Metastasis and the Brain

Adrienne Boire, M.D., Ph.D.

Department of Neurology, Human Oncology and Pathogenesis Program,

Brain Tumor Center

Memorial Sloan Kettering Cancer Center

11 April 2024

Agenda

Part I:

Introduction to CNS metastasis
- Anatomy of CNS metastases
- Clinical scope of the problem
- Clinical management of CNS metastases

Current concepts and questions in CNS metastasis
- Modeling CNS metastasis
- Cancer cell evolution
- Microenvironmental selective pressure
- CNS barriers
- CNS immune surveillance

Part 2:

Paper discussion: Ma et al 2023 Nat Comm. 14(1):2632 “Type I interferon response in astrocytes promotes brain metastasis by enhancing monocytic myeloid cell recruitment”
- Student introduction
- Student presentation of paper
- Discussion

Novel work in CNS metastasis
- Adaptation of neuroscience techniques and approaches to CNS mets
- Liquid biopsy in CNS mets
- Microenvironmental-based therapeutic approaches
- “Omics” limitations and opportunities in CNS mets

Final Q&A, Discussion

Memorial Sloan Kettering Cancer Center

Central Nervous System Metastases:
An understudied problem

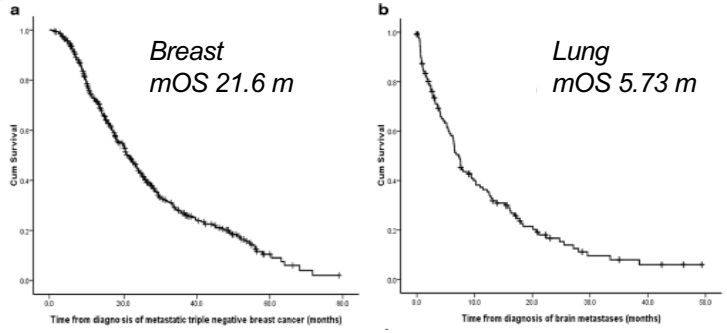
A

CNS Metastasis at Autopsy*

Site	Incidence
Intracranial	24%
Dural	20%
Leptomeningeal	8%
Brain Parenchyma	15%

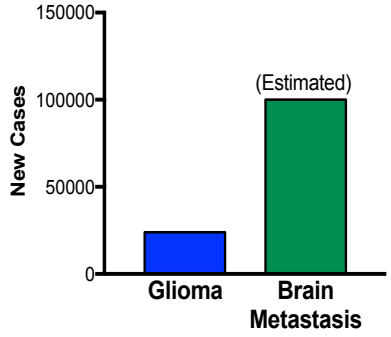
B

Survival**



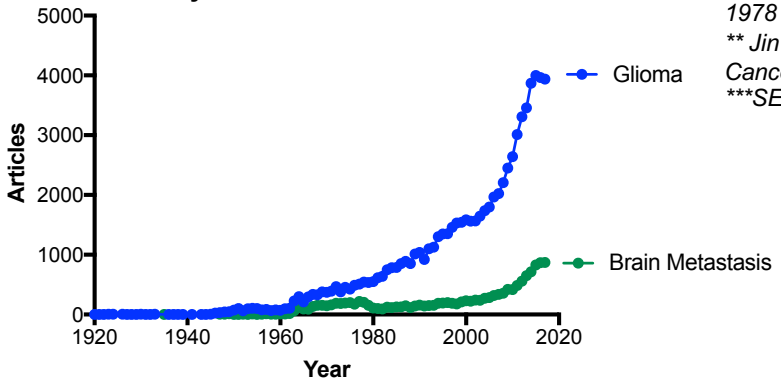
C

New Cases US 2017***



D

Primary Research Articles: PubMed



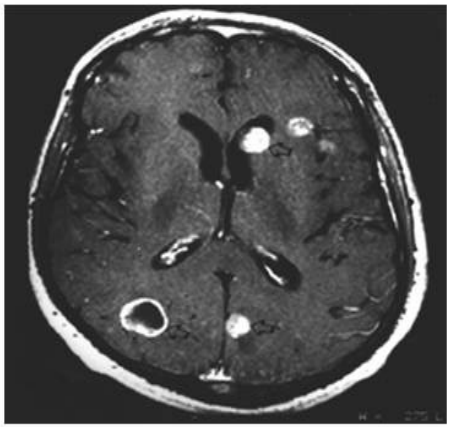
**Posner JB and NL Chernik 1978 Adv Neurol 19: 575–587*
*** Jin J et al 2018 BMC Cancer 18:446*
****SEER Query July 29, 2018*

Memorial Sloan Kettering Cancer Center

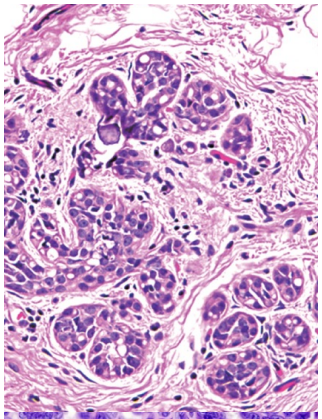
Incidence of brain metastasis is increasing

Incidence of brain metastasis is increasing:

- Improvements in imaging (e.g. MRI)
- Improvements in systemic cancer control (brain is a sanctuary site)
- Clinical Trial Pre-Screening
- Rates of brain metastasis may be influenced by changing treatments
  - 25% of lung cancer patients have brain metastasis at initial presentation
  - 80% of lung cancer patients surviving >2 years will develop brain metastases



Primary Tumors Resulting in CNS Metastases:



Non-Small Cell Lung Cancer

Small Cell Lung Cancer

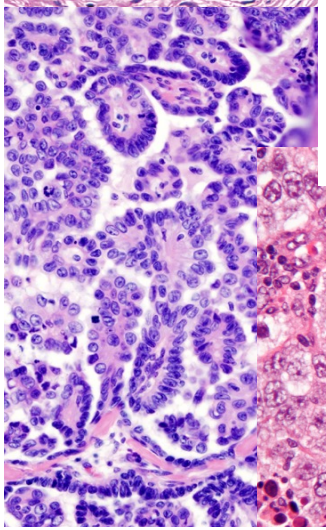
Breast Cancer

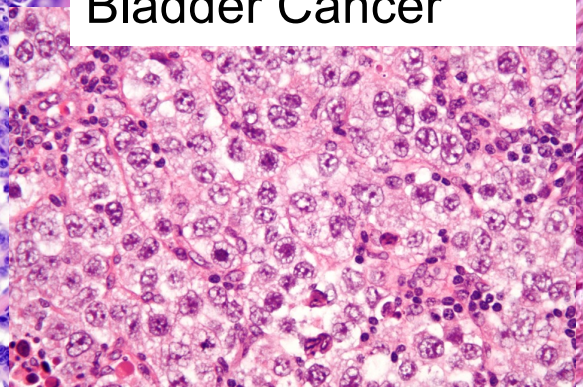
Melanoma

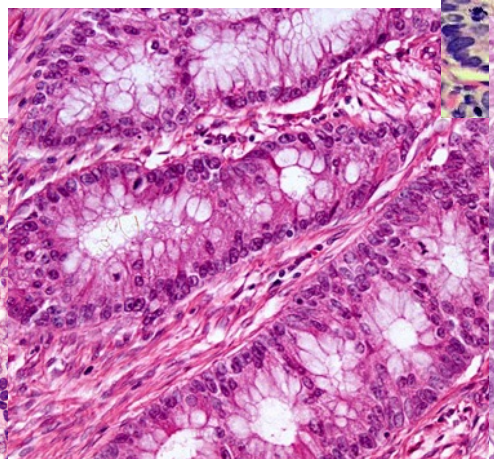
Genito-urinary Cancer

Renal cell carcinoma

Bladder Cancer







Bladder Cancer

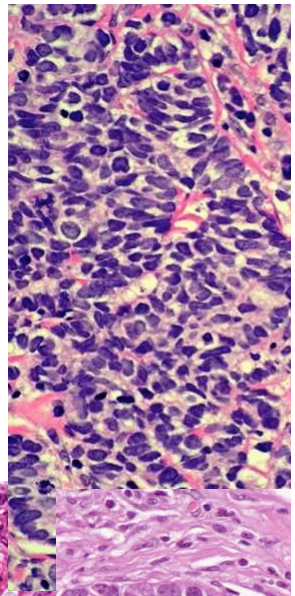
Prostate

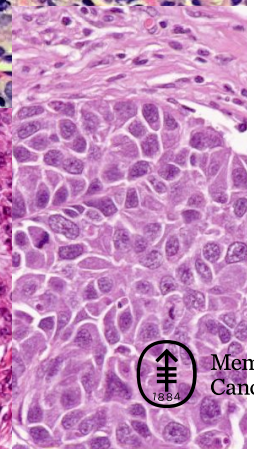
Testicular

Uterine

Ovarian

Gastrointestinal





Memorial Sloan Kettering Cancer Center

Why do I need to know clinical information?

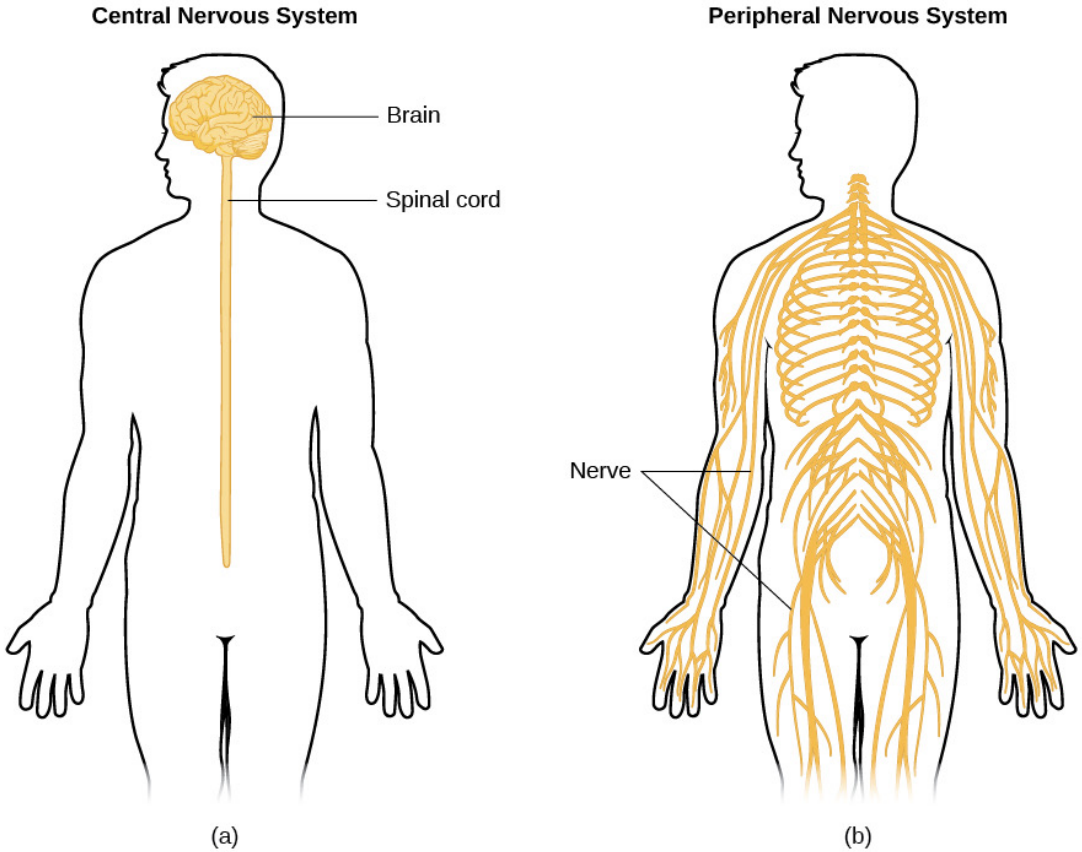
Understand the clinical samples that are (and are NOT) available.

Gain access to clinical literature, datasets

View clinical presentations with a mechanistic eye

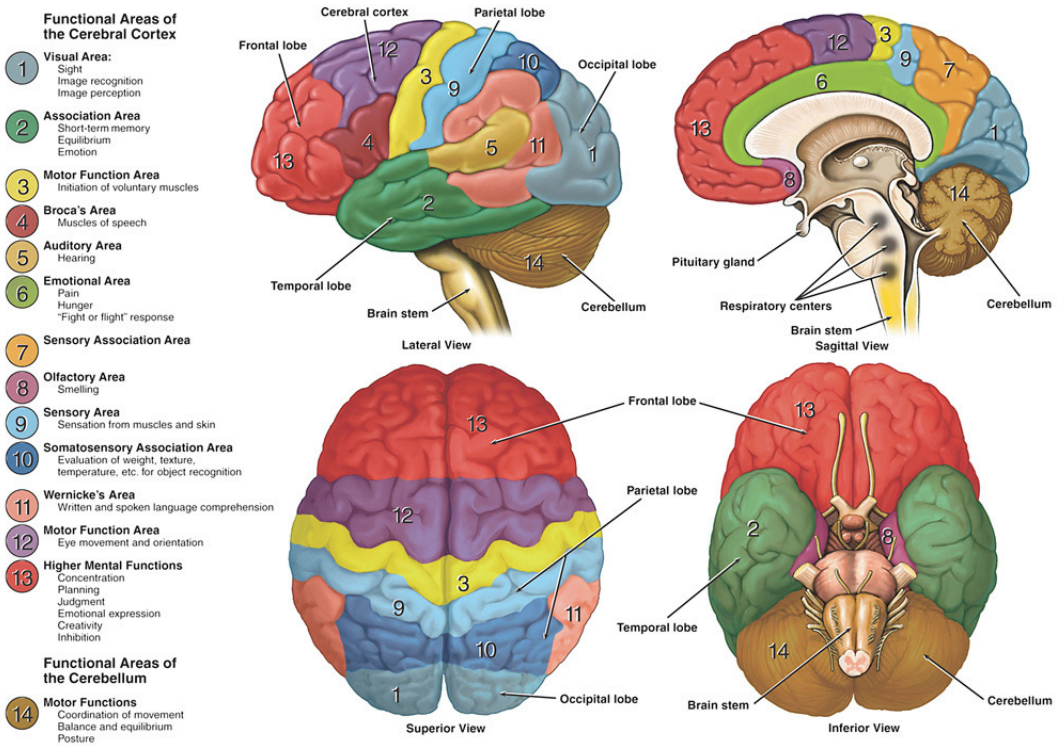
New territories for bench research

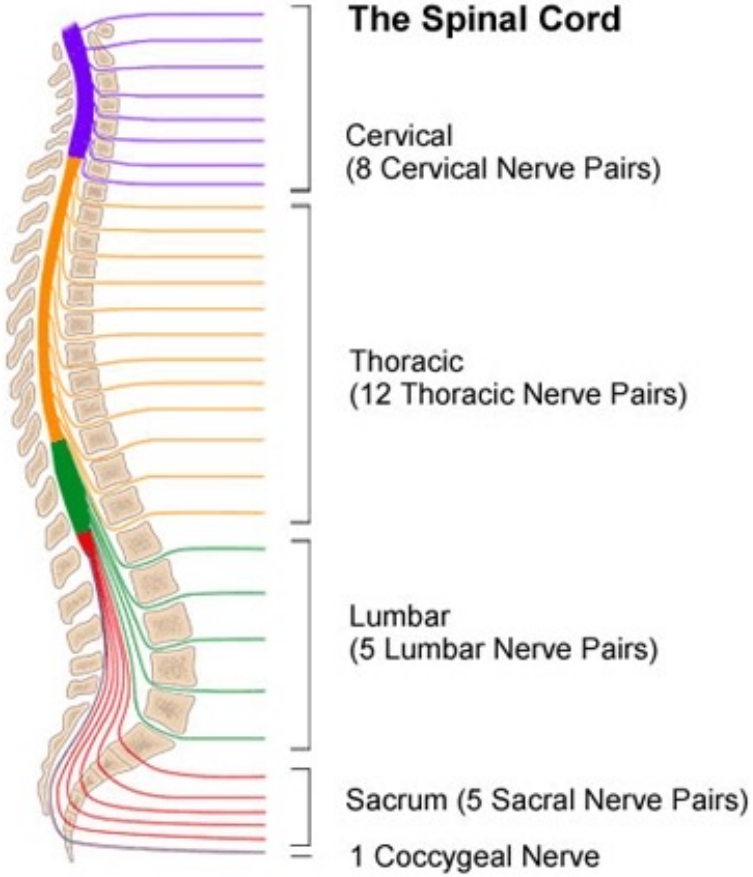
Anatomy of CNS Metastases: central nervous system



