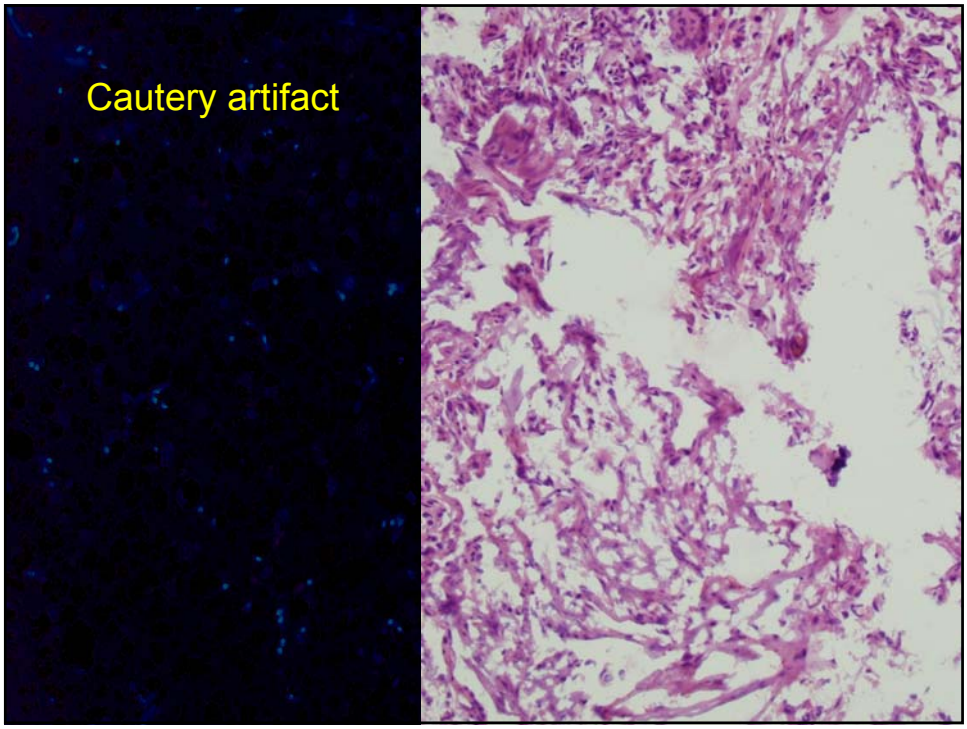
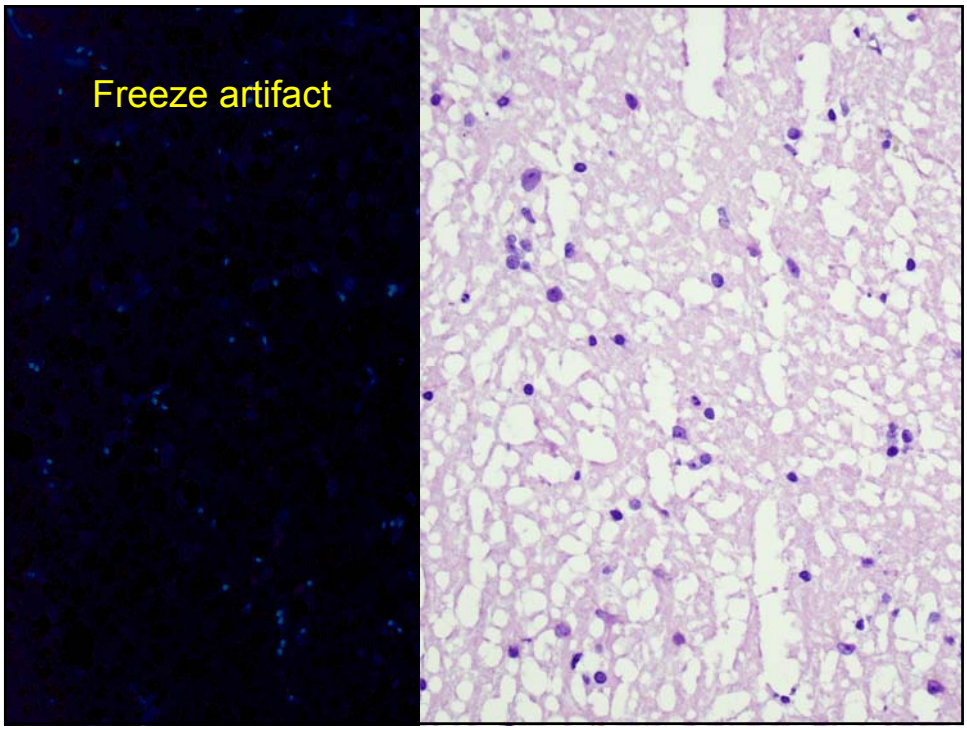
What we will talk about

- Artifacts
- Use of smears
- How to look at smears and FS
- Sampling issues: "Next to"
- Immunochemistry for neuropathology
- Abscess v GBM
- Tumor v Demyelinating
- Recurrence v Treatment effect
- Meningioma and SFT

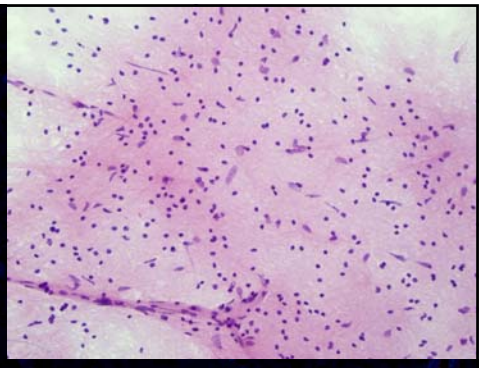

Cautery artifact

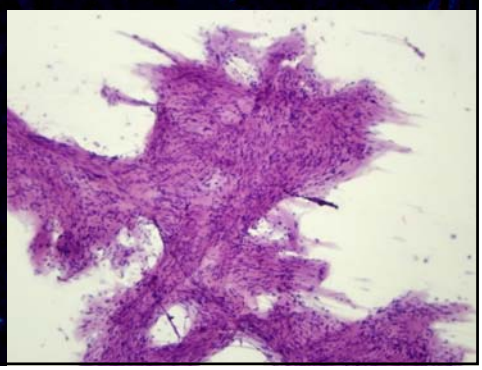

Freeze artifact

Principles of the smear/crush preparation

Amount: ≈ 1mm^3
- Never more than 1/3 of biopsy

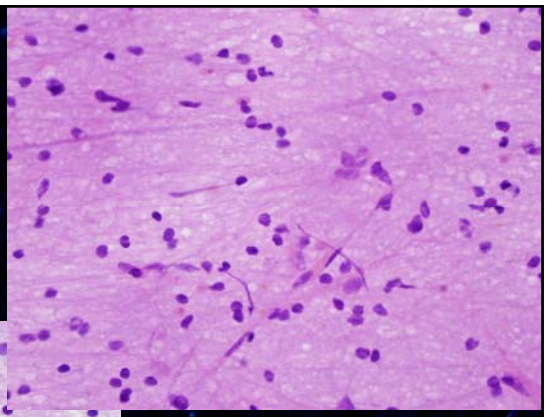
Goal: Lineage/cell population(s)
Interpretation:
- Does it smear well?
  - Yes = brain
  - No = fibrous (e.g. meningioma, schwannoma)
- Are their processes?
  - Yes → Astrocytes
  - No → Oligodendrocytes, epithelial, etc.
- Single cells?
  - Yes → Glial/Brain
  - Clusters →Epithelial, meningothelial
- Predominant population?
  - Does it belong?
  - Atypia?
- Look at the background
  - Necrosis, hemorrhage, inflammation, neuropil, etc.
- Don't try to do too much
  - Cellularity hard to predict
- Account for everything!
  - Don't ignore the naked nuclei because you see some cells with processes

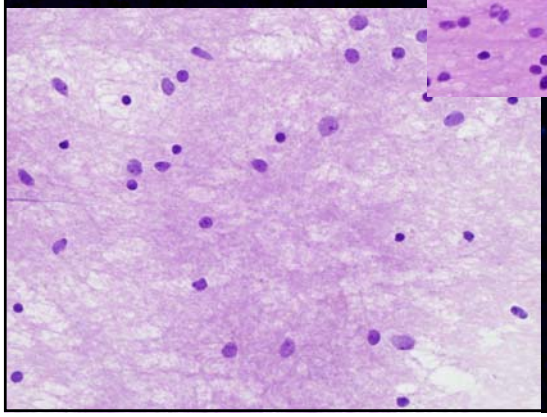




White matter →

- Smears easily/well
- Predominant population of small round nuclei w/ even chromatin
- Granulo-fibrillary background with vague linear orientation





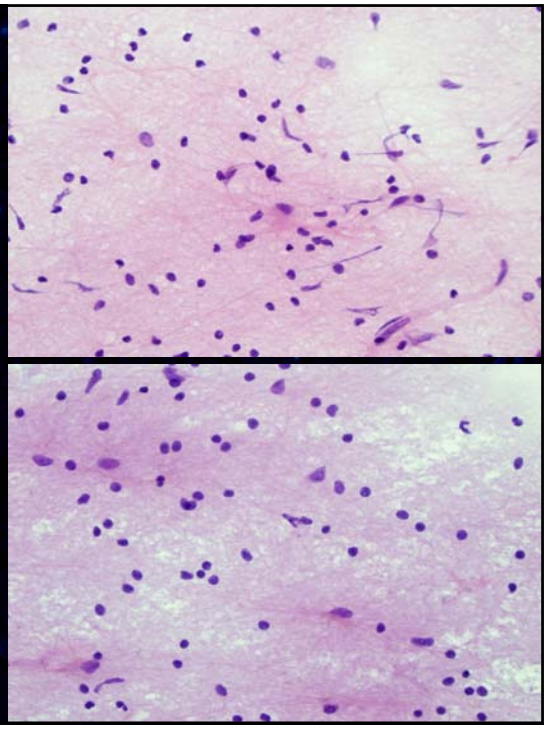
- Smears easily/well
- Smooth to wispy granulo-fibrillary background with diffuse distribution

← **Gray matter**

- Mixed population of small round nuclei, larger ovoid nuclei and large round nuclei

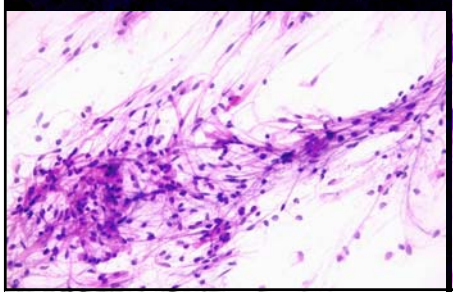
Astrocytic processes

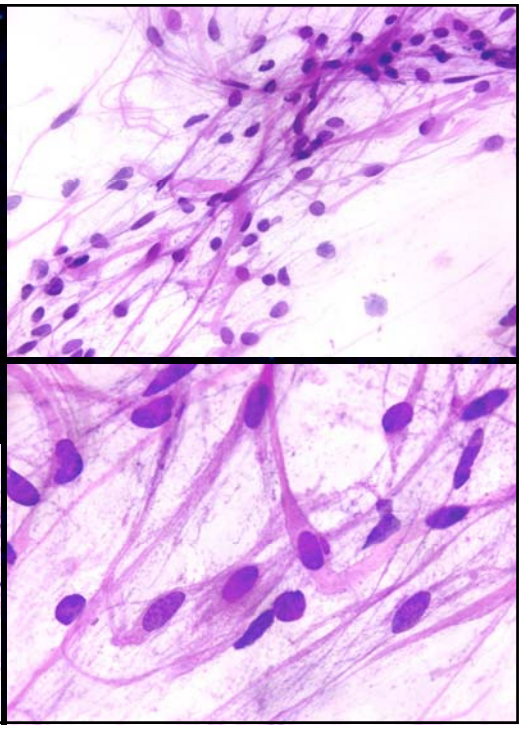
- Identification of the presence or absence of glial/ astrocytic processes is one of most important observations in smear/crush preparations.
  - Astrocytic (+) v non-astrocytic (−) tumors
  - Gliosis (seen here)
    - Typical brain granulo-fibrillary pale background with mixed cell populations (gray matter) or oligodendrocyte-predominant (white matter)
    - Scattered cells with larger ovoid nuclei and numerous obvious processes extending from eosinophilic cell body



Glial processes - Astrocytomas

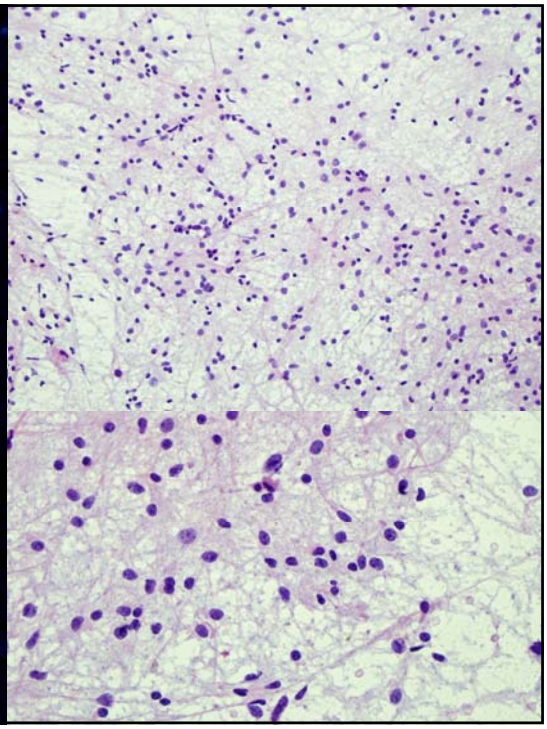
- Most cells have prominent dense/solid eosinophilic processes.
  - May be bipolar, unipolar
  - With or without prominent cell body



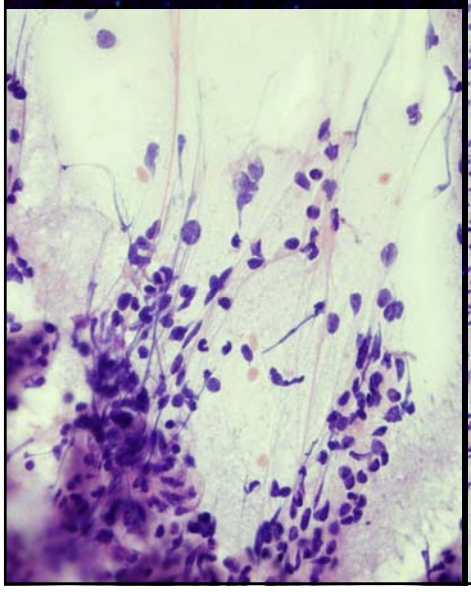


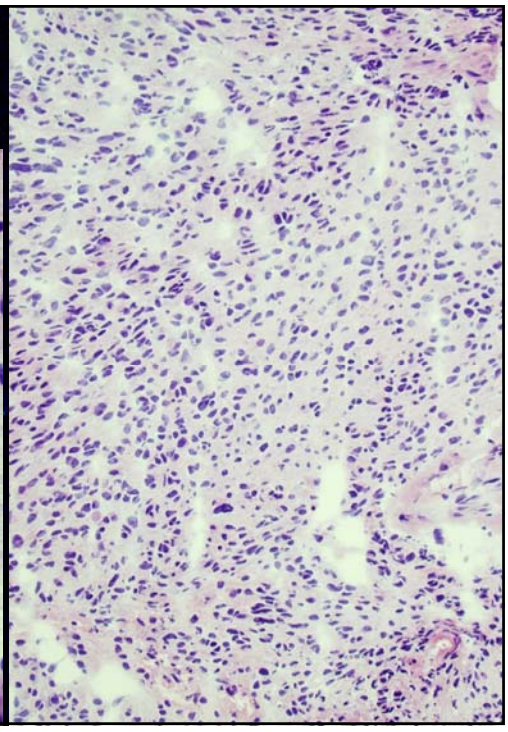
Oligodendroglioma – No processes

- Predominant population of monotonous round-to-oval nuclei without processes
- Background is typical granulo-fibrillary matrix of white matter
  - These are NOT glial processes:
    - pale not strongly eosinophilic
    - Wispy or granulo-fibrillary, not dense/solid



