ADVANCES IN MOLECULAR DIAGNOSTICS AND TARGETED THERAPY FOR CENTRAL NERVOUS SYSTEM TUMORS

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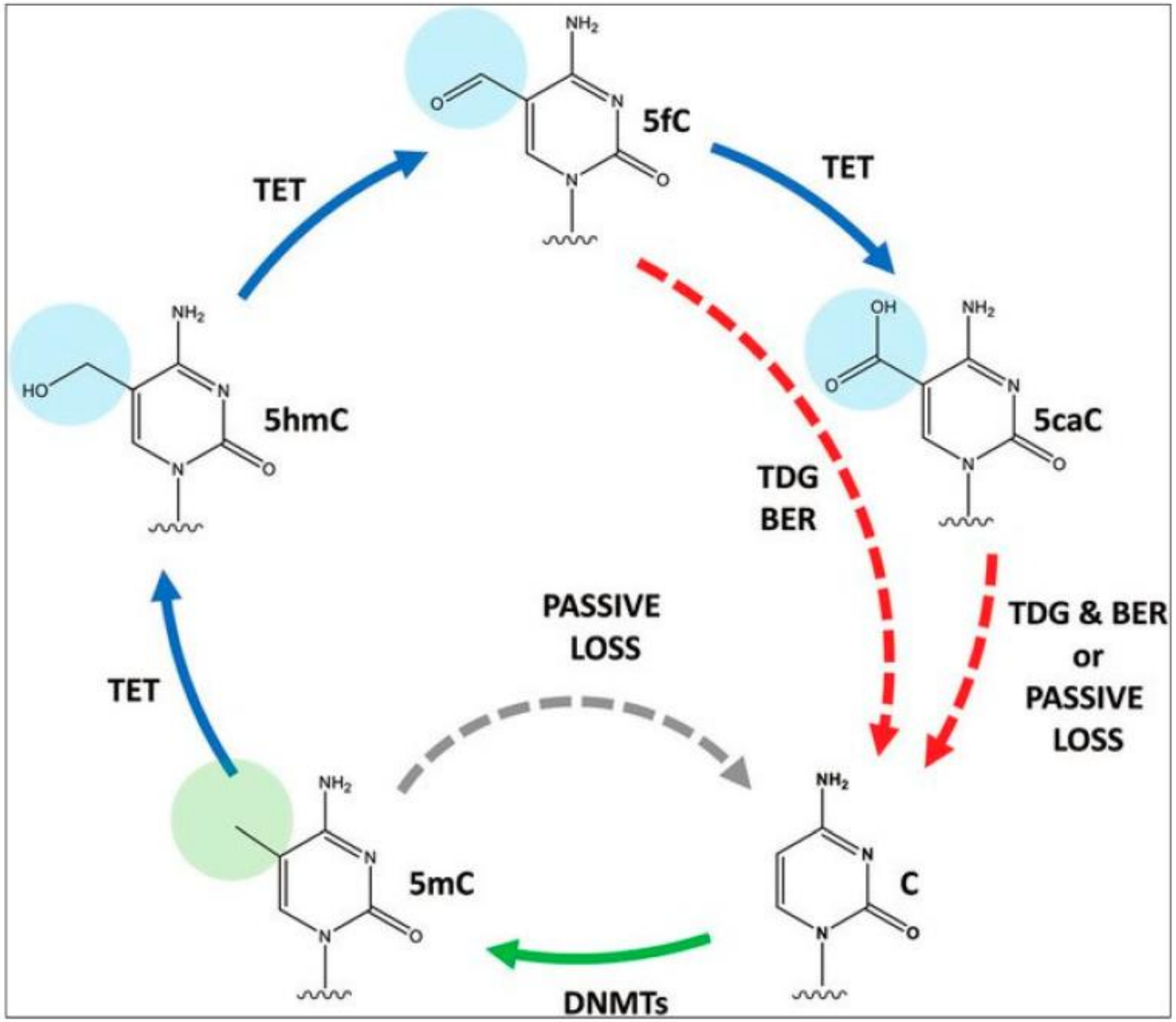
CYTOSINE METHYLATION: THE FOUNDATION OF EPIGENETICS

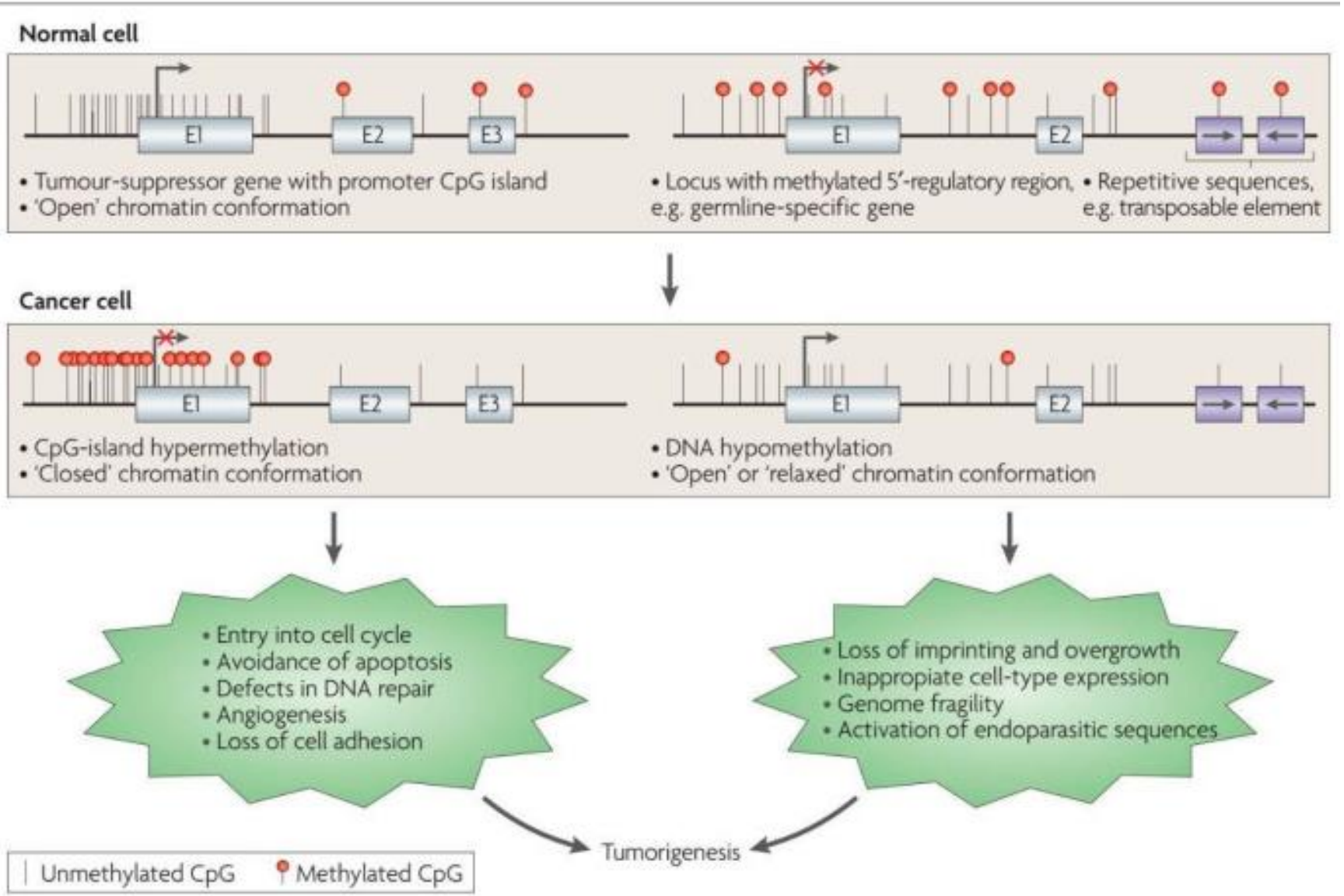
- Cytosine (C) is a core nucleotide
- Methylation (-CH3) by DNA Methyl-Transferases (DNMT) to produce 5-methyl-cytosine is a core alteration
  - "The fifth DNA base"
- Demethylation can be:
  - Passive
  - Active via Ten-Eleven Transferase (TET) and DNA repair activity
    - TET: alpha-ketoglutarate-dependent



Ozair (2023) *Cancers (Basel)*

EPIGENETIC MODIFICATION BASICS

- Epigenetics was first mostly understood on a microcosmic level
  - **DNA promoter epigenetics:**
    - Hypermethylation: blocks promoter and gene transcription
    - Hypomethylation: opens promoter and allows gene transcription
  - **Gene body epigenetics:**
    - Hypermethylation: increases gene transcription
    - Hypomethylation: decreases gene transcription
  - **Histone epigenetics:**
    - Complicated dynamics of acetylation and methylation that is dependent on histone, residue, and amount
      - Looser chromatin structure: more access to DNA for gene transcription
      - Tighter chromatin structure: less access to DNA for gene transcription
- Other modifications include phosphorylation, ubiquitination, sumoylation, ADP-ribosylation



CNS TUMORS CHARACTERIZED BY GLOBAL DNA EPIGENETIC CHANGES

- CpG: cytosine-guanine sequence
- CpG islands are enriched with this sequence
  - 5' promoter regions
  - Untranslated regions
  - Gene bodies to lesser degree

Esteller (2007) *Nature Revi Genetics*

PROFILING DNA METHYLATION USING THE ILLUMINA INFINIUM PLATFORM

- The Illumina Infinium platform with its high-density DNA methylation arrays, more specifically the **HumanMethylation450** with 485 577 CpGs (released in 2011) and its successor, the **Methylation EPIC** with 853 307 CpGs (released in 2015-2016) has been experienced as simple to use and generating reproducible data.

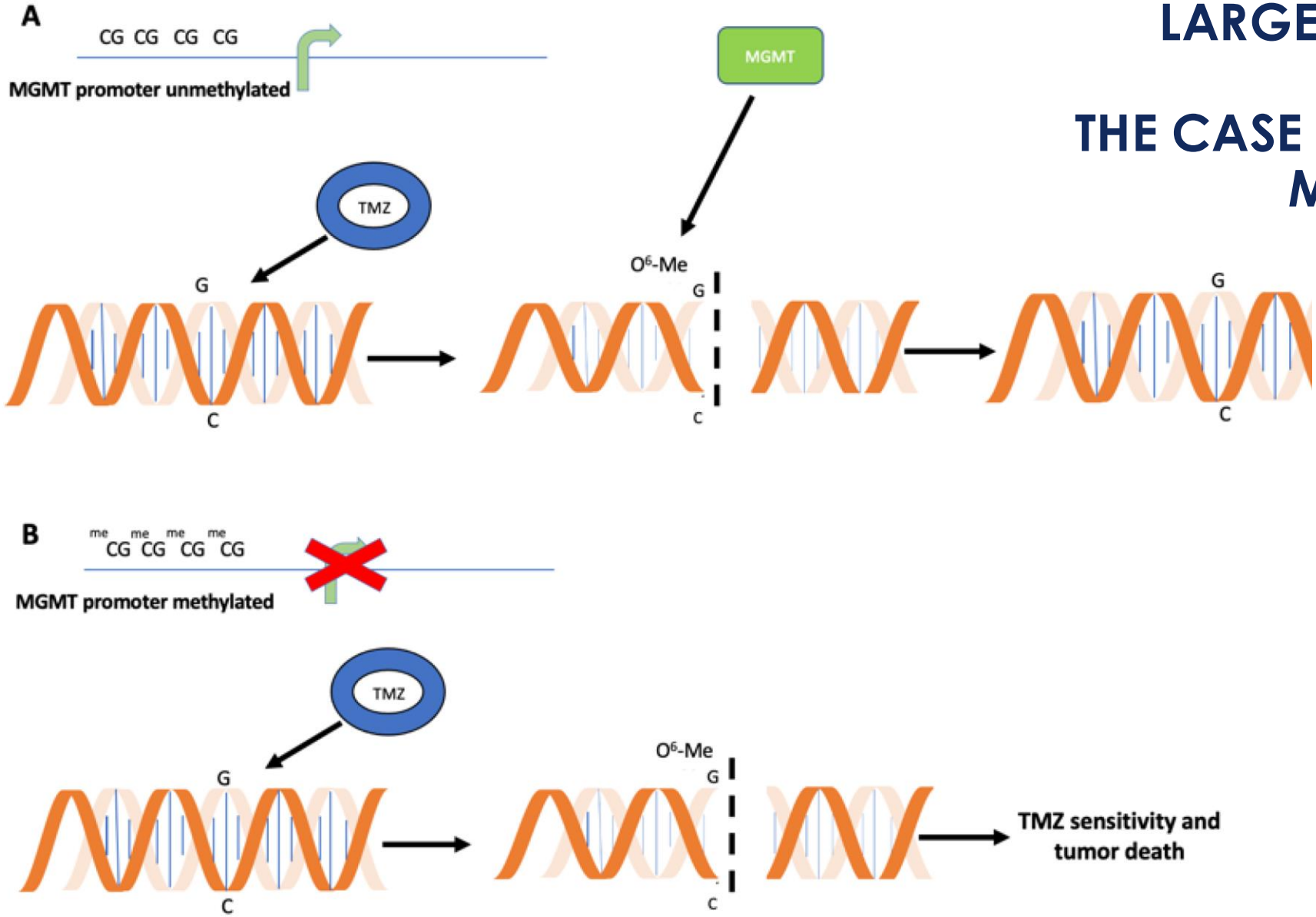
- Several tissue sources, fresh, frozen, or FFPE embedded samples, are suitable for analysis.