Memorial Sloan Kettering Cancer Center

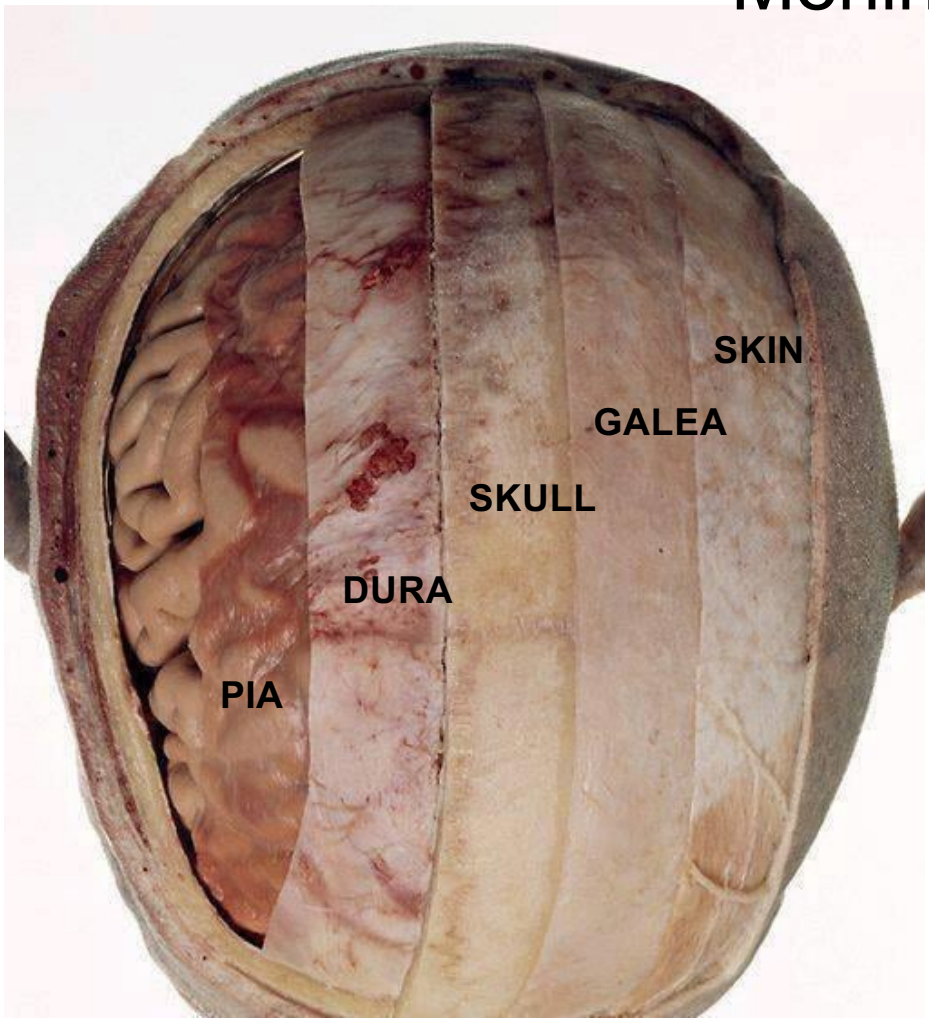
Brain, Spinal Cord

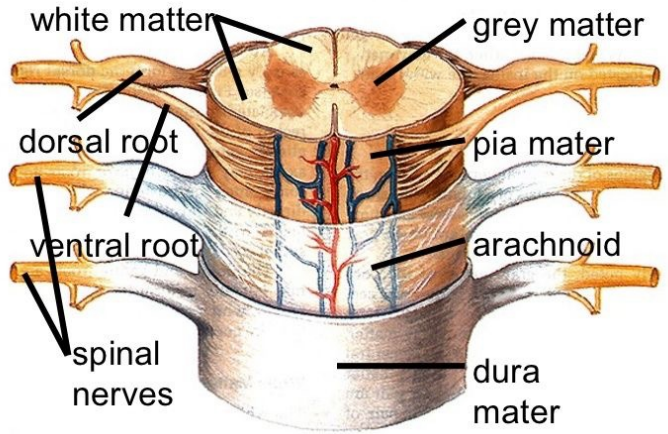


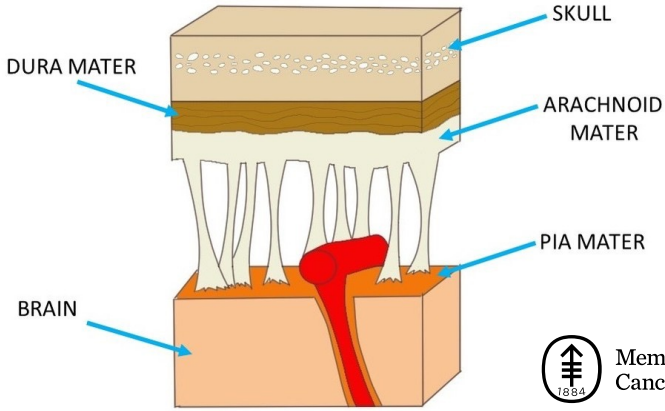


Memorial Sloan Kettering Cancer Center

Meninges

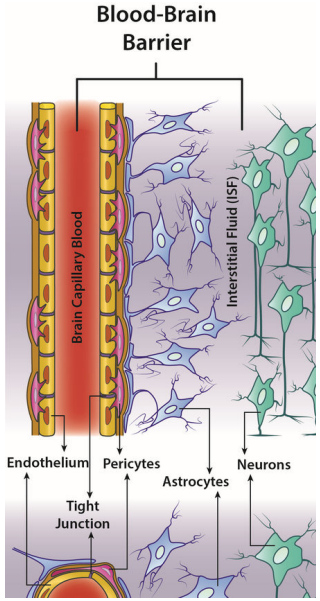


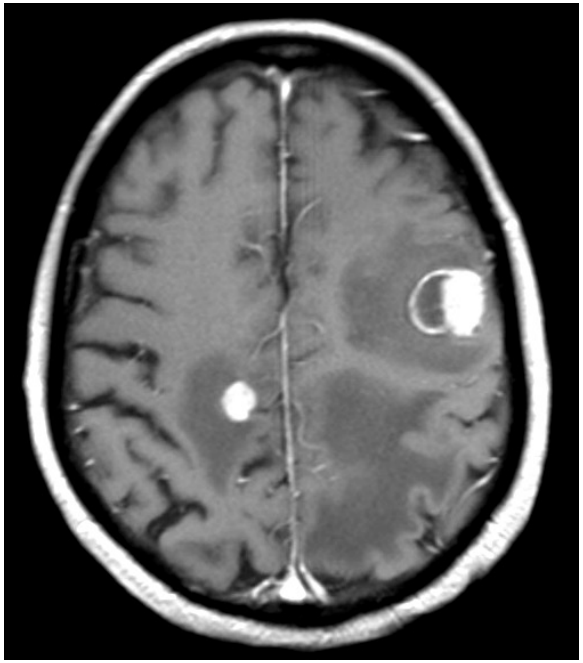


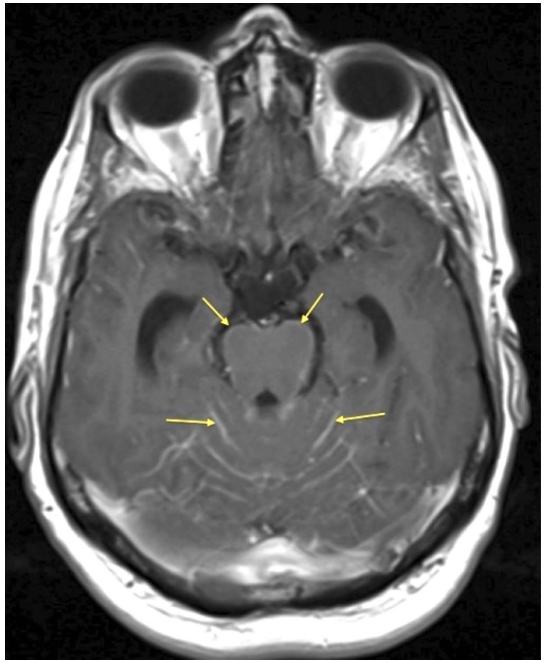


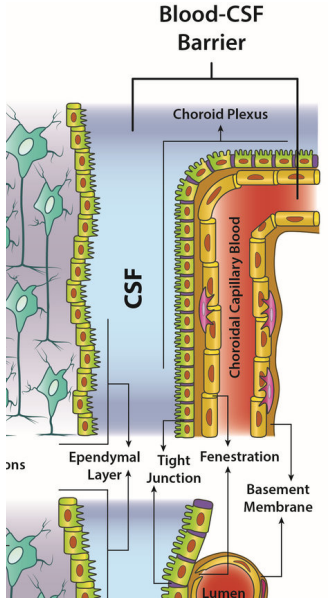
Memorial Sloan Kettering Cancer Center

Metastasis to the CNS reflect microenvironments in the CNS







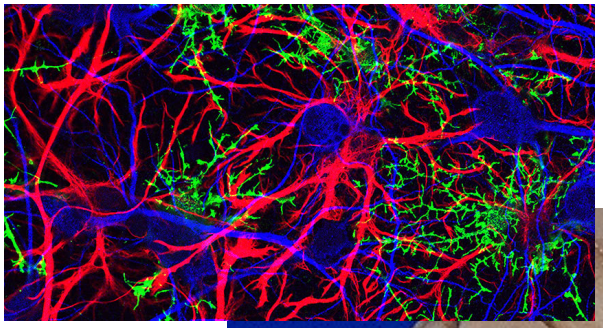


D'Agata F et al 2017 Molecules 23:9

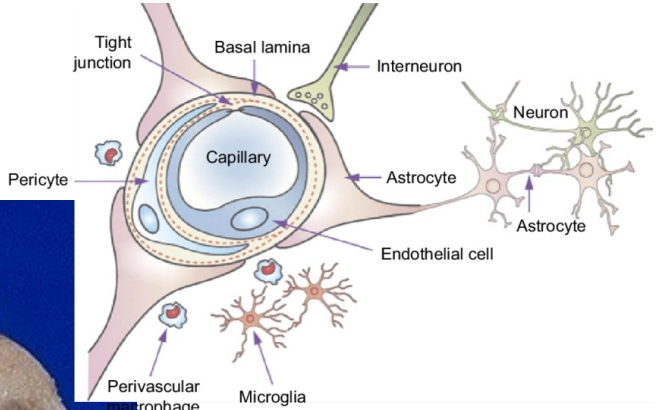
Parenchymal *Leptomeningeal*

Memorial Sloan Kettering Cancer Center

Parenchymal Space

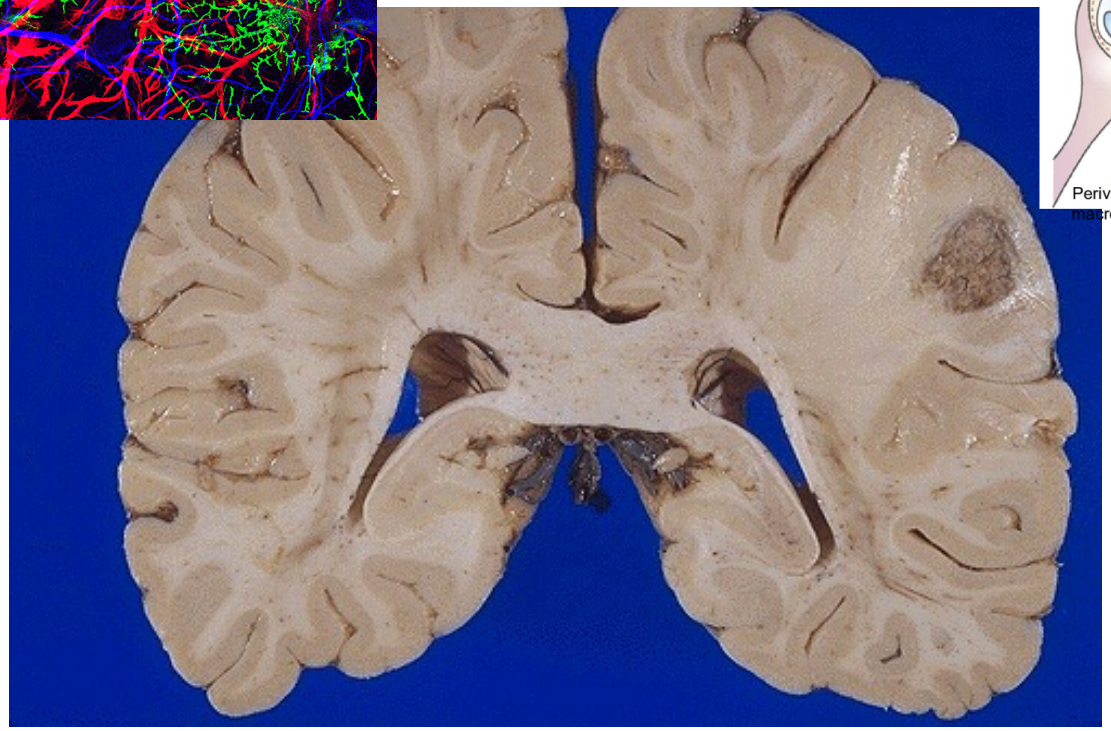


Electrically active
Tightly regulated blood flow



Densely cellular:
Astrocytes
Oligodendrocytes
Microglia
Neurons
Endothelium
Pericytes

Unique players



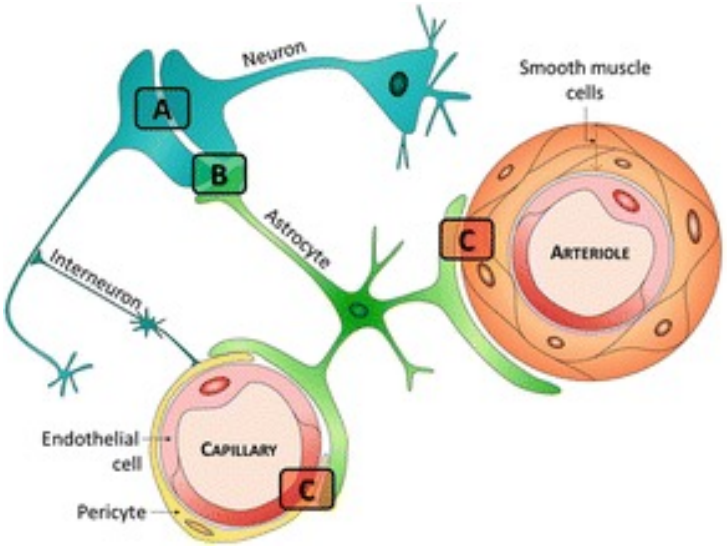
Entry into the space
is *tightly* regulated:

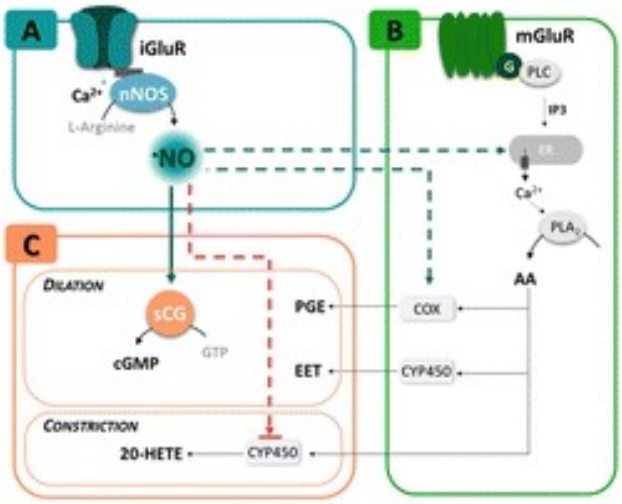
Immune cells
Rx
Metabolites

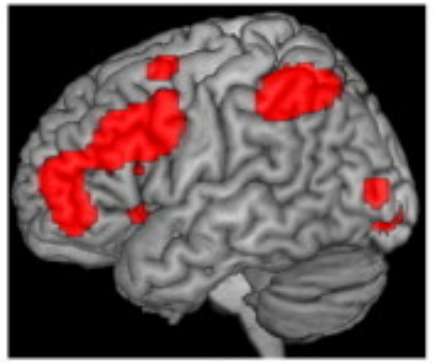
Blood-Brain-Barrier

Memorial Sloan Kettering Cancer Center

Neuro-vascular Coupling: Unique to the Brain



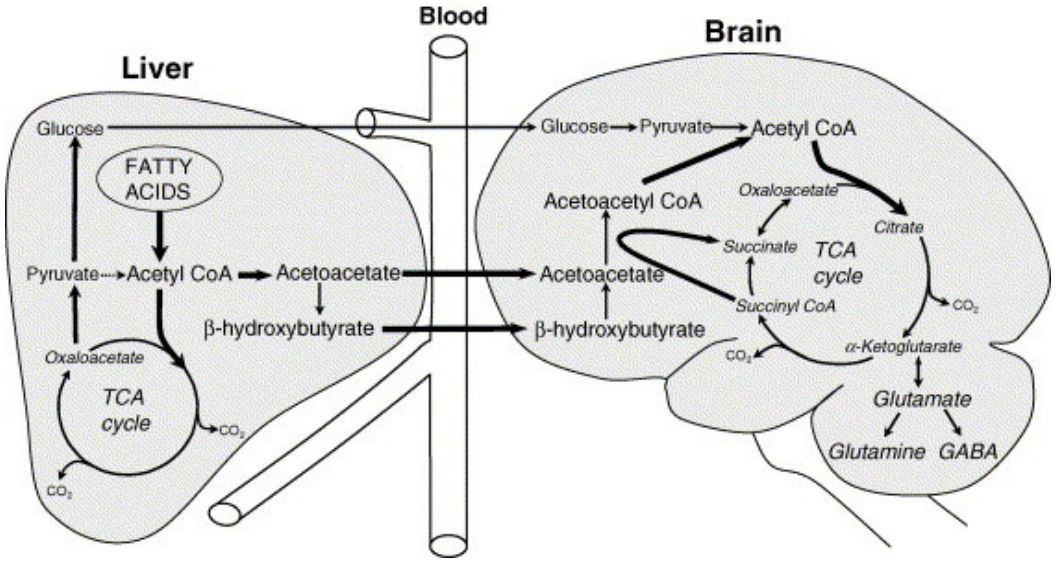




- More neural activity, more blood flow needed
- State-dependent blood flow
- Basis for fMRI/BOLD signal