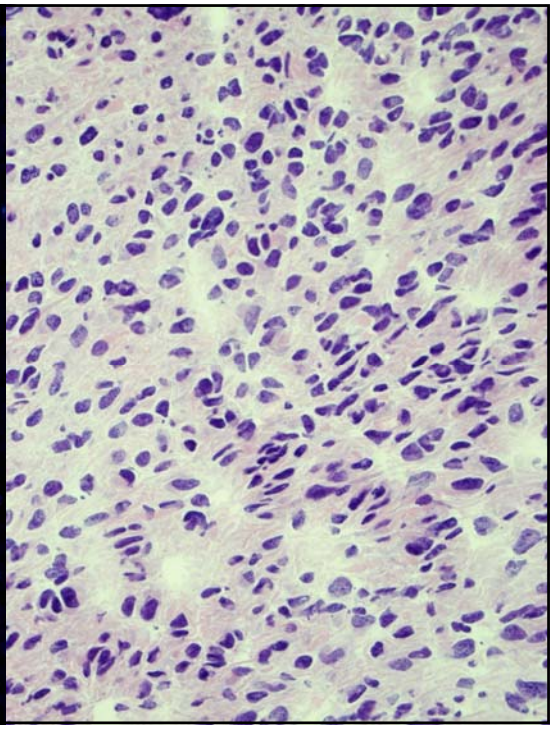
Test our new skills



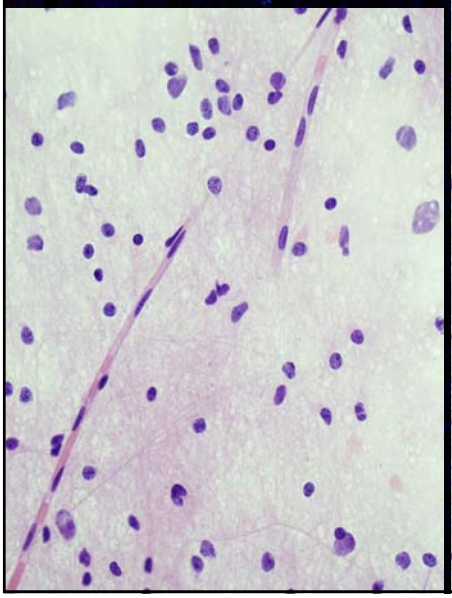


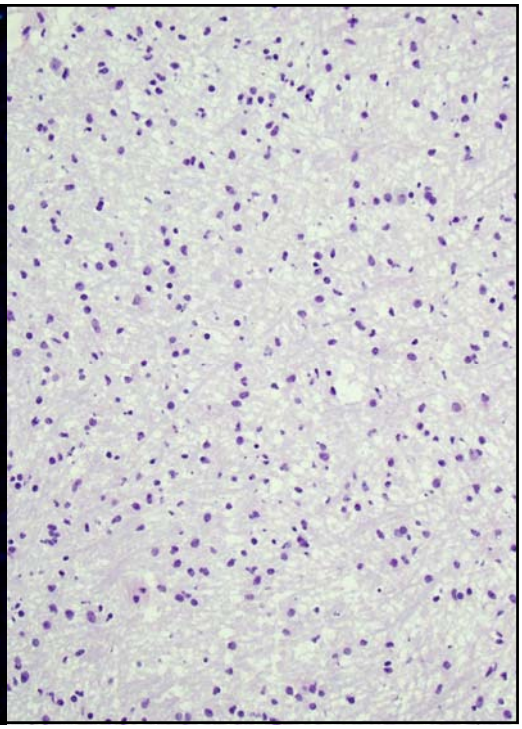
Anaplastic astrocytoma

- Smear: Although not as widespread or prominent as our examples, some cells had definite solid eosinophilic processes.
- Frozen section: Markedly hypercellular population of diffusely distributed atypical cells.



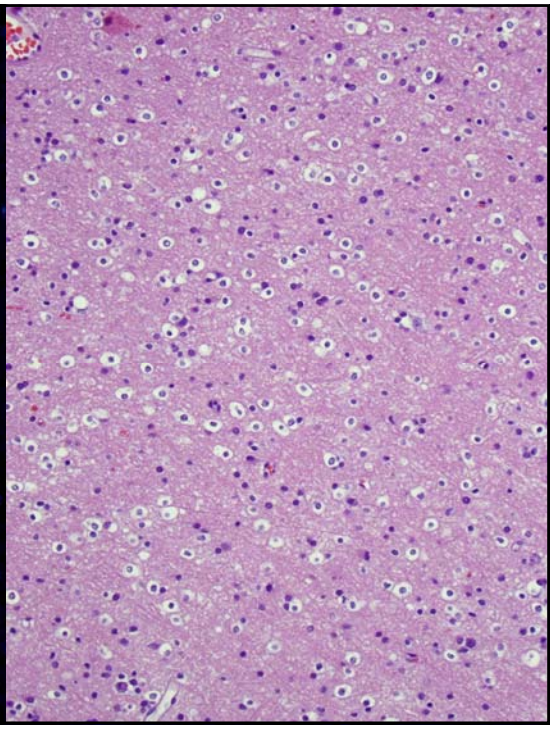
And again





Oligodendroglioma

- Smear: Round cells without processes.
  - Cells are larger than background oligodendrocytes and with more open nuclei
- Frozen section: Diffuse moderately hypercellular proliferation/infiltrate in a background of preserved parenchyma
  - Predominant population matches the smear



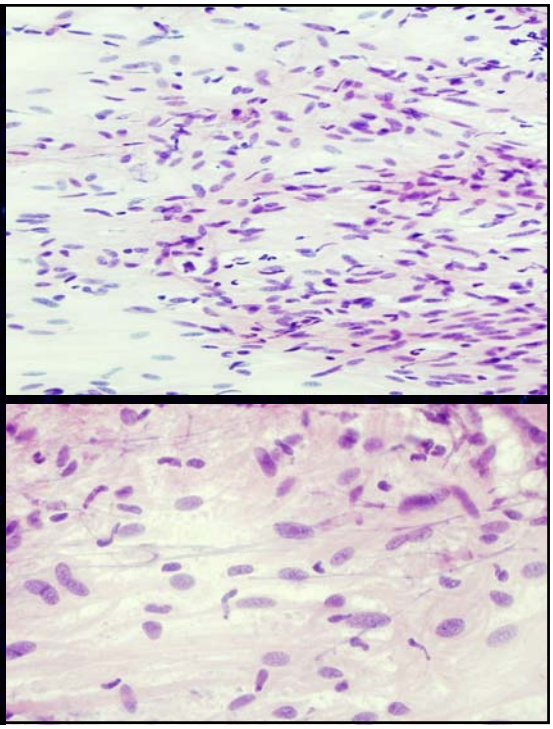
Processes or Stretched cell body?

Non-glial tumors with a fibrous stroma will occasional smear well, particularly when the tumor is highly cellular (less stroma).

In this setting, the smear process will stretch the cell body cytoplasm in a pattern that mimics glial processes. Although sometimes tricky, this differentiation is critical to determining tumor lineage particularly in cases with obscuring artifacts on the sections.

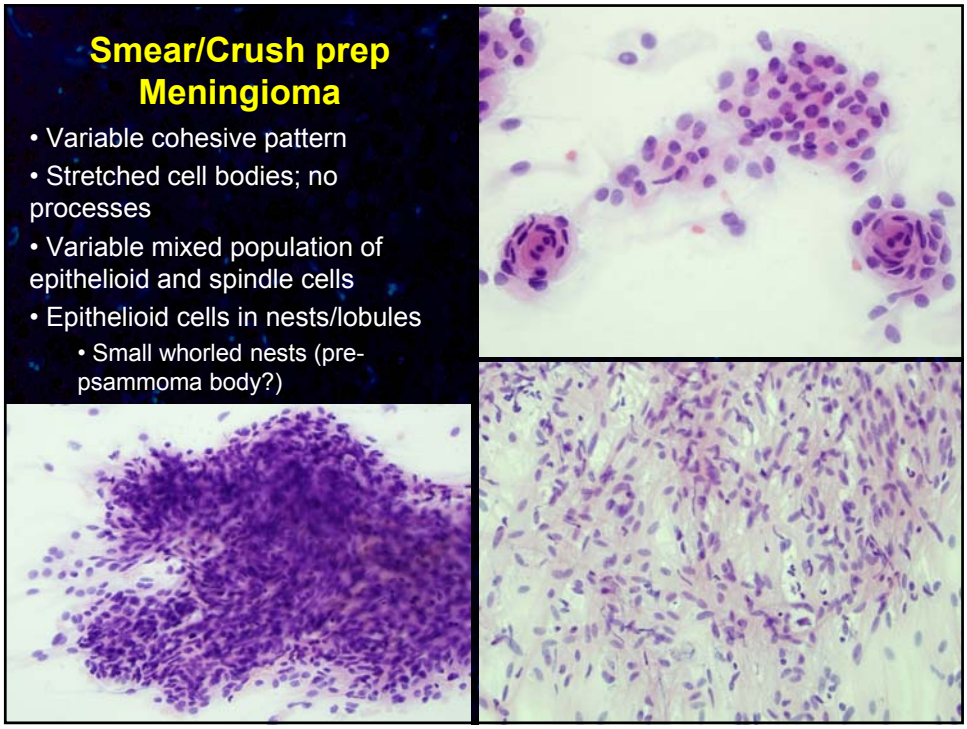
Glial processes: dense strongly eosinophilic

Cytoplasm/cell body: Pale, wispy. May have “railroad track” dual densities along edges



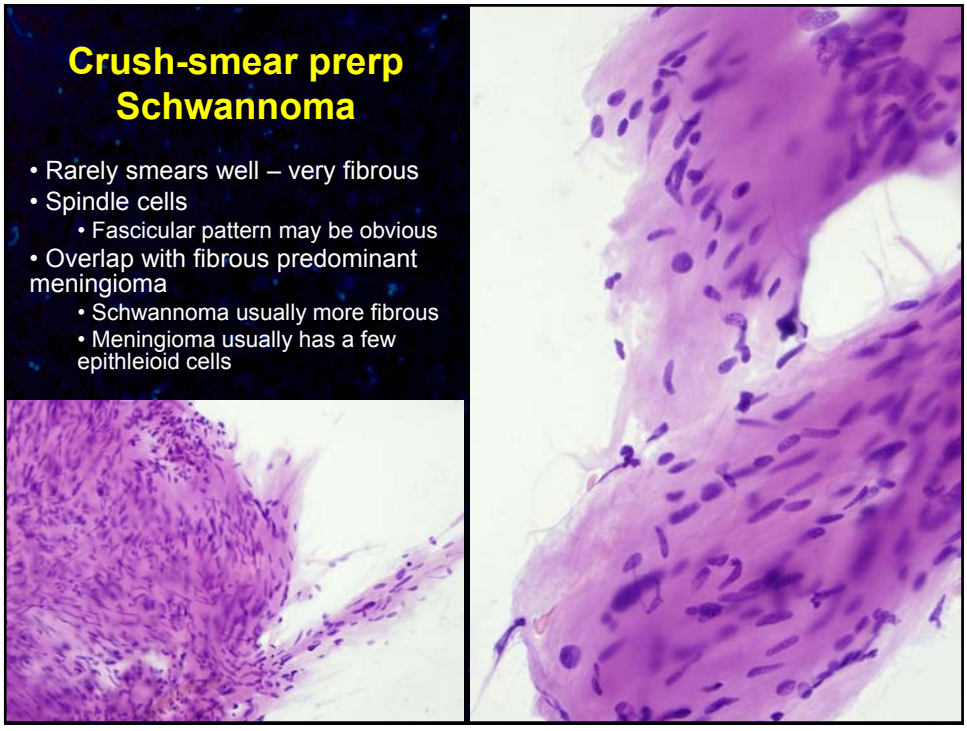
Smear/Crush prep Meningioma

- Variable cohesive pattern
- Stretched cell bodies; no processes
- Variable mixed population of epithelioid and spindle cells
- Epithelioid cells in nests/lobules
  - Small whorled nests (pre-psammoma body?)



