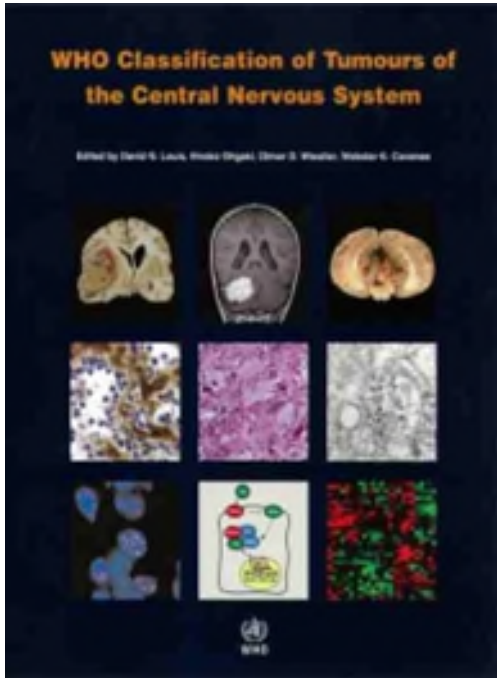
Disclosures

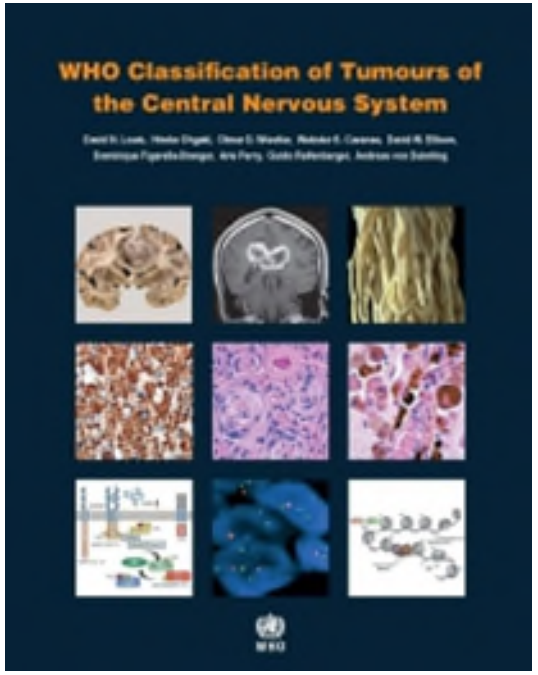
- I have no relevant financial relationships to disclose

Learning Objectives

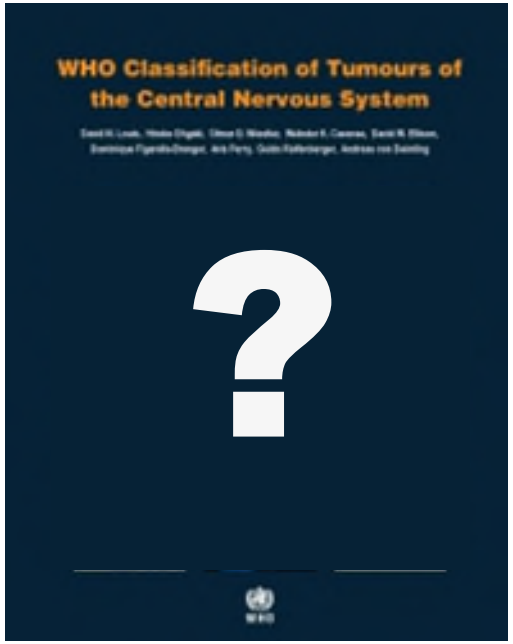
- Outline the main molecular alterations in adult and pediatric diffuse gliomas
- Select immunohistochemical and molecular tests required for accurate classification of diffuse gliomas
- Classify the diffuse gliomas based on the results of the immunohistochemical stains

Classification of CNS Tumors

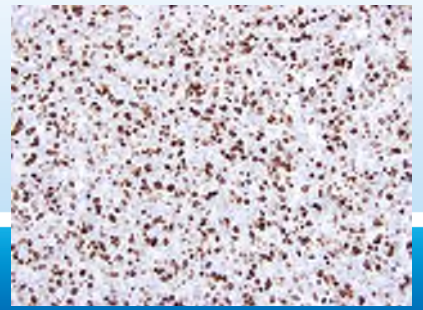


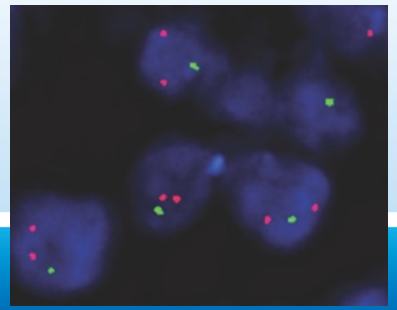


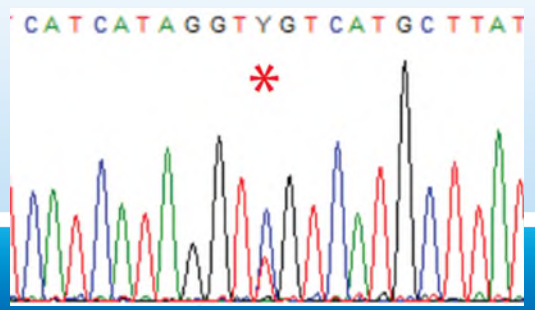
cIMPACT-NOW
Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy–Not Official WHO

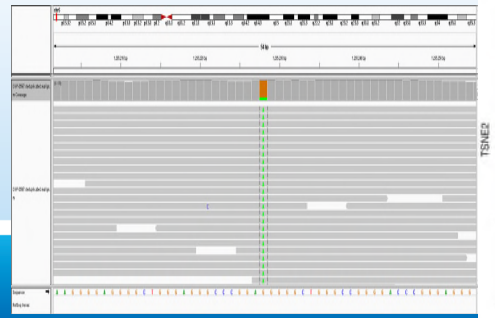


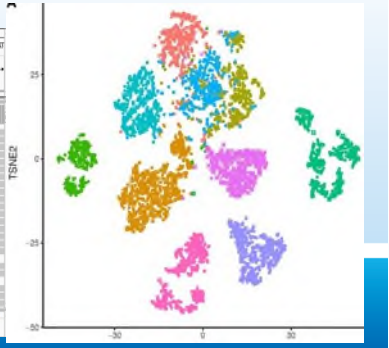
2007 2016 2016-2020 2021







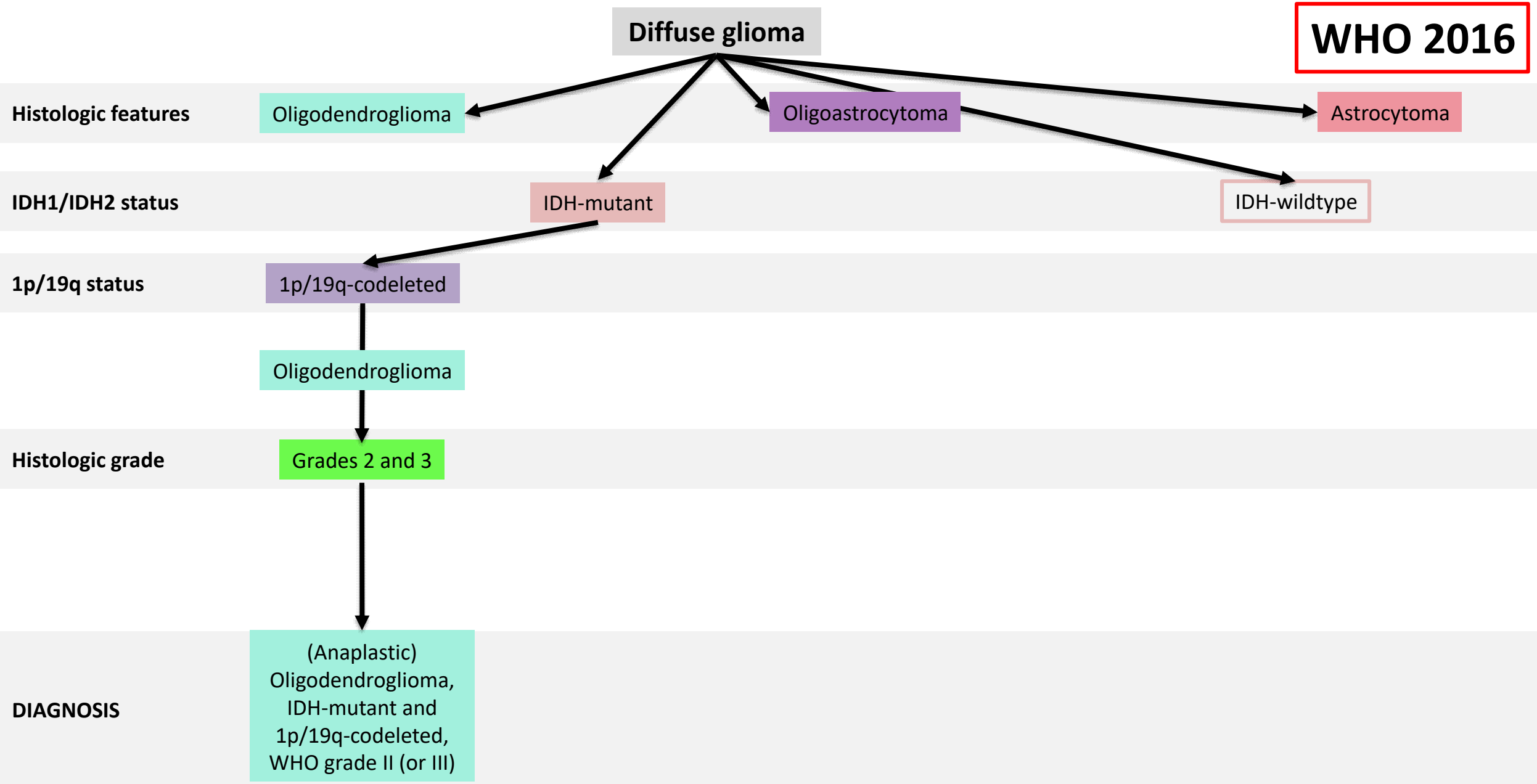




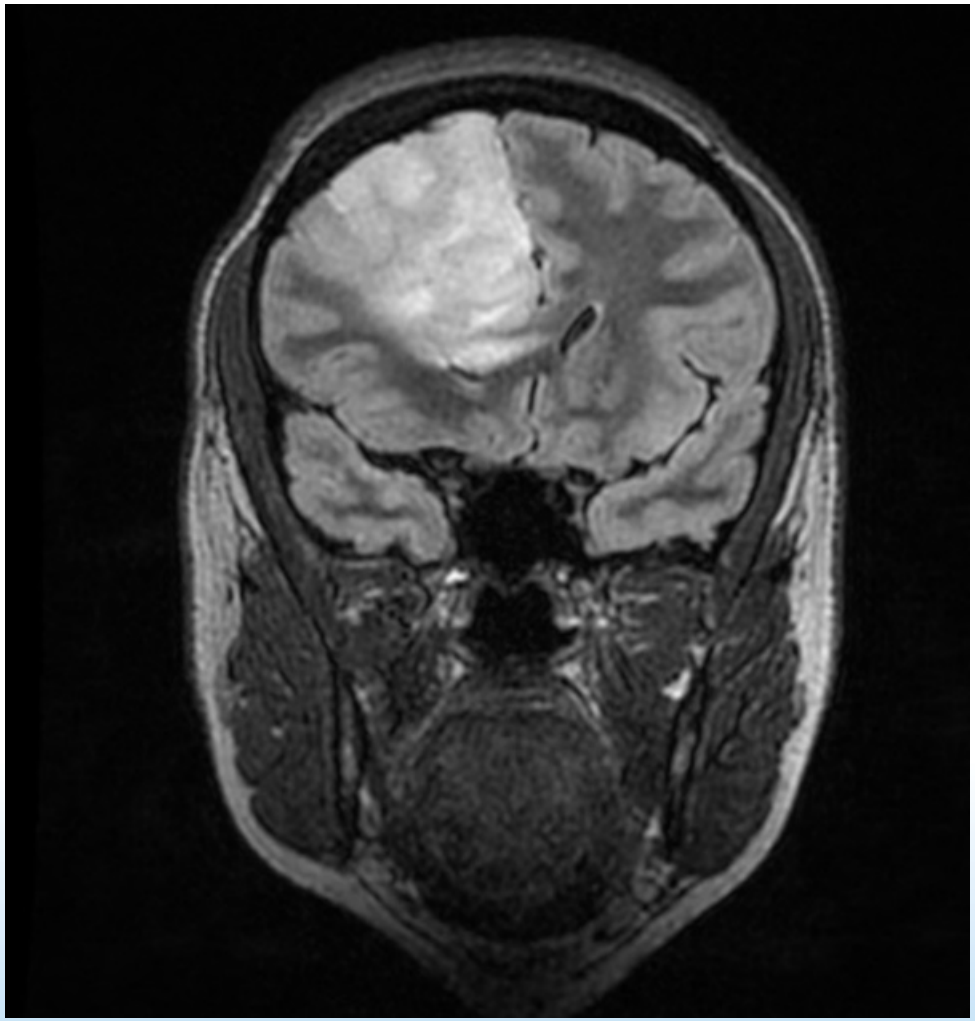
Work-up of diffuse gliomas

- Age, Location and Imaging
- Confirm that it is a diffuse glioma (IHC if needed)
- IDH1 R132H, ATRX, p53, Ki-67
- H3 K27M, H3K27me3, H3G34R/V
- Molecular tests

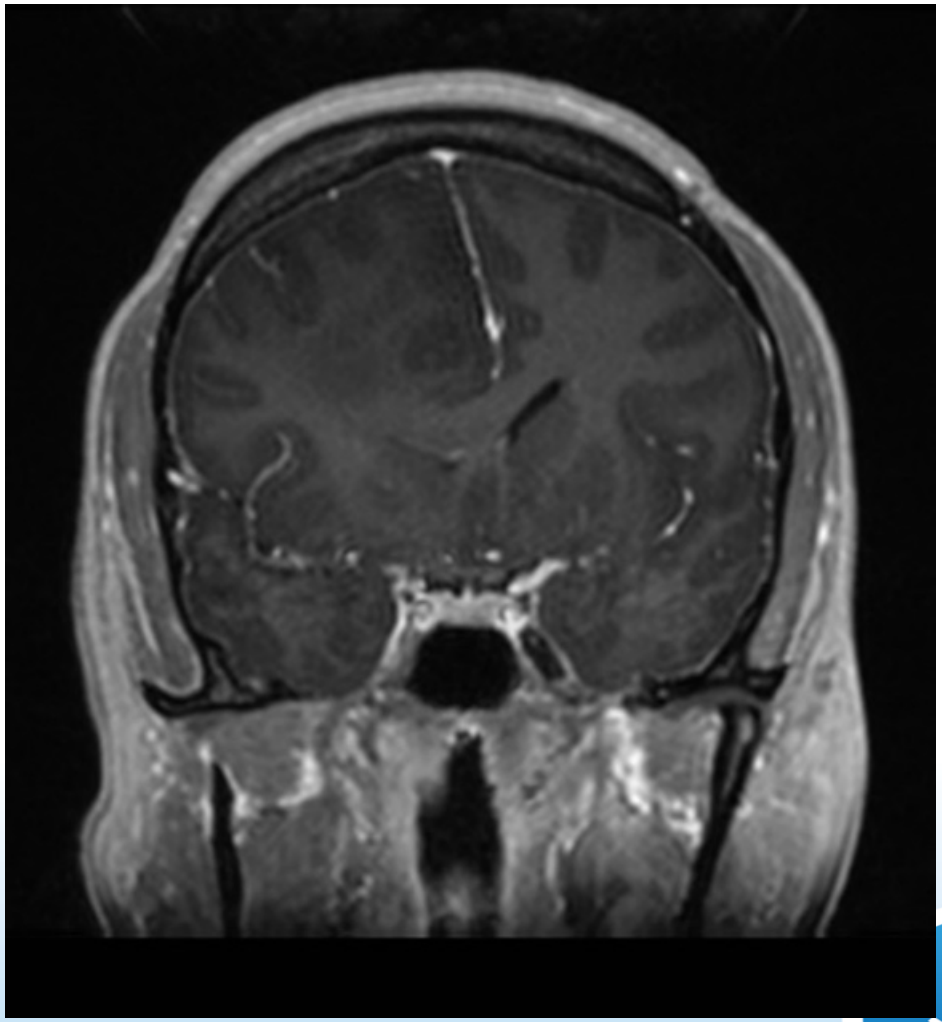
WHO 2016


Diffuse glioma
Histologic features
Oligodendroglioma
Oligoastrocytoma
Astrocytoma
IDH1/IDH2 status
IDH-mutant
IDH-wildtype
1p/19q status
1p/19q-codeleted
Oligodendroglioma
Histologic grade
Grades 2 and 3
DIAGNOSIS
(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)

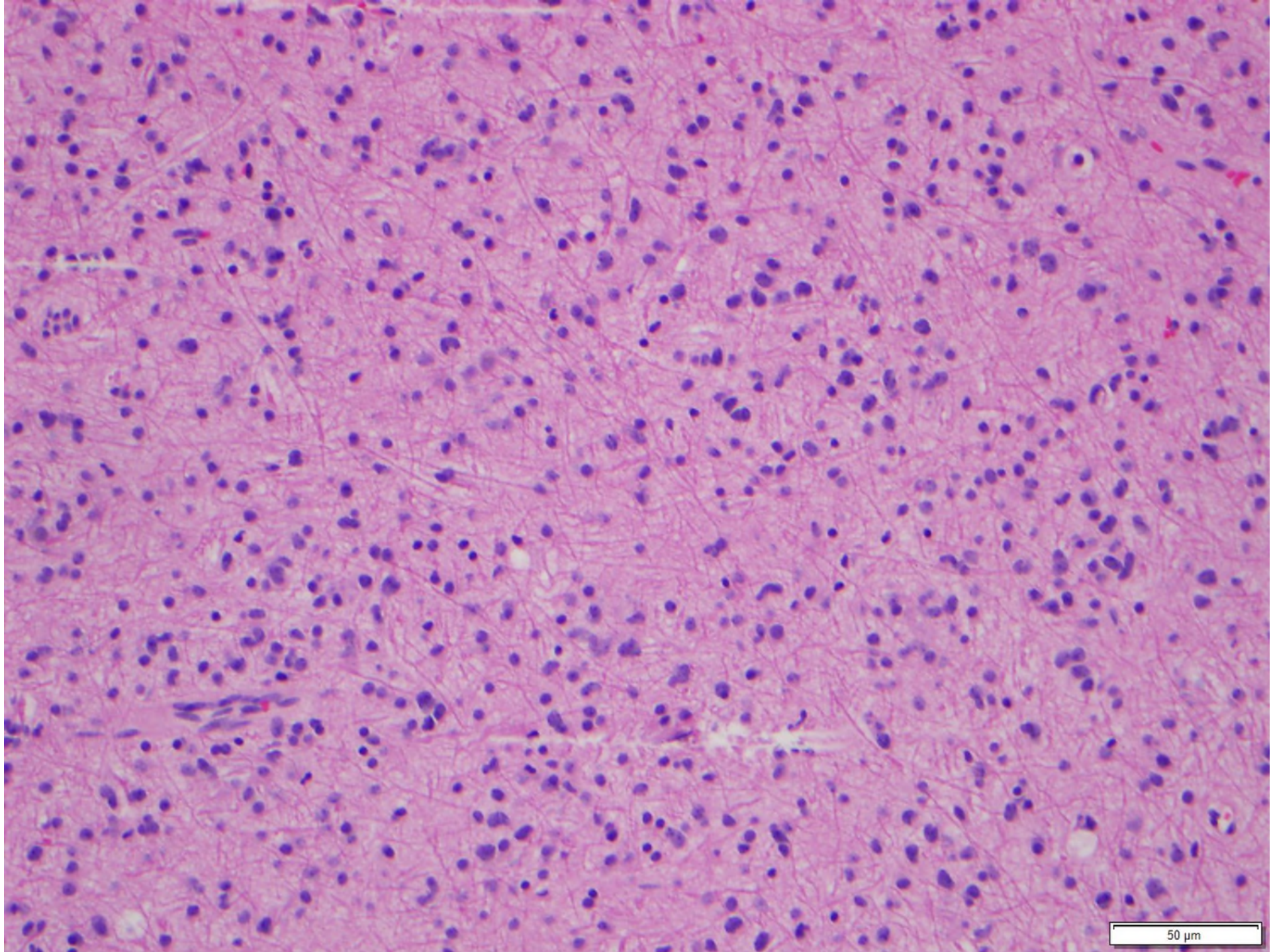
Case 1: 39-year-old woman with headaches

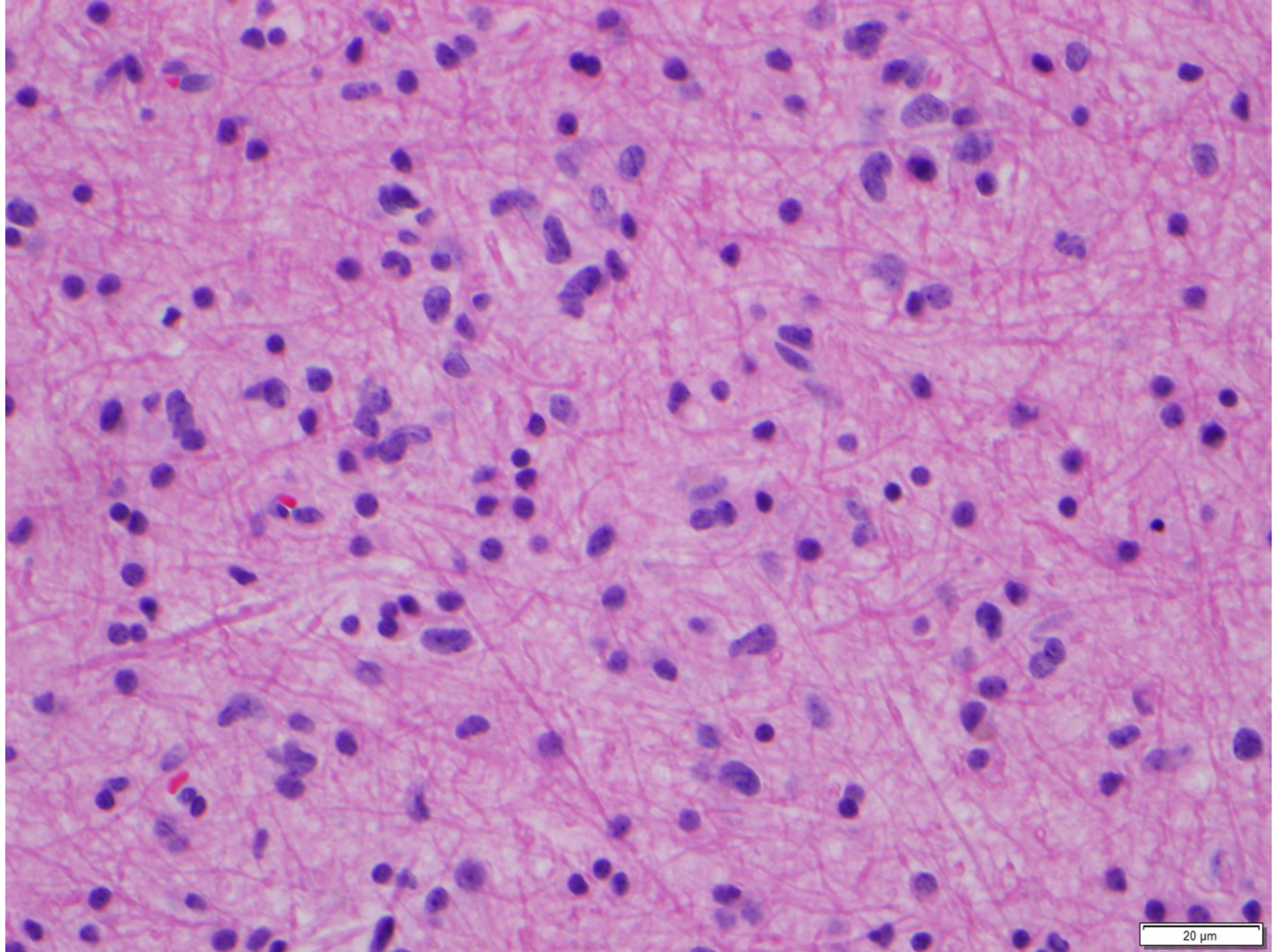


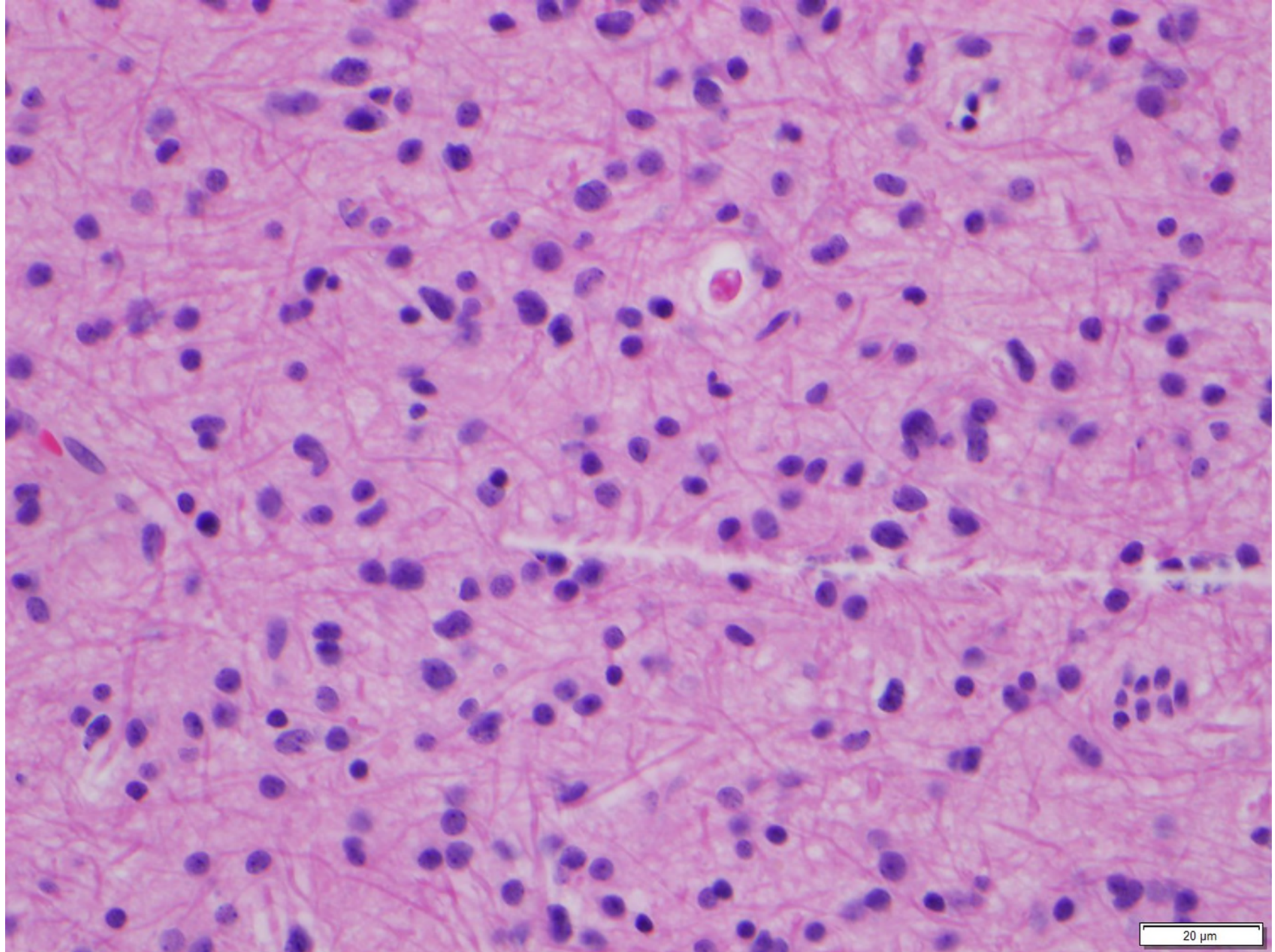
Coronal T2-FLAIR

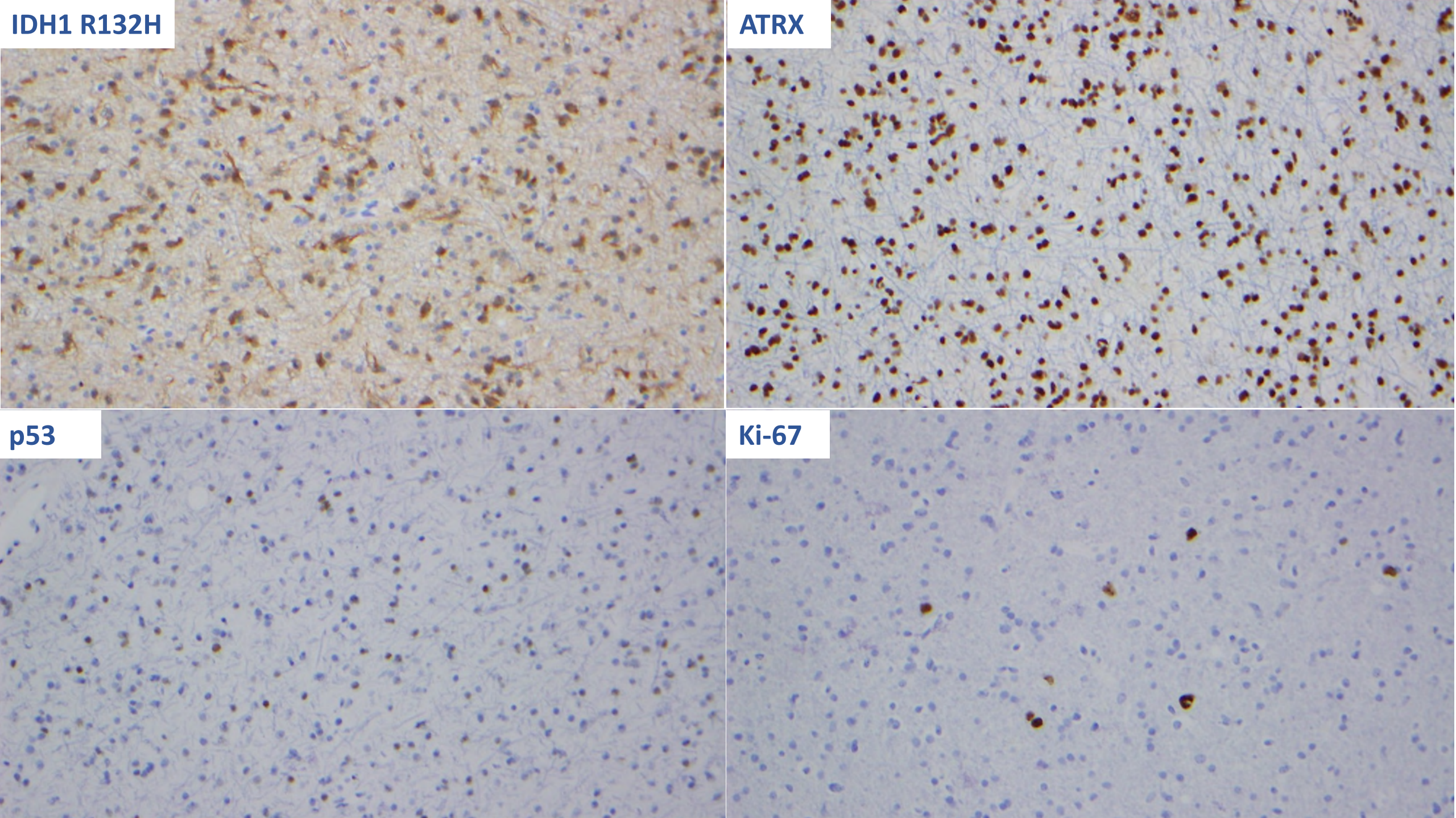


Coronal T1-Contrast


50 µm


20 µm

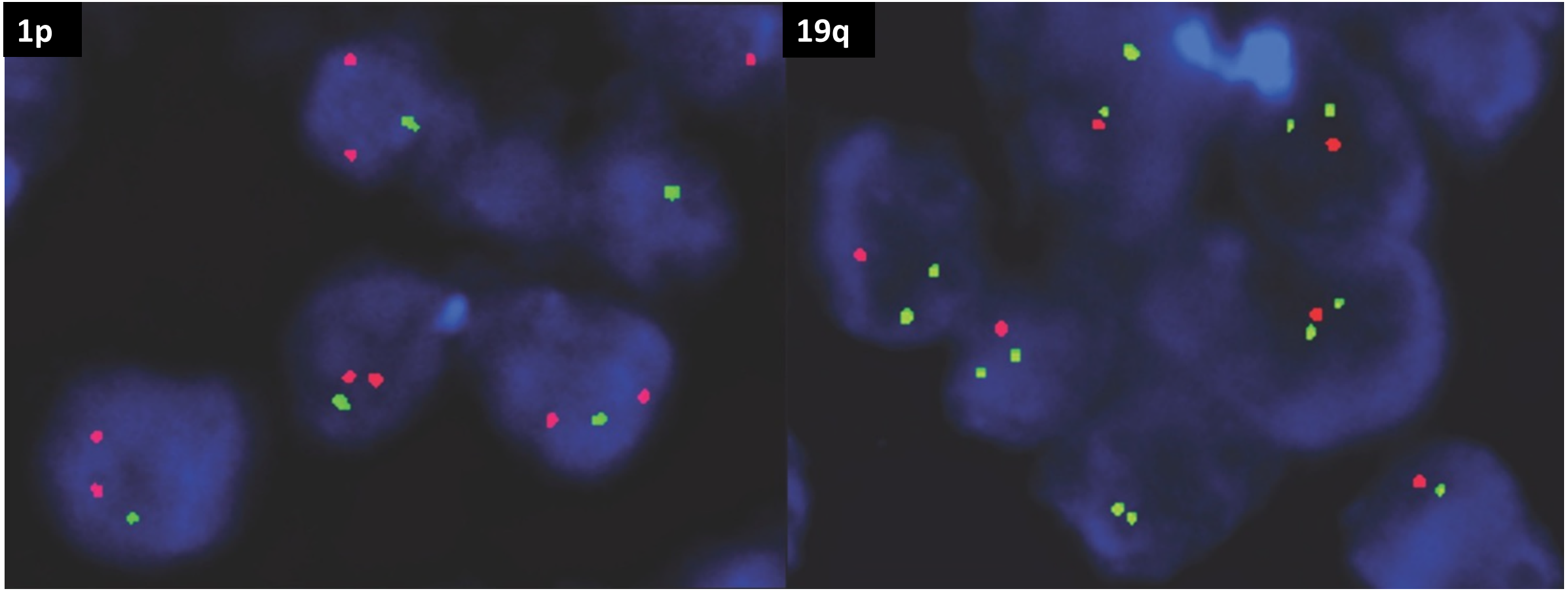

20 µm


IDH1 R132H
ATRX
p53
Ki-67

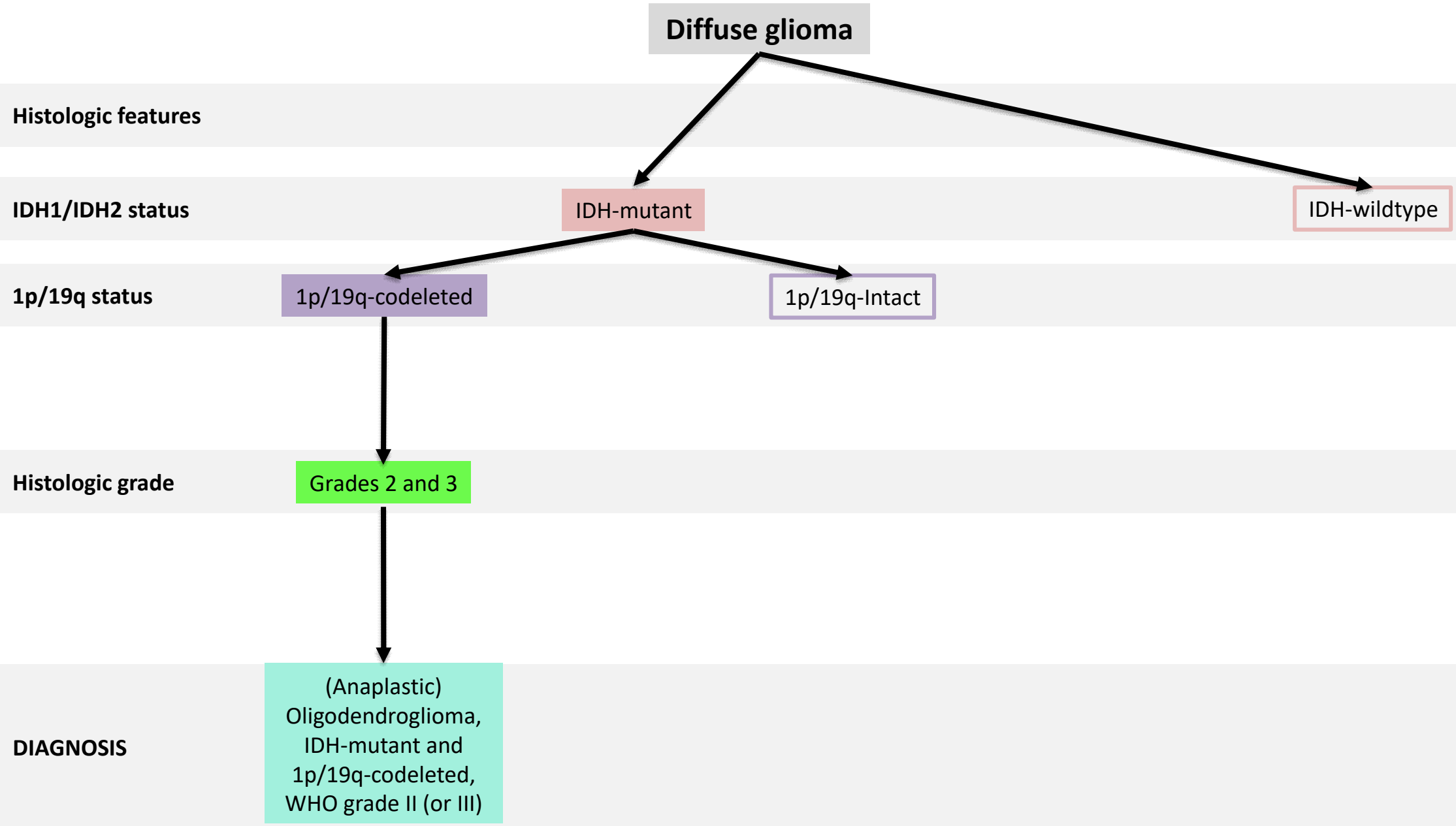
Adult, hemispheric, enhancement (-/+)

- Low-grade histology
- IDH1 R132H (+), ATRX-intact, p53 (-/rarely+)
- 1p/19q codeletion + (FISH, aCGH, NGS, methylation)