- Input of 250 ng DNA allows processing of small specimens such as from stereotactic biopsies or obtaining DNA from a few unstained slides with thickness, diameter, and cellularity of the sample, determining the number of sections required.

- DNA methylation array is a 4 day process

SMALL EPIGENETIC CHANGES AND LARGE CONSEQUENCES:

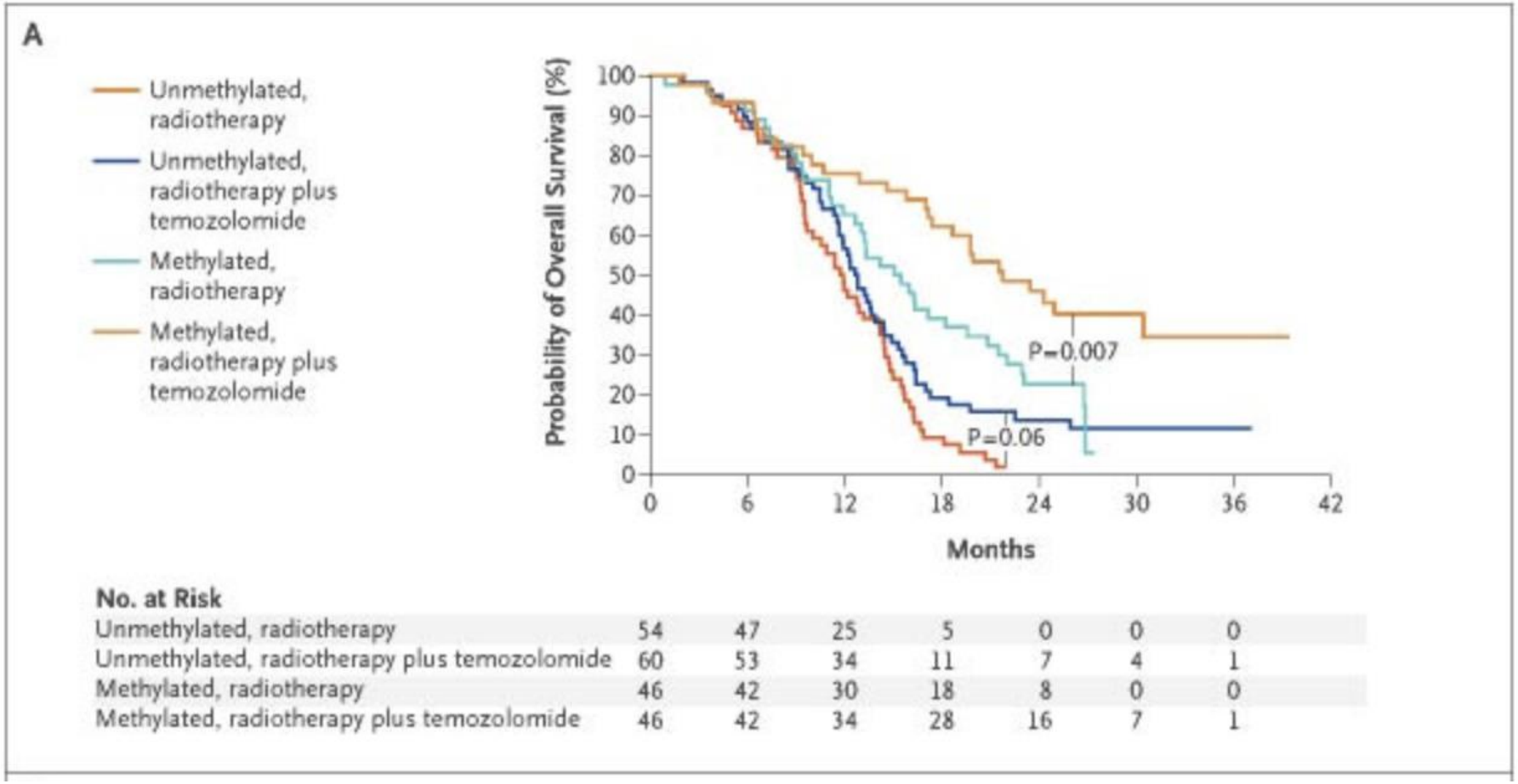
THE CASE OF MGMT PROMOTER METHYLATION



- TMZ chemotherapy damages DNA by methylating guanosine
- MGMT repairs methylated guanosine
- MGMT promoter methylation reduces MGMT expression
  - Makes TMZ more effective

Della Monica et al (2022) *Int J Mol Sci*

CONSEQUENCES OF MGMT PROMOTER METHYLATION IN MALIGNANT GLIOMAS



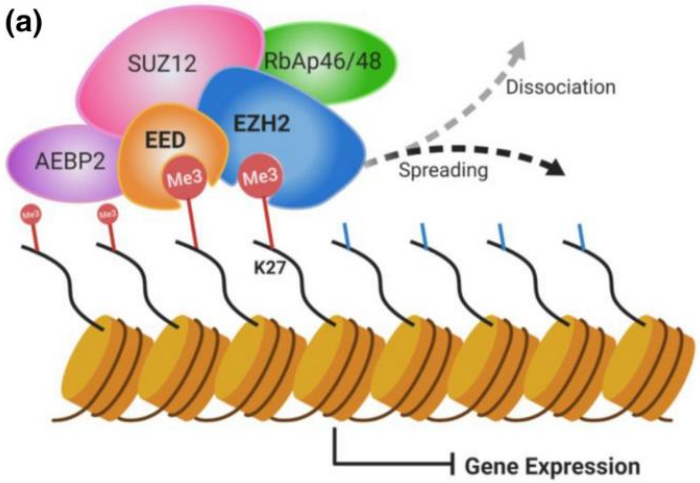
No. at Risk

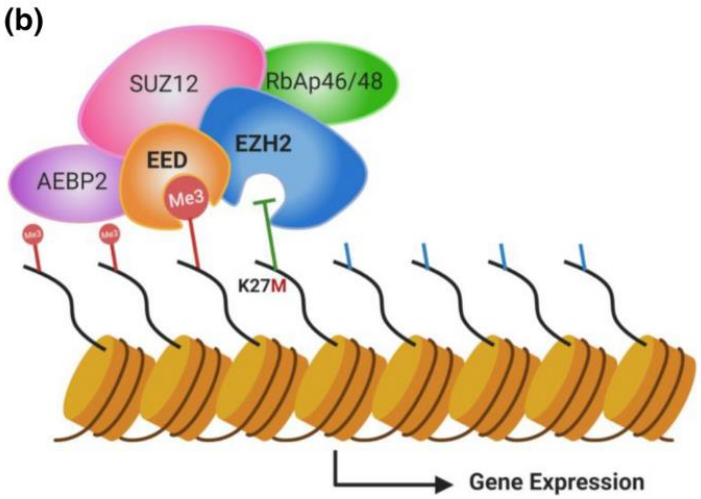
Unmethylated, radiotherapy	54	47	25	5	0	0	0
Unmethylated, radiotherapy plus temozolomide	60	53	34	11	7	4	1
Methylated, radiotherapy	46	42	30	18	8	0	0
Methylated, radiotherapy plus temozolomide	46	42	34	28	16	7	1

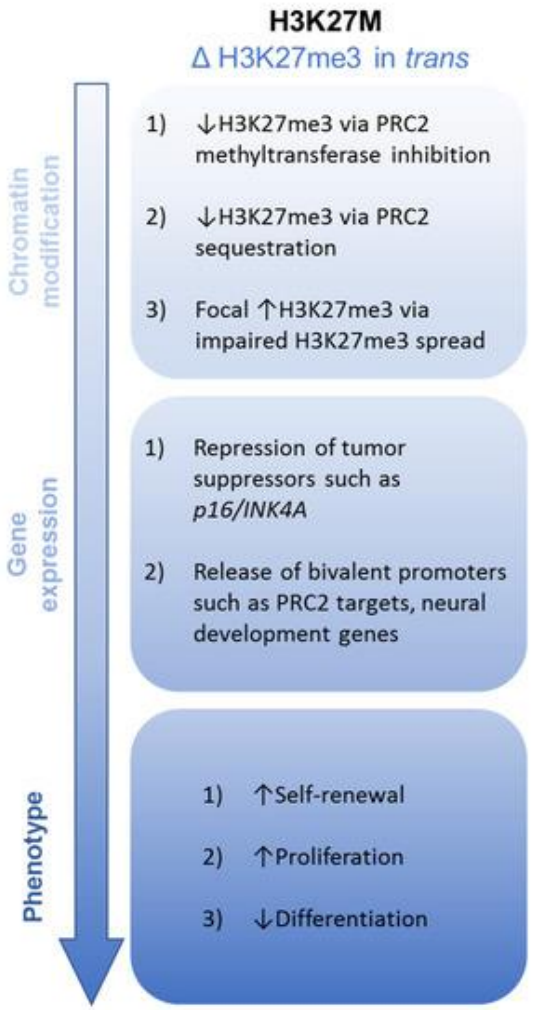
Hegi et al (2005) *NEJM*

SMALL EPIGENETIC CHANGES AND LARGE CONSEQUENCES:

THE CASE OF HISTONE MUTATION







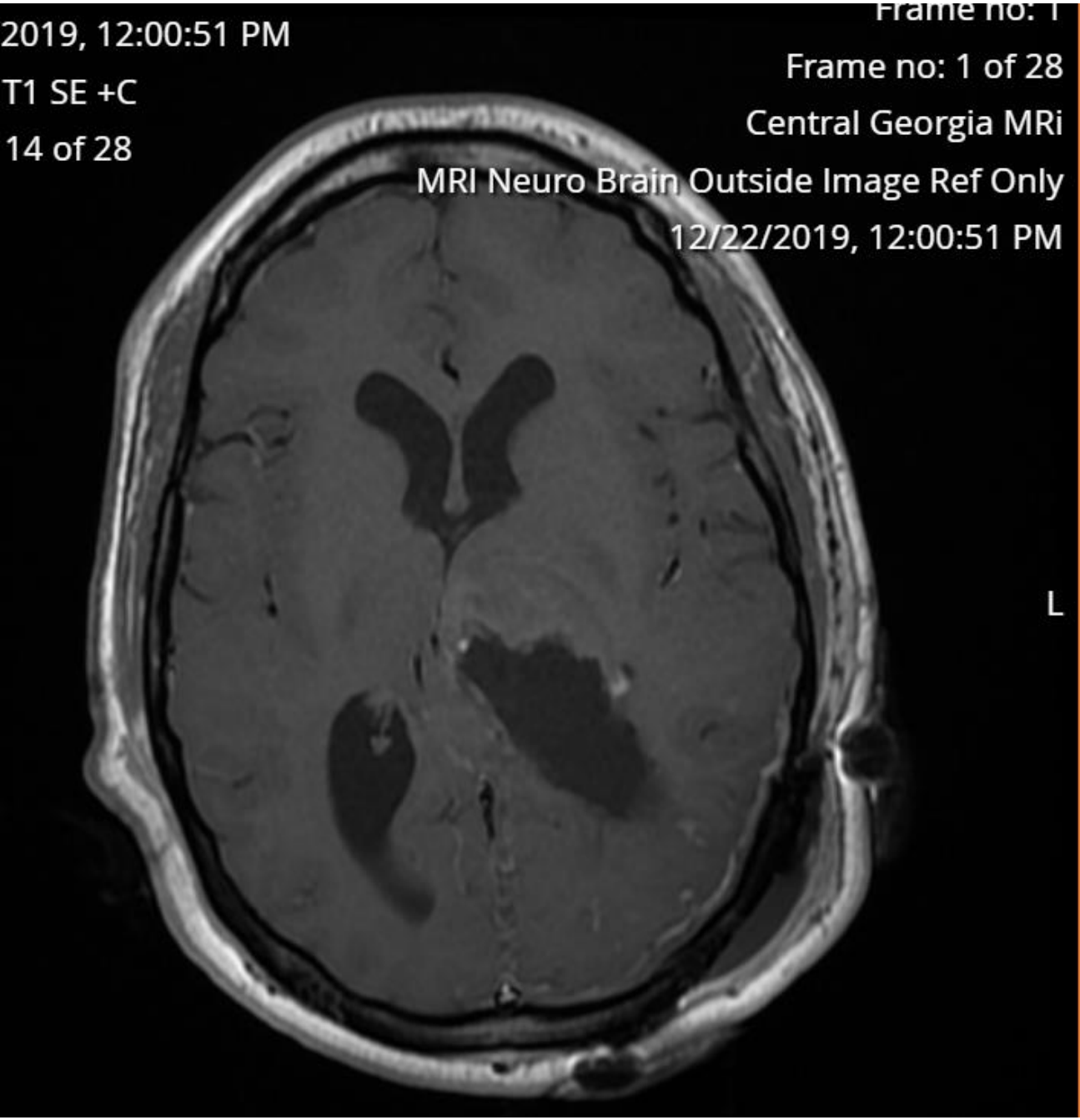
- Defines a set of malignant gliomas in a midline location
  - Pontine (DIPG)
  - Thalamic (DMG)
- Mutation causes loss of methylation on Histone 3
  - H3F3A mutation (H3.3)
  - HIST1H3B mutation (H3.1)
- Captures/inhibits methyltransferase machinery PRC2
  - Global decrease of histone 3 trimethylation
  - Global increase in pro-tumor gene expression