Crush-smear prerp Schwannoma

- Rarely smears well – very fibrous
- Spindle cells
  - Fascicular pattern may be obvious
- Overlap with fibrous predominant meningioma
  - Schwannoma usually more fibrous
  - Meningioma usually has a few epithleioid cells



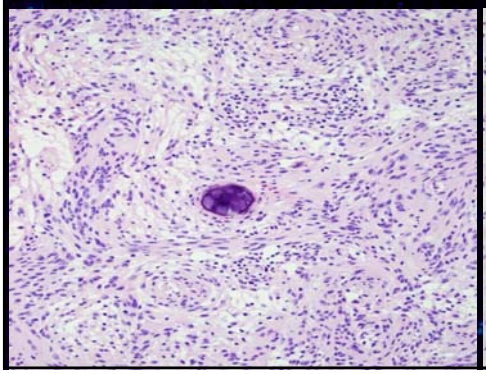
Meningioma v Schwannoma

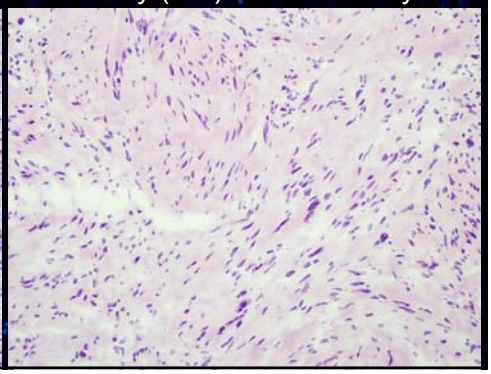
Meningioma

- Lobules and fascicles in fibrous stroma
  - Collagen bundles often discrete
- Nests/whorls/psammoma bodies

Schwannoma

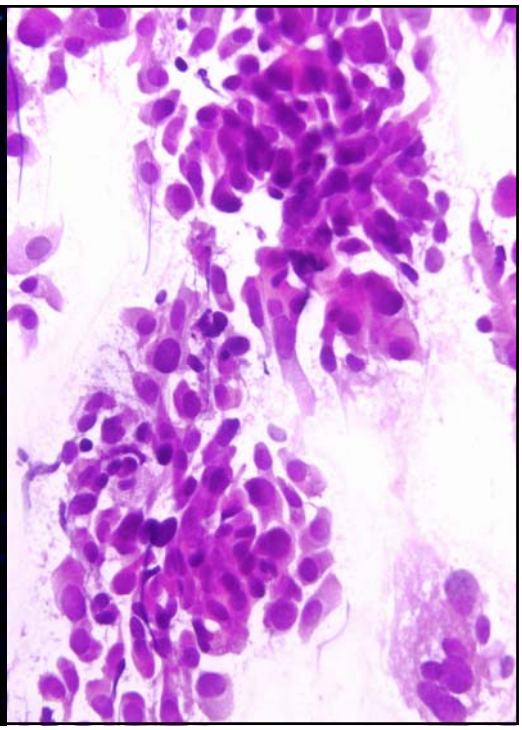
- Fascicles throughout
- Fibrous stroma is diffuse not discrete
- Verocay (like) bodies if lucky





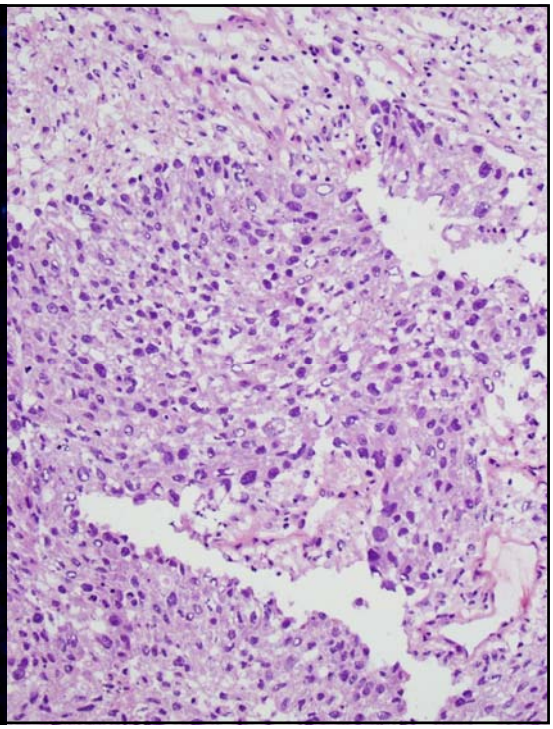
Metastasis
Crush/smear prep

- Clusters of cells, usually epithelioid
  - May be obviously cohesive (or not)
- Lack fibrillary processes
  - Beware of stretched cytoplasm
  - **Beware of admixed reactive astrocytes**
- Naked nuclei
  - At edge of tumor, reactive changes may predominate
  - Small numbers of tumor cells may be ignored, especially if crushed naked nuclei
  - Account for everything



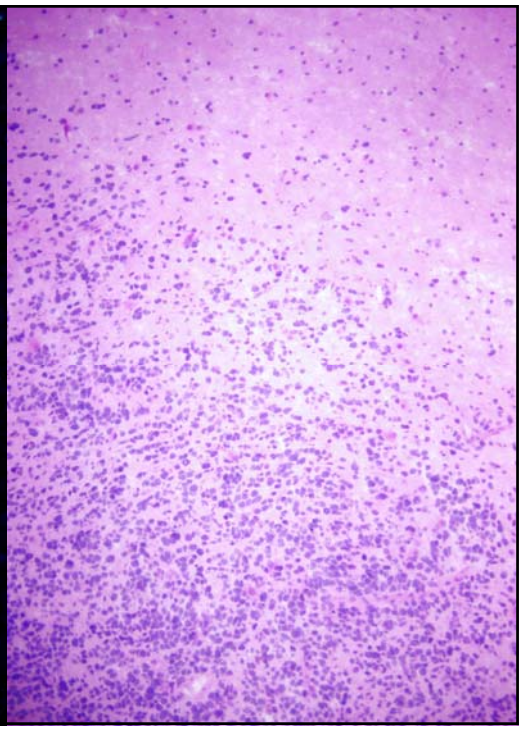
Metastasis

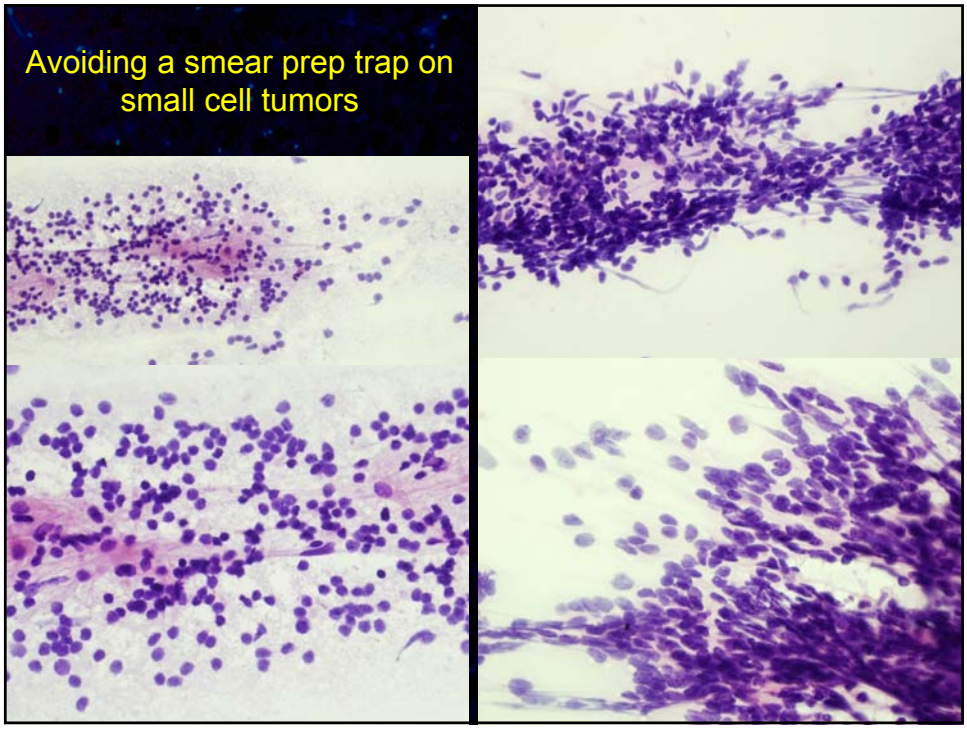
- Metastatic non-small cell carcinoma
- Cohesive nests
- Expansive interface with brain

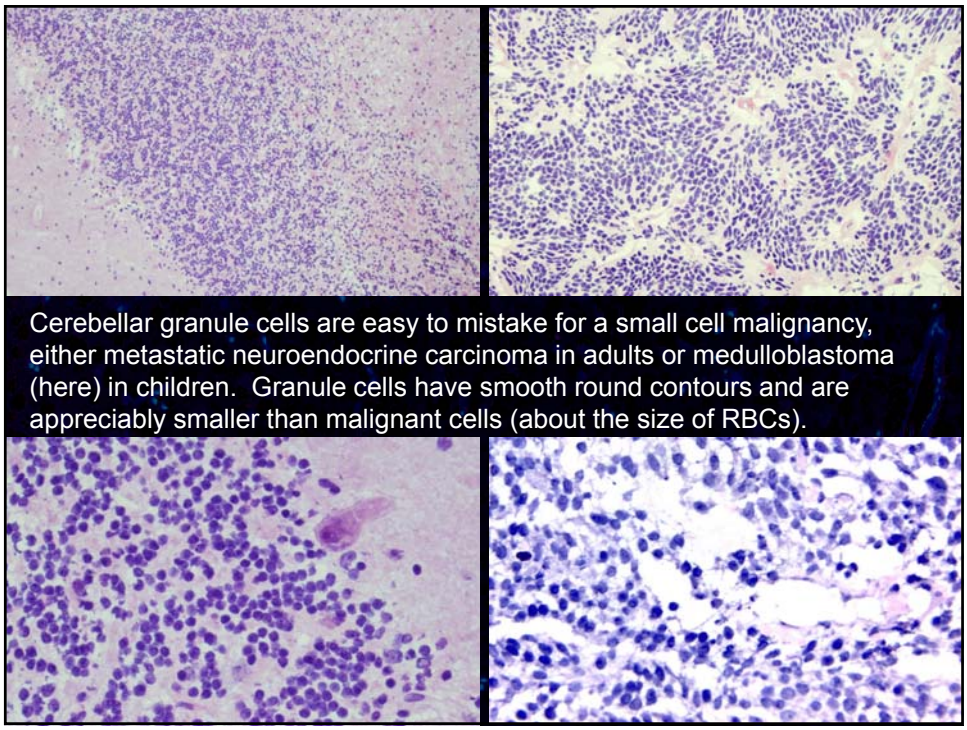


Metastasis
Small cell carcinoma

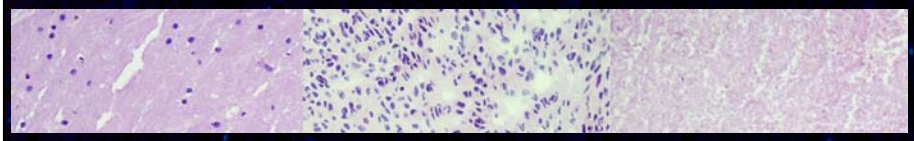
- Neuroendocrine/small cell tumors may raggedly infiltrate from a densely cellular core.
  - Limited extent of invasion
  - Tendency to form short cords
- Cytology may be indistinguishable from small anaplastic astrocytes
- Hopefully, the smear had enough processes!



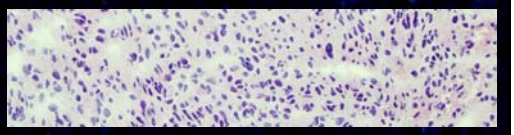

Avoiding a smear prep trap on small cell tumors

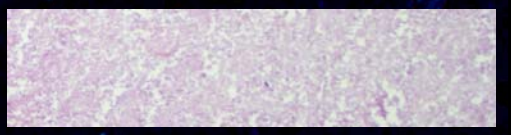

Cerebellar granule cells are easy to mistake for a small cell malignancy, either metastatic neuroendocrine carcinoma in adults or medulloblastoma (here) in children. Granule cells have smooth round contours and are appreciably smaller than malignant cells (about the size of RBCs).

Sampling issues- Pathology

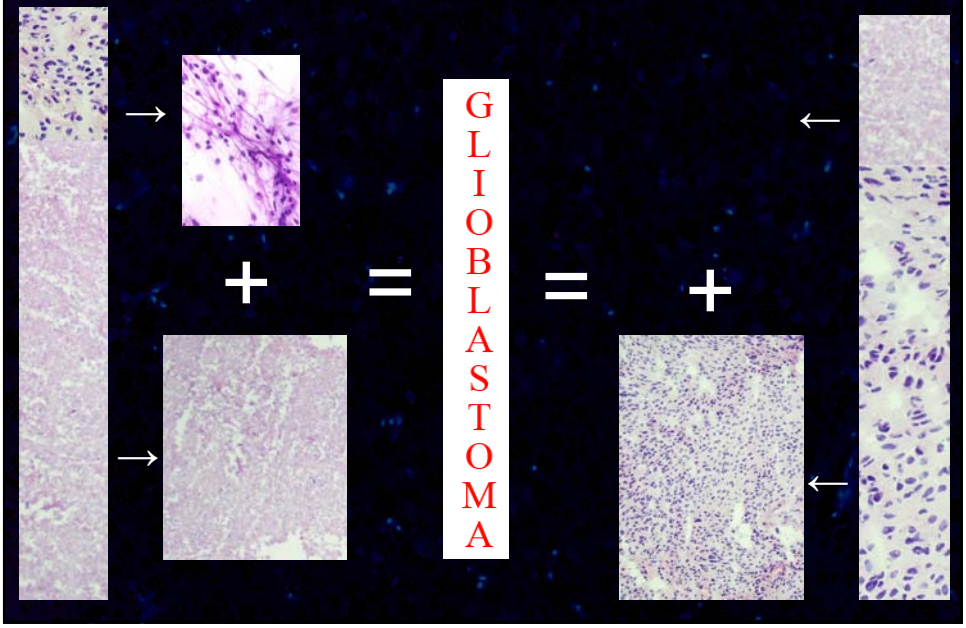


- There is no way to know if the sample chosen for the smear prep is representative
- In the examples at right, using either end would give the same "correct" result (matching the FS)
- In the example at the top, the results would be different and in fact neither would match the frozen section

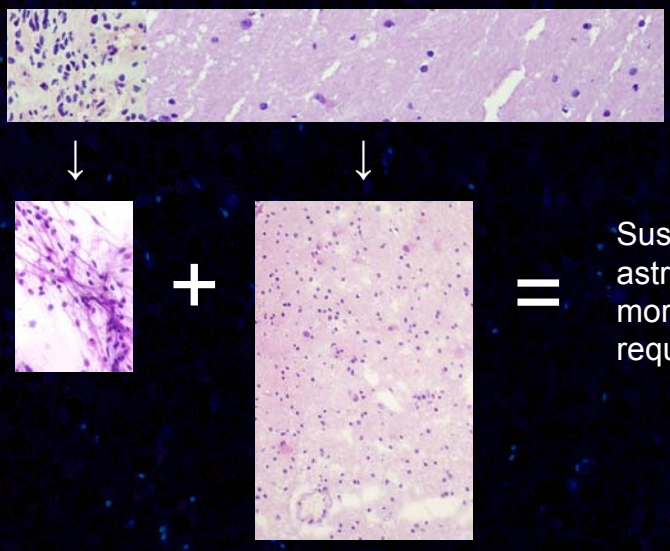




1 + 1 = GBM



It's worth the risk



Suspicious for astrocytoma; more tissue requested

Note that the risk of a non-correlating smear and section or of failing to make a definitive diagnosis is minimal if the surgeon provides an appropriately representative biopsy!

Sampling - Surgeon

We talked about sampling issues that are created in the lab.

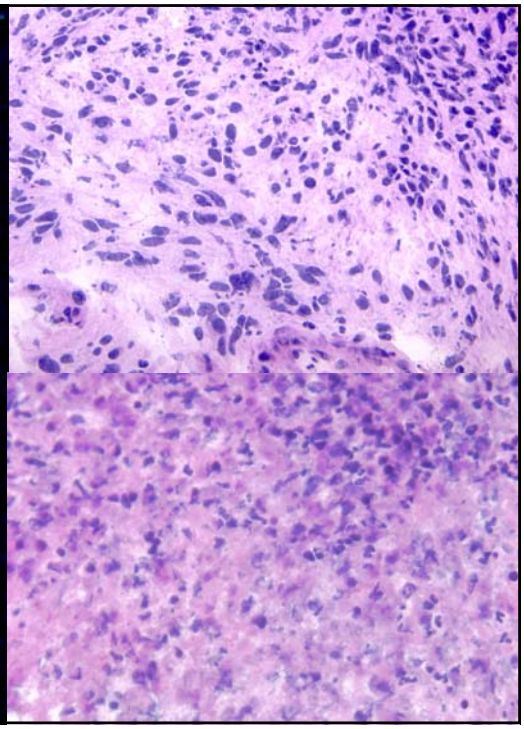
Some are created by the surgeon – but that is why we got the tissue: to find out what it is for sure.

