Improving central nervous system tumor diagnostics through methylation profiling

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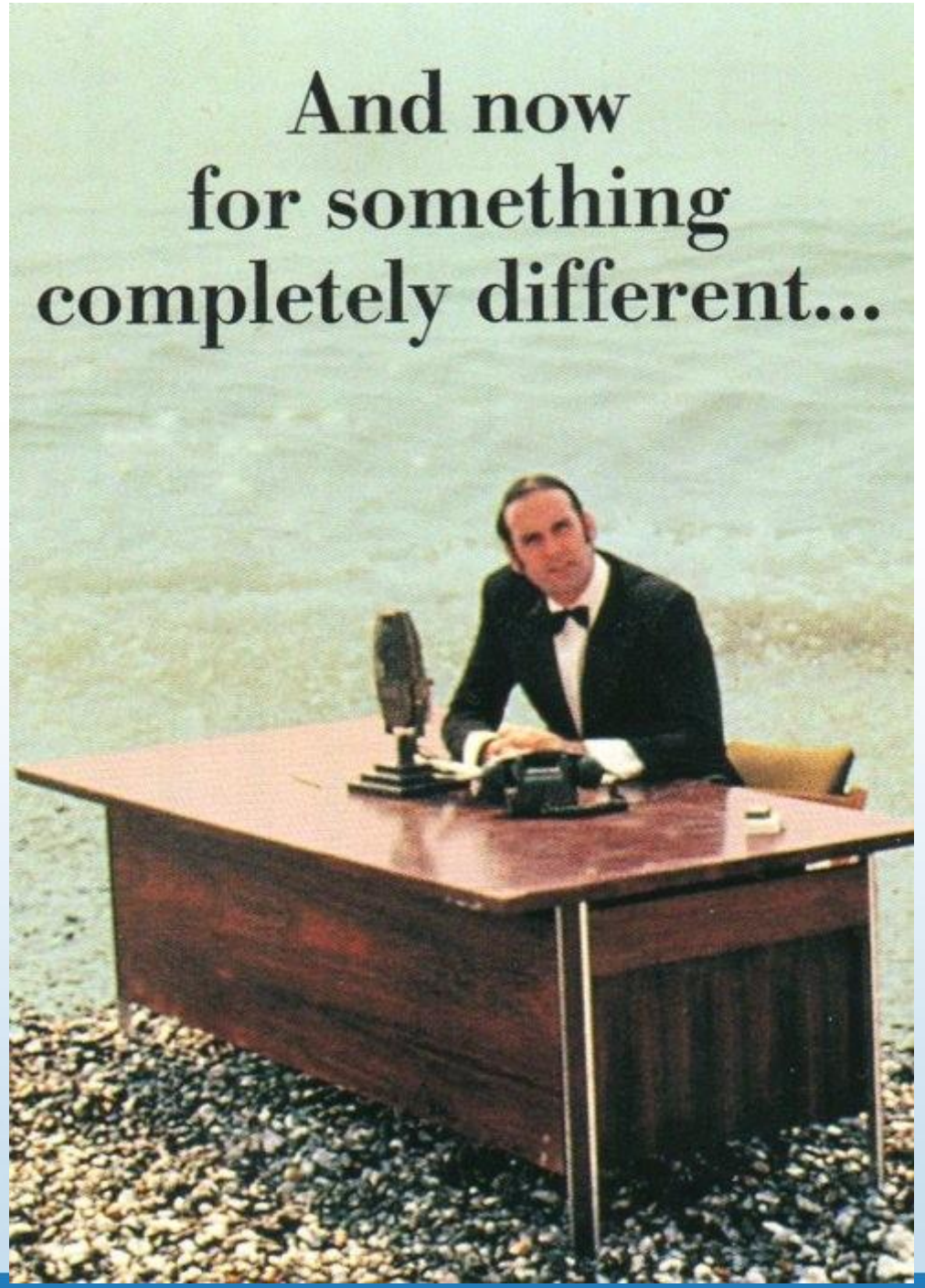
Disclosures

- I have no relevant financial relationships to disclose

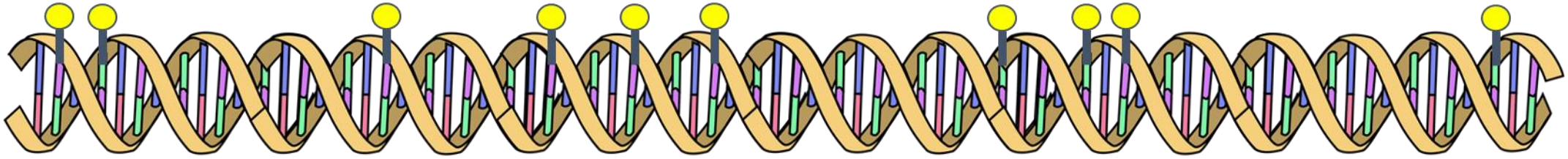
Learning Objectives

1. Describe the general principle of genomic DNA methylation profiling
2. Explain why genomic DNA methylation profiling works as a diagnostic tool
3. List some of the limitations of genomic DNA methylation profiling and why complementary molecular tests like next generation sequencing are still needed

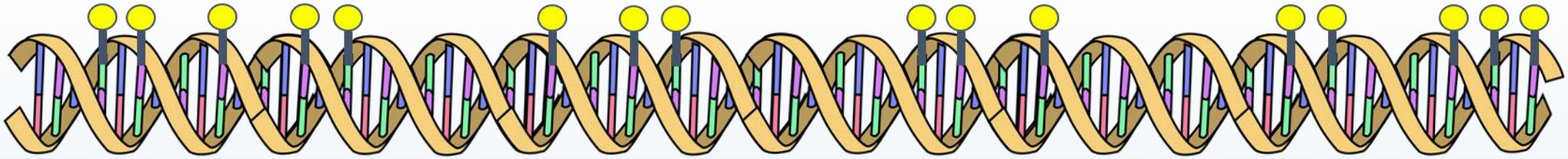

And now
for something
completely different...

methylation fingerprinting

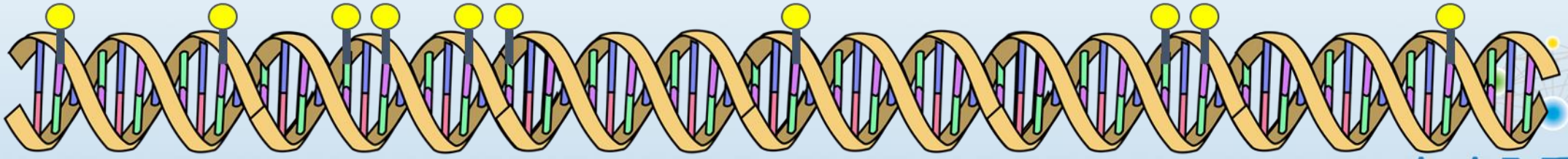
- DNA methylation is a “fingerprint” that shows cell-of-origin
- helps classify difficult tumors
- generates invaluable data for research



IDH^{wt} glioblastoma



IDH^{mut} astrocytoma



posterior fossa ependymoma type B

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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.

classifier development

- ~2800 CNS tumors
- Infinium 450K methylation
- random forest algorithm
  - combines many weak classifiers to make a strong one
- results
  - sensitivity = 0.989
  - specificity = 0.999
  - overall error rate = 1.14%

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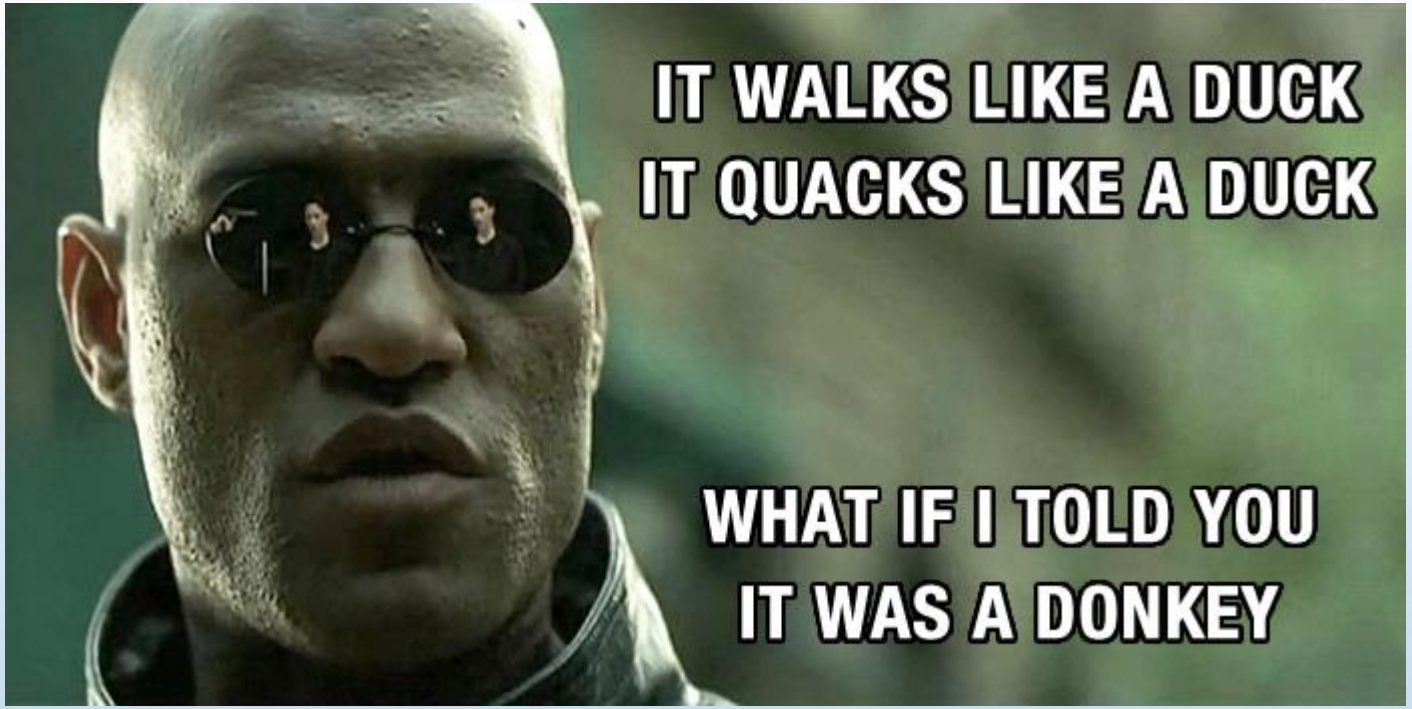
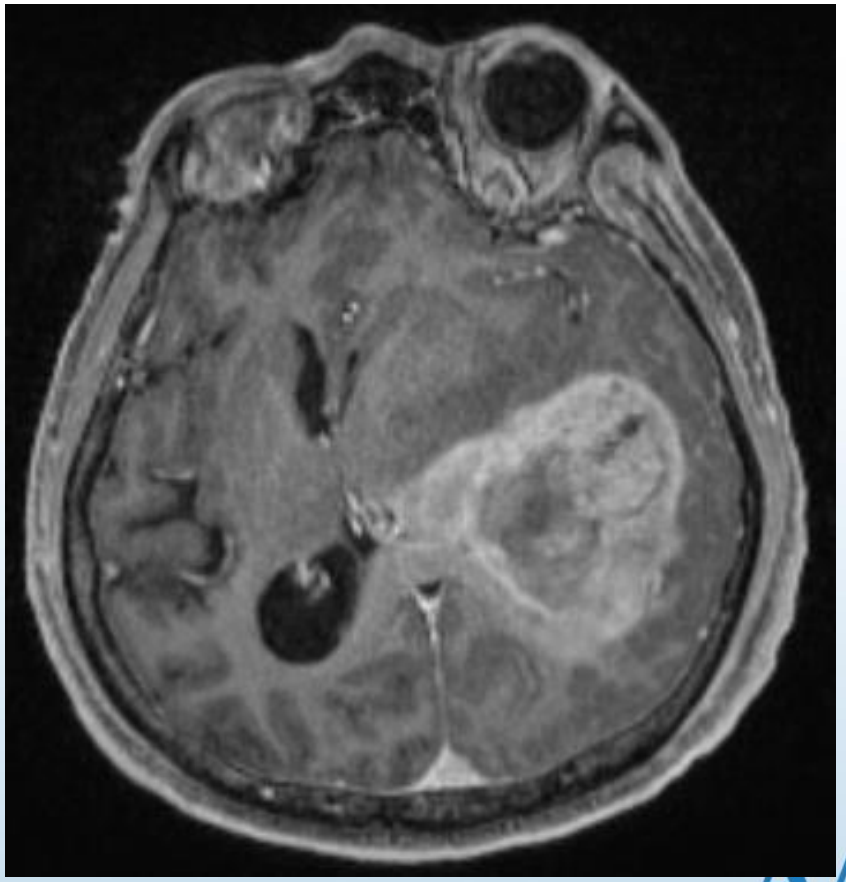
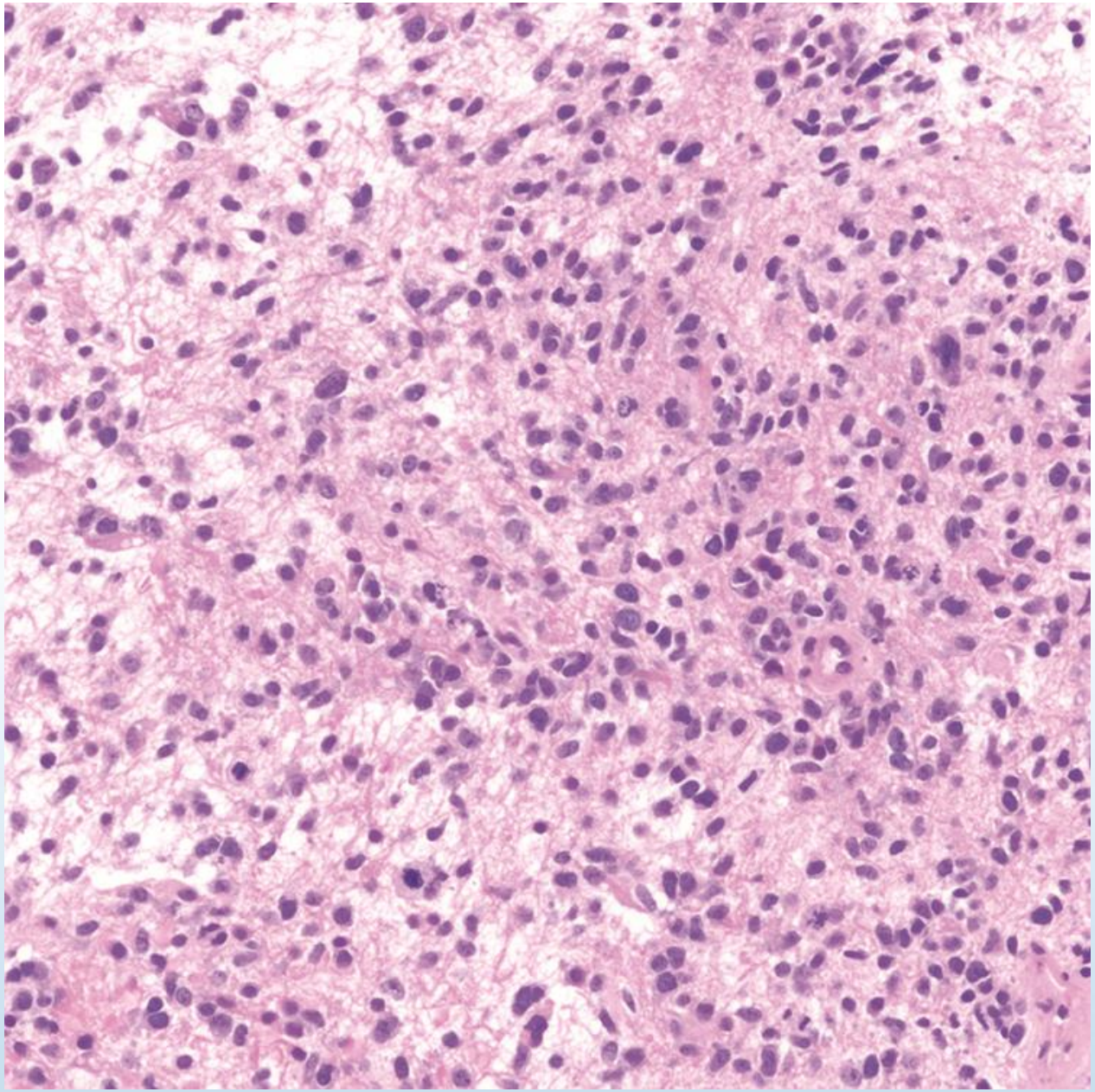
case 1:
if it looks like a duck and methylates like a duck…