Cooney (2020) *J Neurooncol*

Graham et al. (2020) *Int J Mol Sci*

CASE PRESENTATION

- 24yo woman presents with recurrent lesion
- History of presentation with facial weakness and headaches
- Debulking – H3K27Mutant lesion. Initially referred to Emory for proton therapy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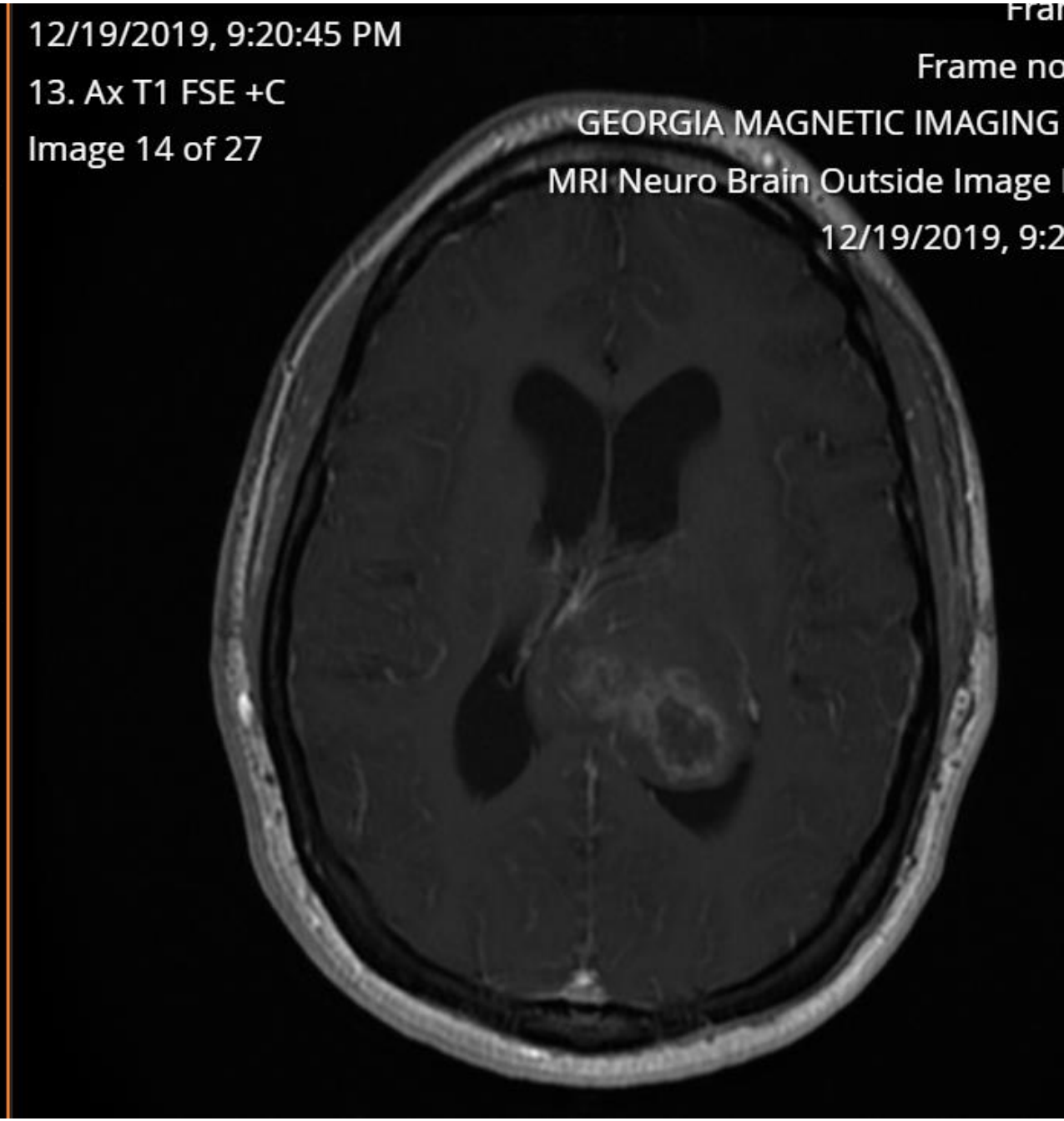
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GEORGIA MAGNETIC IMAGING
MRI Neuro Brain Outside Image
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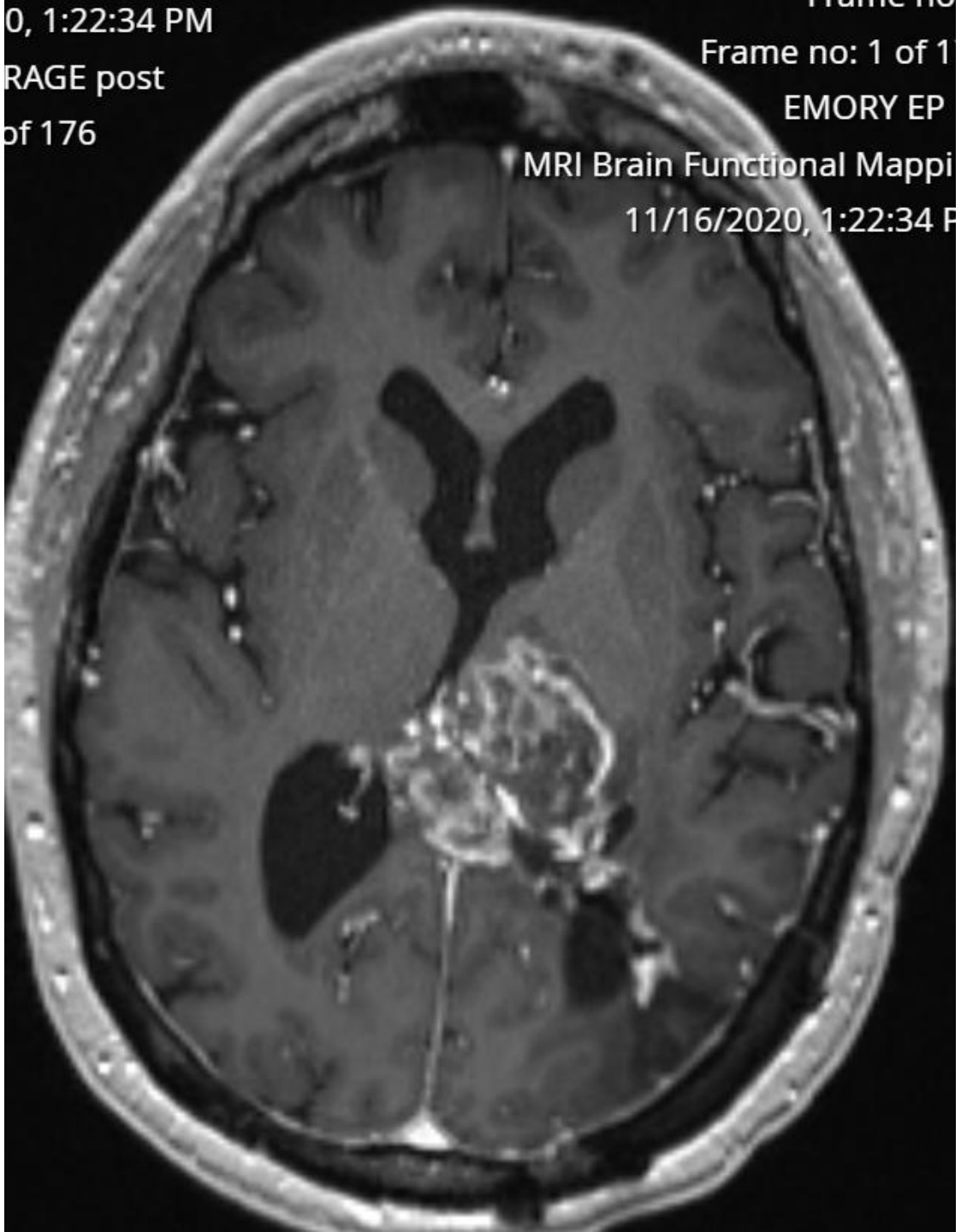
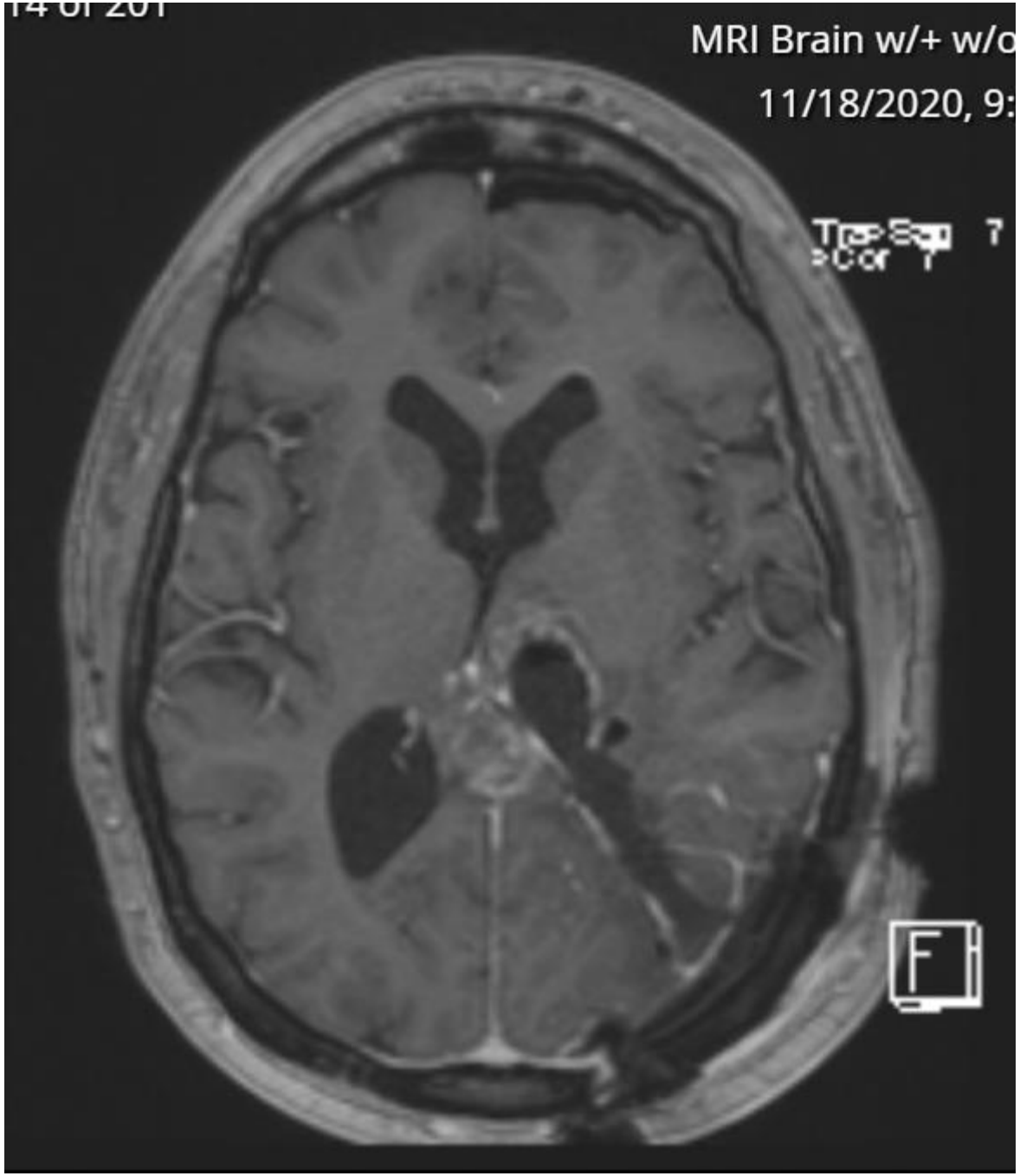
- 2020 presents to proton center