Biopsies "next to" the lesion typically require more time than lesional samples and have the added bonus of finding a way to tell the neurosurgeon that he/she missed.

Some biopsies are lesional but misleading.

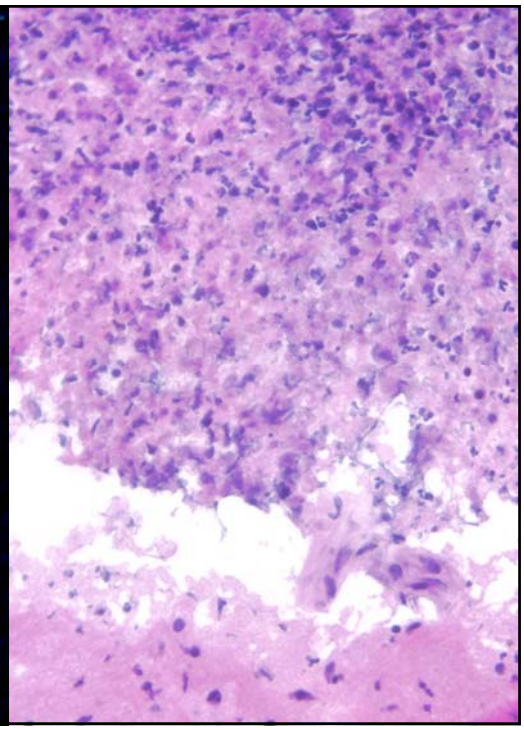
Glioblastoma

- Cellular tumor + Necrotizing inflammation
  - Necrosis in GBM can occurs from vascular injury leading to infarction.
    - May involve tumor and/or brain
  - Just like ordinary infarcts, sometimes massive PMN infiltrates occur at 1-2 days
- Not a problem if both are present, BUT…



Abscess

- Sometimes that nemesis Sampling rears its ugly head and you just get a lot of necrosis and neutrophils
- Nothing to indicate this is not an ordinary abscess
- So this conversation takes place



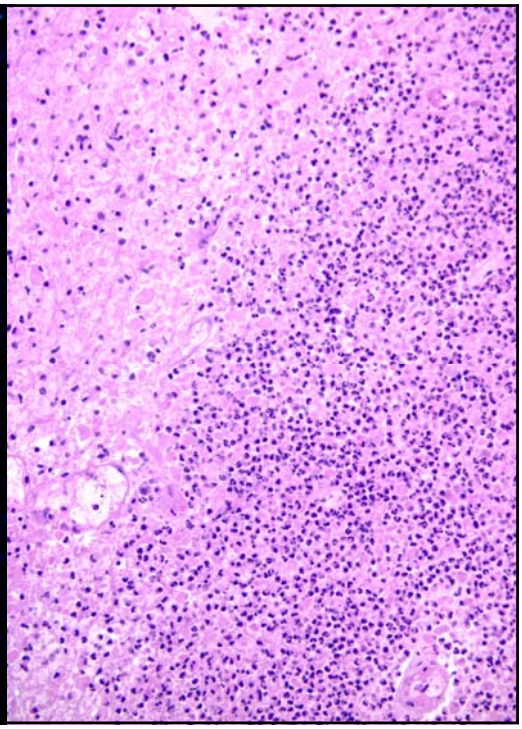
Abscess?GBM

Pathologist: We've got marked necrotizing inflammation – looks like an abscess

Neurosurgeon: No way! This is a tumor (you idiot).

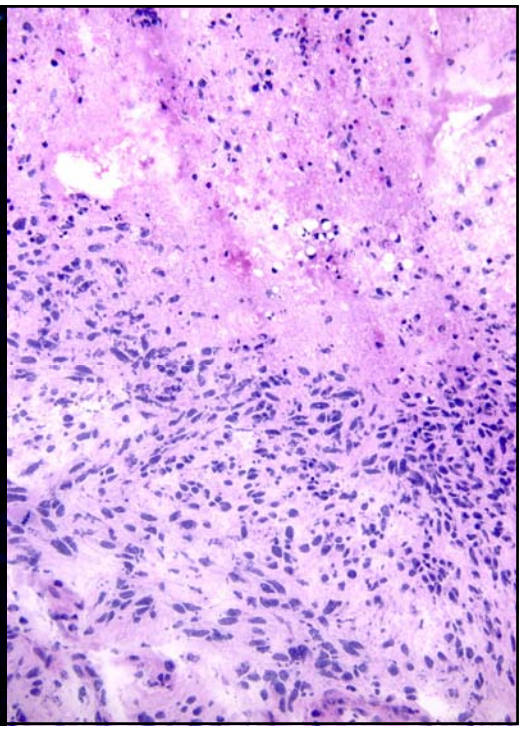
Pathologist (In a firm calm voice): Yeah that happens sometimes with acute tumor necrosis. Just send me some tissue from a viable area.

Neurosurgeon: OK (Wow! This pathologist really knows what they are doing!)

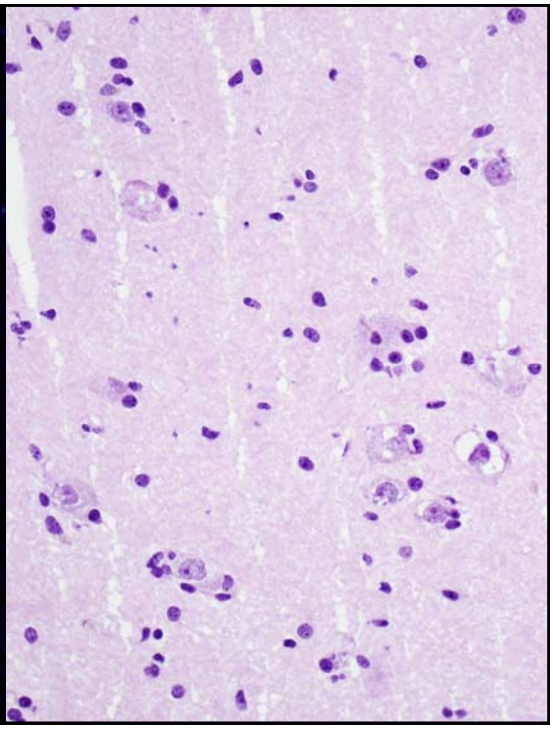


Glioblastoma

- The new biopsy does in fact have cellular tumor.
- The surgeon is happy and has a newfound respect for pathologists (I can dream).



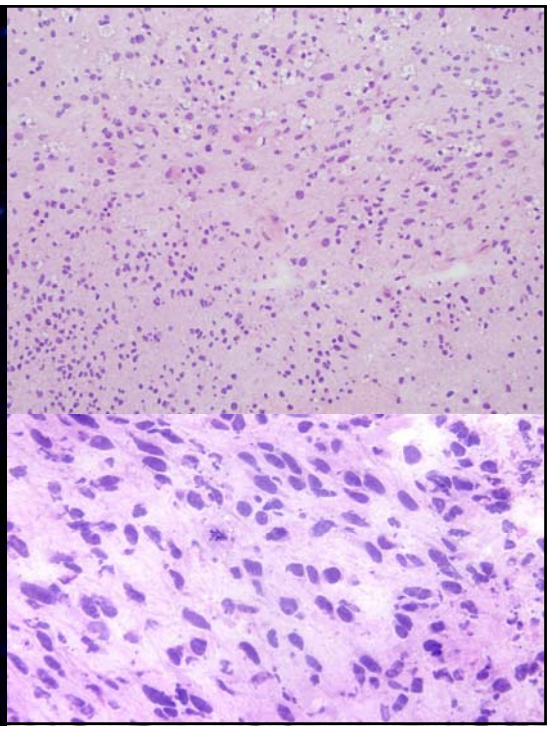
Perineuronal satellitosis



Recurrent tumor v radiation effect

A frequent biopsy question as new enhancing lesions on MRI can reflect either recurrent tumor or radiation-induced vascular injury.

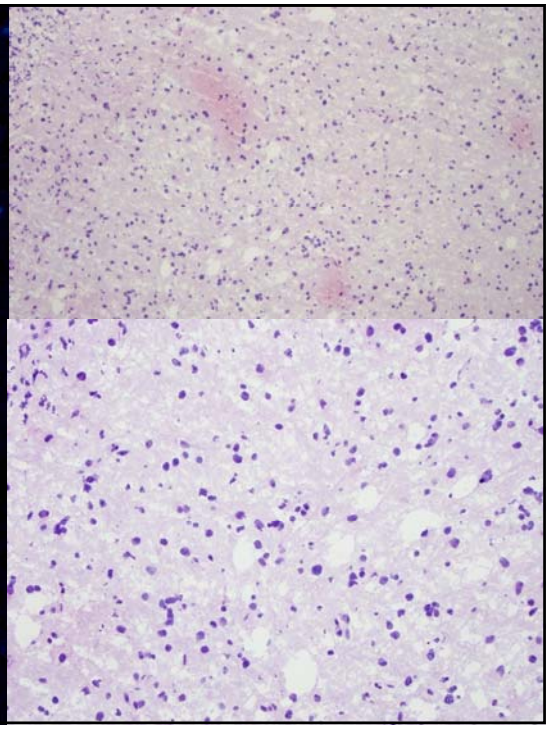
Recurrent tumors are usually characterized by the presence of cellular tumor. Mitoses are strong evidence or actively growing recurrent neoplasm. This pattern is diagnostic even if there are changes of recurrence elsewhere.



Recurrent infiltrating

The other pattern of unqualified recurrence involves infiltrating diffuse growth.

This will be less cellular but the background will be relatively normal brain. In fact, were it not for the history, this pattern be indistinguishable from the infiltrating edge of a primary glioma.

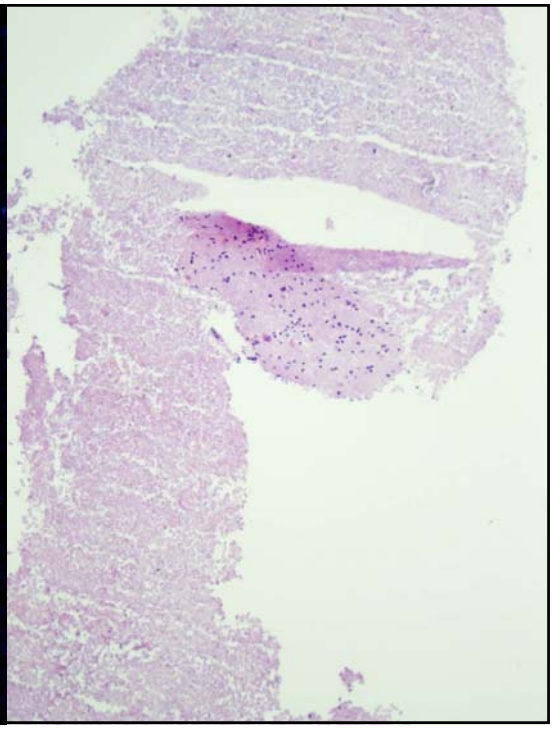


Diffuse necrosis

At the other extreme is the biopsy that shows diffuse necrosis or minimal semi-viable tumor.

Whereas this is also identical to the necrotic centers of untreated glioblastomas, the history of a new or enlarging lesion on MRI indicates that it represents treatment effect.

In cases at either extreme, the answer is easy but as we shall see, that is not always the case.



A side note on types of necrosis

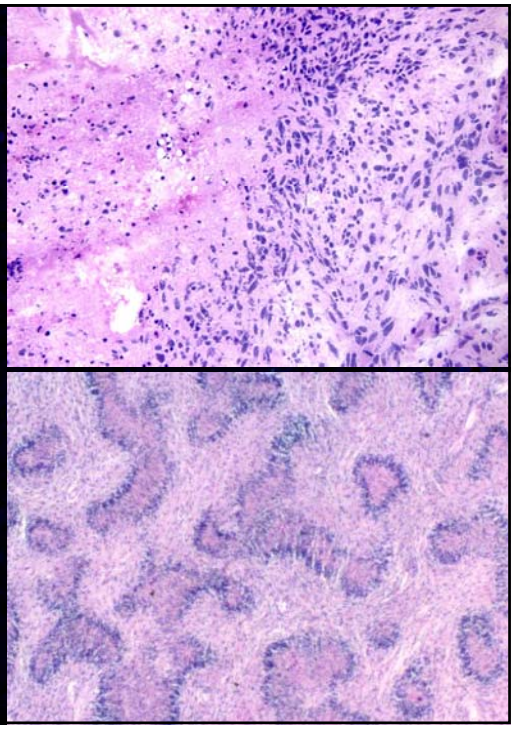
Brain tumor necrosis is described as either “Infarct-type” or (pseudo)palisading.

In primary/untreated tumors they have the same significance.

Infarct necrosis may be due to RT or spontaneous/intrinsic.

However, by convention, pseudopalisading necrosis is considered spontaneous/ intrinsic.

In this regard, the presence of palisading necrosisindicates recurrent tumor.

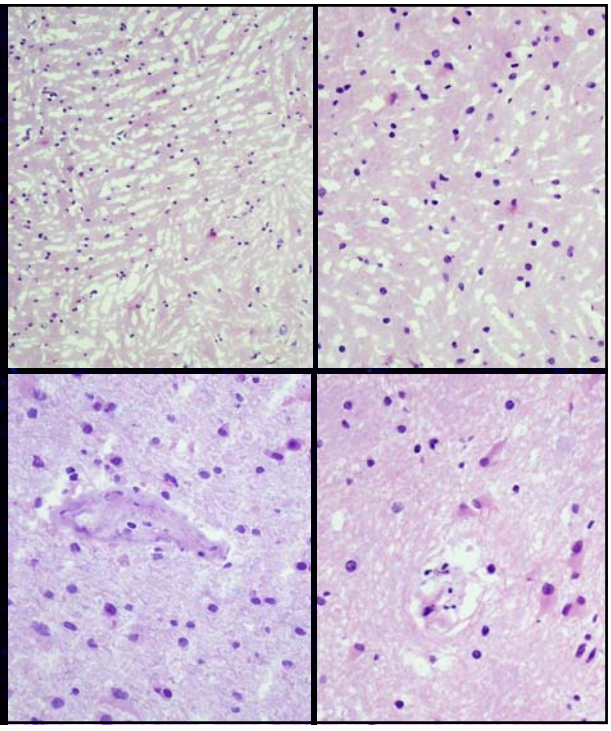


Typical RT changes: Gliosis, edema & vascular fibrosis

Typical changes associated with RT include:

Edema and gliosis in various proportions and severity. The astrocytes often demonstrate reactive or radiation induced atypia.