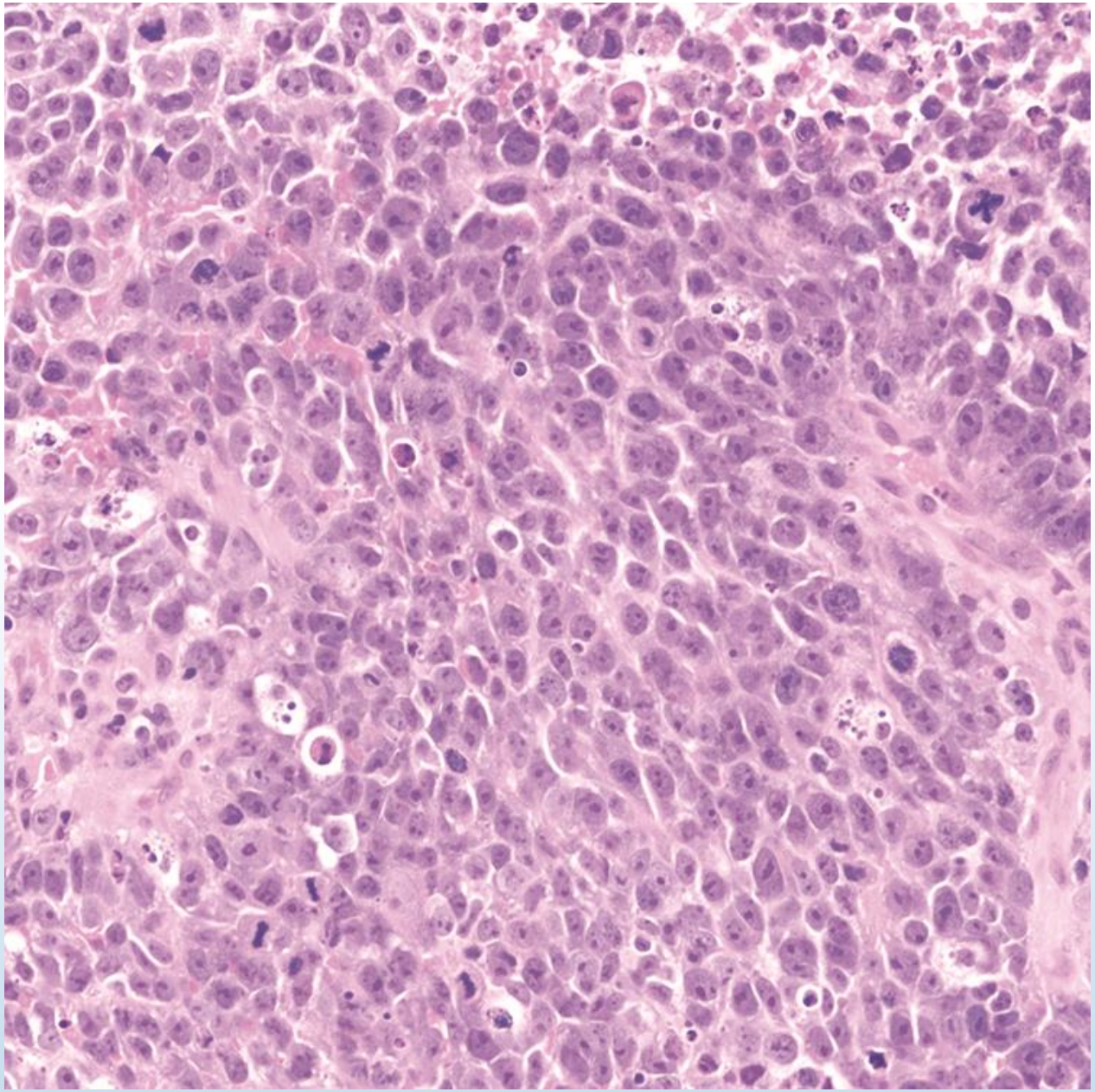


case 1

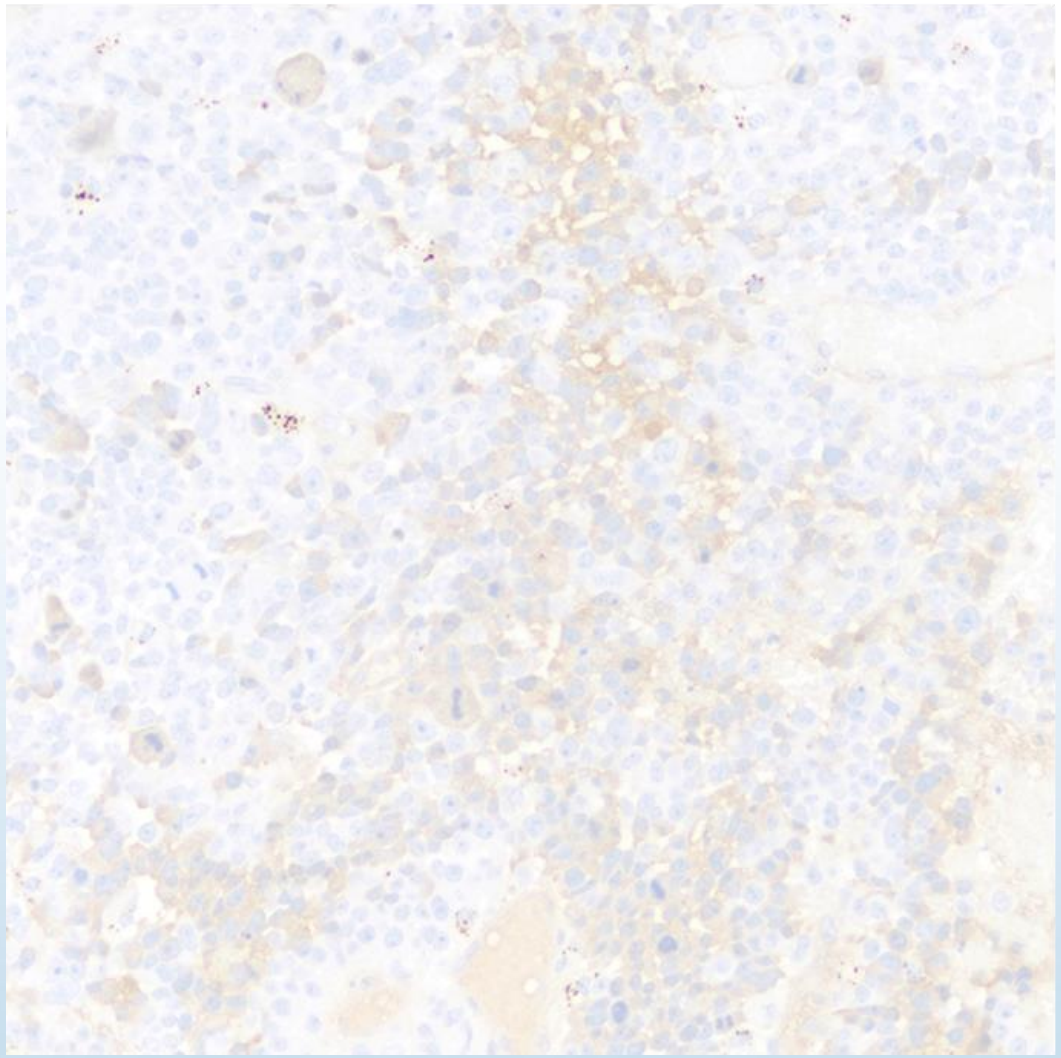
- 65 year-old man
- left brain mass



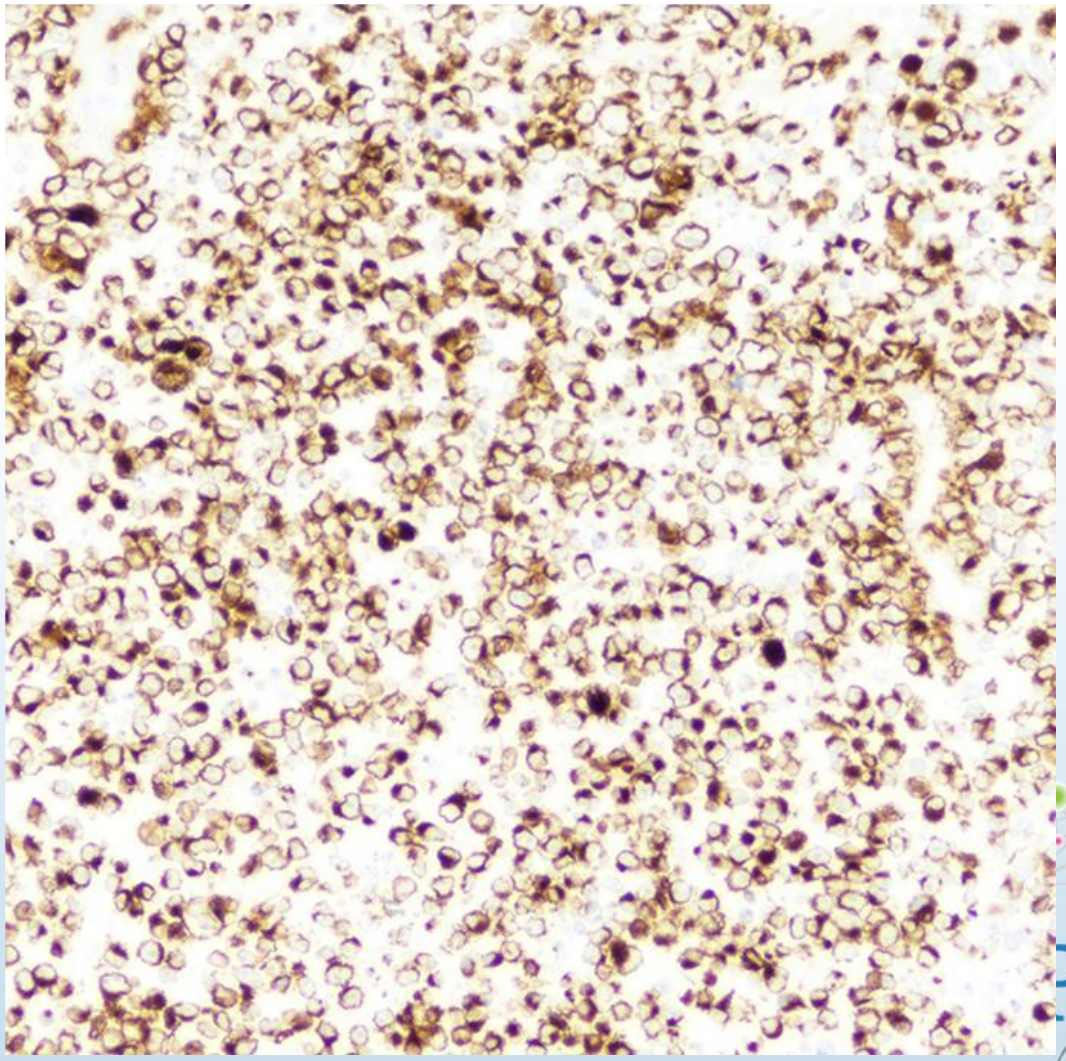




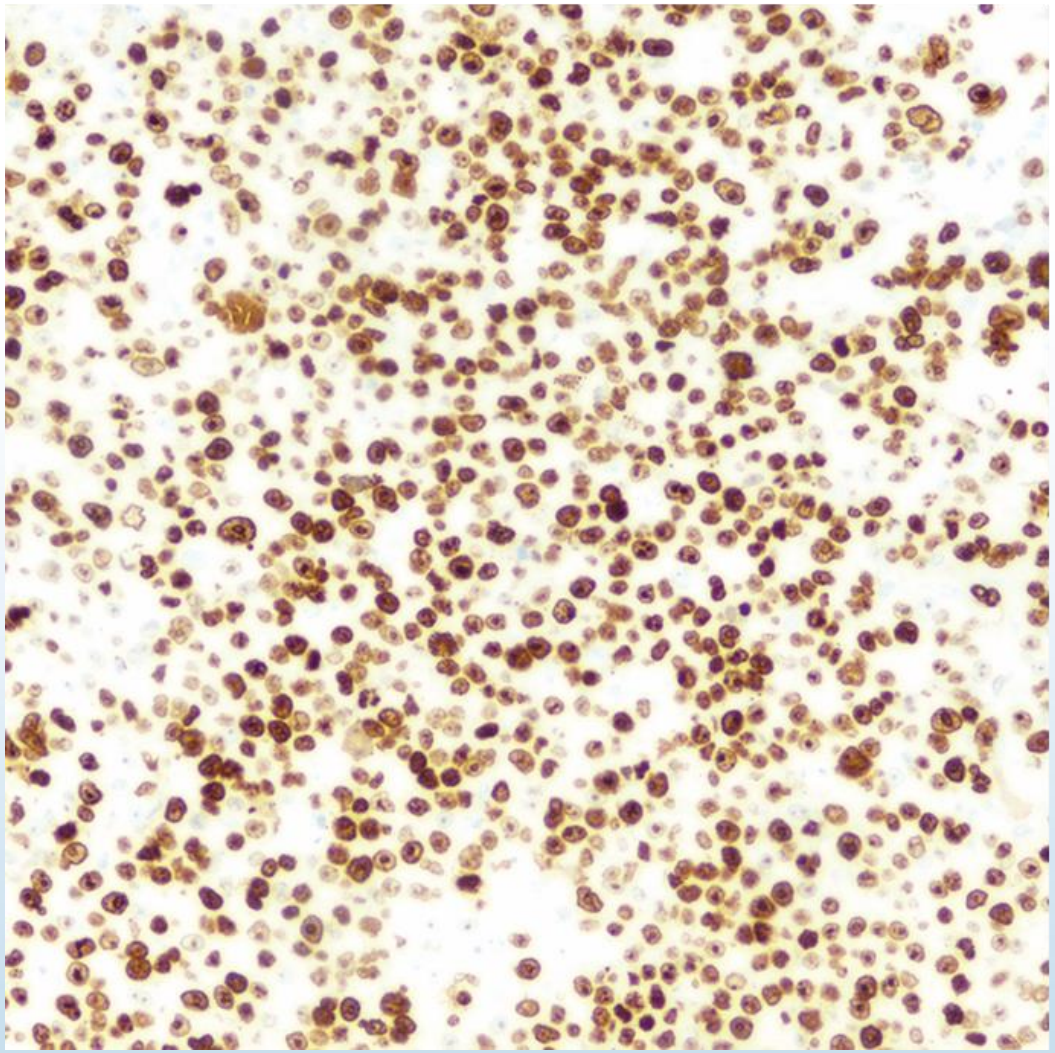
GFAP



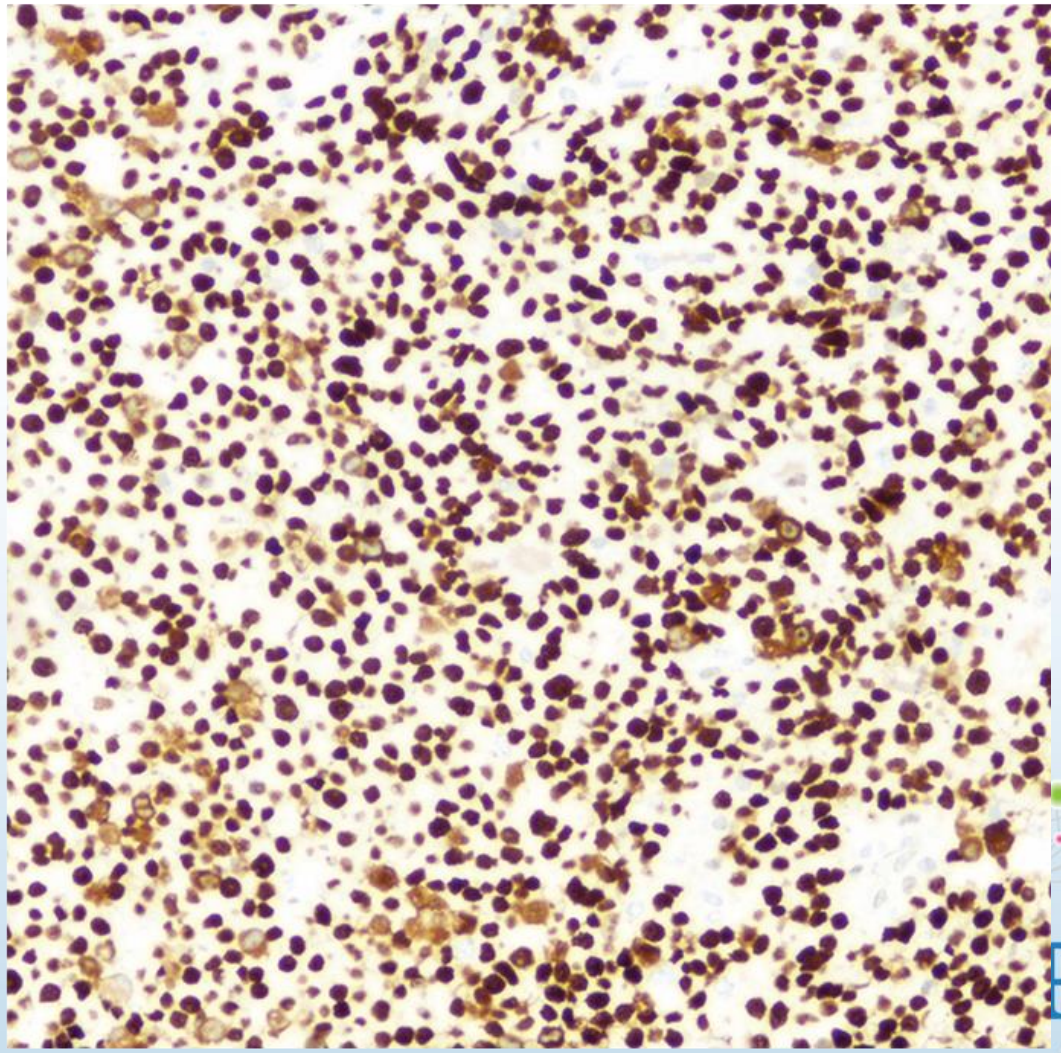
CAM5.2



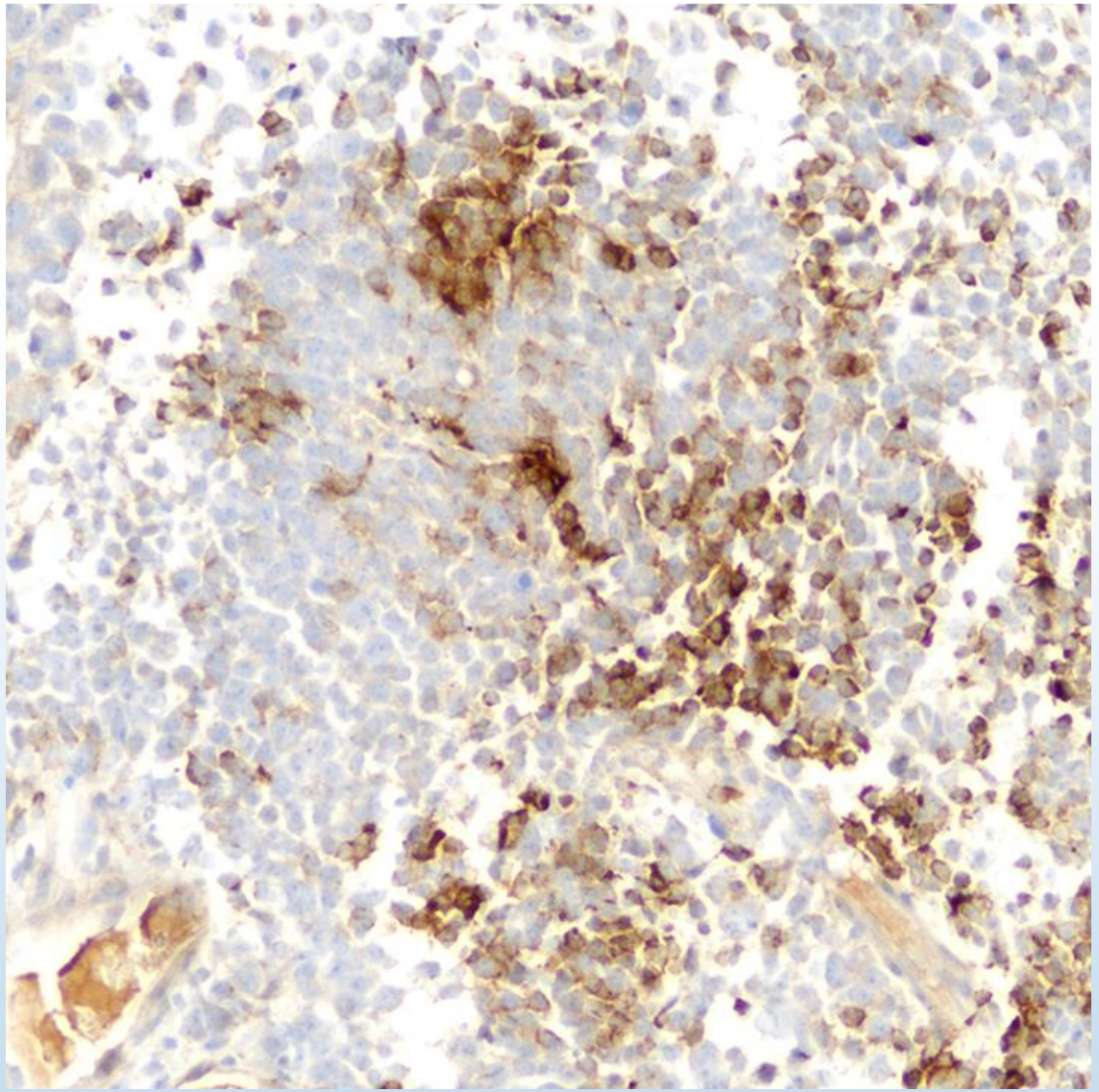
Ki67



P53



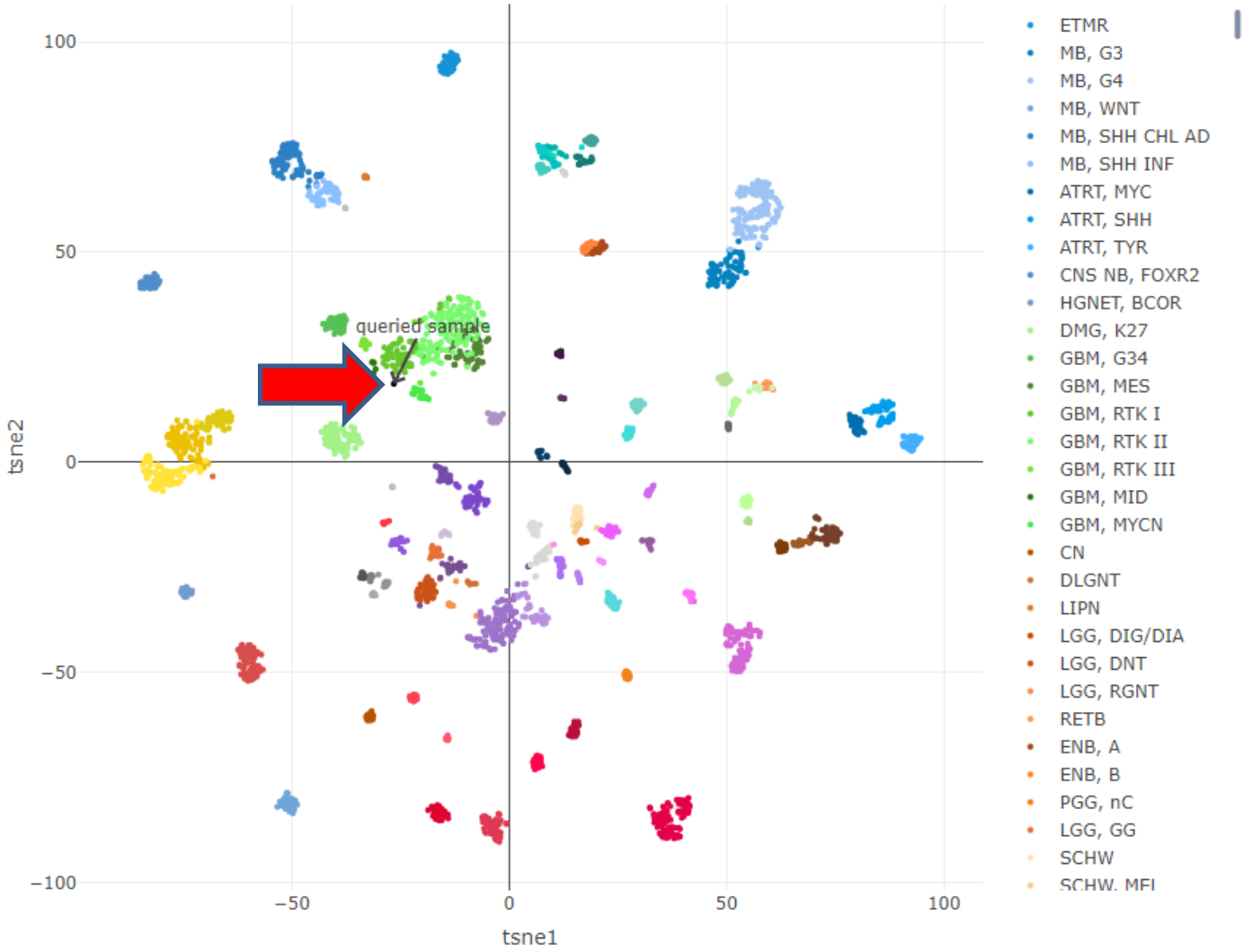
synaptophysin



NGS results

- *TP53* mut
- *MET* amp
- *CDK6* amp

TSNE Clustering



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Epithelial and Pseudoepithelial Differentiation in Glioblastoma and Gliosarcoma

A Comparative Morphologic and Molecular Genetic Study

Fausto J. Rodriguez, MD[1]
Bernd W. Scheithauer, MD[1]
Caterina Giannini, MD, PhD[1]
Sandra C. Bryant, MS[2]
Robert B. Jenkins, MD, PhD[1]

frequent characteristics:

1. varying degrees of CAM5.2 immunoreactivity
2. high Ki67 proliferation index
3. strong p53 IHC
4. *TP53* mutations and *CDKN2A* deletions

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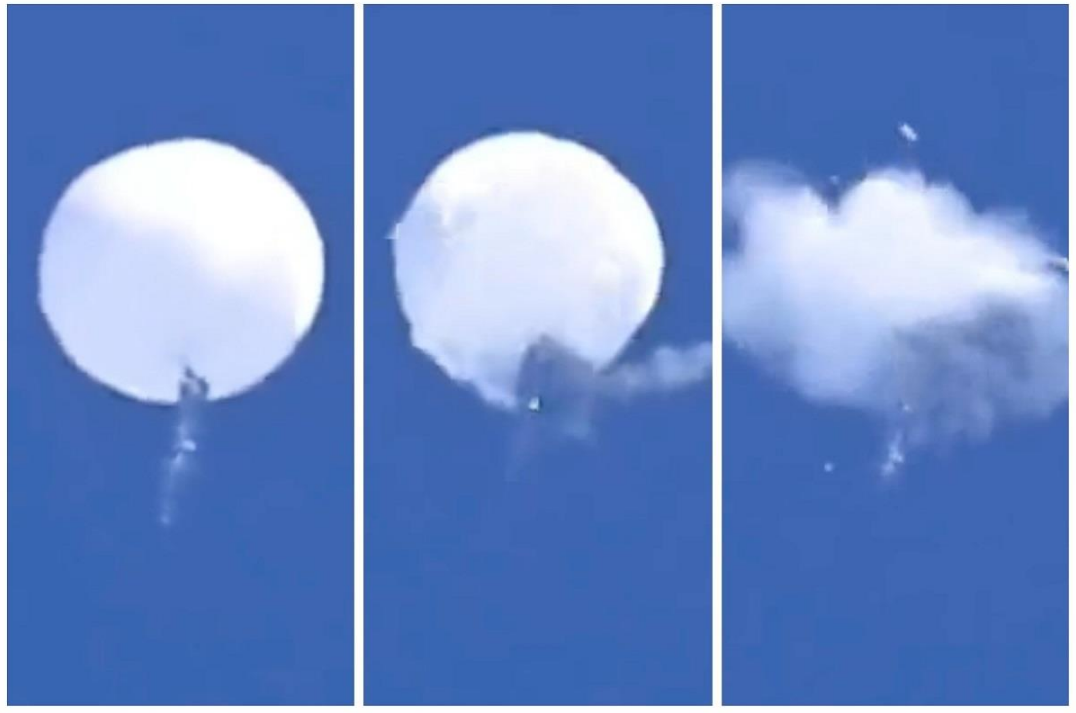
diagnosis

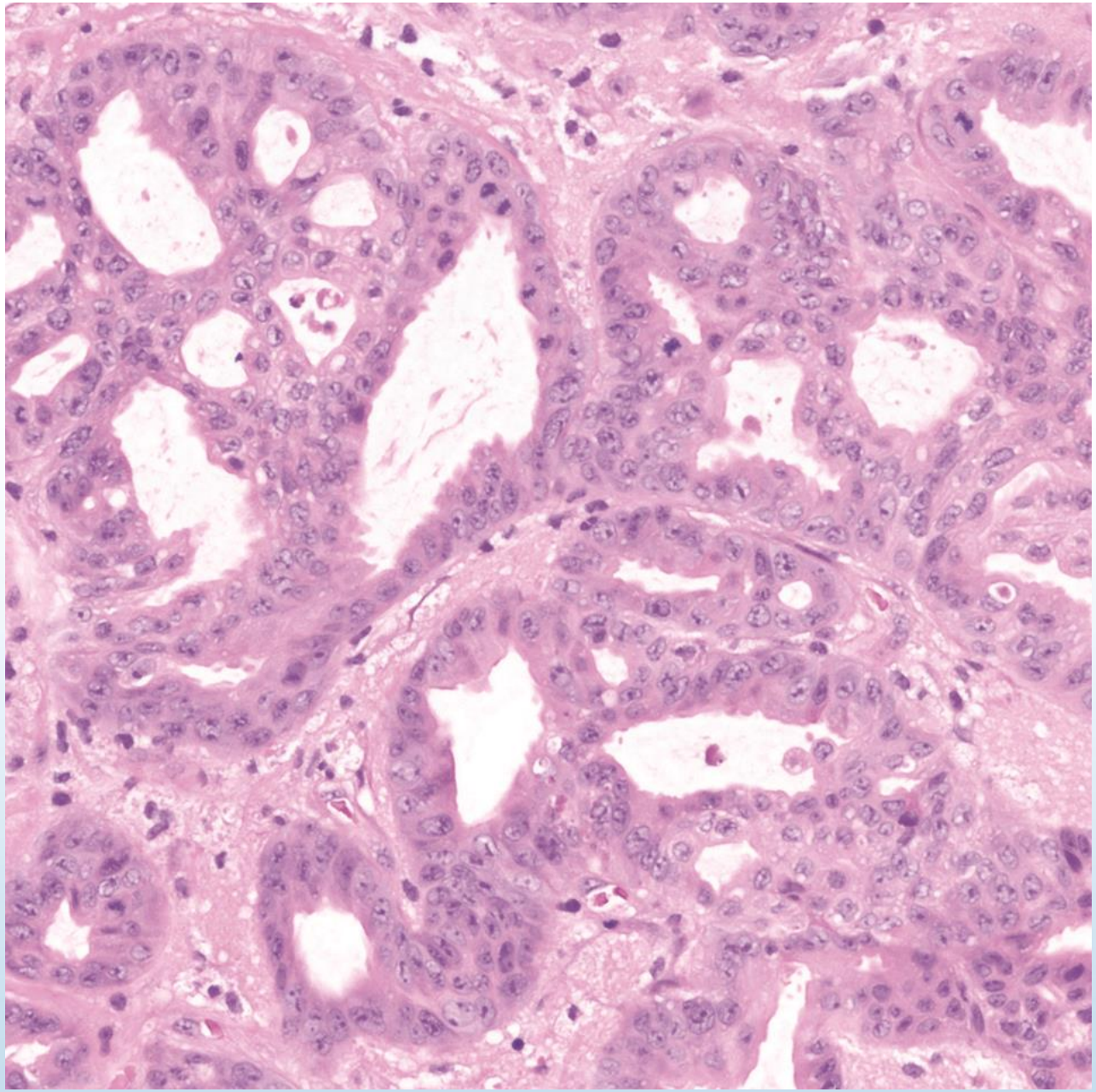
- glioblastoma, IDH wild-type, WHO grade 4, with pseudoepithelial differentiation
- died several weeks later