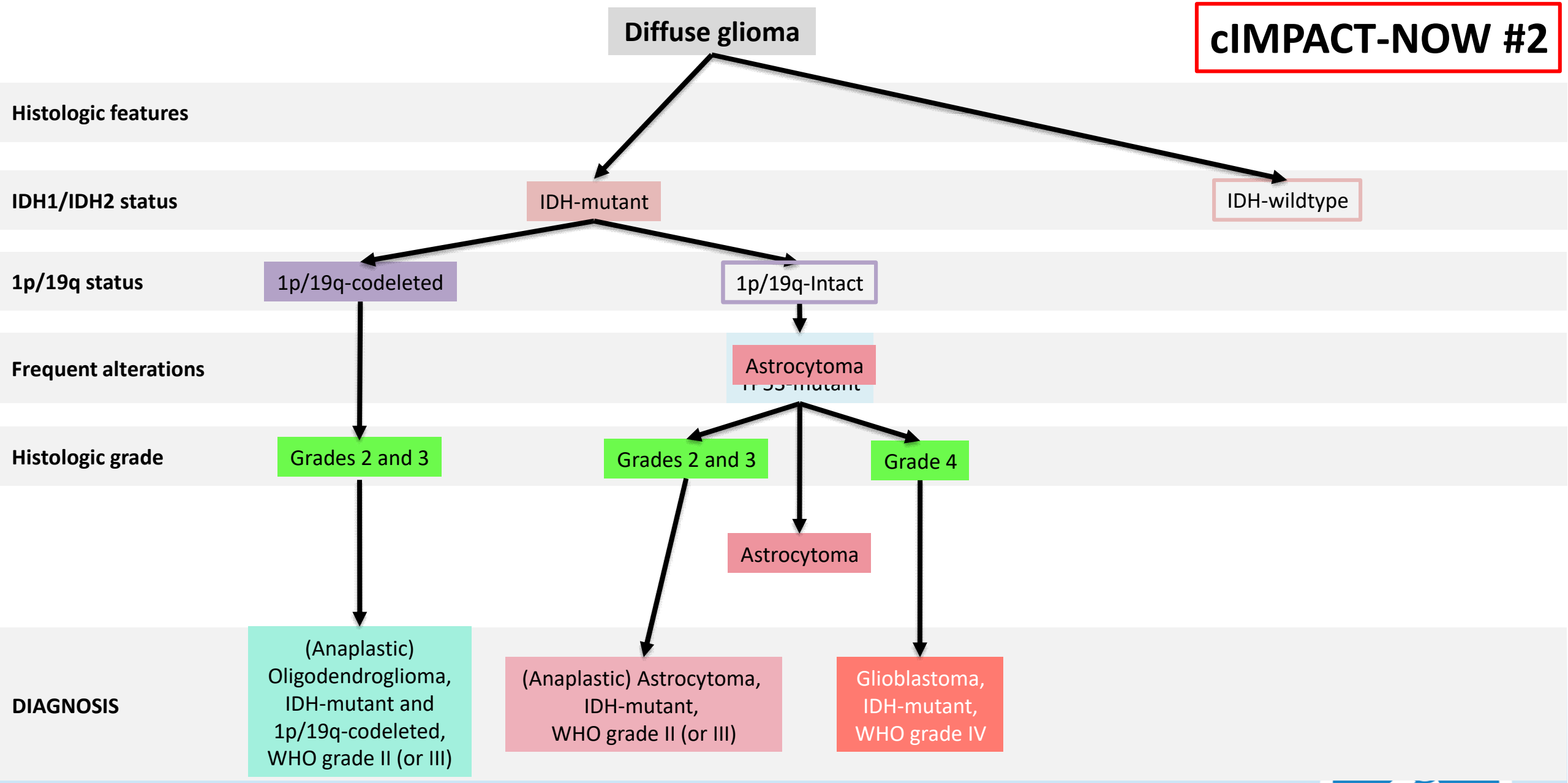
Oligodendroglioma, IDH-mutant and 1p/19q co-deleted



Oligodendroglioma, IDH mutant & 1p/19q codeleted, WHO grade II

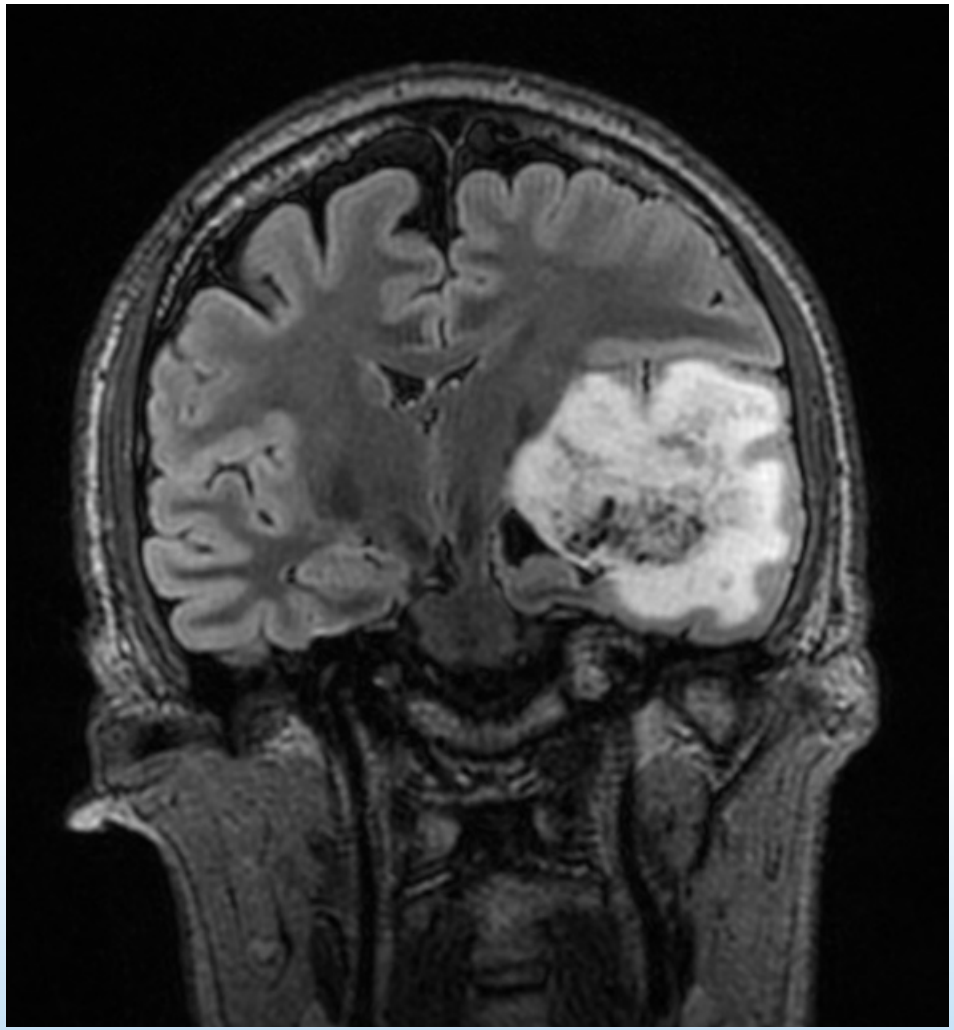

Diffuse glioma
Histologic features
IDH1/IDH2 status
IDH-mutant
IDH-wildtype
1p/19q status
1p/19q-codeleted
1p/19q-Intact
Histologic grade
Grades 2 and 3
DIAGNOSIS
(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)

cIMPACT-NOW #2

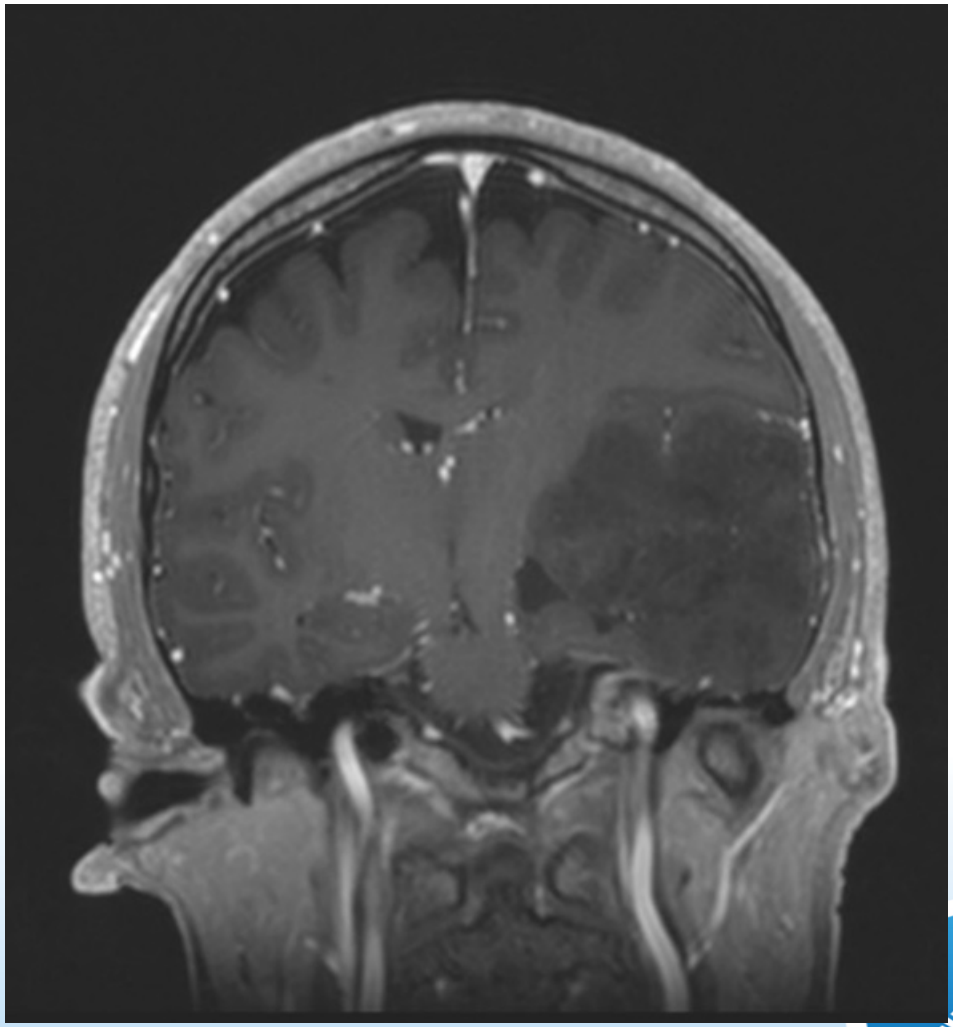


Louis DN, ,et al (2018) Acta Neuropathologica 135(4):639-642. PMID: 29497819

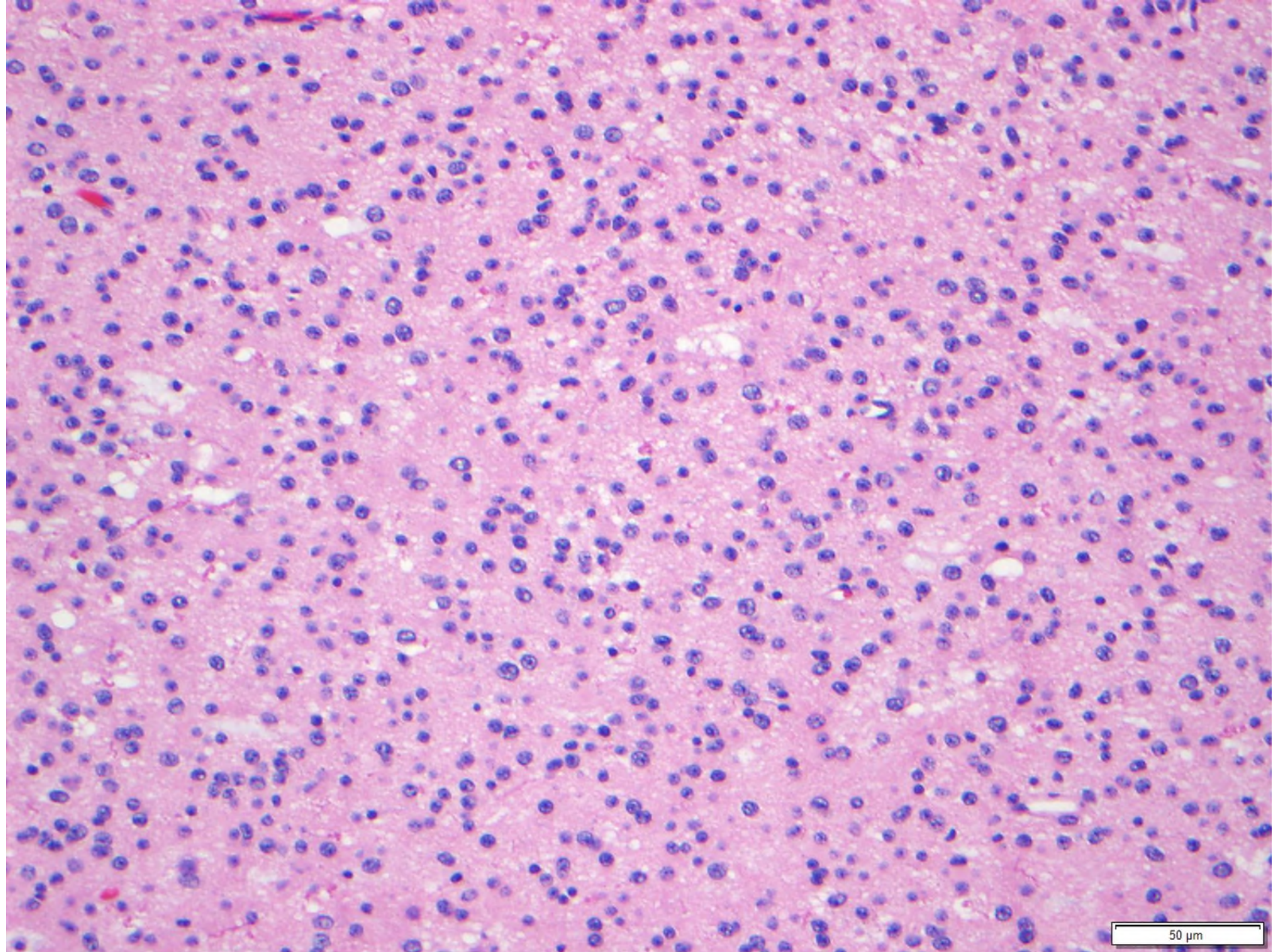
Case 2: 34-year-old man with visual field cuts

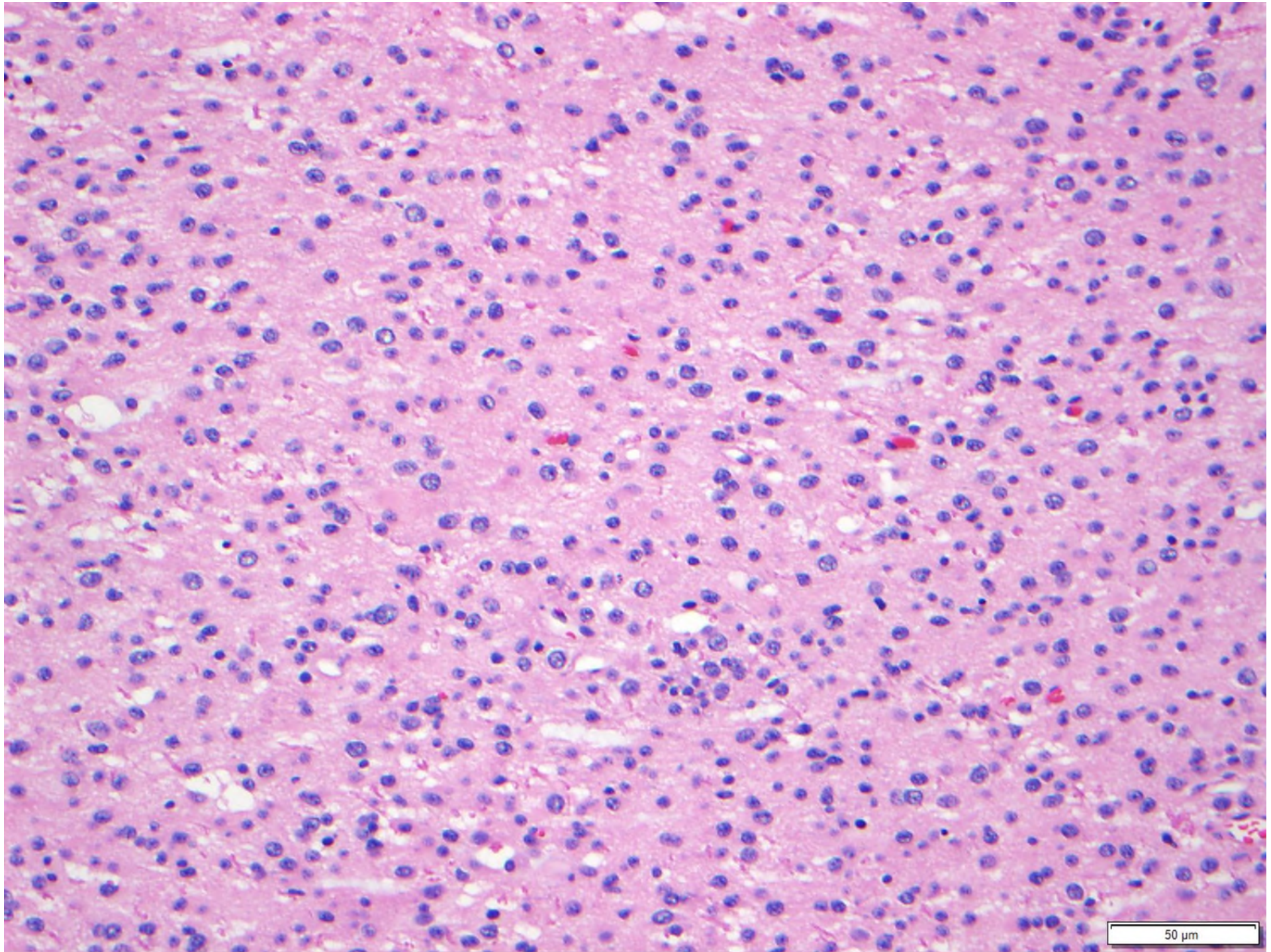


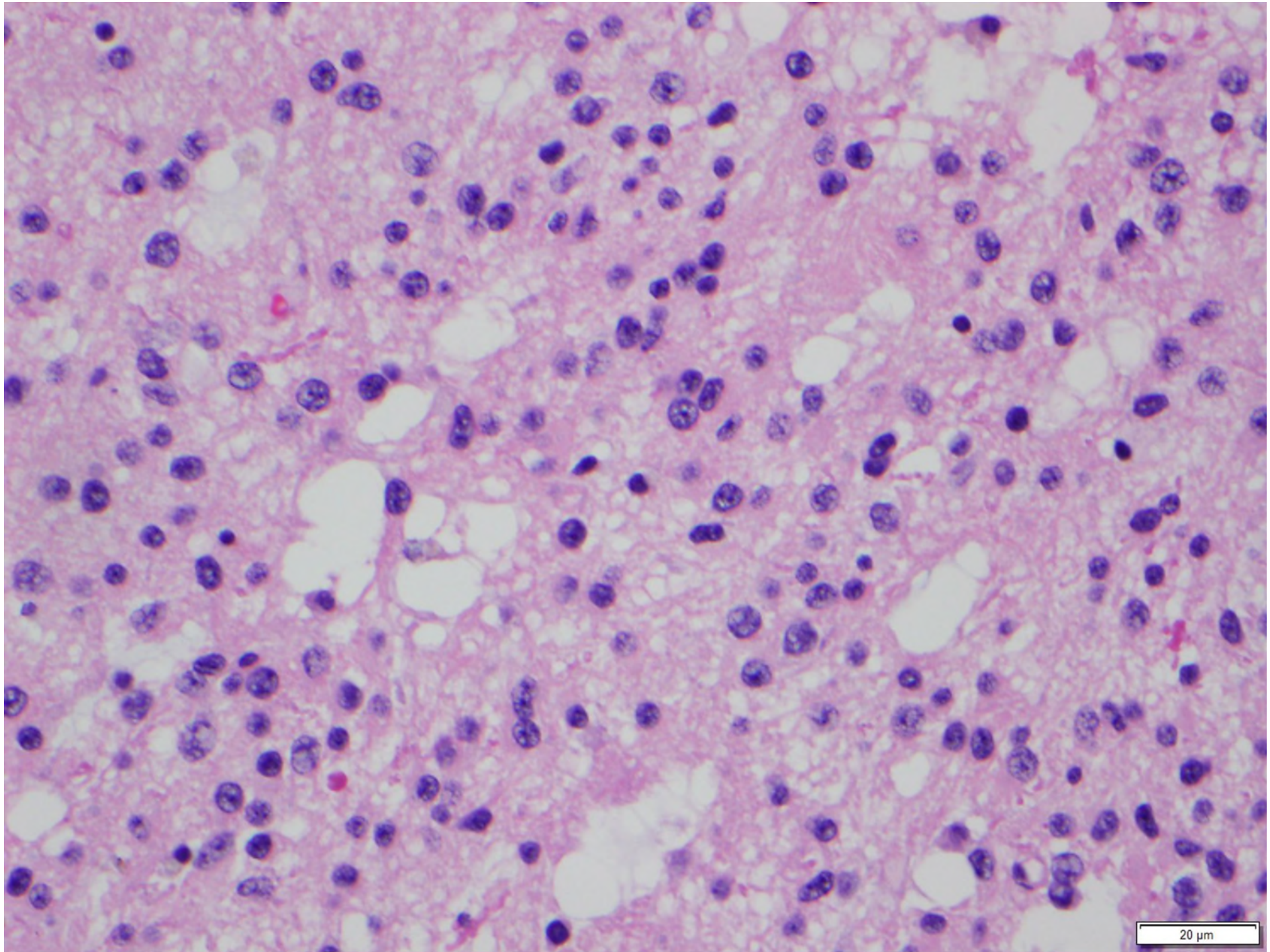
Coronal T2-FLAIR

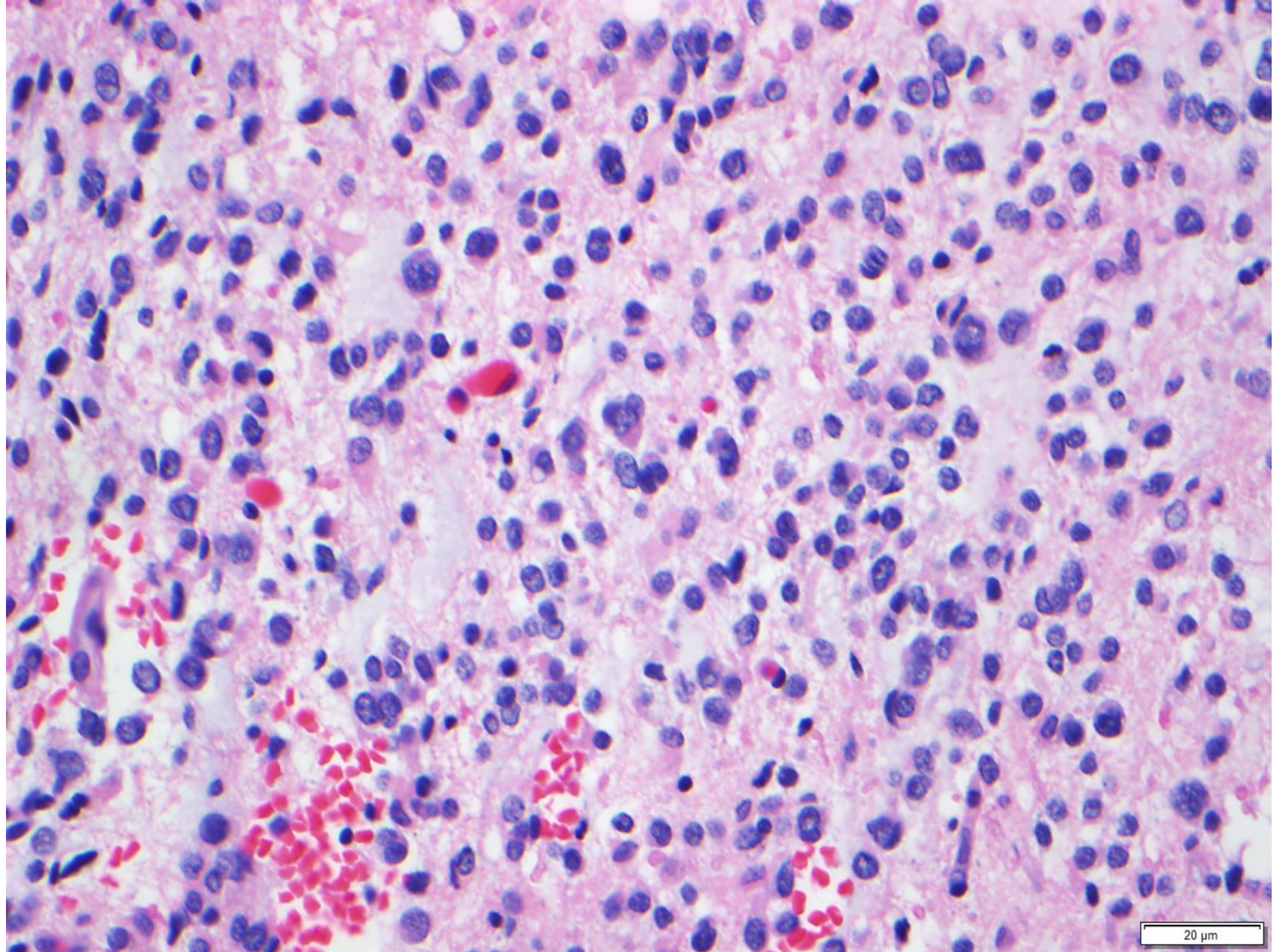


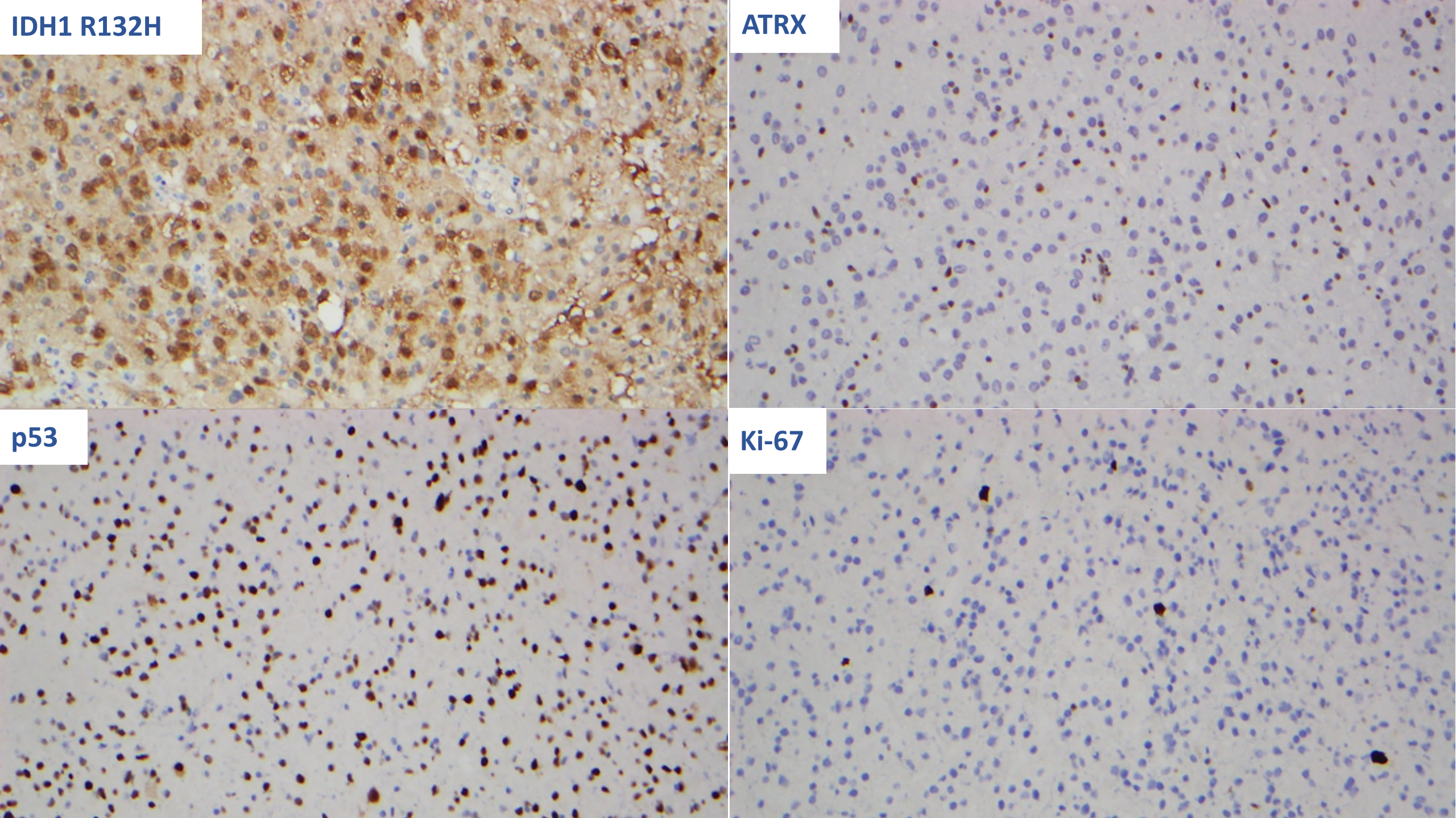
Coronal T1-contrast


50 µm


50 µm


20 µm

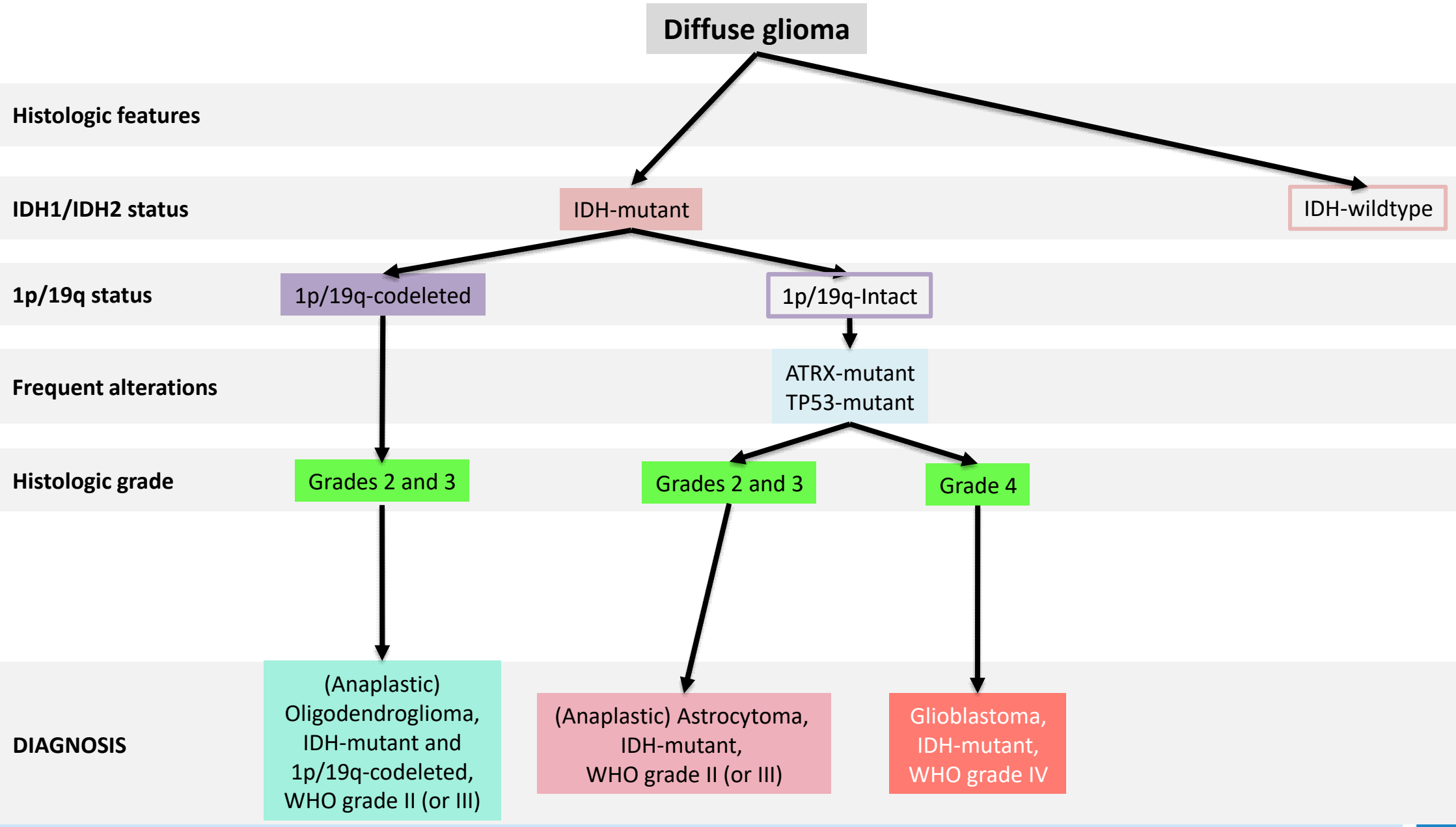

20 µm


IDH1 R132H
ATRX
p53
Ki-67

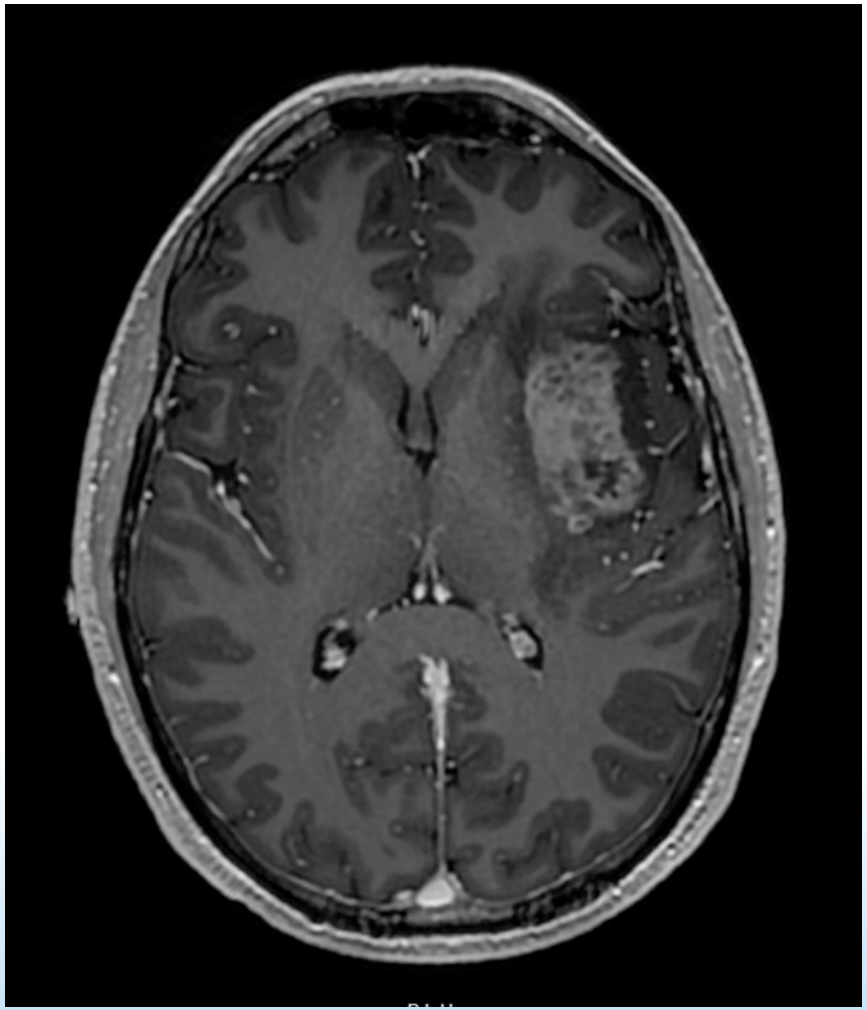
Adult, hemispheric, enhancement (-/+)

- Low-grade histology
- IDH1 R132H (+), ATRX-loss, p53 (+)

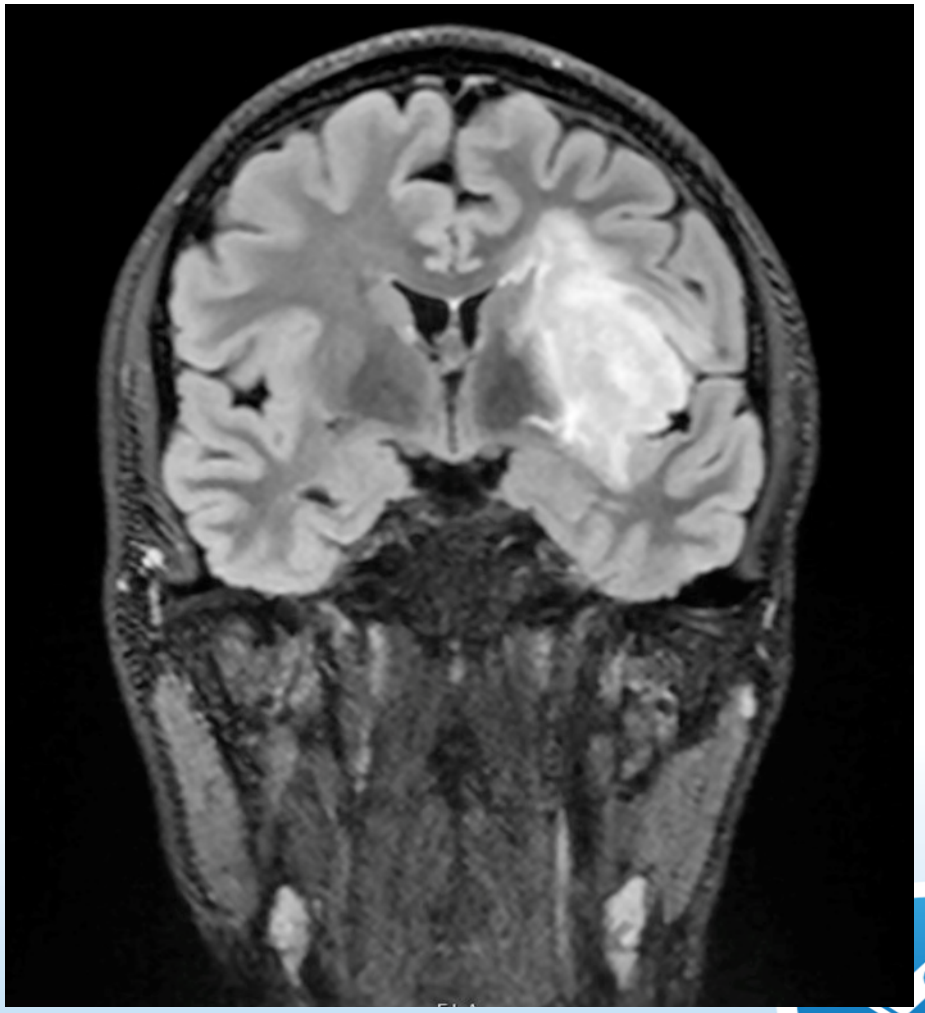
Diffuse astrocytoma, IDH-mutant, WHO grade II


Diffuse glioma
Histologic features
IDH1/IDH2 status
IDH-mutant
IDH-wildtype
1p/19q status
1p/19q-codeleted
1p/19q-Intact
Frequent alterations
ATRX-mutant
TP53-mutant
Histologic grade
Grades 2 and 3
Grades 2 and 3
Grade 4
DIAGNOSIS
(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
(Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
Glioblastoma, IDH-mutant, WHO grade IV