Memorial Sloan Kettering Cancer Center

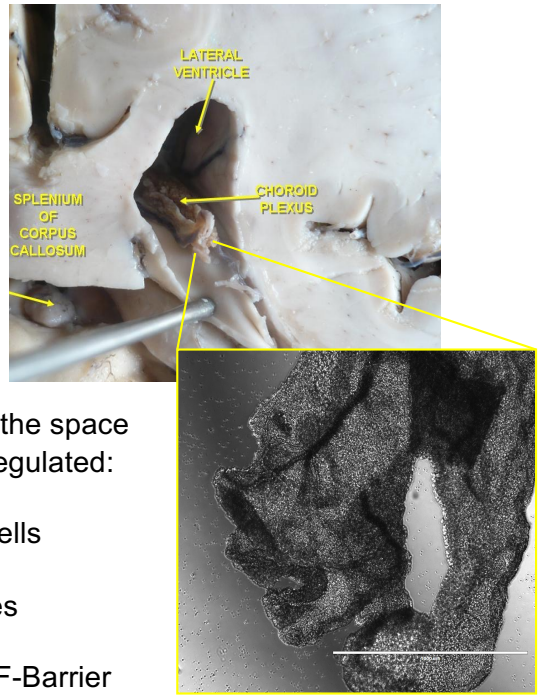
Energy Metabolism: Unique to the Brain



F-18 FDG PET

Recall: Glucose or keytone bodies for fuel; no gluconeogenesis
Recall: Glucose transport is HIGHLY regulated in CNS

Leptomeningeal Space

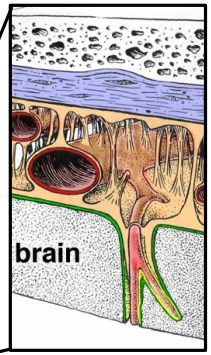


Entry into the space is *tightly* regulated:

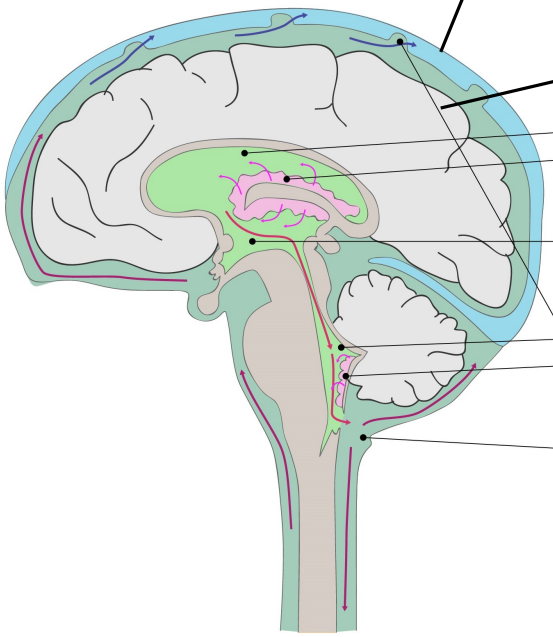
Immune cells
Rx
Metabolites

Blood-CSF-Barrier

Fluid-filled space (CSF)
Pauci-cellular
Minimal Nutrients



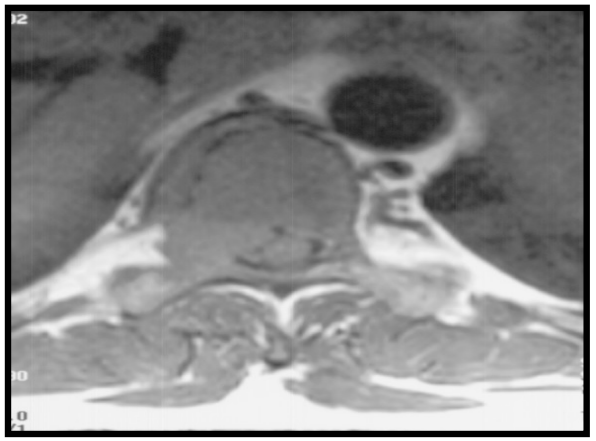
Virchow-Robin Spaces:
Potential spaces
Extending into cortex (but not beyond)
CSF-containing in pathologic contexts



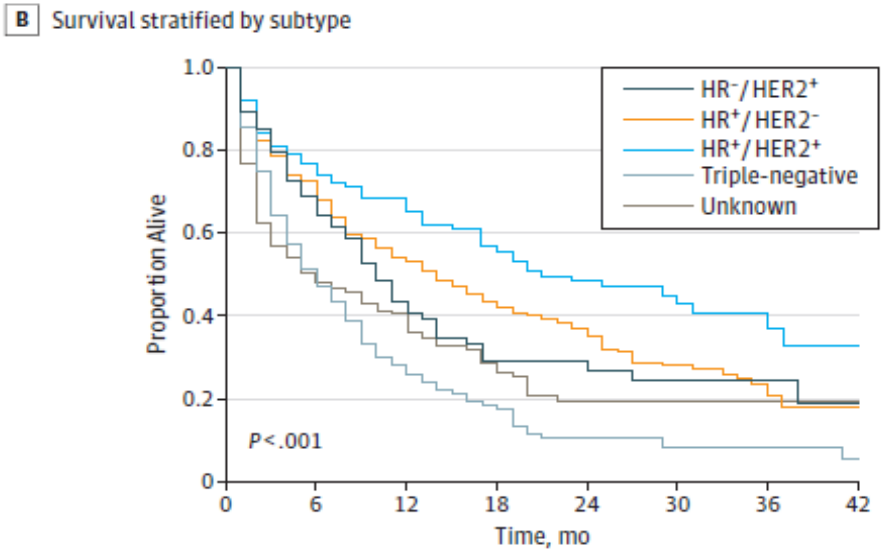
lateral ventricles
plexus choroideus
foramina Monroi
third ventricle
aquaeductus Sylvii
fourth ventricle
plexus choroideus
foramen Magendii
foramina Luschkae
subarachnoid space
arachnoid granulations
venous sinuses

Spinal Metastases

- Spinal tumors account for 15% of all central nervous system neoplasms with most being from metastases
- 70% of bone metastases are in the spine (secondary to the amount of bone marrow)
- 70,000 cases per year
- Pain is a common presentation, but cord compression may be seen and can present with neurological deficits
- Common histologies include breast, lung, **prostate, thyroid, and renal cell.**



Brain Metastases in Newly Diagnosed Breast Cancer: A Population-Based Study



No. at risk

HR⁻/HER2⁺	95	56	32	19	15	9	4	3
HR⁺/HER2⁻	328	196	121	78	54	33	17	5
HR⁺/HER2⁺	127	85	68	50	39	21	12	6
Triple-negative	153	67	34	20	10	6	4	2
Unknown	145	66	43	27	13	10	8	3

Memorial Sloan Kettering Cancer Center

M Ahluwalia, Cleveland Clinic

Primaries resulting in Leptomeningeal Metastasis: MSKCC

Patients seen at MSKCC ('75-'78) with Lepto (n = 140)

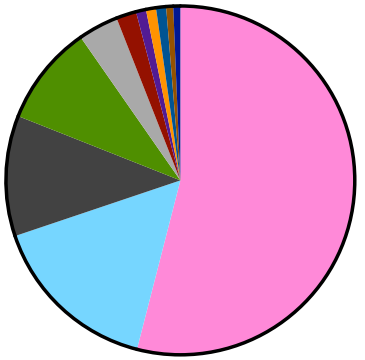
Primary Tumor	Number of Patients
Breast	81 (58)
Lung	24 (17)
Melanoma	17 (12)
Lymphoma	14 (10)
Unknown Primary	5 (4)
Renal	3 (2)
Prostate	2 (1)
Pancreas	2 (1)
Sarcoma	2 (1)
Nasopharynx	1 (0.7)
Esthesioneuroblastoma	1 (0.7)

Autopsy series 1978

Primary Tumor	Autopsies	Number with Lepto (%)
Leukemia	287	28 (10)
Lymphoma	309	15 (4)
Breast	324	11 (3)
Melanoma	125	6 (5)
Lung	297	4 (1)
Gastro-intestinal	311	3 (1)
Sarcoma	126	1 (1)

Retrospective Series 2016

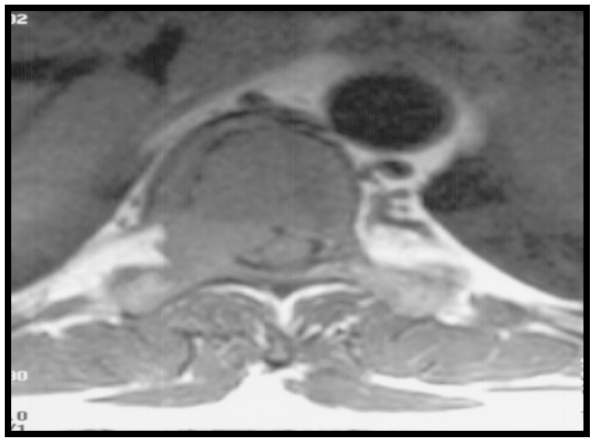
Primary Tumor	**Incidence**
Breast	58 %
Lung	17 %
Melanoma	12 %
Lymphoma	10 %
Unknown Primary	4 %
Renal	2 %
Prostate	1 %
Pancreas	1 %
Sarcoma	1 %
Nasopharynx	0.7 %
Neuroblastoma	0.7 %



Memorial Sloan Kettering Cancer Center

Spinal Metastases

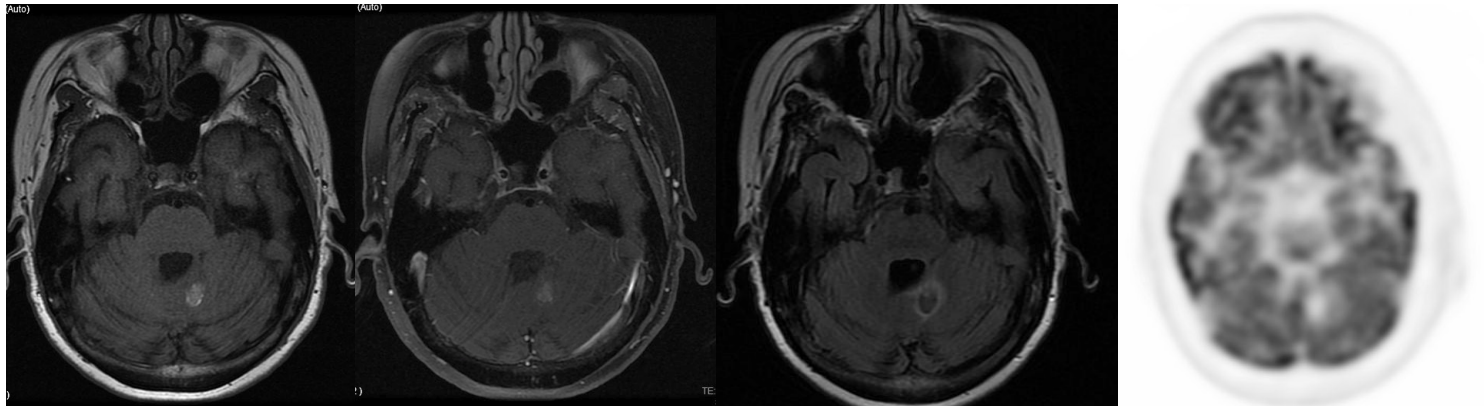
- Spinal tumors account for 15% of all central nervous system neoplasms with most being from metastases
- 70% of bone metastases are in the spine (secondary to the amount of bone marrow)
- 70,000 cases per year
- Pain is a common presentation, but cord compression may be seen and can present with neurological deficits
- Common histologies include breast, lung, **prostate, thyroid, and renal cell.**



Memorial Sloan Kettering Cancer Center

Brain Metastasis Diagnosis

68 yo RH woman with ER/PR +/+ breast cancer, NED x 5 y, presents with sudden onset projectile emesis.



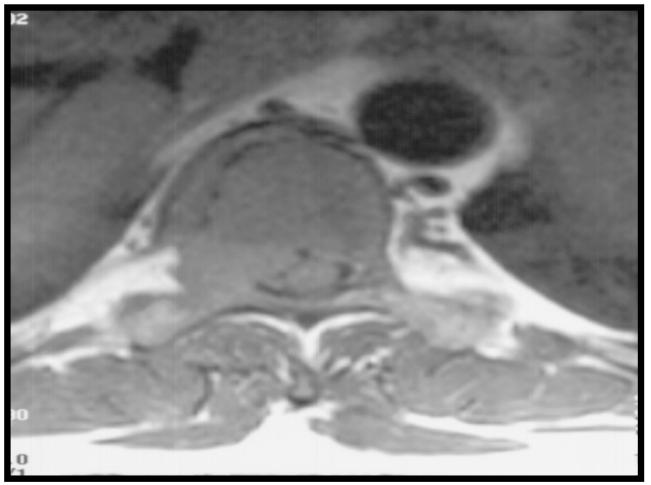
T1 Pre | T1 Post | FLAIR | PET

AVM not Metastasis!

Memorial Sloan Kettering Cancer Center

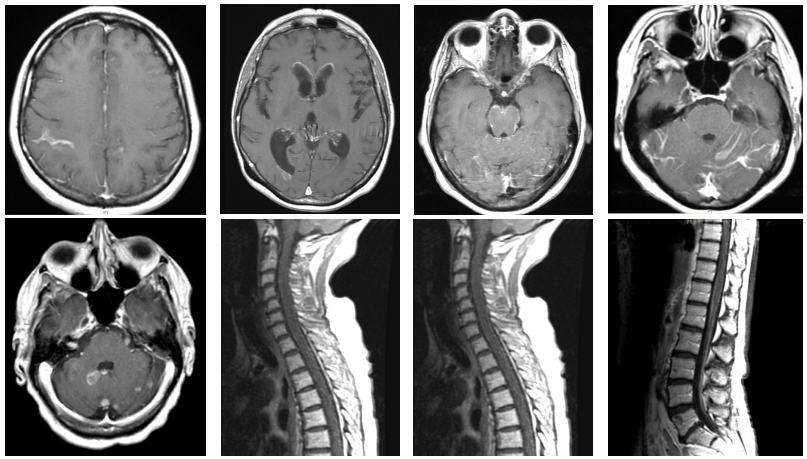
Spine Metastases

- Workup includes plain films, bone scan, CT, or MRI
- MRI is the best to visualize bone marrow involvement, visualize soft tissue extension, and evaluate cord/nerve root compression
- Location
  - Bony spine
  - Dura / Leptomeningeal disease
  - Spinal cord (rare)



LM Diagnosis

Neuroaxis imaging + CSF Examination = Complete LMD staging



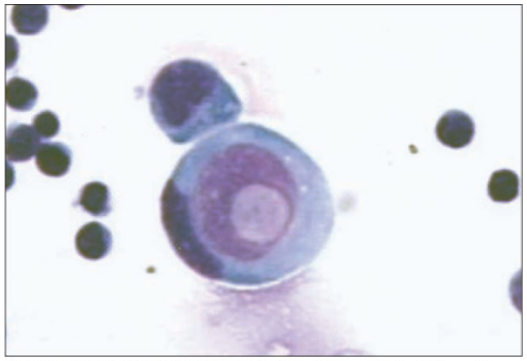
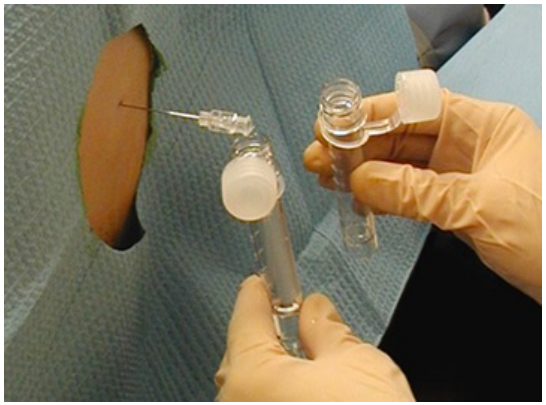


Fig 1. CSF, breast adenocarcinoma cell, Light microscopy (May-Grünwald/ Giensa stain, 1,000X). Presence of degenerative vacuoles in the cytoplasm, the nucleus has hyperchromasia. The cell in the center is phagocyting other cell (cell autophagy). There are normal lymphocytes around the malignant cell (note the difference in size between the cells).

Almeida SM et al 2007 Arq. Neuro-Psquiatr65(3):1678

CSF Sampling

- Where?
  - Bedside LP is usually fastest, easiest.
  - Fluro does not give accurate OP.
- When?
  - After MRI is best, but not essential.
  - During the week, anytime.
  - On the weekend for symptomatic relief
- Send What?
  - Cytology
  - Circulating tumor markers
  - Circulating tumor cells (rare cell capture technology) cancer centers only
  - Research tests?
- Again?!?
  - If negative, may repeat in two weeks
  - If negative, may repeat in two weeks at a different site (C-tap).
- Risks?
  - What are you worried about?
  - What is the patient worried about?



Goals of Treatment

- Control
  - Macroscopic disease
  - Microscopic disease
  - Systemic disease
- Preserve
  - Neurologic function
  - QOL

Caring for patients with CNS metastases combines palliative care with tumor-directed treatments.

Symptomatic lesions drive initial treatment:

Neurologic Exam is essential

1. Localize symptoms

Diplopia, Urinary retention, Weakness:

Q. *localizable to a radiographic lesion?*

2. Establish ICP & Manage

Fundoscopic exam, Direct Measurement by LP for LMD or suspicious exam

3. Stop Seizures

Plaques of LMD, cortical parenchymal lesions, bleeding lesions may be epileptogenic,

Loading dose AED followed by maintenance dose (LEV, PHT)

May need cEEG

4. Local Treatment

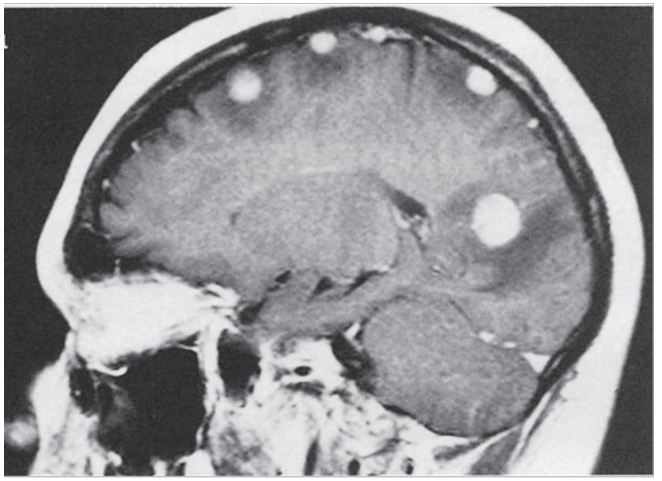
(Treat the tumor you see, the symptomatic sites)

5. Systemic Treatment

(Treat the tumor you don't see)

Surgical Management of Brain Metastasis:

- Symptomatic, accessible:
  - Resect
- 1-3 surgically accessible metastases:
  - Resect.
  - Improves survival by 1-4 mo (depending on study).
- Surgically Accessible, Brain is *only site of active disease*:
  - Biopsy vs. resect



Neurologic Exam and Localization is Essential.