case 2:
Unidentified Metastatic Object

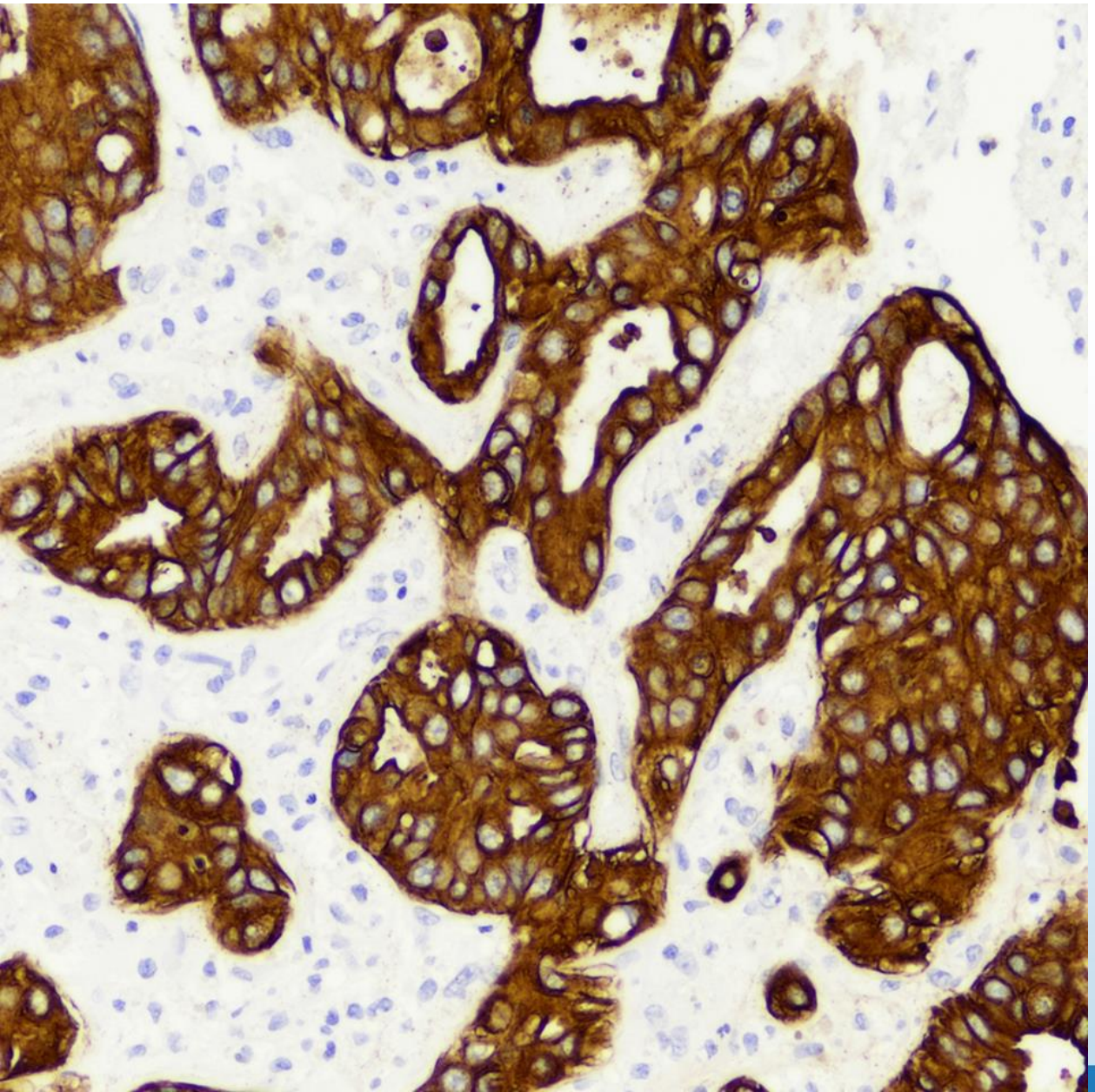


case 2

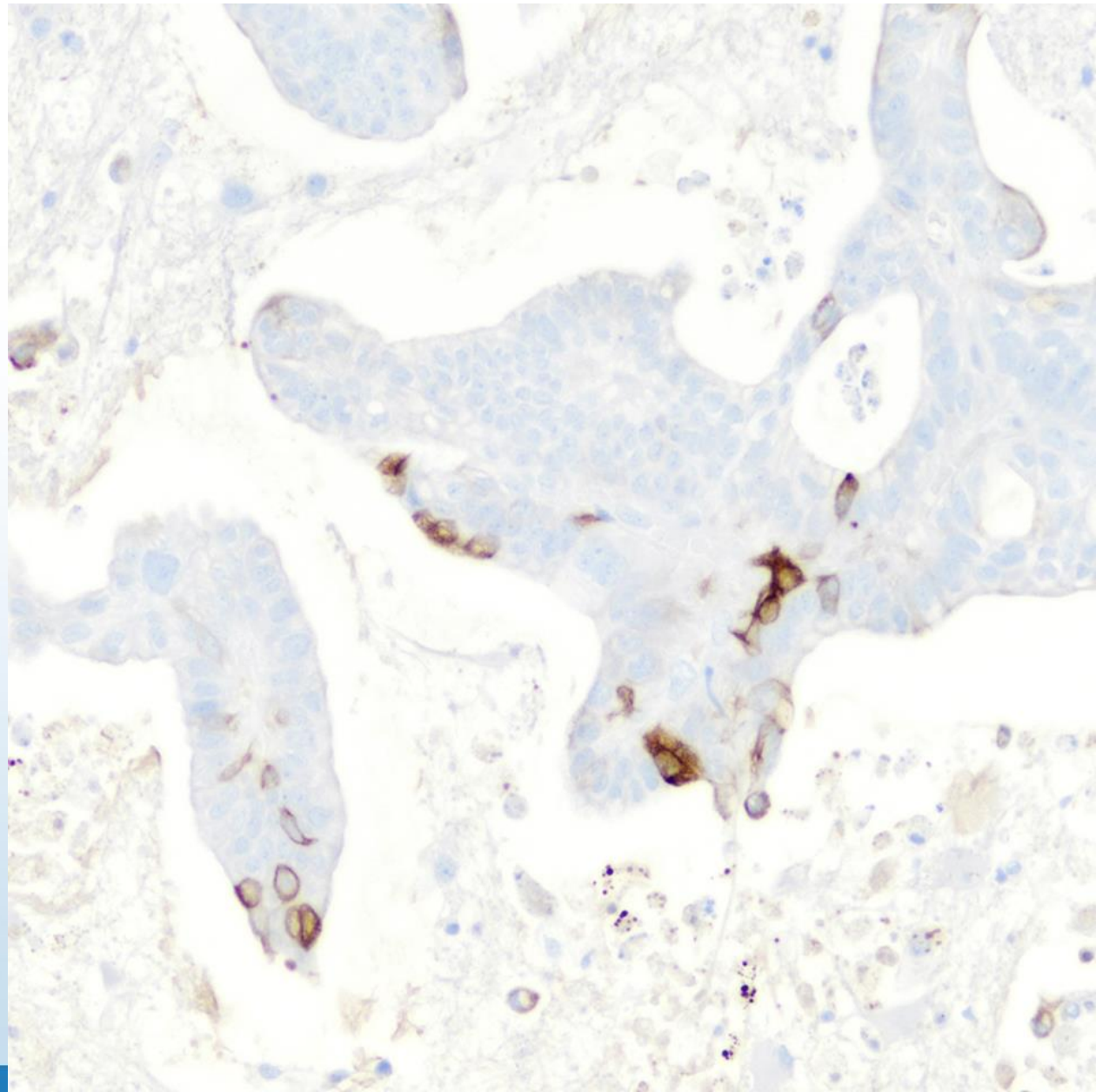
- 67 y/o F
- right frontoparietal mass



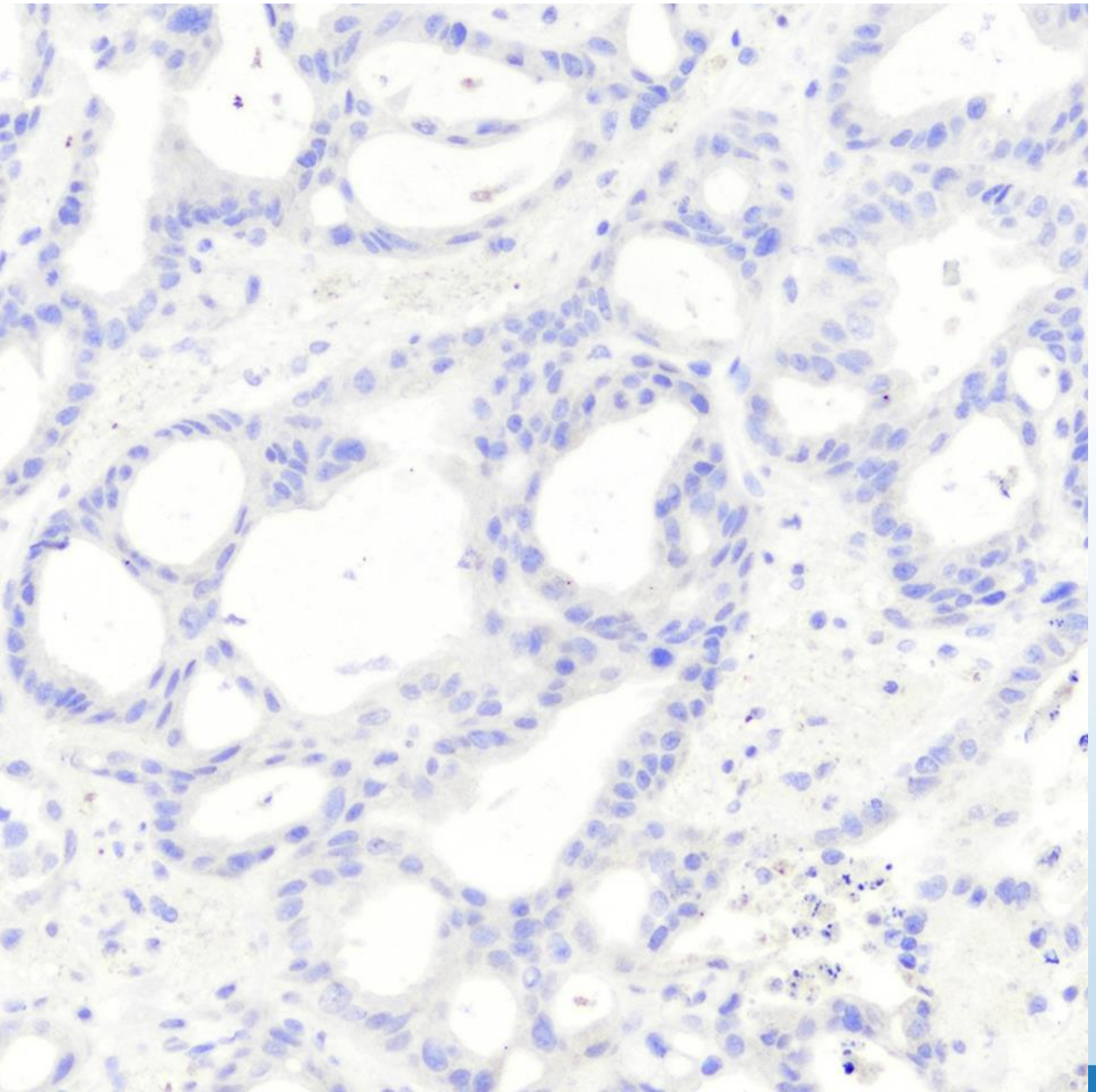
cytokeratin 7



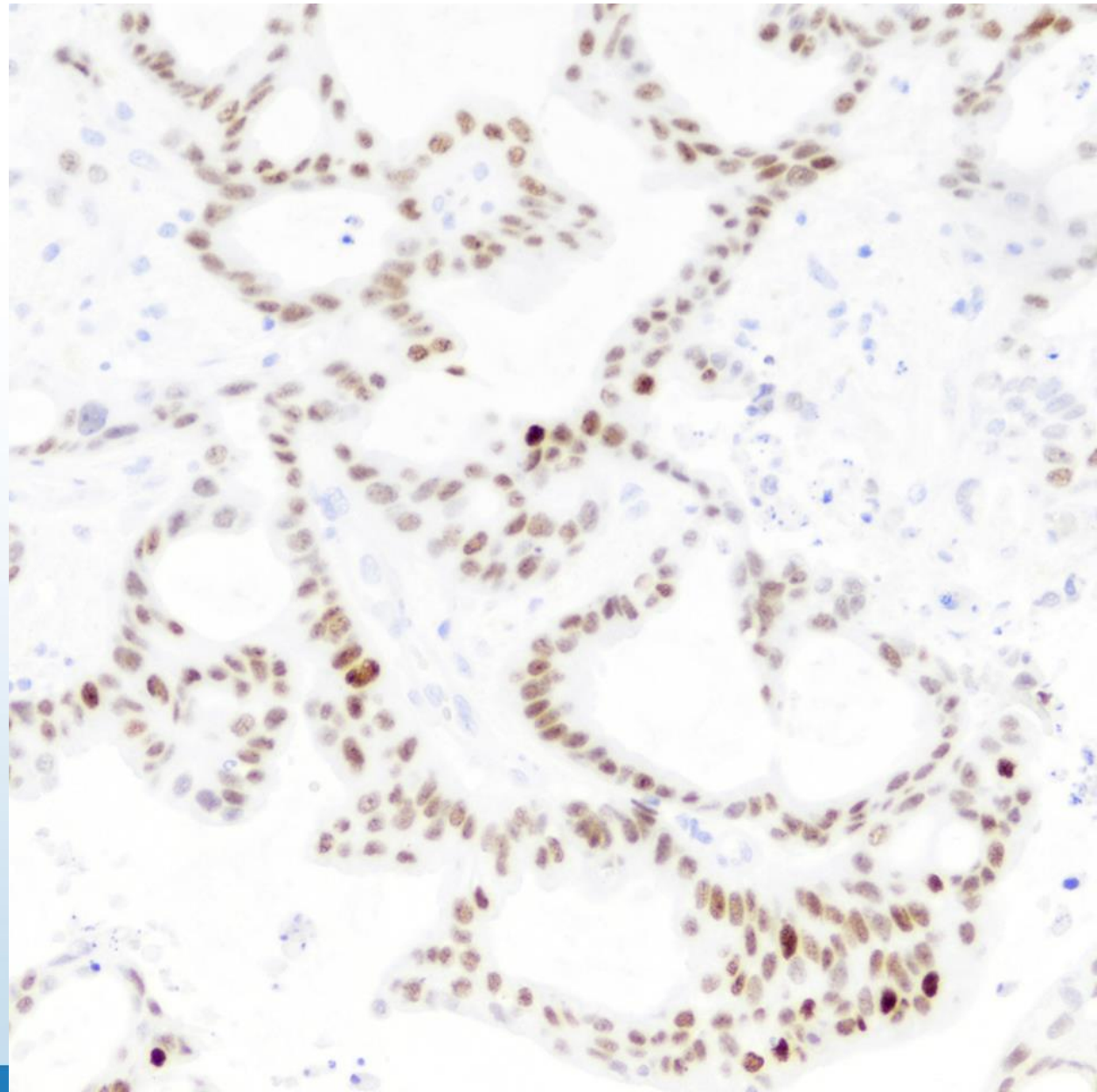
cytokeratin 20



TTF1



CDX2



preliminary diagnosis

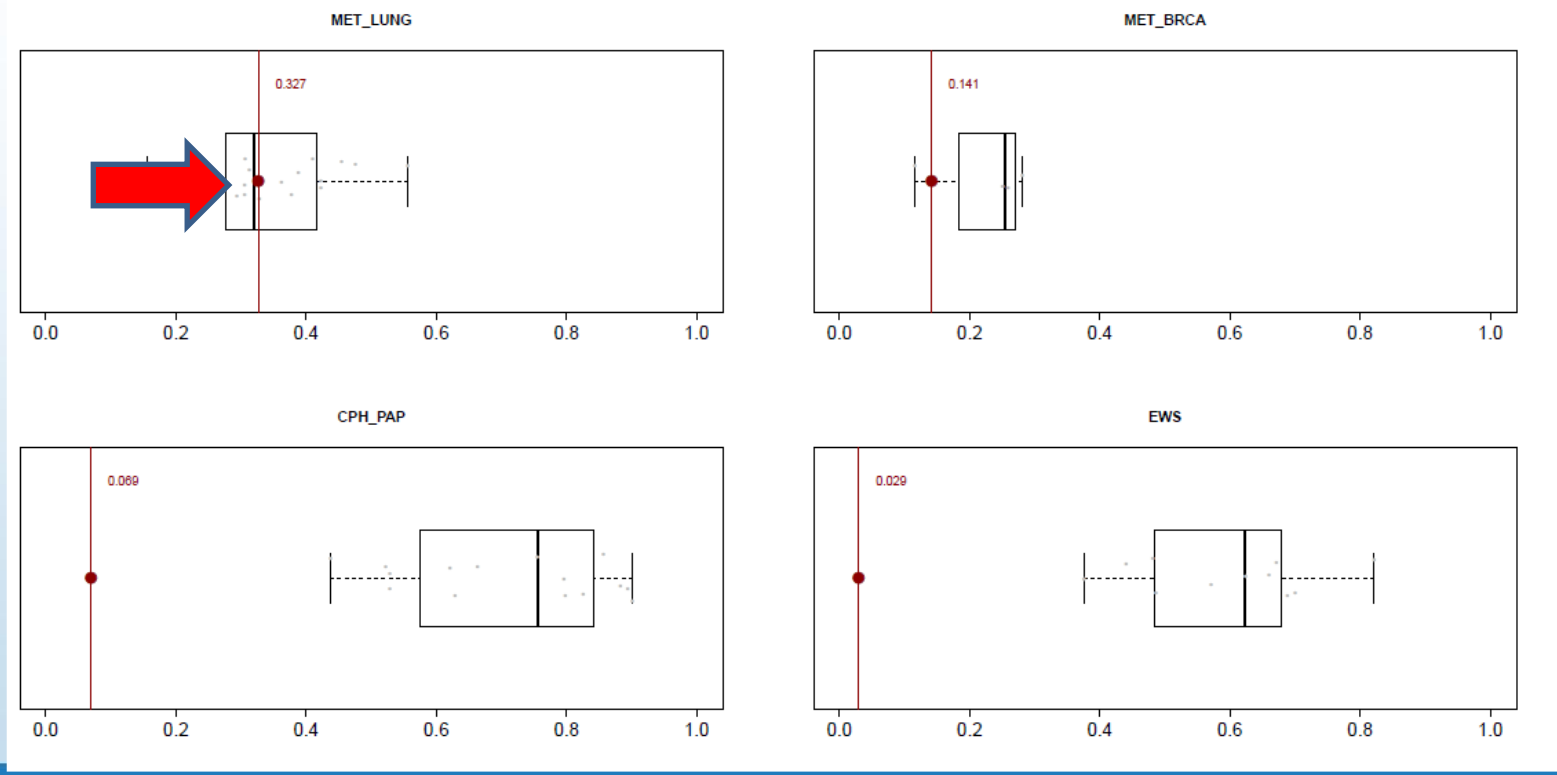
- metastatic carcinoma of unclear origin

NGS

- mutations in
  - *PIK3CA*
  - *ARID1A*
  - *KRAS*
  - *GNAS*

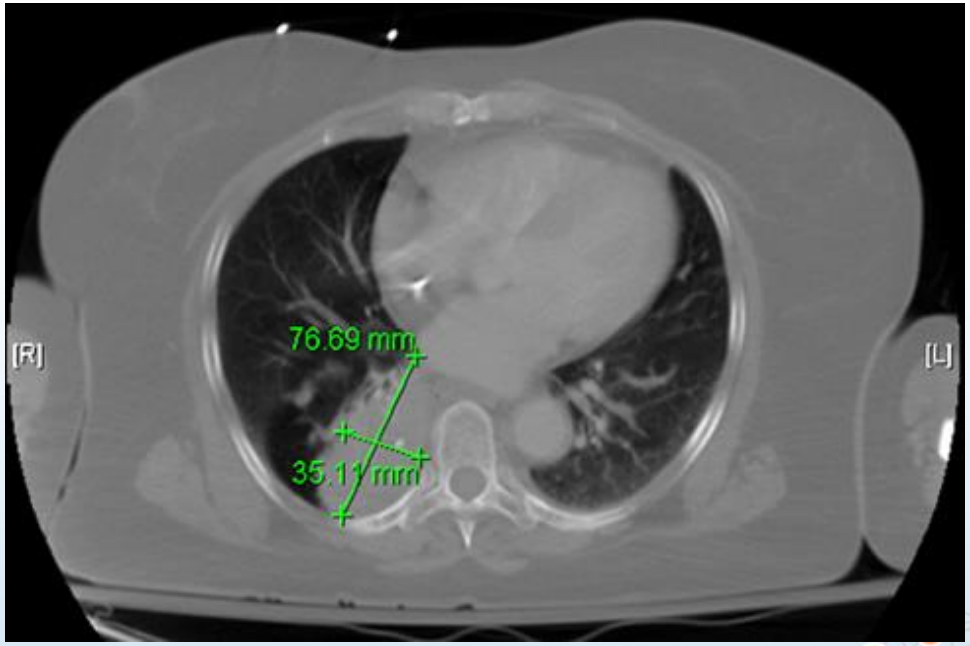
450k Classifier Scores (mnpprediction version 0.1.9)

Score	Abbreviation	Tumor subclass
0.3266	MET_LUNG	NA
0.1411	MET_BRCA	NA
0.0691	CPH_PAP	Papillary craniopharyngioma
0.0293	EWS	Ewing sarcoma



radiology

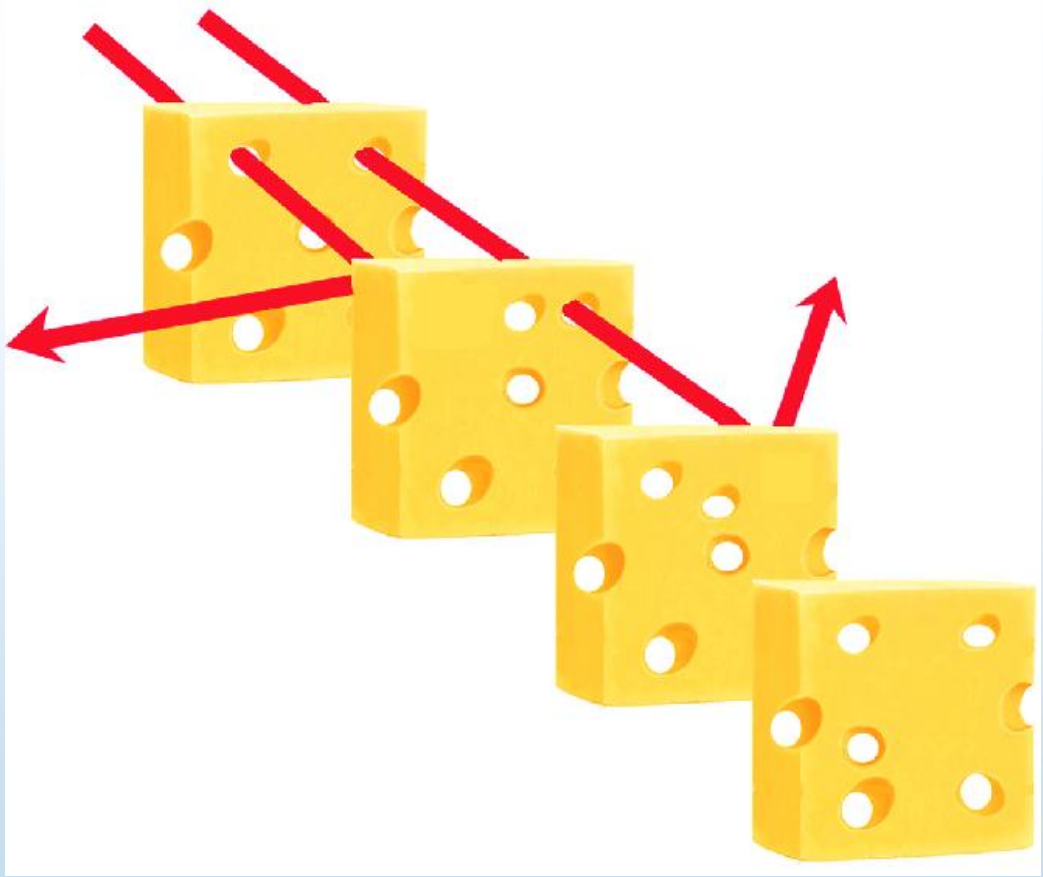
- large RLL pleural-parenchymal tumor with hilar lymphadenopathy
- nothing abnormal in the biliary tree, liver, spleen, or pancreas



final diagnosis

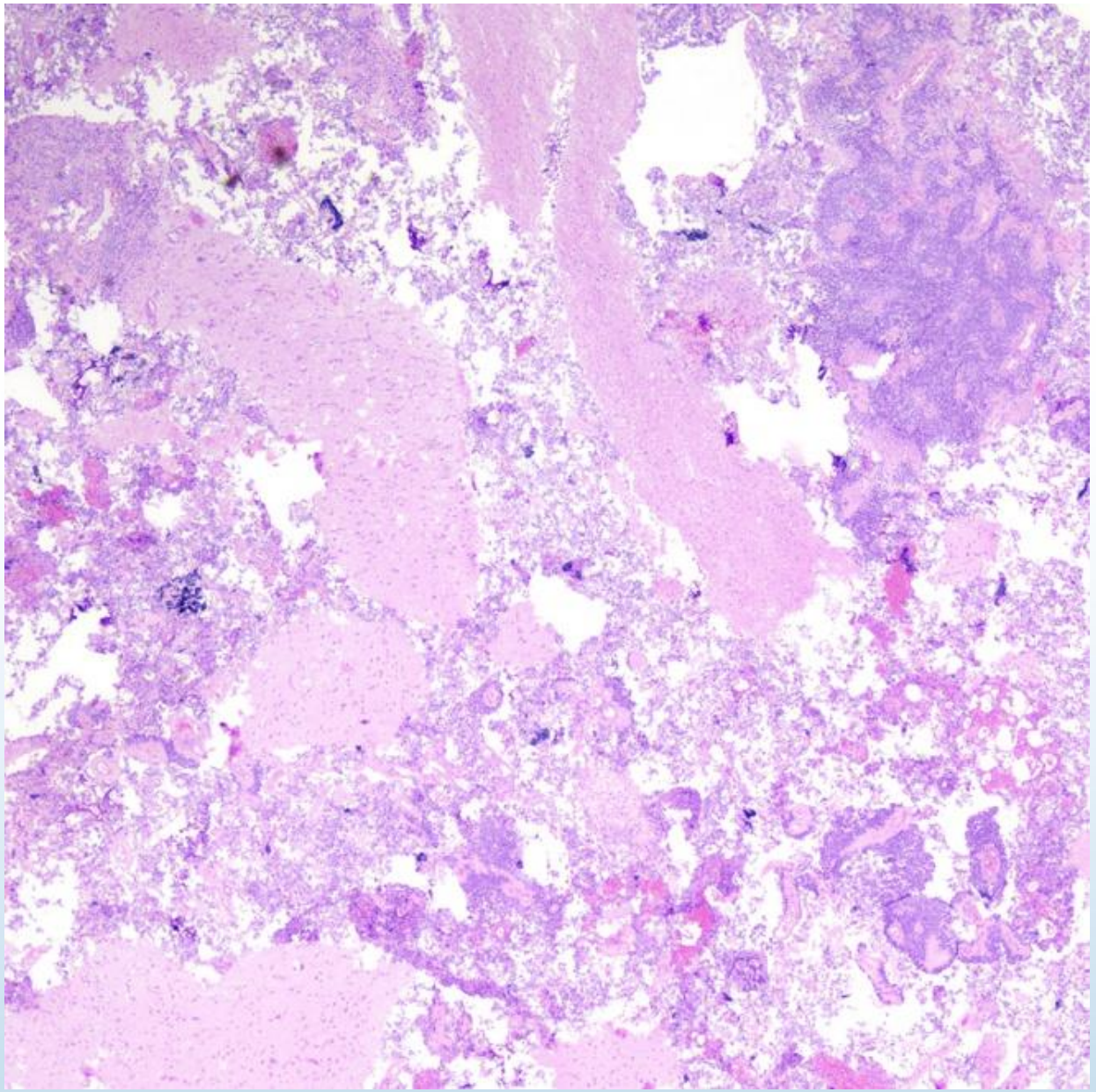
- metastatic lung adenocarcinoma

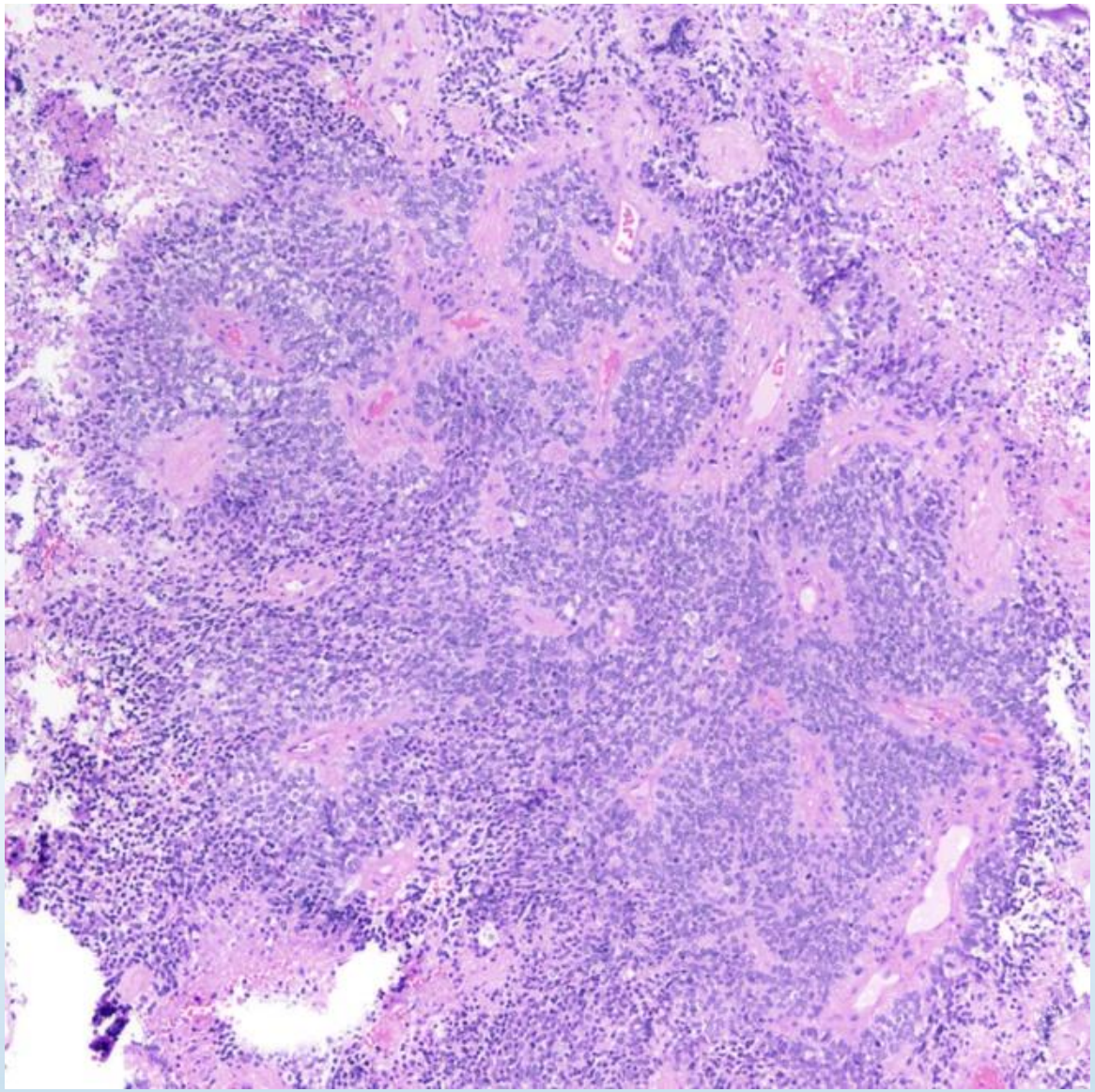
case 3:
the Swiss Cheese model in action