Vascular changes including hyaline fibrosis, loss of endothelial cells, fibrinoid degeneration and even thrombosis.

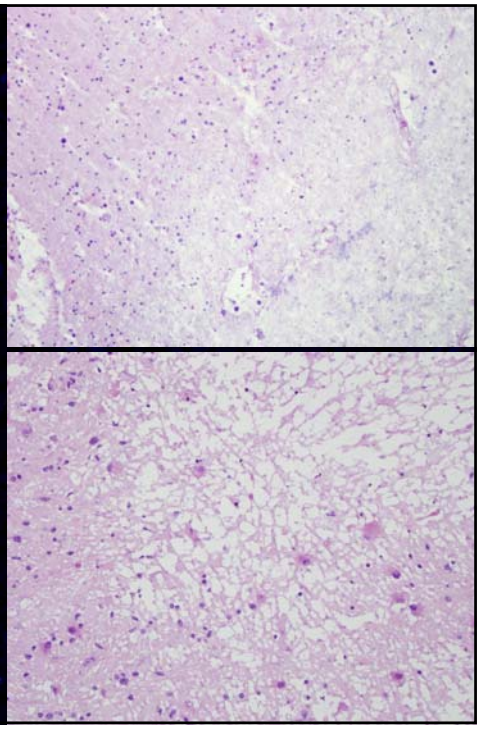


Parenchymal infarcts

Small circumscribed foci of severe edema with axonal loss or overt necrosis represent microvascular infarcts due to radiation vascular injury (the mechanism of "clinical radiation necrosis").

They are distinguished from tumor necrosis by the lack of ghost outlines of cellular tumor (parenchymal architecture may be apparent) and the surrounding background of normal or reactive brain.

A very specific indicator of treatment effect.



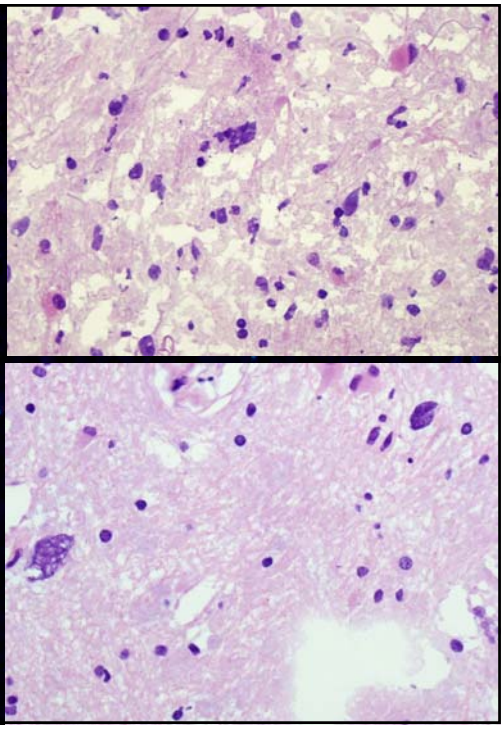
Individual Tumor Cells

The cells seen here are too bizarre to be radiation-induced atypia in reactive astrocytes so they are almost certainly tumor cells.

There are always residual tumor cells in treated gliomas whether you see them or not or they are in the biopsy or not.

ITC are NOT recurrent tumor.

In a background of reactive changes, they are most likely residual senescent tumor cells.

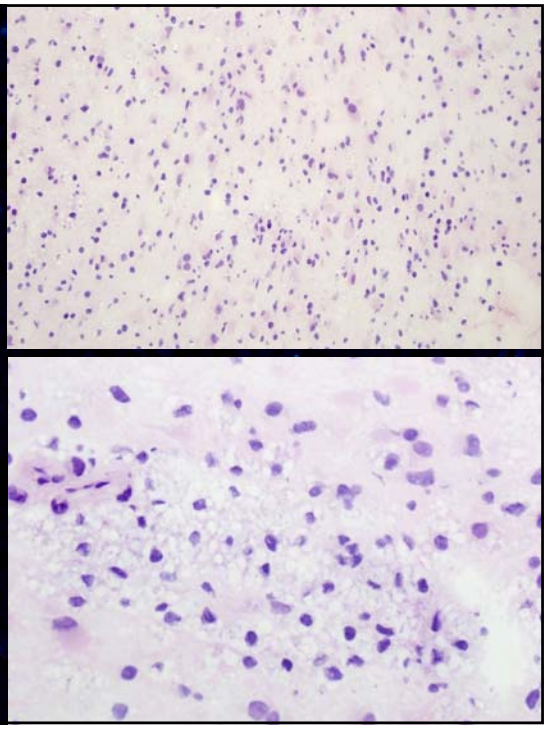


Macrophages

A few individual macrophage are scattered throughout (high grade) gliomas

Large numbers are only rarely seen and clusters, parenchymal or perivascular, are so exceptional as to seriously challenge a diagnosis of recurrent tumor (or of tumor at all).

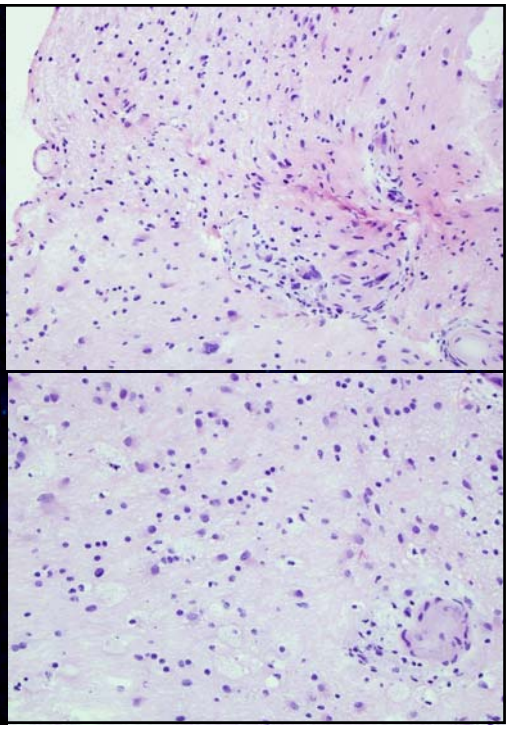
As seen here, they are all but diagnostic of treatment effect over recurrence.



???

As newly minted experts, what do you think?

- Recurrent?
- Treatment?

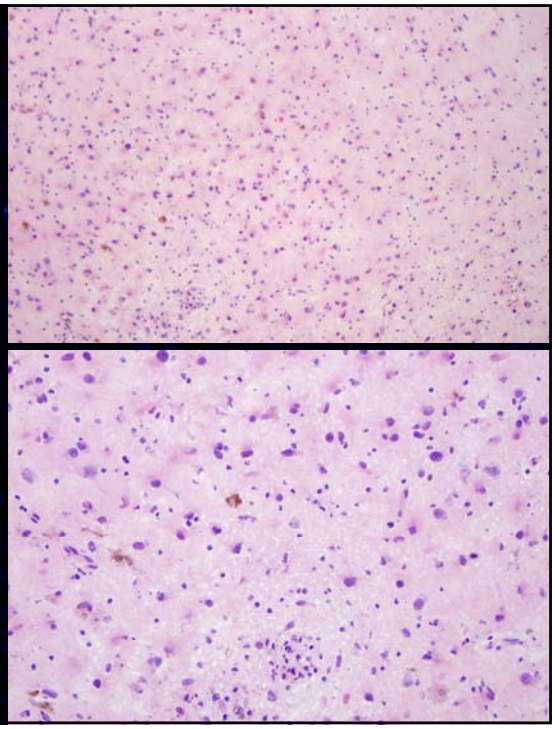


???

How about this one?

Recurrent?

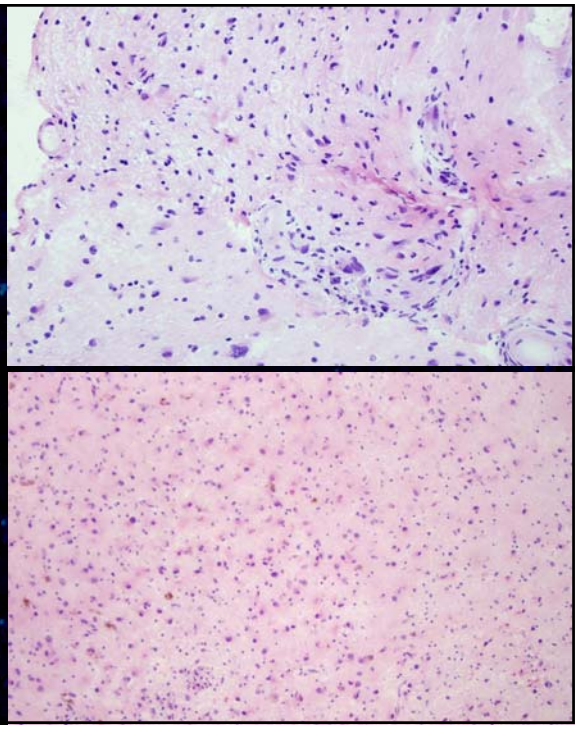
Treatment



???

Sometimes there is no “right” answer

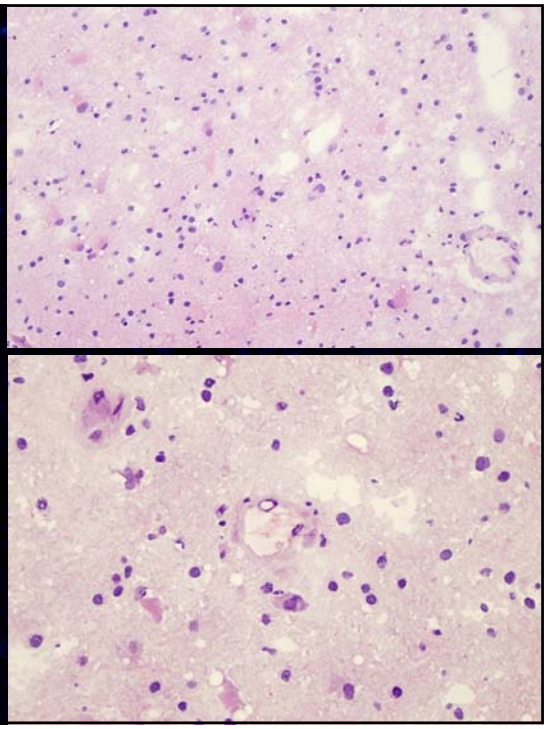
I have gone back and forth on these cases from when I first saw them to when I put the pictures in the talk.



Recurrent v treatment Next-to-last word

- Mild hypercellularity
- Scattered atypical cells
- Edema
- Scattered small cells
- Scattered reactive appearing astrocytes
- Vascular changes

- Would favor treatment but not straightforward …unless the surgeon gave you the history that they were looking for recurrent *metastatic carcinoma*
  - RT can cause a lot of atypia
  - Lack of Hx can cause a lot of confusion

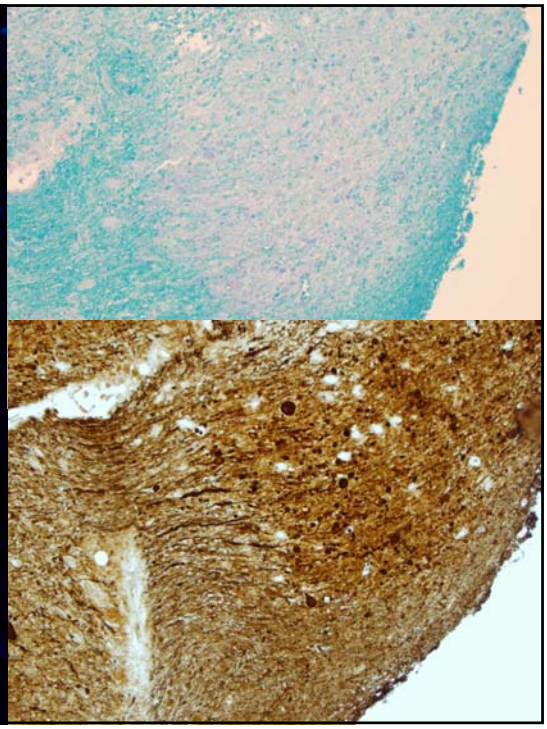


Recurrent tumor or treatment?

- Extremes – cellular tumor or diffuse necrosis easy but…
- Milieu
  - Scattered atypical cells in a healthy-appearing stroma suggests tumor
  - Scattered atypical cells in a background of macrophages and necrosis suggests treatment – allow a few more atypical cells here
- Macrophages are your friends
  - Clustered macrophages are almost never seen in gliomas
- Parenchymal infarcts are specific for treatment effect
- Individual tumor cells does not mean recurrence
  - There are always tumor cells left behind; eventually the treatment will always fail and the tumor will recur unless the patient dies first from the treatment itself, a heart attack, a car accident….
- The answer is often BOTH
  - The patient did receive treatment for an incurable tumor. There will (almost) always be some treatment-related changes and the tumor will recur
  - Remember the question is really "Has the treatment failed/Is the tumor winning?"
    - Definite cellular or infiltrating tumor indicates treatment failure even in a preponderant background of treatment effect
- The answer is sometimes a shrug.
  - How many atypical cells do you need to call it "cellular"?
  - Sometimes pathology doesn't have the "right" answer – pick a side and comment that the case is borderline/not definitive/or whatever term you prefer for equivocation
    - That's why tumor boards were created

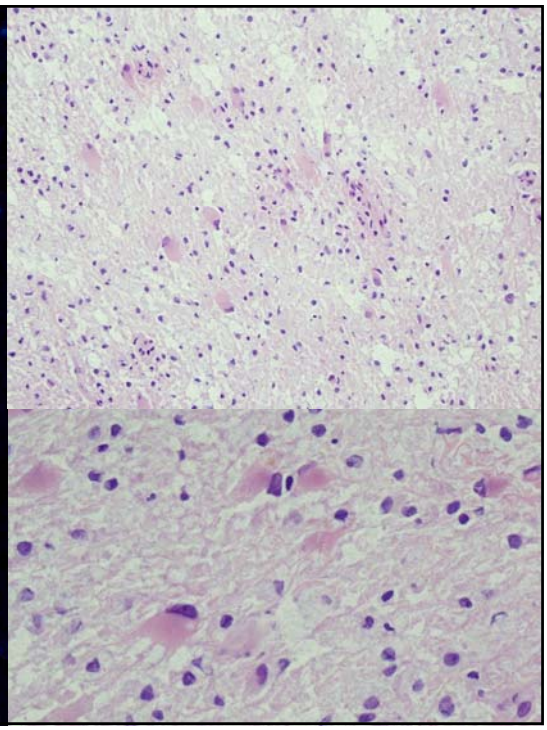
Inflammatory/reactive tumor-like lesions – Multiple sclerosis

- Permanent sections usually straightforward
  - White matter
  - Circumscribed v diffusely infiltrative
  - Reactive astrocytes though often atypical
  - Macrophages in clusters parenchymal & perivascular
  - Myelin loss with relative axonal preservation