Symptomatic, Inaccessible lesions: RT alone

1. RT to symptomatic lesion(s)

SRS or IMRT: Outpatient

If ICP is a concern, in-house, possibly post VPS

If urinary retention +/or new leg weakness, in-house

2. Steroid pulse with RT: Especially if large territory

WBRT, RT to post fossa or C-T spine

Dexamethasone 4 then 2 BID.

3. Consider bevacizumab

Steroid-sparing agent

Re-RT

Involved field adjacent to previously treated

Consider possible/probable trips to OR

After local, symptomatic treatment, Systemic treatment

(For patients with "good" KPS)

1. Systemic

Enhancing disease

Treatment with good CSF penetration

2. Intrathecal: Leptomeningeal Metastasis only*

MRI negative (or nearly neg) LM disease. Normal ICP, CSF flow

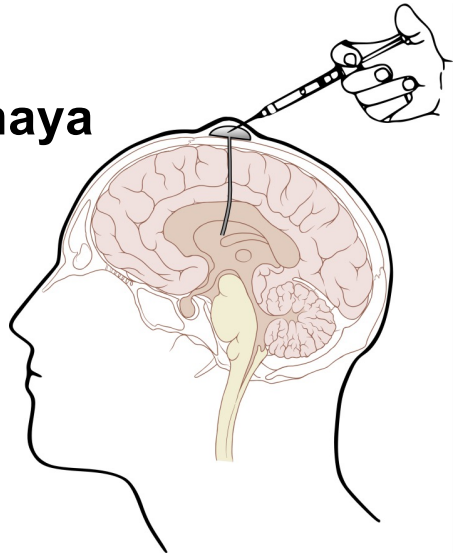
*IT/IO treatment penetration of bulky disease is poor.

3. Trial

These are becoming more common!

Intrathecal Treatment

Ommaya



***Must have good CSF Flow**

In doubt, establish with dedicated study

Access:

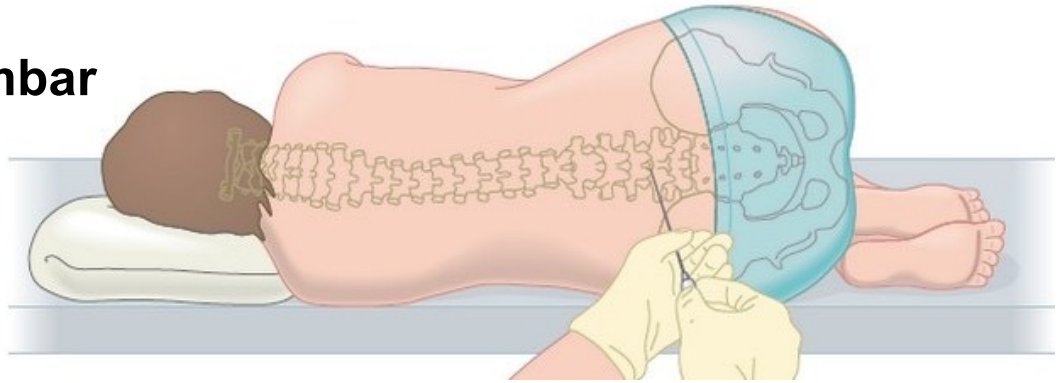
Ommaya Port:

- Access CSF, requires OR trip
- Excellent Rx circulation

Lumbar Treatment:

- No OR required
- Imperfect circulation
- Rx infiltration outside CSF 10%

Lumbar



Memorial Sloan Kettering Cancer Center

Intrathecal Treatment: Rx

Methotrexate

12 mg/dose, 2 times weekly x 1 month, then weekly x 1 month (if responsive)

Concurrent oral leucovorin 10 mg PO BID x 3 days

Cytarabine (not preferred for solid tumors; liposomal formulation no longer available)

50 mg/dose 2 times weekly x 1 month, then weekly x 1 month

Thio-TEPA

10 mg/dose 2 times weekly x 1 month, then weekly x 1 month

Investigational:

Etoposide, Dacarbazone, Busulfan, Mephelan, Topotecan…

All:

1. Measure pressure after accessing space (LP)
2. Remove equal volume CSF, + rinse
3. Instill Rx slowly, mix
4. Rinse with reserved CSF

Risks:

1. Chemical Meningitis
2. Infection
3. Transient elevated ICP

Systemic, CNS-penetrant Treatments:

EGFR
Erlotinib
Gefitinib
Osimertinib

ALK
Crizotinib
Ceritinib
Alectinib
Lorlatinib

Her2
Lapatanib
Trametinib
Enhertu

VEGF
Bevacizumab

CD20
Rituxumab

PD-1/PDL-1
Pembrolizumab
Nivolumab

MEK
Trematinib
Cobimetinib

CTLA4 MoAb
Ipilimumab

Untargeted:
HD MTX, with leucovorin rescue
HD Cytarabine

Capecitabine
Carboplatin
Temozolomide

**This list is not exclusive and is expanding

Current concepts and questions in CNS metastasis

- Modeling CNS metastasis
- CNS barriers
- Microenvironmental selective pressure
- Interactions with glia: Journal Club

How to study Metastasis

Generate Mouse Metastatic Models

↓

Discover Key Genes

↓

Find Mechanism

↓

Validate in Human Tissues

vs.

Interrogate Human Tissues

↓

Discover Key Genes

↓

Validate in Mouse Metastatic Models

↓

Find Mechanism

The ideal model

Primary tumor

Mets to CNS (+/- other sites)

Intact immune system

Easy to generate large n

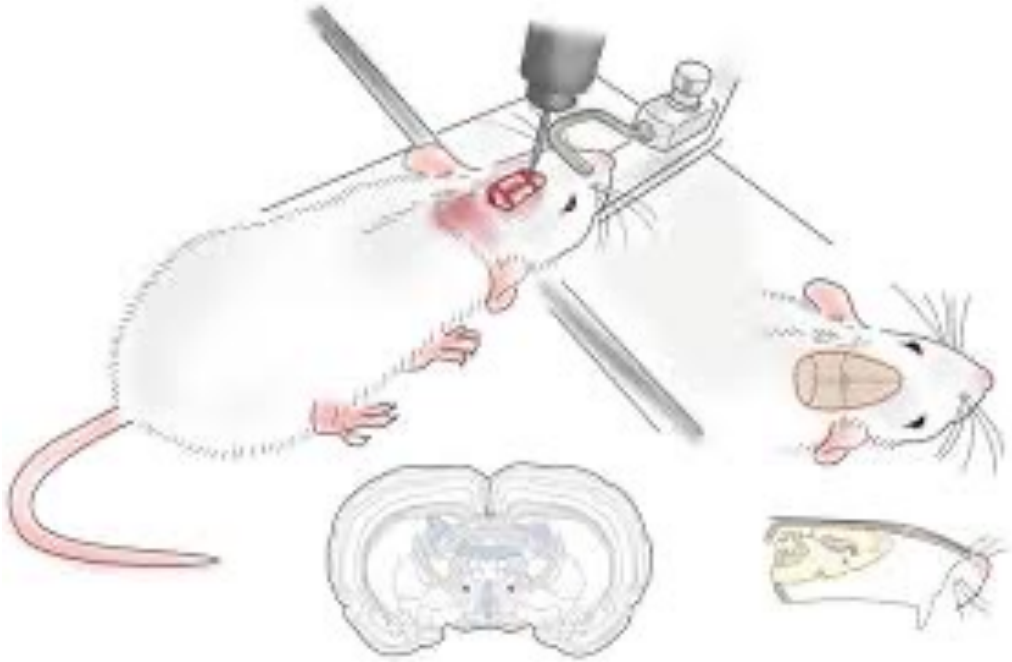
Reproducible

… does not exist

Memorial Sloan Kettering Cancer Center

Intracranial Implant

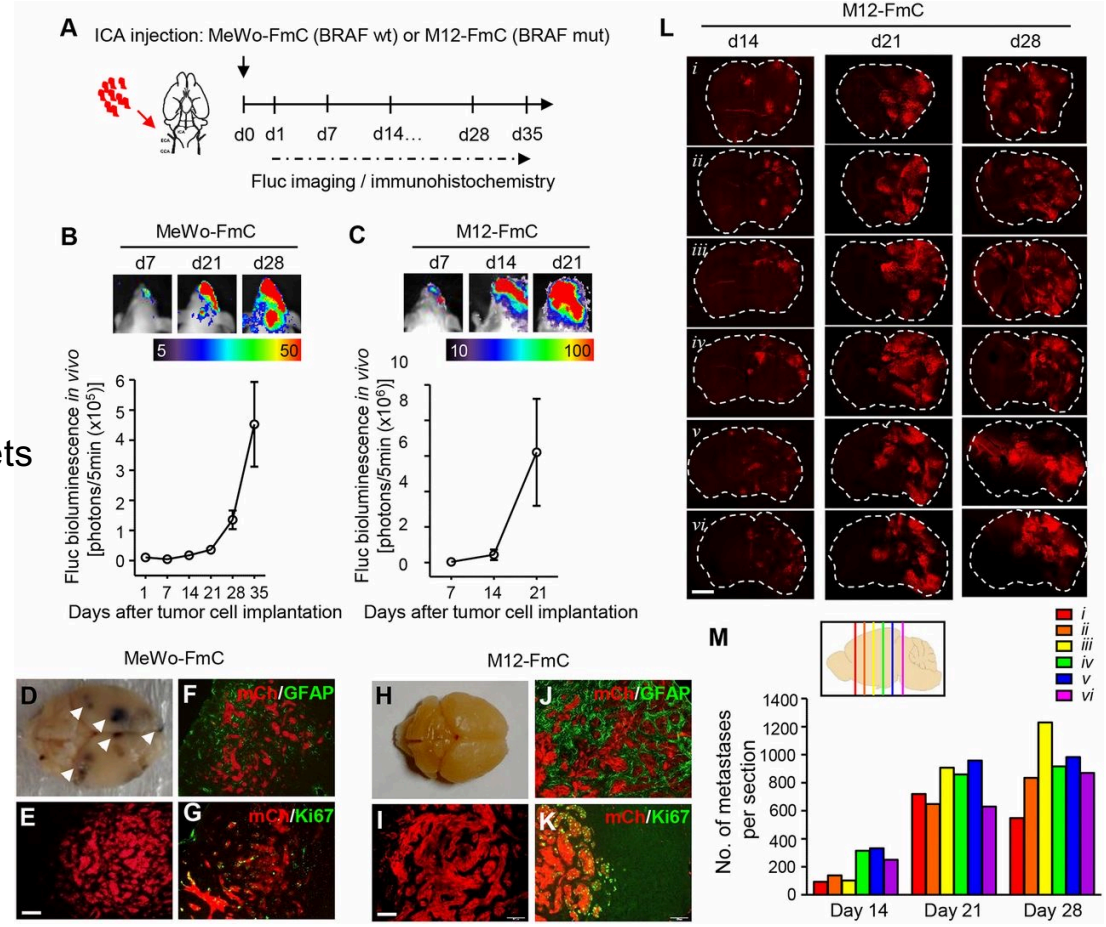
- High take rate
- Large number of cells needed
- Disturbs microenvironment
- No primary tumor
- Typically use nude mice
- Xenografts (PDX) possible



ICA Injection

(Fidler & scientific progeny)

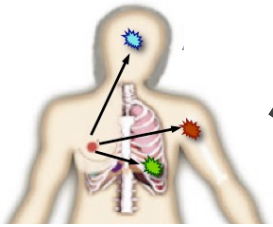
- Very likely to generate brain mets
- Nice contralateral control
- Difficult to learn, master
- Does not allow for “below the neck” mets
- No primary tumor



Memorial Sloan Kettering Cancer Center

Iterative in vivo selection

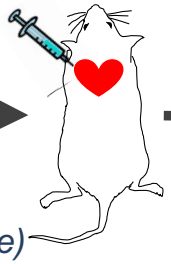
Advanced cancer patient

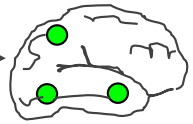


Transgenic mouse

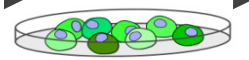
Parental cells
(GFP/Luciferase)

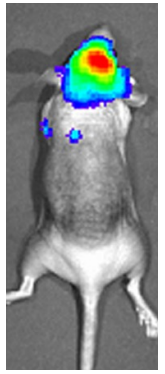
"cell sorter"



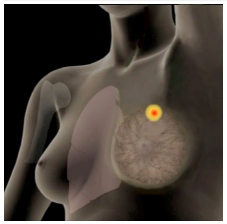


BrM





Breast Carcinoma



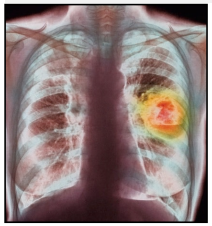
Models:

- MDA231-BrM2 (Triple negative)

MMTV-neu

- ErbB2-BrM2 (ErbB2 hyperactive)

Lung Adenocarcinoma



Models:

- H2030-BrM3 (*KRAS*^{G12C})
- PC9-BrM (*EGFR*$^{\Delta exon19}$)

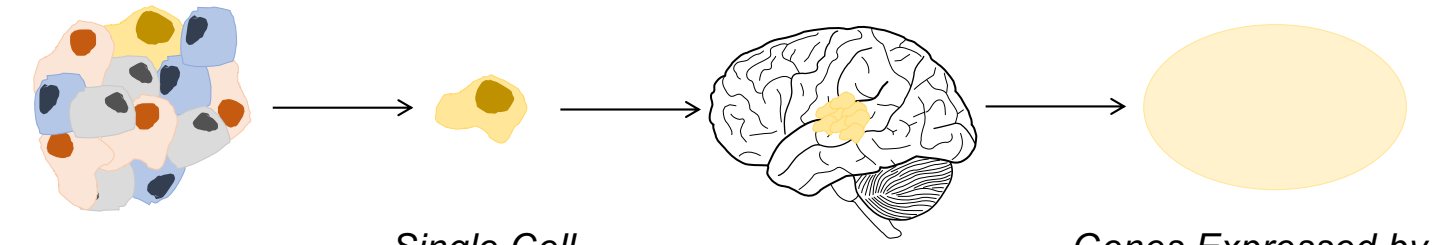
Kras^{G12D};*p53*$^{-/-}$

- High brain metastasis:
  Kras/p53-393N1
  Kras/p53-482N1
- Low brain metastasis:
  Kras/p53-373N1
  Kras/p53-2691N1