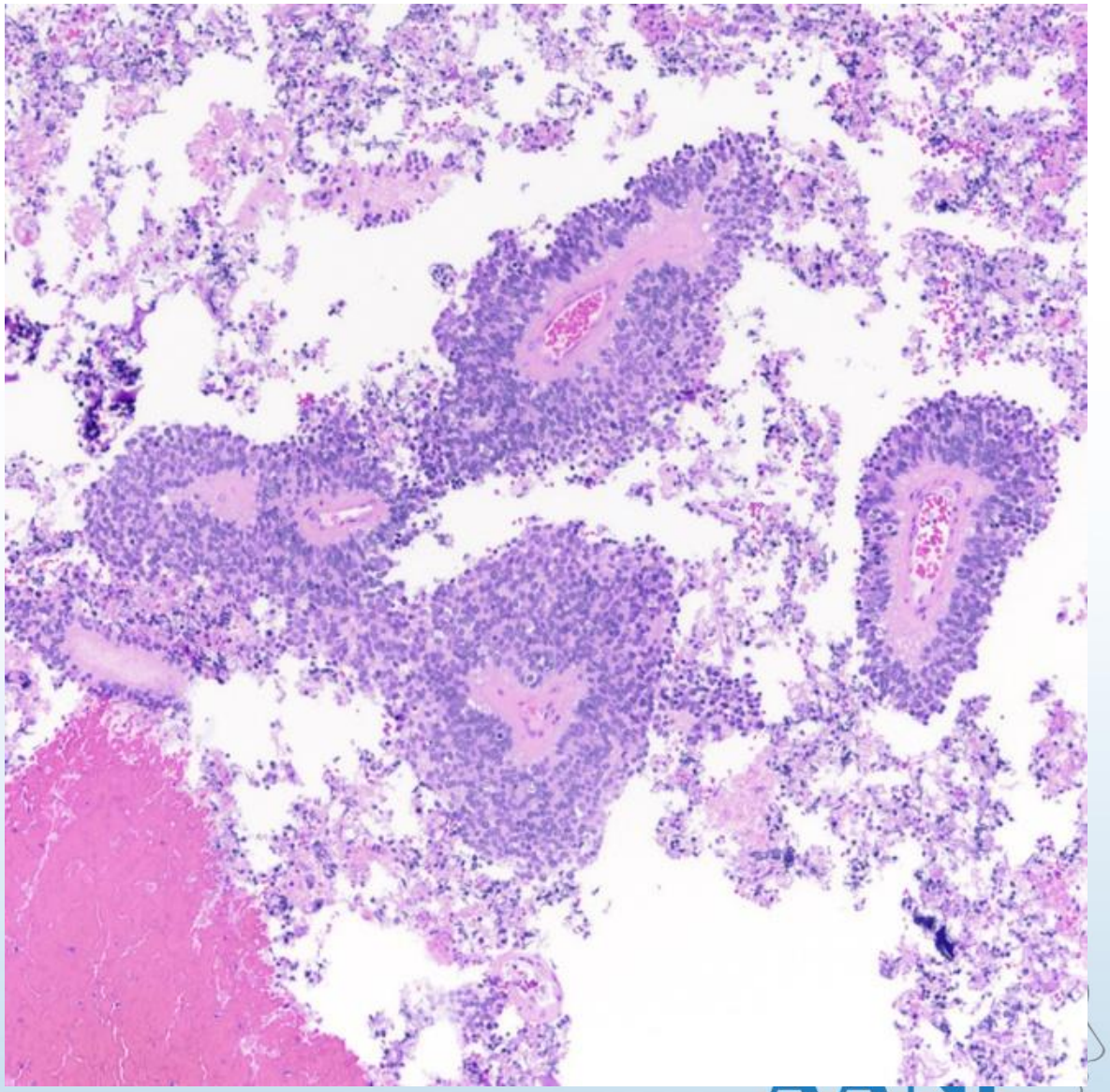


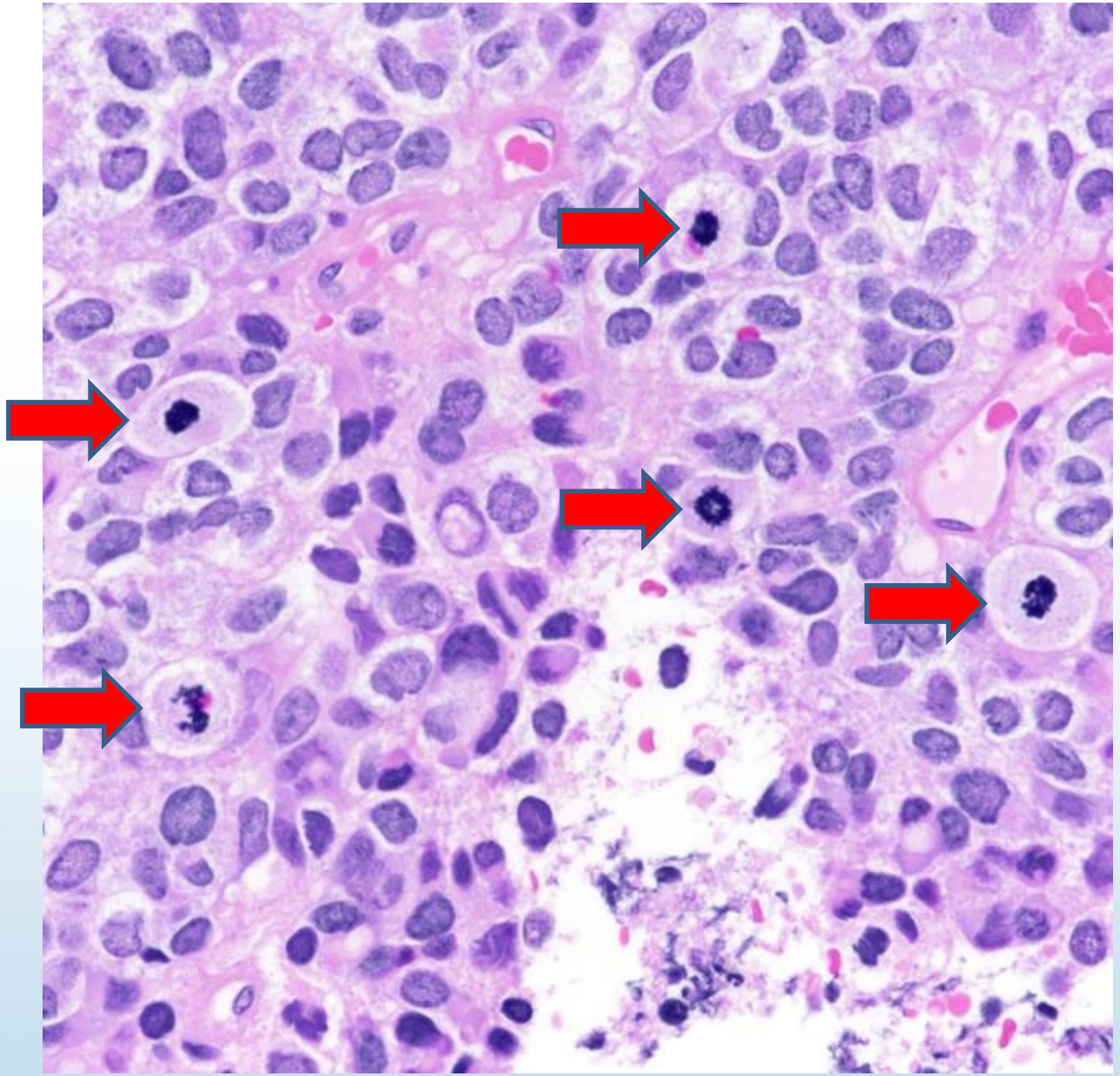
case 3

- 40 year-old man
- right frontal tumor
- diagnosed as “high grade glioma” at OSH





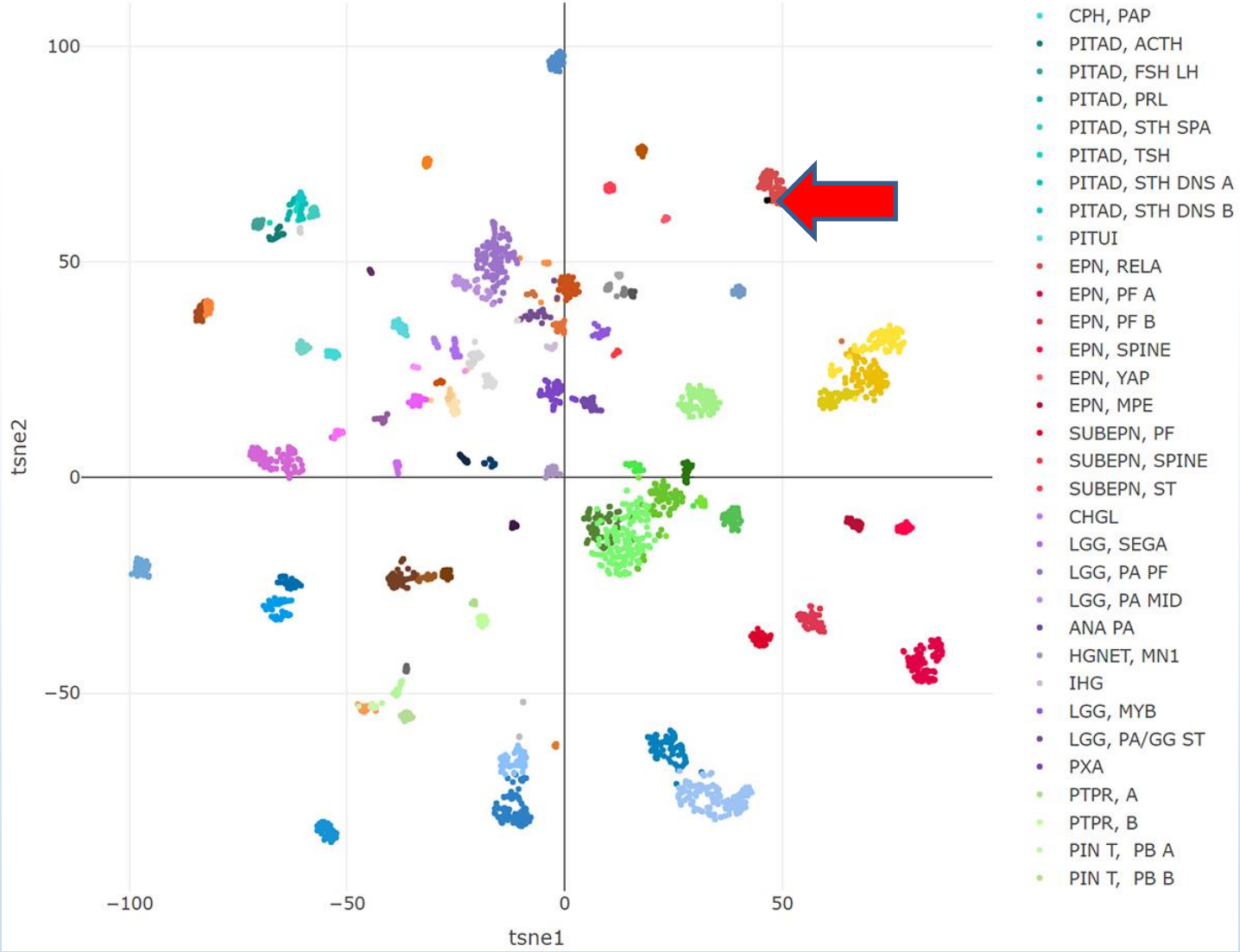




NGS results

- *ZFTA* fusion
- homozygous deletion of *CDKN2A/B*

Infinium 850K methylation profile



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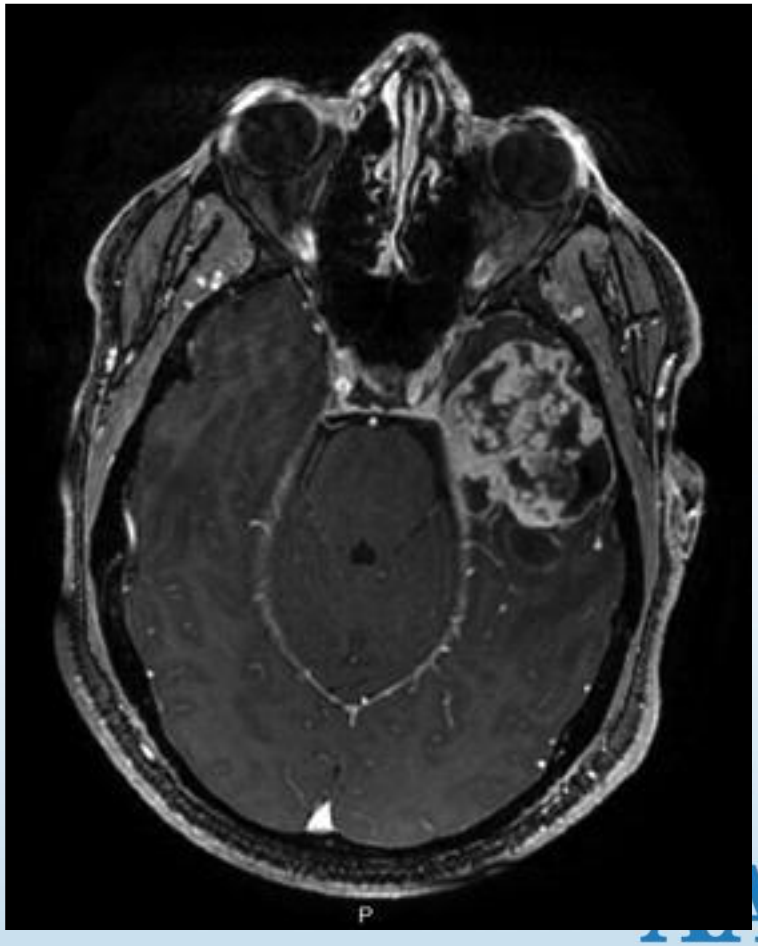
revised diagnosis

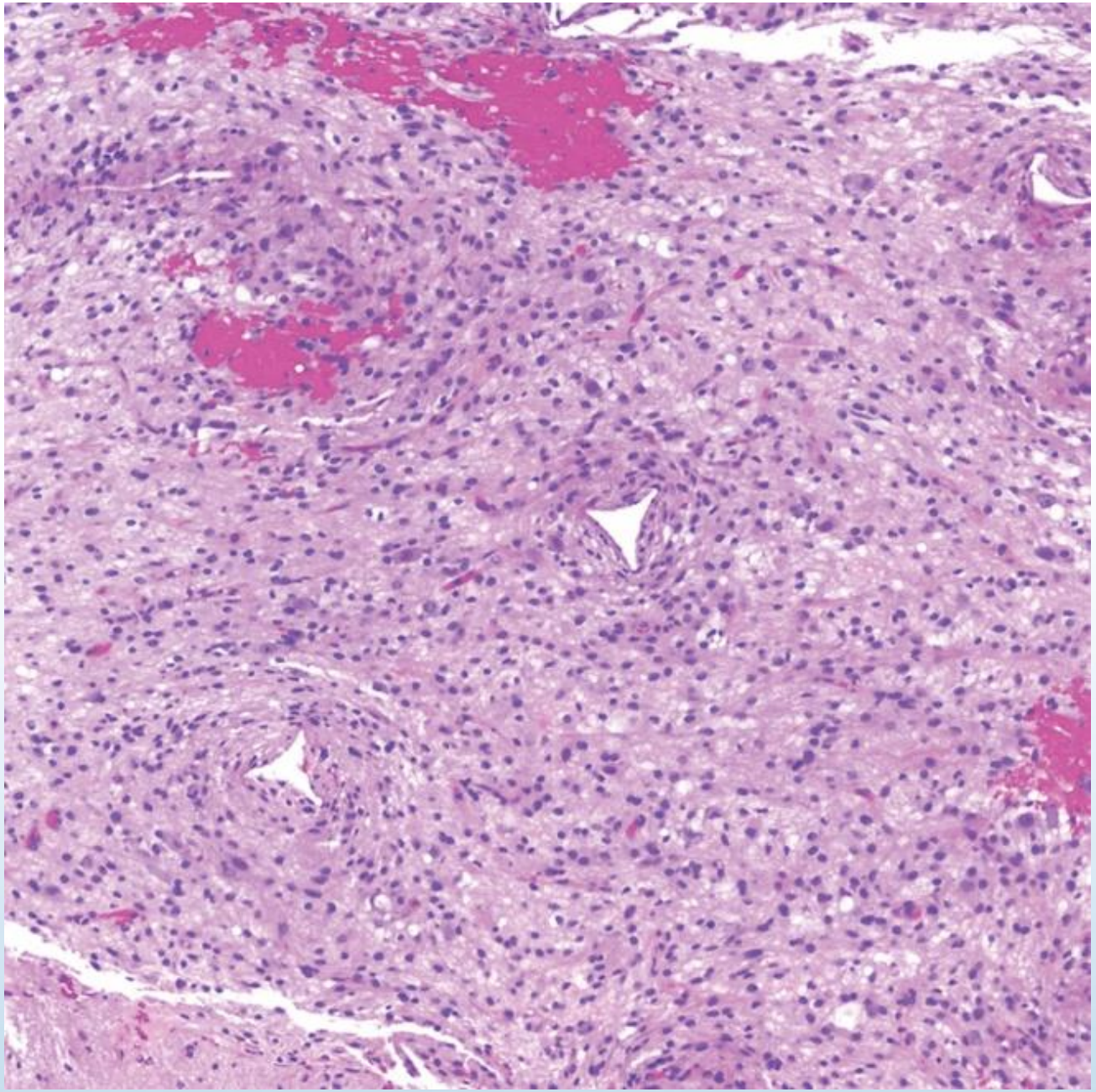
- ependymoma, *ZFTA* fusion-positive, WHO grade 3