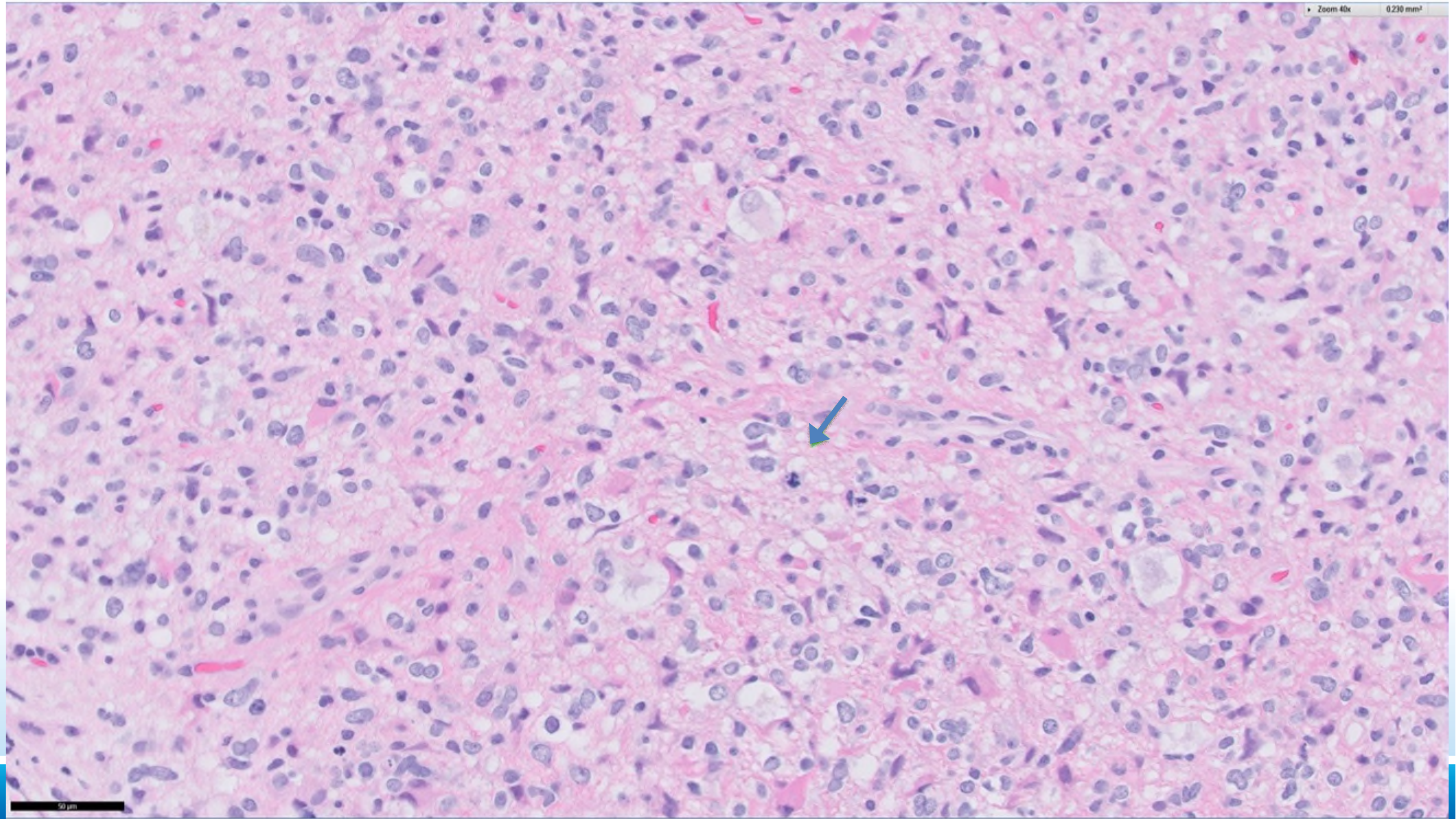
Case 3: 37-year-old man with recurrence

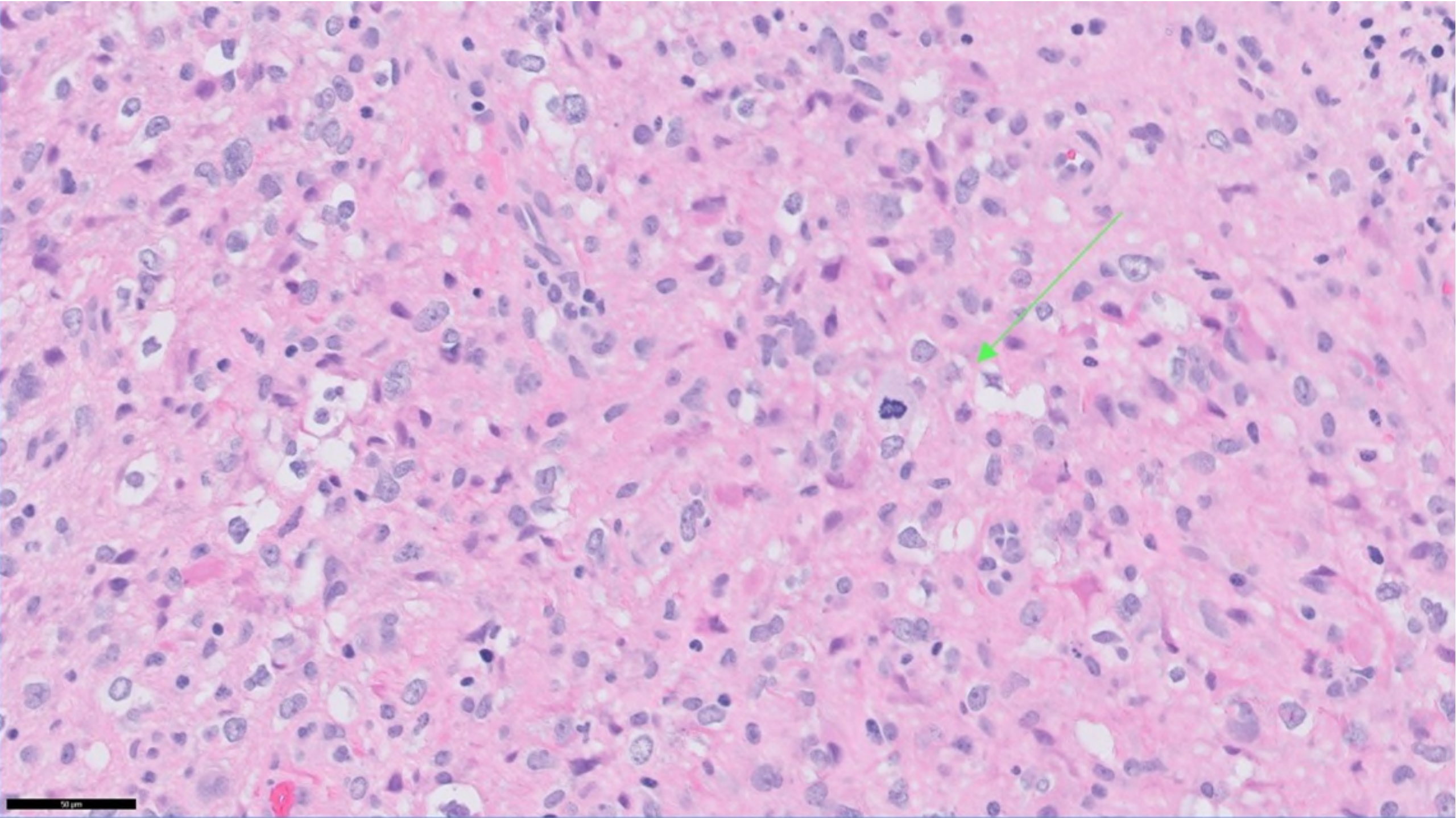


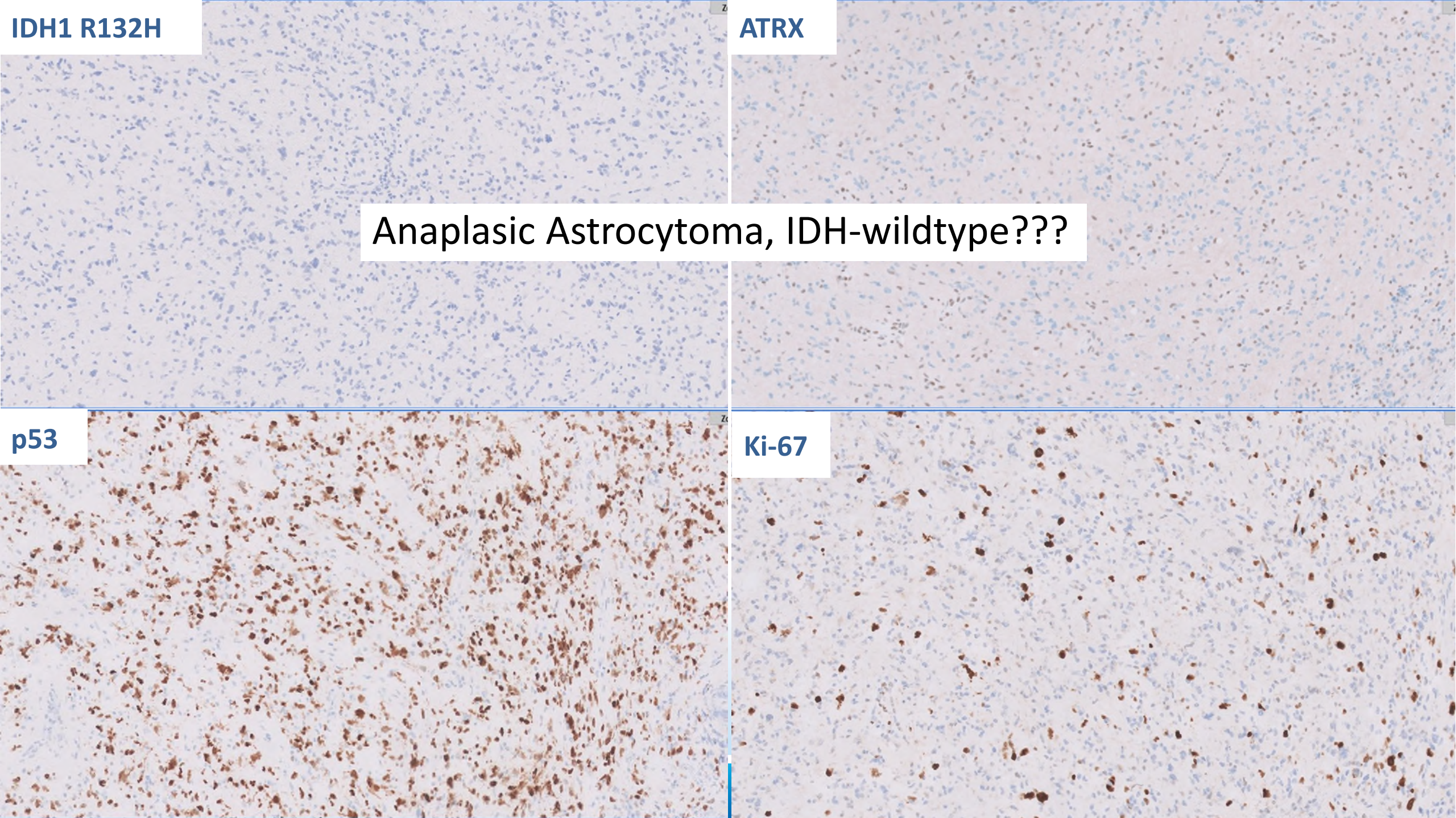
Axial T1-contrast



Coronal T2 FLAIR


Zoom 40x
0.230 mm²
50 µm

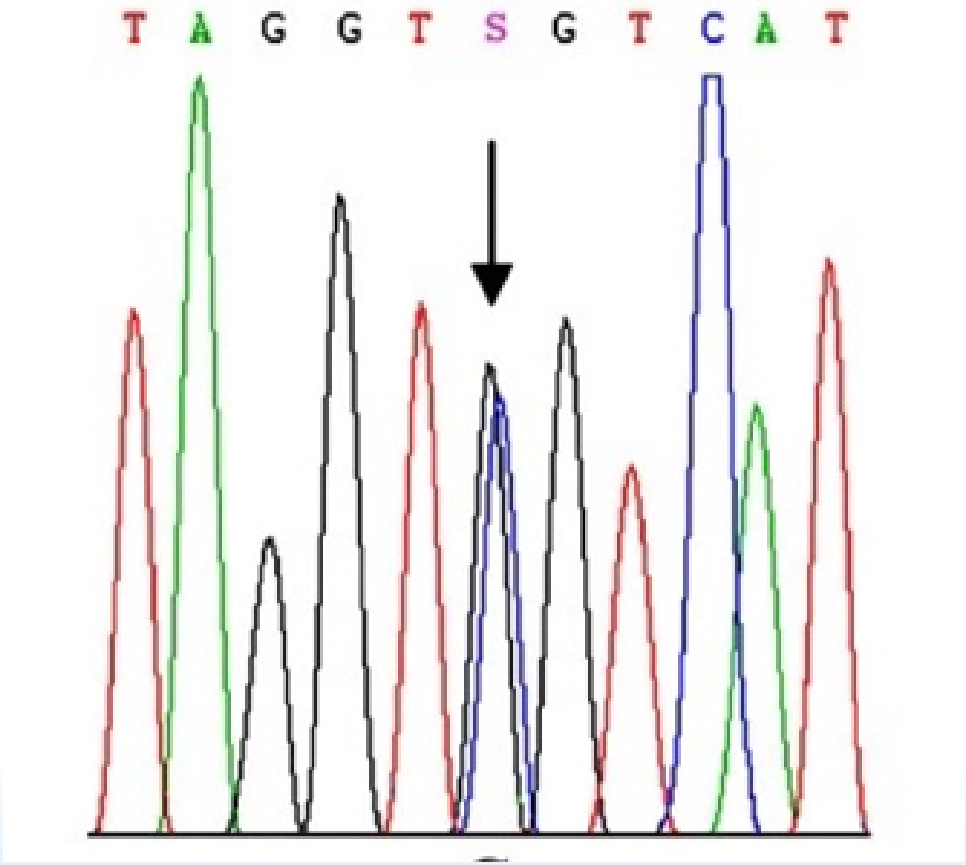

50 µm


IDH1 R132H
ATRX
Anaplasic Astrocytoma, IDH-wildtype???
p53
Ki-67

Adult, hemispheric, enhancement (+/-)

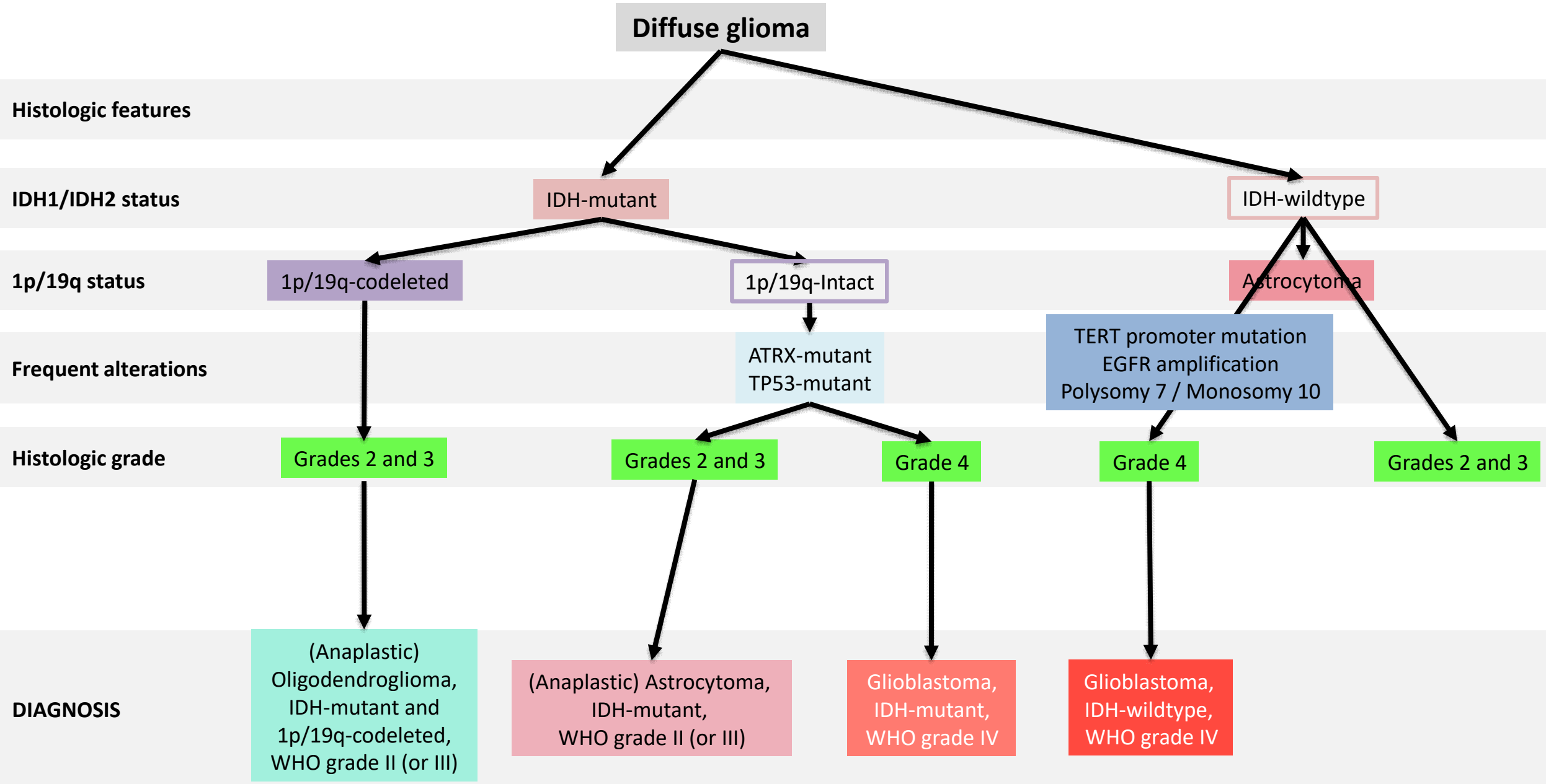
- Lower-grade histology (or high-grade)
- IDH1 R132H (-), ATRX-loss, p53 (+)
- IDH1/2 sequencing (or UCSF500 NGS)

IDH1 Sanger sequencing

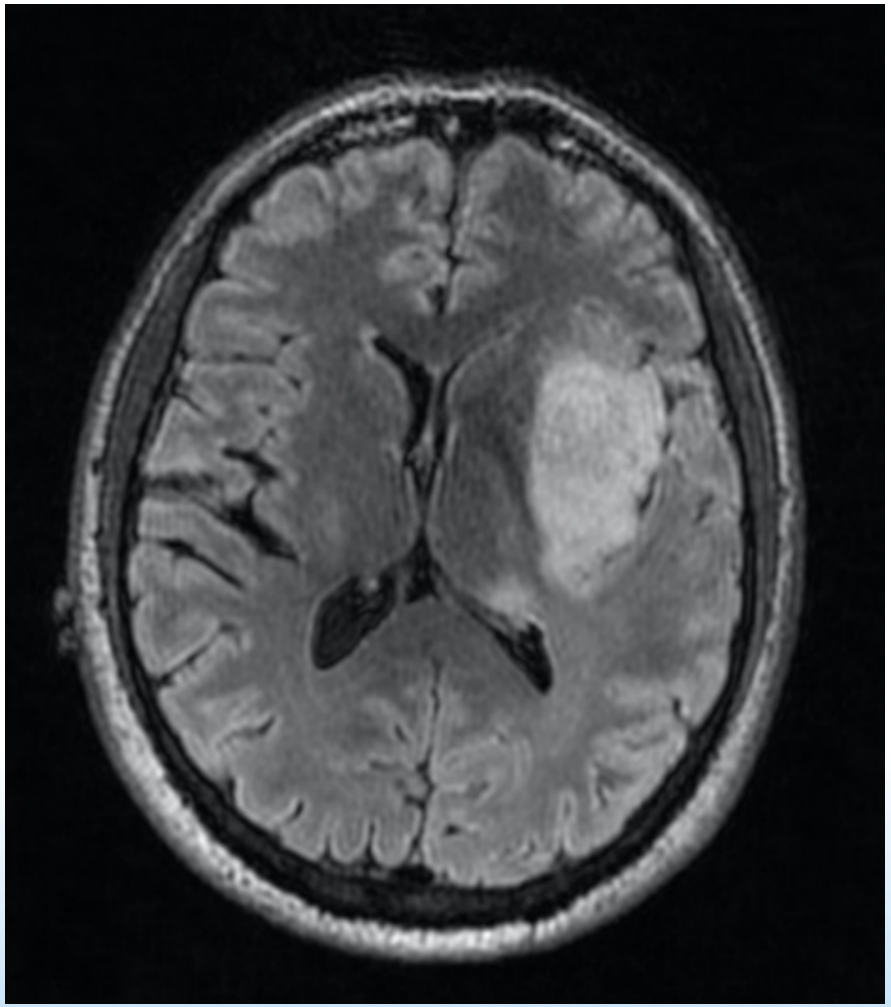


IDH1 R132G
c.C394G

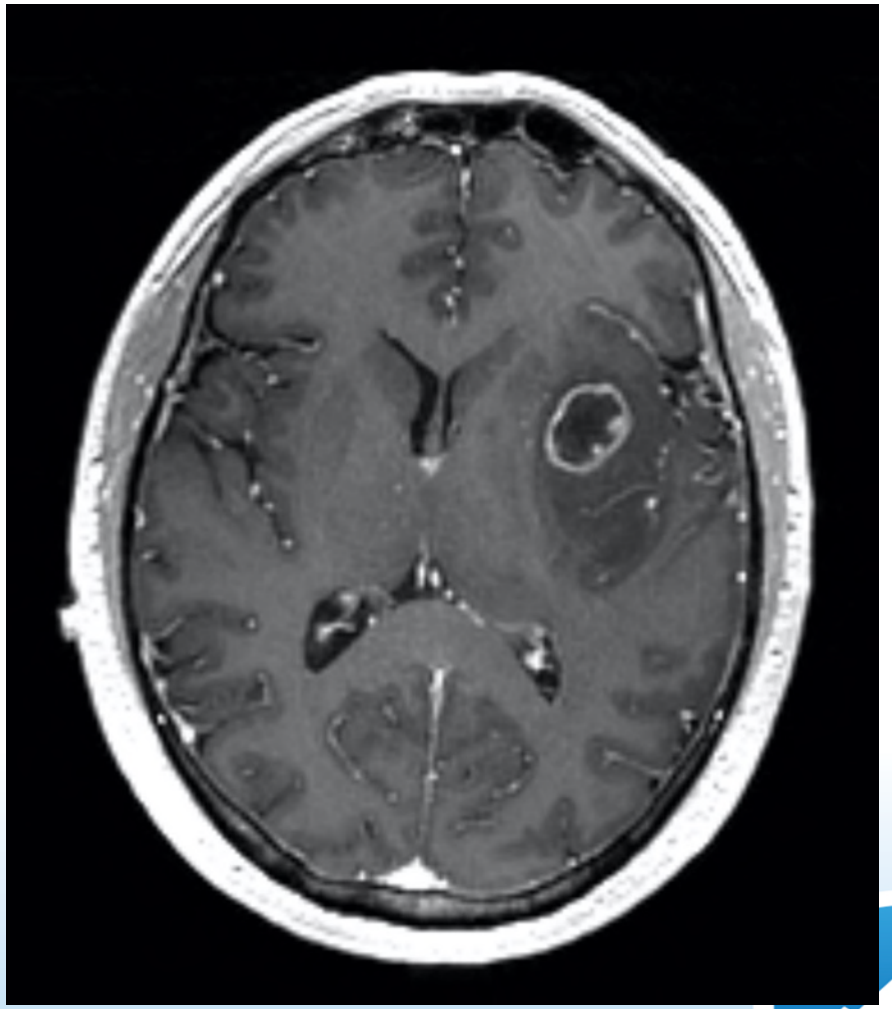
Anaplastic astrocytoma, IDH mutant, WHO Grade III


Diffuse glioma
Histologic features
IDH1/IDH2 status
IDH-mutant
IDH-wildtype
1p/19q status
1p/19q-codeleted
1p/19q-Intact
Astrocytoma
Frequent alterations
ATRX-mutant
TP53-mutant
TERT promoter mutation
EGFR amplification
Polysomy 7 / Monosomy 10
Histologic grade
Grades 2 and 3
Grades 2 and 3
Grade 4
Grade 4
Grades 2 and 3
DIAGNOSIS
(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
(Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
Glioblastoma, IDH-mutant, WHO grade IV
Glioblastoma, IDH-wildtype, WHO grade IV

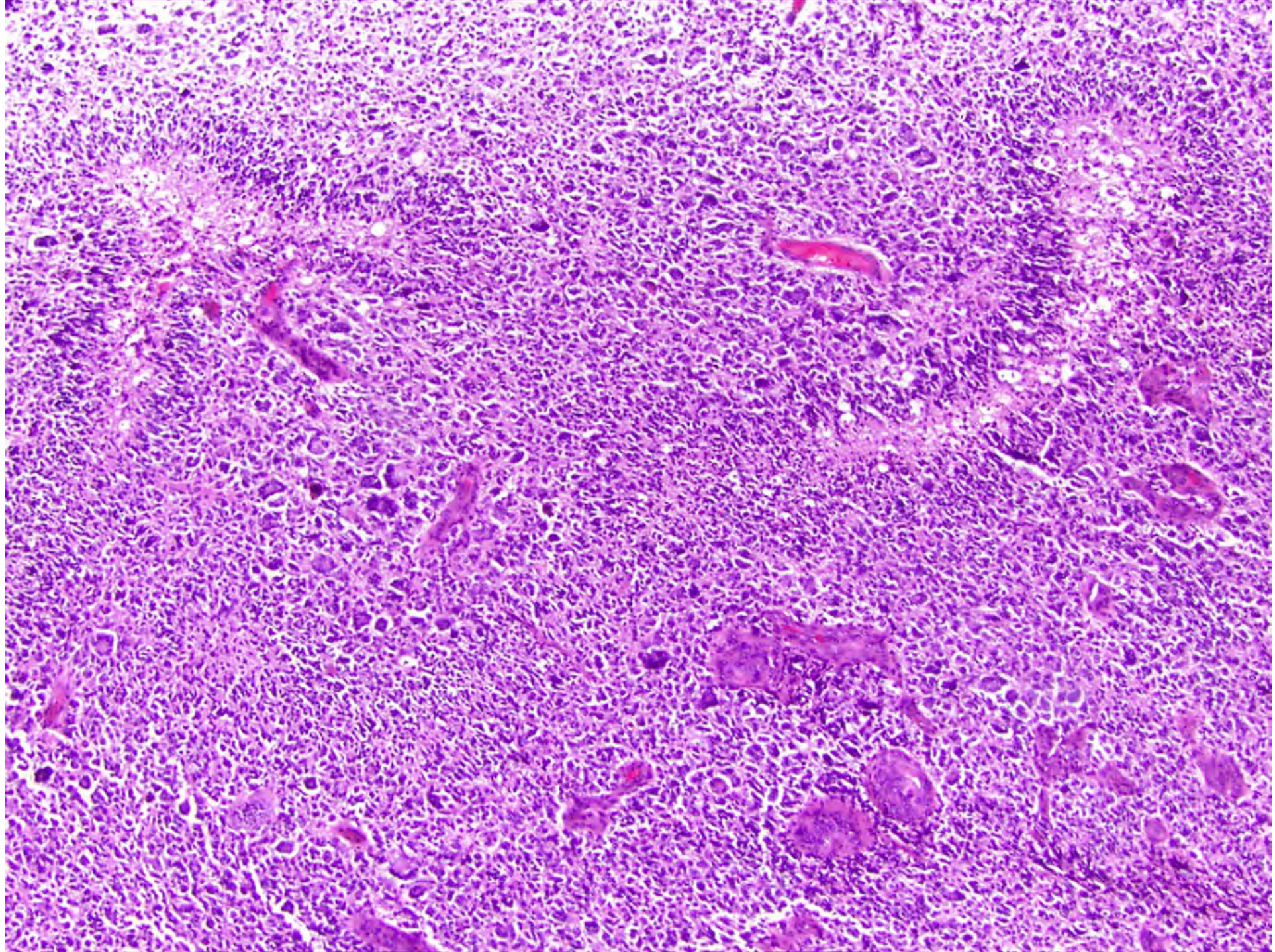
Case 4: 62-year-old man with seizure



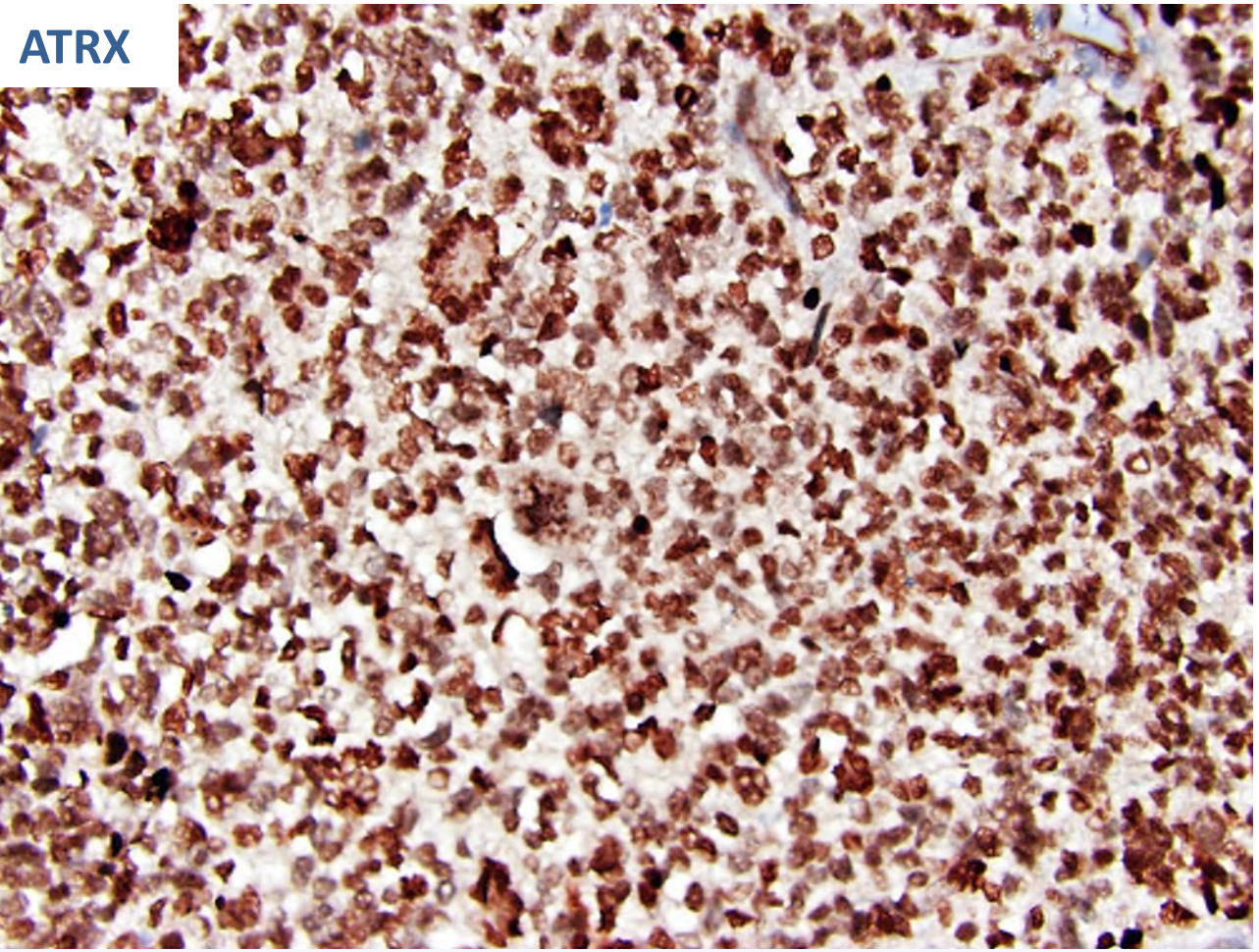
Axial T2 FLAIR



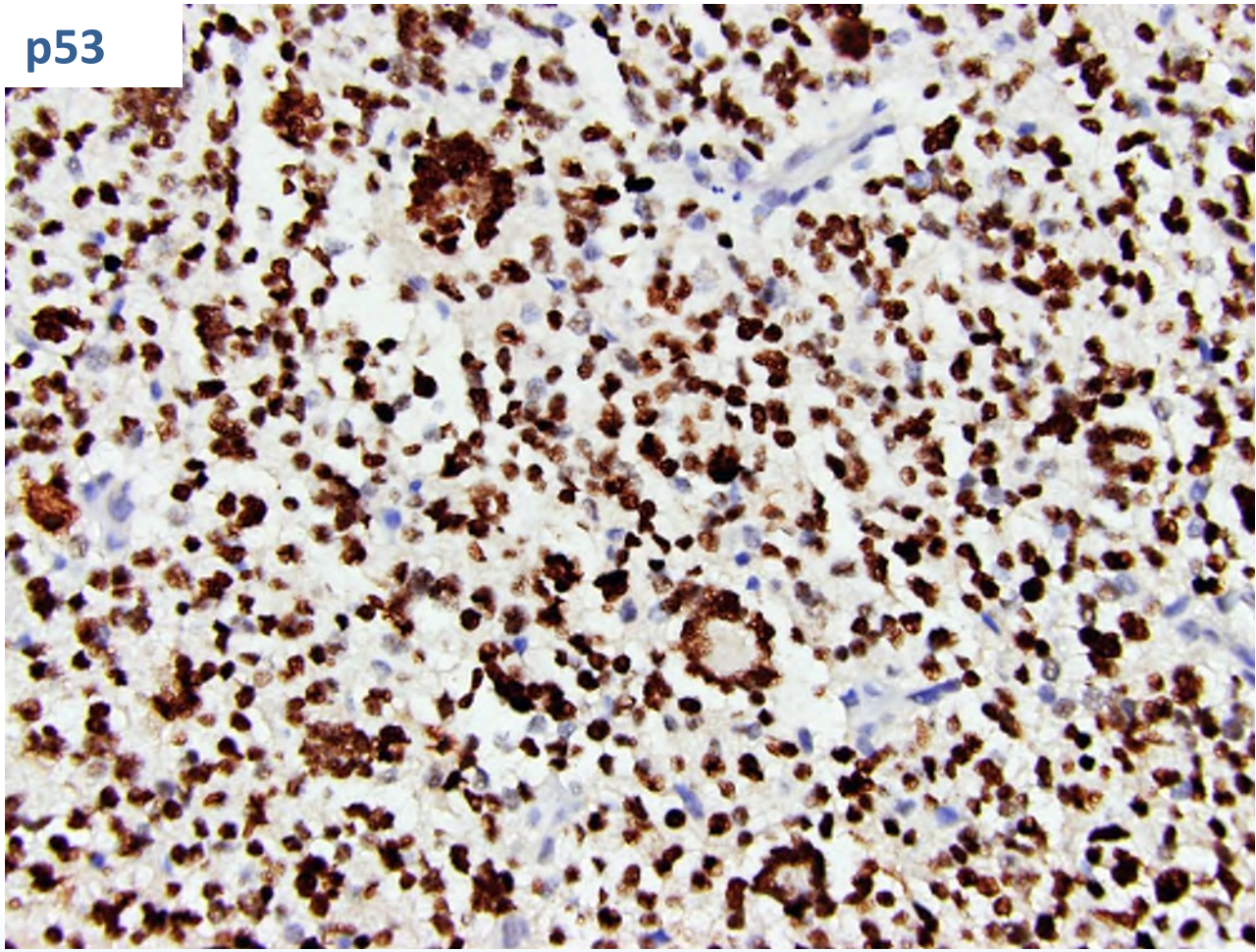
Axial T1 contrast



ATRX



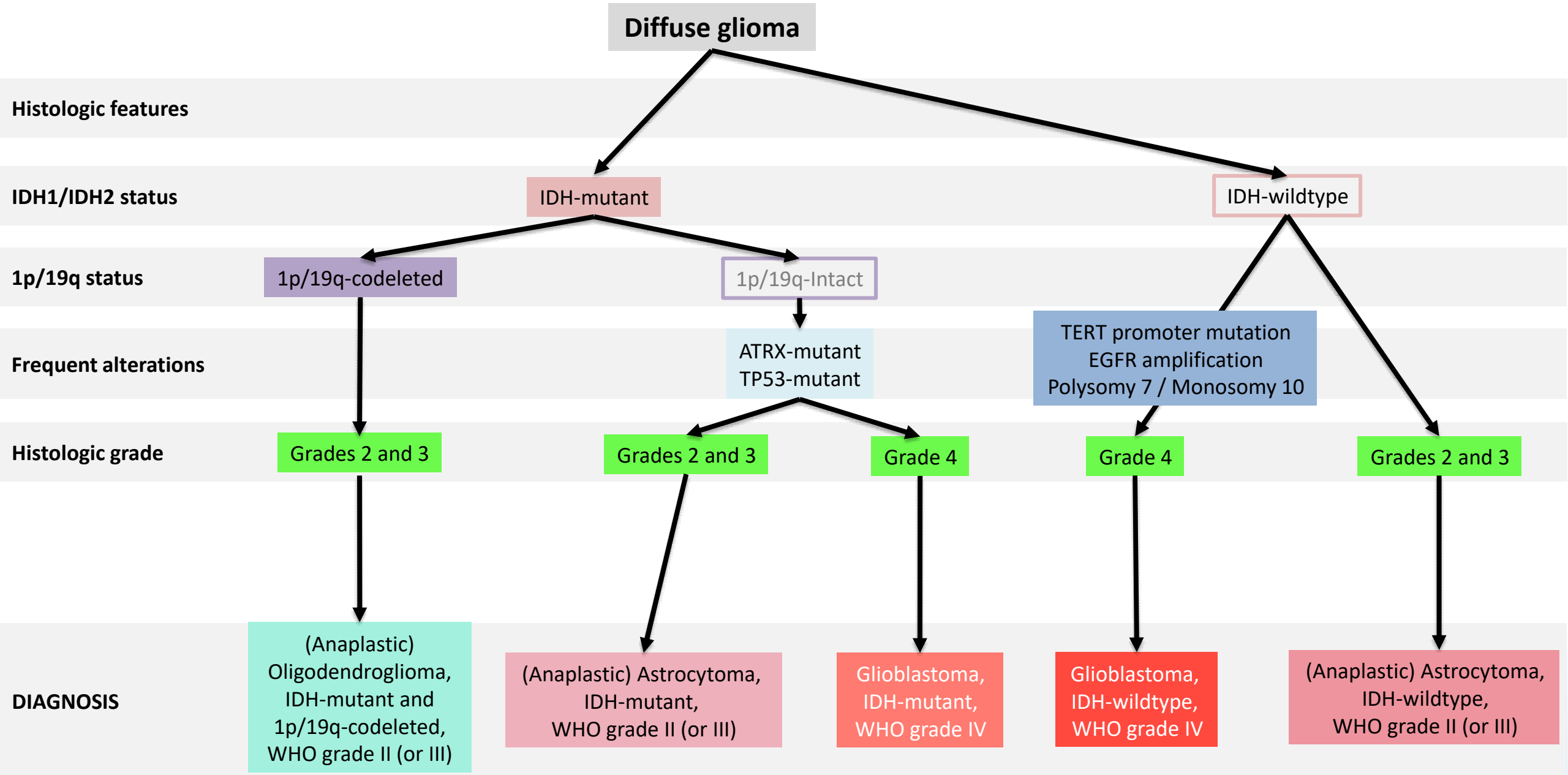
p53



Older adult, hemispheric, enhancing

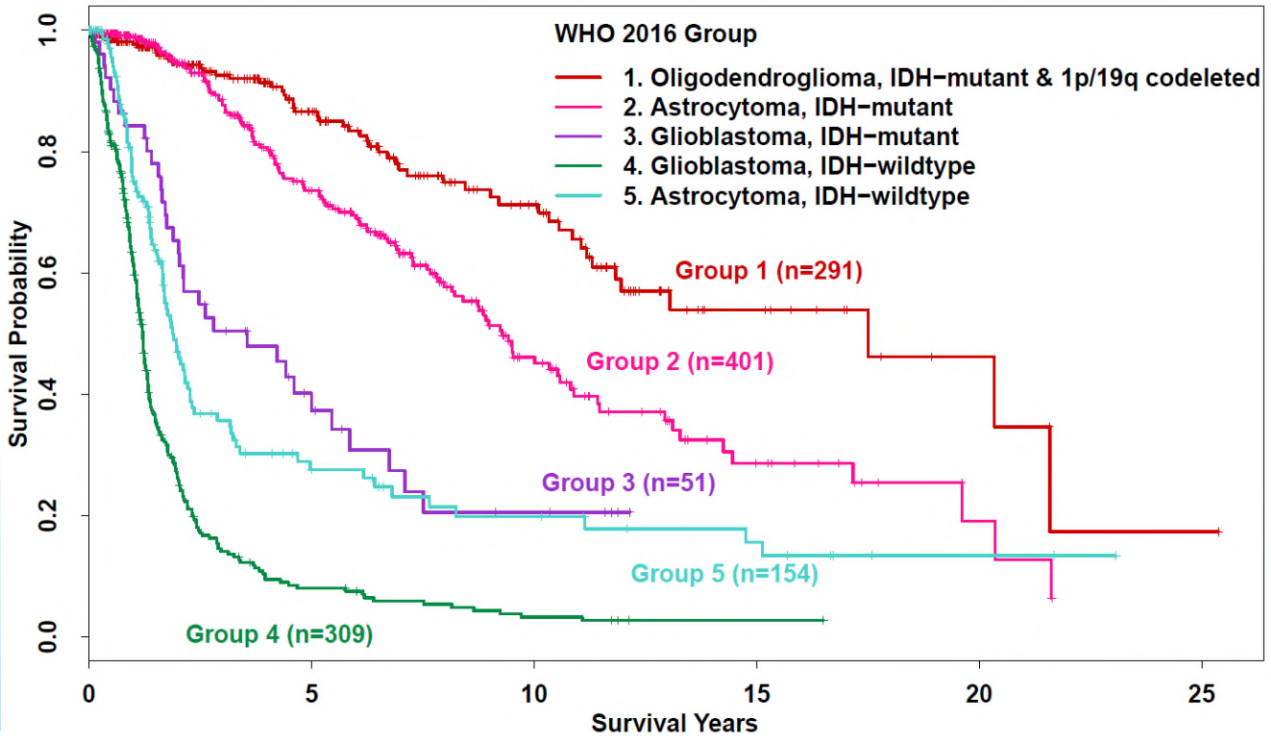
- High-grade histology
- IDH1 R132H (-), ATRX-intact, p53 (+/-)