Nguyen et al *Cell* 2009
Bos et al *Nature* 2009
Valiente et al *Cell* 2014

Memorial Sloan Kettering Cancer Center

Exploit Cancer's Diversity and Adaptability



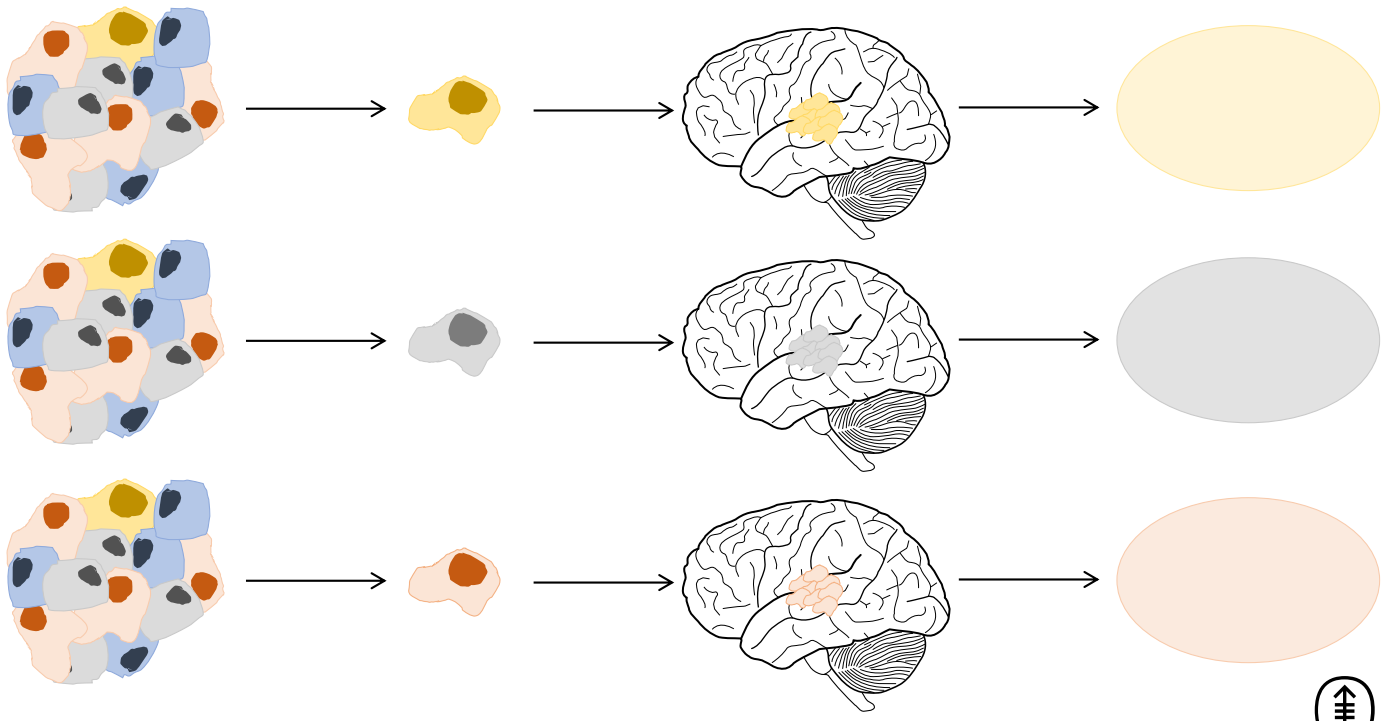
Primary Tumor

Single Cell Dissemination

Brain Metastasis

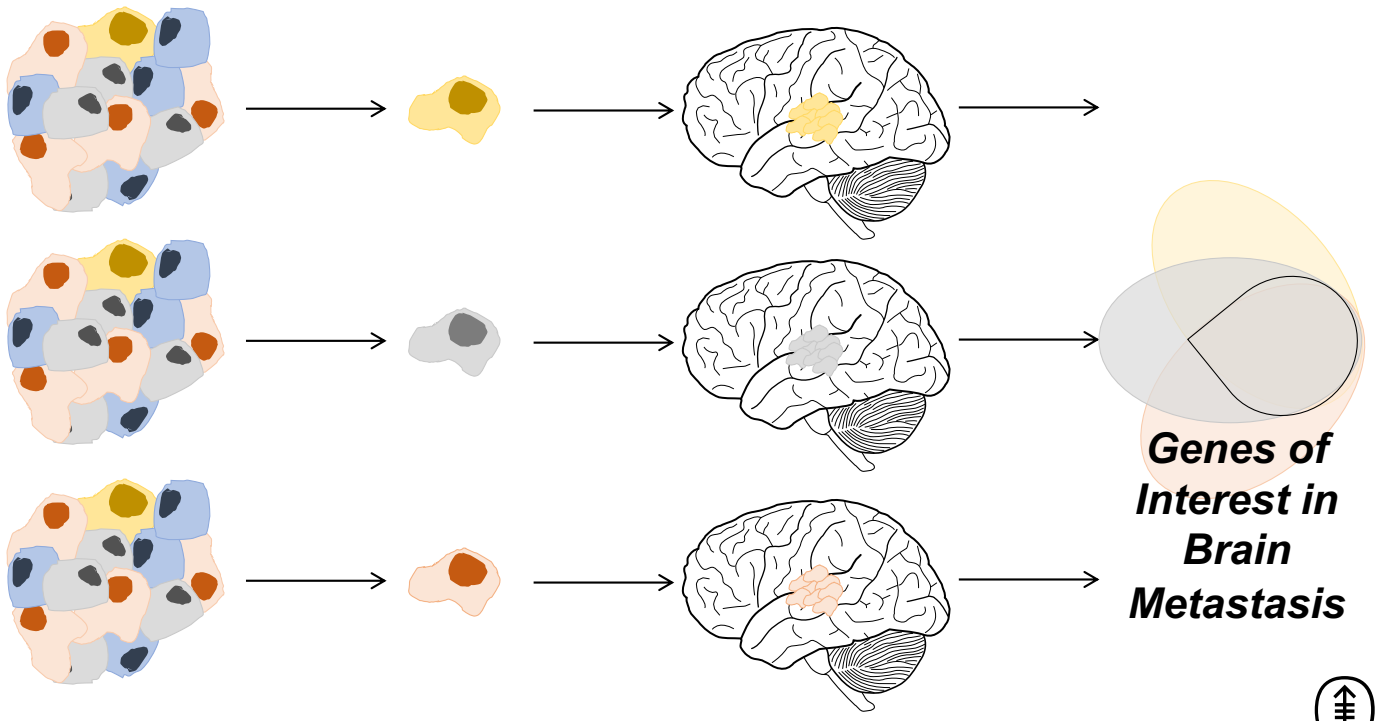
Genes Expressed by Brain Metastasis

Exploit Cancer's Diversity and Adaptability



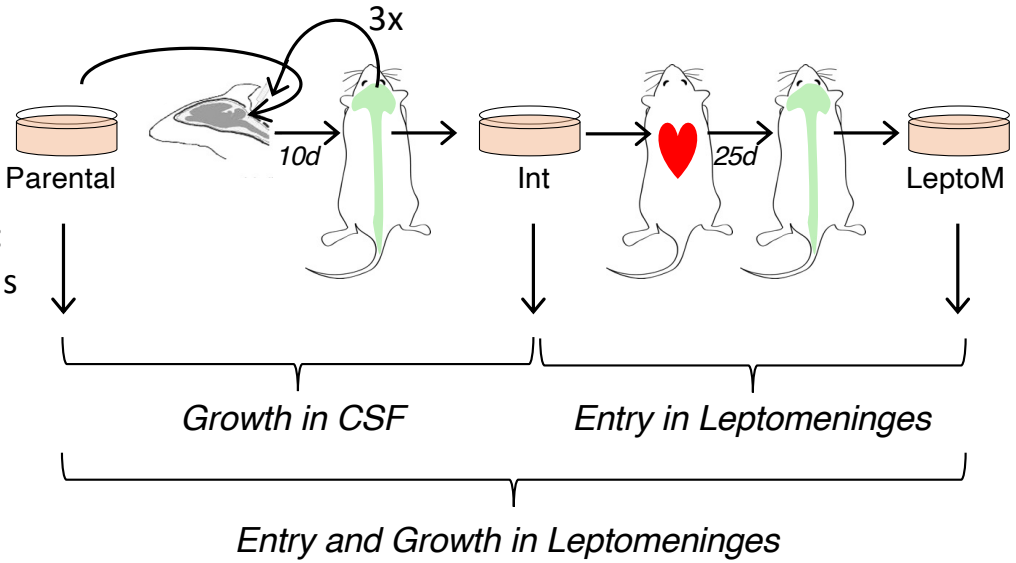
Memorial Sloan Kettering Cancer Center

Exploit Cancer's Diversity and Adaptability



Memorial Sloan Kettering Cancer Center

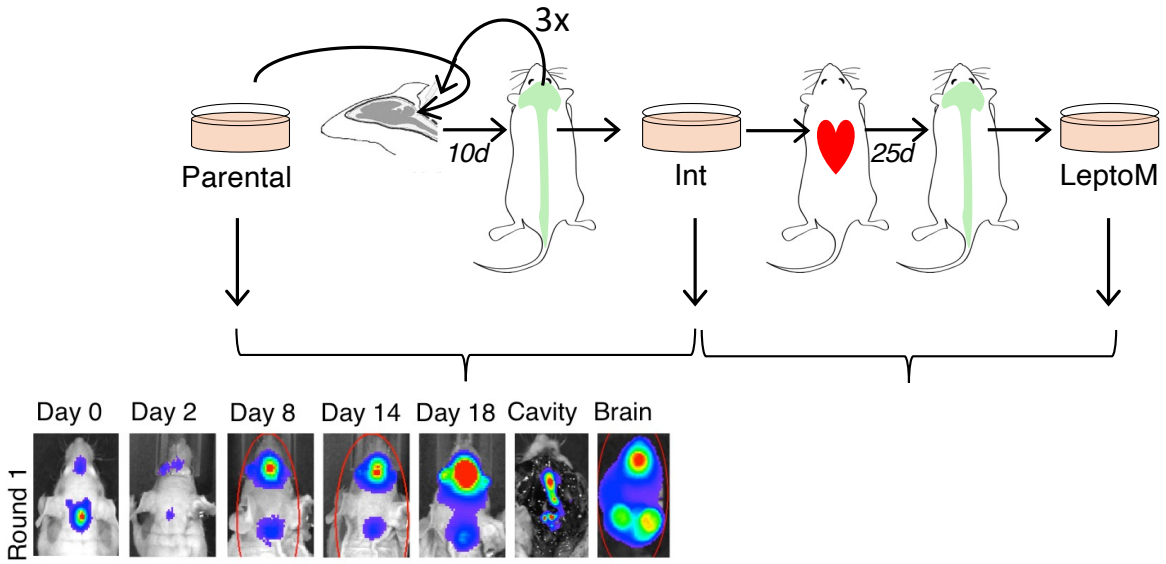
Mouse Modeling of LM



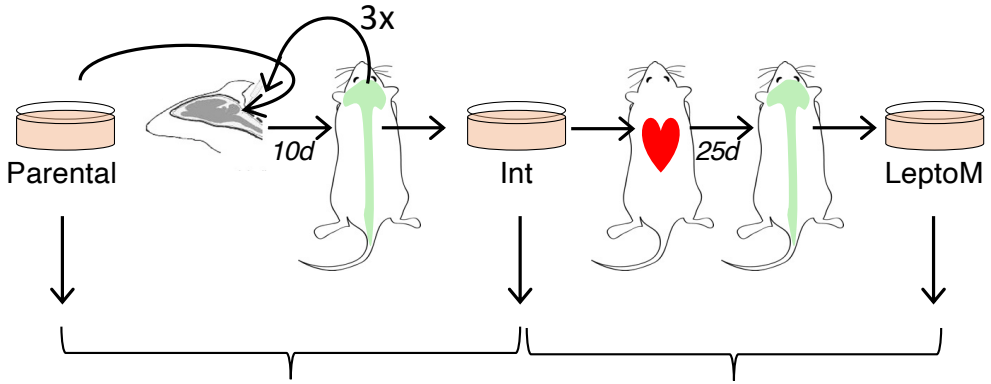
Parental Cells: Heterogeneous pool of cells

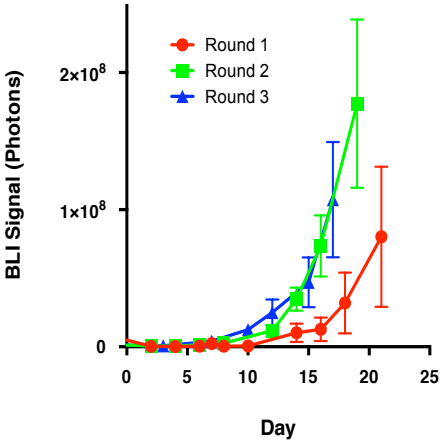
LeptoM Cells: Subset of Parental Cells

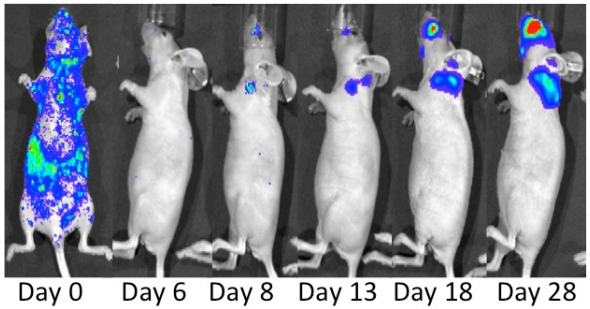
Mouse Modeling of LM



Mouse Modeling of LM



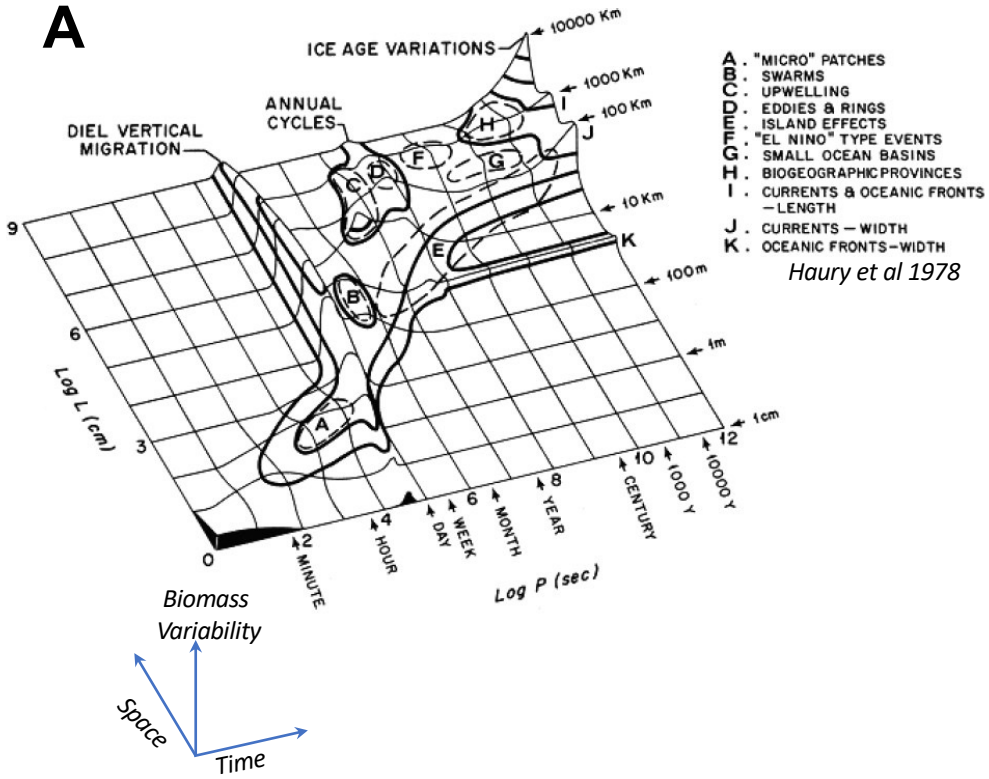




Memorial Sloan Kettering Cancer Center

Mouse vs. Man:
Metastasis as Evolutionary Problem

A



Haury et al 1978

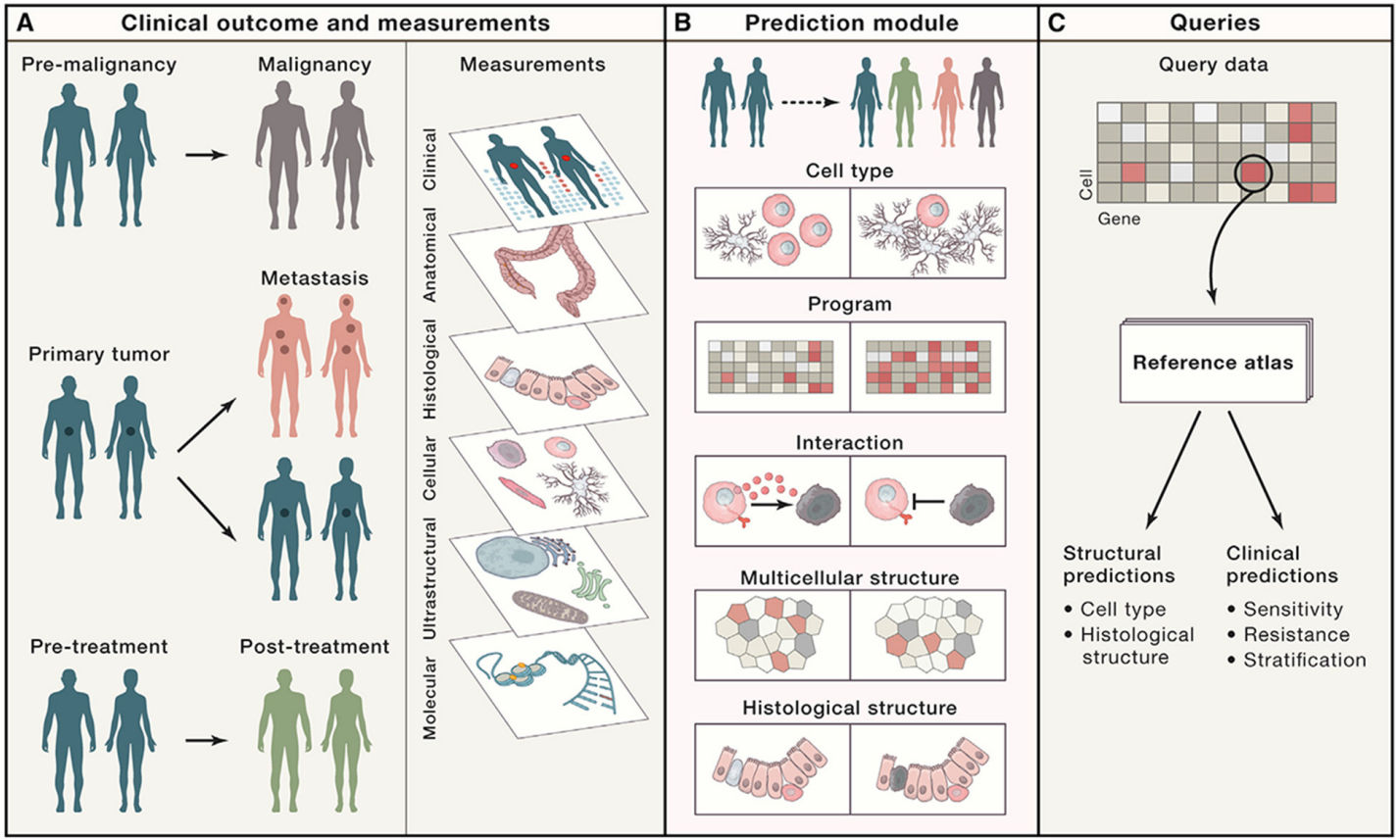
B

Human:
3.7 x 10^{13} cells
70-80 years
Cancer disease course 2-10 years

Mouse:
1.2 x 10^{10} cells
2-3 years
Cancer disease course: 1-4 months

Memorial Sloan Kettering Cancer Center

Strategy for interrogation of human metastatic disease



HTAN 2020 Cell Apr 16;181(2):236-249

Careful!!

”So you can be the President
I'd rather be the Pope
Yeah, you can be the side effect
I'd rather be the dope”
- Prince 1993

Post hoc ergo propter hoc

(After this, therefore because of this)

"the rooster crows immediately before sunrise; therefore the rooster causes the sun to rise."