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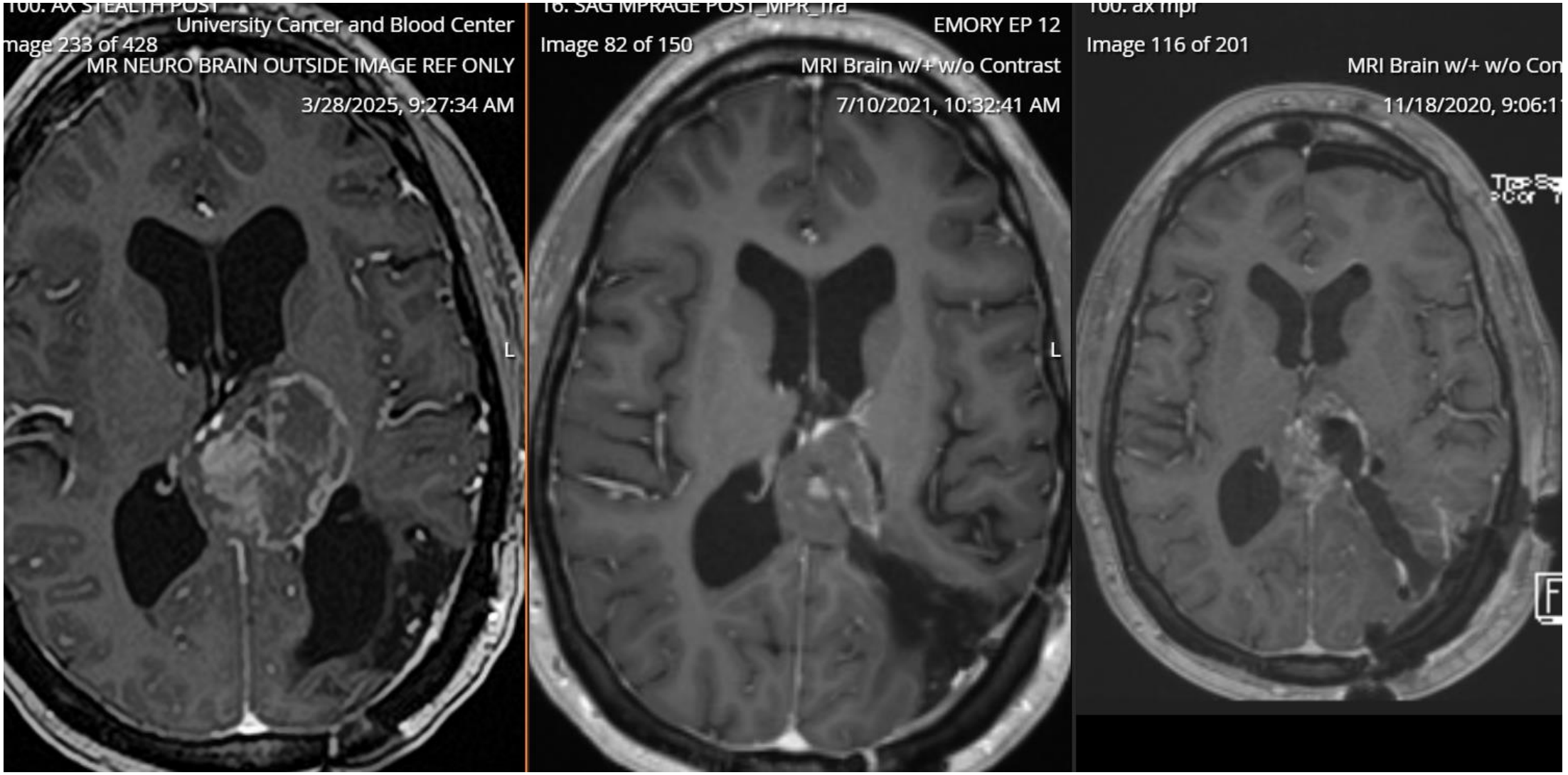
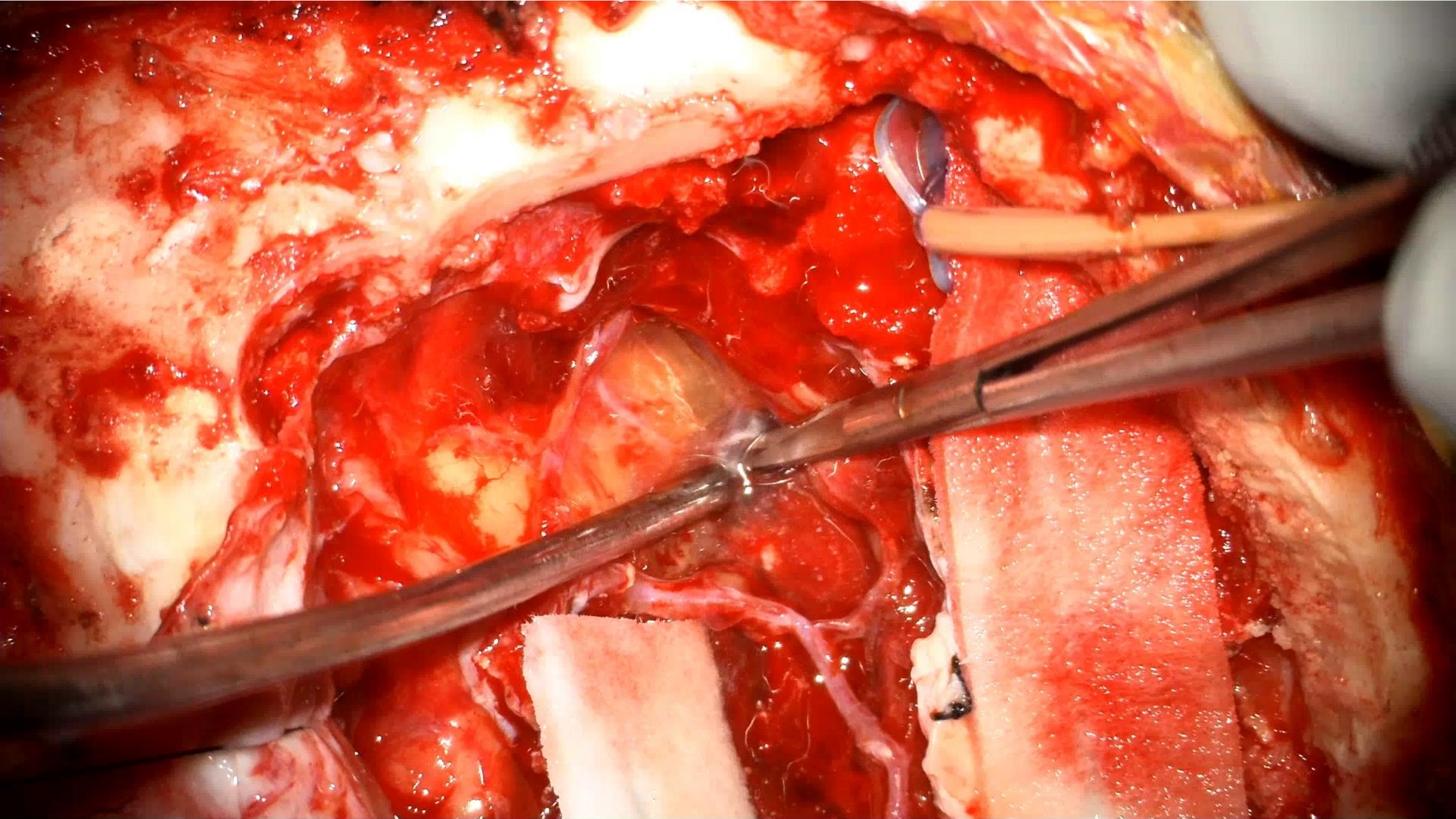
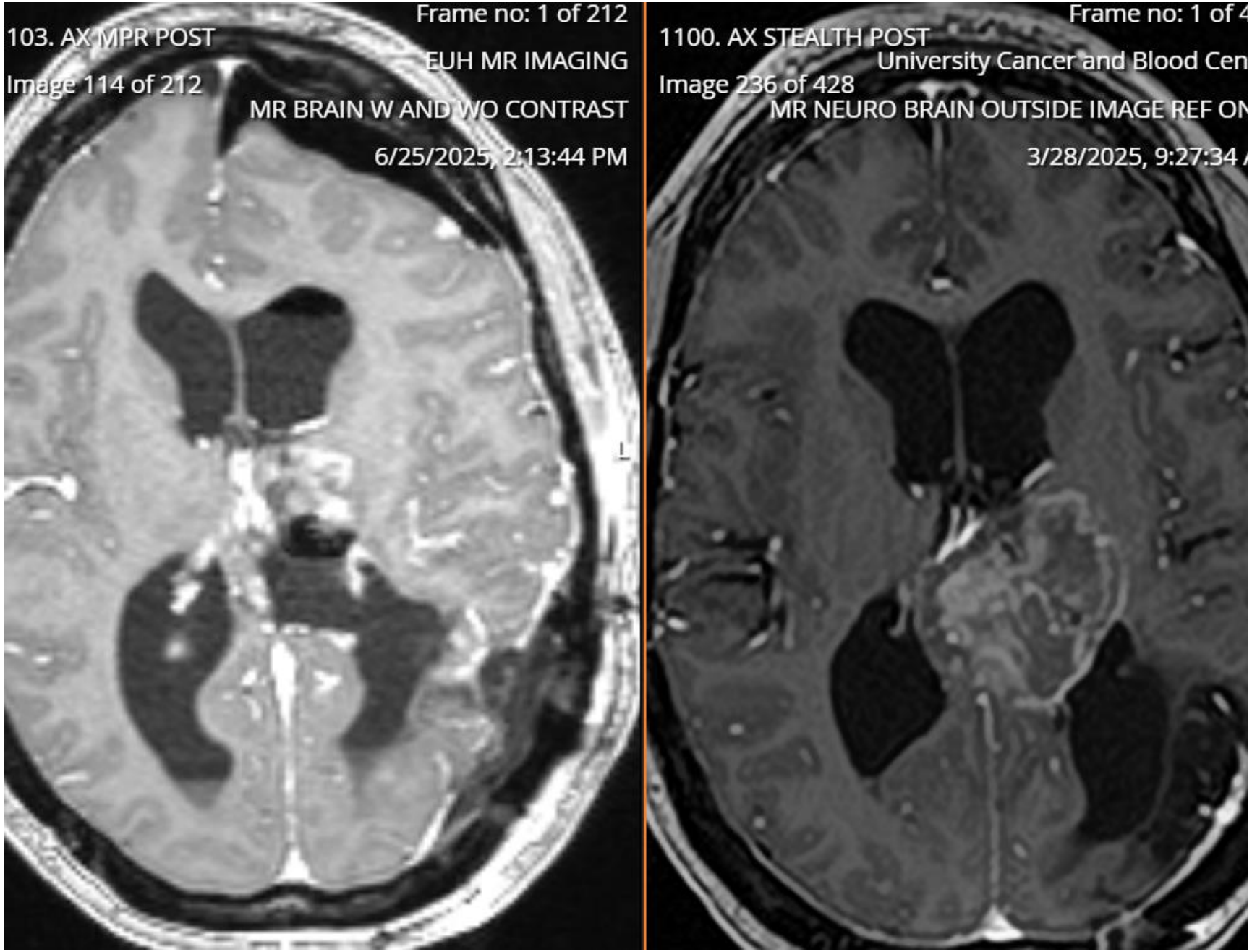
- Has proton therapy, then ONC201 trial
- Does well initially
- Then put on bevacizumab plus ONC201 and is holding steady, but signs of progression start to mount

4.5 YEARS FROM SECOND SURGERY



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ONC201 (Dordaviprone) in Recurrent H3 K27M–Mutant Diffuse Midline Glioma

Authors: Isabel Arrillaga-Romany, MD, PhD, Sharon L. Gardner, MD, Yazmin Odia, MD, MS, FAAN, Dolly Aguilera, MD, Joshua E. Allen, PhD, Tracy Batchelor, MD, Nicholas Butowski, MD, Clark Chen, MD, PhD, Timothy Cloughesy, MD, Andrew Cluster, MD, John de Groot, MD, Karan S. Dixit, MD, Jerome J. Graber, MD, MPH, Aya M. Haggiagi, MD, Rebecca A. Harrison, MD, Albert Kheradpour, MD, Lindsay B. Kilburn, MD, Sylvia C. Kurz, MD, PhD, Guangrong Lu, MBBS, Tobey J. MacDonald, MD, Minesh Mehta, MD, Allen S. Melemed, MD, Phioanh Leia Nghiemphu, MD, Samuel C. Ramage, PhD, Nicole Shonka, MD, Ashley Sumrall, MD, Rohinton S. Tarapore, PhD, Lynne Taylor, MD, Yoshie Umemura, MD, and Patrick Y. Wen, MD SHOW FEWER | AUTHORS INFO & AFFILIATIONS