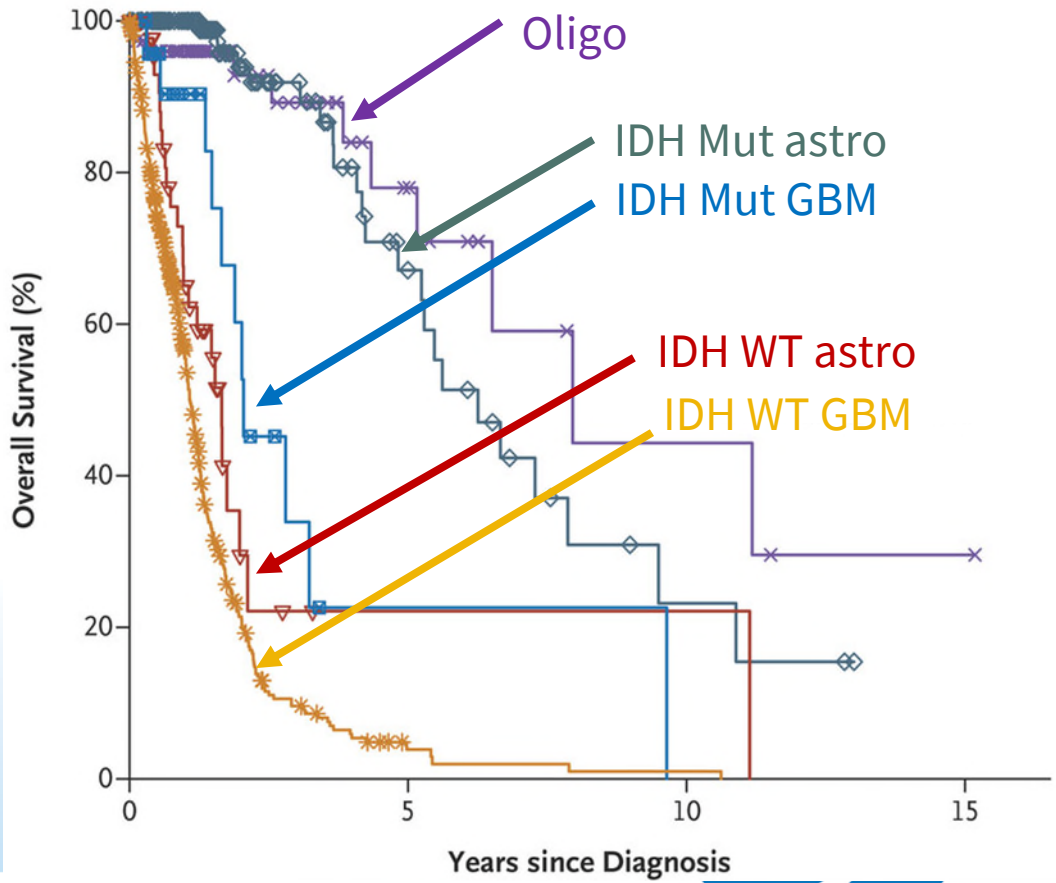
  Glioblastoma, likely IDH-wildtype; WHO grade IV
- Expect TERTp, EGFR ampl, Polysomy 7/ monosomy 10
- Others: PTEN loss, NF1, SETD2, etc…
- MGMT promoter methylation testing
- Should we do IDH1/2 sequencing?
  - Likelihood less than 1%


Diffuse glioma
Histologic features
IDH1/IDH2 status
IDH-mutant
IDH-wildtype
1p/19q status
1p/19q-codeleted
1p/19q-Intact
Frequent alterations
ATRX-mutant
TP53-mutant
TERT promoter mutation
EGFR amplification
Polysomy 7 / Monosomy 10
Histologic grade
Grades 2 and 3
Grades 2 and 3
Grade 4
Grade 4
Grades 2 and 3
DIAGNOSIS
(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
(Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
Glioblastoma, IDH-mutant, WHO grade IV
Glioblastoma, IDH-wildtype, WHO grade IV
(Anaplastic) Astrocytoma, IDH-wildtype, WHO grade II (or III)

Diffuse Astrocytoma, IDH-wildtype

- Provisional entity in 2016 WHO
- It just says what is absent, not what is present
- Mix bag of tumors
- Significant subset behaves aggressively



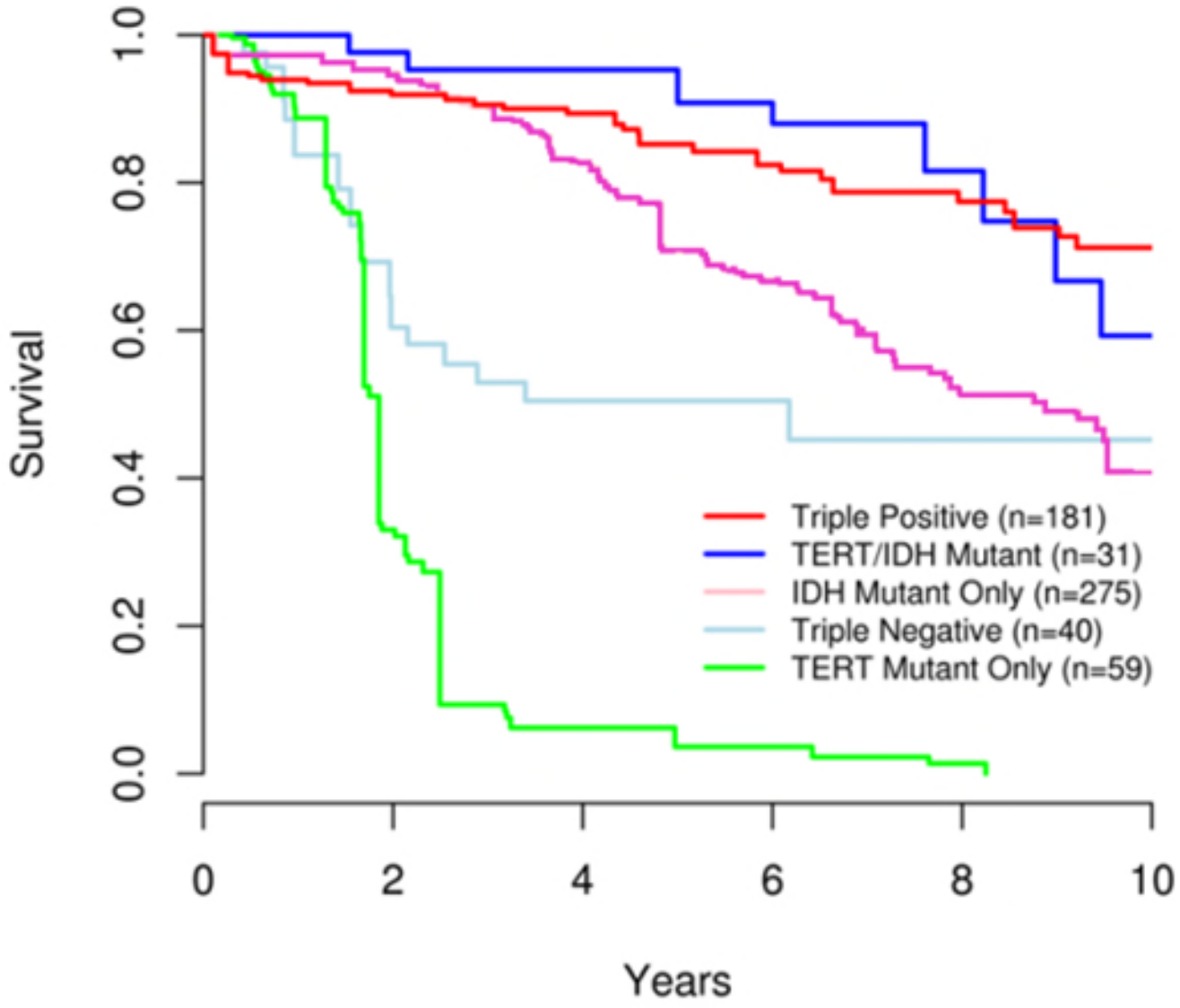


TCGA. Comprehensive, Integrative Genomic Analysis of Diffuse Lower-Grade Gliomas. N Engl J Med. 2015;372(26):2481-98. PMID: 26061751.
Pekmezci M, et al. Adult infiltrating gliomas with WHO 2016 integrated diagnosis: additional prognostic roles of ATRX and TERT. Acta Neuropathol. 2017;133(6):1001-1016. PMID: 28255664.

Diffuse Astrocytoma, IDH-wildtype

- Provisional entity in 2016 WHO
- It just says what is absent, not what is present
- Mix bag of tumors
- Significant subset behaves aggressively
- What are the molecular alterations in these tumors correlating with worse clinical outcomes?

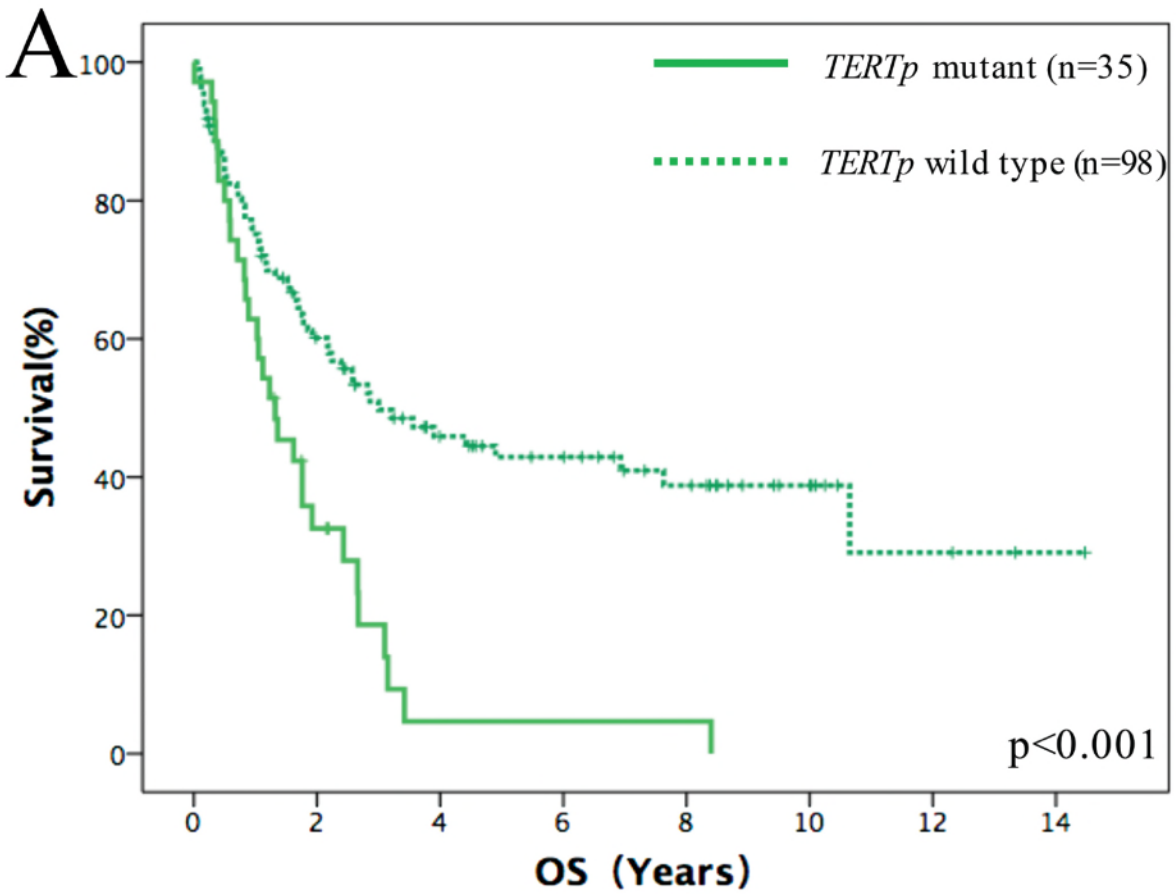
TERT promoter mutation → poor prognosis

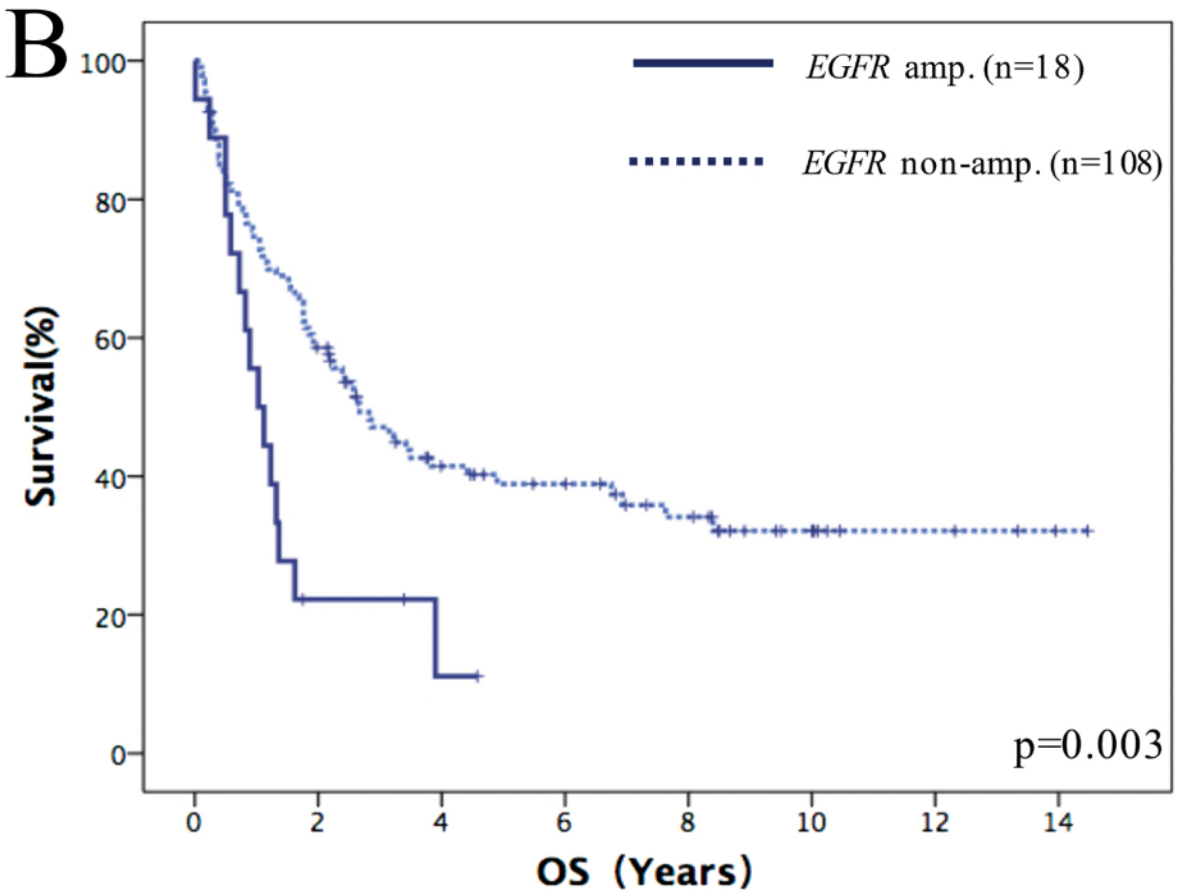


Among lower grade (II-III) gliomas

Eckel-Passow JE, et al. Glioma Groups Based on 1p/19q, IDH, and TERT Promoter Mutations in Tumors. N Engl J Med. 2015:25;372(26):2499-508. PMID: 26061753.

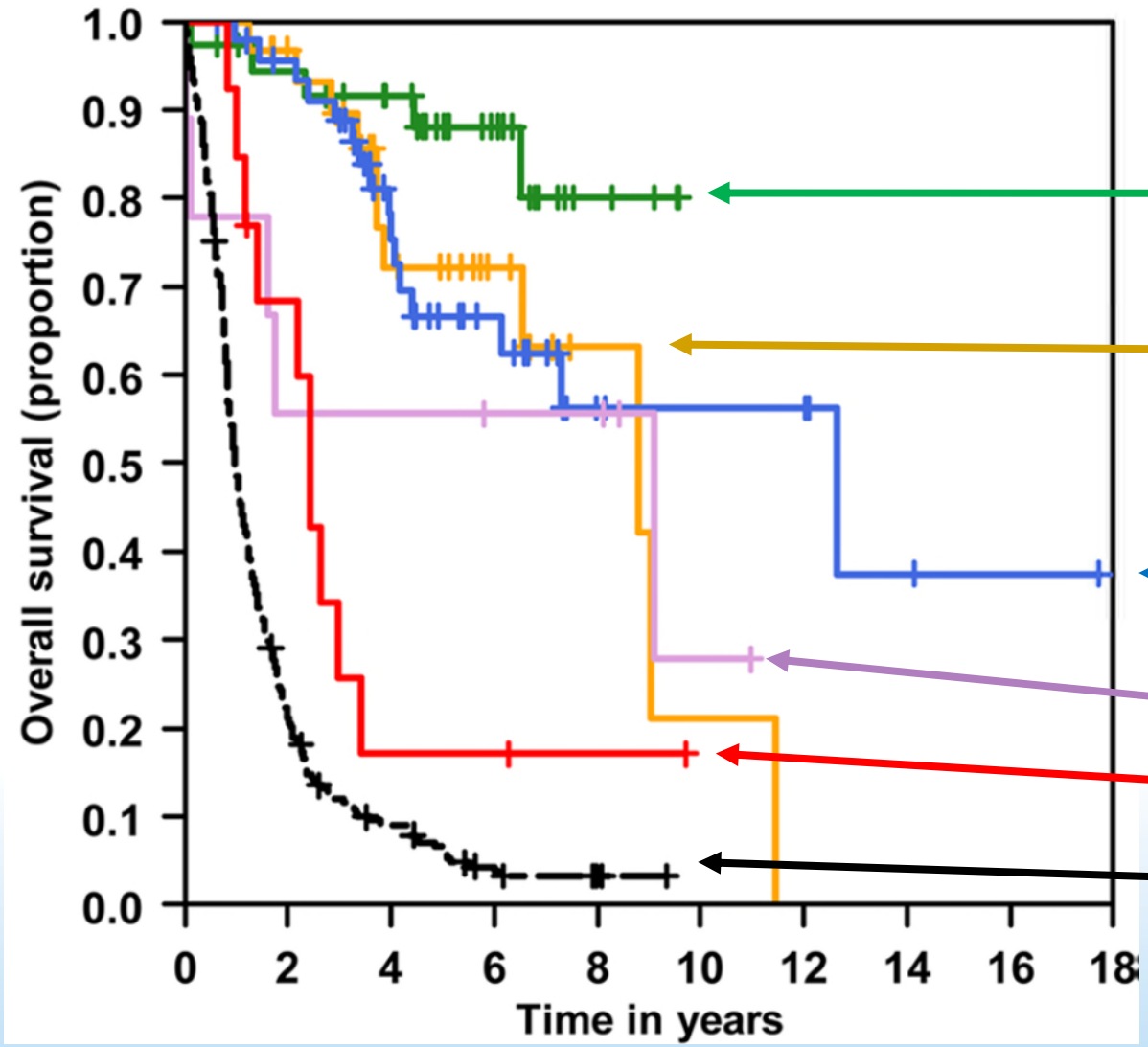
and EGFR amplification → poor prognosis





Aibaidula A, et al. Adult IDH wild-type lower-grade gliomas should be further stratified. Neuro Oncol. 2017 Oct 1;19(10):1327-1337. doi: 10.1093/neuonc/nox078. PMID: 28575489.

Copy number alterations in diffuse gliomas



Oligodendroglioma

IDH mutant astrocytoma with 7q gain

IDH mutant astrocytoma with multiple gains/losses

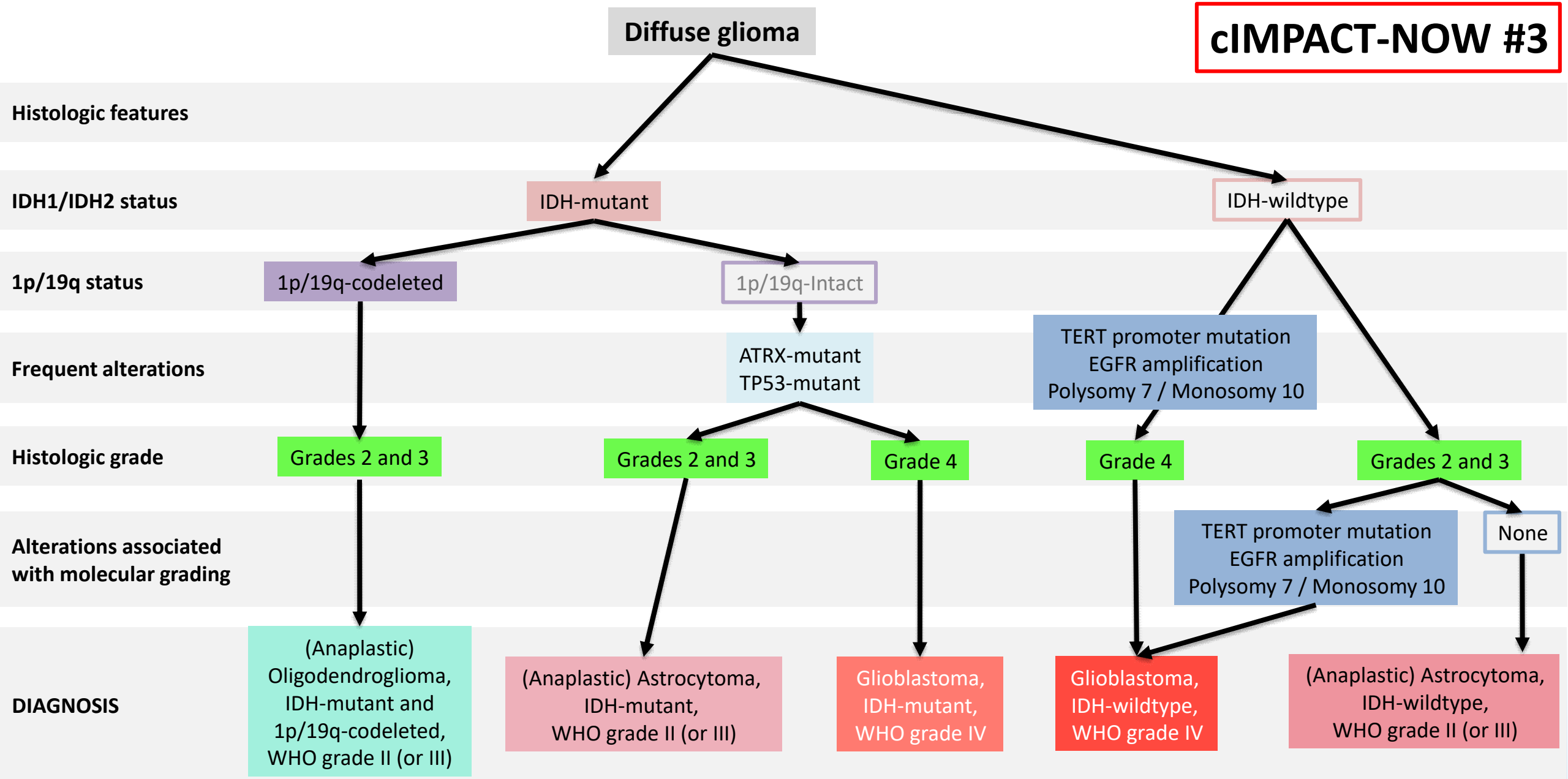
IDH WT astrocytoma with few changes

IDH WT astrocytoma with 7 gain and 10 loss

IDH WT GBM

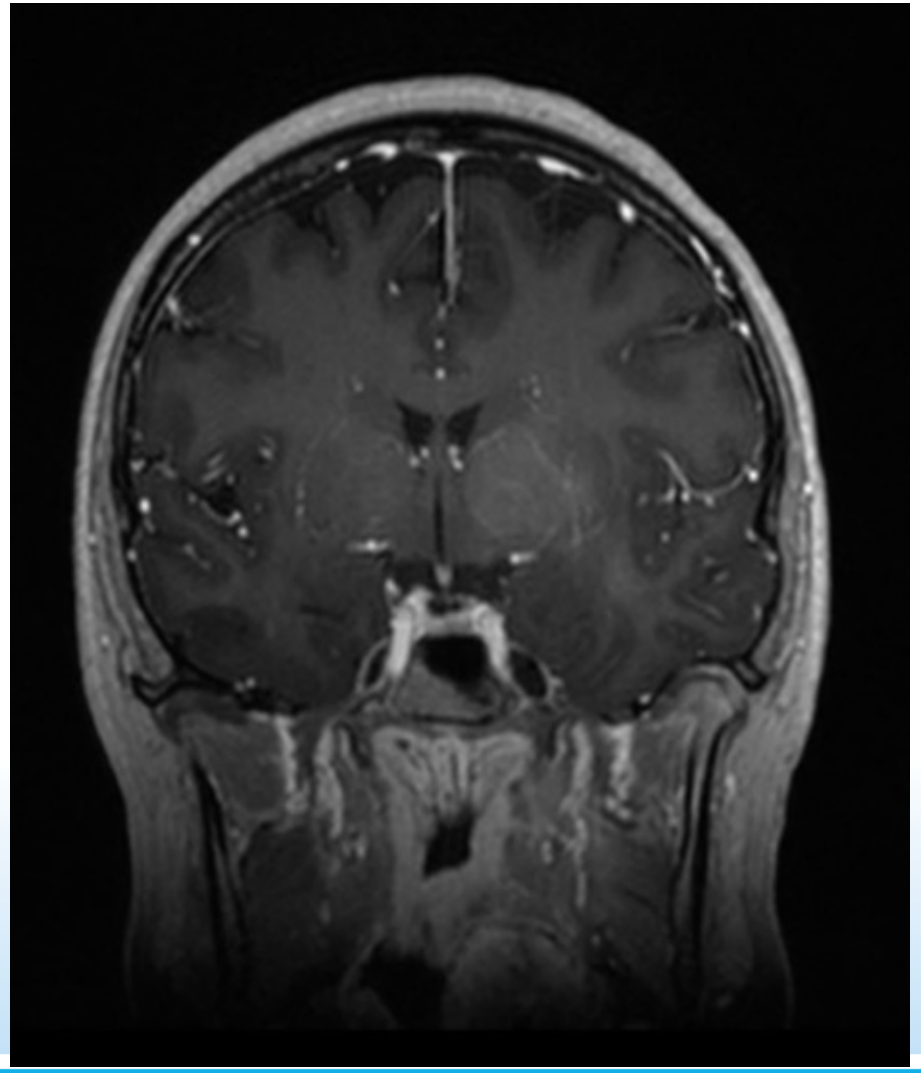
Weller M, et al. Molecular classification of diffuse cerebral WHO grade II/III gliomas using genome- and transcriptome-wide profiling improves stratification of prognostically distinct patient groups. Acta Neuropathol. 2015;129(5):679-93. PMID: 25783747.

cIMPACT-NOW #3

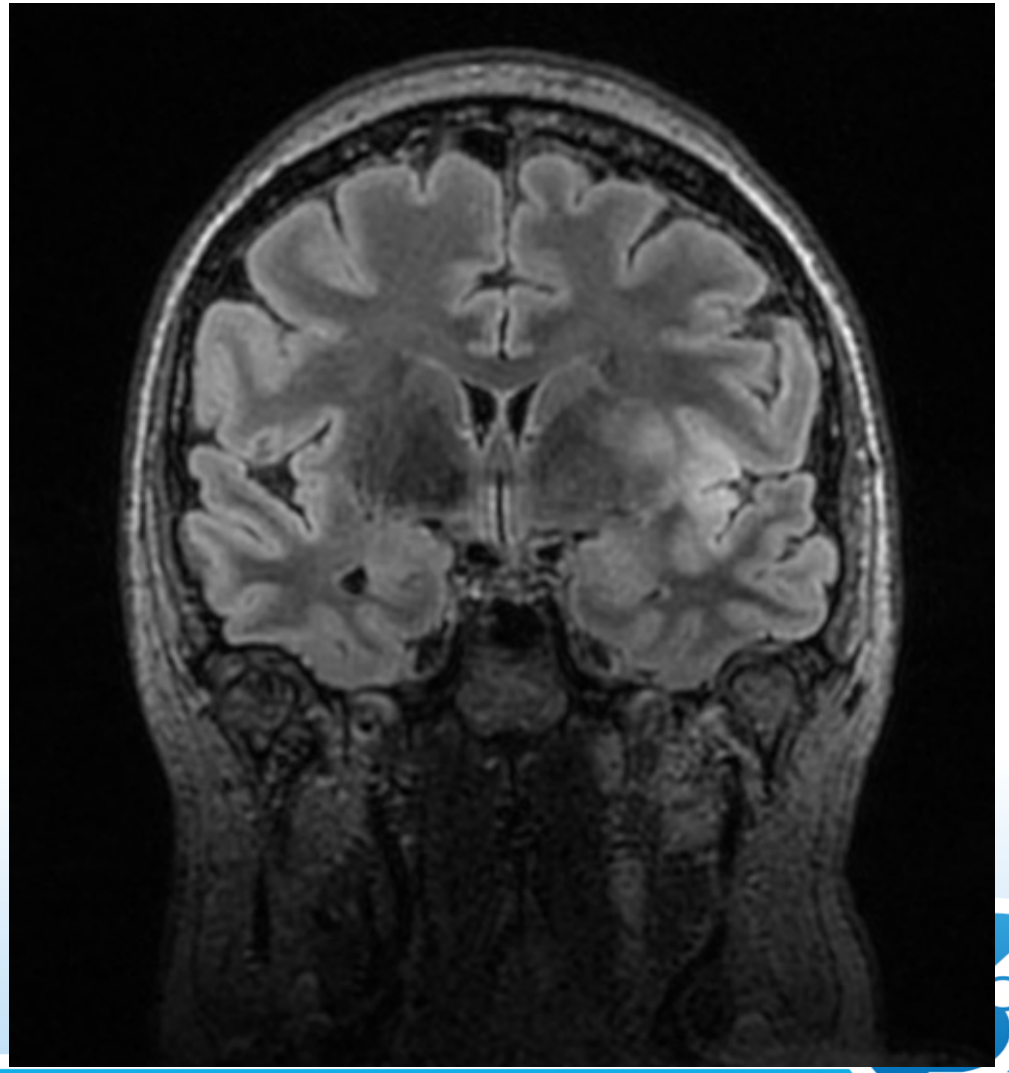


Brat DJ, et al. cIMPACT-NOW update 3: recommended diagnostic criteria for "Diffuse astrocytic glioma, IDH-wildtype, with molecular features of glioblastoma, WHO grade IV". Acta Neuropathol. 2018;136(5):805-810. PMID 30259105

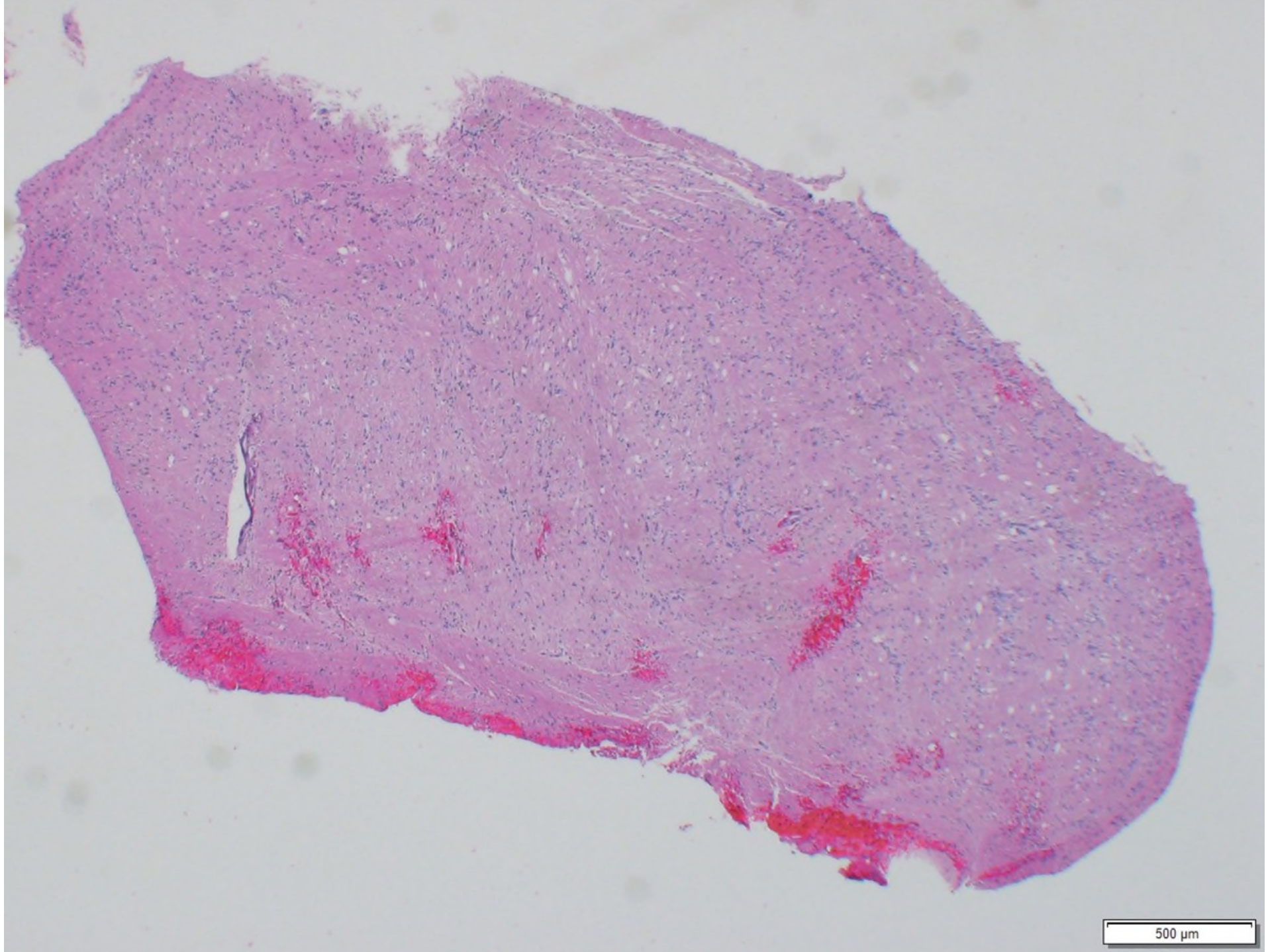
Case 5: 57-year-old woman with new-onset seizures