Current concepts and questions in CNS metastasis

Modeling CNS metastasis

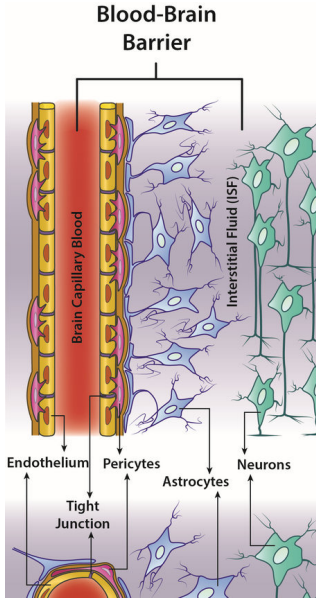
CNS barriers

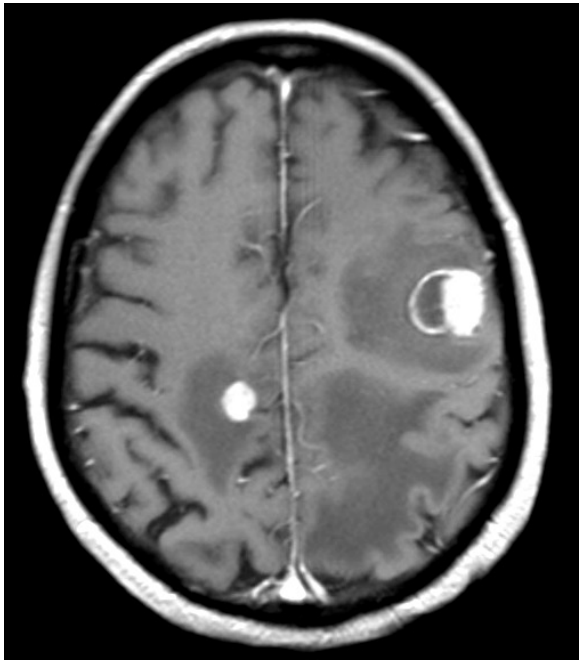
Microenvironmental selective pressure

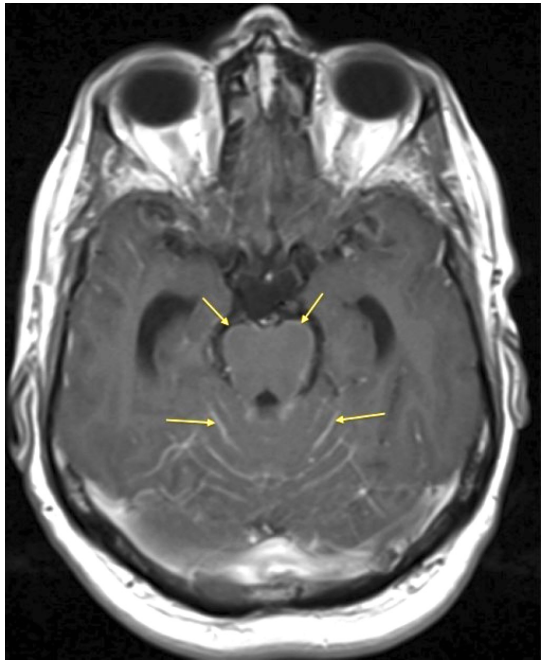
Interactions with glia: Journal Club

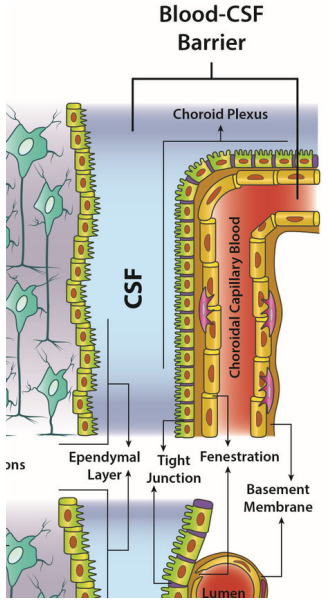
Blood Brain Barrier vs. Blood CSF Barrier:

Metastasis Edition









D'Agata F et al 2017 Molecules 23:9

Parenchymal *Leptomeningeal*

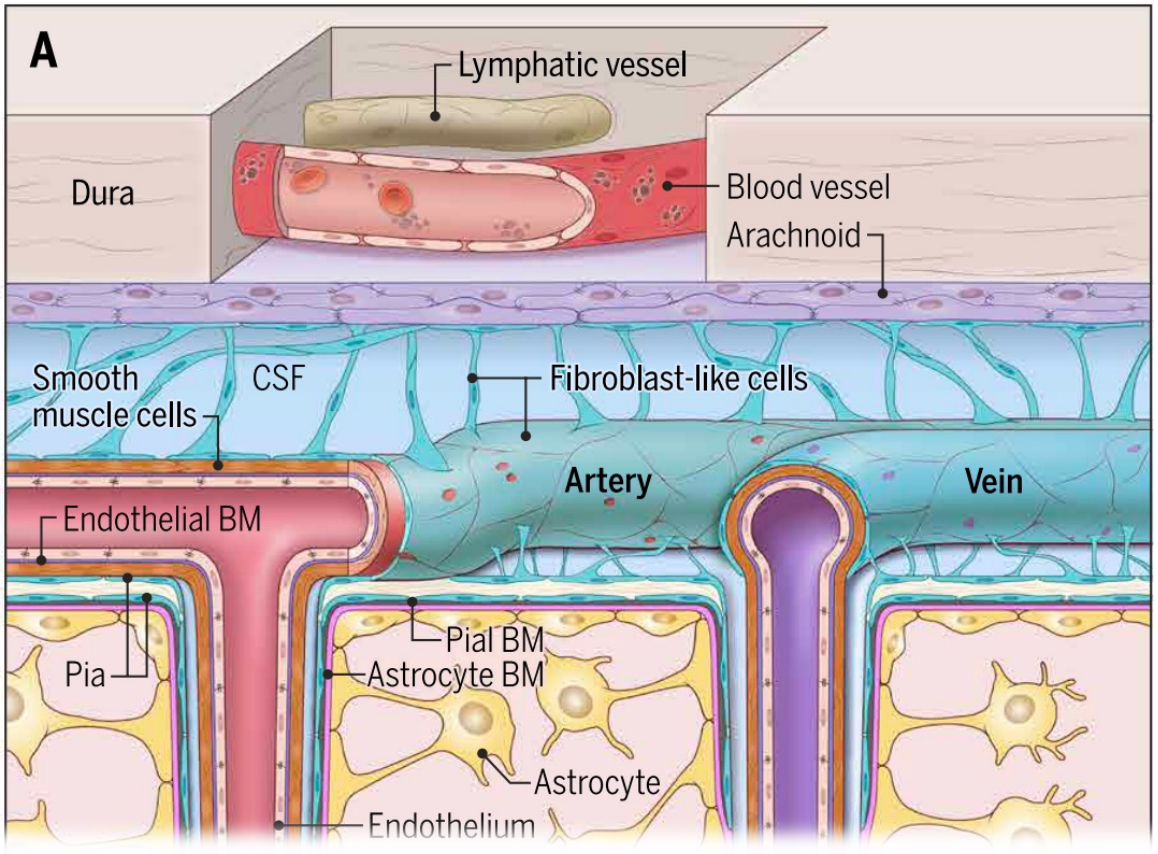
Memorial Sloan Kettering Cancer Center

The leptomeninges

Pachymeninges

Leptomeninges

Brain Parenchyma

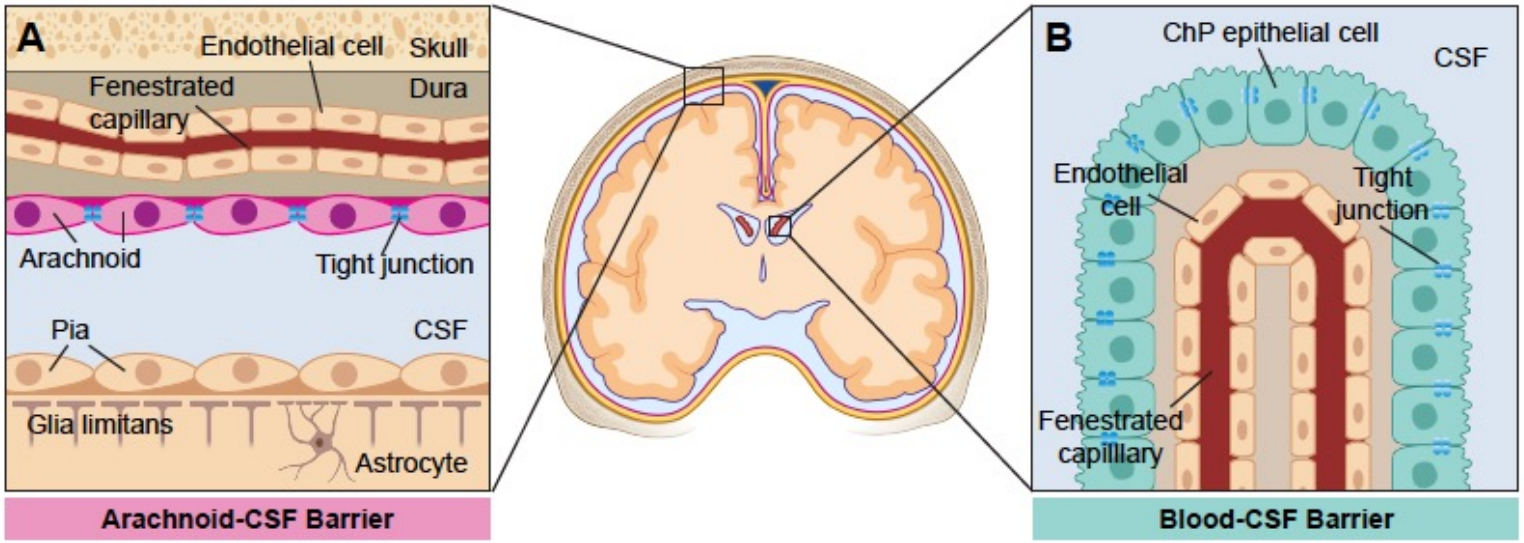


Pauci-cellular
Hypoxic
Low nutrient

Memorial Sloan Kettering Cancer Center

adapted from Mastorakos, *et al*. 2019

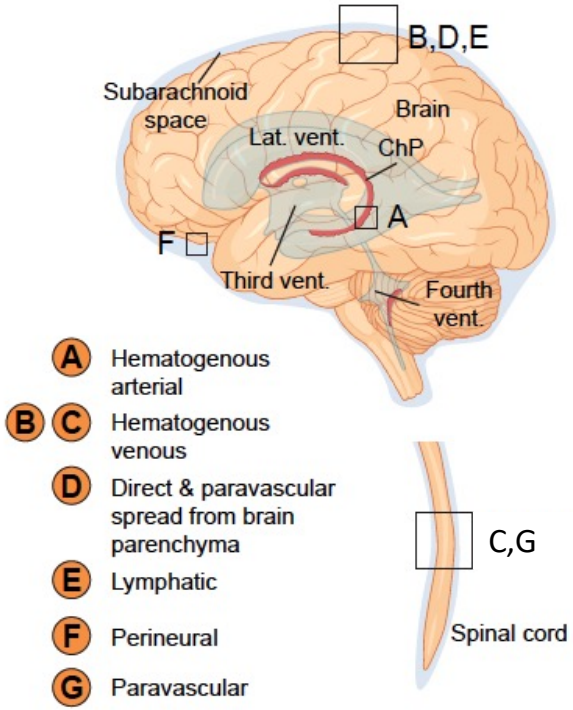
Major Blood-CSF-Barriers

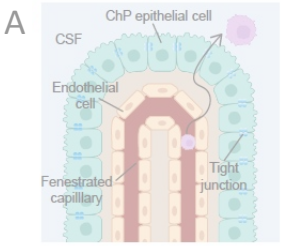


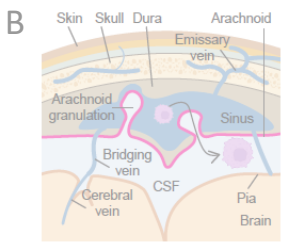
Memorial Sloan Kettering Cancer Center

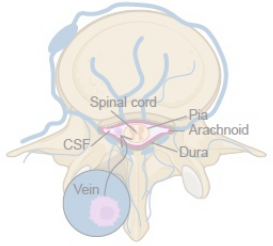
Freret M and Boire A 2023 in press

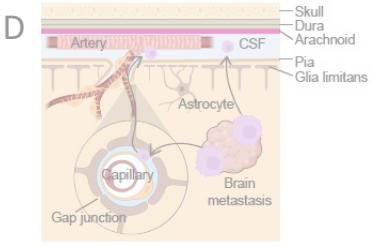
Routes of Leptomeningeal Entry

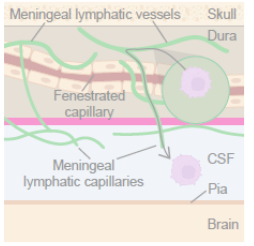


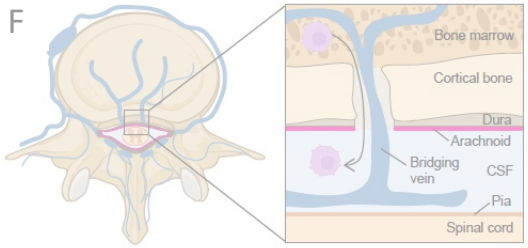


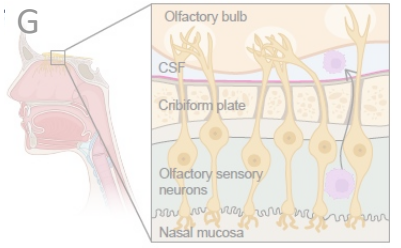












Memorial Sloan Kettering Cancer Center

Freret M and Boire A 2023 in press

Blood-CSF-Barriers:

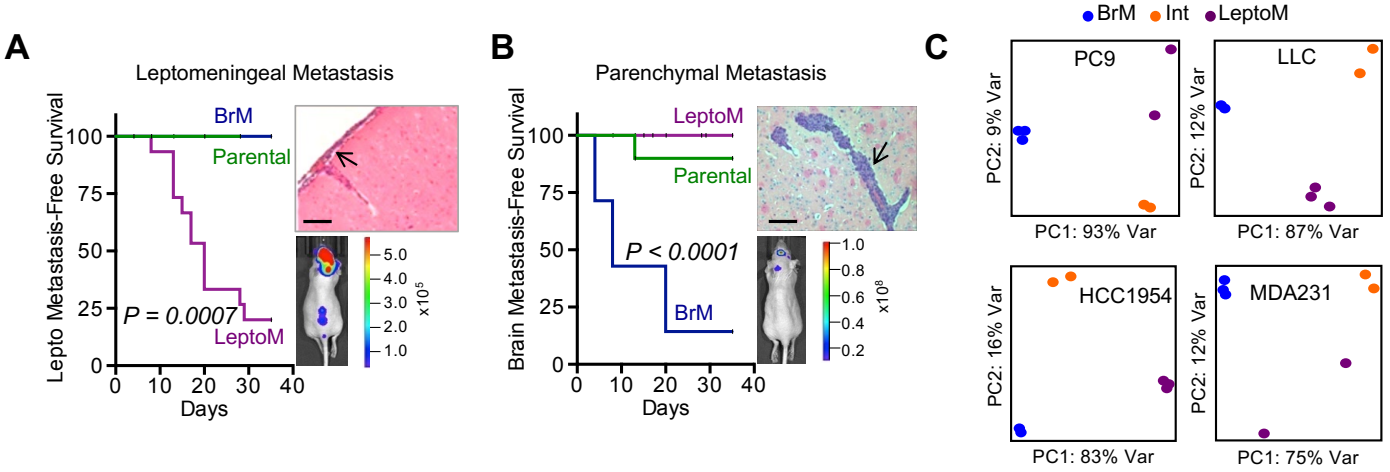
A More Modern View

Castle with Moat

TSA at Airports

Memorial Sloan Kettering Cancer Center

LeptoM derivatives are phenotypically and transcriptomally distinct from BrM derivatives