J Clin Oncol **42**, 1542-1552(2024) • Volume 42, Number 13 • DOI: 10.1200/JCO.23.01134

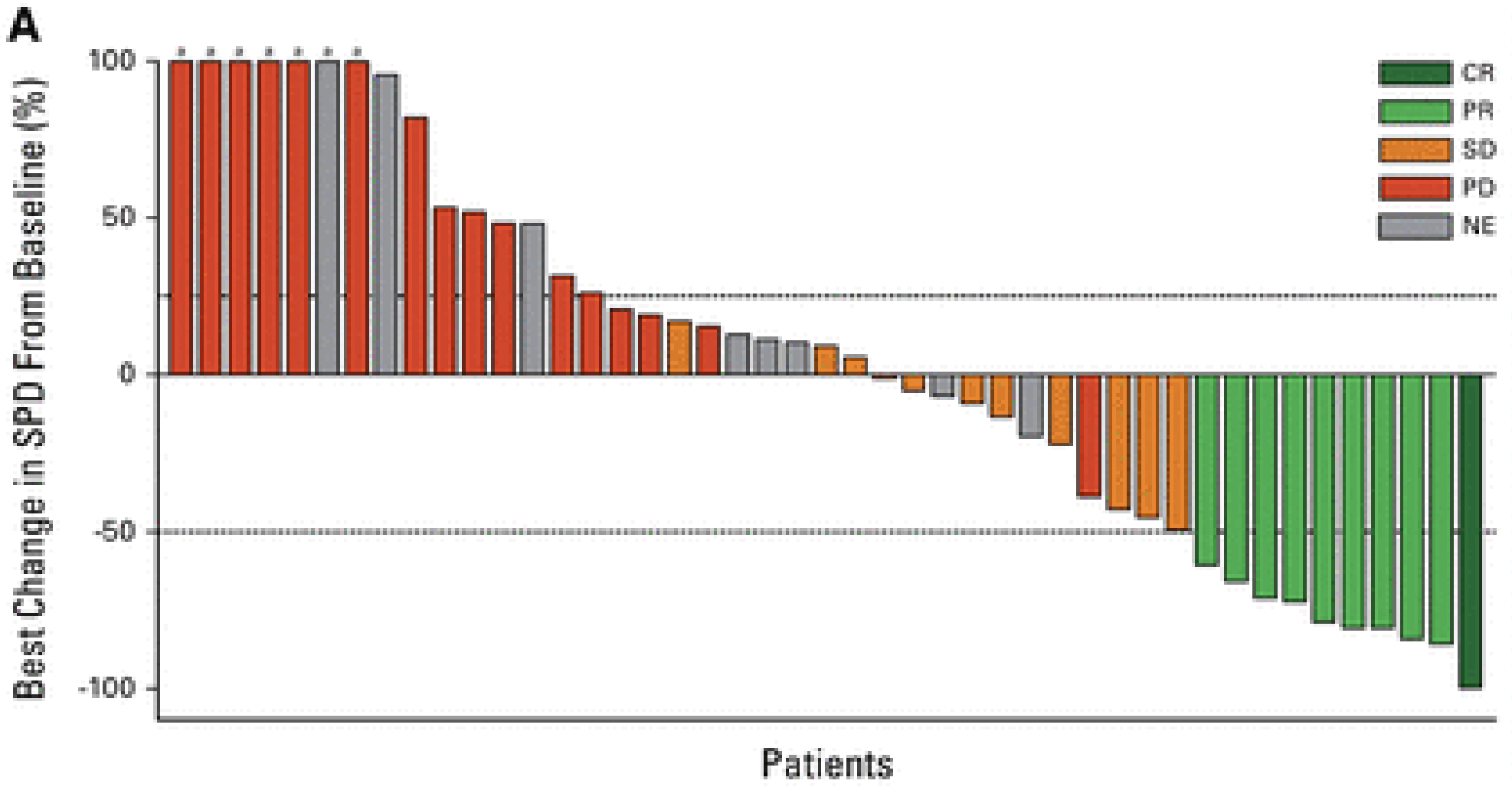

FIG 2. Change in tumor size by RANO-HGG criteria in the efficacy population. (A) Swimmer and (B) spider plots of patients in the efficacy population assessed by BICR while receiving monotherapy ONC201. Three patients did not have on-treatment MRIs available for BICR; one patient censored before first on-treatment MRI because of concurrent therapy; one patient did not have measurable target lesion by BICR. aChange >100%. BICR, blinded independent centralized review; CR, complete response; MRI, magnetic resonance imaging; NE, not evaluable; NR, not reached; PD, progressive disease; PR, partial response; SD, stable disease; SPD, sum of products of perpendicular diameters (target-enhancing lesions per BICR).

Published in: Isabel Arrillaga-Romany; Sharon L. Gardner; Yazmin Odia; Dolly Aguilera; Joshua E. Allen; Tracy Batchelor; Nicholas Butowski; Clark Chen; Timothy Cloughesy; Andrew Cluster; John de Groot; Karan S. Dixit; Jerome J. Graber; Aya M. Haggiagi; Rebecca A. Harrison; Albert Kheradpour; Lindsay B. Kilburn; Sylvia C. Kurz; Guangrong Lu; Tobey J. MacDonald; Minesh Mehta; Allen S. Melemed; Phioanh Leia Nghiemphu; Samuel C. Ramage; Nicole Shonka; Ashley Sumrall; Rohinton S. Tarapore; Lynne Taylor; Yoshie Umemura; Patrick Y. Wen J Clin Oncol 2024 02-9
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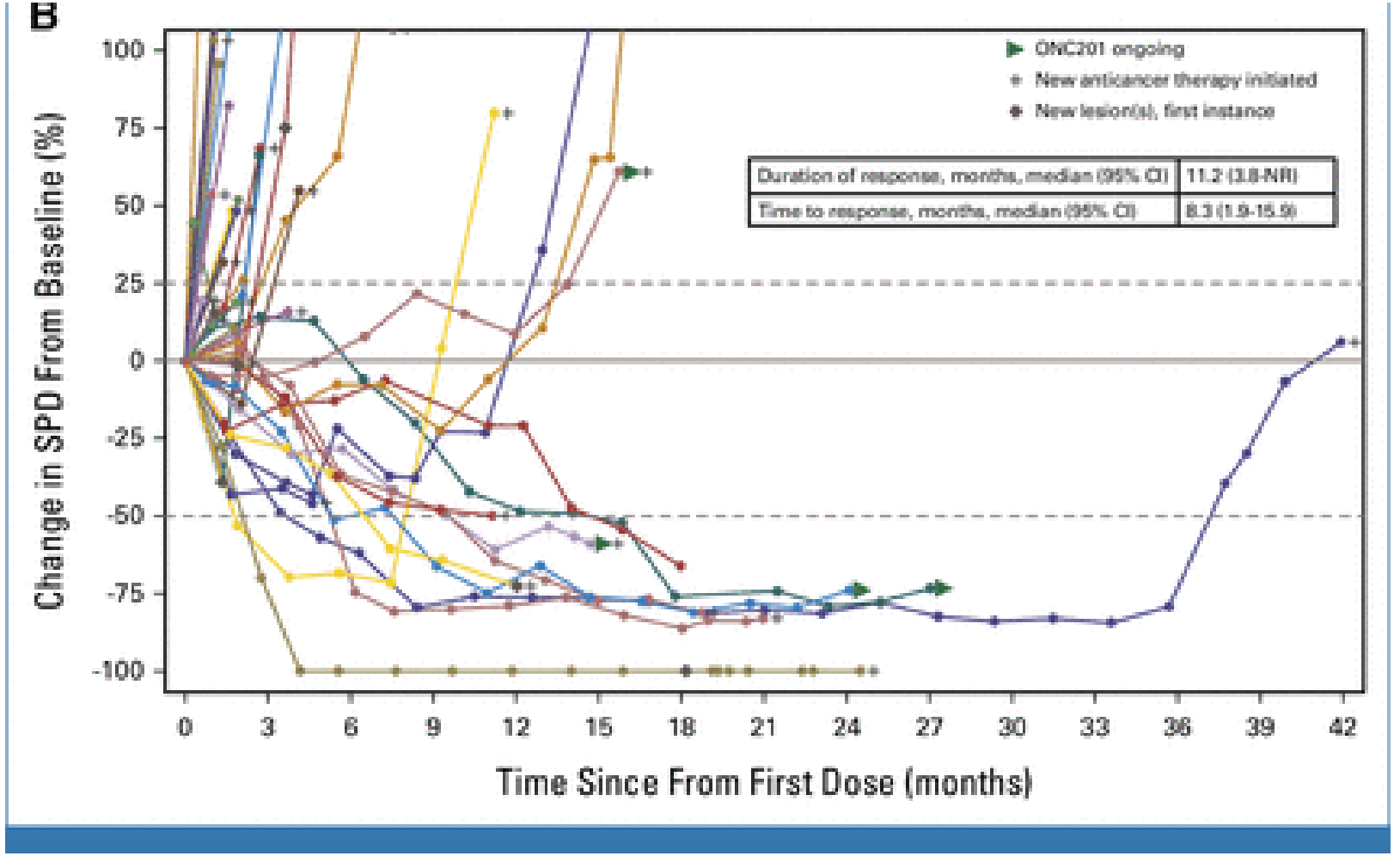

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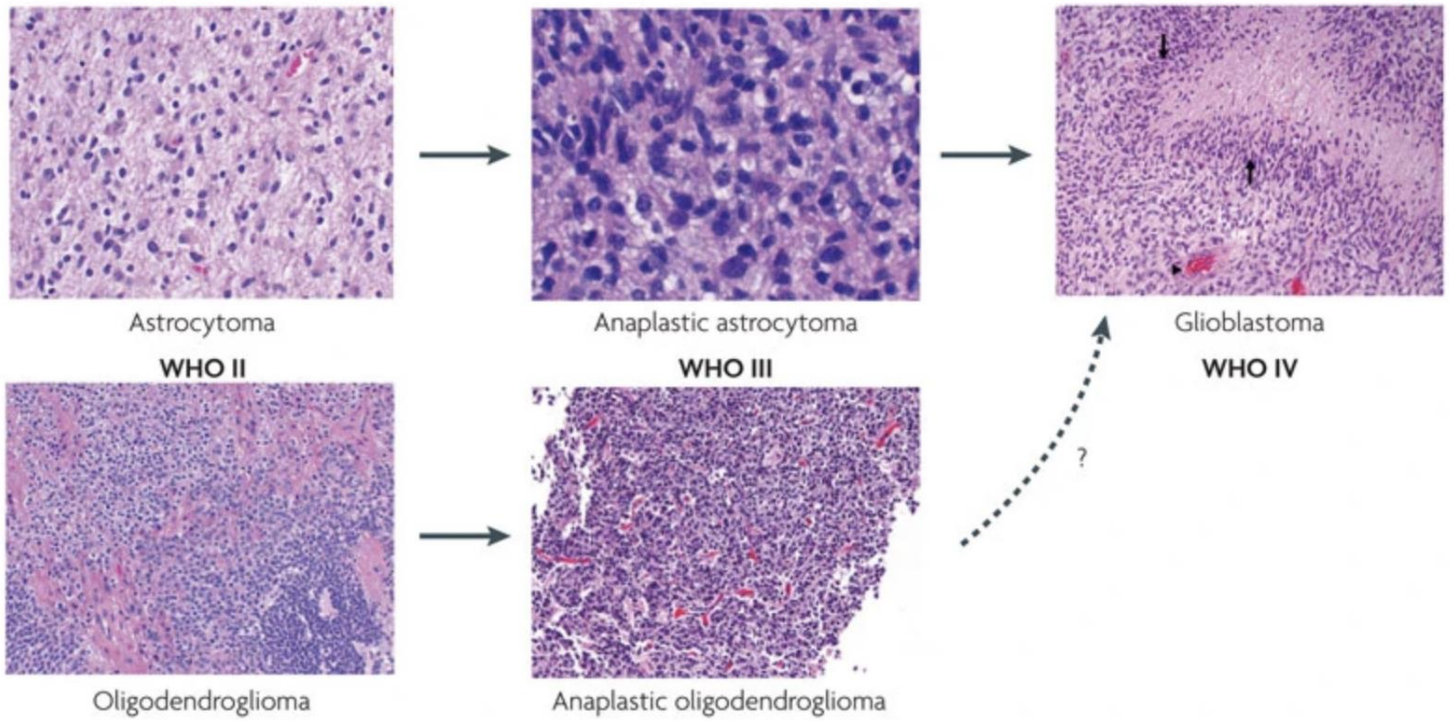
DOI: 10.1200/JCO.23.01134