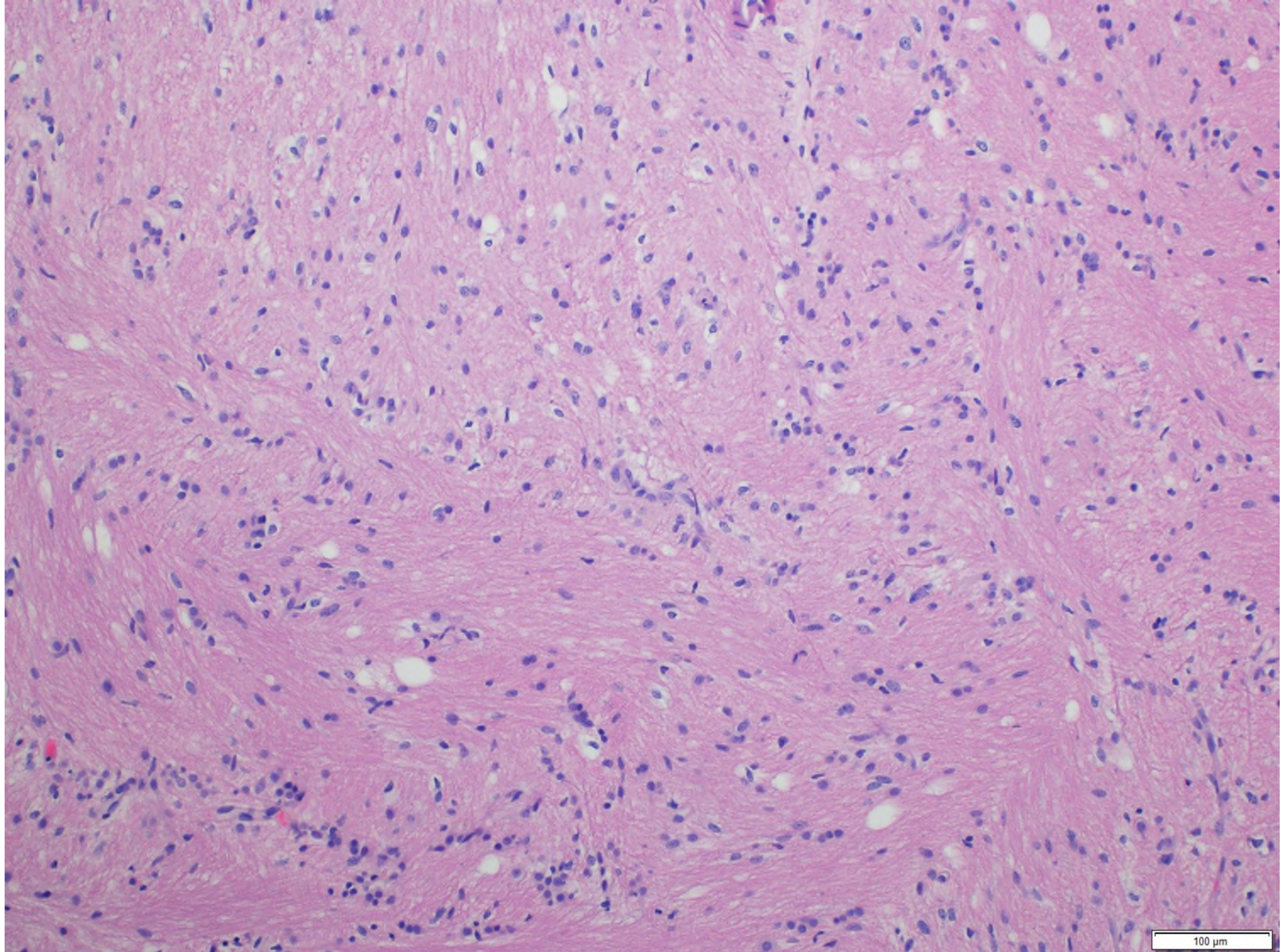


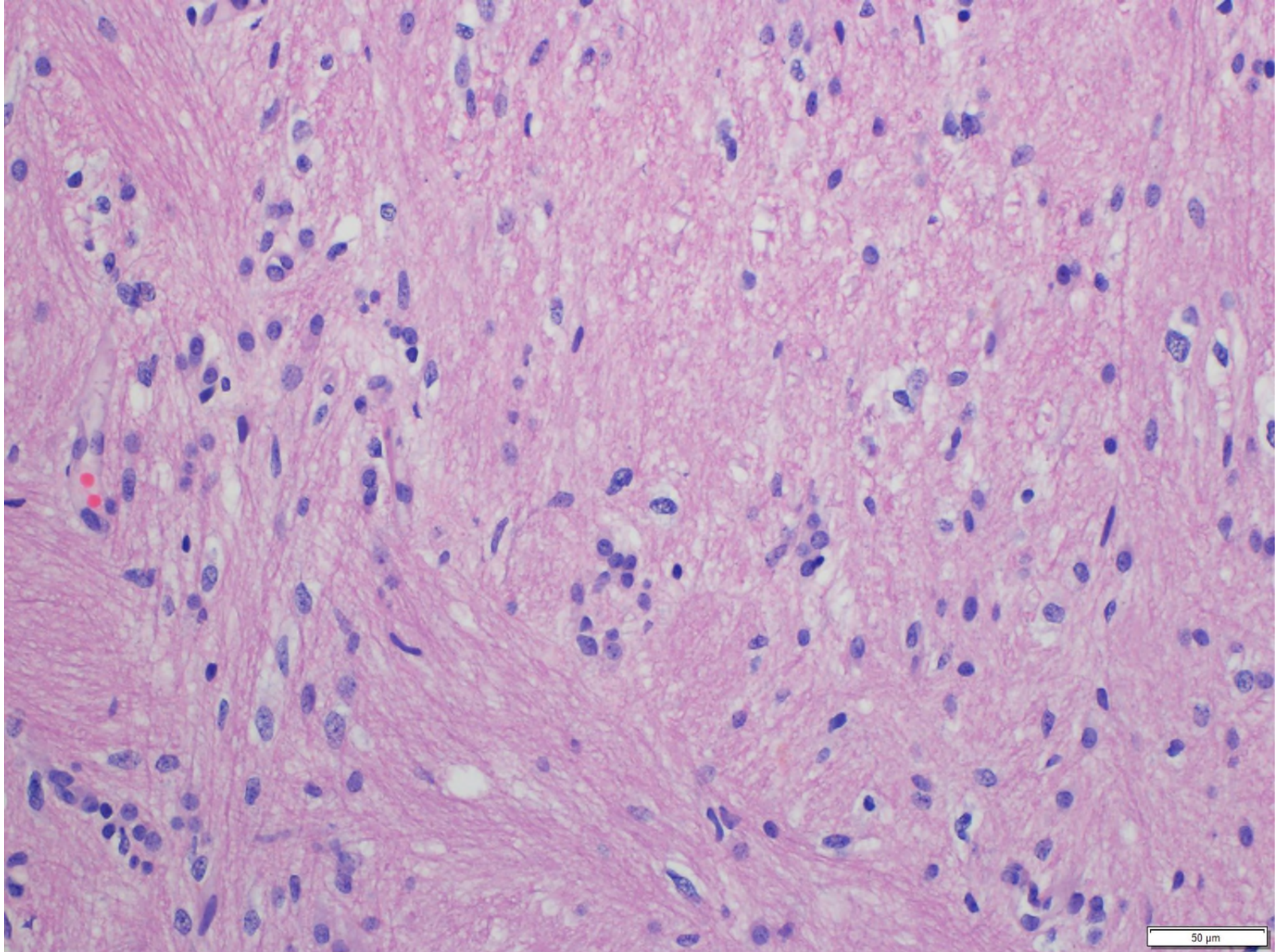
Coronal T1-contrast

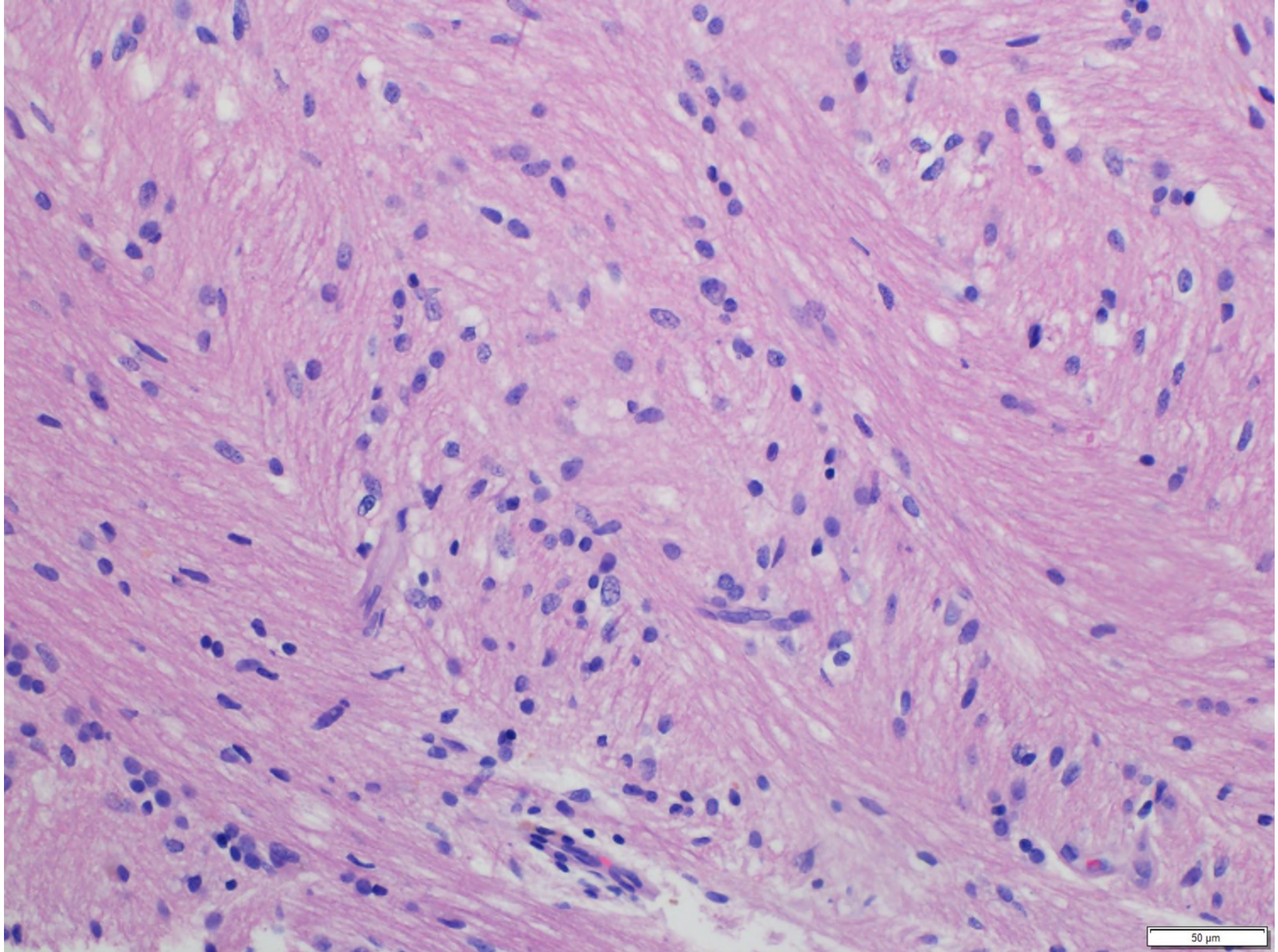


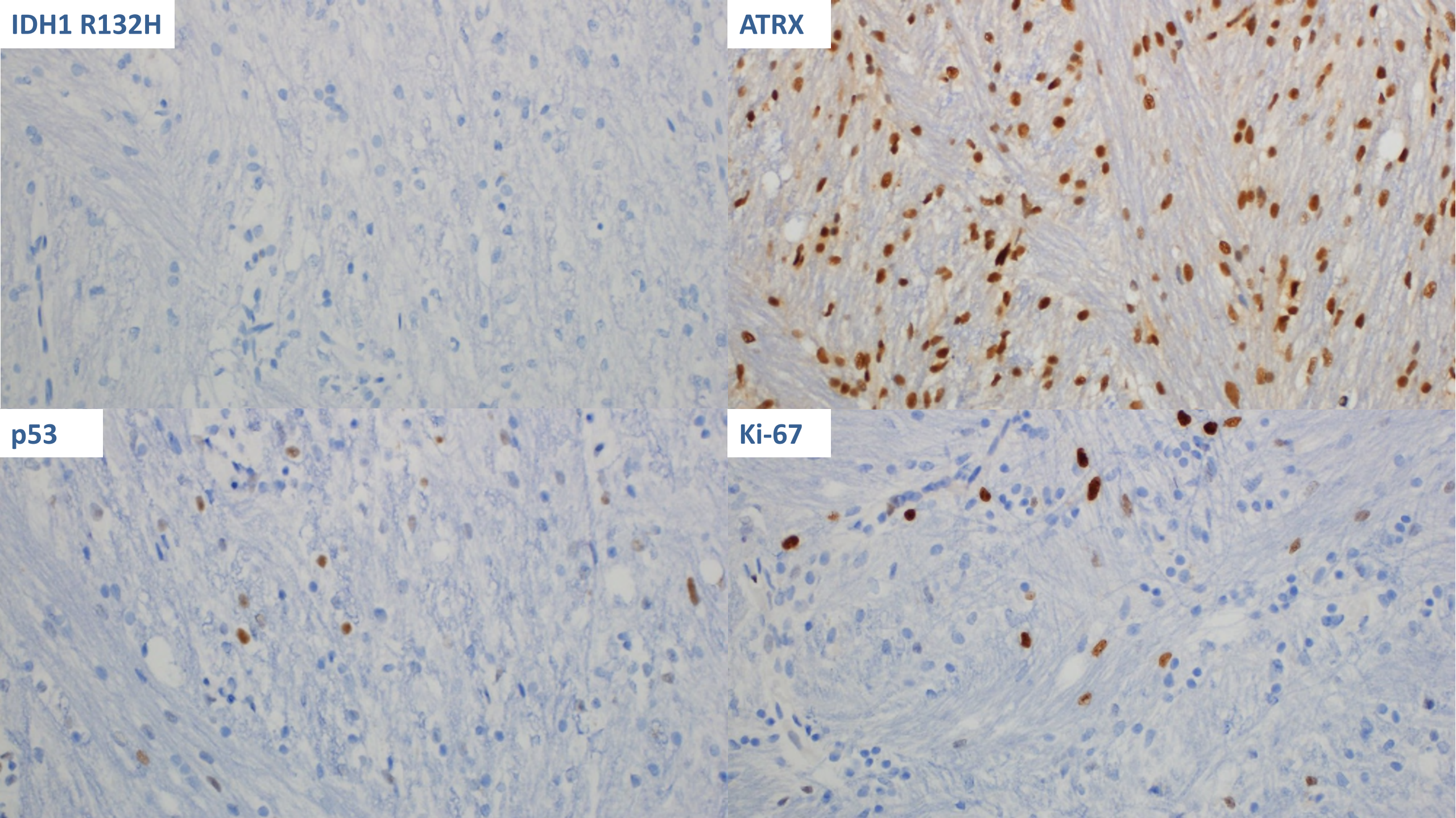
Coronal T2-FLAIR


500 µm


100 µm


50 µm


50 µm


IDH1 R132H
ATRX
p53
Ki-67

Older adult, hemispheric, enhancing

- Low-grade histology
- IDH1 R132H (-), ATRX-intact, p53 (+/-)

Diffuse astrocytoma, IDH-wildtype, WHO grade II

???

Older adult, hemispheric, enhancing

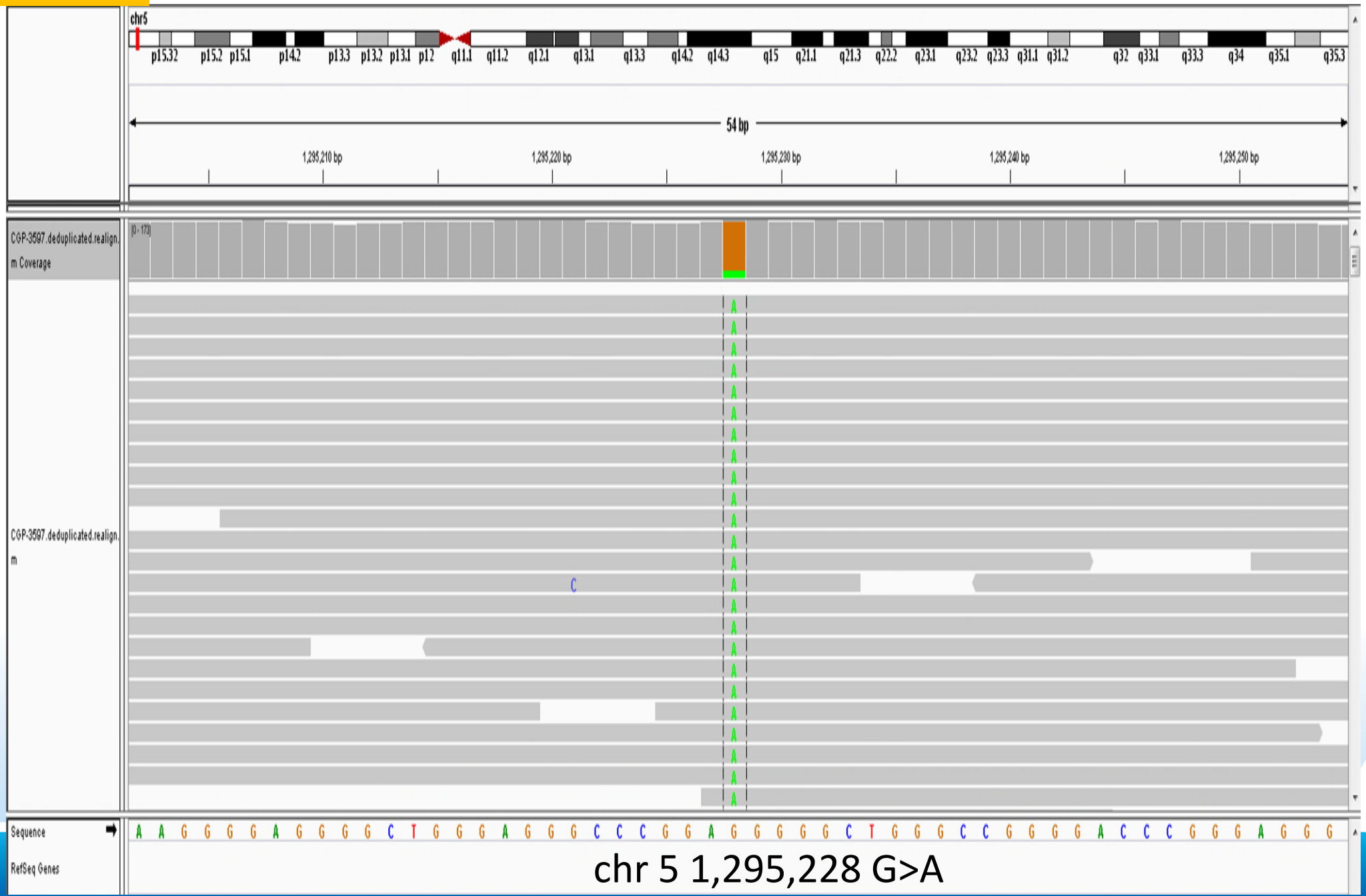
- Low-grade histology
- IDH1 R132H (-), ATRX-intact, p53 (+/-)

  Diffuse astrocytic glioma, likely IDH-wildtype; see comment

  (Molecular) glioblastoma, IDH wildtype, grade IV
- TERTp or EGFR ampl or Polysomy 7/monosomy 10
- UCSF500 next generation sequencing

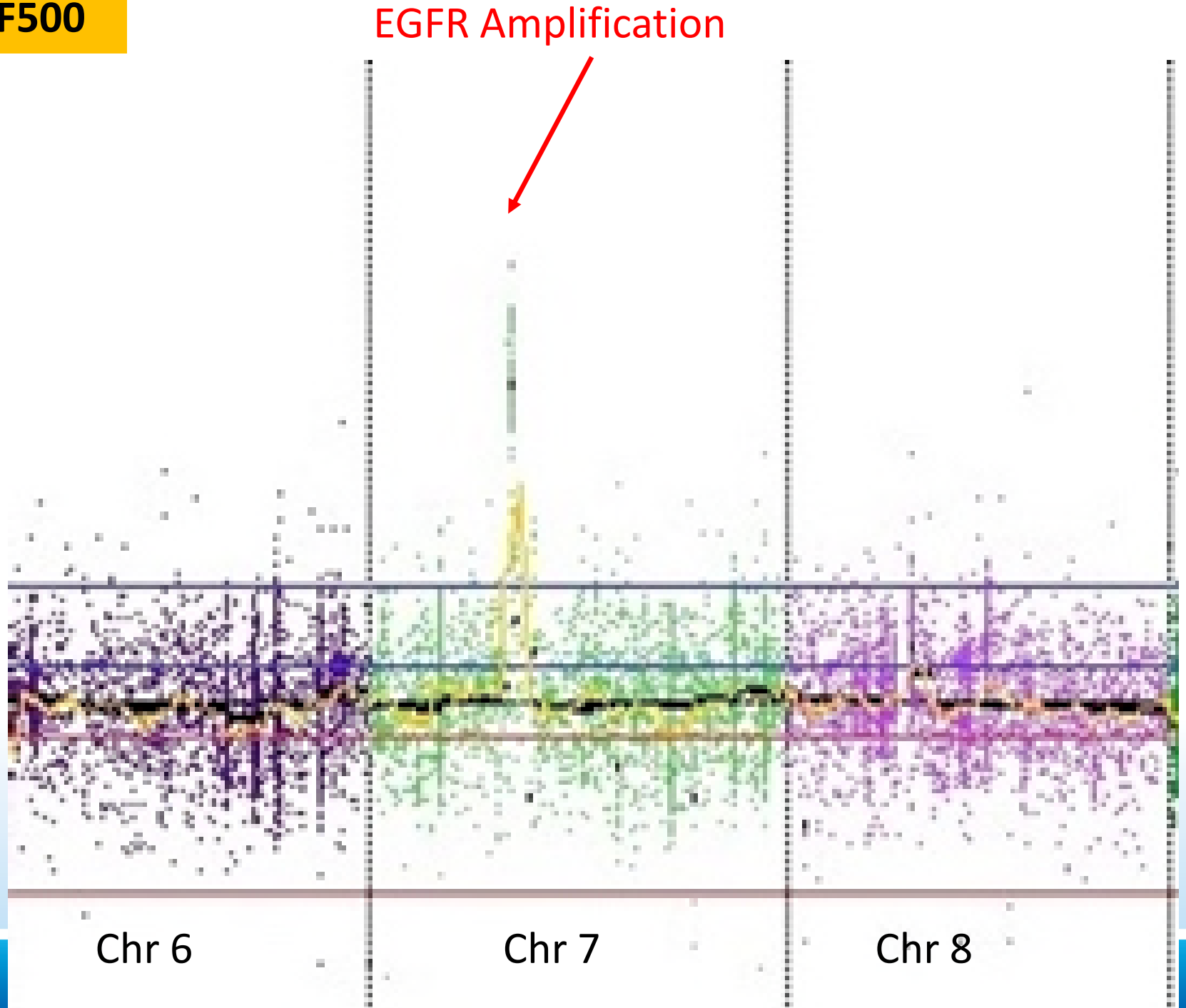
UCSF500

TERT Promoter mutation



chr 5 1,295,228 G>A

UCSF500



UCSF500

PATHOGENIC AND LIKELY PATHOGENIC ALTERATIONS				
VARIANT	TRANSCRIPT ID	CLASSIFICATION	READS	MUTANT ALLELE FREQUENCY
TERT upstream chr 5: g.1,295,228 G>A	N/A	Pathogenic	165	15%
EGFR high-level amplification	any	Pathogenic	>6000	N/A
EGFR p.A289V	NM_005228	Pathogenic	3030	28%
EGFR p.S227F	NM_005228	Pathogenic	1508	8%
CDKN2A deep deletion	any	Pathogenic	N/A	N/A
PTPRD p.D884N	NM_130391	Likely pathogenic	479	40%

Glioblastoma, IDH-wildtype, WHO grade IV

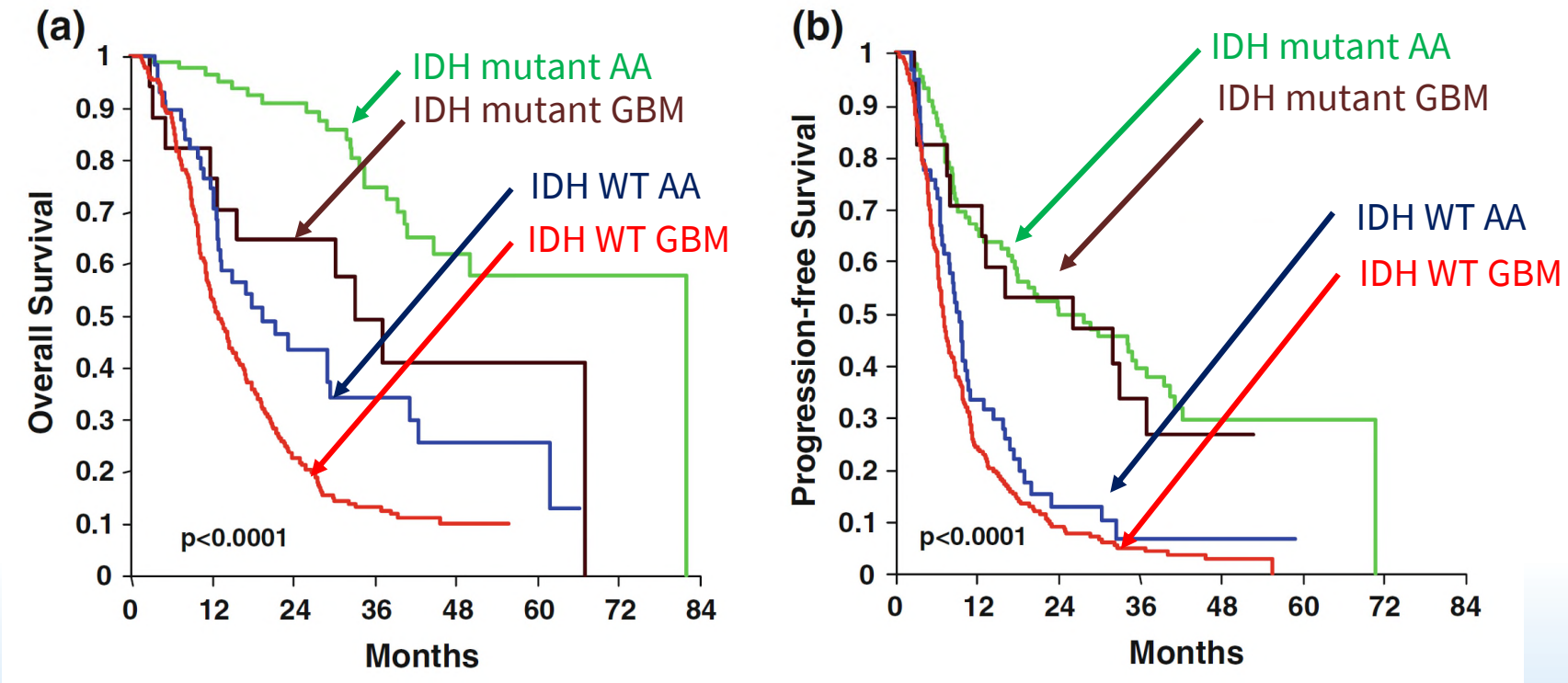
Caveat: These markers are not 100% specific

Sensitivity and specificity for 544 *IDH*wt GBM in a series of 2417 brain tumors

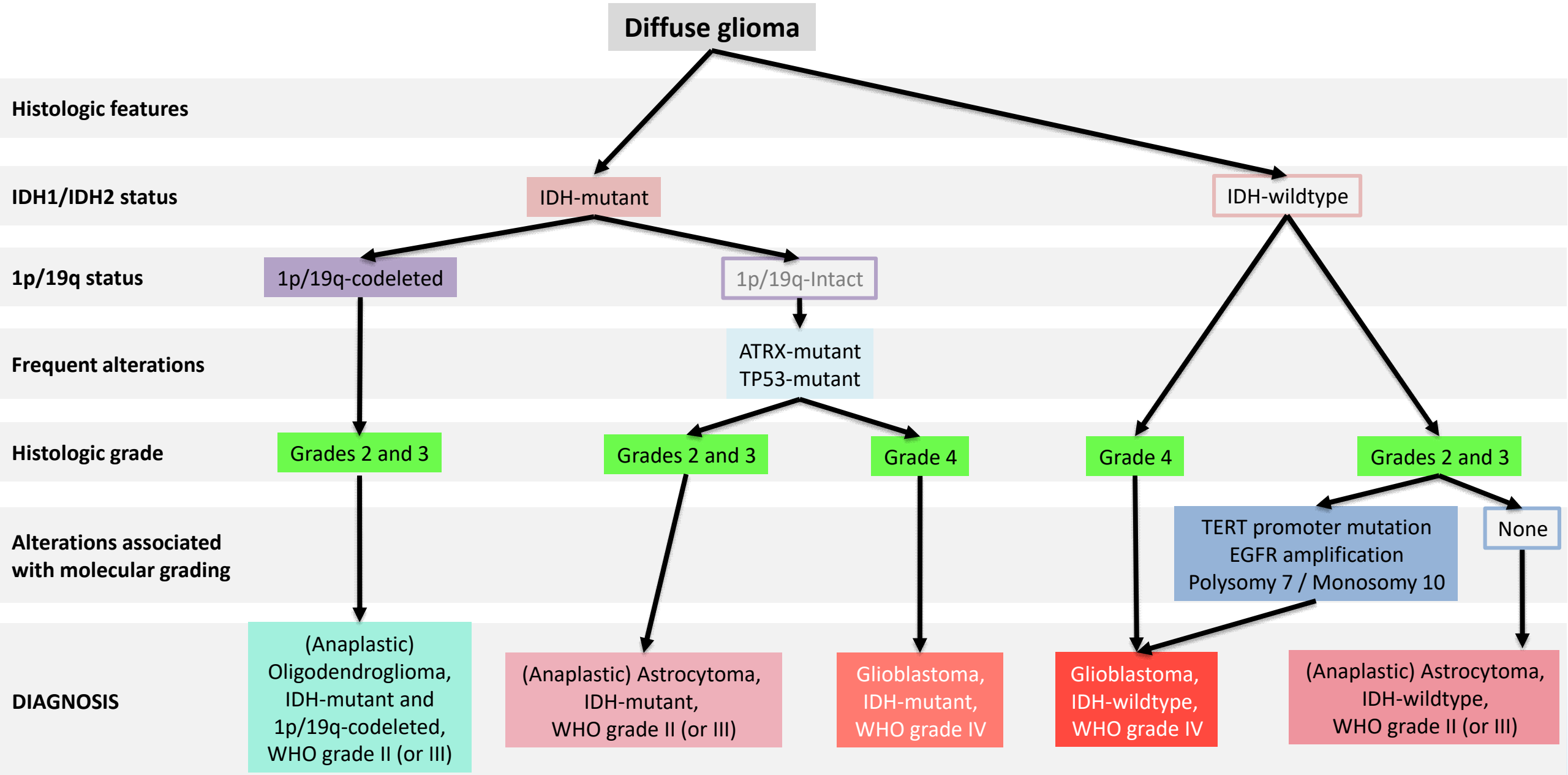
	Single			Double			Triple	Any double /triple
	TERT	*EGFR*	7/10	7/10 *EGFR*	7/10 *TERT*	*EGFR TERT*	7/10 *EGFR TERT*	
True positive	363	196	323	29	136	124	124	317
True negative	1674	1870	1835	1872	1873	1873	1873	1862
False positive	199	3	38	1	0	0	0	11
False negative	181	348	221	515	516	420	420	227
Sensitivity	66.7%	36.0%	59.4%	5.3%	5.1%	22.8%	22.8%	58.3%
Specificity	89.4%	99.8%	98.0%	99.9%	100.0%	100.0%	100.0%	99.4%

Brat DJ, et al. cIMPACT-NOW update 3. Acta Neuropathol. 2018;136(5):805-810. PMID 30259105

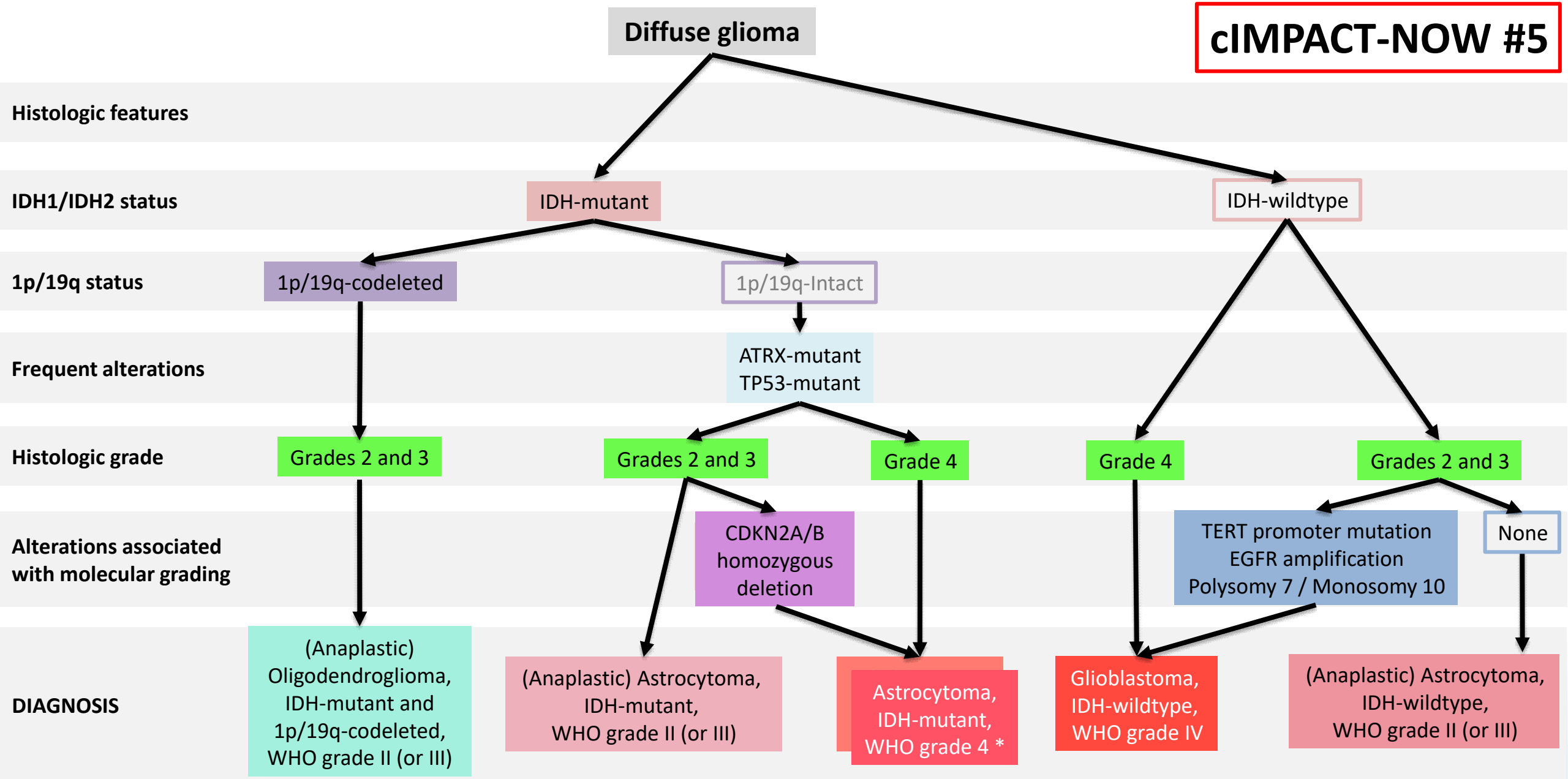
Caveat: How aggressive is molecular GBM



Hartmann C, et al. Patients with IDH1 wild type anaplastic astrocytomas exhibit worse prognosis than IDH1-mutated glioblastomas, and IDH1 mutation status accounts for the unfavorable prognostic effect of higher age: implications for classification of gliomas. Acta Neuropathol. 2010;120(6):707-18. PMID: 21088844.


Diffuse glioma
Histologic features
IDH1/IDH2 status
IDH-mutant
IDH-wildtype
1p/19q status
1p/19q-codeleted
1p/19q-Intact
Frequent alterations
ATRX-mutant
TP53-mutant
Histologic grade
Grades 2 and 3
Grades 2 and 3
Grade 4
Grade 4
Grades 2 and 3
Alterations associated with molecular grading
TERT promoter mutation
EGFR amplification
Polysomy 7 / Monosomy 10
None
DIAGNOSIS
(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
(Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
Glioblastoma, IDH-mutant, WHO grade IV
Glioblastoma, IDH-wildtype, WHO grade IV
(Anaplastic) Astrocytoma, IDH-wildtype, WHO grade II (or III)

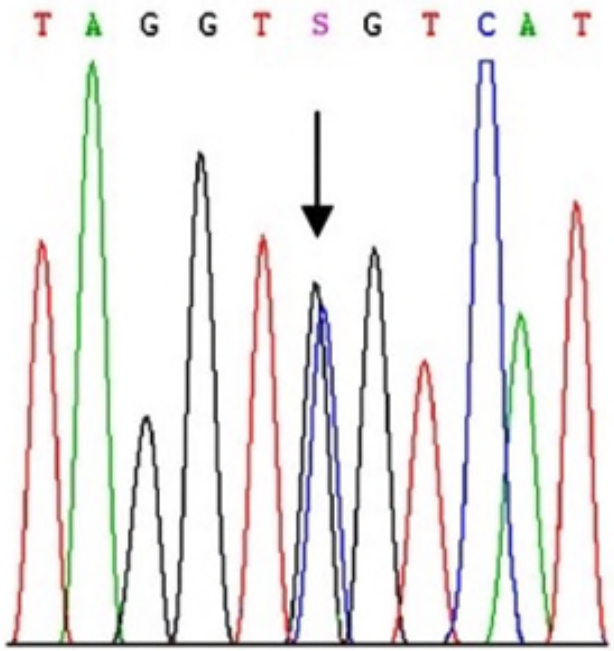
cIMPACT-NOW #5



Brat DJ, Aldape K, Colman H, et al. cIMPACT-NOW update 5: recommended grading criteria and terminologies for IDH-mutant astrocytomas. *Acta Neuropathol.* 2020;139(3):603-608

Case 2: Adult, hemispheric, enhancement (-/+)

- Lower-grade histology (or high-grade)
- IDH1 R132H (-), ATRX-loss, p53 (+)

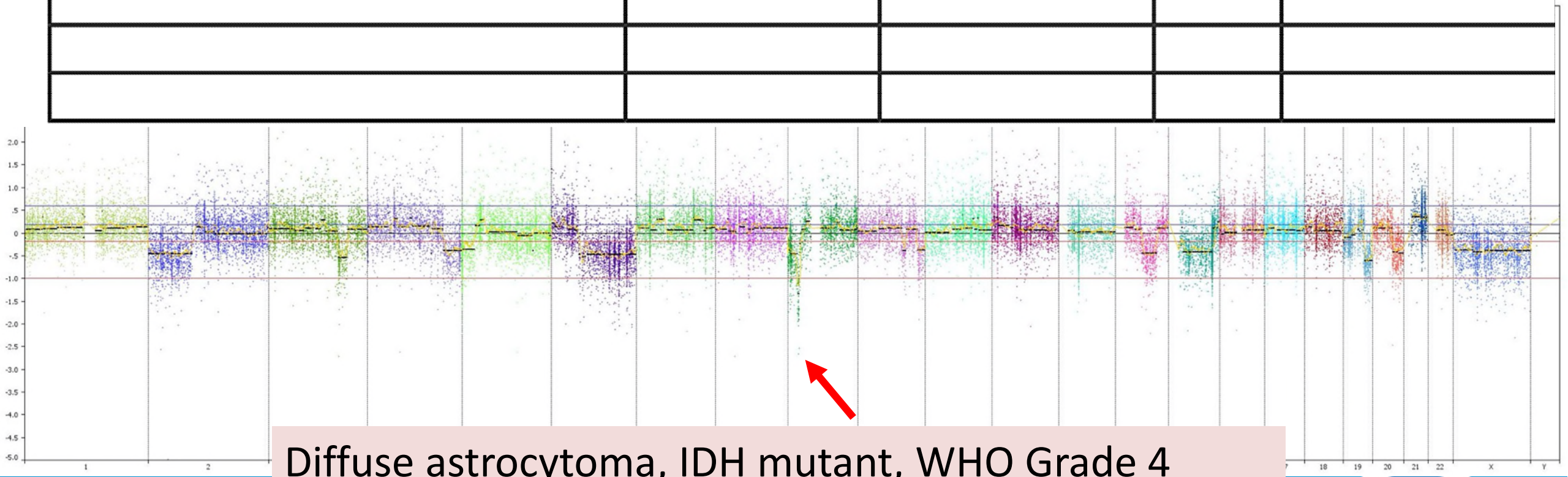


IDH1 R132G

c.C394G

Anaplastic astrocytoma, IDH mutant, WHO Grade III

Targeted NGS panel



Diffuse astrocytoma, IDH mutant, WHO Grade 4

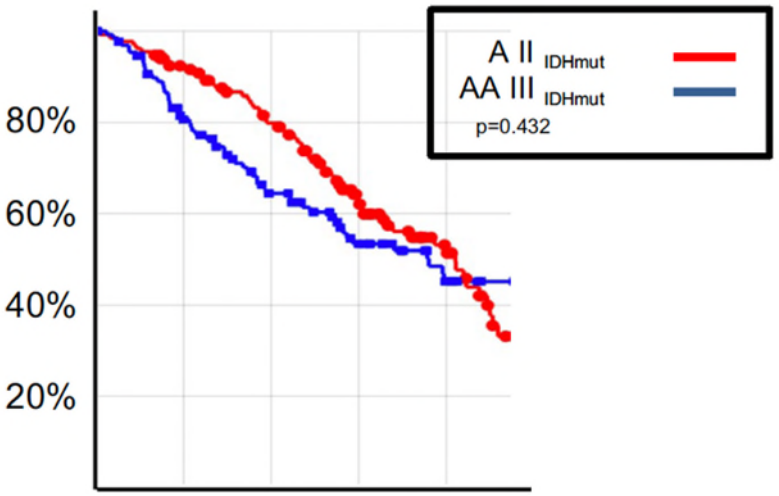
Adult, hemispheric, enhancement (-/+)

- Low-grade or high-grade histology
- IDH1 mutant, ATRX-loss, p53 (+)

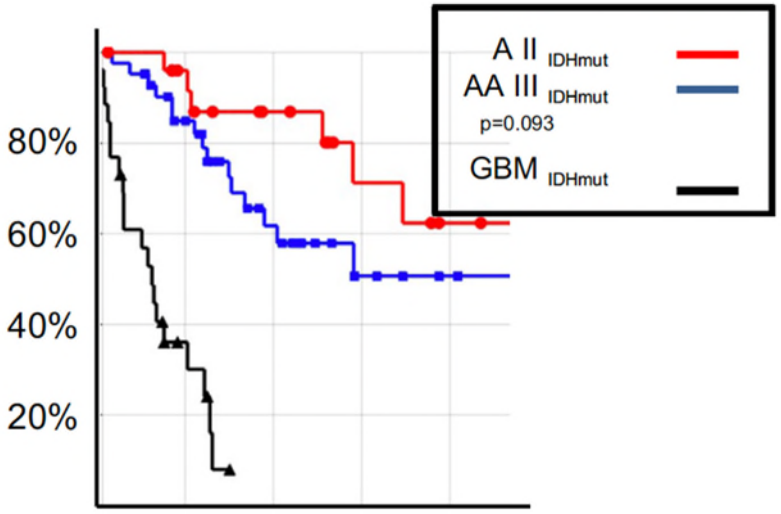
  Diffuse astrocytoma, IDH-mutant, WHO grade (2 or 3)
- When to test for CDKN2A/B homozygous deletion to exclude grade 4?
  - imaging suggests high grade but histology lower grade
  - Increased mitoses and/or Ki-67 to raise suspicion for high grade
  - Clinical concern for high-grade or progression to high-grade
  - Or all?