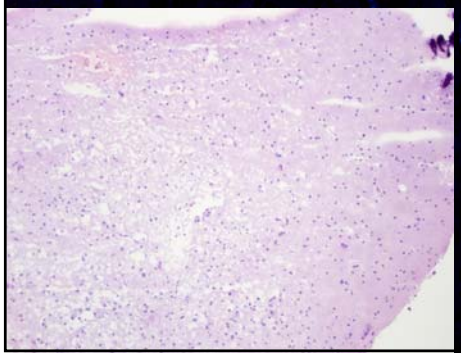
Multiple sclerosis

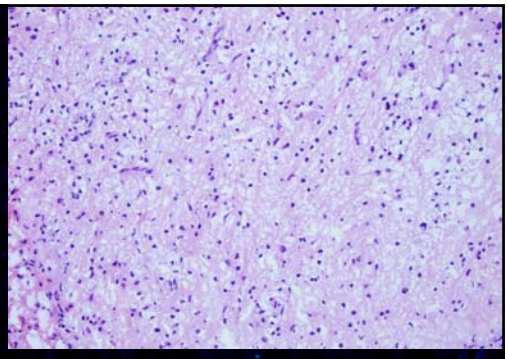
- Frozen sections often not straightforward
- Hypercellular and edematous white matter
  - Reactive astrocytes though often atypical
    - Atypia is clue because of low number
  - Small mononuclear cells account for most of hypercellularity

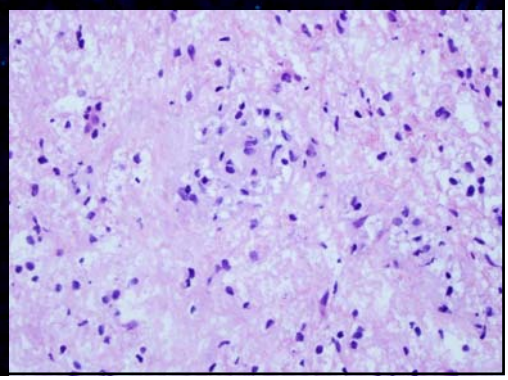


Multiple sclerosis

- At low power circumscribed pattern often apparent if biopsy big enough
- At higher power, mononuclear cells are seen to be macrophages in clusters - parenchymal & perivascular

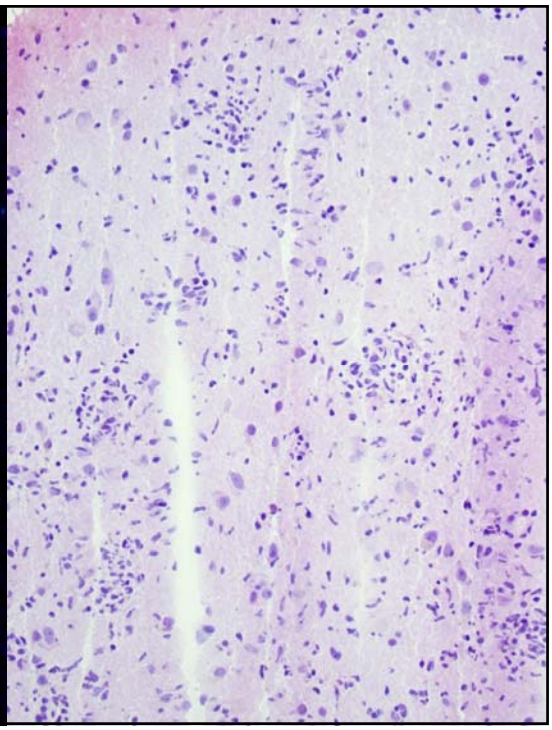






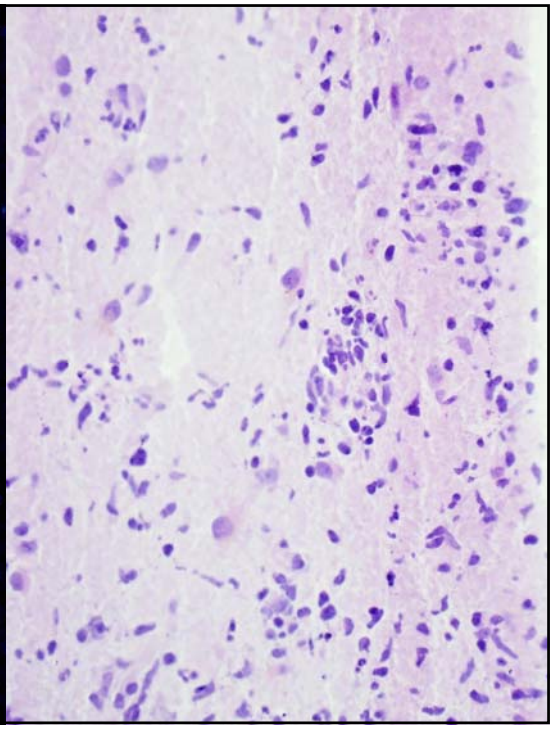
Inflammatory/Reactive v Tumor

- Hypercellular gray and white matter
- Obvious astrocytes with some atypia
- Cells with round nuclei ?mononuclear
- Cells with slender elongate/oblong nuclei w/o obvious processes/ cytoplasm



Inflammatory/Reactive v Tumor

- Changes primarily in gray matter
  - White matter less cellular – edema and a few astrocytes
- Round and slender oblong nuclei both diffusely distributed and clustered around vessels or neurons
  - Distribution suggests inflammatory
    - Lymphocytes and microglia/histiocytes

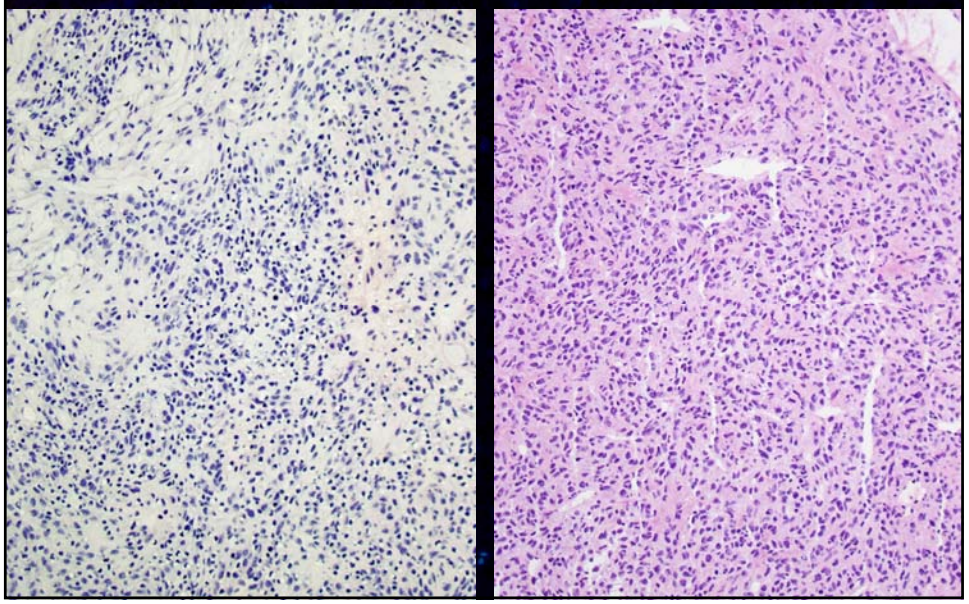


Inflammatory/Reactive v Tumor

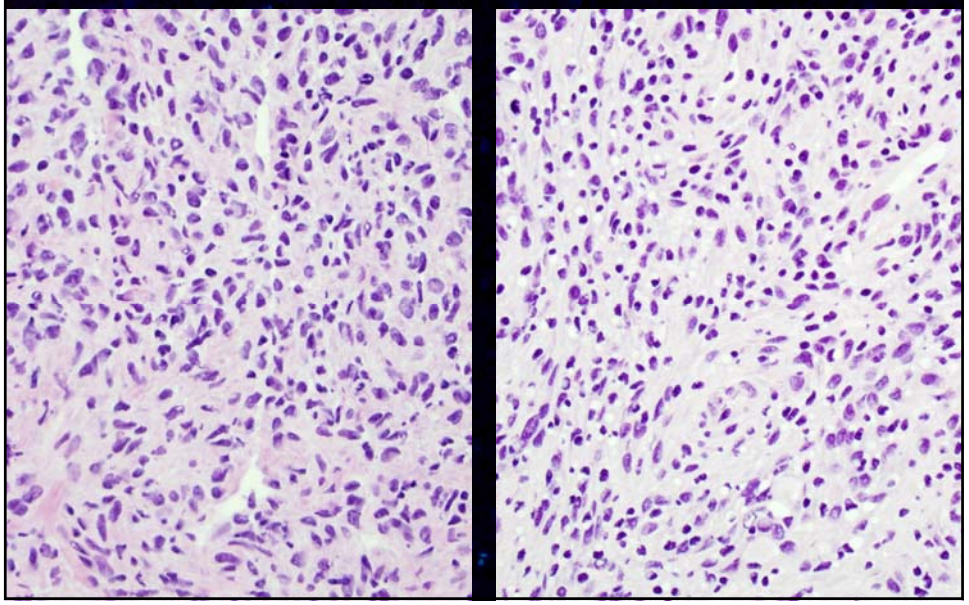
- Cell number
  - Overall
  - By type
- Cell type
  - Astrocytes
  - Mononuclear cells
  - Microglia v astrocyte
  - MACROPHAGE
    - Clustered macrophages are almost never seen in tumor
- Cell/Lesion distribution
  - Diffuse WM – Tumor
  - Circumscribed WM – MS
  - Grey matter - Inflammatory
  - Perivascular - Inflammatory

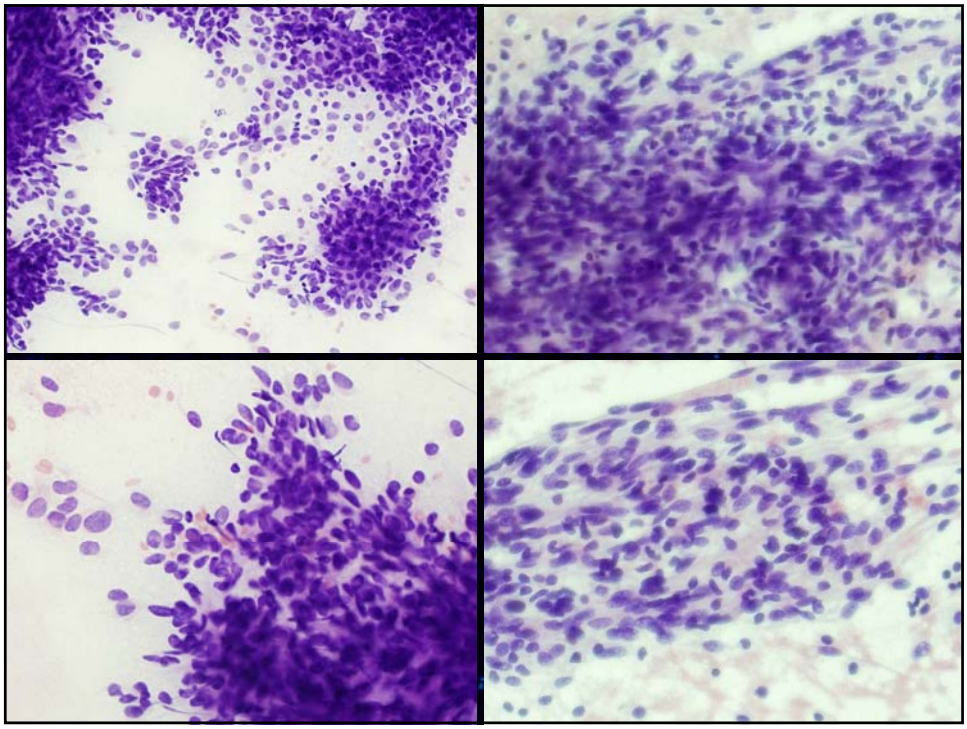
No organisms on biopsy but autopsy confirmed Balmuthia/Acanthamoeba meningoencephalitis

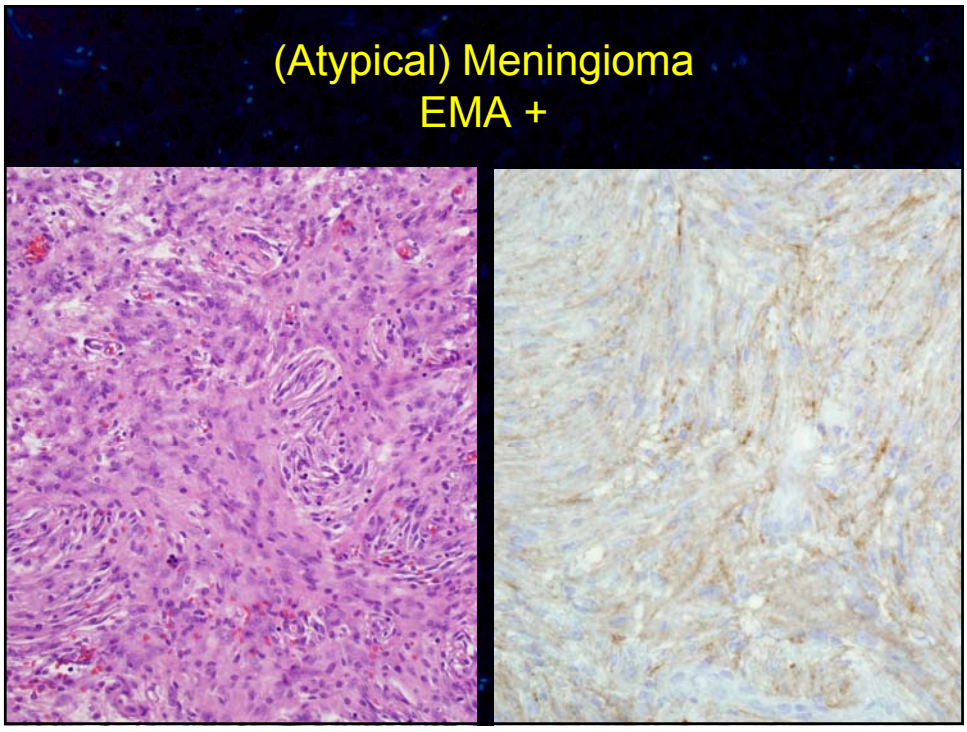
Atypical) Meningioma v Hemangiopericytoma/SFT