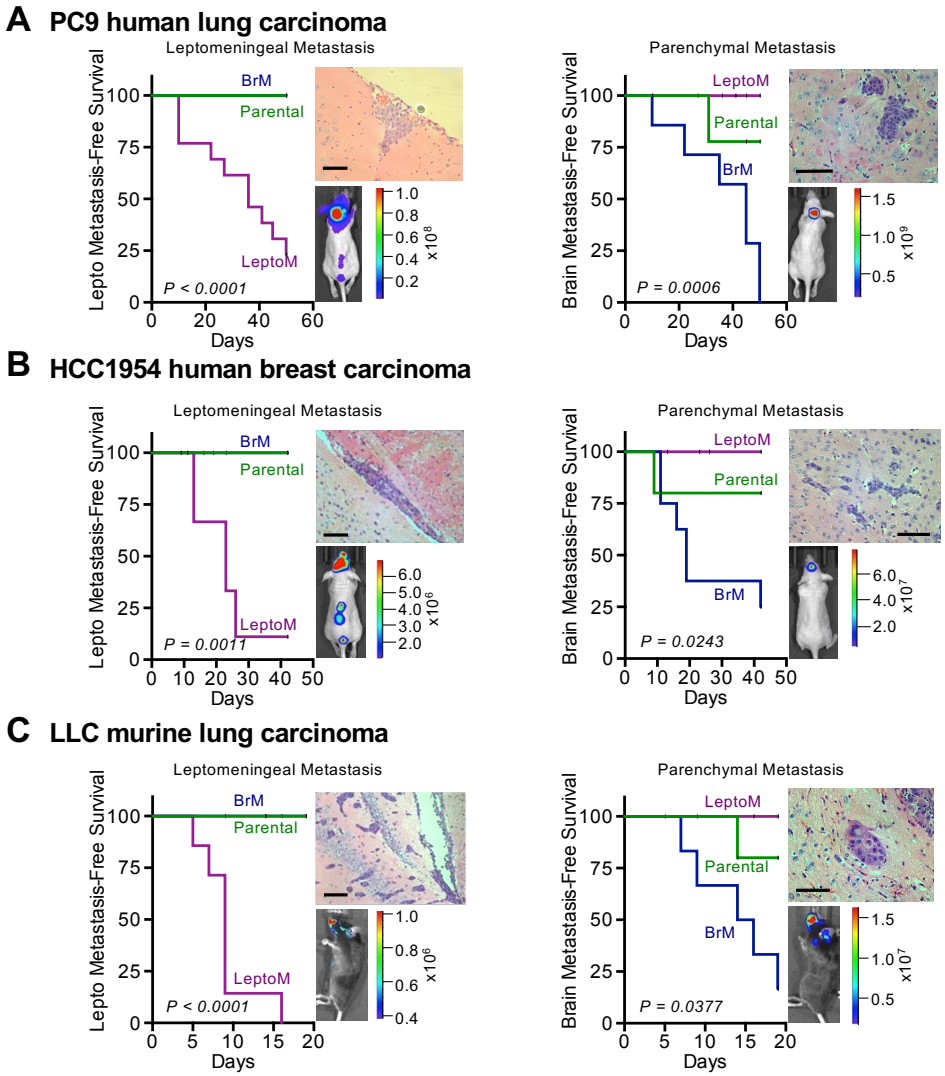


D

Cell Line	Primary Cancer	Subtype	Mouse	Ref
MDA231	Human Breast	ER(-)/PR(-)/Her2(-)	Nu/nu	Bos PD et al 2009
HCC1954	Human Breast	ER(-)/PR(-)/Her2(+)	Nu/nu	Malladi S et al 2016
Lewis Lung Carcinoma	Mouse Lung	Non-small cell	C57/Bl6	Chen Q et al 2016
PC9	Human Lung	EGFR	Nu/nu	Nguyen DX et al 2009

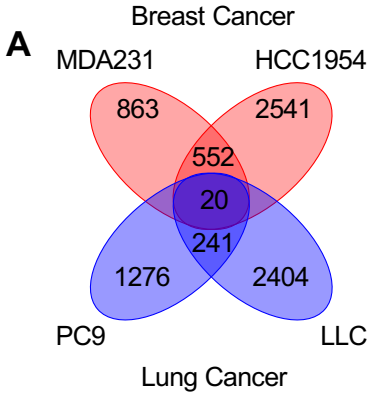
Boire A, Zou Y, Shieh J, Macalino D, Pentsova E and J Massagué. 2017 Complement Component 3 Adapts the Cerebrospinal Fluid for Leptomeningeal Metastasis. *Cell* 168(6):1101-1113

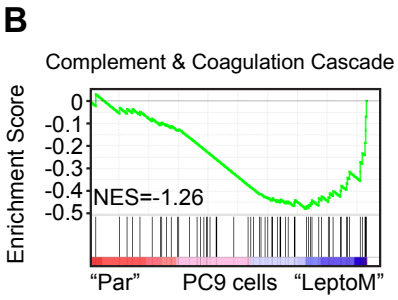
Memorial Sloan Kettering Cancer Center

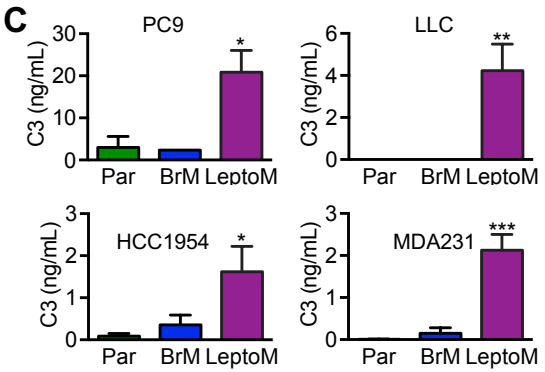


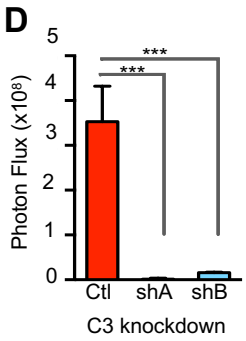
Boire 2017 *Cell* 168(6):1101

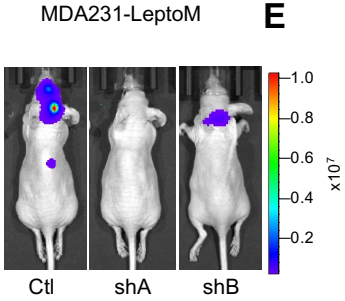
Complement is C3 associated with cell growth in CSF