Value of histologic grading for IDH-mutant astrocytomas

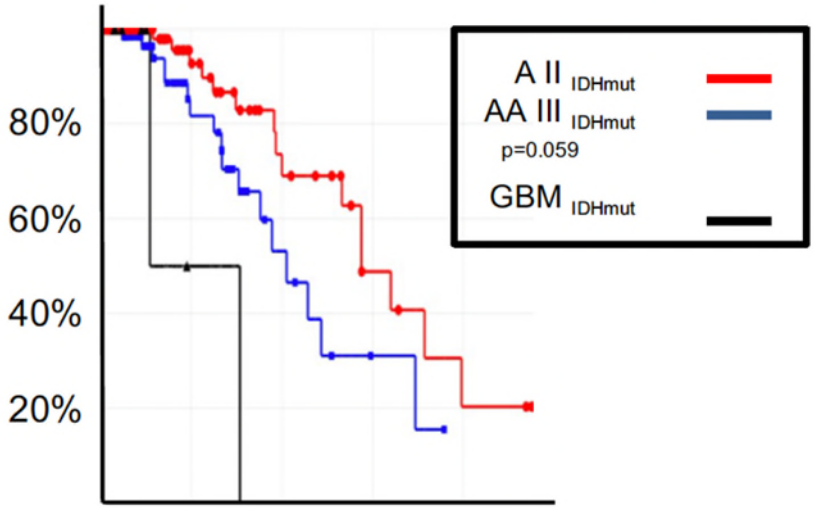
OS- MD Anderson



OS- Heidelberg



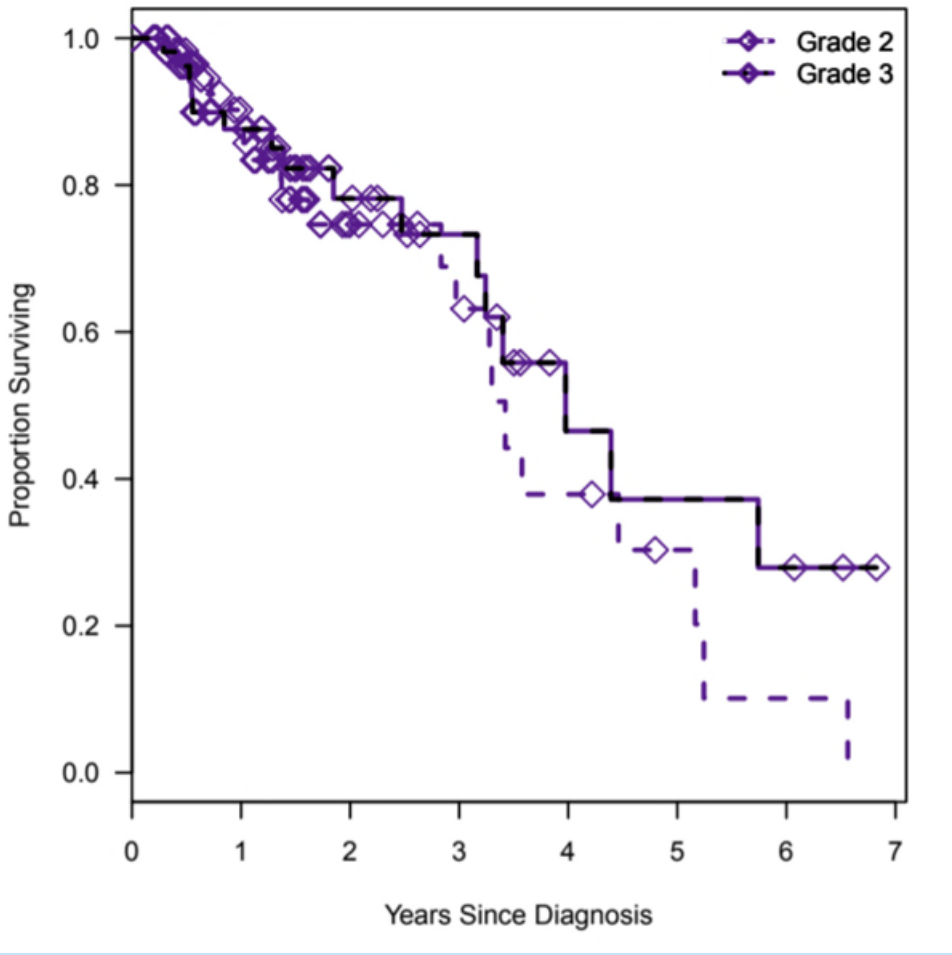
OS- TCGA



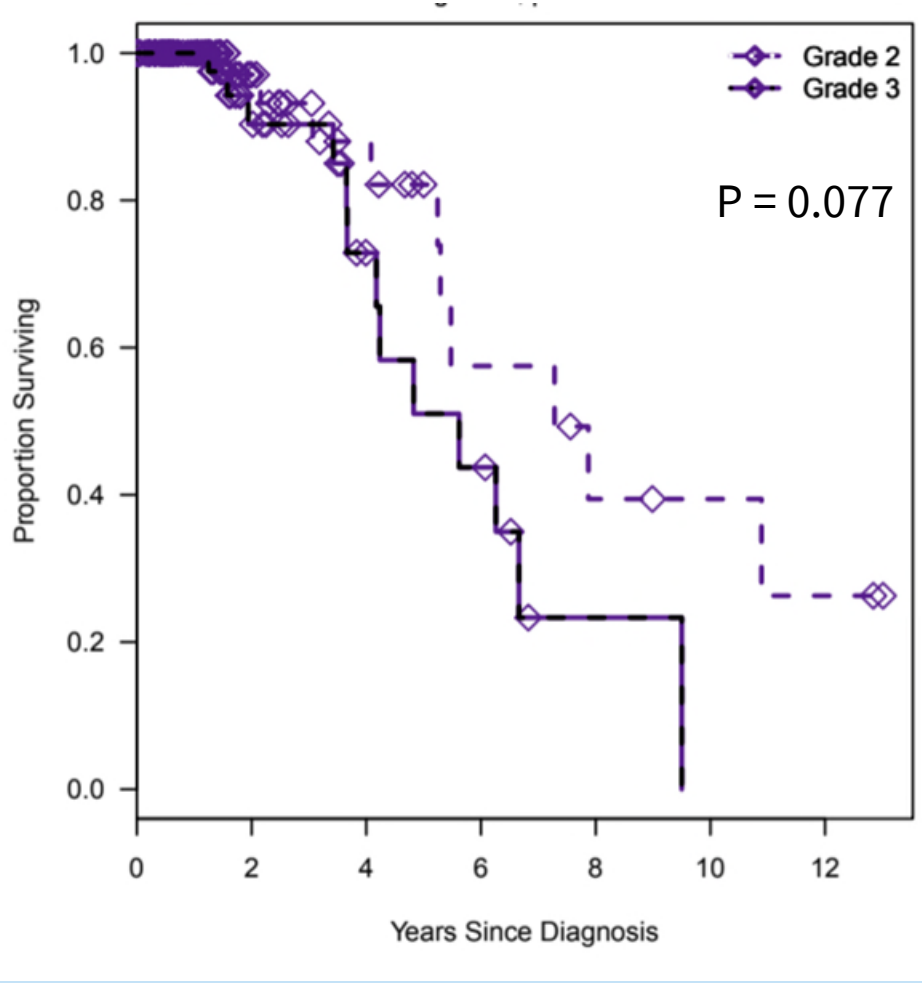
Reuss DE, et al. IDH mutant diffuse and anaplastic astrocytomas have similar age at presentation and little difference in survival: a grading problem for WHO. Acta Neuropathol. 2015;129(6):867-73. PMID: 25962792.

Value of histologic grading for IDH-mutant astrocytomas

Progression free survival

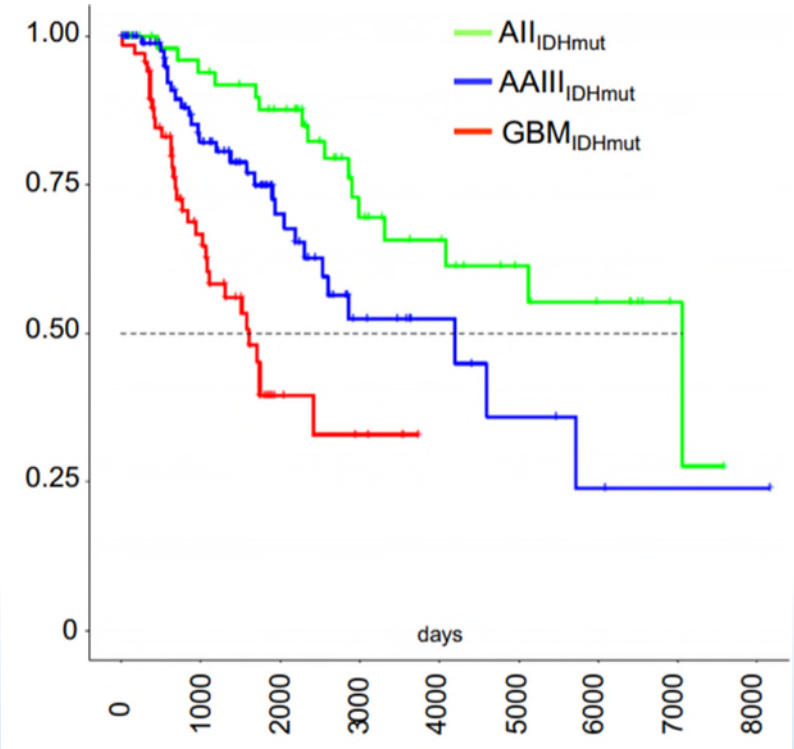


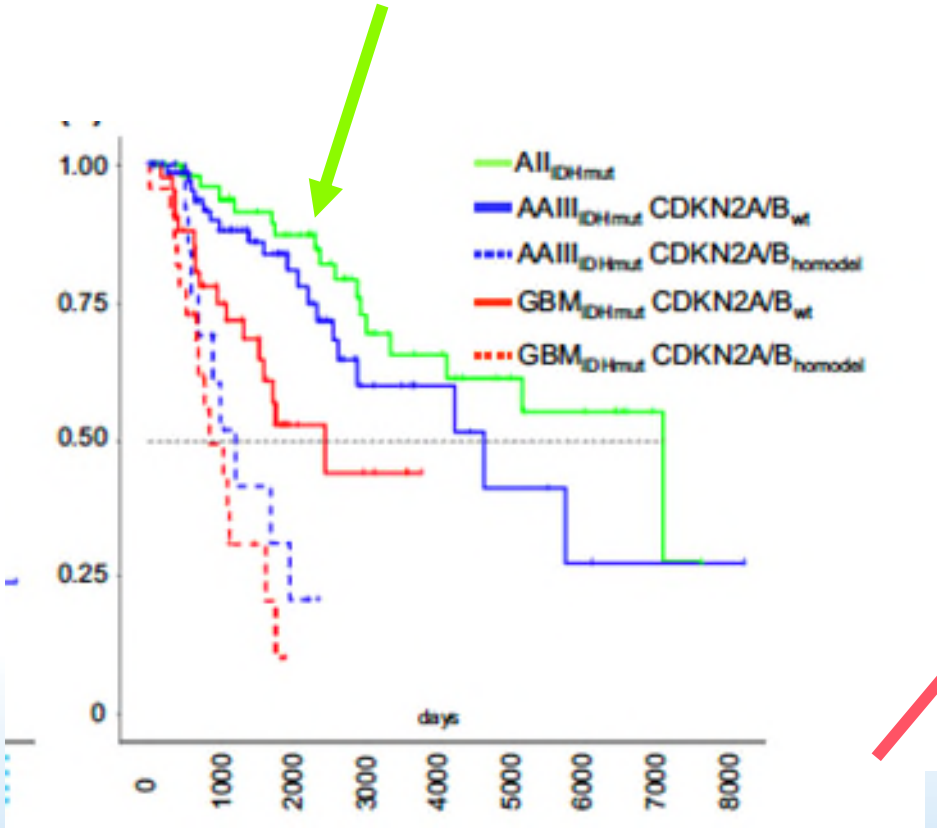
Overall survival

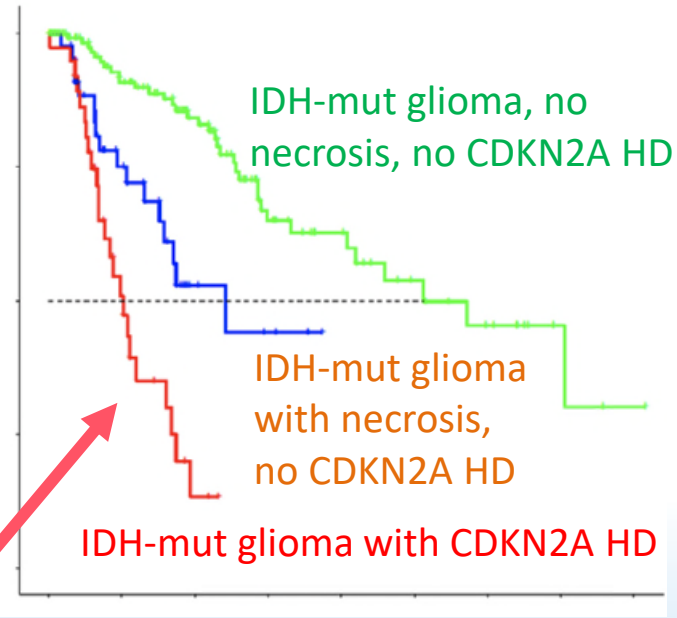


TCGA. N Engl J Med 2015; 372:2481-98. PMID: 26061751.

Value of histologic grading for IDH-mutant astrocytomas

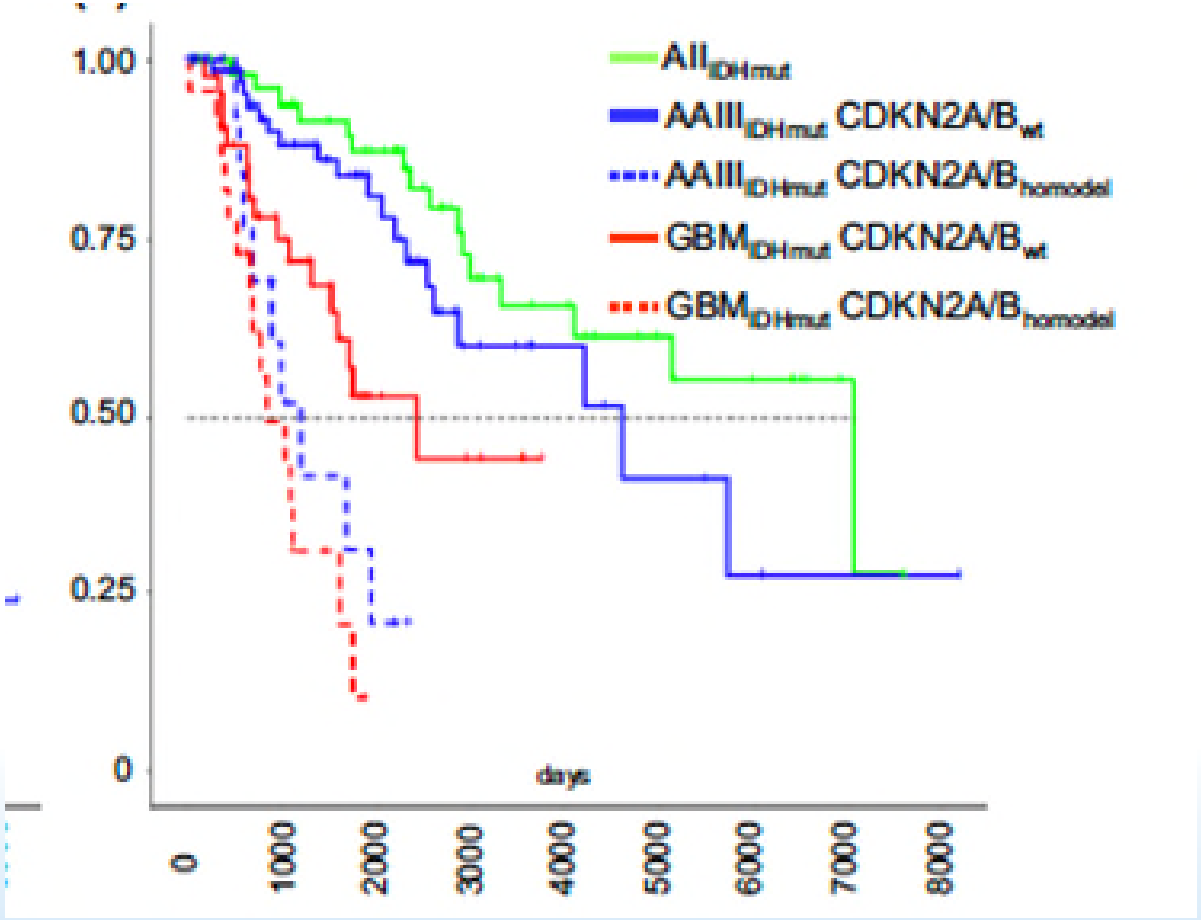


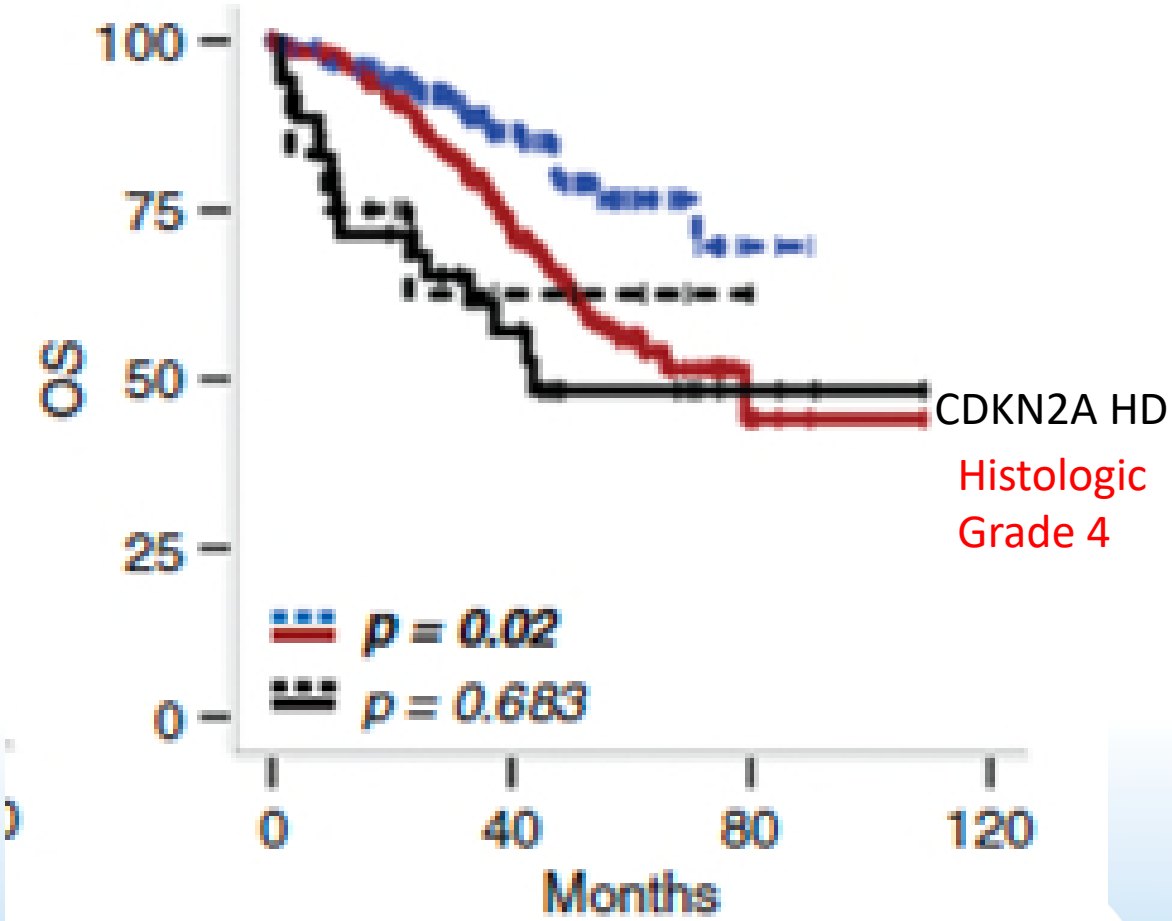




Shirahata M, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. Acta Neuropathol. 2018;136(1):153-166. PMID: 29687258

Caveat: Majority of the studies showed a prognostic effect for grade 3 tumors only

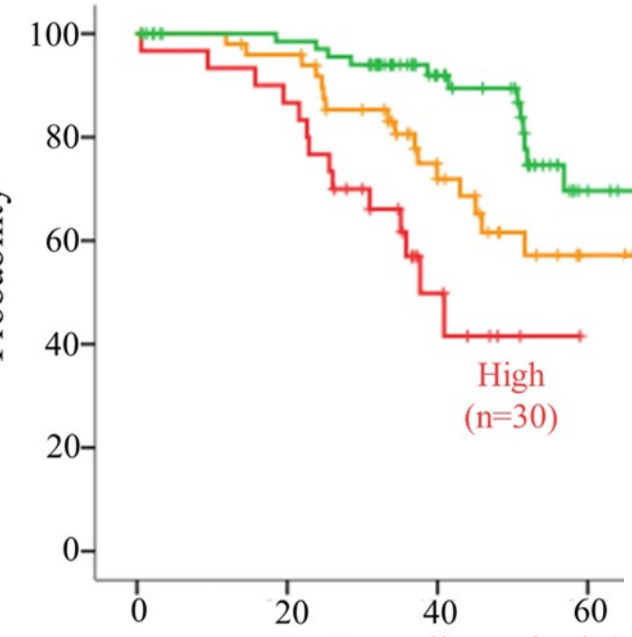




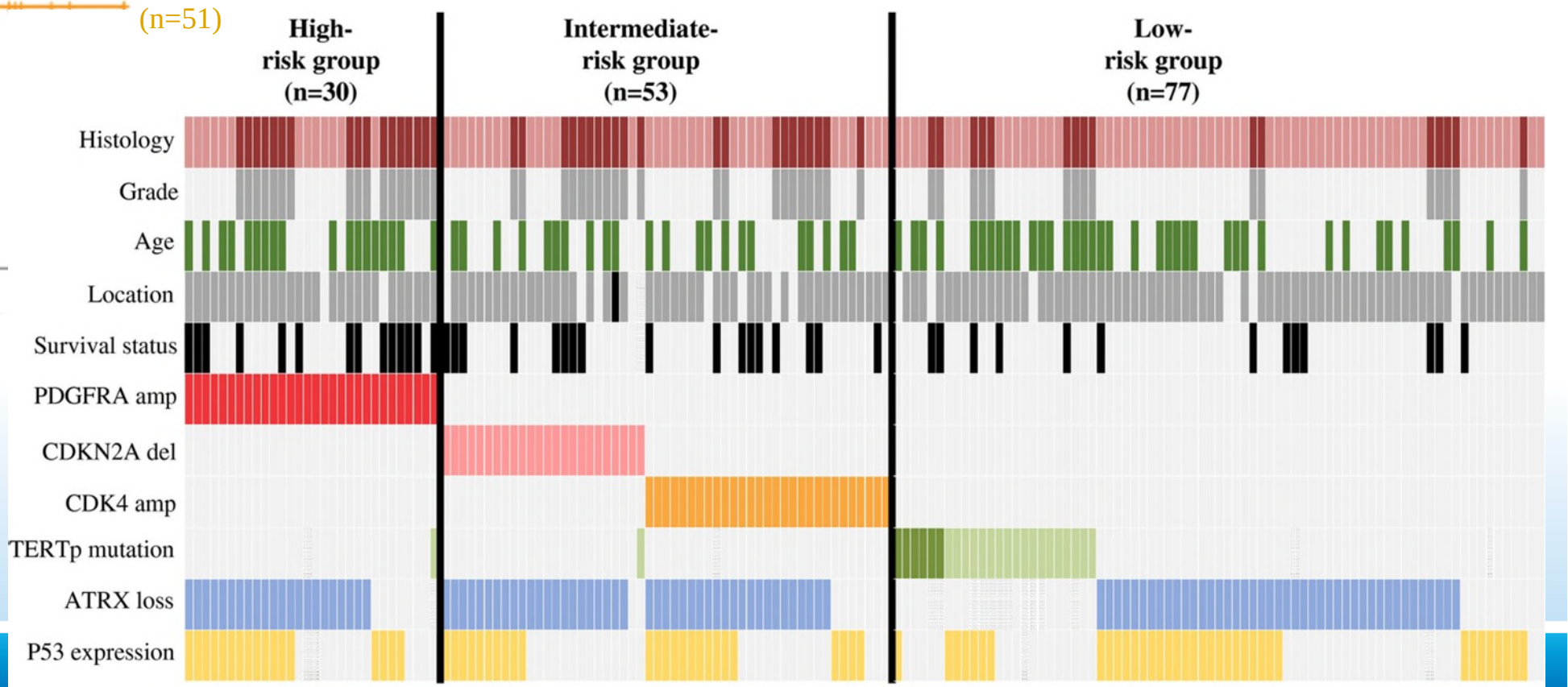
Shirahata M, et al. Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. Acta Neuropathol. 2018;136(1):153-166. PMID: 29687258.

Appay R, et al. CDKN2A homozygous deletion is a strong adverse prognosis factor in diffuse malignant IDH-mutant gliomas. Neuro Oncol. 2019;17;21(12):1519-1528. PMID: 31832685.

Caveat: CDKN2A HD may not even be the worst group

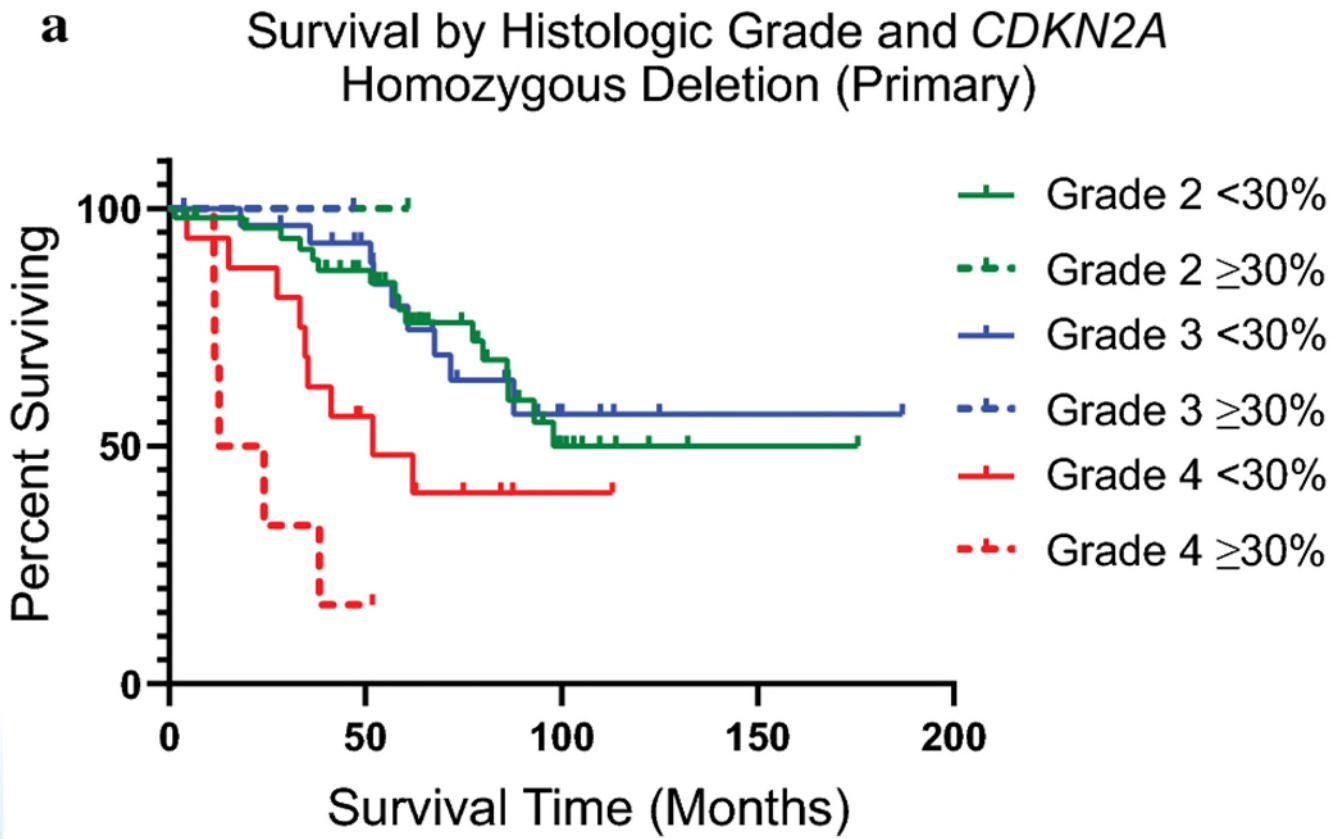


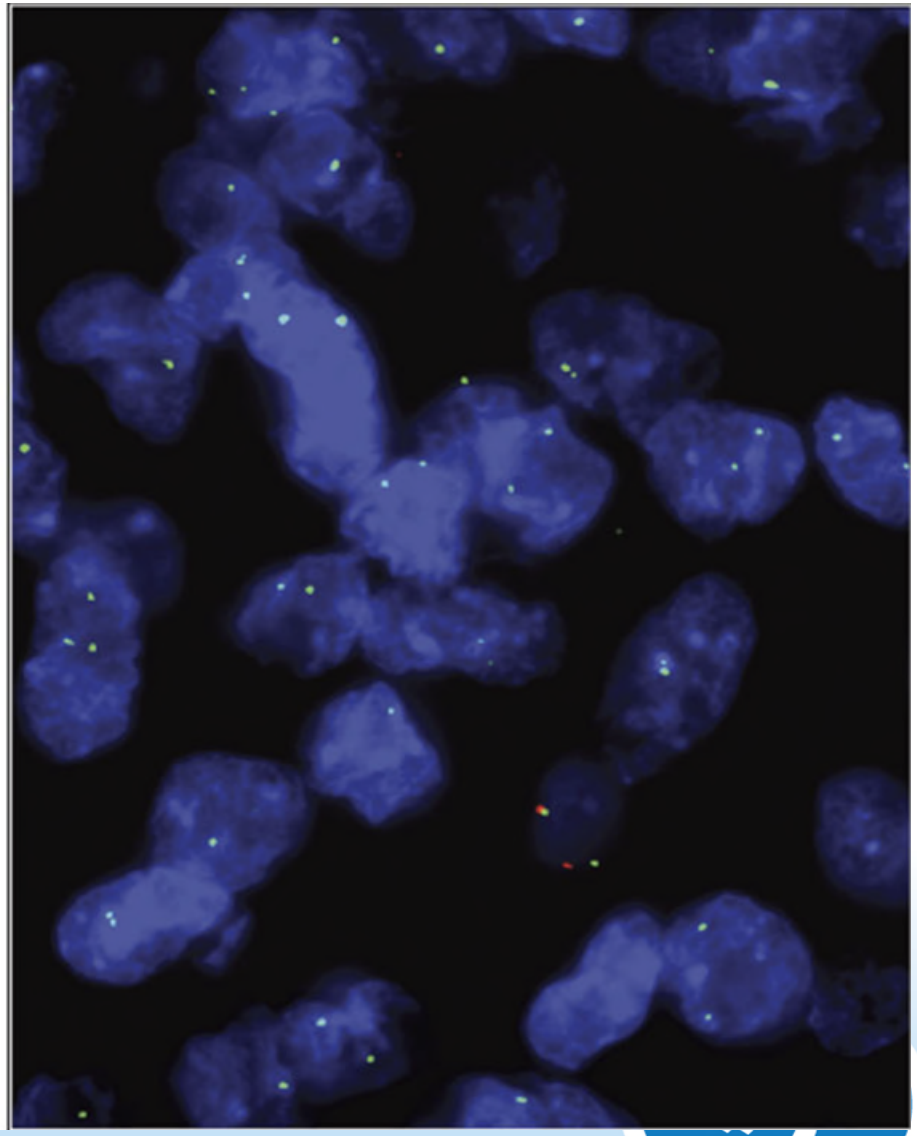
13 of 24 with CDKN2A deletion were grade 2



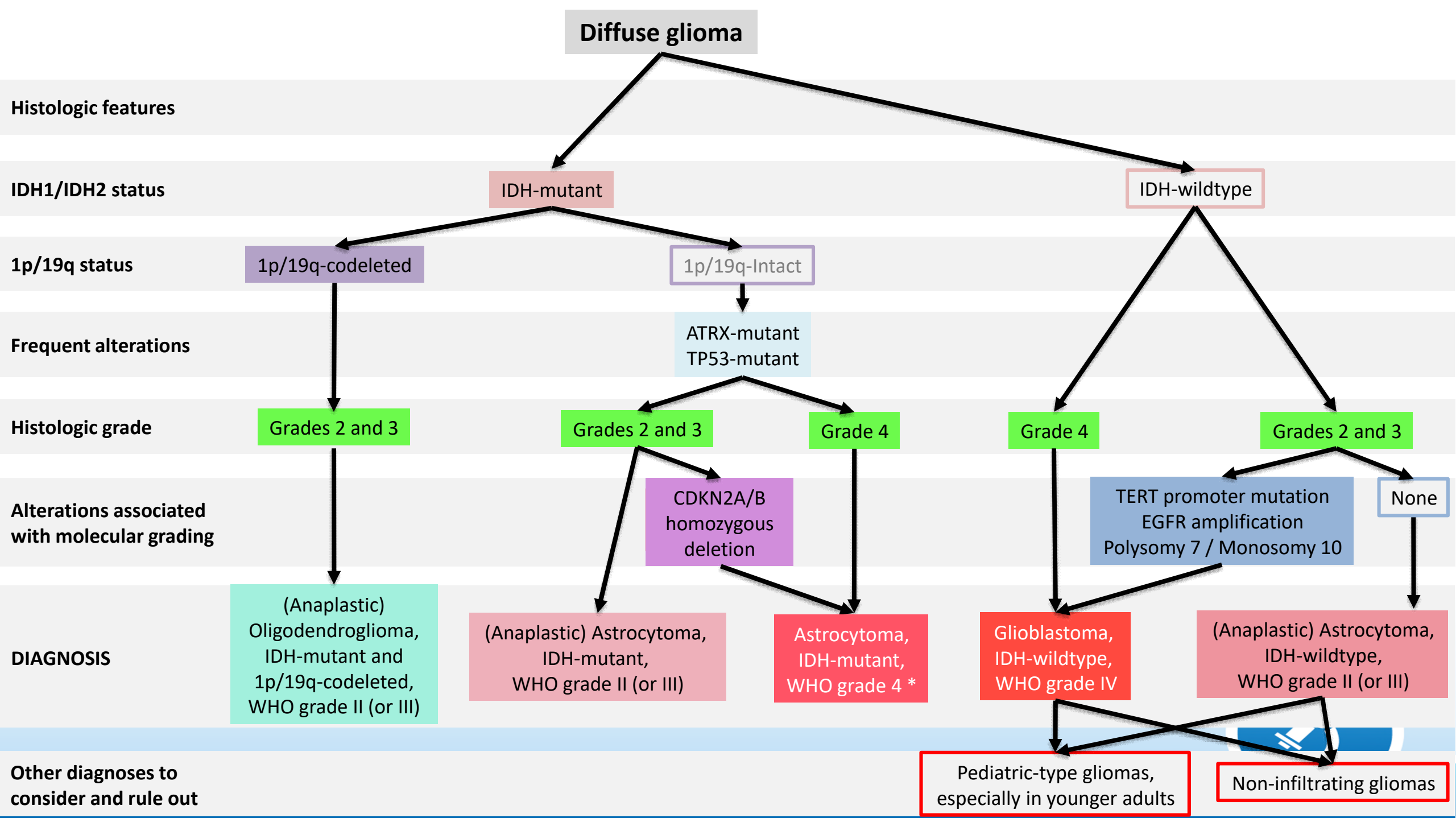
Yang RR, et al. Brain Pathol. 2020; 30(3):541-553. PMID: 31733156

Caveat: Assessing homozygous deletion by FISH can be difficult with unclear cut-off values





Marker DF, Pearce TM. Homozygous deletion of CDKN2A by fluorescence in situ hybridization is prognostic in grade 4, but not grade 2 or 3, IDH-mutant astrocytomas. Acta Neuropathol Commun. 2020 Oct 20;8(1):169. PMID: 33081848.