First Treatment Approved for Rare Pediatric and Adult Brain Tumor Type: FDA Grants Accelerated Approval to Dordaviprone

Primary tumor location	
Nonthalamus	2/17 (11.8)
Thalamus	8/33 (24.2)

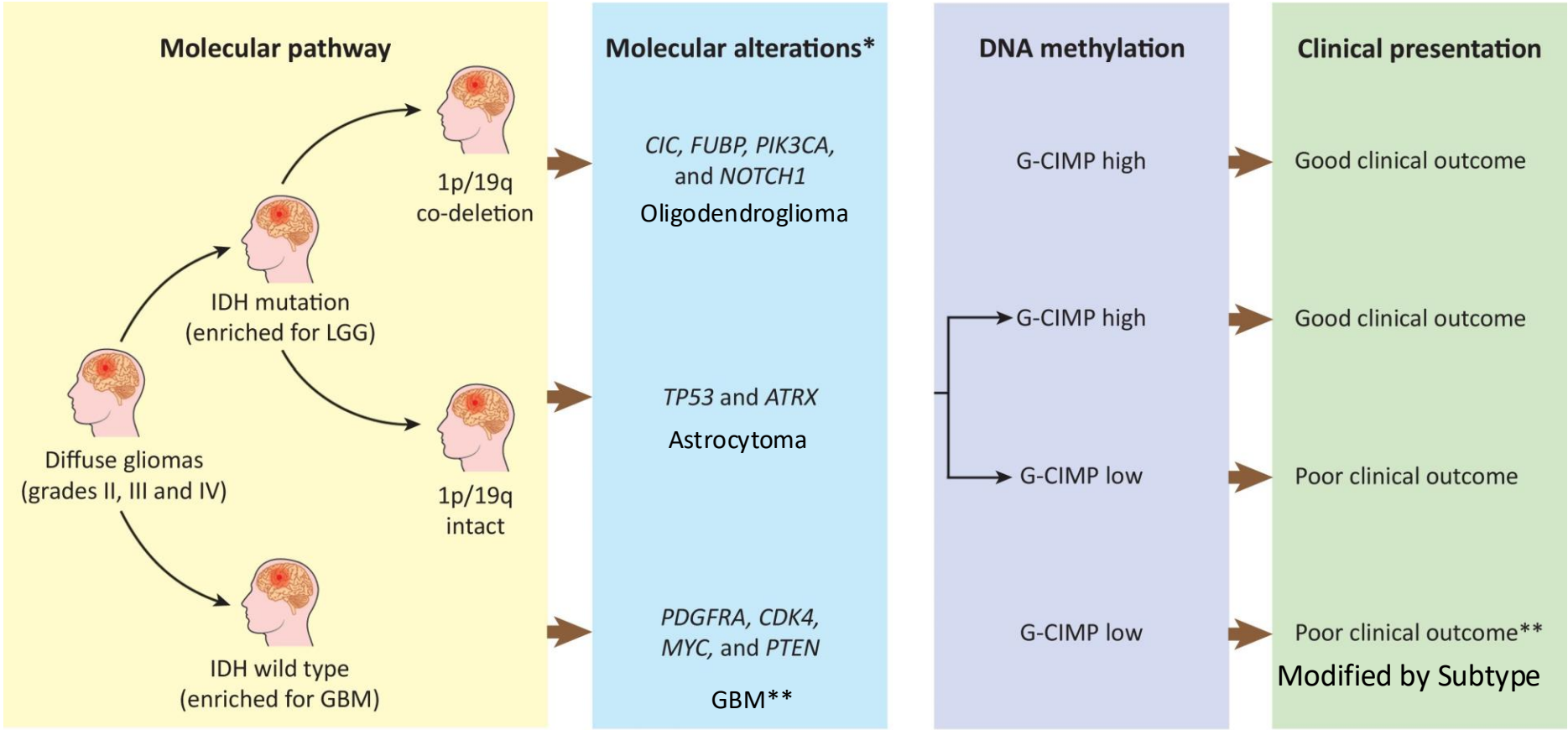
SUMMARY: HISTORICAL CHARACTERIZATION OF GLIOMAS



- Histological and Clinical Diagnoses
  - Primary vs Secondary
- All Treated Equally
  - Initially: Surgery Only
  - Radiation subsequently found to improve outcomes
  - Major chemotherapy breakthrough in 2005: Temozolomide

Huse and Holland (2010) *Nature Reviews Cancer*

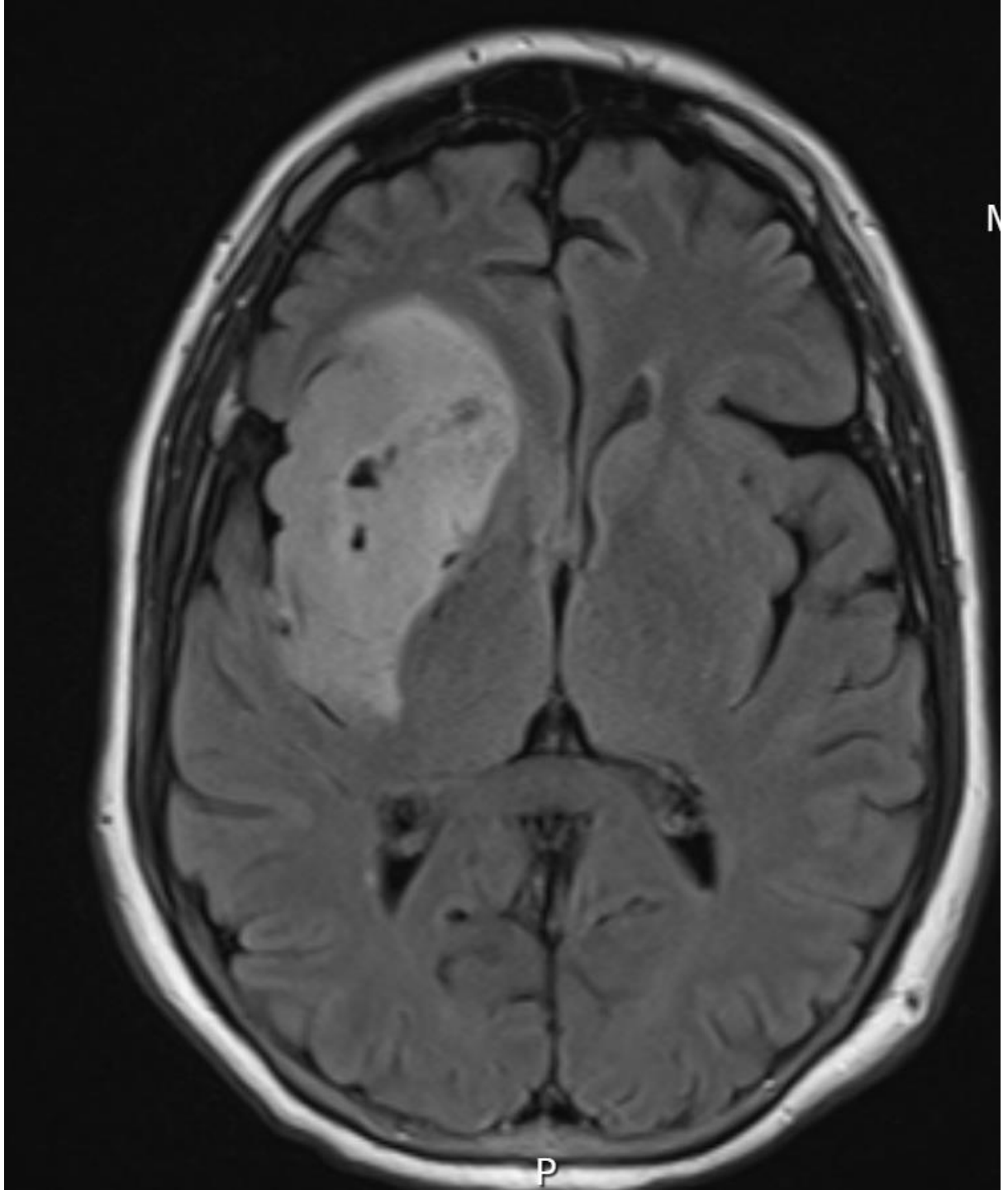
SUMMARY: MOLECULAR/EPIGENOMIC ERA OF GLIOMA CHARACTERIZATION



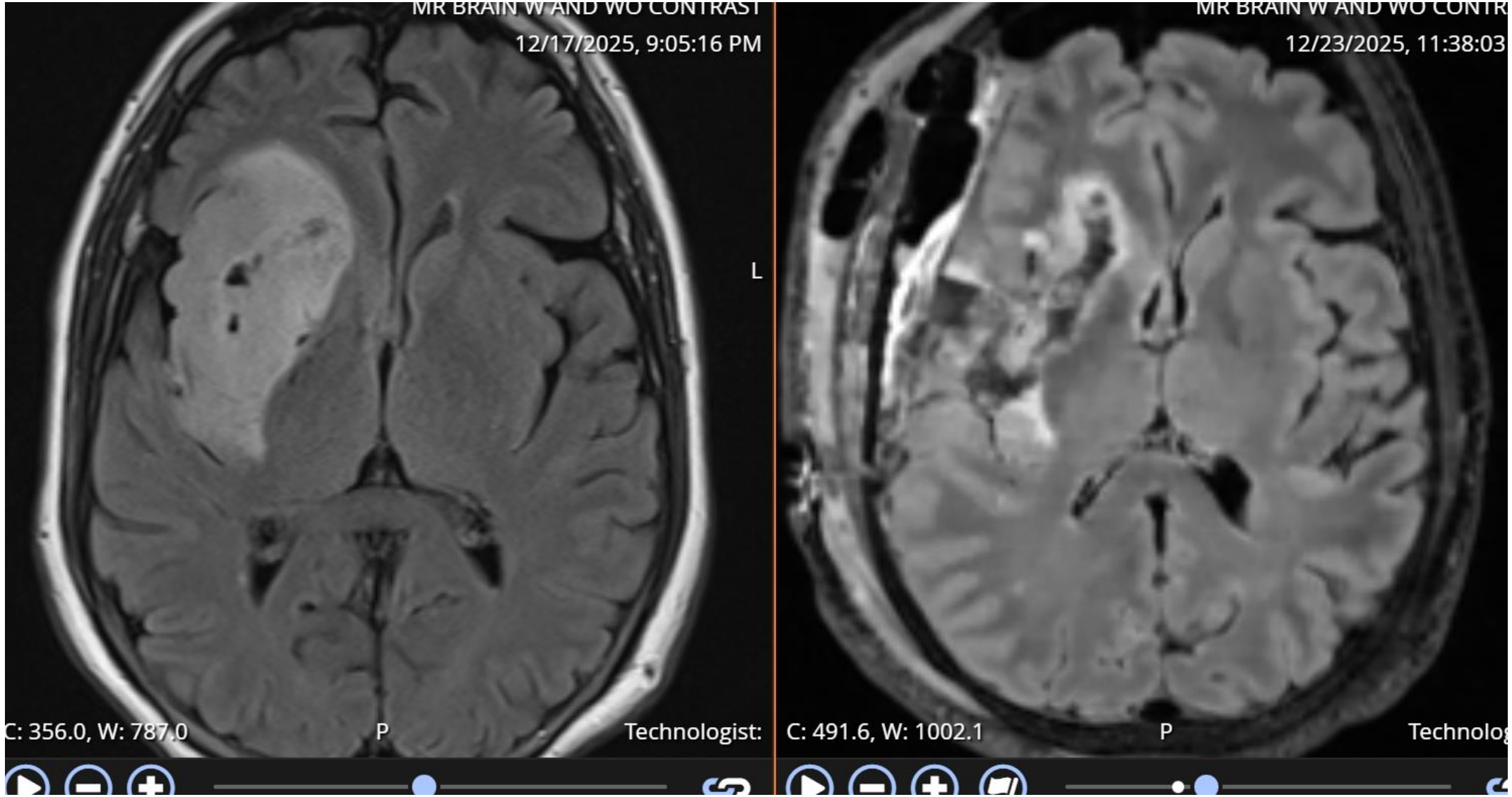
Modified from Su et al (2018) *Trends Cancer*