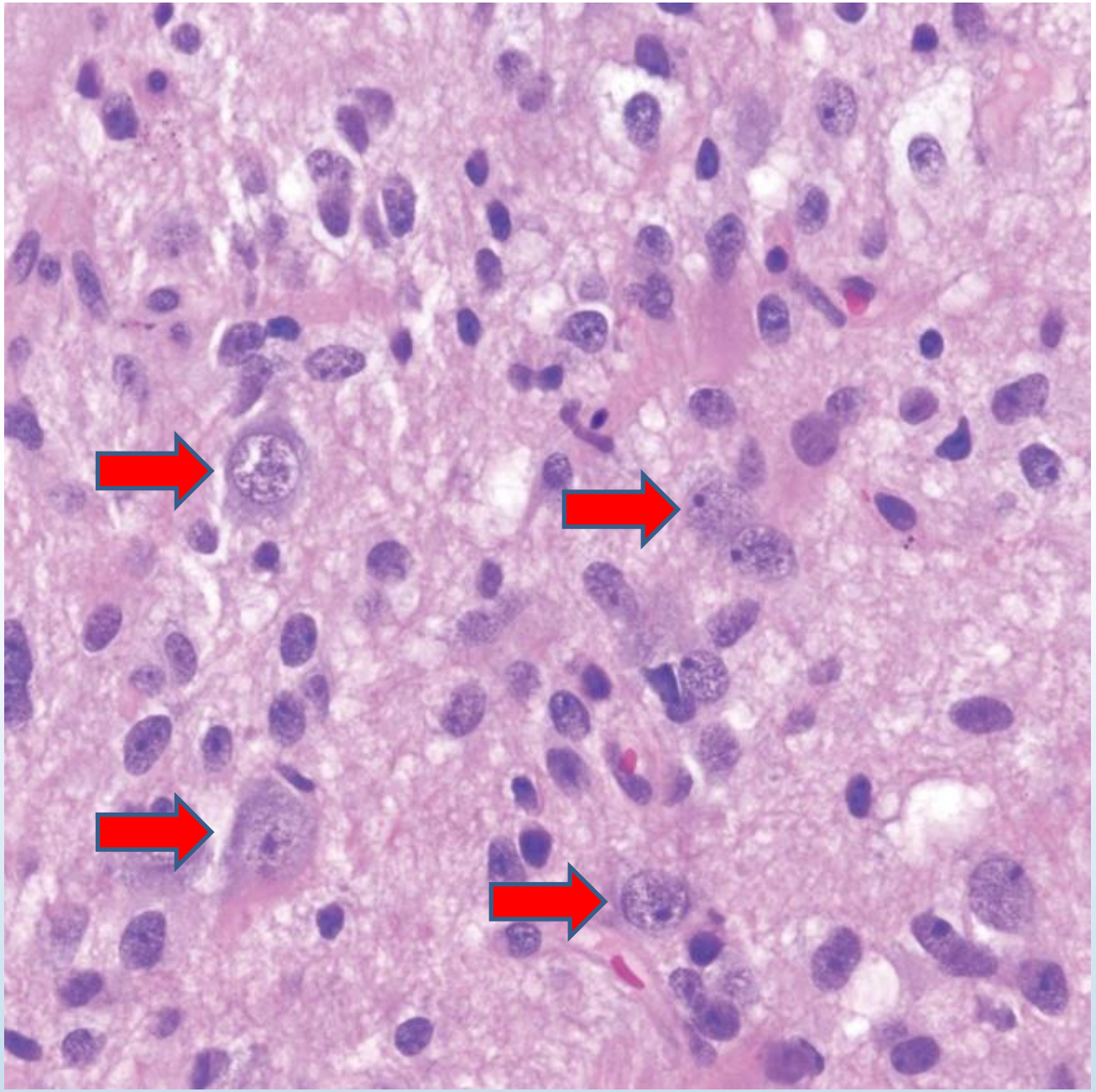
case 4:
correcting past mistakes

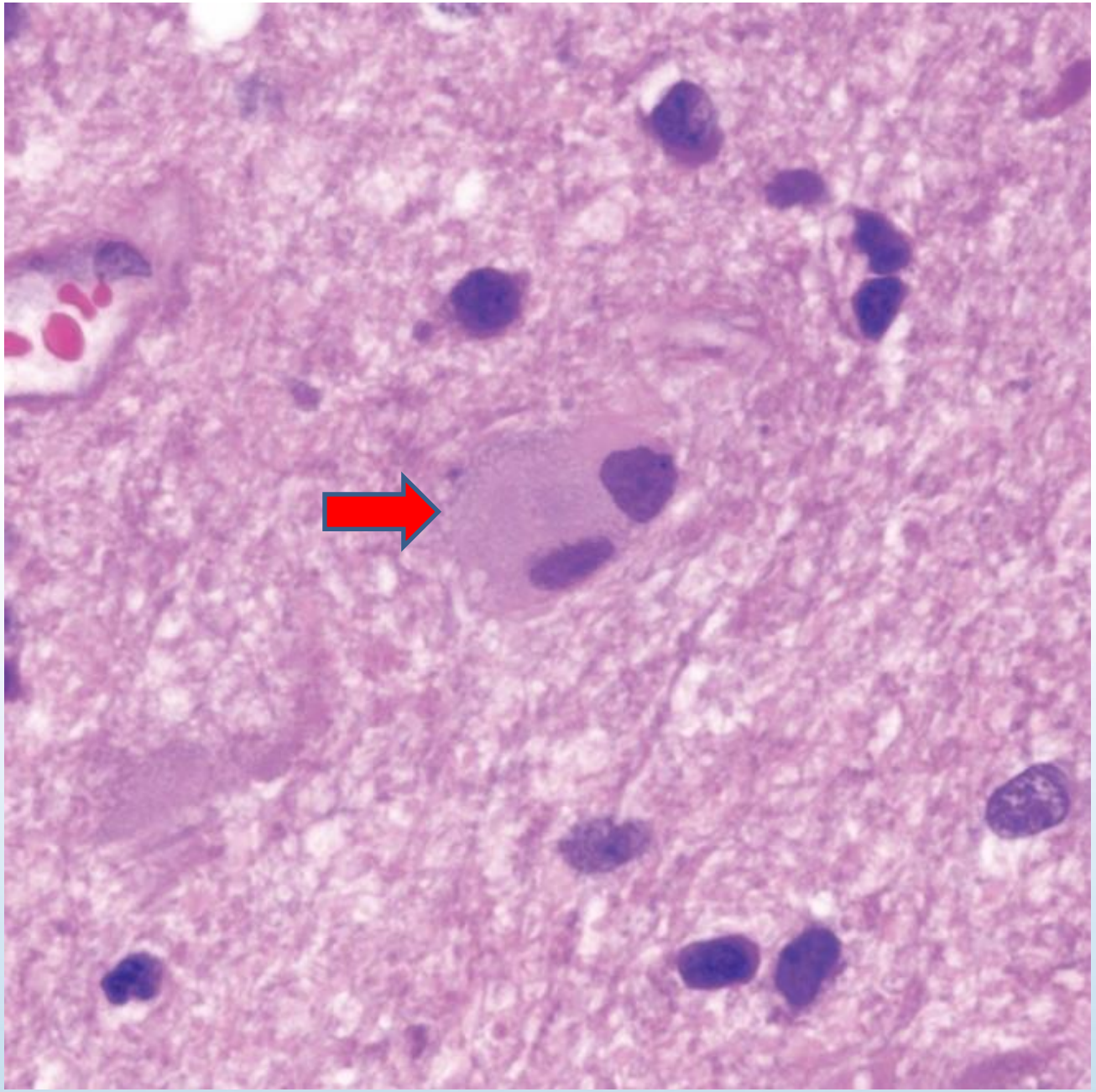
case 4

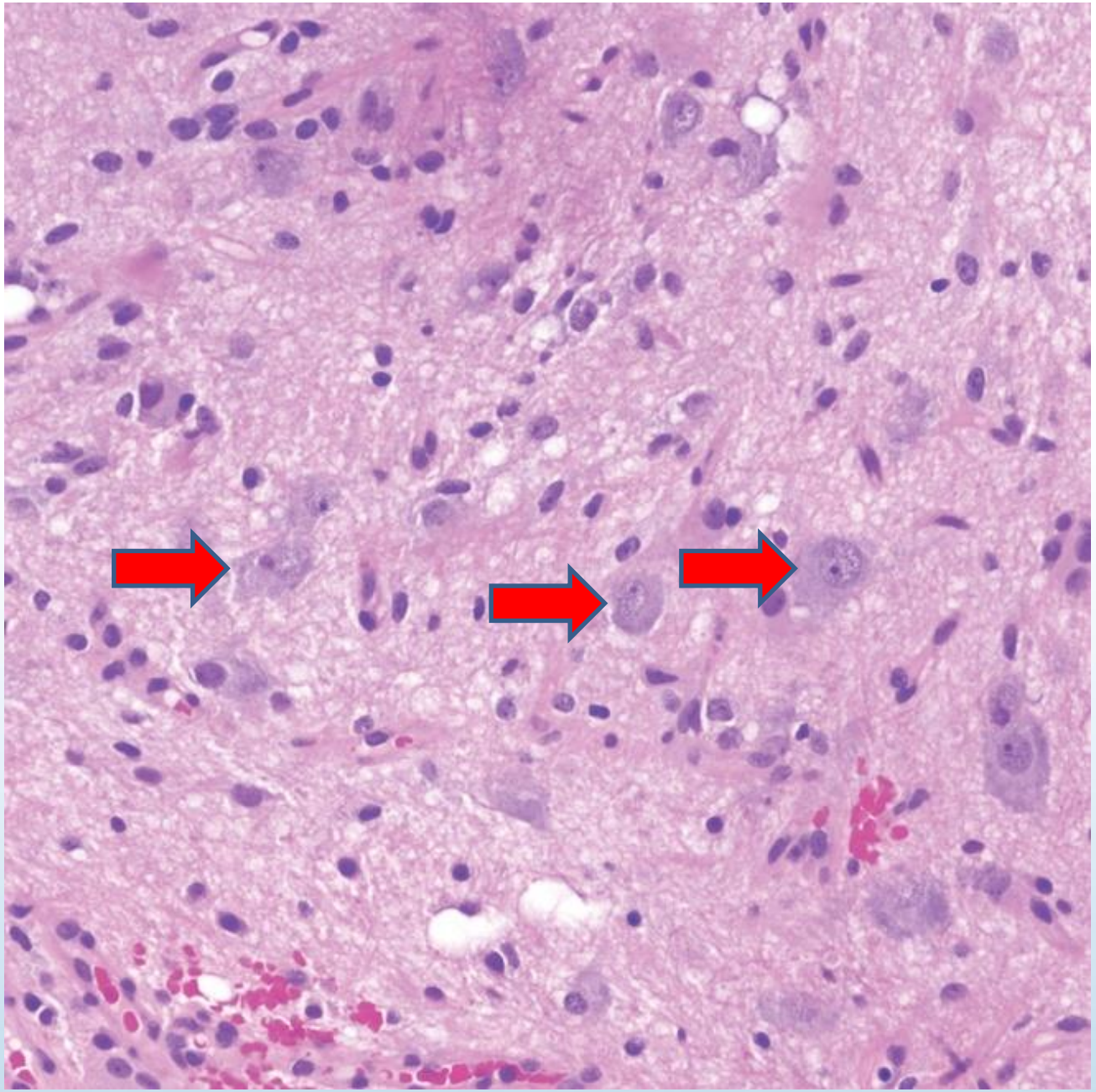
- 47 year-old man
- left temporal lobe tumor
- diagnosed as GBM at OSH

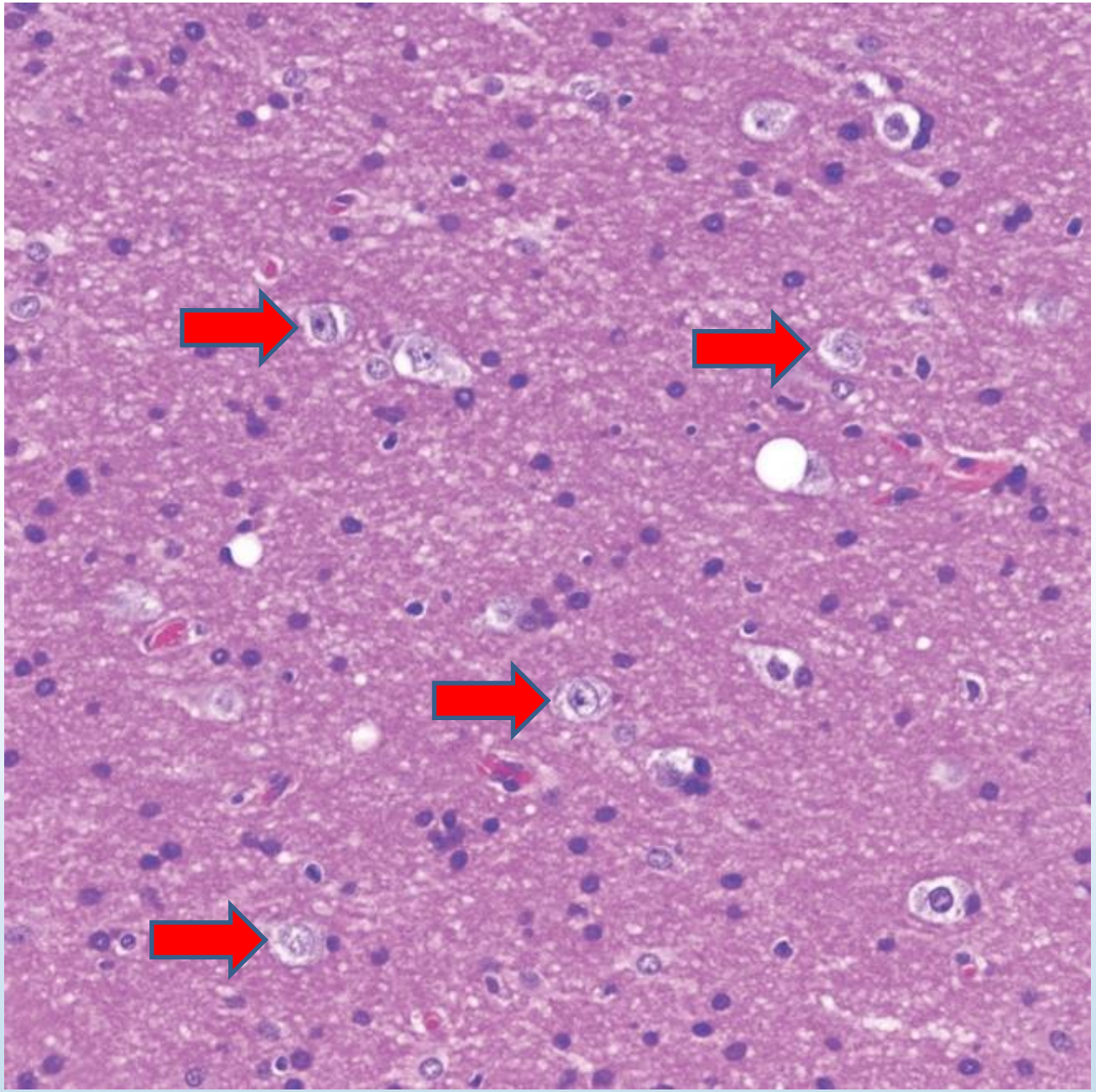




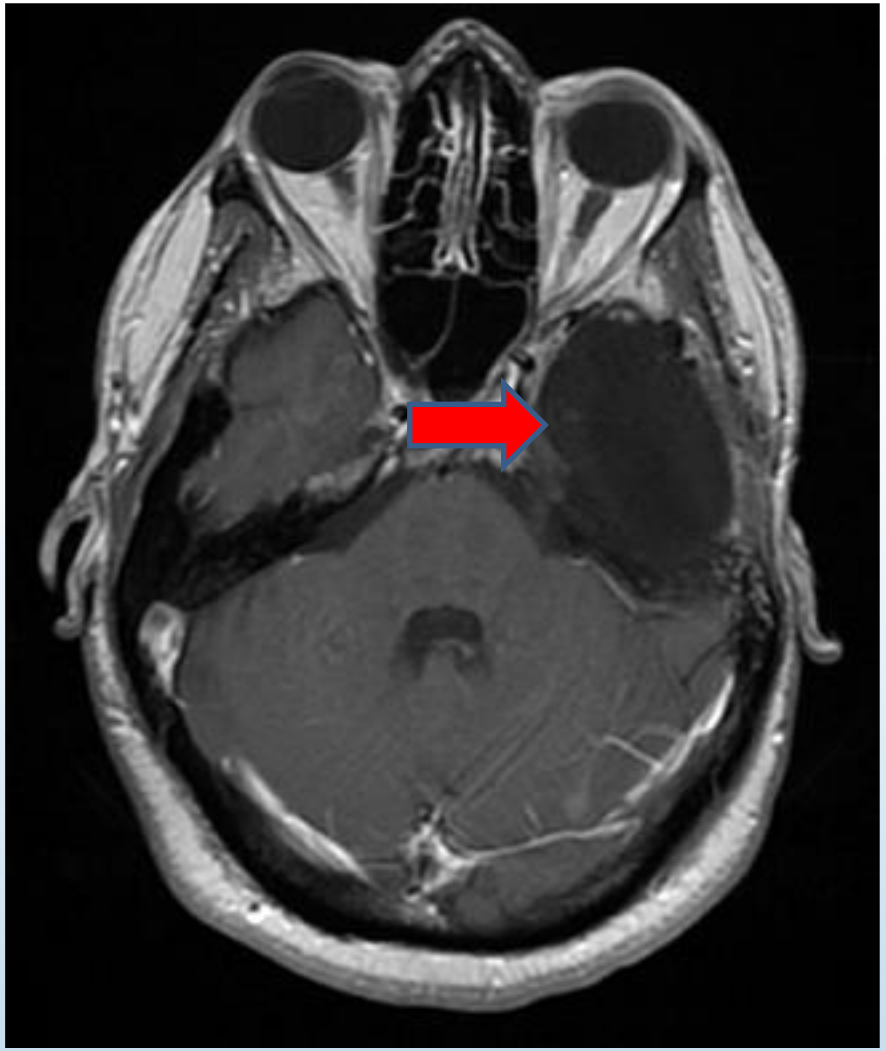




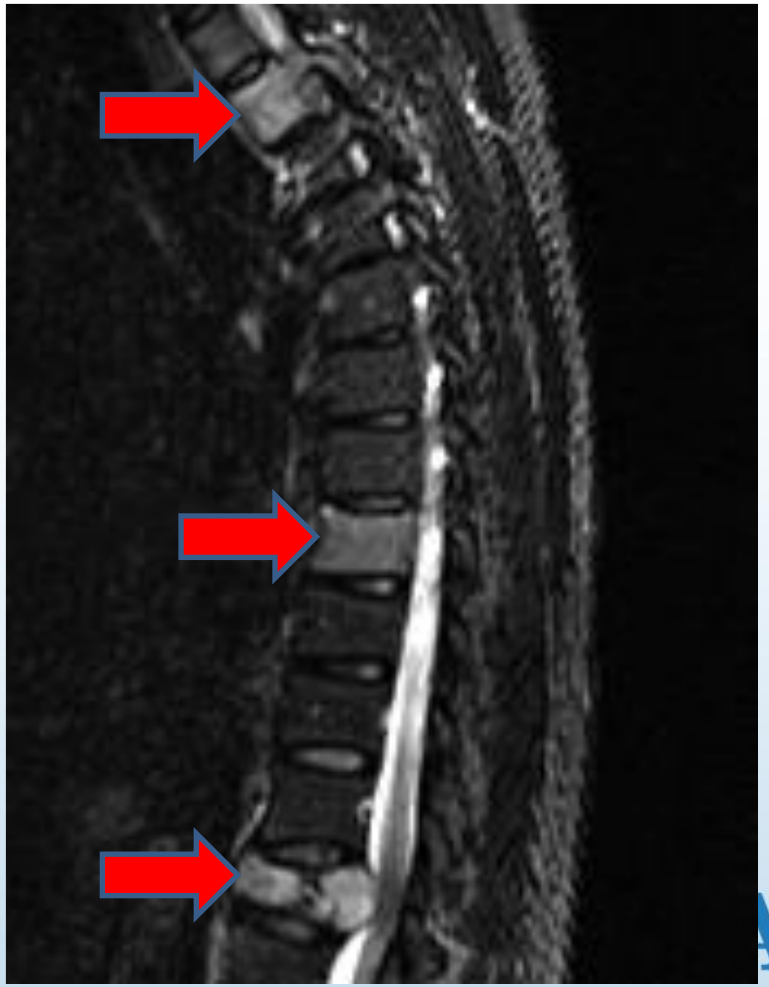




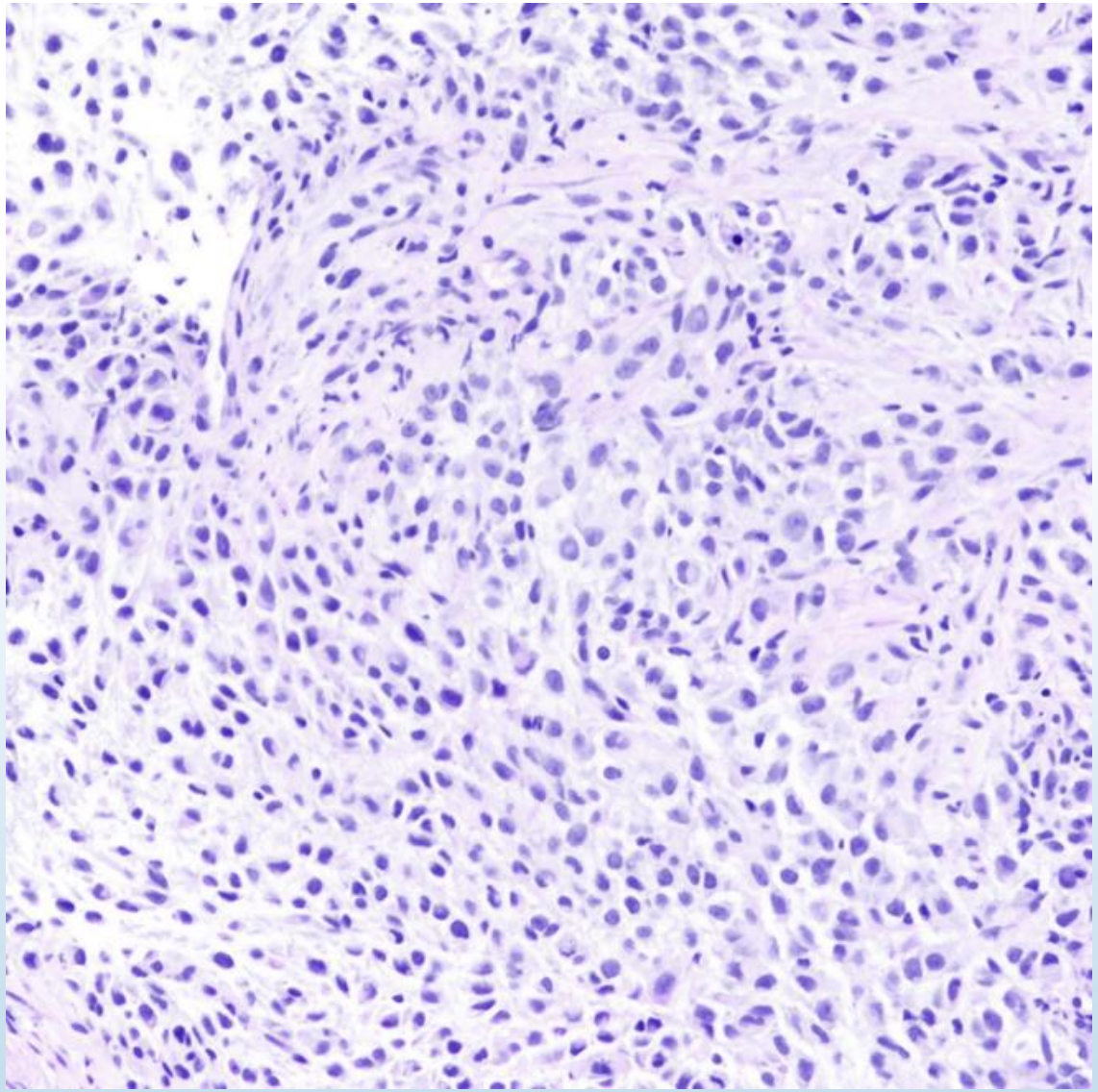
two years later



chest wall and spine

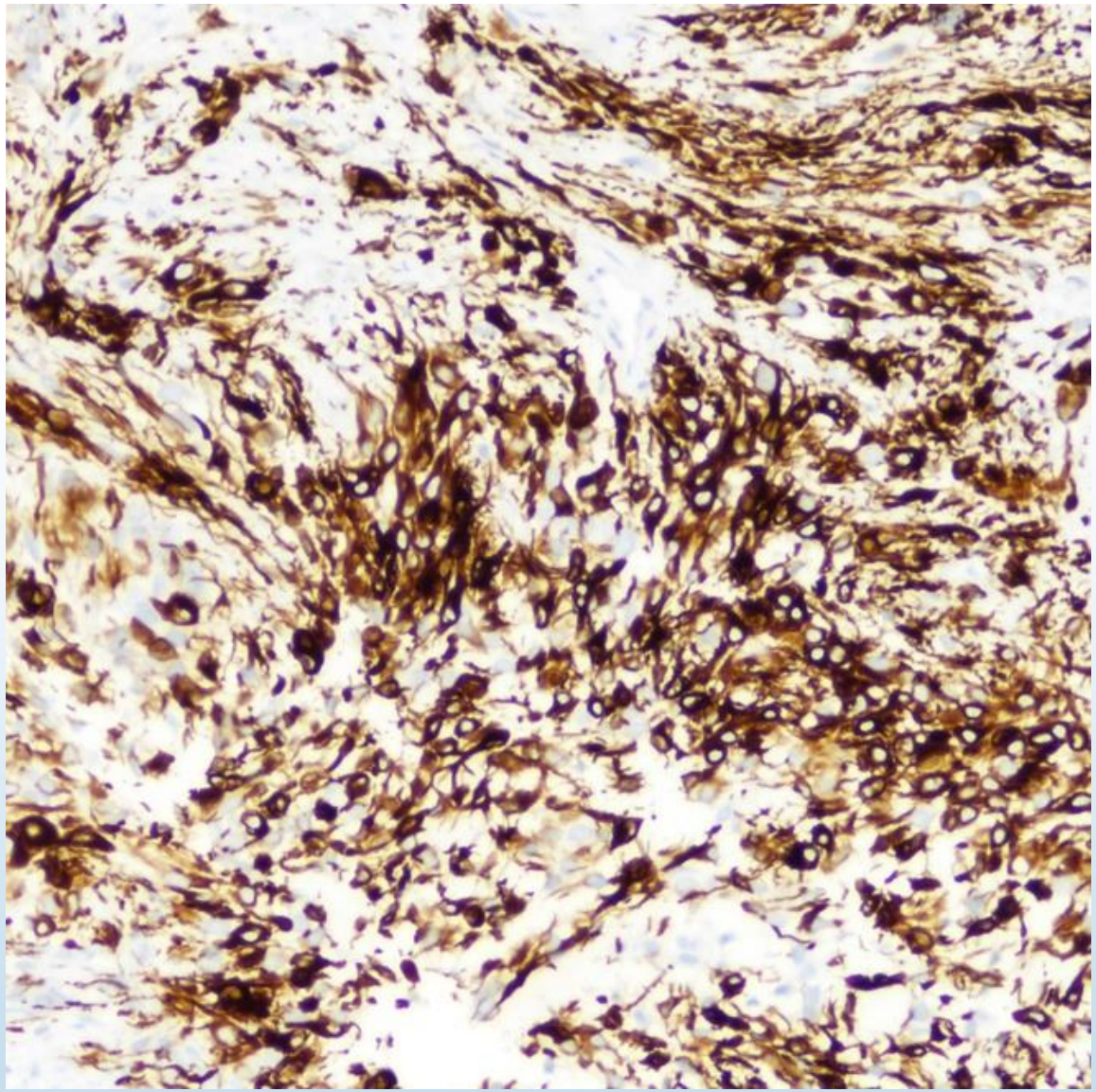


chest wall mass

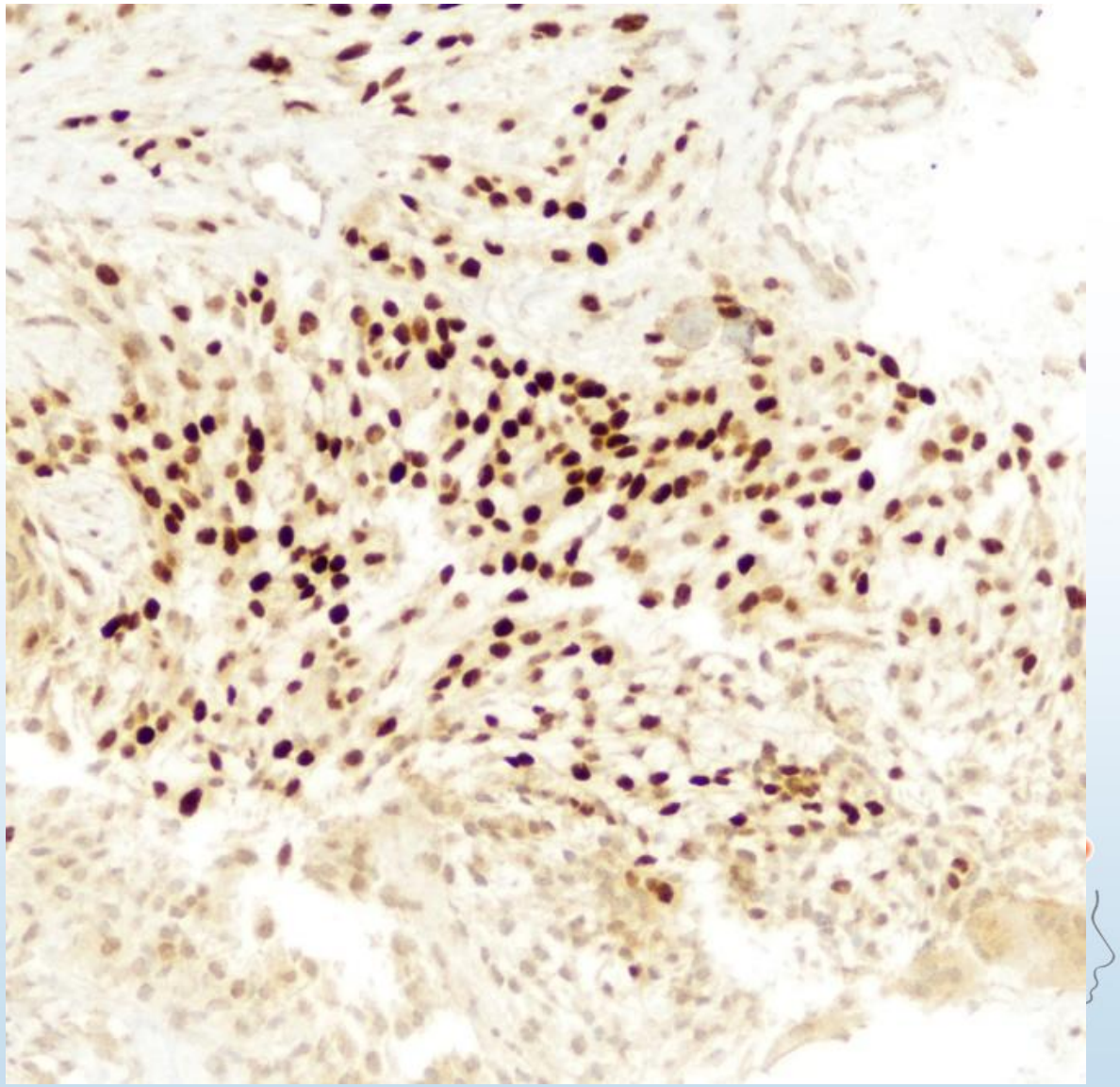


chest wall mass

GFAP



olig2



NGS

brain tumor

- BRAF V600E
- *TERT* promoter mutant
- *SETD2* mutant

chest wall mass

- BRAF V600E
- *TERT* promoter mutant
- *SETD2* mutant
- *TP53* mutant

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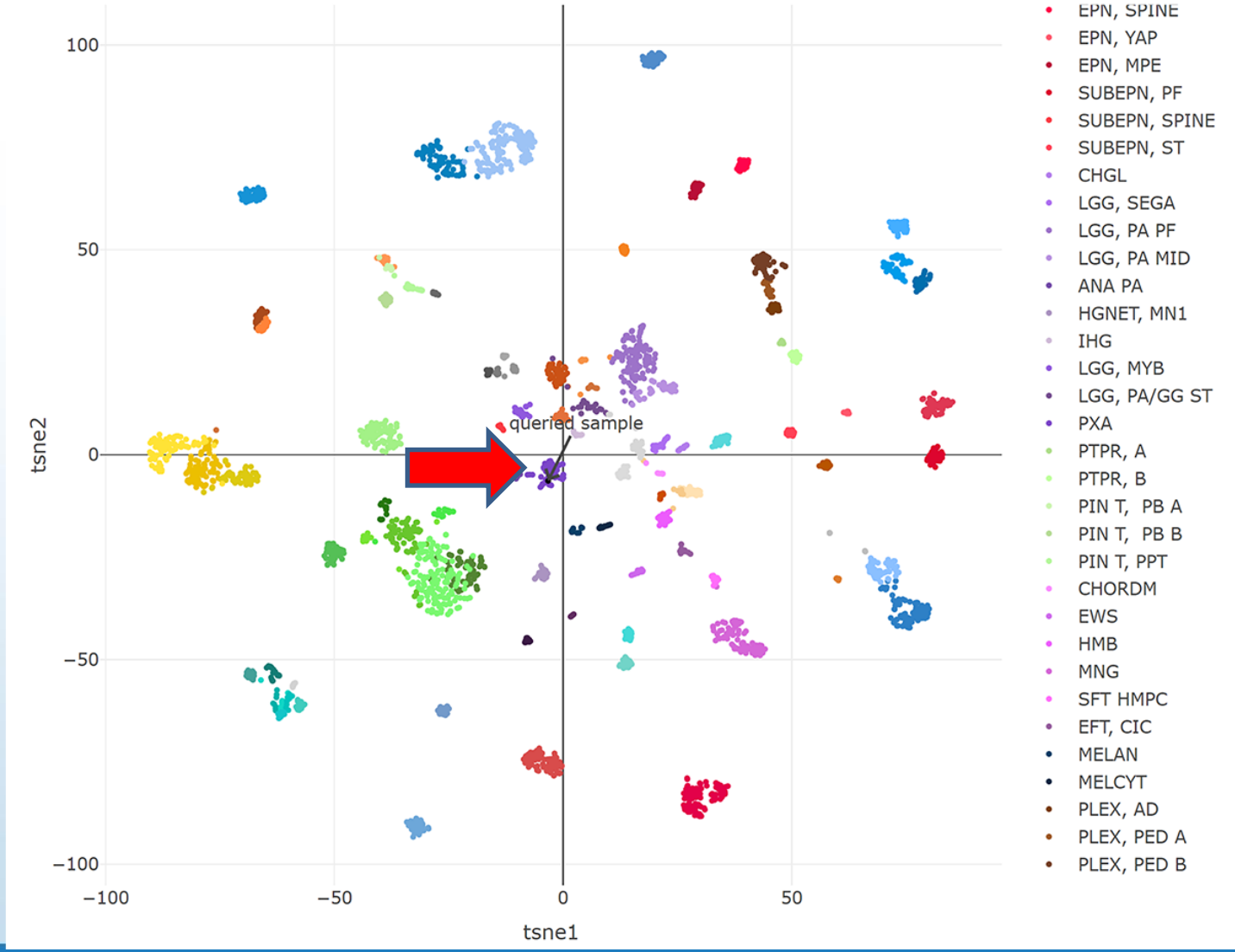
Infinium 850K methylation profile