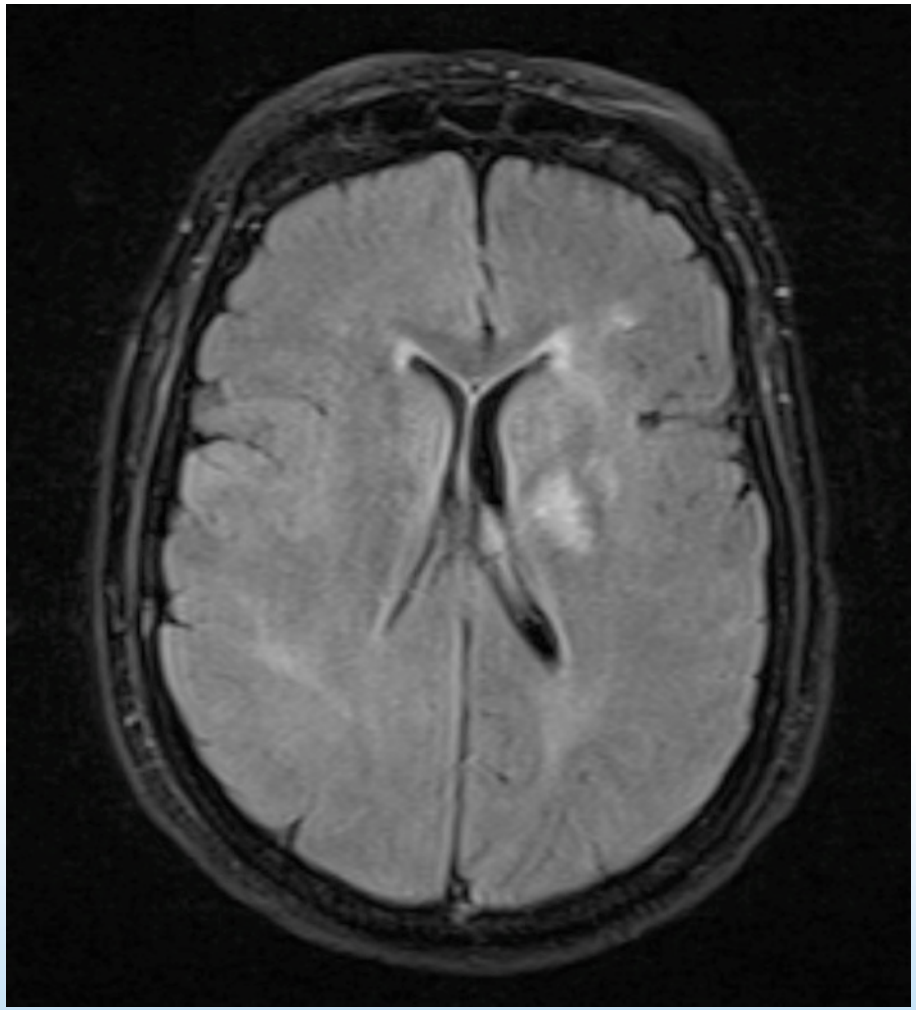

Diffuse glioma
Histologic features
IDH1/IDH2 status
IDH-mutant
IDH-wildtype
1p/19q status
1p/19q-codeleted
1p/19q-Intact
Frequent alterations
ATRX-mutant
TP53-mutant
Histologic grade
Grades 2 and 3
Grades 2 and 3
Grade 4
Grade 4
Grades 2 and 3
Alterations associated with molecular grading
CDKN2A/B homozygous deletion
TERT promoter mutation
EGFR amplification
Polysomy 7 / Monosomy 10
None
DIAGNOSIS
(Anaplastic) Oligodendroglioma, IDH-mutant and 1p/19q-codeleted, WHO grade II (or III)
(Anaplastic) Astrocytoma, IDH-mutant, WHO grade II (or III)
Astrocytoma, IDH-mutant, WHO grade 4 *
Glioblastoma, IDH-wildtype, WHO grade IV
(Anaplastic) Astrocytoma, IDH-wildtype, WHO grade II (or III)
Other diagnoses to consider and rule out
Pediatric-type gliomas, especially in younger adults
Non-infiltrating gliomas

Diffuse glioma (pediatric type)

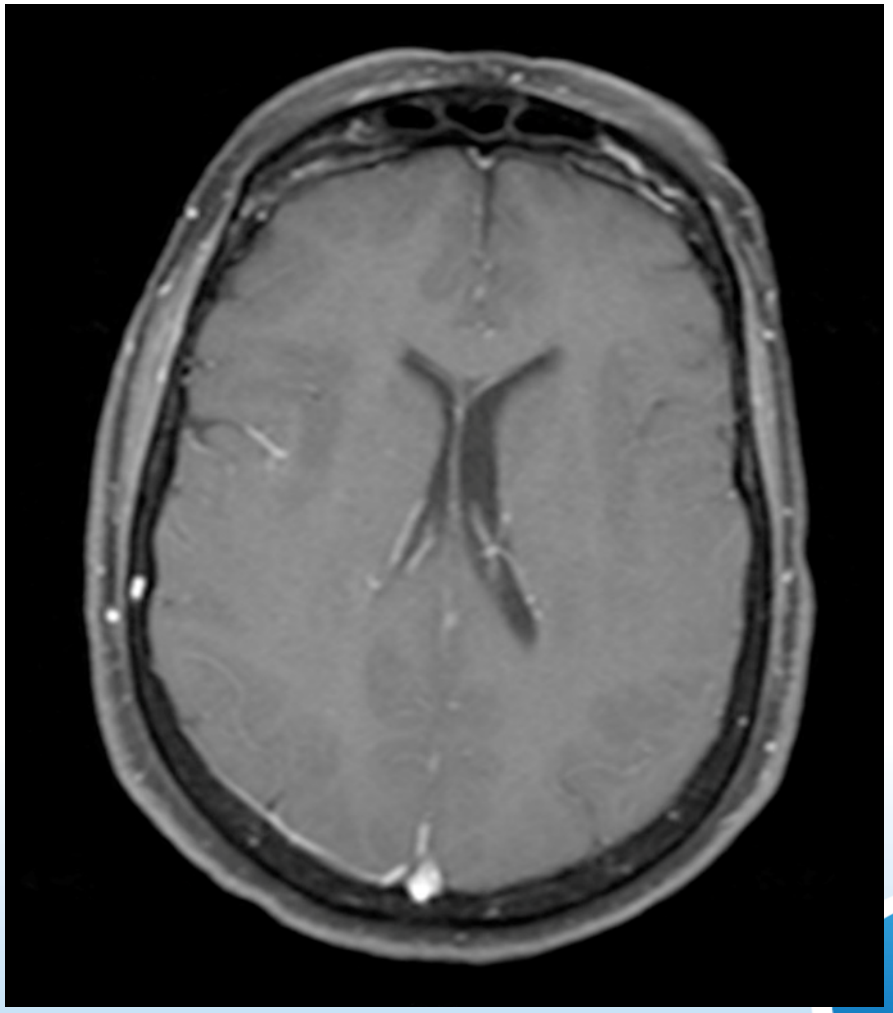
cIMPACT-NOW #2

Louis DN, et al. cIMPACT-NOW update 2. Acta Neuropathologica 2018;135(4):639-642. PMID: 29497819

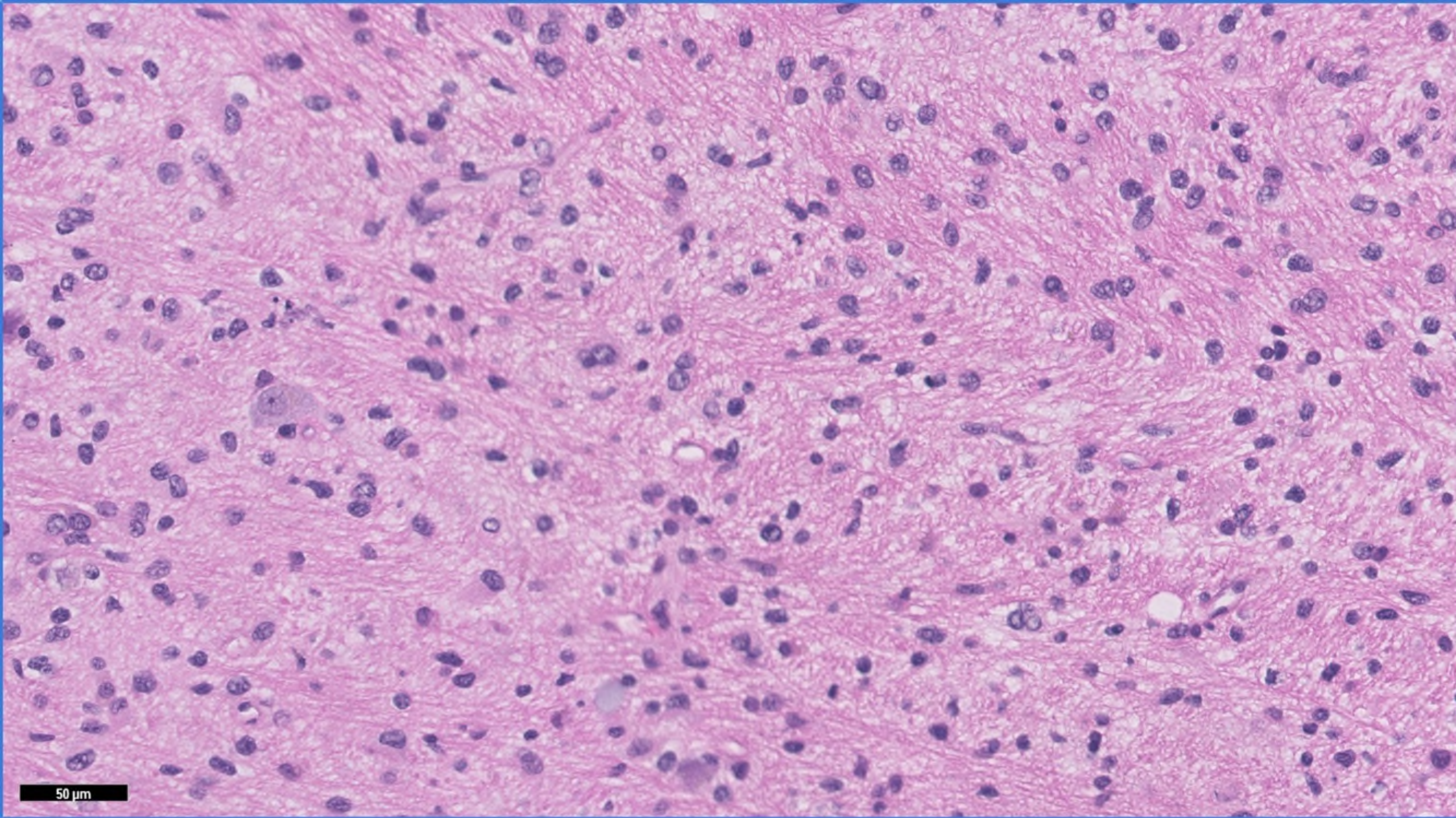
Case 6: 72-year-old man with altered mental status

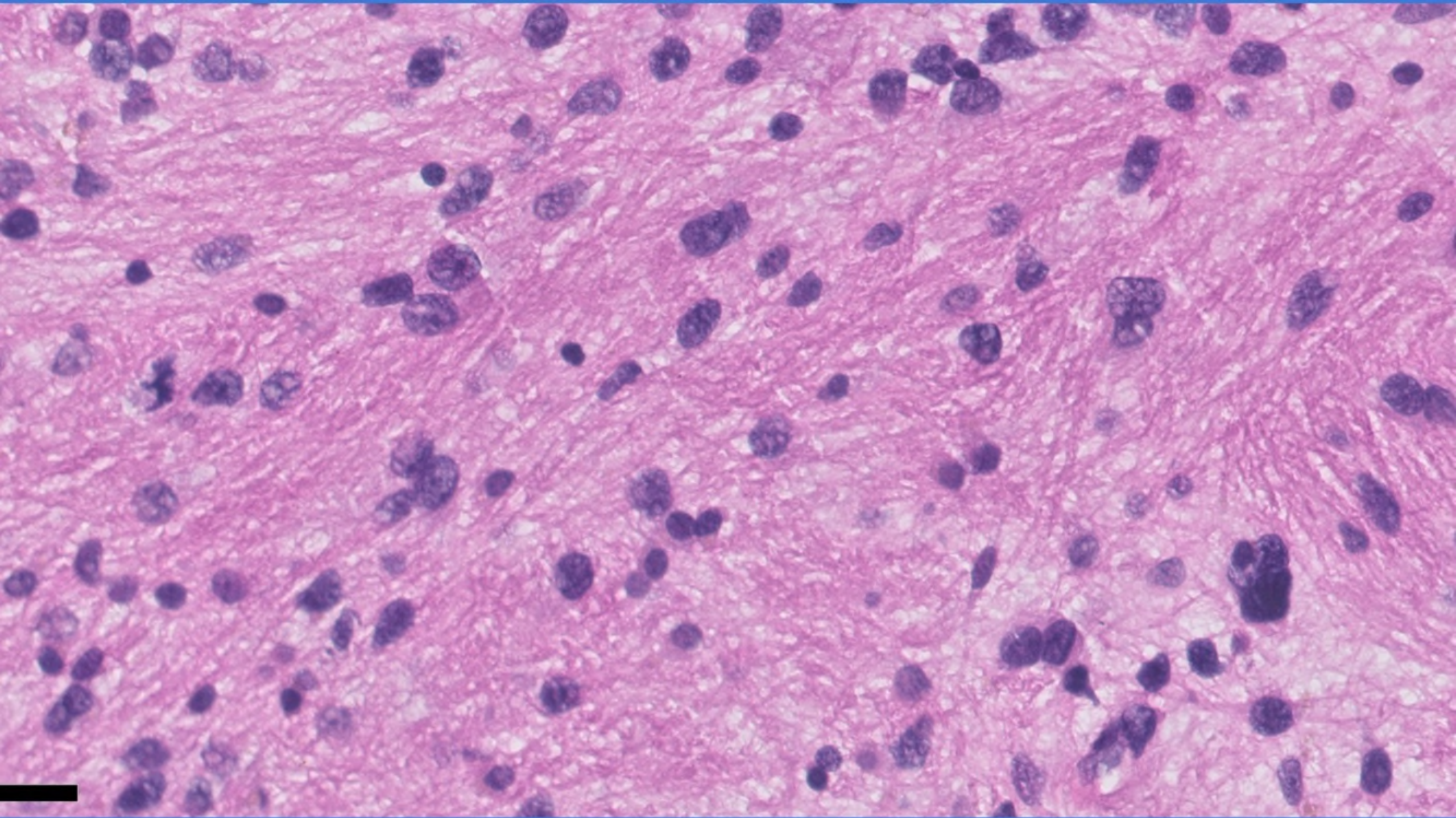


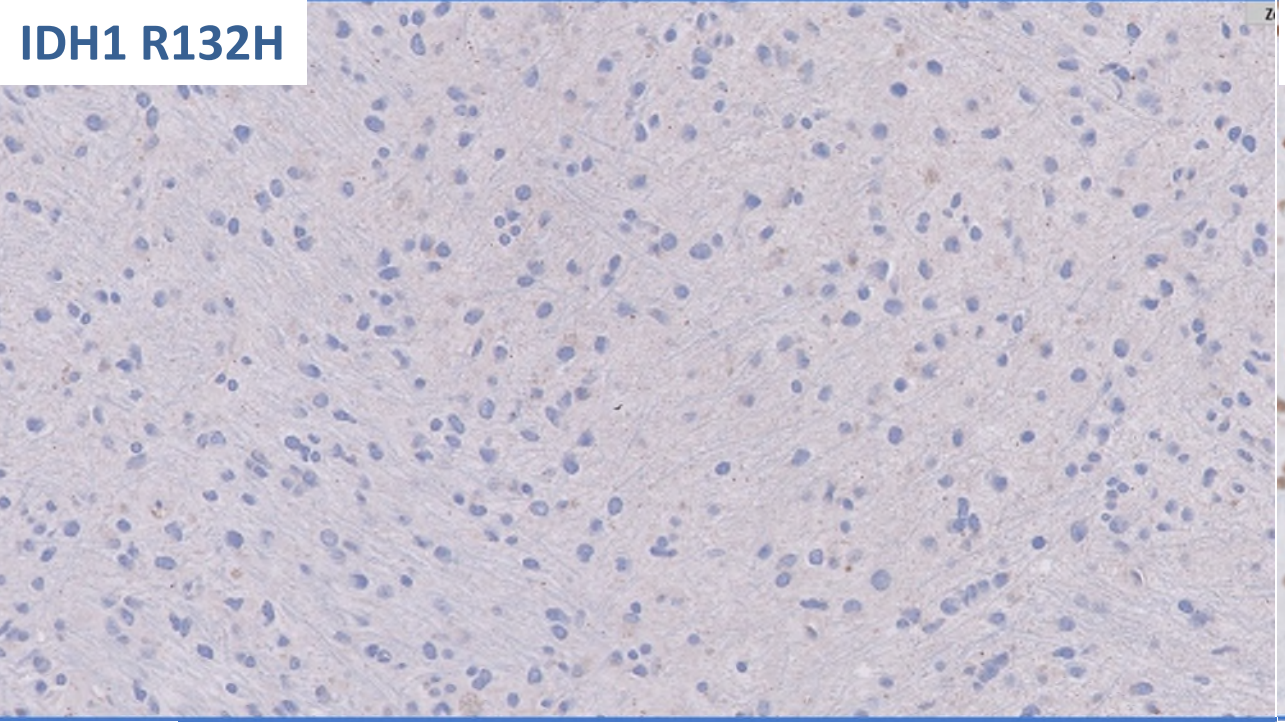
Axial T2-FLAIR

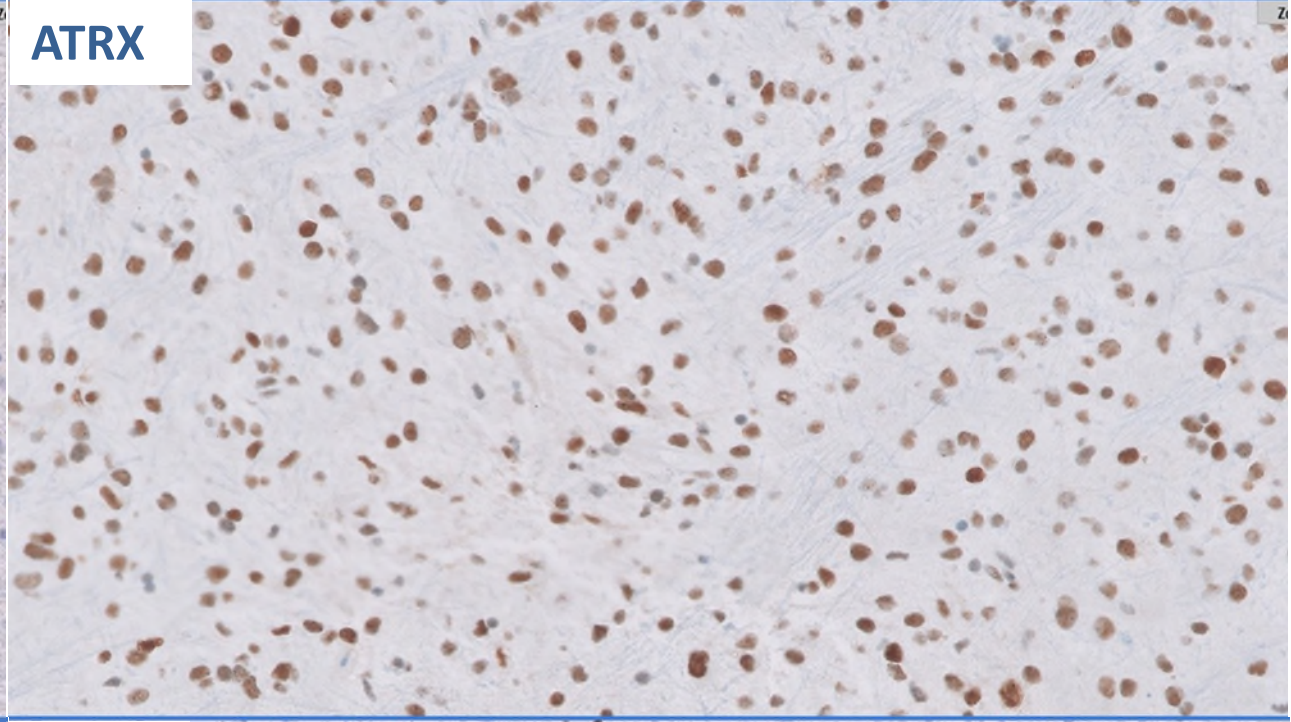


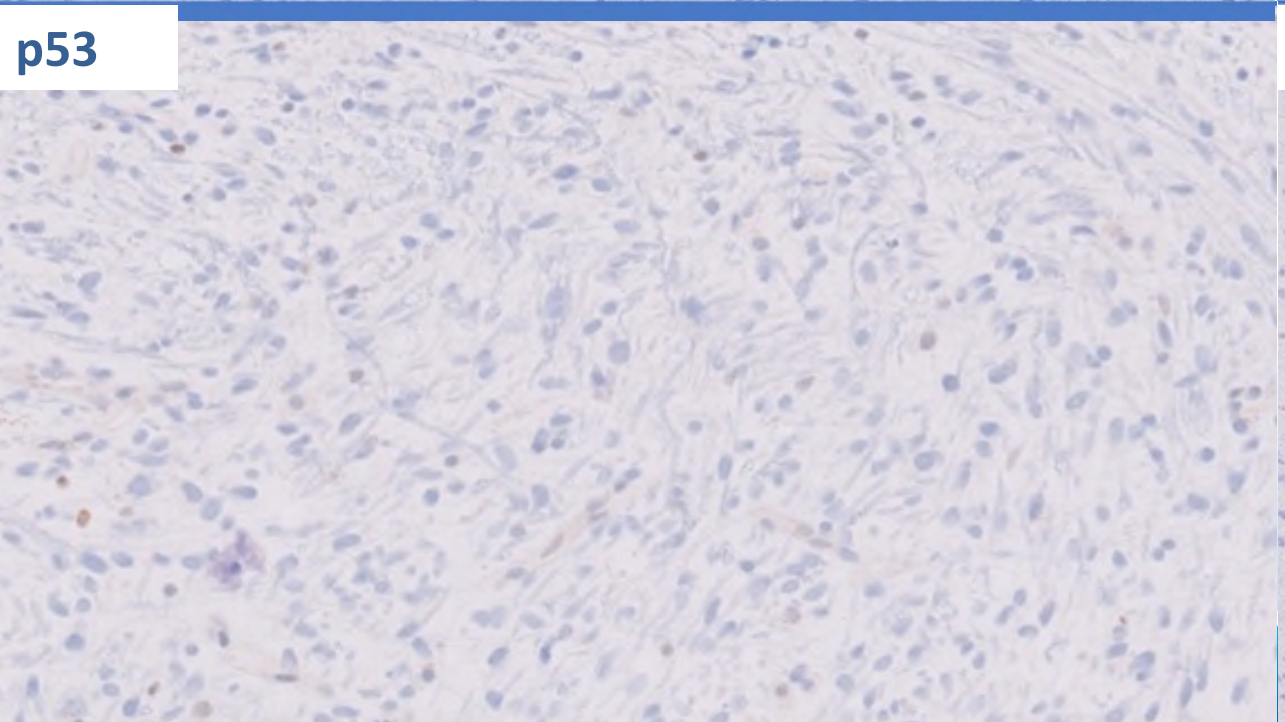
Axial T1-contrast

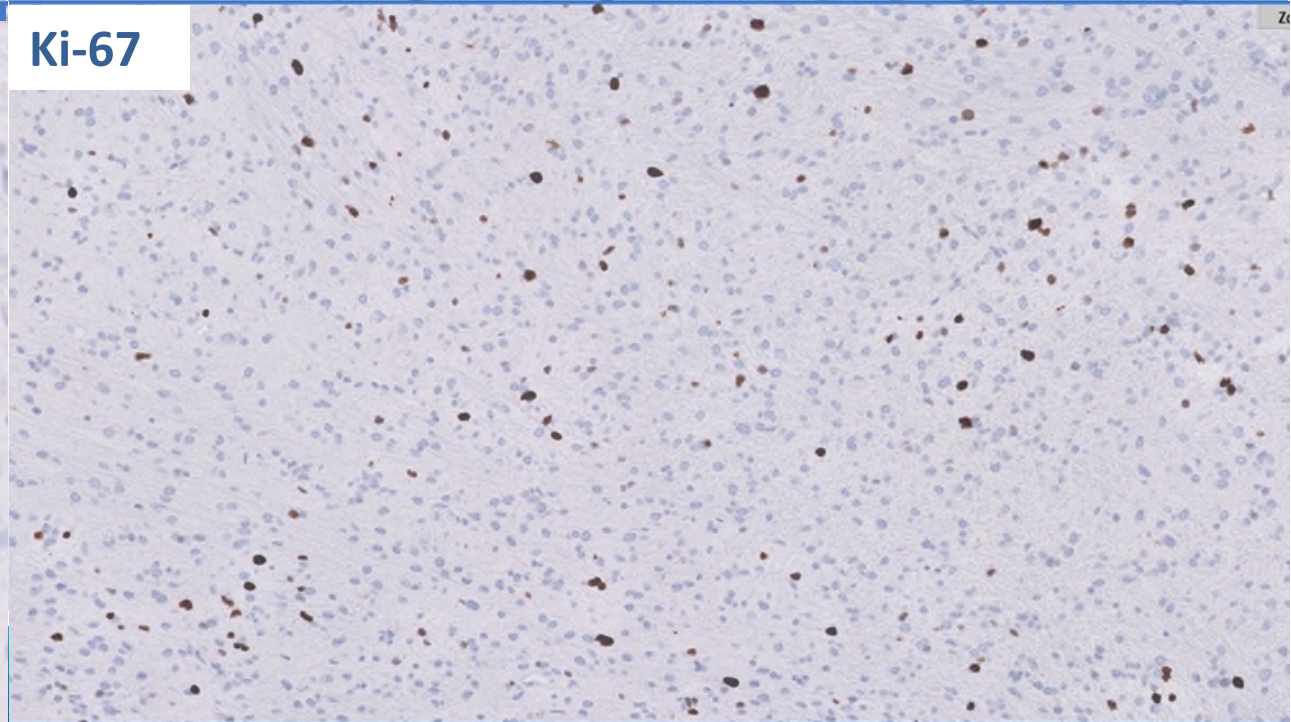

50 µm



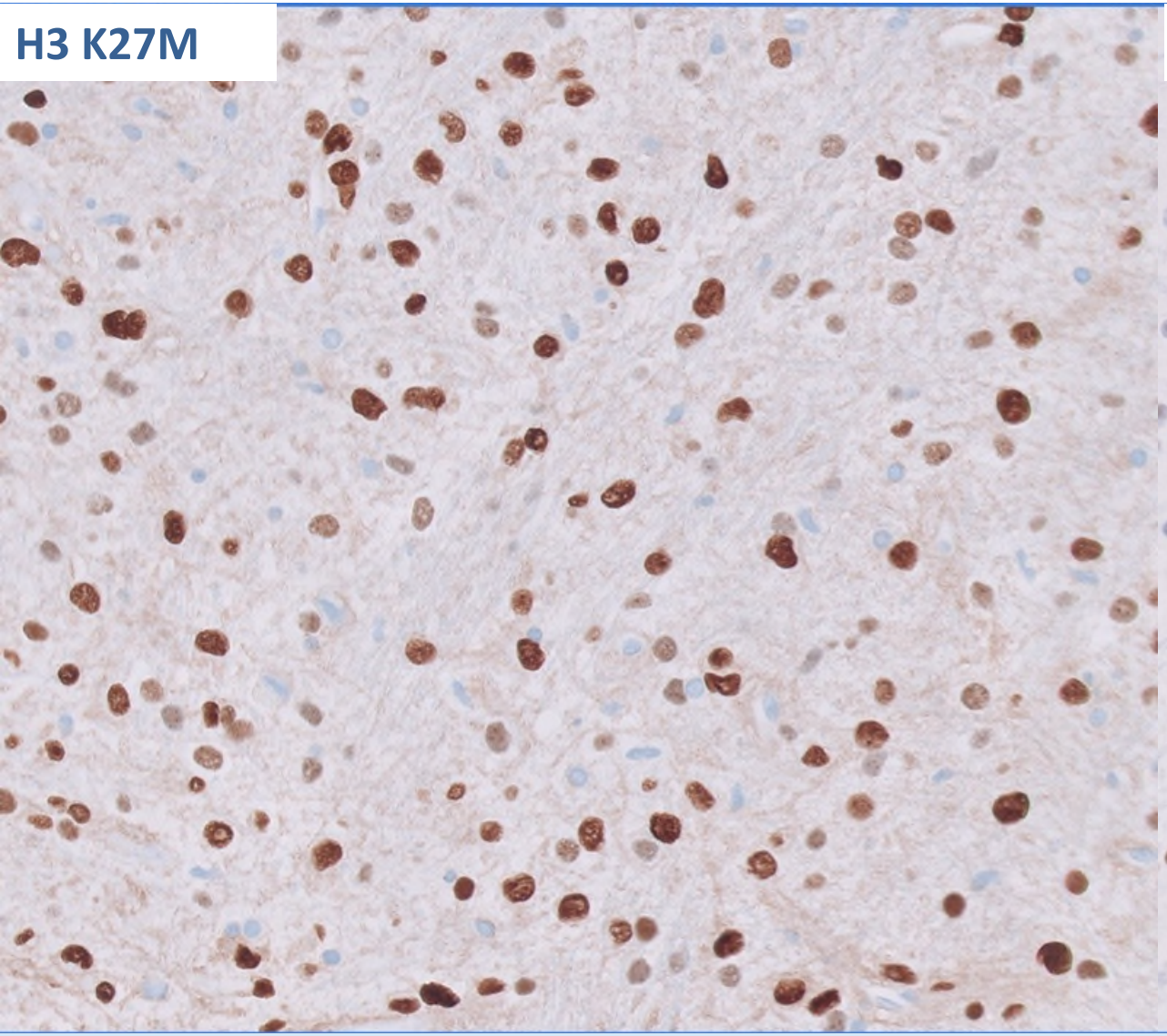

IDH1 R132H


ATRX

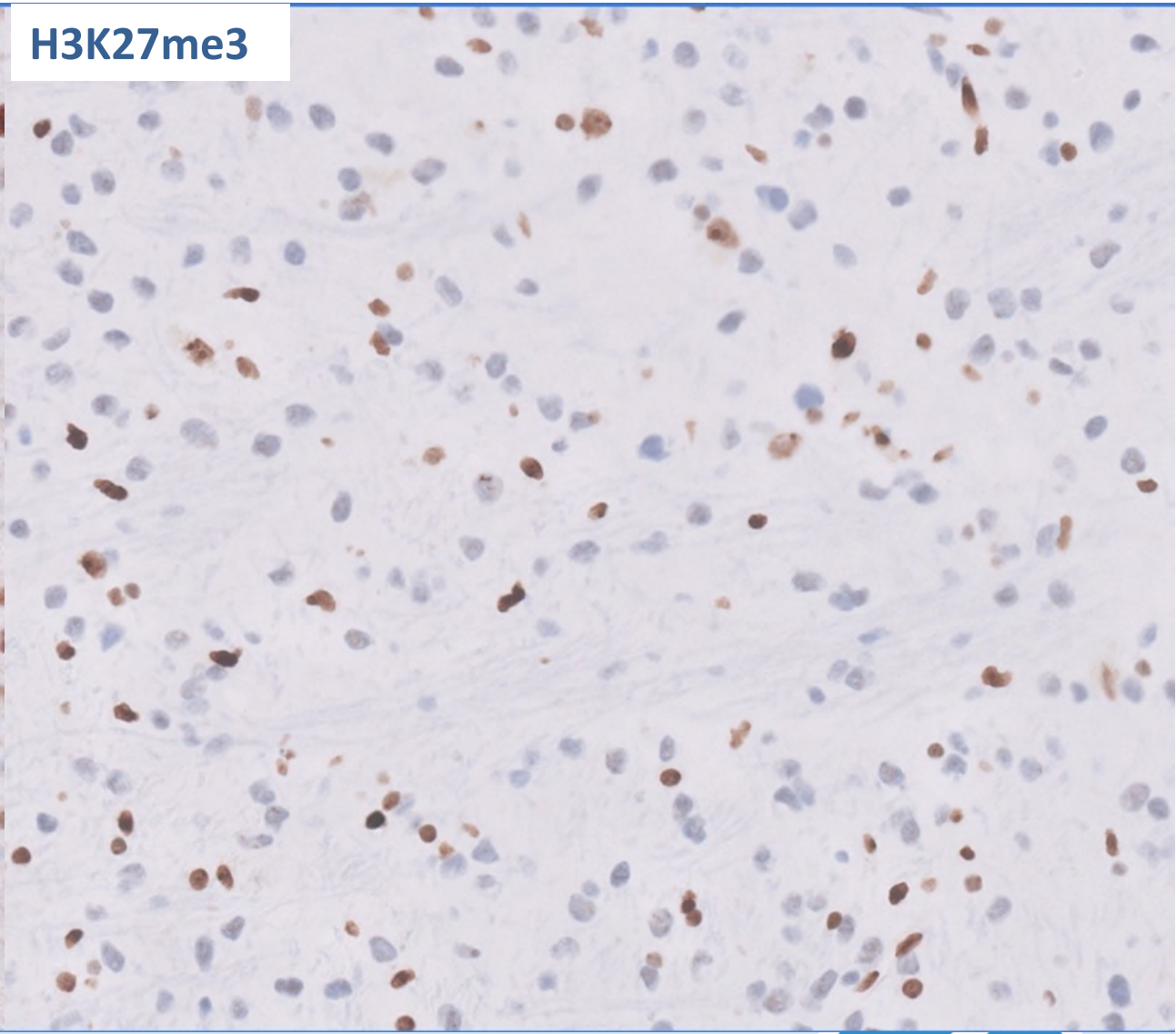

p53


Ki-67

H3 K27M



H3K27me3

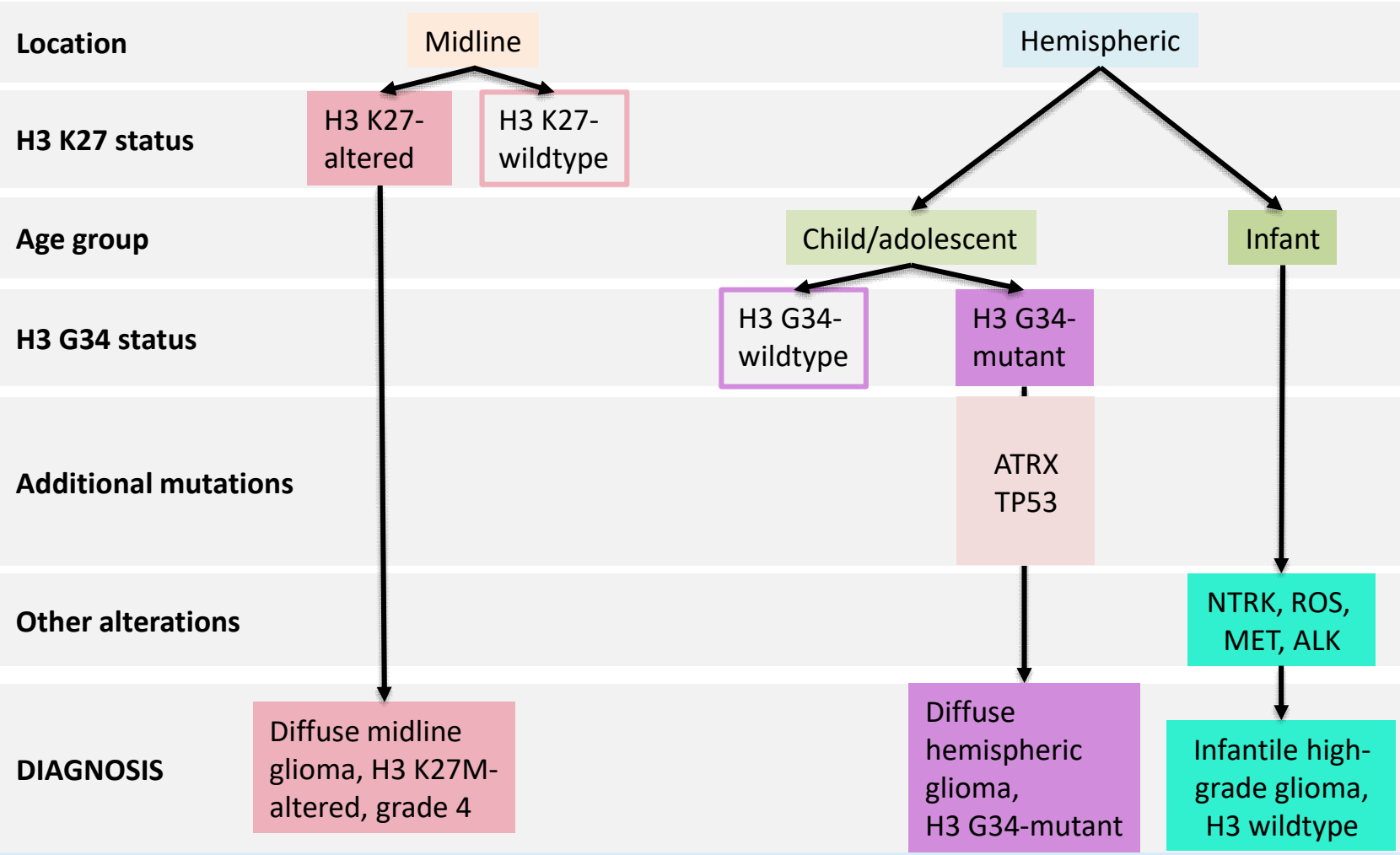


Any age, midline

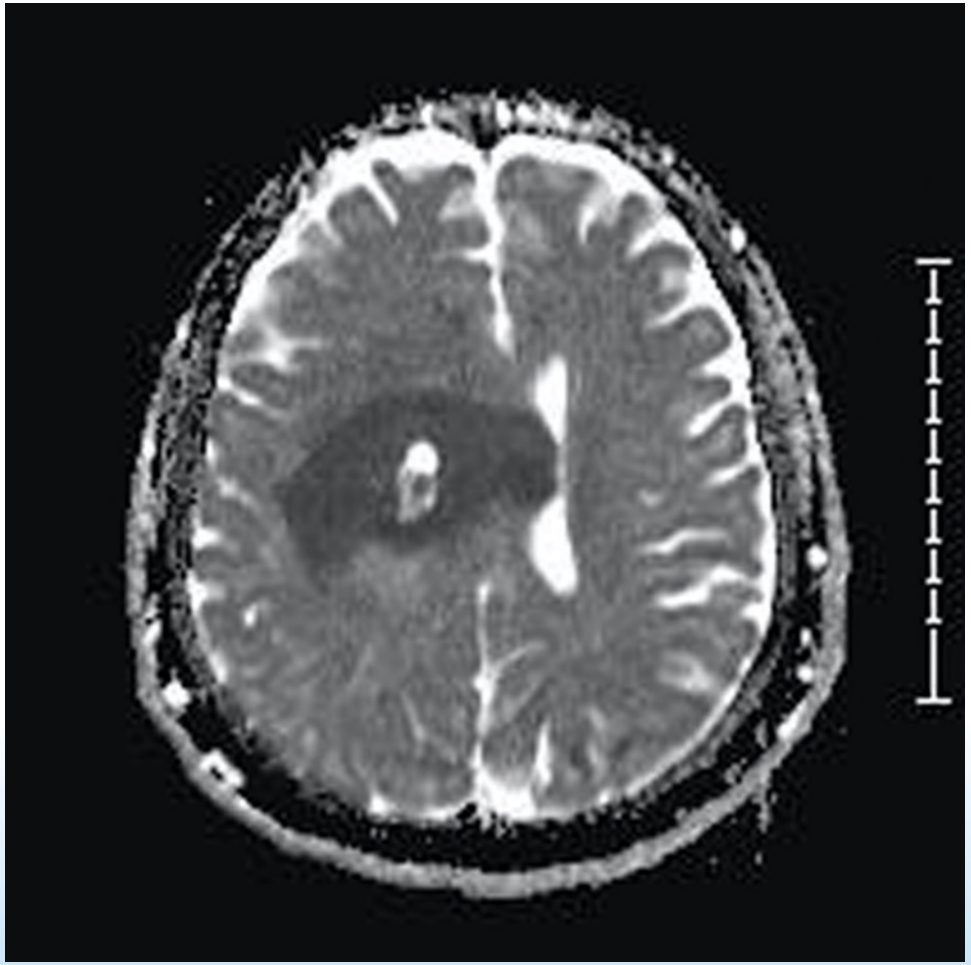
- Low-grade or high-grade histology
- IDH1R132H (-), ATRX (+/-), p53 (+/-)
- H3 K27M (+), H3K27me3-loss