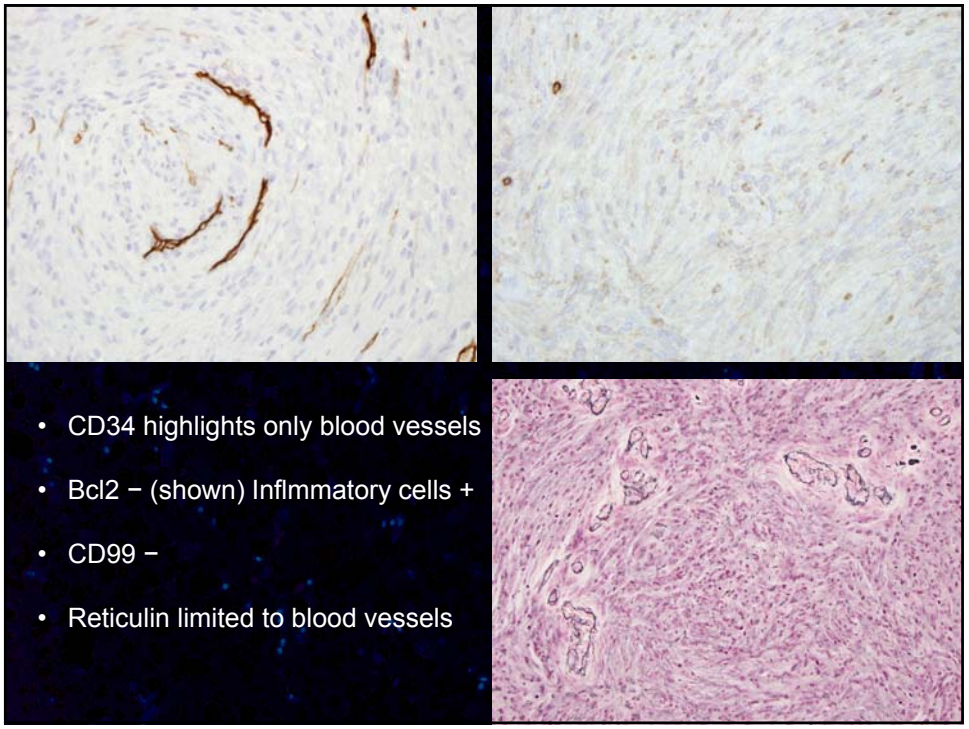


(Atypical) Meningioma v Hemangiopericytoma/SFT



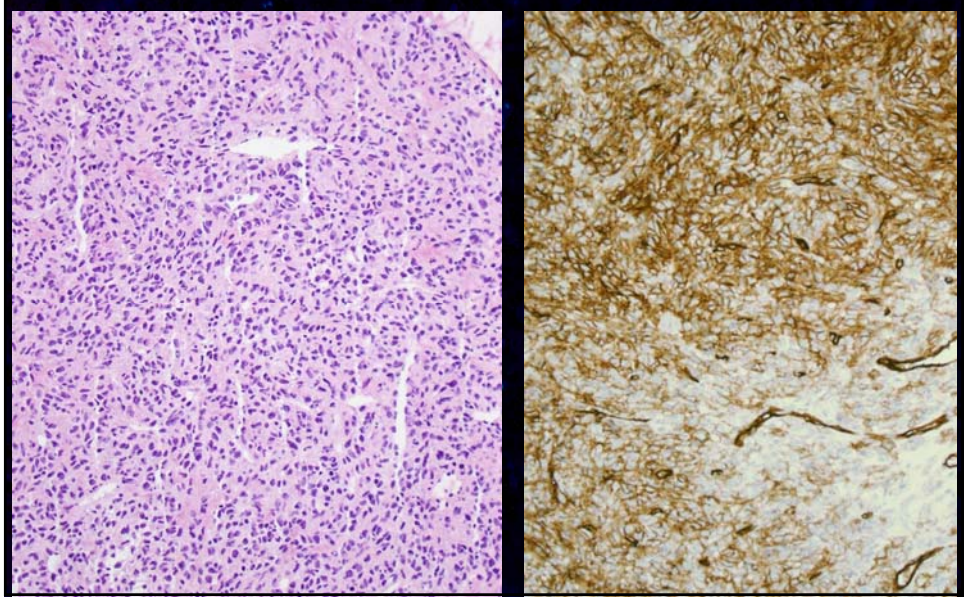


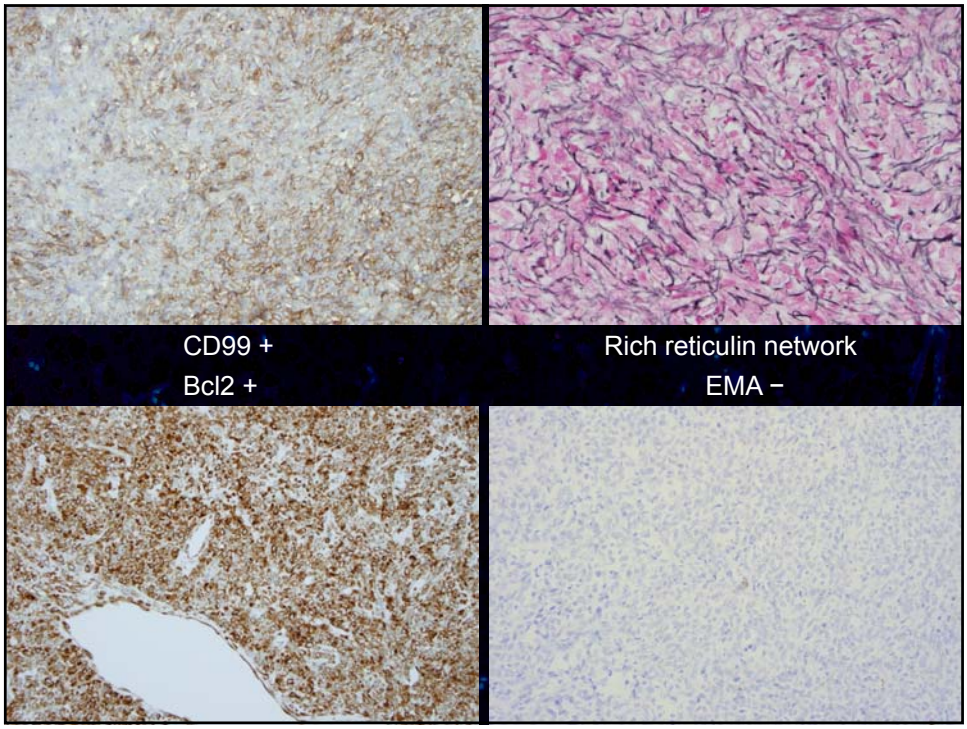

(Atypical) Meningioma
EMA +



- CD34 highlights only blood vessels
- Bcl2 − (shown) Inflmmatory cells +
- CD99 −
- Reticulin limited to blood vessels

Hemangiopericytoma/SFT
CD34 +





CD99 +
Bcl2 +

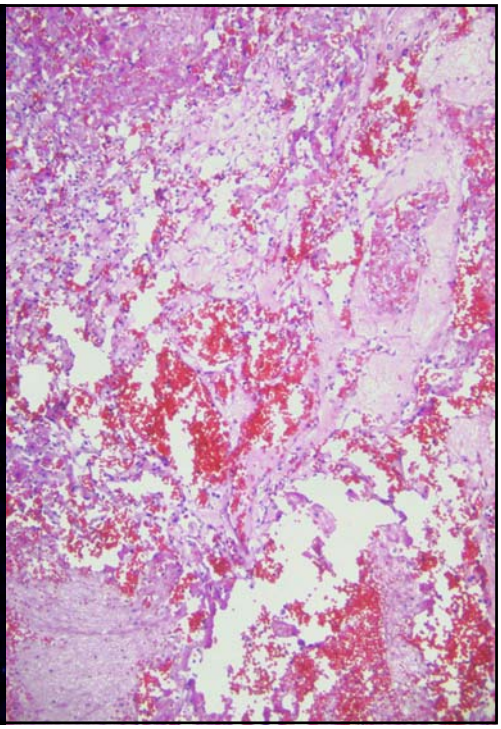
Rich reticulin network
EMA −

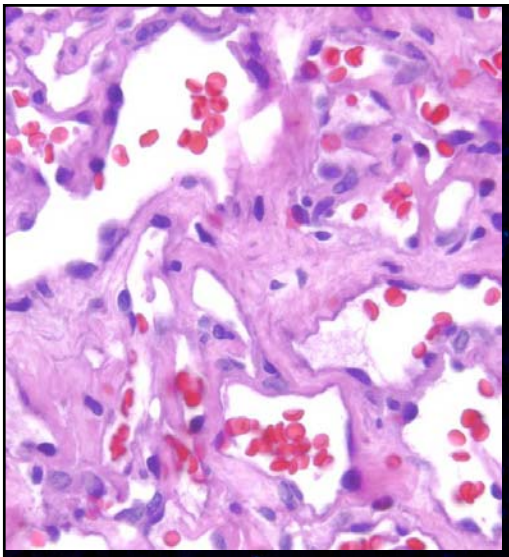
Hemangiopericytoma/SFT

- Invasive, aggressive tumors that almost always recur
  - Grading based primarily on proliferative activity which correlates with cellularity and atypia
    - Timing of recurrence, not if
- At FS, any time you see an “I think it’s a meningioma, but it’s ‘atypical’, ‘funny’, ‘worrisome’ ” consider HPC/SFT
  - Operative management the same
  - Surgeons often recognize tumor is more invasive – may ask if it is atypical or an HPC
- Old name: Angioblastic meningioma based on high number of atypical microvessels.
  - Not currently used but still causes confusion

Angiomatous meningioma

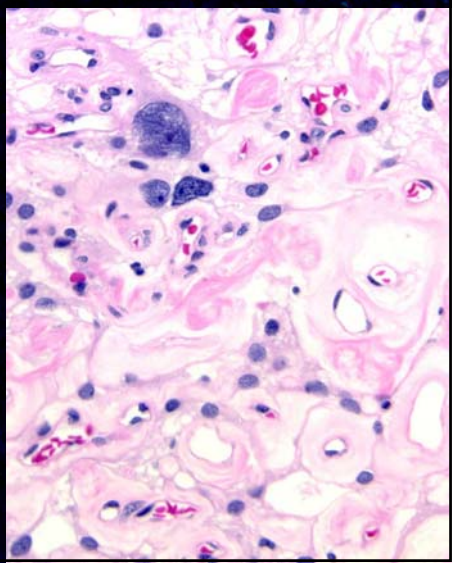
- Highly vascular, poorly cellular
  - Cell loss and collapse leads to approximation of hyalized blood vessels
  - More vessels than tumor cells
- Degenerative/involutional change
  - (Almost) always grade I
    - Hard to be aggressive and degenerative at the same time
- Closely related to microcystic meningioma
  - Similar cell loss but mucopolysaccharide-rich edema fills the space so less collapse





- Hyalinized closey approximated vessels
- Few residual tumor cells
- Degenerative atypia common often pronounced

Angiomatous meningioma



IMMUNOHISTOCHEMISTRY THAT ACTUALLY HELPS

Lineage IHC

Essential	Specialized/Limited
❖ S100	❖ Beta-tubulin
❖ GFAP	❖ MAP-2
❖ Synaptophysin	❖ Nestin
❖ Neu-N	❖ CD-133
❖ EMA	
❖ CD34	
▪ CD99	
▪ BCL-2	
❖ Cam 5.2	
▪ NOT AE1/3	

Glioma IHC

- α-internexin
- Cathepsin B
- EGFR/EGFR vIII
  - ch 7
- IDH1
- INI-1
- MGMT
  - O6 methyl guanine methytransferase
- Nestin
- p16
- p53
- PDGFR
- PTEN
  - PI3K/Akt
- YKL-40

Glioma IHC that actually helps

- α-internexin
  - High correlation with 1p 19q codeletion
  - Usefulness as surrogate ?
- IDH1
- INI-1
  - RTK/ATRT
  - Valuable/limited
- p53

IDH1 and p53

IDH1

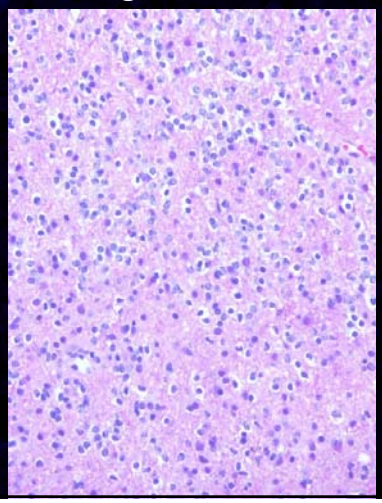
- Most common mutation in diffuse gliomas – 75%
  - ? Initial tumorigenic mutation
  - IDH2 mutationa less common
- Antibody is specific to mutated gene
  - Highly sensitive
  - 100% specific for diffuse glioma (Oligo/Astro)

p53

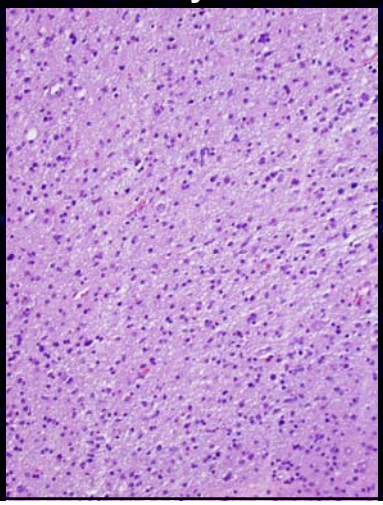
- Widely expressed tumor suppresser gene/pathway
- p53 mutation or other defect in p53 pathway leads to accumulation (mutant or wild type) of normally transient protein
  - >50% diffuse astrocytomas
  - Rare in oligodendrogliomas
- Antibody detects both wild type and mutant