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revised diagnosis

- pleomorphic xanthoastrocytoma, WHO grade 3, with metastases to the chest wall and spine
- responded to combination of BRAF and MEK inhibitors
- still alive 3+ years later

Association of Pleomorphic Xanthoastrocytoma with Cortical Dysplasia and Neuronal Tumors

A Report of Three Cases

Boleslaw Lach, M.D., Ph.D.[1]
Neil Duggal, M.D.[2]
Vasco F. DaSilva, M.D.[3]
Brien G. Benoit, M.D.[3]

A rare clinical presentation: a pleomorphic xanthoastrocytoma presenting with intracerebral haemorrhage and metastasizing vigorously—case report and review of the literature

Gülden Demirci Otluoğlu[1] · M. Memet Özek[1]

Metastatic pleomorphic xanthoastrocytoma in the scalp

Joel Foo, Wai Hoe Ng *

Department of Neurosurgery, National Neuroscience Institute, 11 Jalan Tan Tock Seng, Singapore 308433, Singapore

Spinal Pleomorphic Xanthoastrocytoma: Case Report and Literature Review

Darius Tan[1], Leon T. Lai[1,2], Christopher D. Daly[1], Vu Tran[1], Julian Maingard[3,4], Craig Timms[1]

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Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Clinical Observation

Complete Remission of an Extracranially Disseminated Anaplastic Pleomorphic Xanthoastrocytoma With Everolimus: A Case Report and Literature Review

Amanda J. Saraf, DO[a,1], Ghada Elhawary, MBChB[b,1], Jonathan L. Finlay, MD[a], Suzanne Scott, APRN[a], Randal Olshefski, MD[a], Mark Halverson, MD[c], Daniel R. Boue, MD, PhD[d], Mohamed S. AbdelBaki, MD[a,*]

Anaplastic pleomorphic xanthoastrocytoma with leptomeningeal dissemination responsive to BRAF inhibition and bevacizumab

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Neuro-Oncology Advances

2(1), 1–5, 2020 | doi:10.1093/noajnl/vdz057 | Advance Access date 27 December 2019

Using methylation profiling to diagnose systemic metastases of pleomorphic xanthoastrocytoma

Kwok-ling Kam, Matija Snuderl, Osaama Khan, Jean-Paul Wolinsky, Vinai Gondi, Sean Grimm, and Craig Horbinski

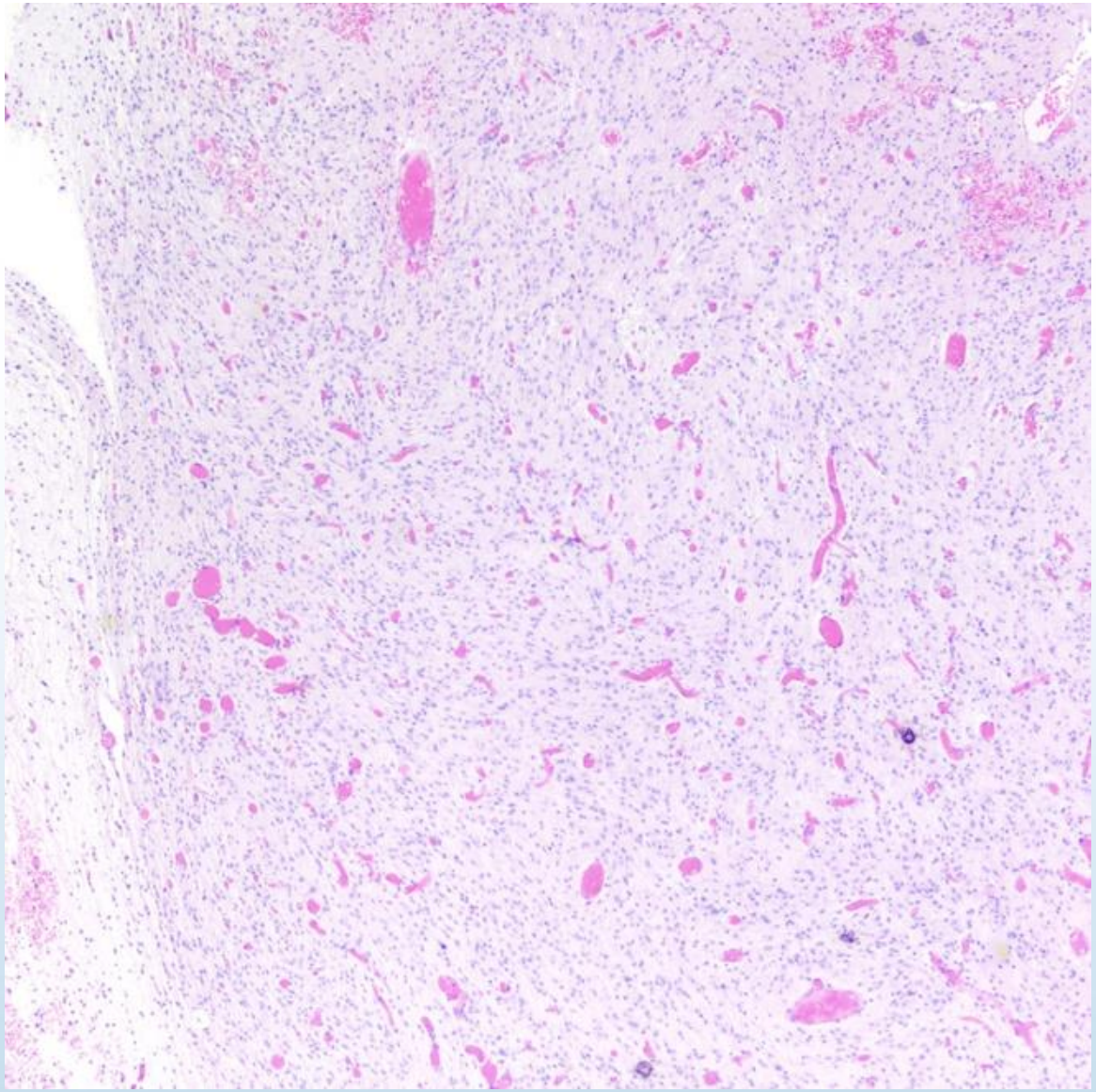
Department of Pathology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois (K-l.K., C.H.); Department of Pathology, NYU Langone Health, New York University, New York (M.S.); Department of Neurosurgery, Feinberg School of Medicine, Northwestern University, Chicago, Illinois (O.K., J-.P.W., and C.H.); Department of Radiation Oncology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois (V.G.); Department of Neurology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois (S.G.)

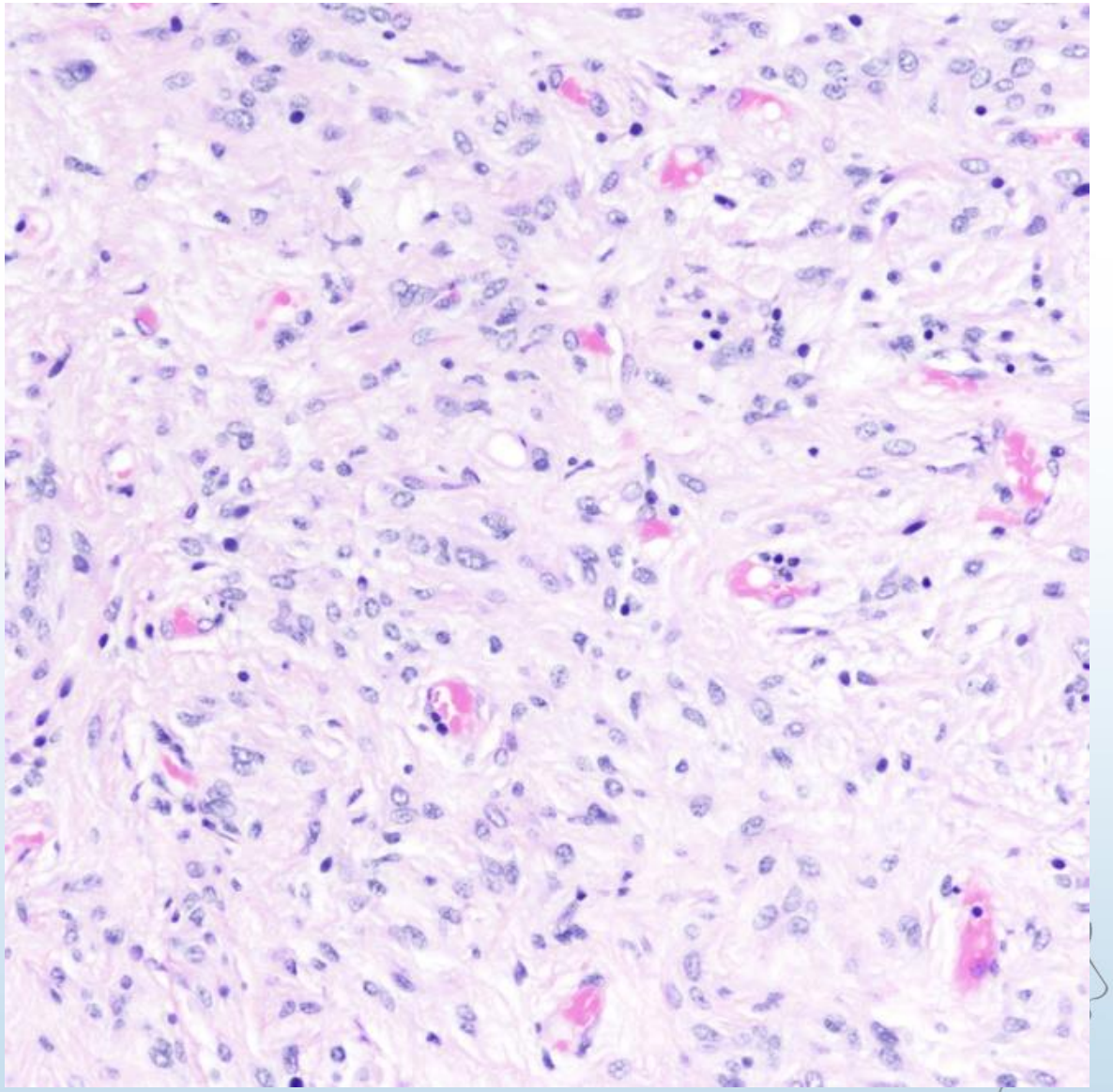
case 5:
nobody's perfect

case 5

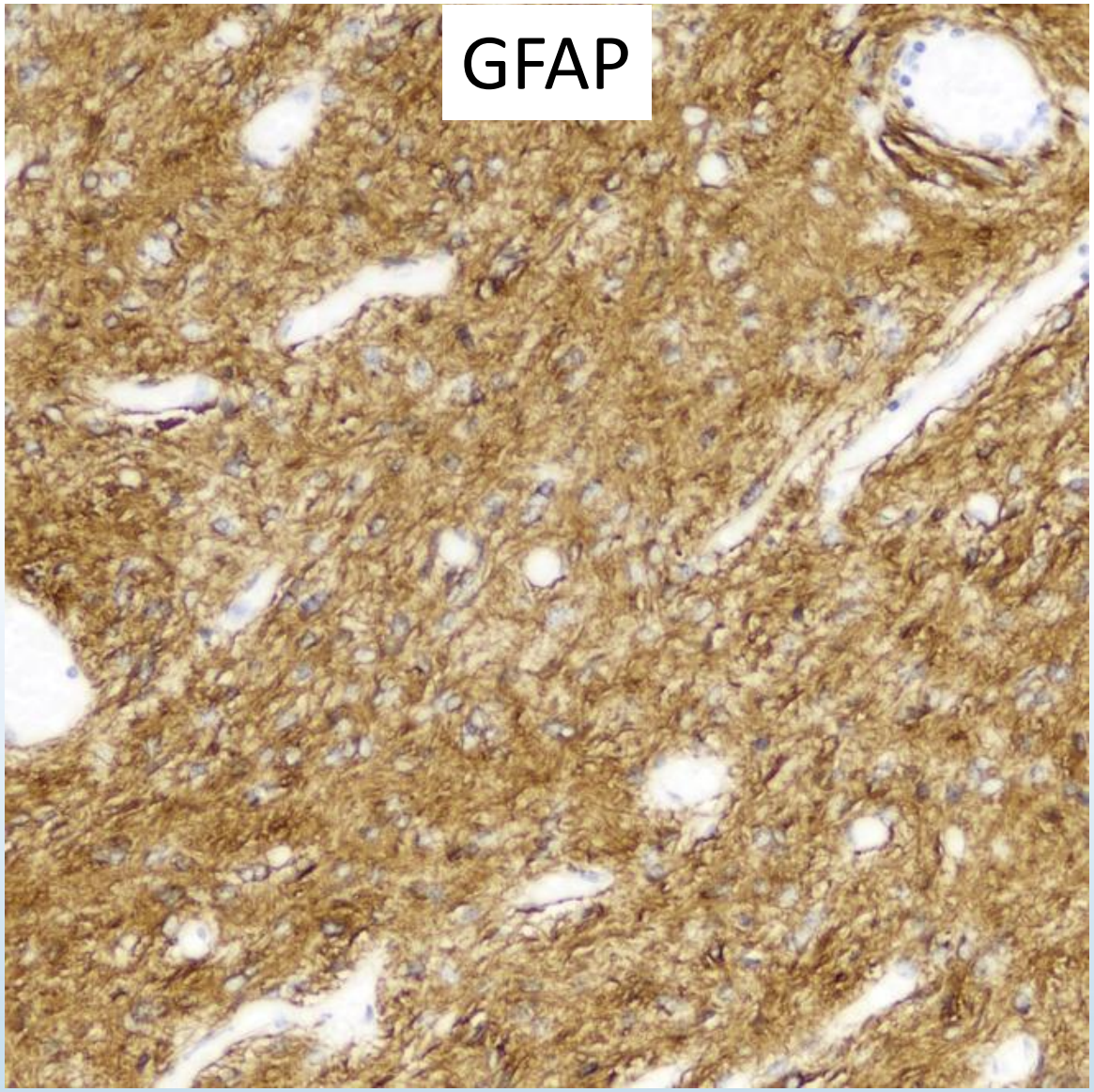
- 30 y/o M
- mass in the pineal region
- original tumor resected 6 years ago, didn’t recur until 6 years later
- consult from OSH—no radiology