Trends in Cancer

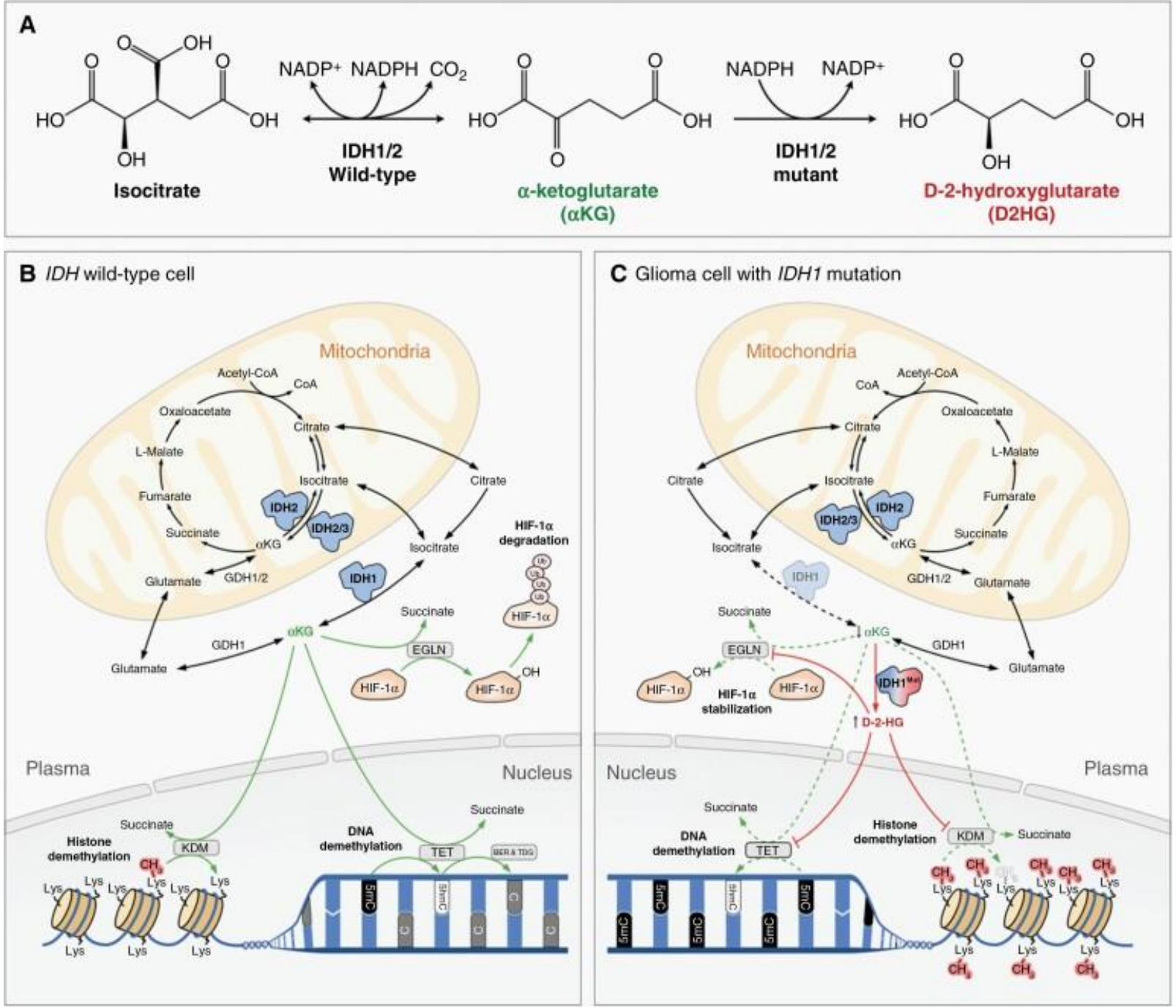
- 59yo teacher
- Intermittent LUE numbness/tingling
- Transient dizziness
- Odd taste of cork in mouth



GRADE 3 OLIGODENDROGLIOMA



IDH MUTATION: A SLOW EPIGENETIC DRIVER OF GLIOMAS



Miller et al. (2020) Neurooncol

DISTINGUISHING BETWEEN IDH MUTATED GLIOMAS

Note about IDH Mutations:

- >90% are IDH1 R132H (canonical)
  - IHC antibody tests only this
- If negative in:
  - <55: get DNA sequencing for non-canonical IDH1 and IDH2 mutations
  - >=55: no need to further sequence (<1% non-canonical)

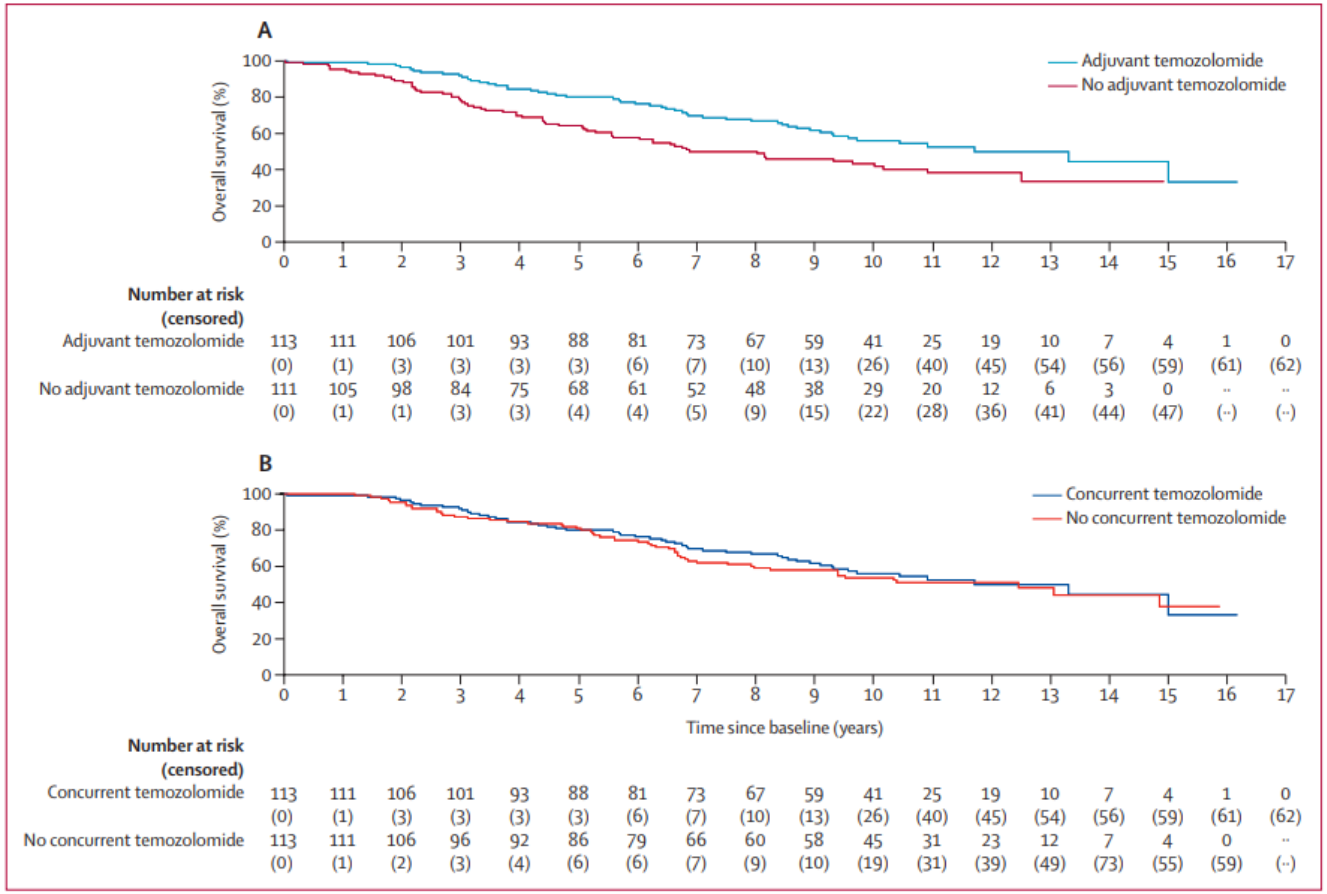
Molecular Hallmarks of IDH-mutant Glioma

IDH-mutant glioma	Molecular hallmarks
Astrocytoma, IDH-mutant	All IDH1 or IDH2 (~ 90% of IDH1R132H-type), ATRX (> 90%), TP53, CDKN2A/B
Infratentorial astrocytoma, IDH.mutant	All IDH1 or IDH2 (< 25% of IDH1R132H-type), ATRX (< 50%), TP53, CDKN2A/B
Primary mismatch repair deficient IDH-mutant astrocytoma (PMMRDIA)	All IDH1 or IDH2 (~ 90% of IDH1R132H-type), ATRX (> 90%), TP53, CDKN2A/B, all MLH1 or MSH6 or MSH2
Oligodendroglioma, IDH-mutant and 1p/19q codeleted	All IDH1 or IDH2, all 1p/19q codel, pTERT, CIC, FUBP1, NOTCH1
Oligosarcoma, IDH-mutant	All IDH1 or IDH2, all 1p/19q codel (in few cases obscured by duplication), pTERT (~ 50%), CDKN2A/B

Miller et al. (2020) Neurooncol

DIFFERENT TREATMENT PARADIGMS FOR DIFFERENT TUMORS: ASTROCYTOMA

Adjuvant TMZ (Blue) vs No Chemo Post-Radiation (Red)



Concurrent TMZ (Blue) vs Radiation Alone (Red)

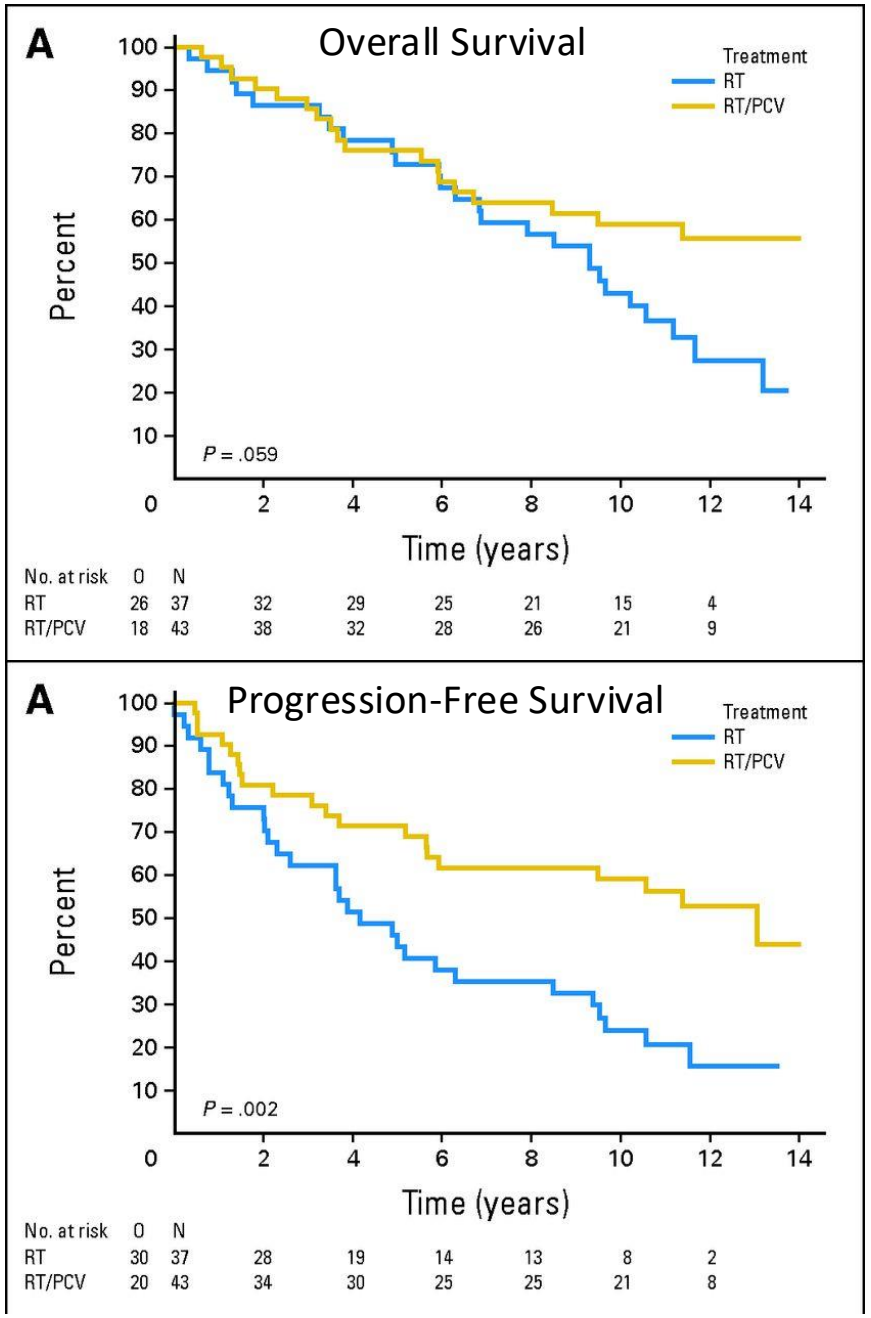
- Treatment paradigm for astrocytomas reflected those for GBM
  - Stupp Protocol: concurrent radiation with TMZ followed by 6-12 cycles of adjuvant TMZ
  - Applied to:
    - All Grade 3 and 4 astrocytomas
    - Recurrent gross total/subtotally resected Grade 2 astrocytomas