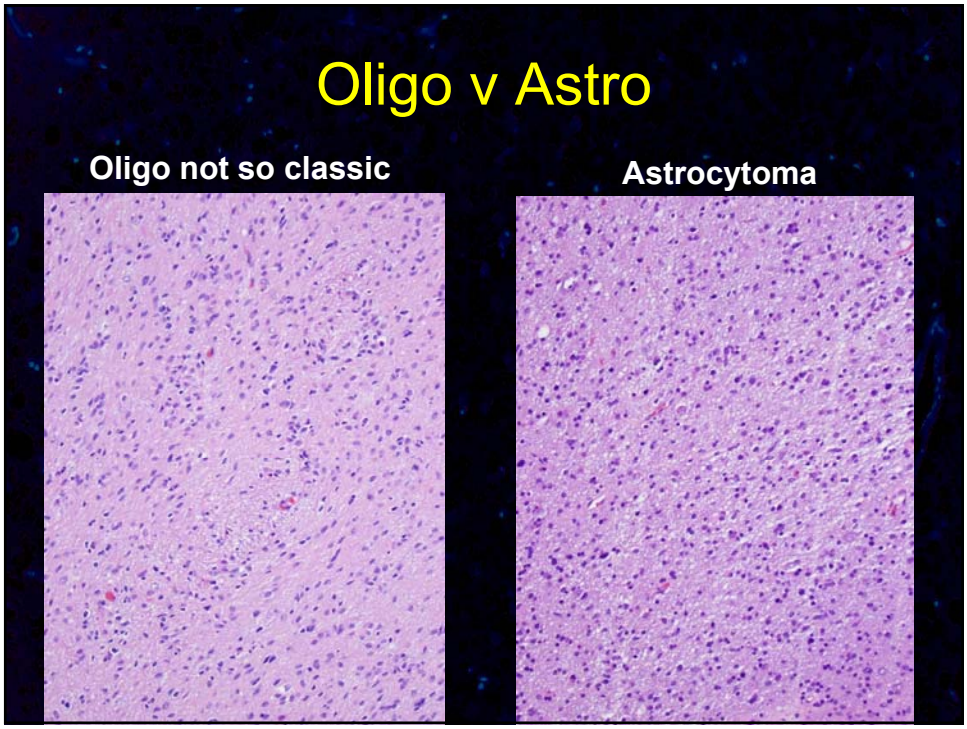
Oligo v Astro

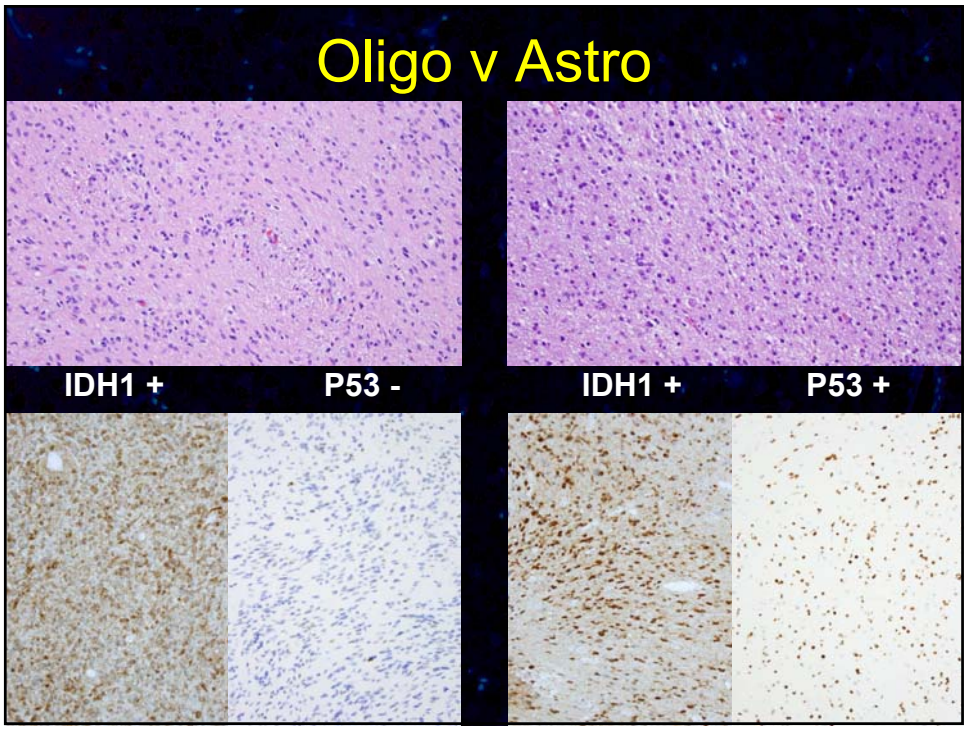
Oligo classic



Astrocytoma

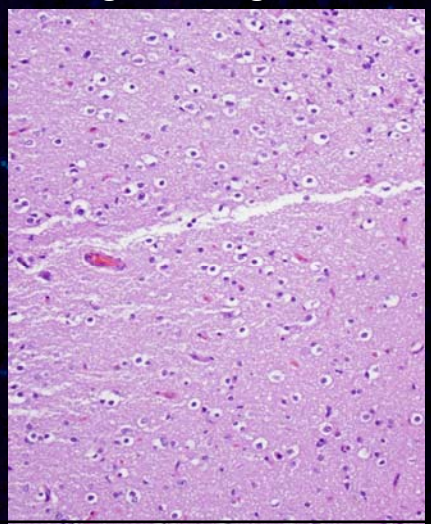



Oligo v Astro
Oligo not so classic
Astrocytoma

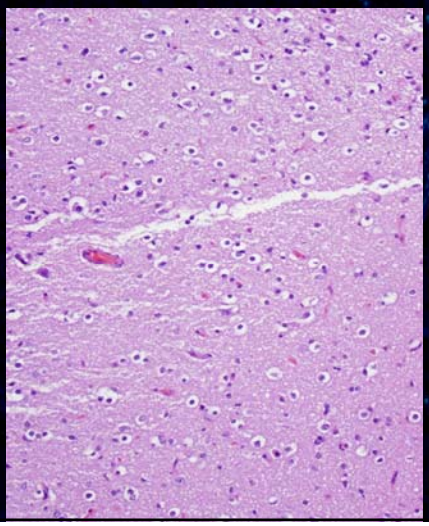

Oligo v Astro
IDH1 +
P53 -
IDH1 +
P53 +

Oligodendroglioma v mimics

Oligodendroglioma

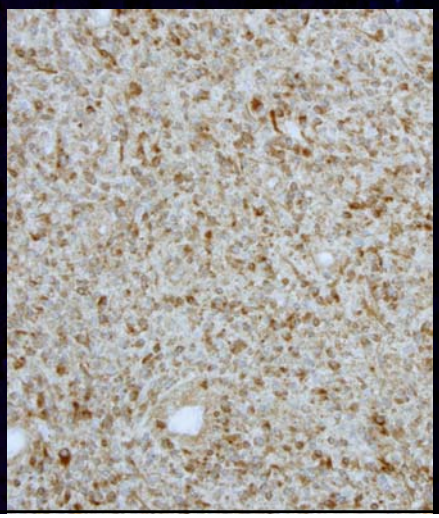


DNT

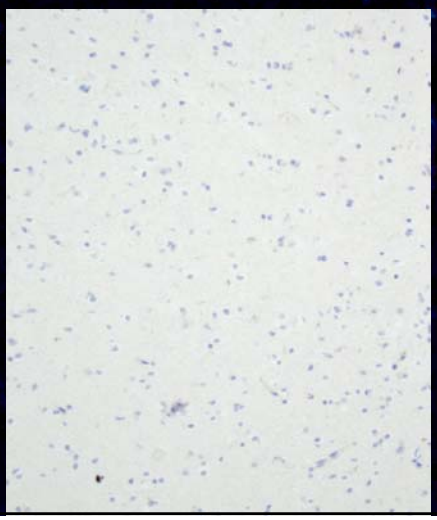


Oligodendroglioma v mimics

Oligodendroglioma



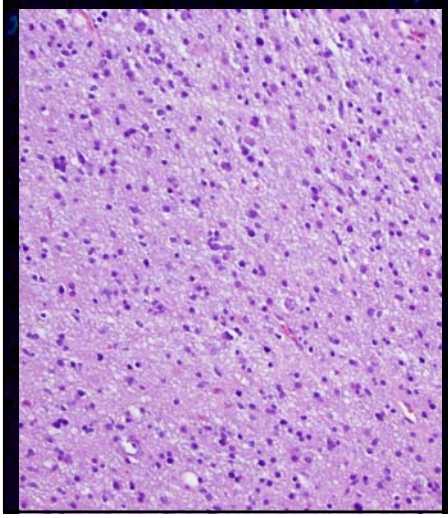
DNT

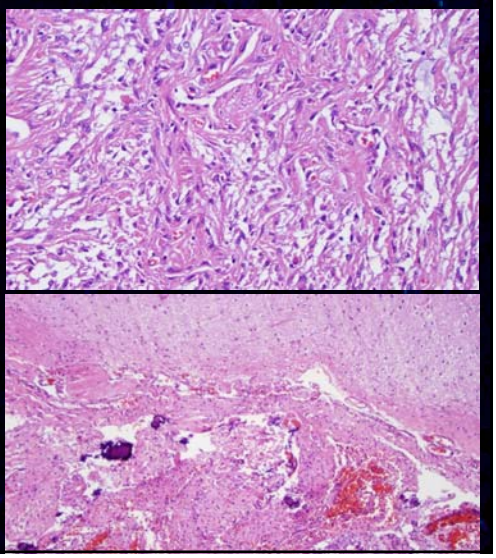


Diffuse astrocytoma v mimics

Diffuse astrocytoma

Pilocytic astrocytoma

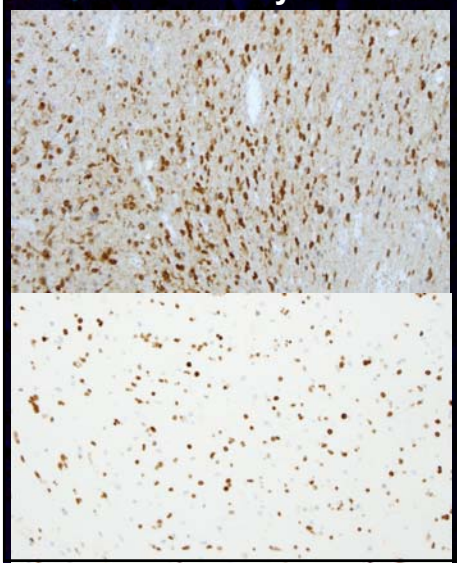


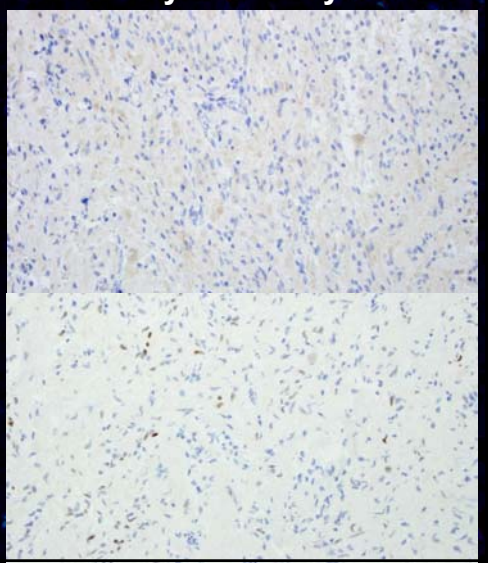


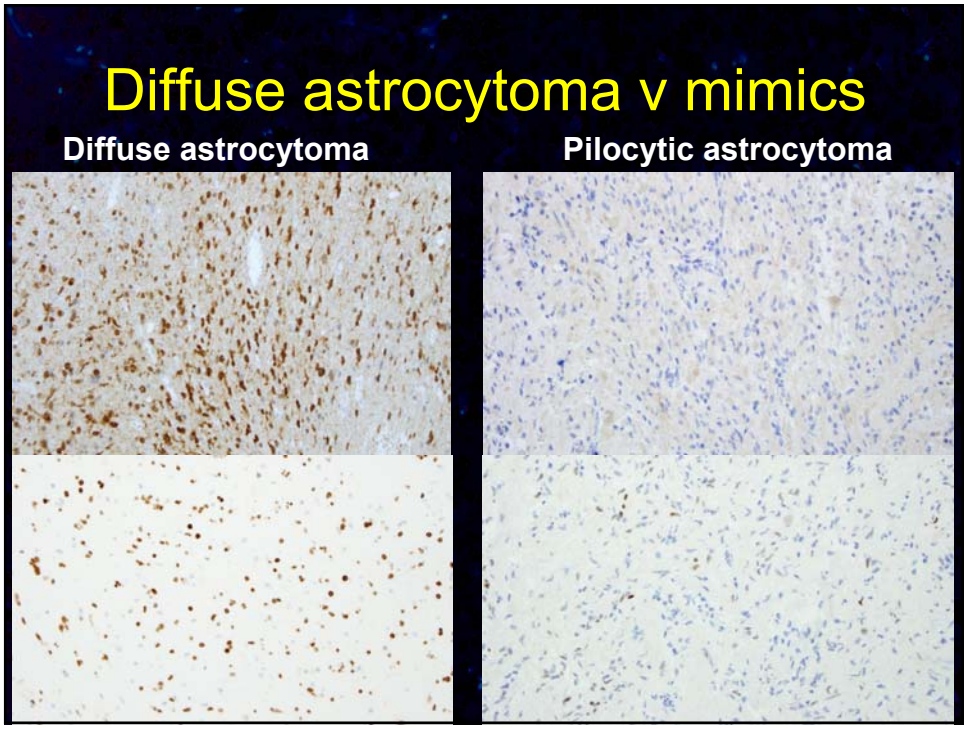
Diffuse astrocytoma v mimics

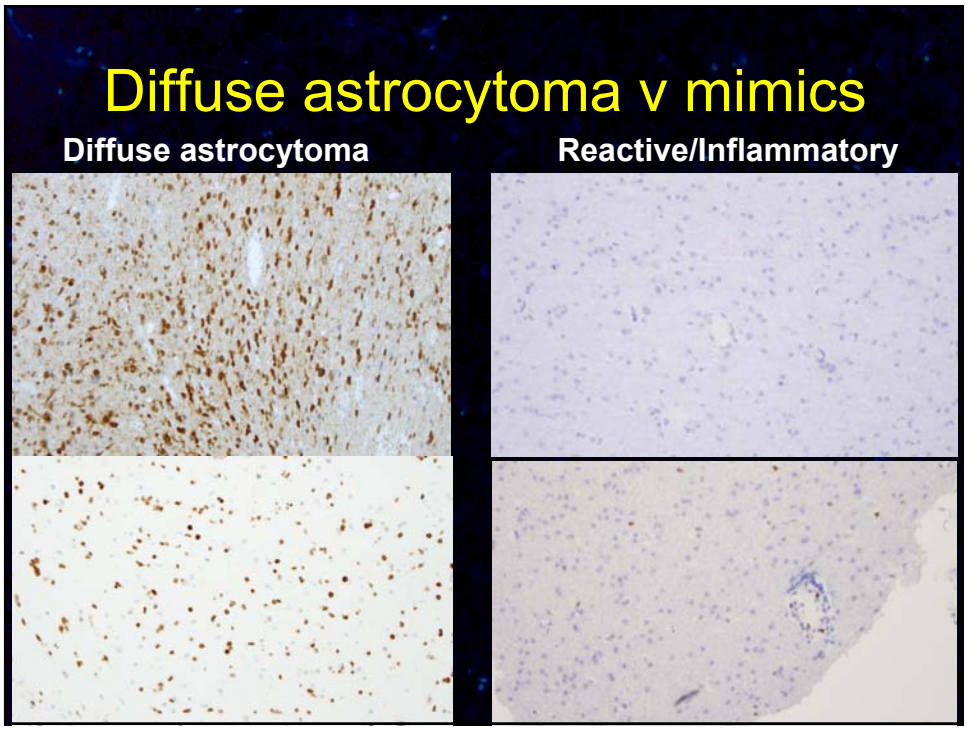
Diffuse astrocytoma

Pilocytic astrocytoma






Diffuse astrocytoma v mimics
Diffuse astrocytoma
Pilocytic astrocytoma


Diffuse astrocytoma v mimics
Diffuse astrocytoma
Reactive/Inflammatory

What we covered

- FS challenges: Artifacts & Neurosurgeons
- Smear/Crush Preparation: Friend or foe
- Glioblastoma?Abscess
- Recurrent?/Treatment?/???
- Tumor v Inflammatory (MS): Beware of Macrophages
- Meningioma/SFT/Hemangiopericytoma
- IHC: IDH1!!!