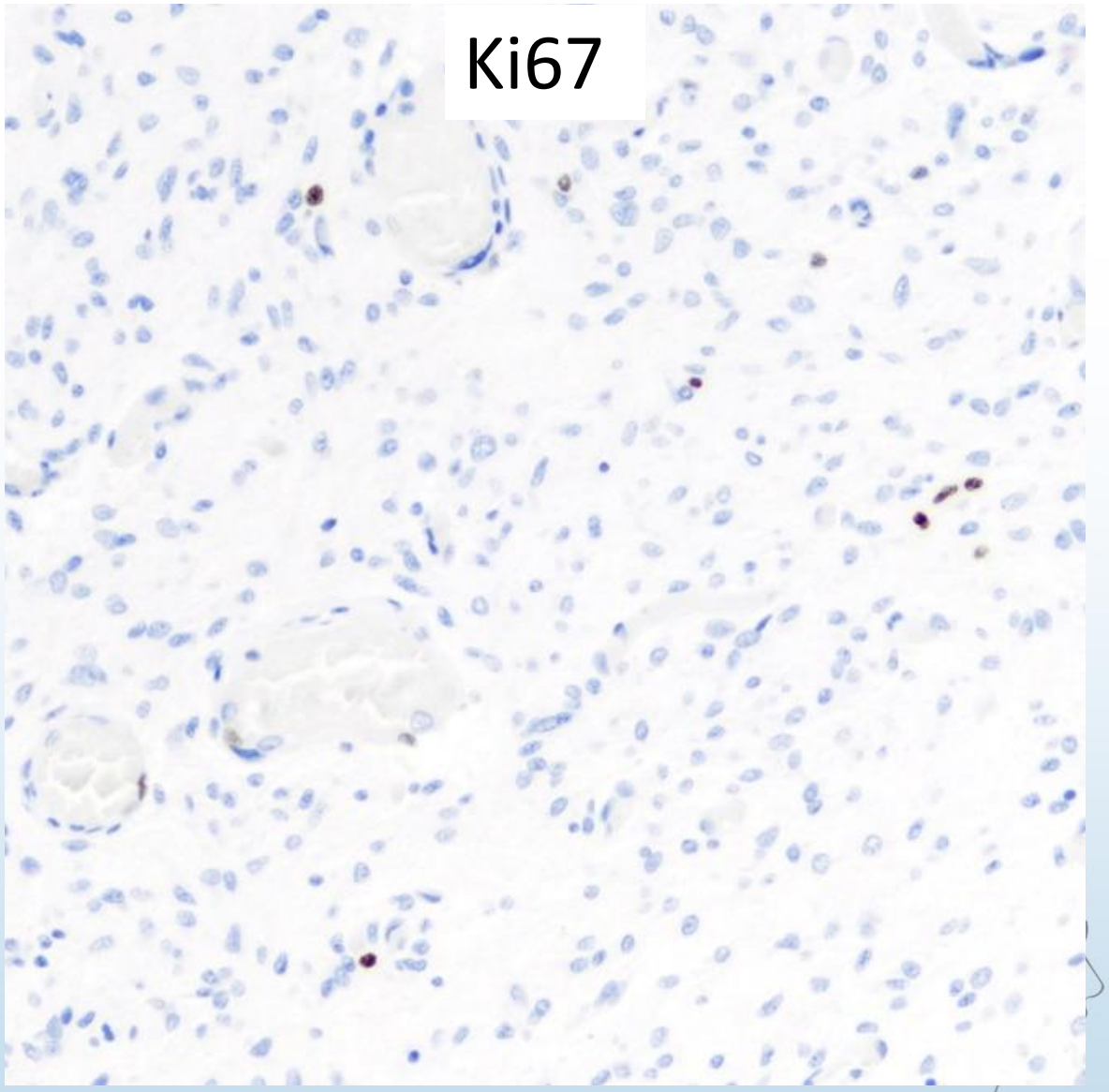
original tumor



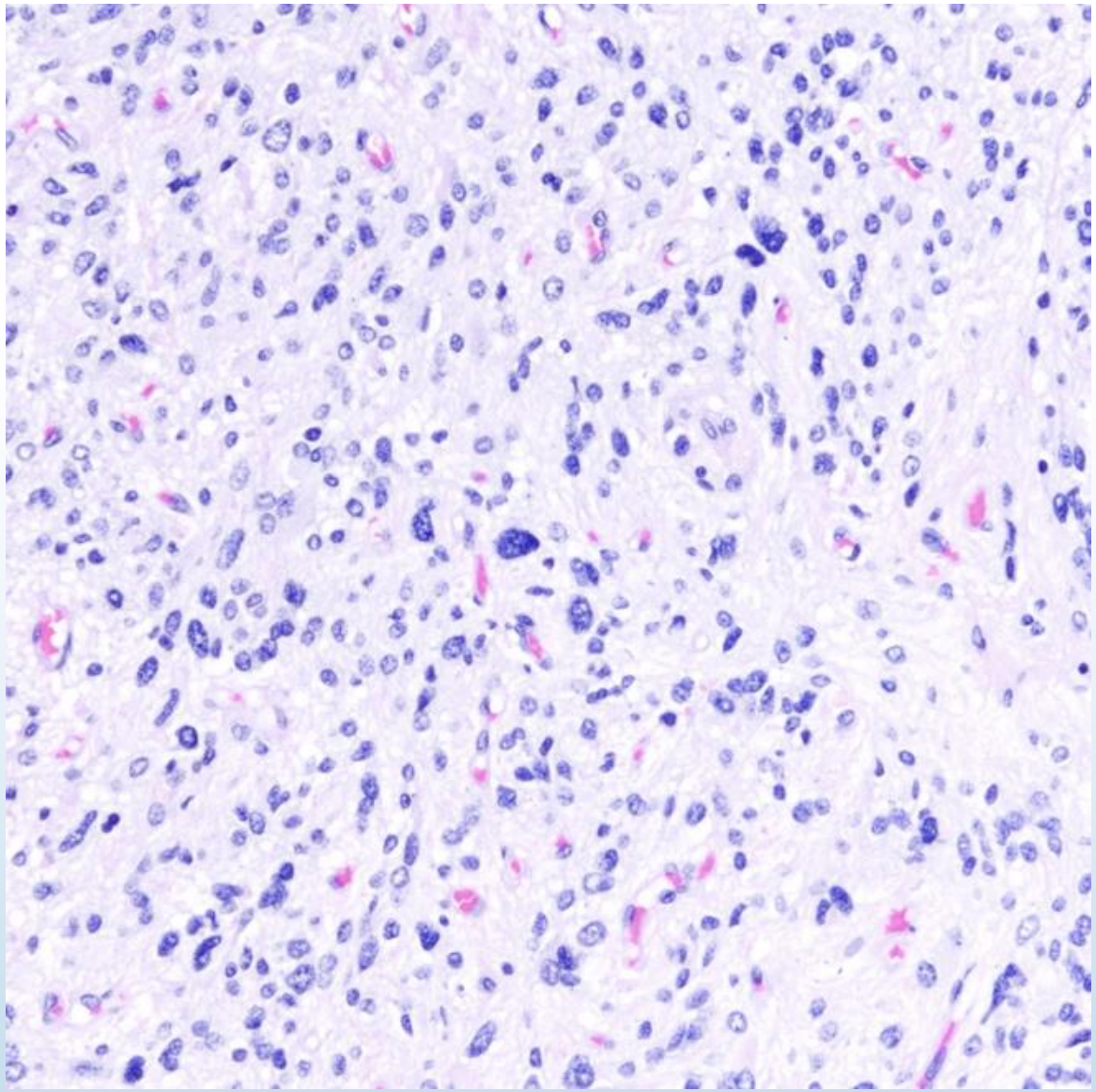


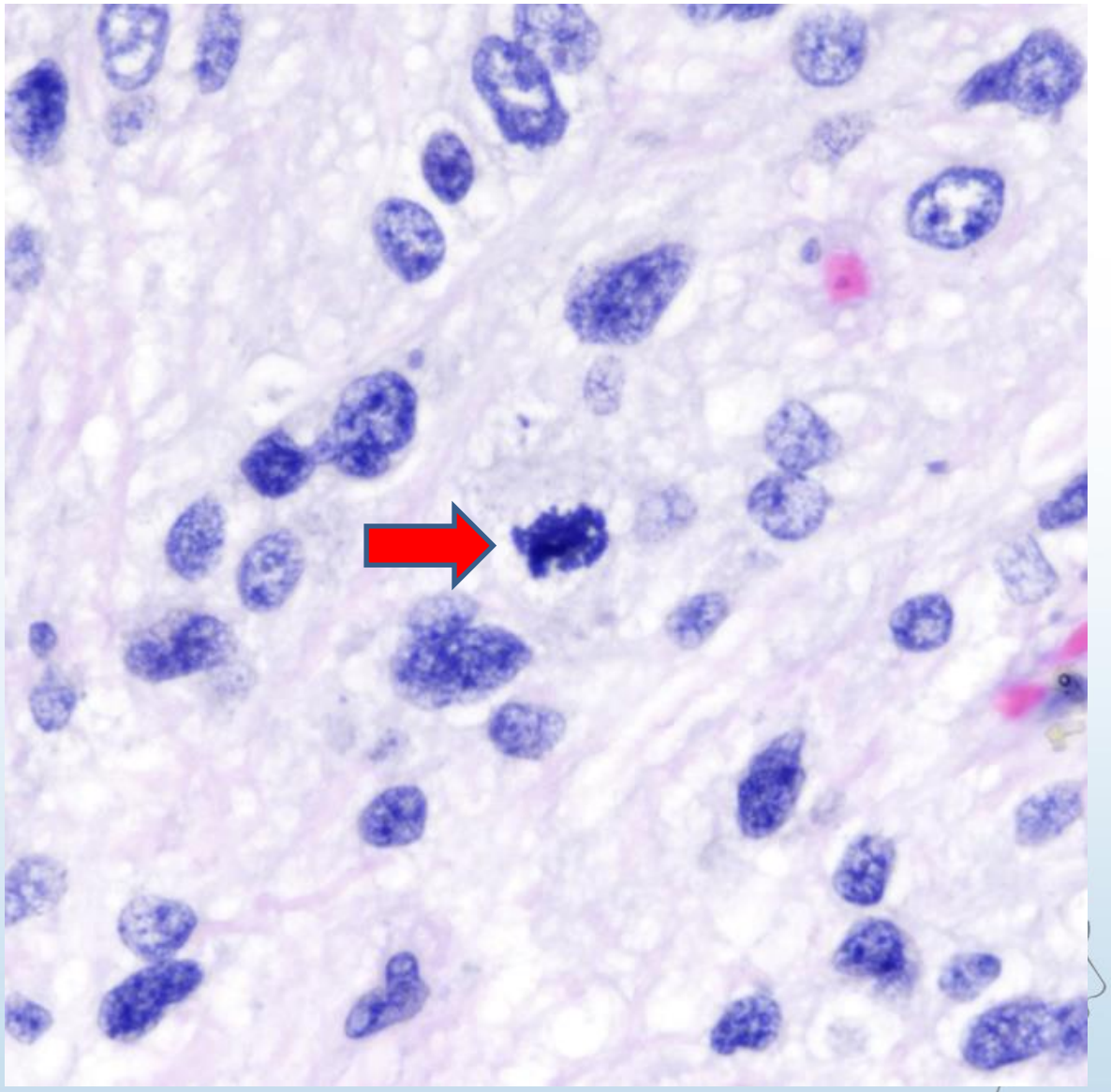
original tumor



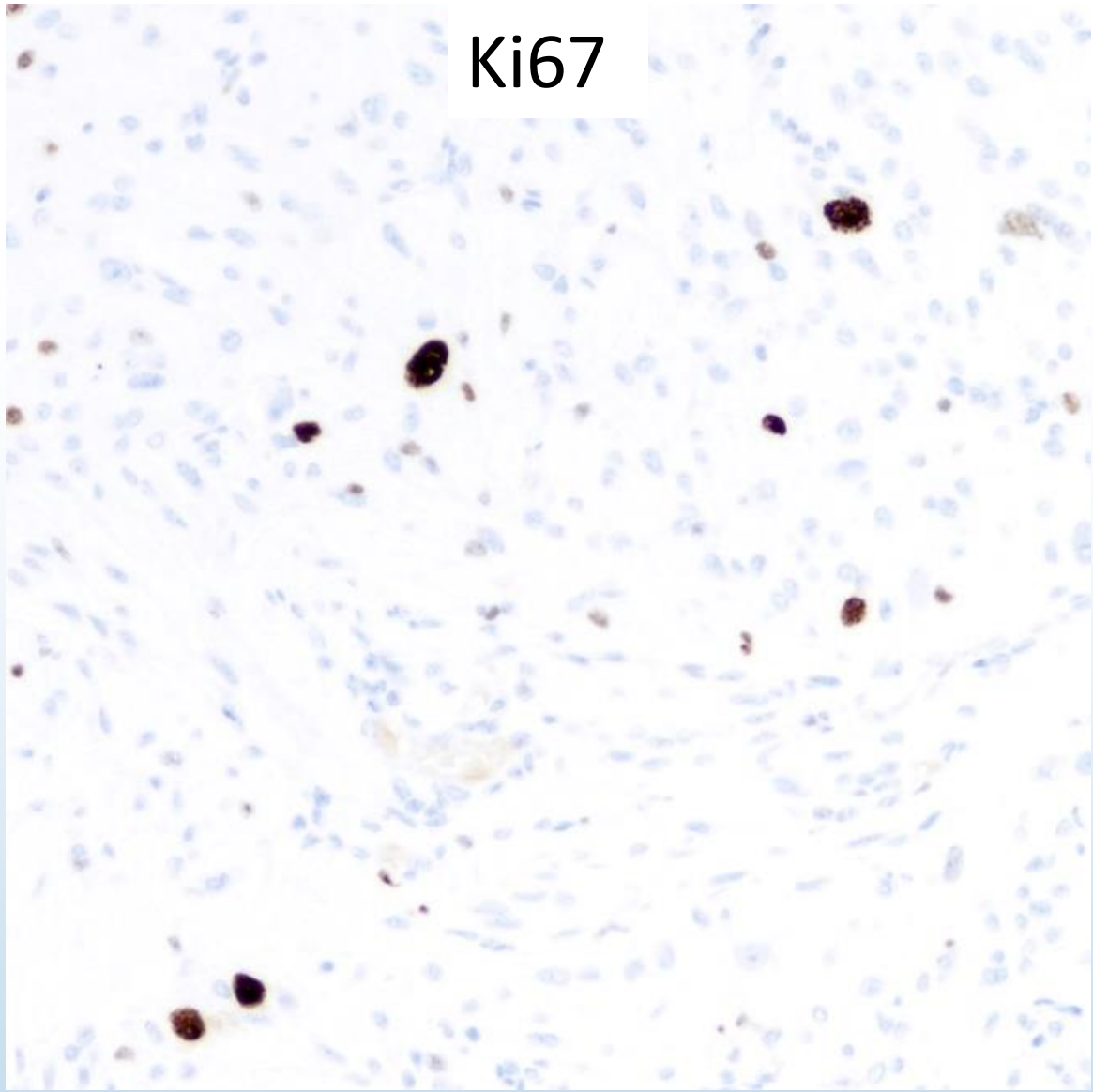


recurrent tumor 6 years later





recurrent tumor 6 years later



molecular results

- NGS
  - **H3-3A K27M**
  - **BRAF K601N**
- oncoscan
  - gain of 6p
  - loss at 1p
  - CN-LOH on 1q and 17q
- methylation profiling
  - “diffuse midline glioma, H3K27M mutant”

Journal of Cancer Research and Clinical Oncology (2021) 147:1365–1378
https://doi.org/10.1007/s00432-021-03545-2

ORIGINAL ARTICLE – CANCER RESEARCH

Personalized oncology and *BRAF*^{K601N} melanoma: model development, drug discovery, and clinical correlation

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Brain Tumor Pathology (2019) 36:162–168
https://doi.org/10.1007/s10014-019-00347-w

CASE REPORT

A long-term survivor of pediatric midline glioma with *H3F3A* K27M and *BRAF* V600E double mutations

Yoshiko Nakano[1,2] · Kai Yamasaki[1,2] · Hiroaki Sakamoto[3] · Yasuhiro Matsusaka[3] · Noritsugu Kunihiro[3] · Hiroko Fukushima[4] · Takeshi Inoue[4] · Mai Honda-Kitahara[1] · Junichi Hara[2] · Akihiko Yoshida[5] · Koichi Ichimura[1]

Brain Pathology ISSN 1015–6305

RESEARCH ARTICLE

Co-occurrence of histone H3 K27M and BRAF V600E mutations in paediatric midline grade I ganglioglioma

Mélanie Pagès[1,2,3], Kevin Beccaria[4], Nathalie Boddaert[5], Raphaël Saffroy[6], Aurore Besnard[1], David Castel[7,8], Frédéric Fina[9], Doriane Barets[10], Emilie Barret[7,8], Ludovic Lacroix[11], Franck Bielle[12], Felipe Andreiuolo[1], Arnault Tauziède-Espariat[1], Dominique Figarella-Branger[10,13], Stéphanie Puget[4], Jacques Grill[7,8], Fabrice Chrétien[1,2,14], Pascale Varlet[1,2,3]

Neuropathology and Applied Neurobiology (2015), **41**, 403–408

Scientific correspondence

Evidence for *BRAF* V600E and *H3F3A* K27M double mutations in paediatric glial and glioneuronal tumours

diagnosis

- midline glioma with H3-3A K27M and BRAF mutations (see comment)

Acta Neuropathologica
https://doi.org/10.1007/s00401-023-02542-8

CORRESPONDENCE

Variant allelic frequency of driver mutations predicts success of genomic DNA methylation classification in central nervous system tumors

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Received: 3 November 2022 / Revised: 19 January 2023 / Accepted: 20 January 2023

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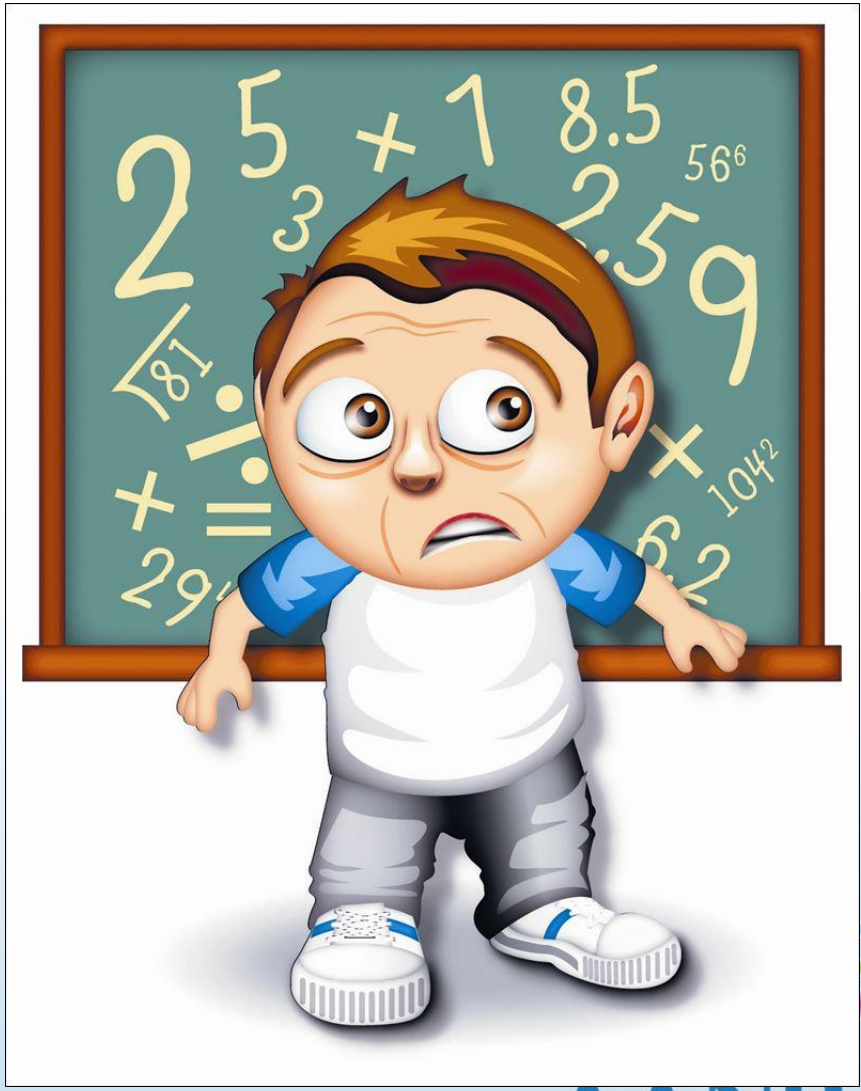
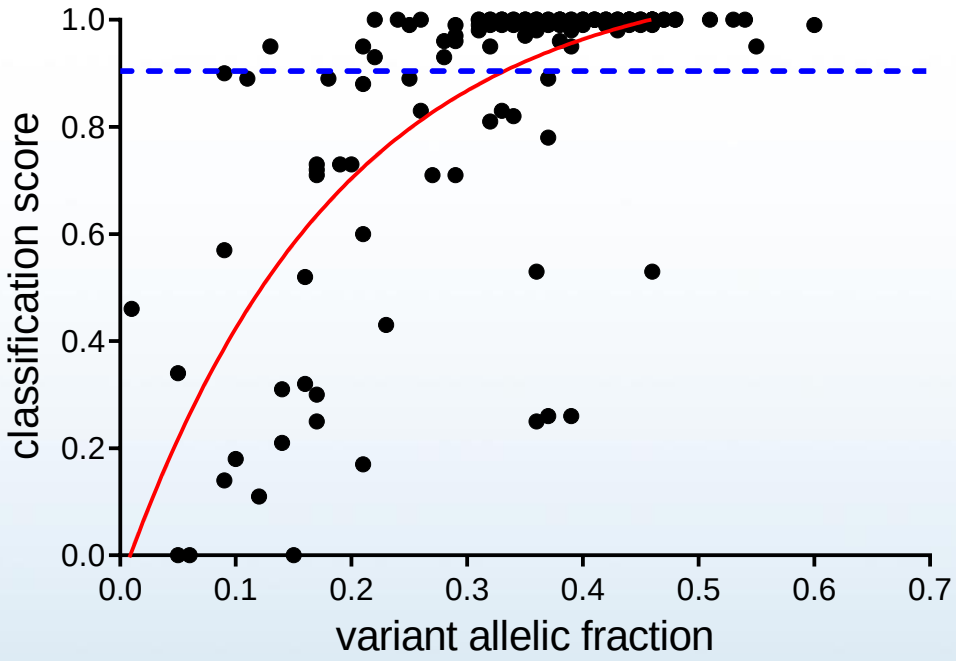
simple math

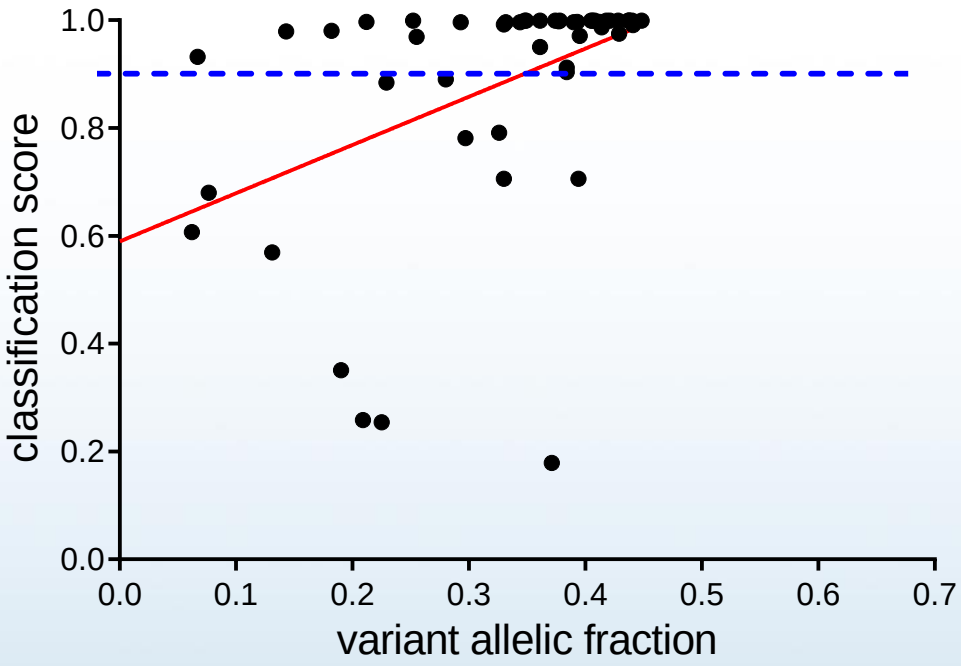
1. *if* a driver mutation is present in virtually 100% of tumor cells…
2. …*and* nearly always exists in a heterozygous state…
3. …*and* there are no copy number variations at that gene locus…
4. …*then* Variant Allelic Fraction x 2 = % tumor cellularity



NMH


classification score
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
variant allelic fraction

NYU


classification score
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0.4
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0.5
0.6
0.7
variant allelic fraction

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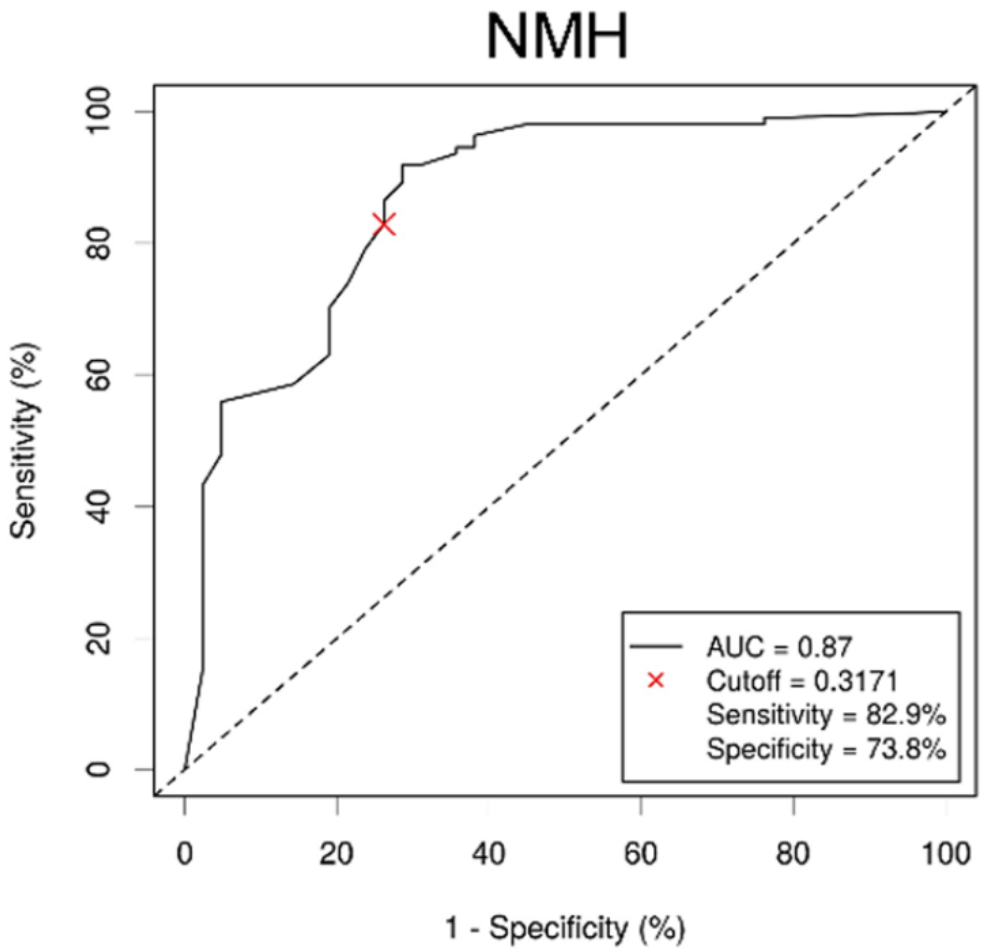
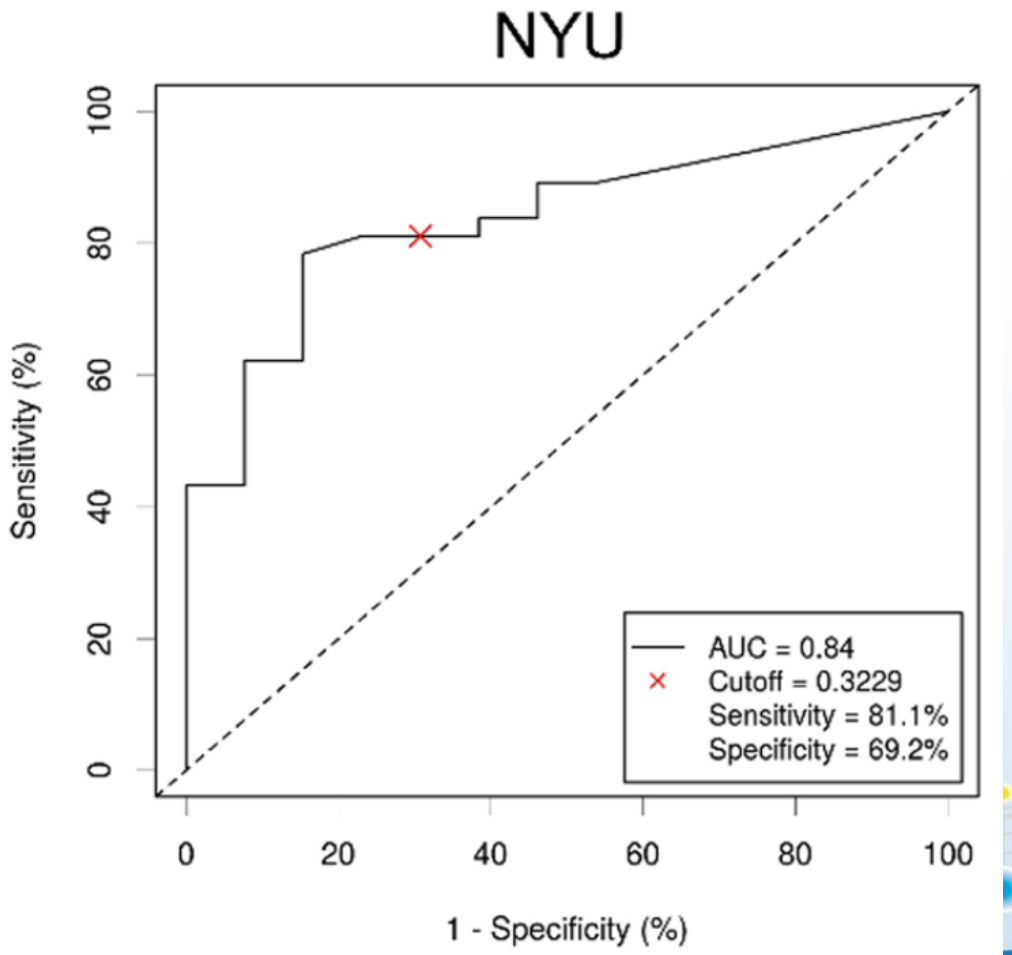

NMH
Sensitivity (%)
1 - Specificity (%)
0
20
40
60
80
100
AUC = 0.87
Cutoff = 0.3171
Sensitivity = 82.9%
Specificity = 73.8%


NYU
Sensitivity (%)
1 - Specificity (%)
0
20
40
60
80
100
AUC = 0.84
Cutoff = 0.3229
Sensitivity = 81.1%
Specificity = 69.2%

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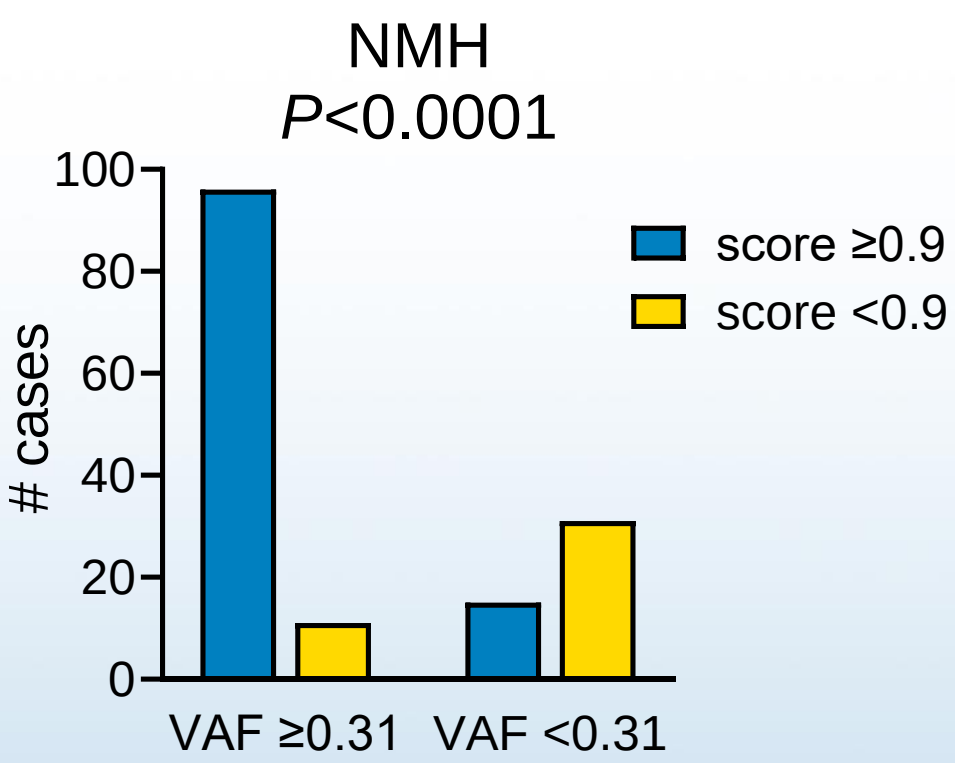
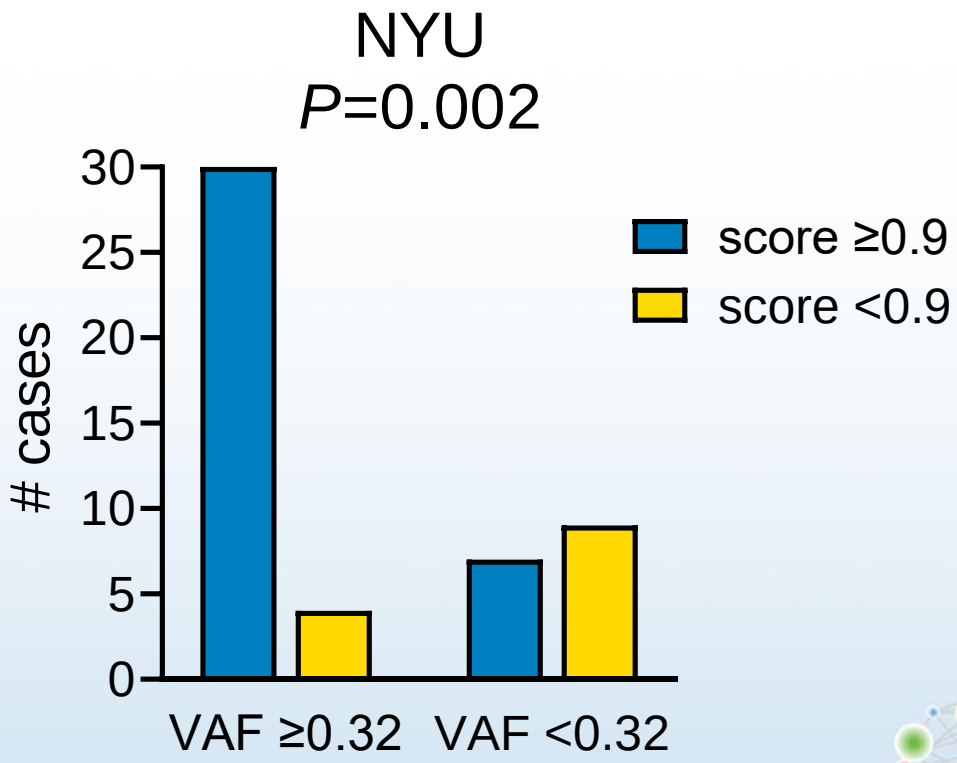
if you don’t get a match, insufficient tumor cellularity might be the problem