Diffuse midline glioma, H3 K27M-mutant, WHO grade 4

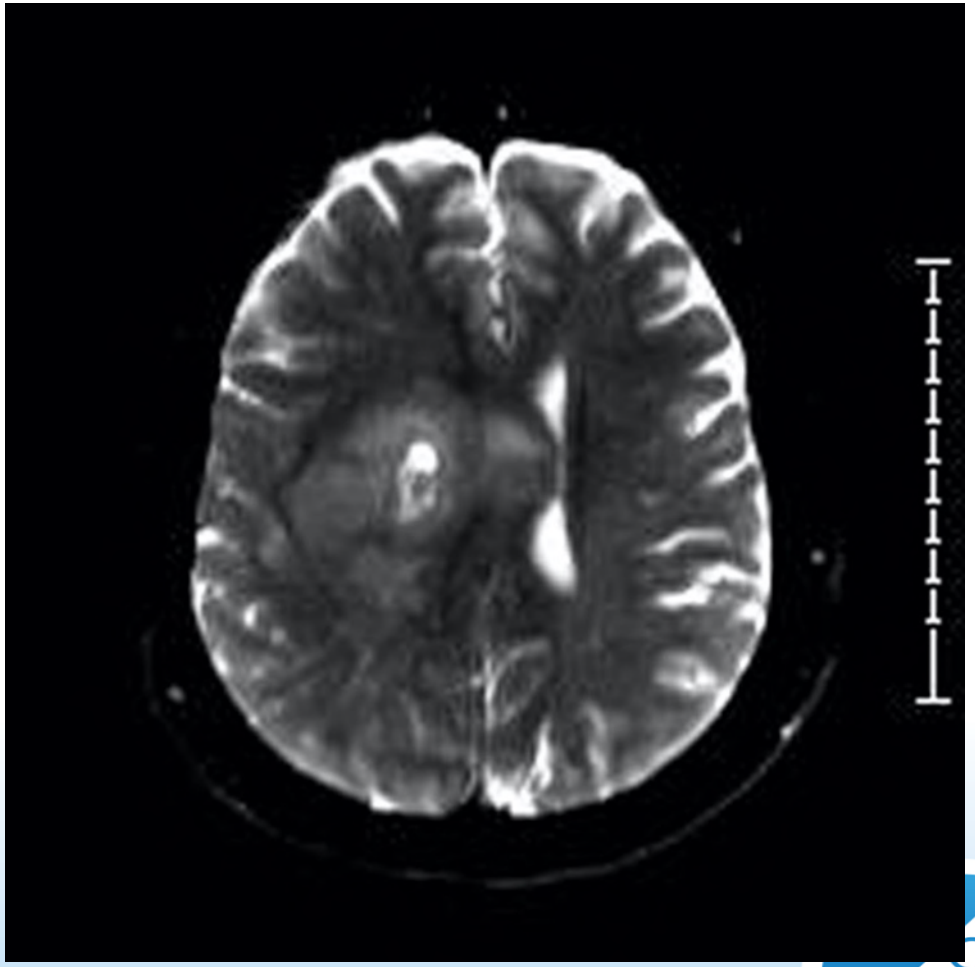
Diffuse glioma (pediatric type)



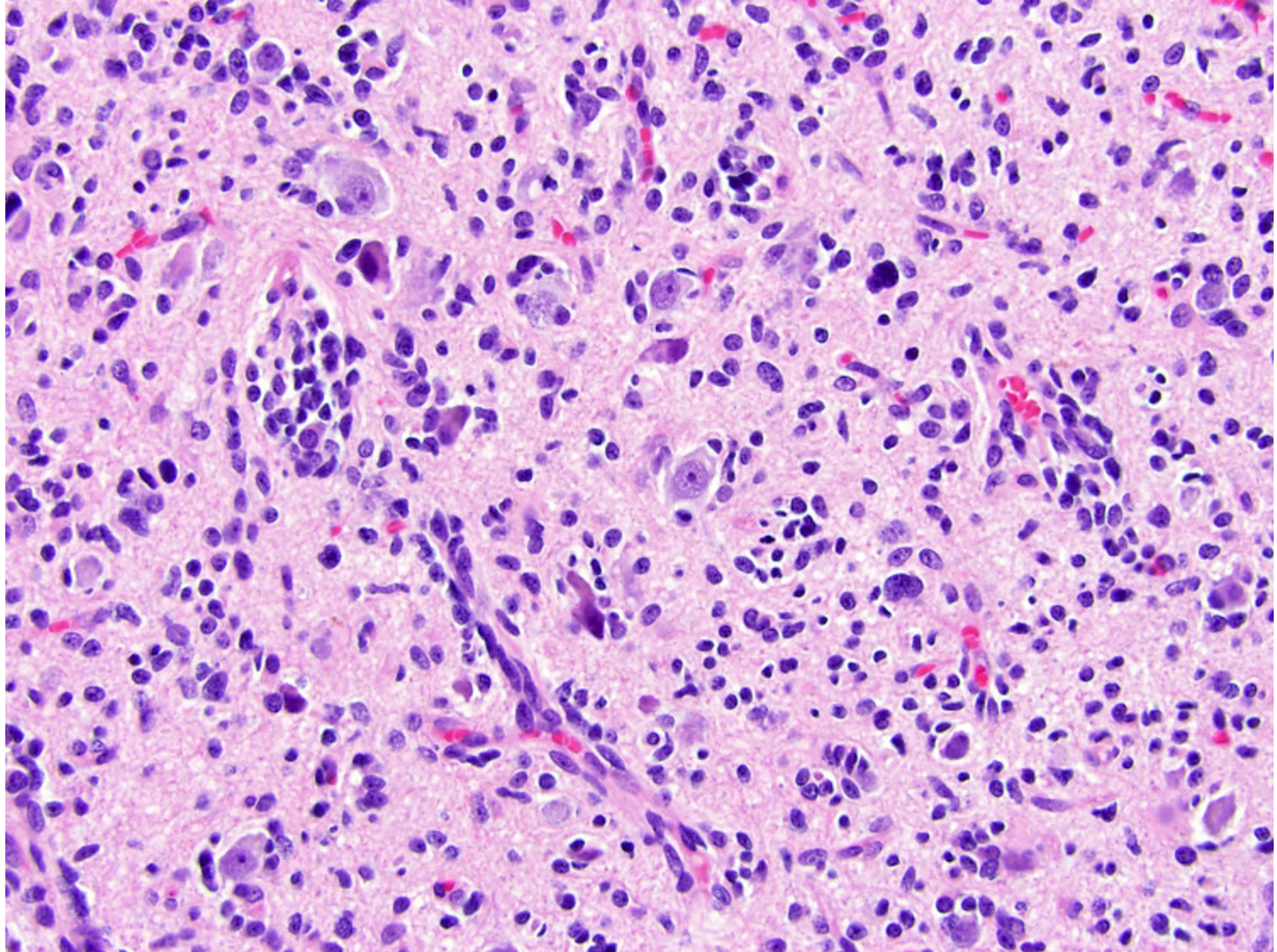
Case 7: 27-year-old woman

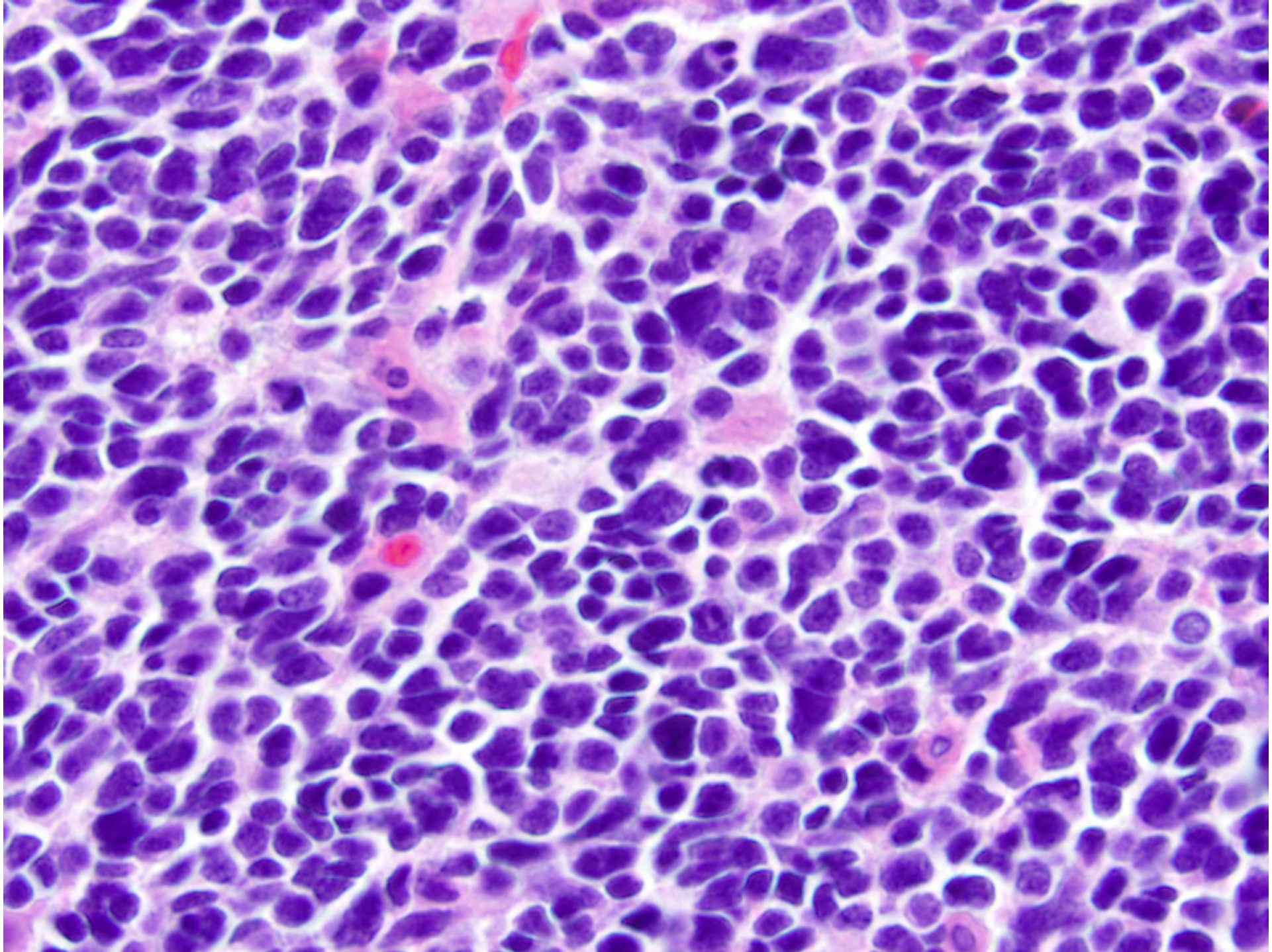


Apparent diffusion coefficient (ADC)

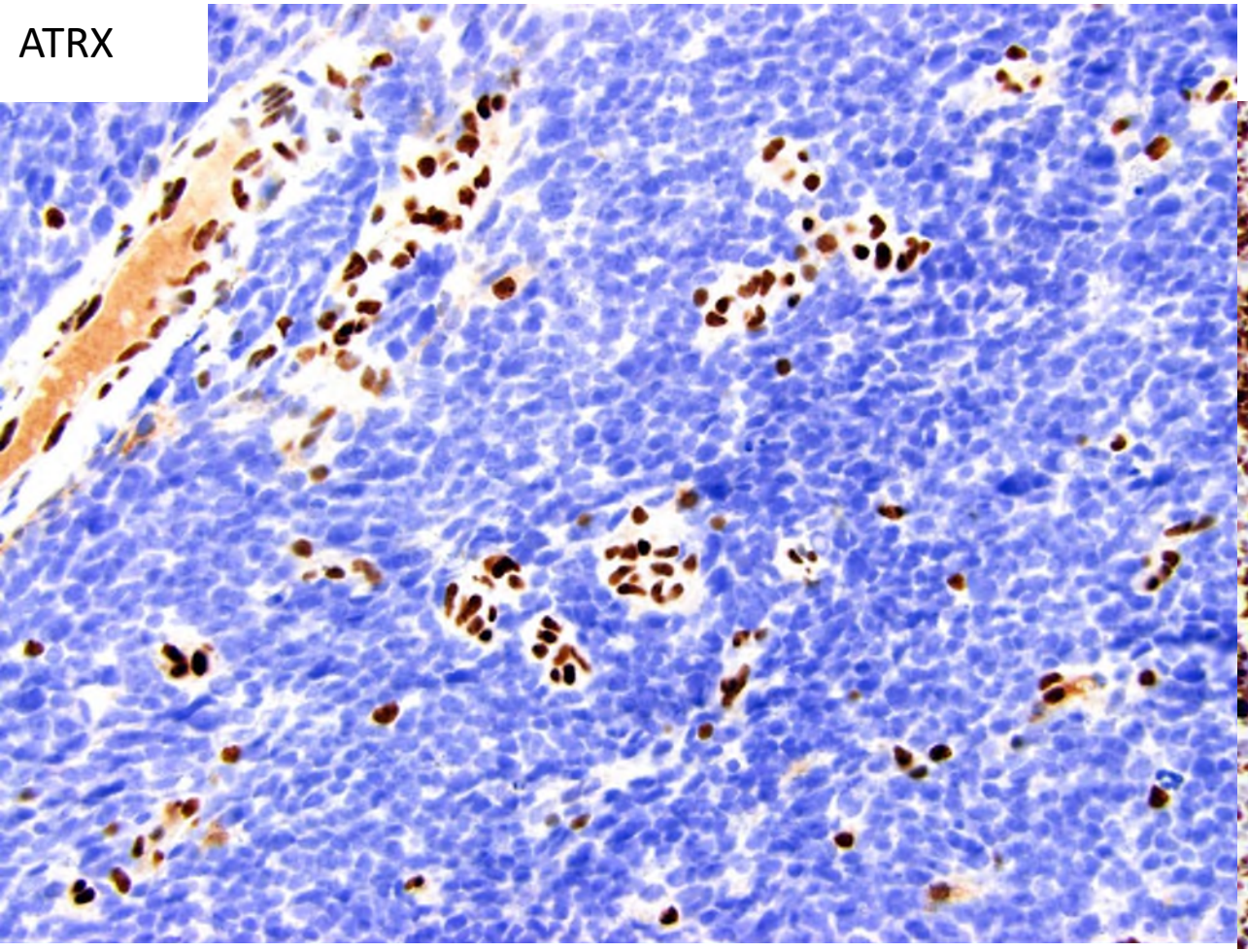


Diffusion weighted Images (DWI)

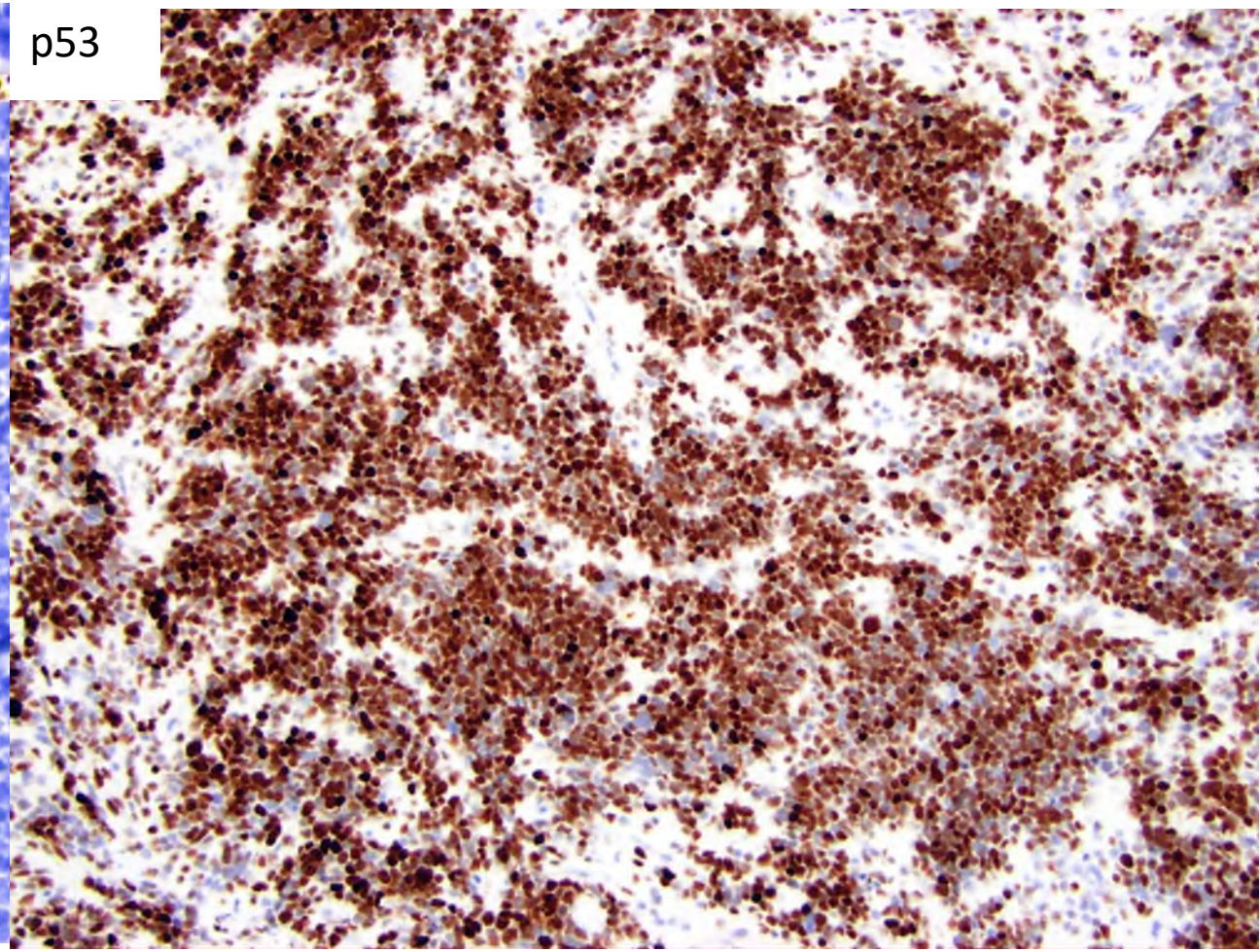




ATRX



p53



Glioblastoma, IDH-wildtype, WHO grade IV ???

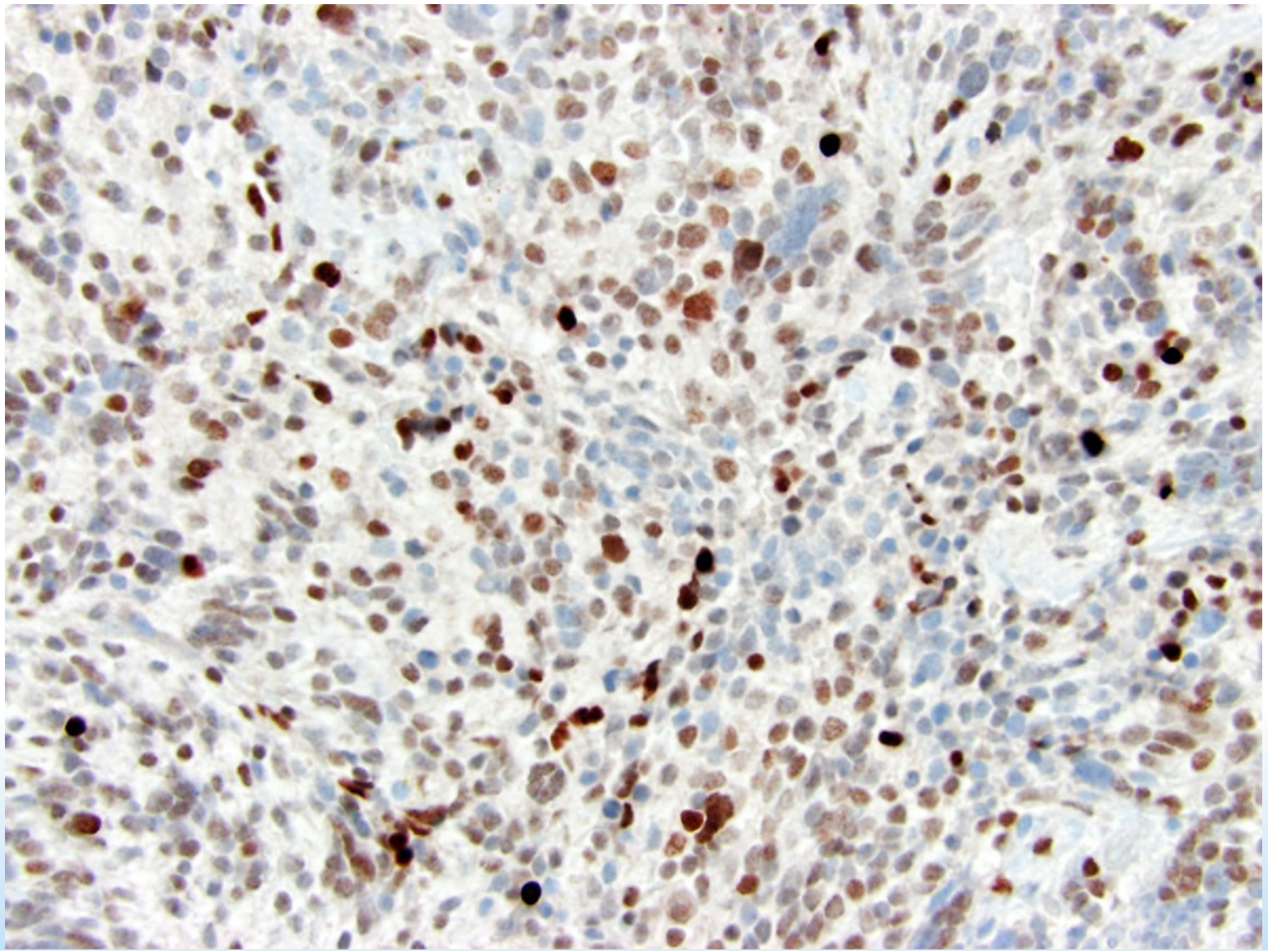
(Young) adult, hemispheric, enhancement (+/-)

- High-grade histology (but may be low-grade)
- IDH1 R132H (-), ATRX-loss, p53 (+)
- IDH1/2 sequencing

OR

- H3 G34R/V ?

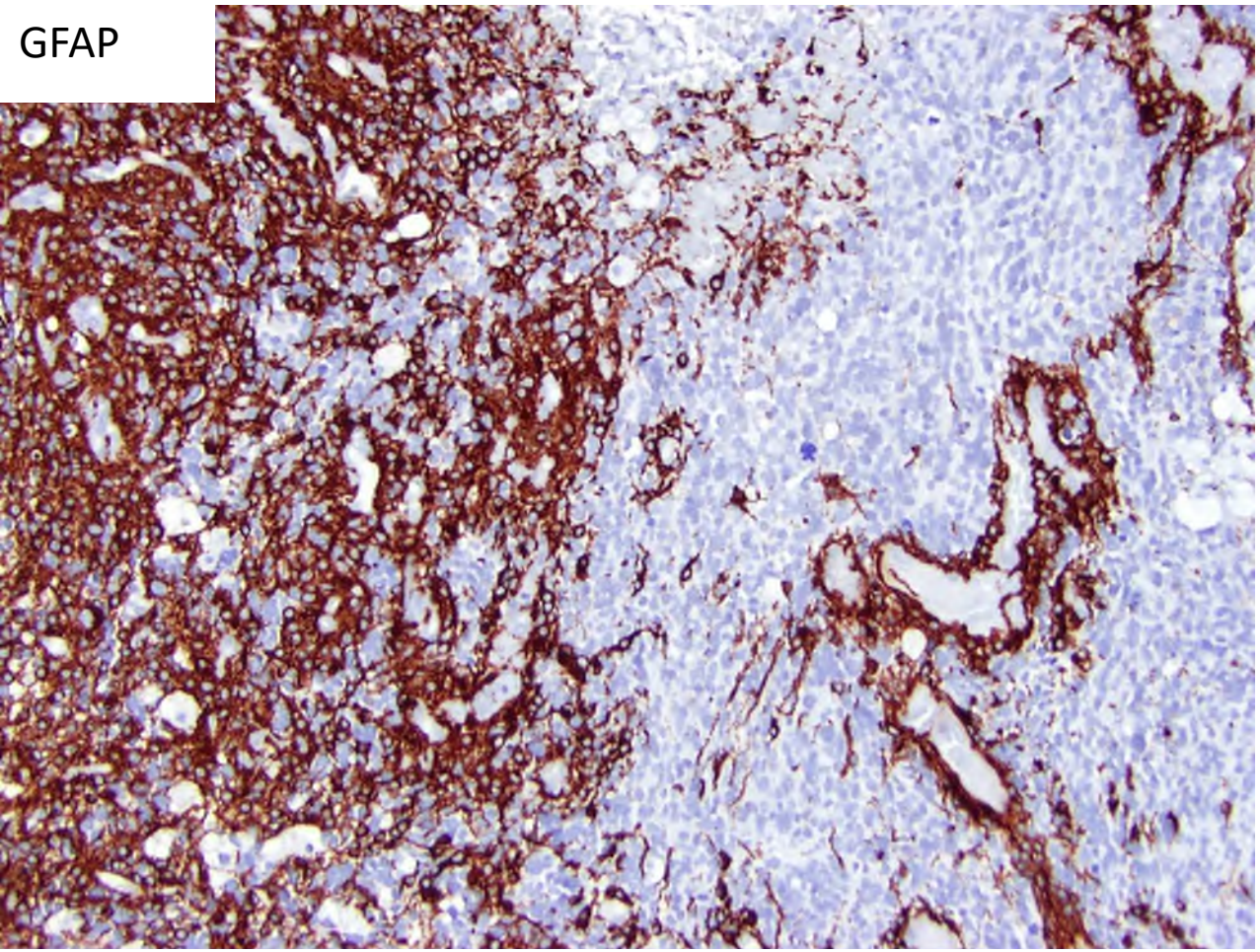
H3 G34 R/V cocktail



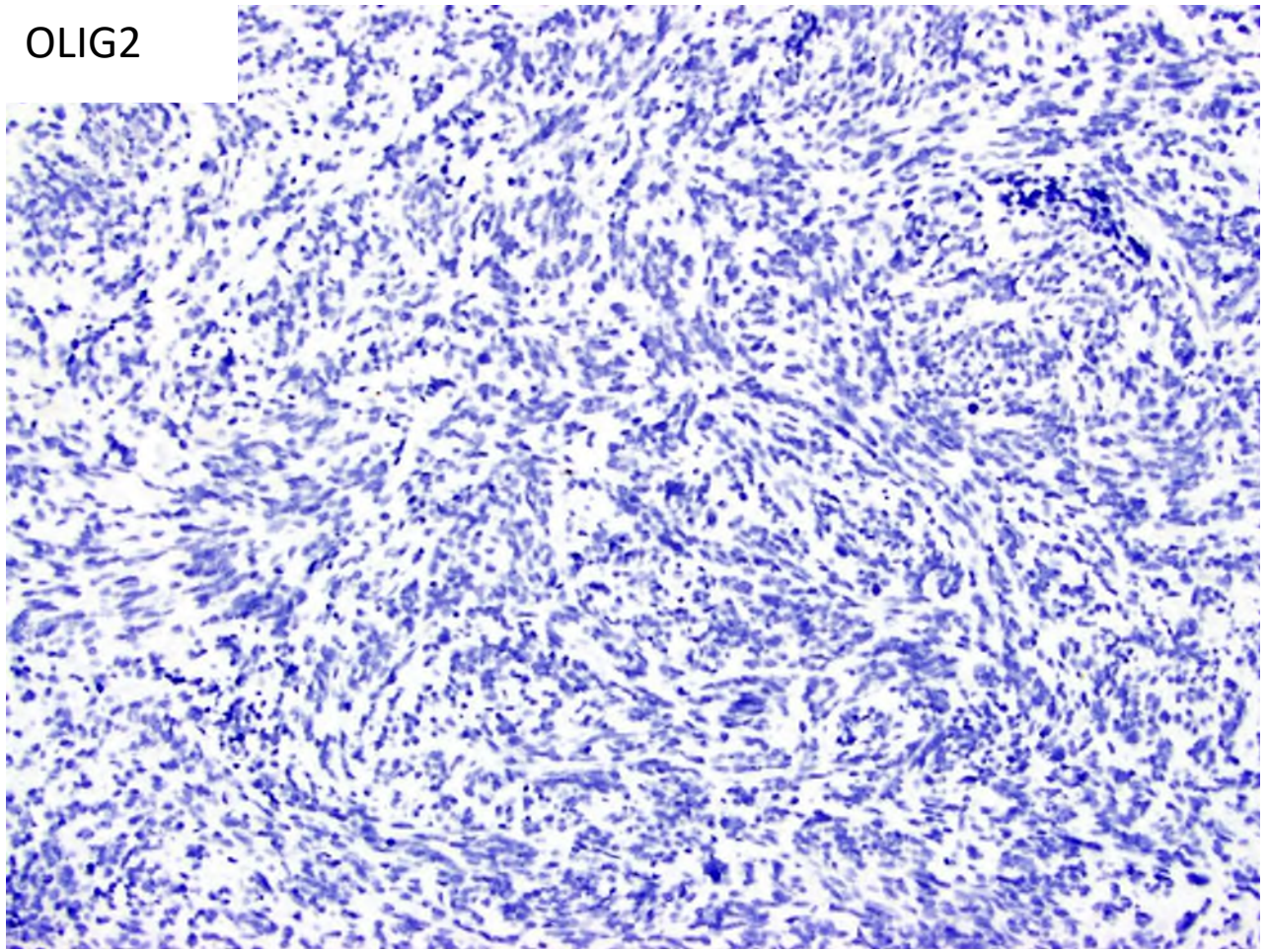
Diffuse hemispheric astrocytoma, H3 G34-mutant

WHO grade 4*

GFAP

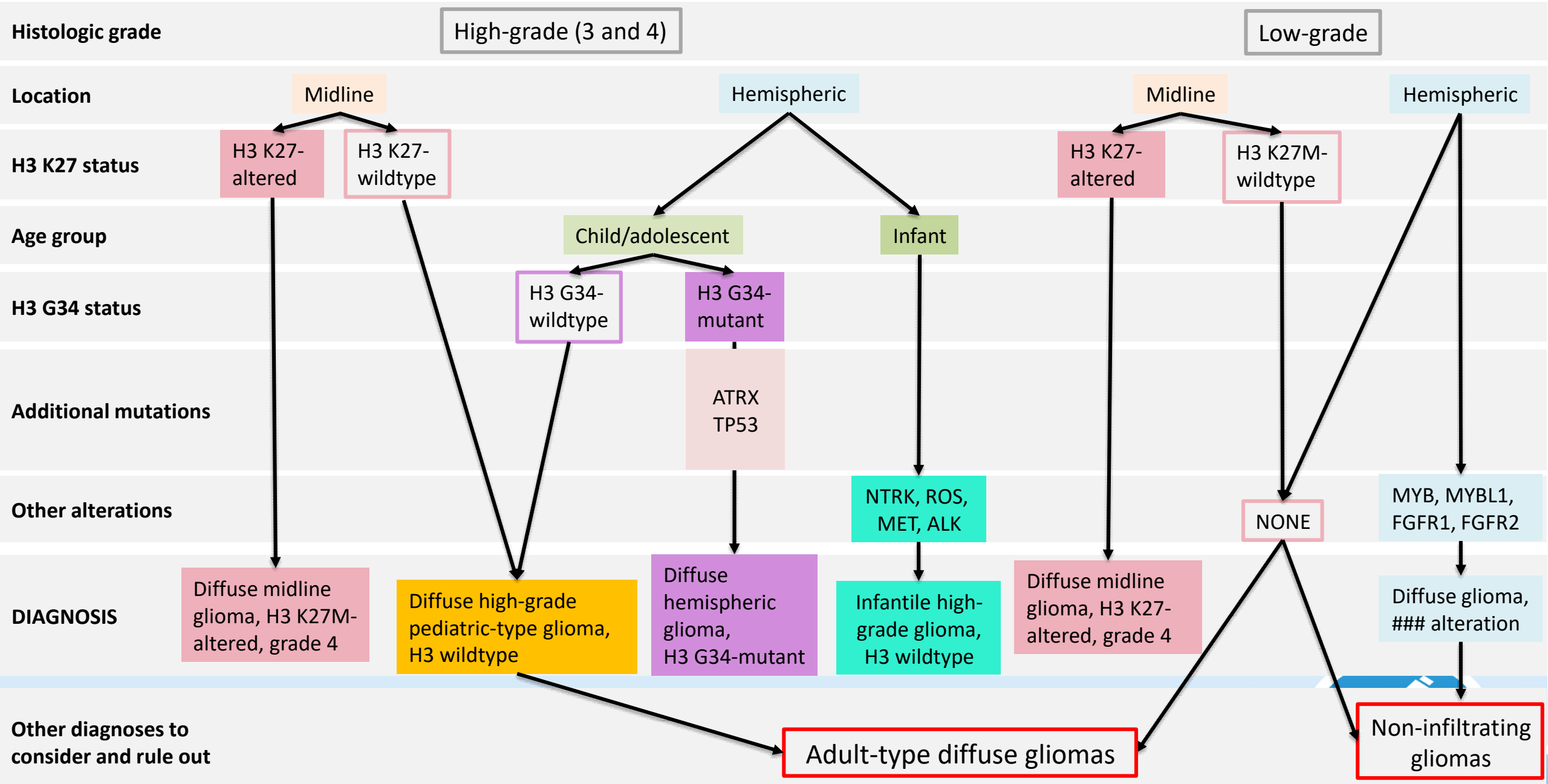


OLIG2



Diffuse glioma (pediatric type)

cIMPACT-NOW #4

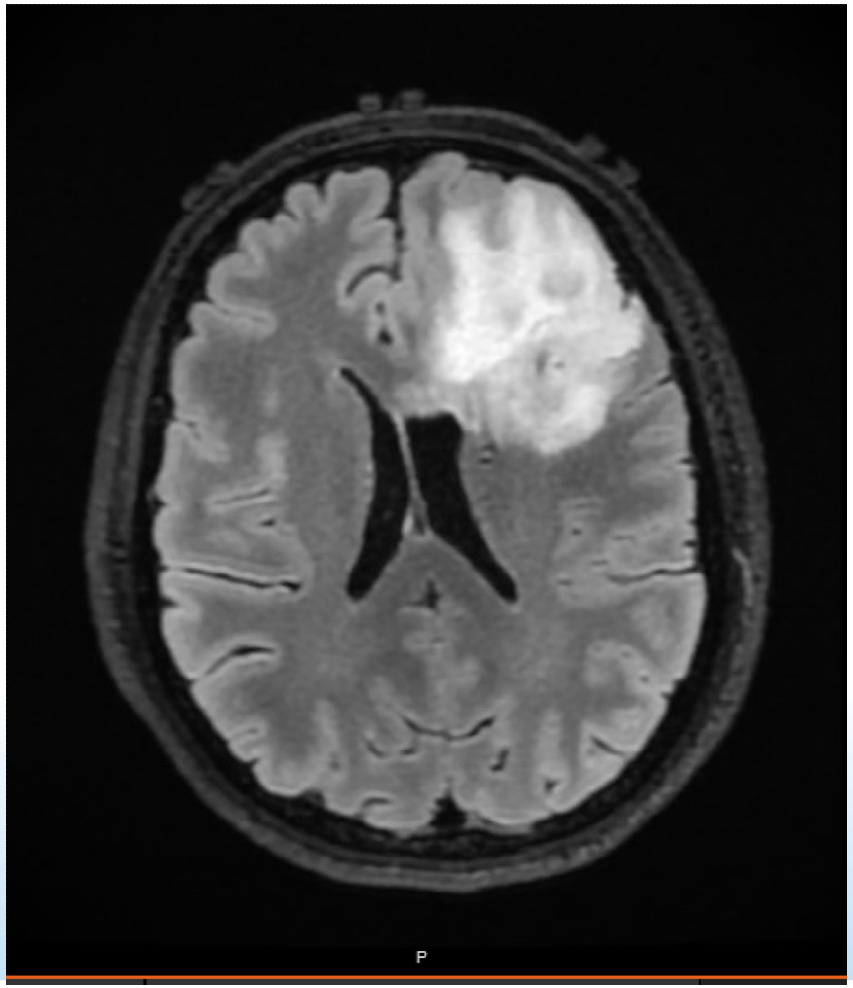


Work-up of diffuse gliomas

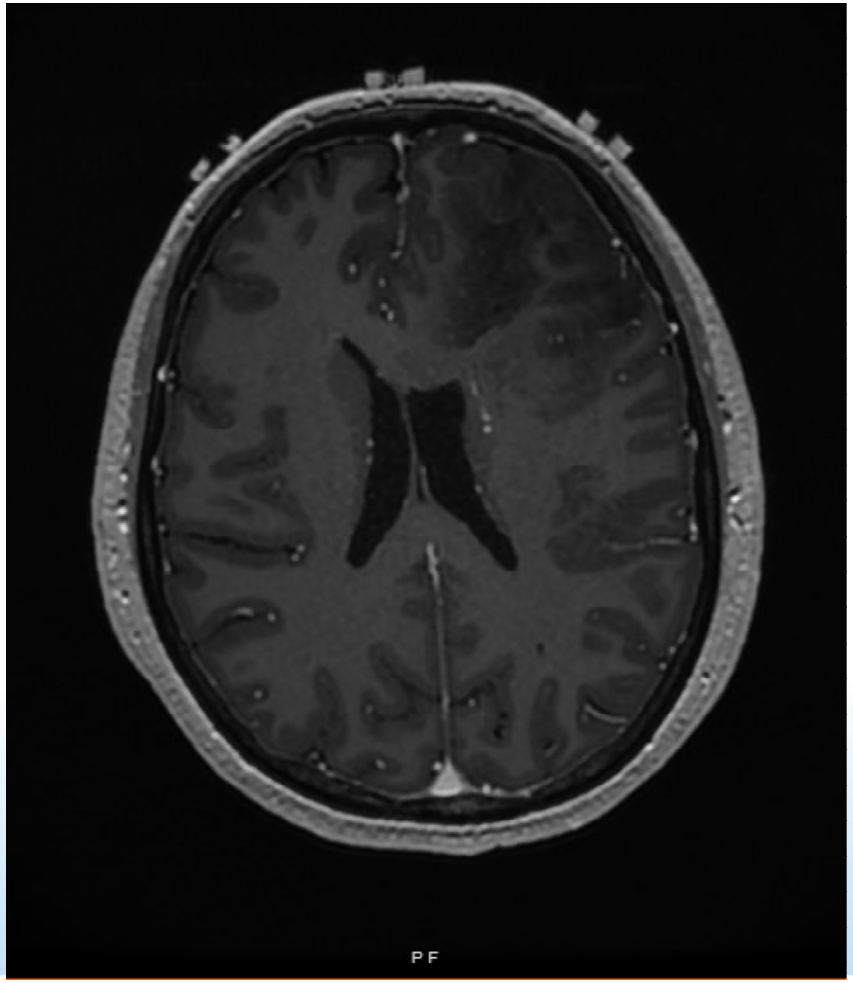
- Age, Location and Imaging
- Confirm that it is a diffuse glioma (IHC if needed)
- IDH1 R132H, ATRX, p53, Ki-67
- H3 K27M, H3K27me3, H3G34R/V
- Molecular tests

Case 8: 47-year-old woman

https://pathology.ucsf.edu/aanp-teaching-session
https://pathpresenter.net/#/public/display?token=bb734709



Axial T2-FLAIR



Axial T1-contrast

THANK YOU…

?

AMERICAN ASSOCIATION
OF NEUROPATHOLOGISTS

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