- Final analysis of **EORTC CATNON trial**, however, reveals:
  - No benefit to concurrent chemotherapy with radiation
    - In fact, patients often did worse symptomatically/clinically
  - Significant benefit with adjuvant chemotherapy
    - More is probably better (aim for 12 cycles)

Van den Bent et al. (2026) Lancet Oncol

DIFFERENT TREATMENT PARADIGMS FOR DIFFERENT TUMORS: OLIGODENDROGLIOMAS

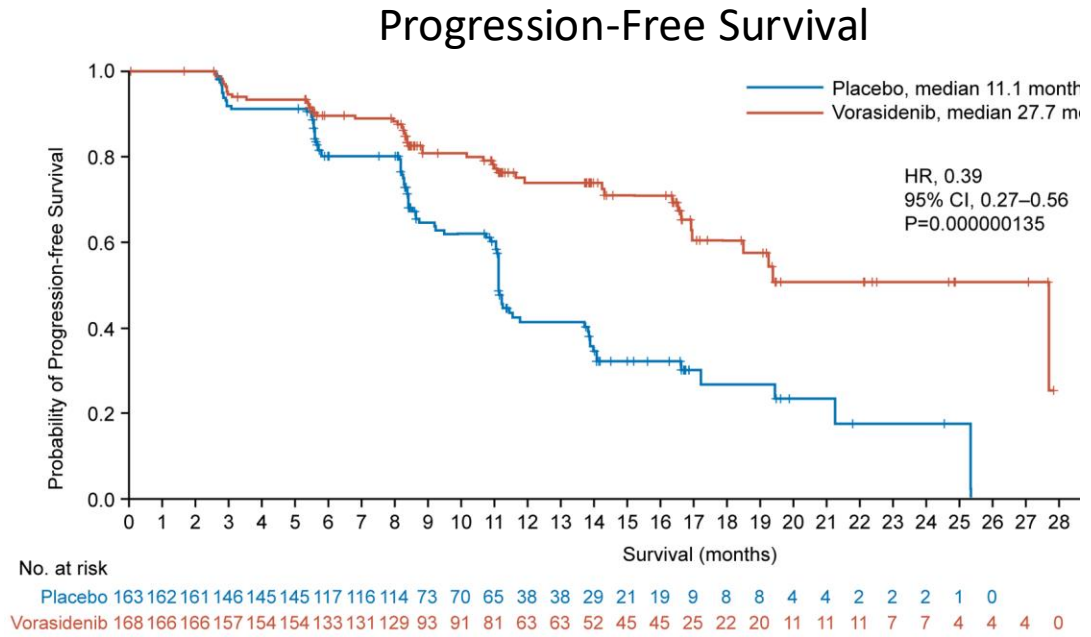
- Treatment paradigms for oligodendrogliomas come from **EORTC study 26951**
  - Radiation vs radiation followed by up to 6 cycles of PCV (Procarbazine + CCNU/Lomustine + Vincristine)
  - Originally applied to all "low grade gliomas"; post hoc analysis when oligo marker 1p/19q co-deletion discovered
    - Note: IDH mutation was emerging as a marker during this time
  - Median cycles 3, only 30% completed all 6 cycles
- Current practice standard:
  - All grade 3 oligodendrogliomas
  - Recurrent gross total or subtotally resected Grade 2 oligodendrogliomas
  - Most neuro-oncologists no longer co-administer vincristine
    - Very neurotoxic, no clear BBB penetrability in adults

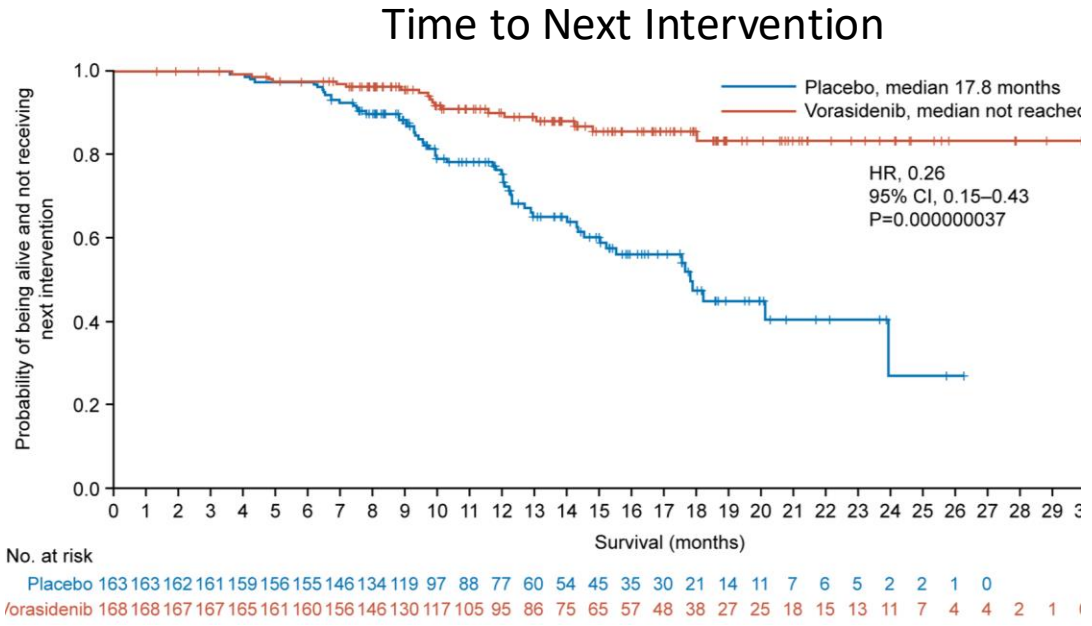


Van den Bent et al. (2013) JCO

THE DISRUPTOR: IDH MUTANT INHIBITOR VORASIDENIB

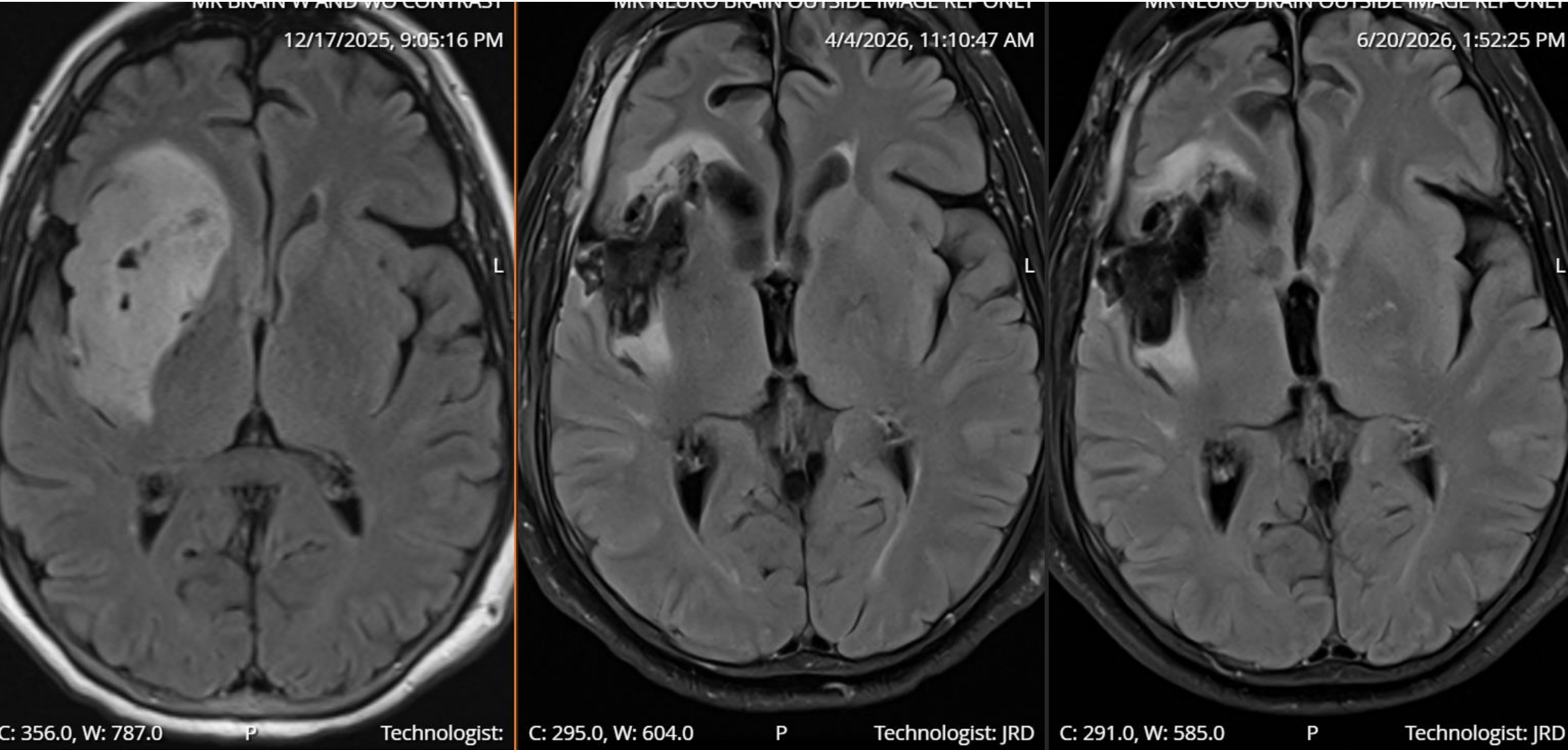
- Daily, oral, bi-specific inhibitor of mutant IDH at the active site to prevent oncometabolite production
  - Agnostic to IDH subtype (IDH1/2)
- **INDIGO trial**: recruited patients with:
  - Proven IDH1 or 2 mutation
  - No previous treatment other than surgery
  - Demonstration of radiological growth
- Interim analysis showed delays in growth and decision for next line of treatment
  - FDA approved in 2024
- Controversy:
  - When to start in relation to initial timing and extent of surgery
  - What about Grade 3 IDH mutant gliomas?





Mellinghoff et al. (2023) NEJM

GRADE 3 OLIGODENDROGLIOMA



OTHER CNS TUMOR/INHIBITOR COMBINATIONS

Tumor Alteration\	CNS Tumor Examples	Inhibitor Class	Inhibitor Examples	Administration Info
BRAF V600E Mutation	• Pleomorphic Xanthoastrocytoma (PXA) • Ganglioglioma (GG) • Epithelioid GBM	Type 1 BRAF Inhibitor + MEK Inhibitor	• Dabrafenib + Trametinib • Encorafenib + Binimetinib	BID + Daily (PO)
KIAA1549::BRAF Fusion	• Pilocytic Astrocytoma (PA) • Diffuse Leptomeningeal Glioneuronal Tumor (DLGNT)	Type 2 BRAF Inhibitor	• Tovorafenib (only agent on market)	Weekly (PO)
H3K27M Mutation	• Diffuse Midline Glioma (DMG) • Diffuse Interstitial Pontine Glioma (DIPG)	Imipridone	• Dordaviprone (only agent on market)	Weekly (PO)
NTRK Fusion	• GBM/HGG (rare)	NTRK Inhibitor	• Entrectinib • Larotrectinib	Daily vs BID (PO) (Solid Tumor indication)

ACKNOWLEDGEMENTS – WINSHIP BRAIN TUMOR CENTER

- **Neuropathology**:
- Stewart Neill

- **Radiation Oncology**:
- Jim Zhong
- Bree Eaton
- Lisa Sudmeier
- Hui-Kuo Shu
- Sunil Dutta
- Zach Buchwald
- Natia Esiashvili

- **Rehab Oncology**
- Jasmine Malhotra
- Noble Jones

- **Neuropsychology**
- Suzanne Penna

- **Neuroplastic Surgery**
- Kerry-Ann Mitchell
- Daniel Cuzzone

- **Neuro Oncology**:
- Byram Ozer
- Laura Donovan
- **Sani Gandhi (New!)**

- **Neurosurgery**:
- Kimberly Hoang
- Chris Deibert
- Guy McKhann

- **Neuroradiology:**
- Brent Weinberg
- Nicole Freedman

- **Research Team**
- Renee Read
- Steven Sloan
- Nicholas Boulis
- Edikan Ogunnaike
- Nassir Mokarram
- Hui Mao
- Hyunsuk Shim

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QUESTIONS?