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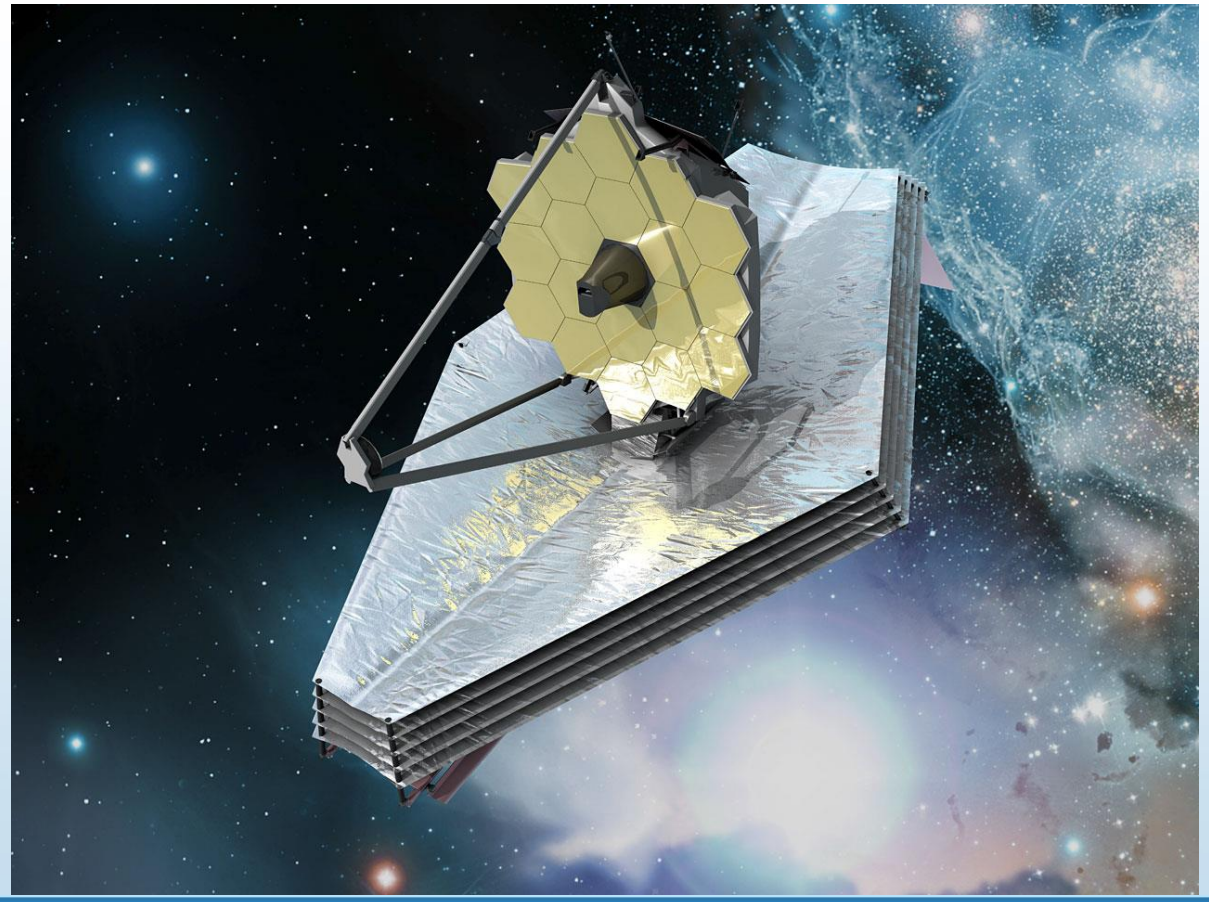
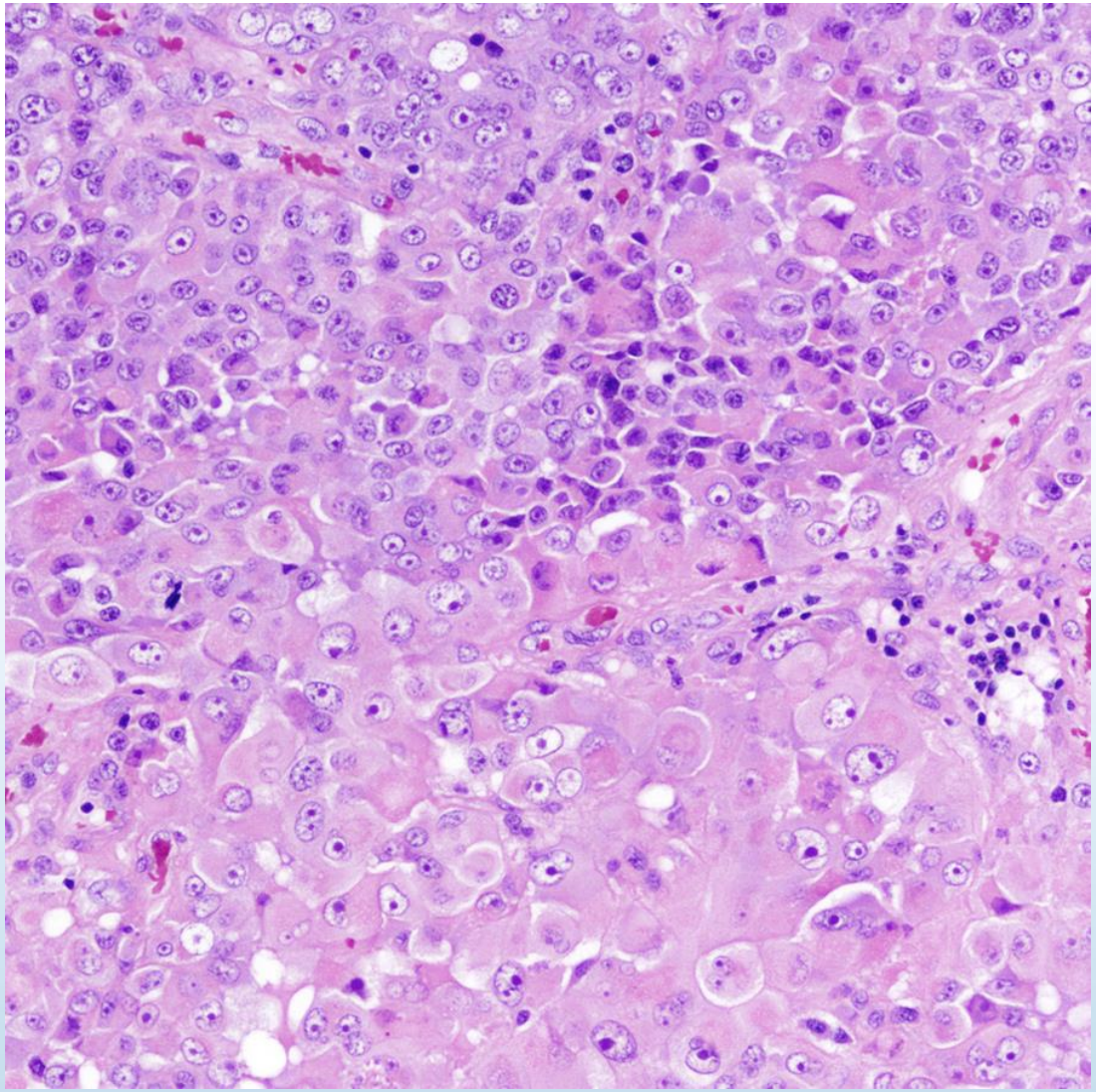
case 6:
uncharted territory



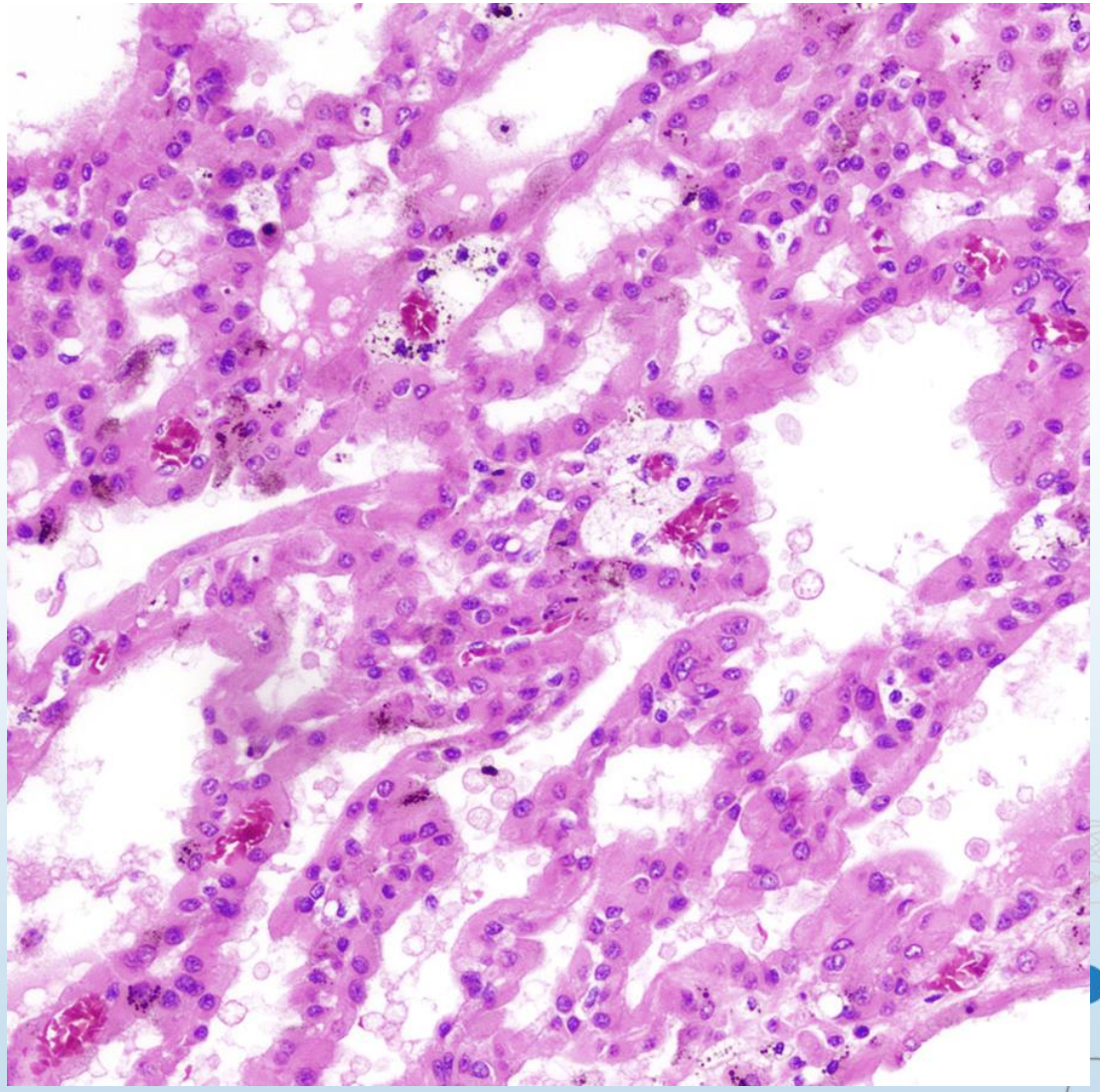
case 6

- 2 year-old boy
- cerebral tumor

block 1

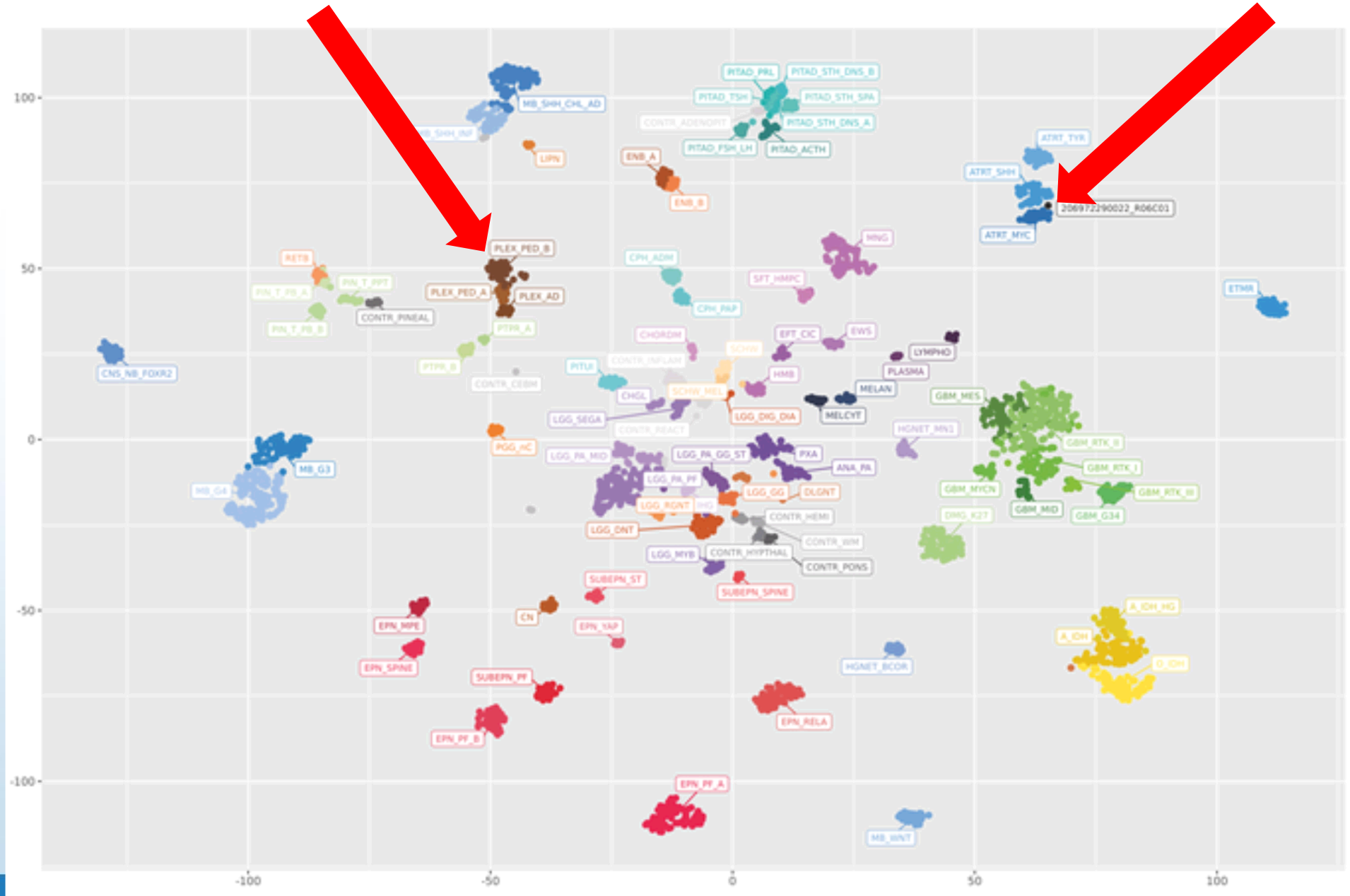


block 2

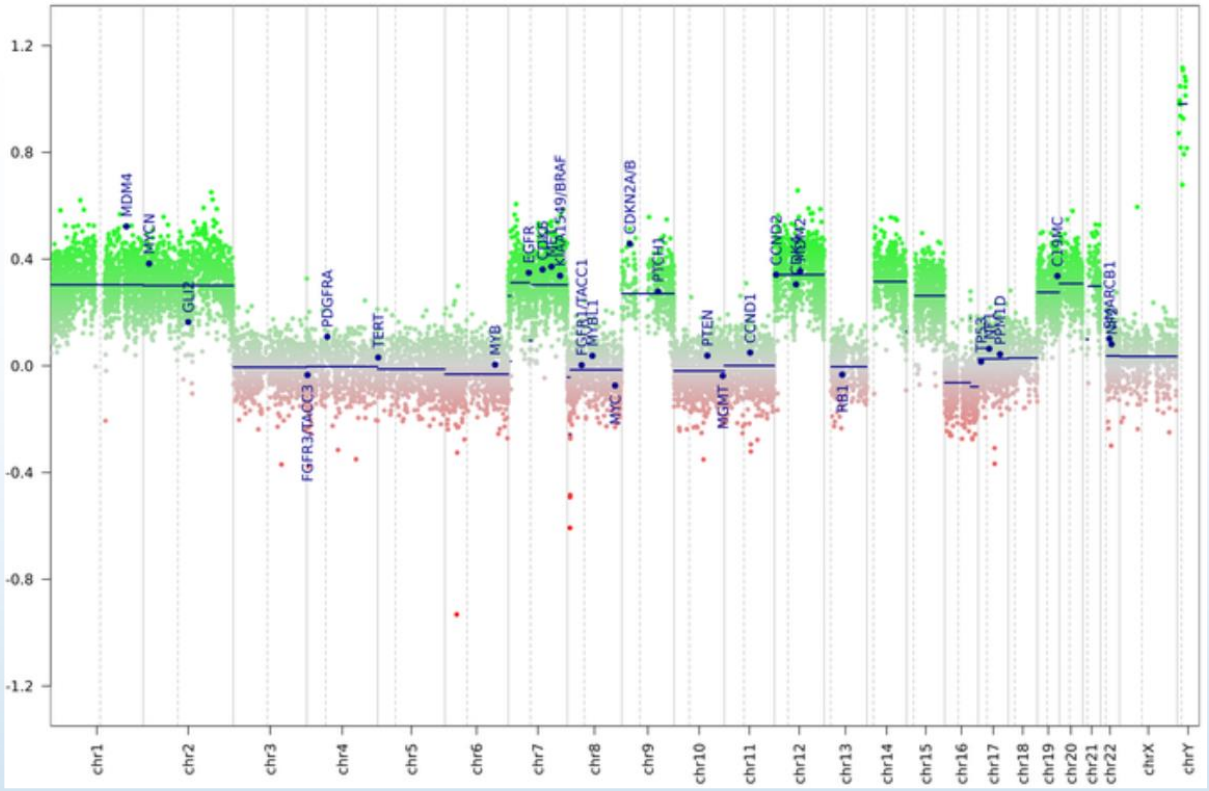


block 2: “choroid plexus tumor”

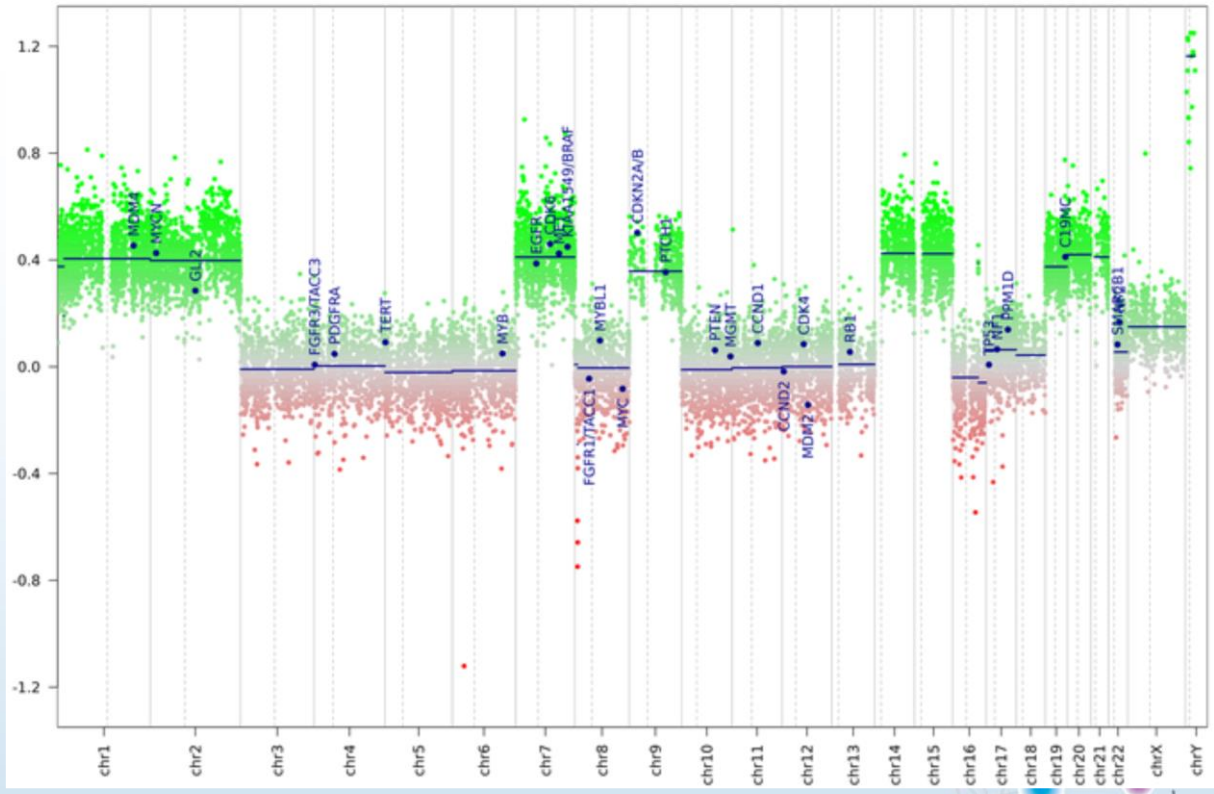
block 1: “ATRT”



block 1:
ATRT



block 2:
choroid plexus tumor



probable hypodiploid genomes

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NGS results

ATRT (block 1)

- *TP53* p.Arg282Trp
- *TSC2* p.Arg1477Glyfs*46
- *KMT2D* p.Pro2354Ser

choroid plexus tumor (block 2)

- *TP53* p.Arg282Trp
- *TSC2* p.Arg1477Glyfs*46
- *KMT2D* p.Pro2354Ser

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diagnosis

- high grade neoplasm with divergent embryonal (ATRT) and CPC subclonal evolution

methylation profiling fosters the discovery of new tumor types

High-grade glioma with pleomorphic and pseudopapillary features (HPAP): a proposed type of circumscribed glioma in adults harboring frequent *TP53* mutations and recurrent monosomy 13

Drew Pratt[1] · Zied Abdullaev[1] · Antonios Papanicolau-Sengos[1] · Courtney Ketchum[1] · Pavalan Panneer Selvam[1] · Hye-Jung Chung[1] · Ina Lee[1] · Mark Raffeld[1] · Mark R. Gilbert[2] · Terri S. Armstrong[2] · Peter Pytel[3] · Ewa Borys[4] · Joshua M. Klonoski[5] · Matthew McCord[6] · Craig Horbinski[6] · Daniel Brat[6] · Arie Perry · Charles Eberhart[8] · Caterina Giannini[9] · Martha Quezado[1] · Kenneth Aldape[1]

Glioneuronal tumor with *ATRX* alteration, kinase fusion and anaplastic features (GTAKA): a molecularly distinct brain tumor type with recurrent *NTRK* gene fusions

Expanded analysis of high-grade astrocytoma with [illegible] identifies an epigenetically and clinically distinct subtype associated with neurofibromatosis type 1

Patrick J. Cimino[1] · Courtney Ketchum[2] · Rust Turakulov[2] · Omkar Singh[2] · Zied Abdullaev[2] · Caterina Giannini[3] · Peter Pytel[4] · Giselle Yvette Lopez[5] · Howard Colman[6] · MacLean P. Nasrallah[7] · Mariarita Santi[8] · Igor Lima Fernandes[9] · Jeff Nirschl[10] · Sonika Dahiya[11] · Stewart Neill[12] · David Solomon[13] · Ellis Perez[14] · David Capper[14] · Haresh Mani[15] · Dario Caccamo[16] · Matthew Ball[17] · Michael Badruddoja[18] · Rati Chkheidze[19] · Sandra Camelo-Piragua[20] · Joseph Fullmer[21] · Sanda Alexandrescu[22] · Gabrielle Yeaney[23] · Charles Eberhart[24] · Maria Martinez-Lage[25] · Jie Chen[26] · Leor Zach[27] · B. K. Kleinschmidt-DeMasters[28] · Marco Hefti[29] · Maria-Beatriz Lopes[30] · Nicholas Nuechterlein[31] · Craig Horbinski[32] · Fausto J. Rodriguez[33] · Martha Quezado[2] · Drew Pratt[2] · Kenneth Aldape[2]

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conclusion

methylation profiling is now an **indispensable** part of neuropathology

references

1. Capper D., et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018 Mar 22;555(7697):469-474. PMCID: PMC6093218.
2. Zhang S, et al. DNA methylation profiling to determine the primary sites of metastatic cancers using formalin-fixed paraffin-embedded tissues. Nat Commun. 2023 Sep 14;14(1):5686. PMCID: PMC10502058.
3. Kam KL, et al. Using methylation profiling to diagnose systemic metastases of pleomorphic xanthoastrocytoma. Neurooncol Adv. 2020 Jan-Dec;2(1):vdz057. PMCID: PMC6978194
4. Santana-Santos L, et al. Validation of Whole Genome Methylation Profiling Classifier for Central Nervous System Tumors. J Mol Diagn. 2022 Aug;24(8):924-934. PMID: 35605901
5. Jamshidi P, et al. Variant allelic frequency of driver mutations predicts success of genomic DNA methylation classification in central nervous system tumors. Acta Neuropathol. 2023 Mar;145(3):365-367. PMID: 36700952.

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Any questions?