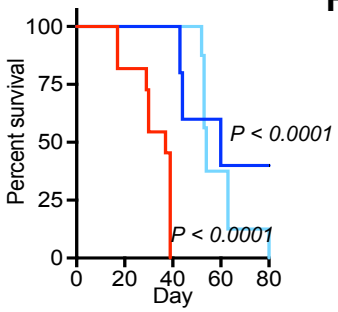


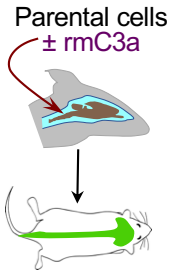


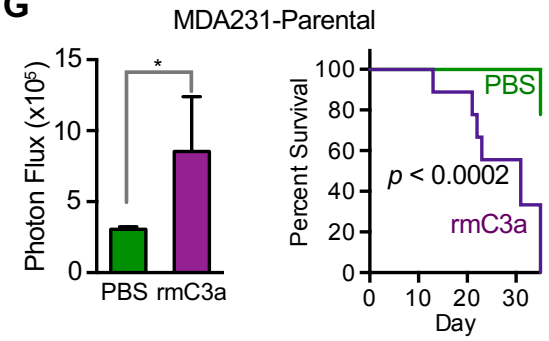








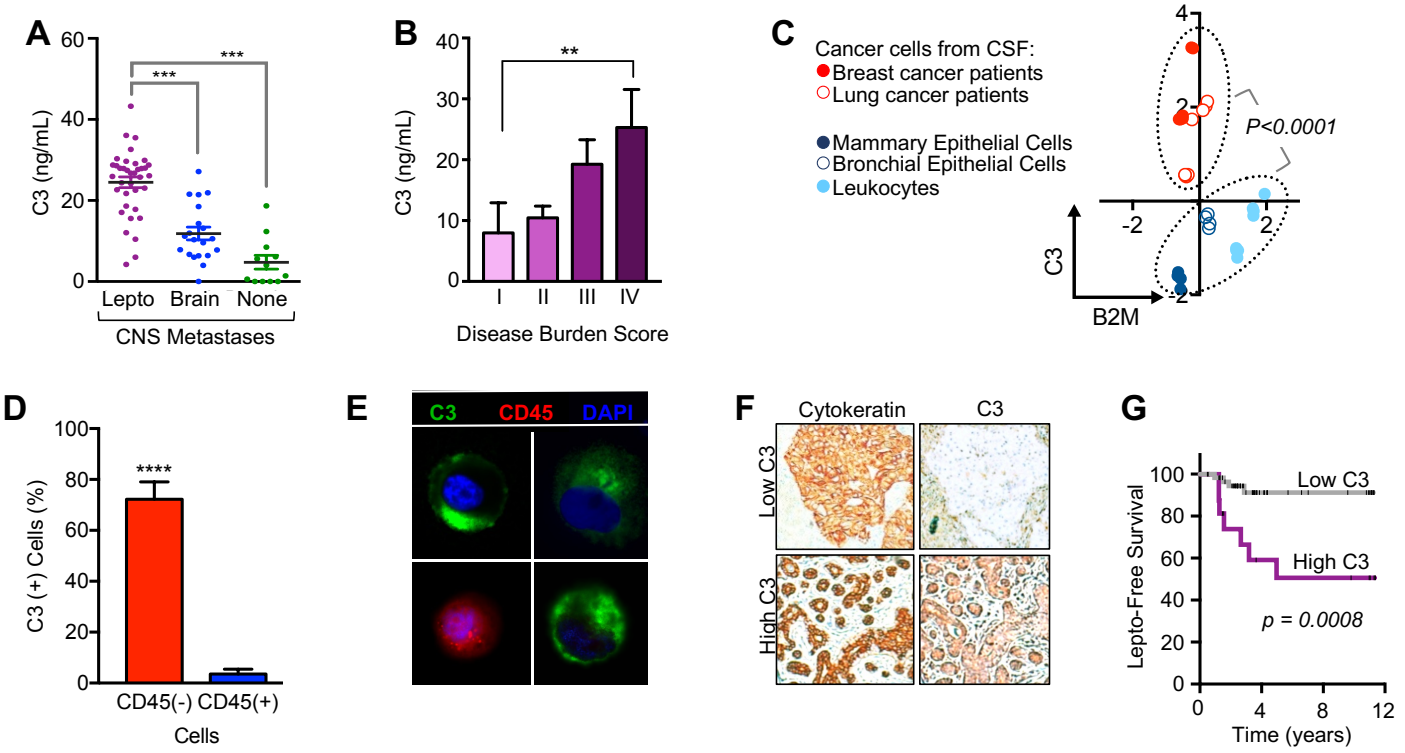




Memorial Sloan Kettering Cancer Center

Boire 2017 *Cell* 168(6):1101

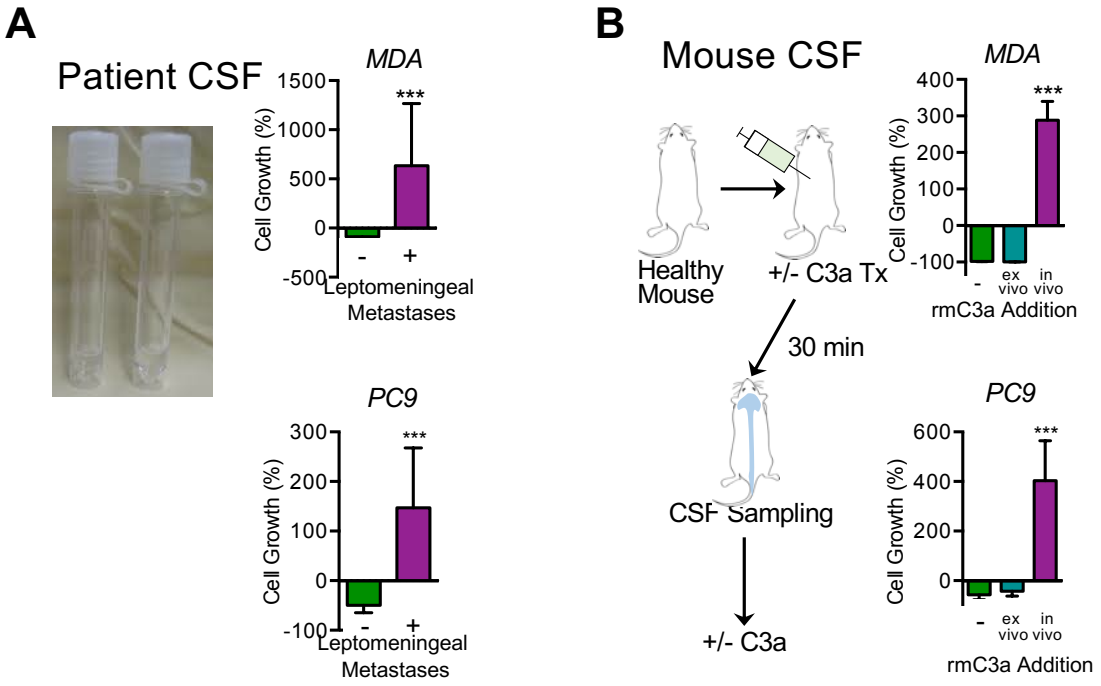
C3 Implicated in Human LM



Boire 2017 *Cell* 168(6):1101

Memorial Sloan Kettering Cancer Center

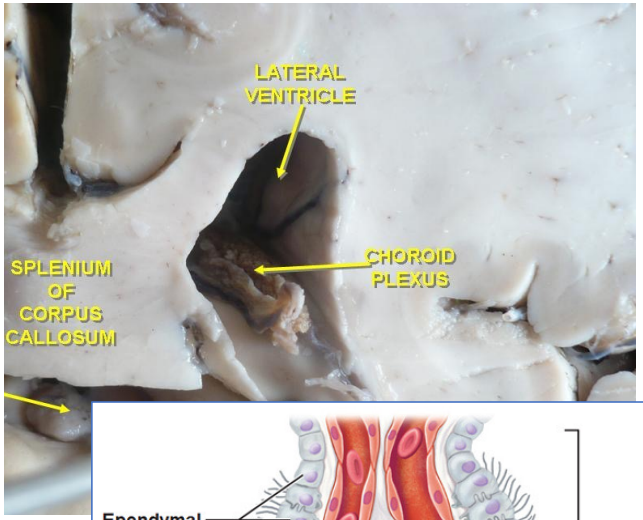
C3 improves cancer cell growth in CSF

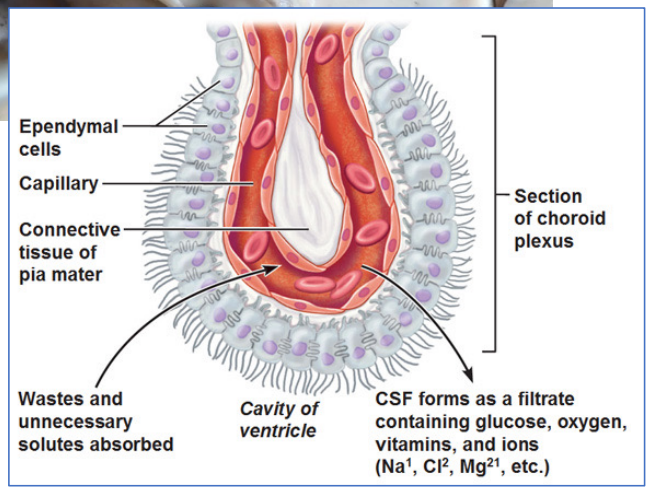


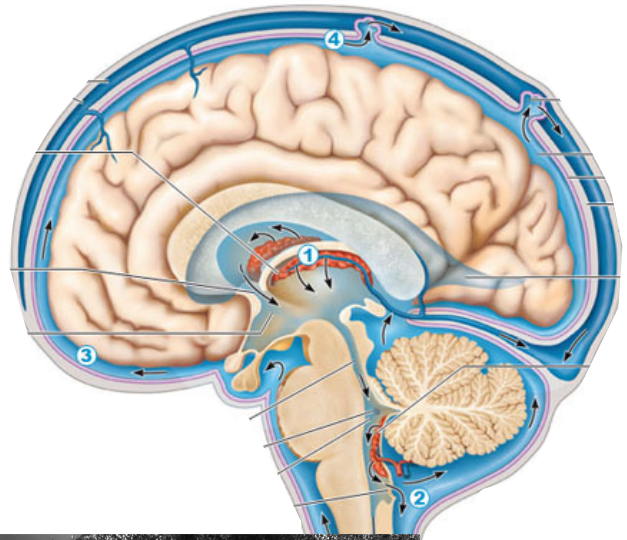
Boire 2017 *Cell* 168(6):1101

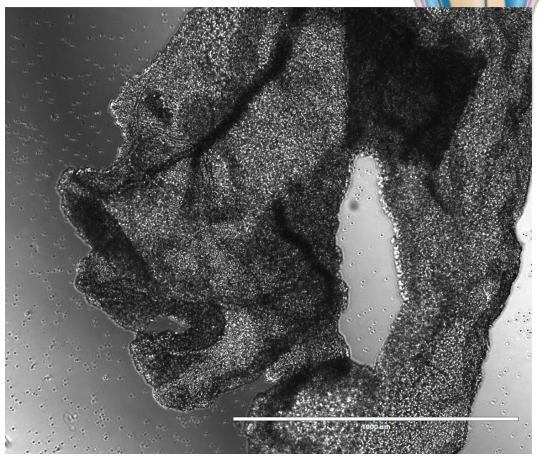
Memorial Sloan Kettering Cancer Center

Choroid Plexus



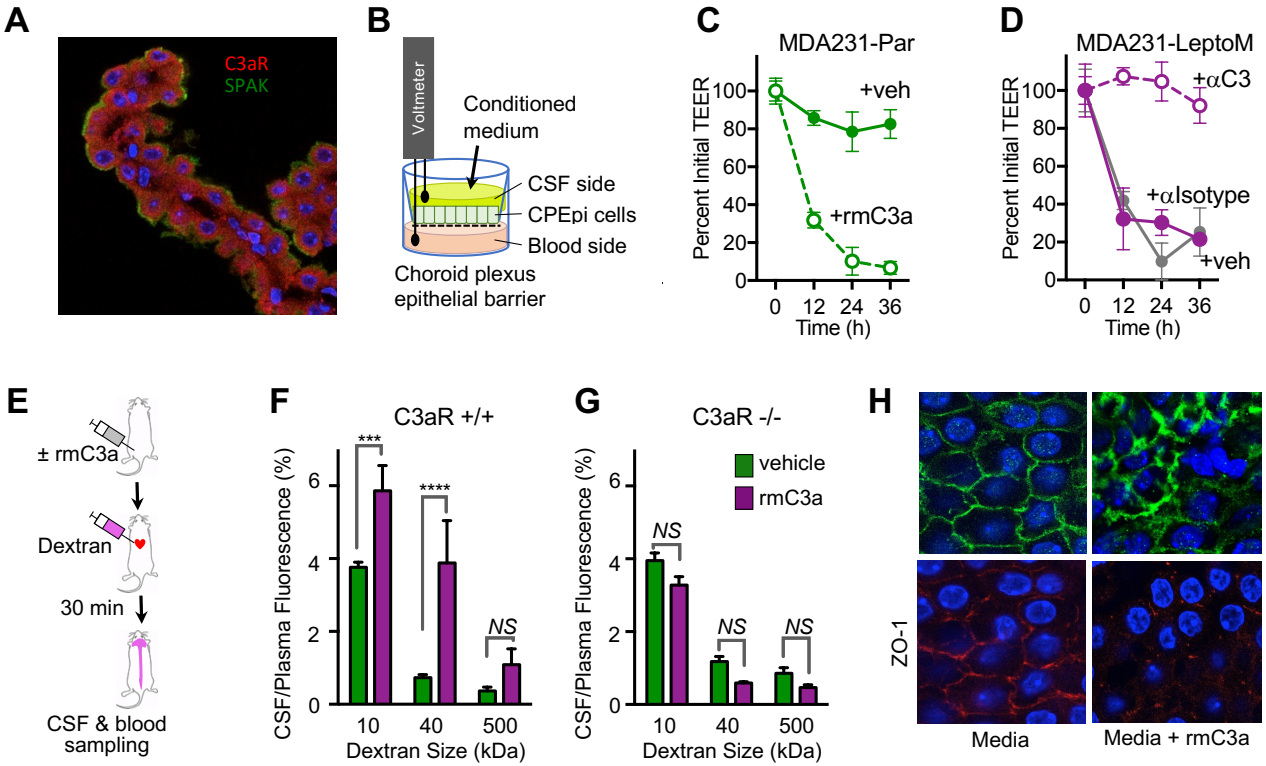






Memorial Sloan Kettering Cancer Center

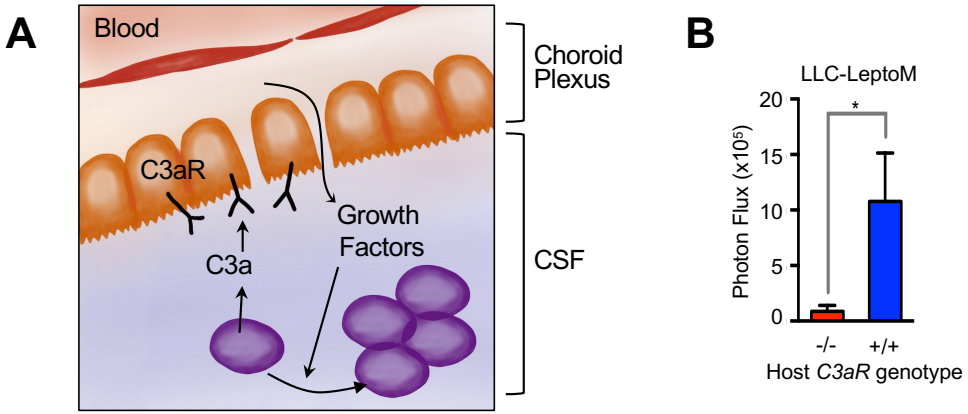
Complement C3 activates C3aR to loosen choroid plexus tight junctions



Boire 2017 *Cell* 168(6):1101

Memorial Sloan Kettering Cancer Center

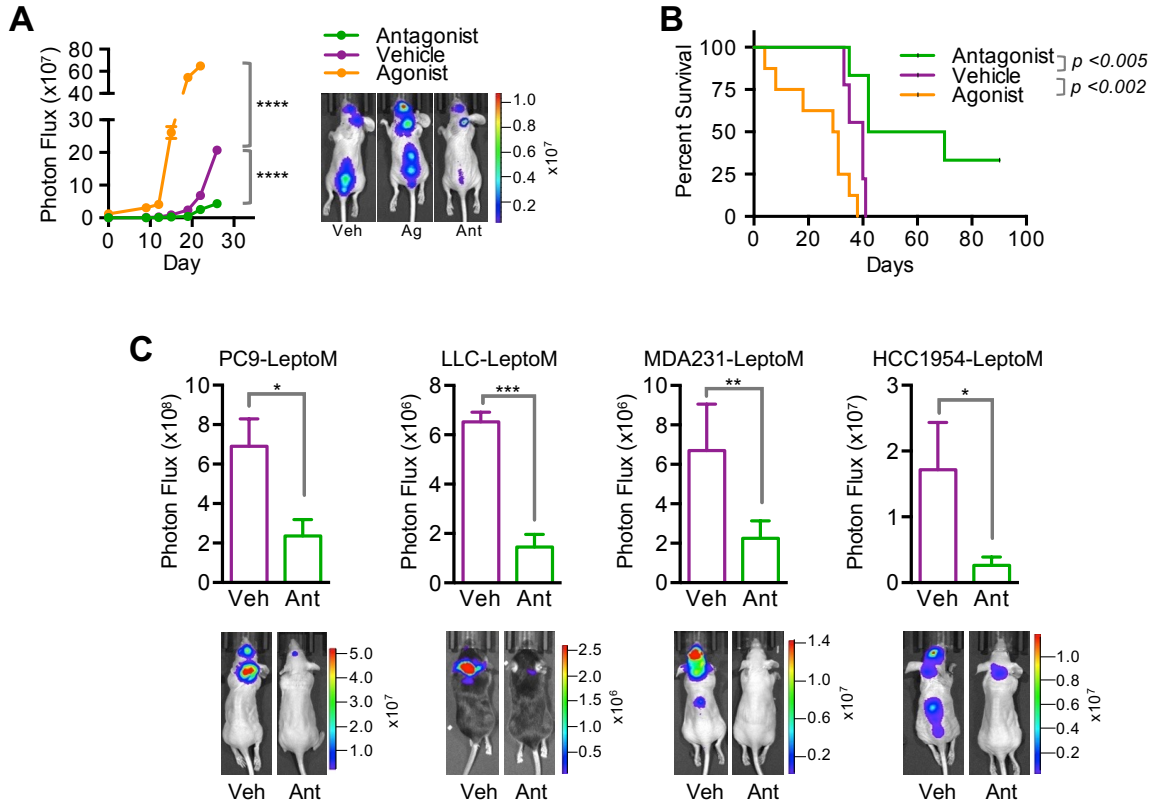
C3aR activation alters composition of CSF



Boire 2017 *Cell* 168(6):1101

Memorial Sloan Kettering Cancer Center

C3aR antagonism inhibits cancer cell growth in the leptomeninges



Boire 2017 *Cell* 168(6):1101

Memorial Sloan Kettering Cancer Center

Current concepts and questions in CNS metastasis

Modeling CNS metastasis

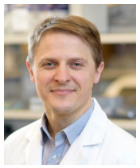
CNS barriers

Microenvironmental selective pressure

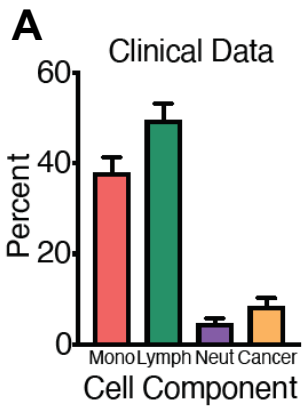
Interactions with glia: Journal Club

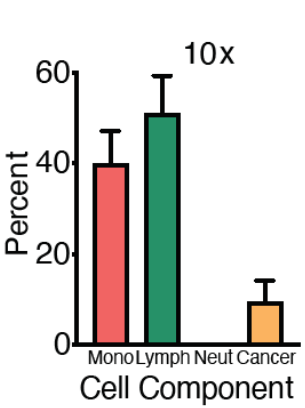
Capturing Leptomeningeal Metastasis by scRNA Seq

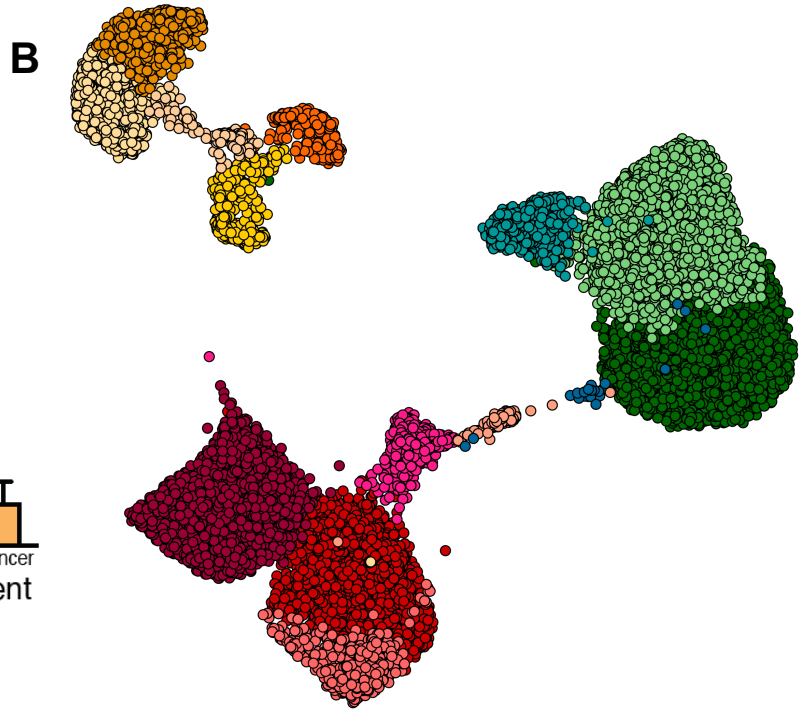
Dana
Pe'er



Linas
Mazutis



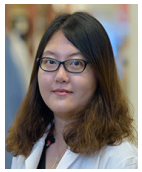




5 patients with
untreated LM
2 breast cancer
3 lung cancer
Total cells: 15K

Chi Y, Remsik J, Kiseliovas V, Derderian C, Sener U, Alghader M, Saadeh F, Nikishina K, Bale T,Iacobuzio-Donahue C, Thomas T, Pe'er D, Mazutis L and A Boire 2020 Cancer cells deploy lipocalin- 2 to collect limiting iron in leptomeningeal metastasis. Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center



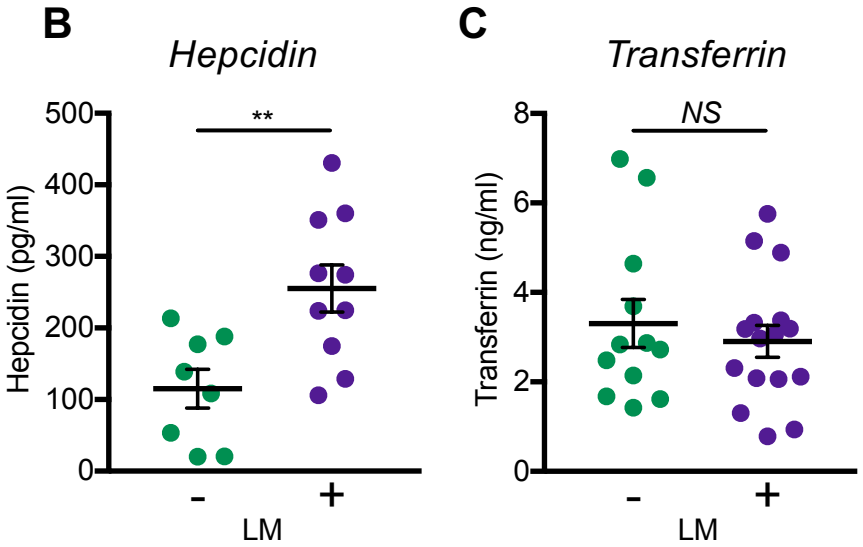
Yudan Chi

Cancer cells and immune cells inhabit an Fe-deficient environment, and are deficient in iron

A

Analyte	CSF	Serum
Osmolarity (mOsm/mL)	295	295
pH	7.33	7.41
Oxygen (mmHg)	43.0	104.0
Glucose (mg/dL)	60.0	90.0
Lactate:Pyruvate	26.0	17.6
Total Protein (mg/dL)	35.0	7000
Free Amino Acids (mol/dL)	80.9	228.0
Total Lipids (mg/dL)	1.5	750.0
Iron (μg/dL)	1.5	15000

Composition of CSF and Serum.
Adapted from Fishman RA 1992

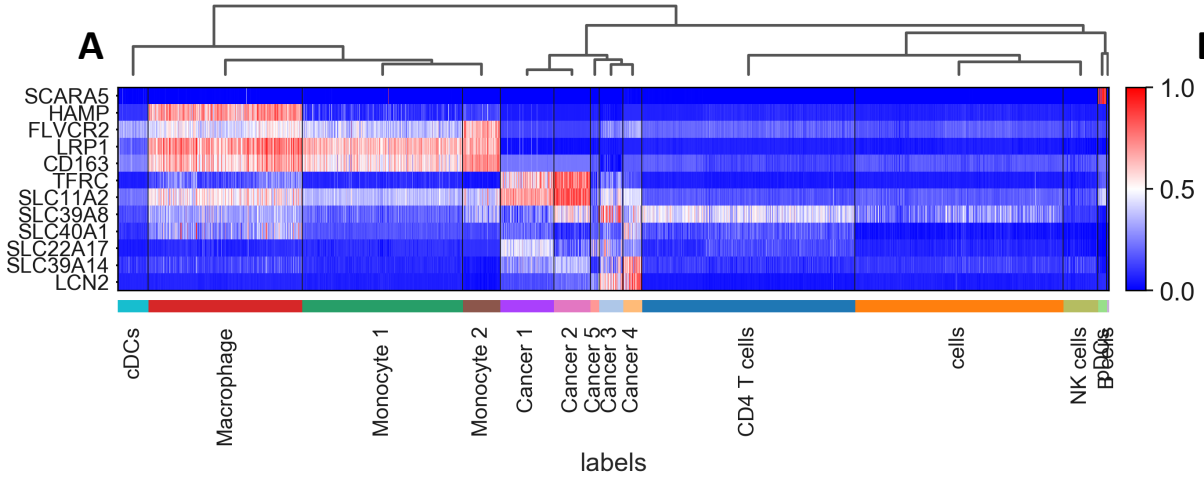


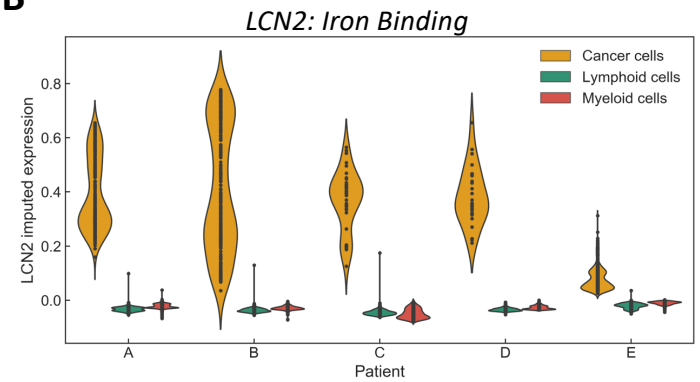
Chi Y et al Science 369(6501):276-282.

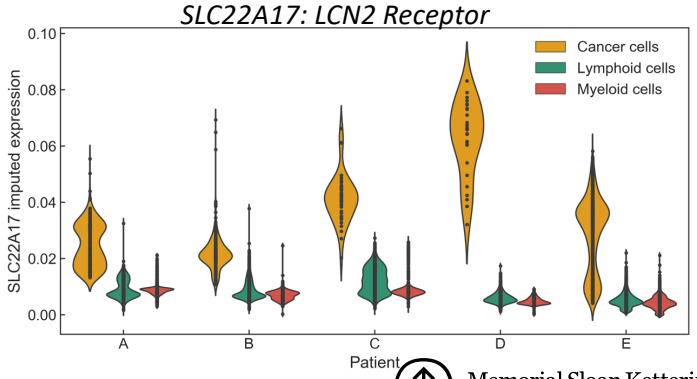
Memorial Sloan Kettering Cancer Center

Vaidotas Kiseliovas

Cancer cells express LCN2 and SLC22A17







Memorial Sloan Kettering Cancer Center

Chi Y et al Science 369(6501):276-282.

Lipocalin 2 *LCN2*

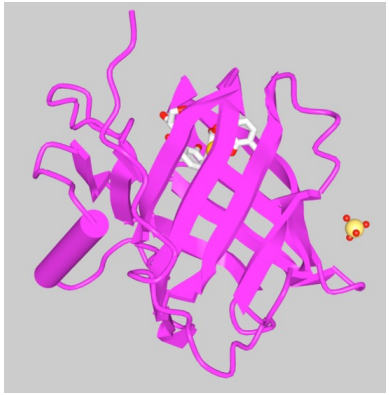
Neutrophil gelatinase-associated lipocalin, 25kDa alpha-2-microglobulin-related subunit of MMP9, Migration-stimulating factor inhibitor, oncogene 24p3, siderocalin

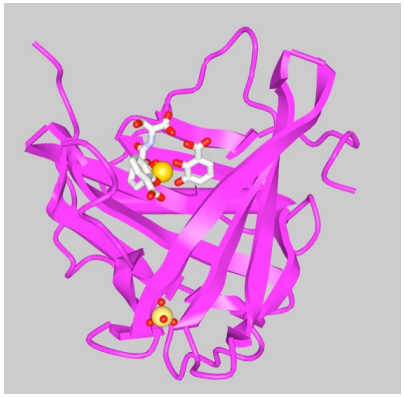
Lipocalins are secreted transporters

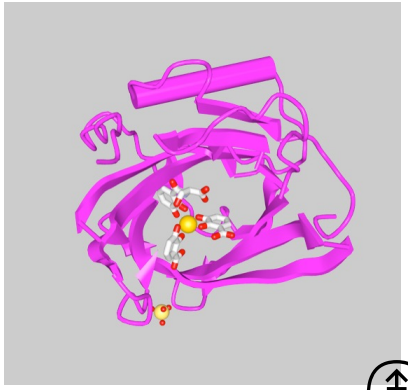
LCN2 -co-purifiying with MMP-9, participates in nutritional immunity though siderophore function (Binds Fe^{3+})

Yan J et al 2002 Mol Cell

Generated by Neutrophils and/or Macrophage in inflammatory processes







Goetz DH et al 2002 Mol Cell

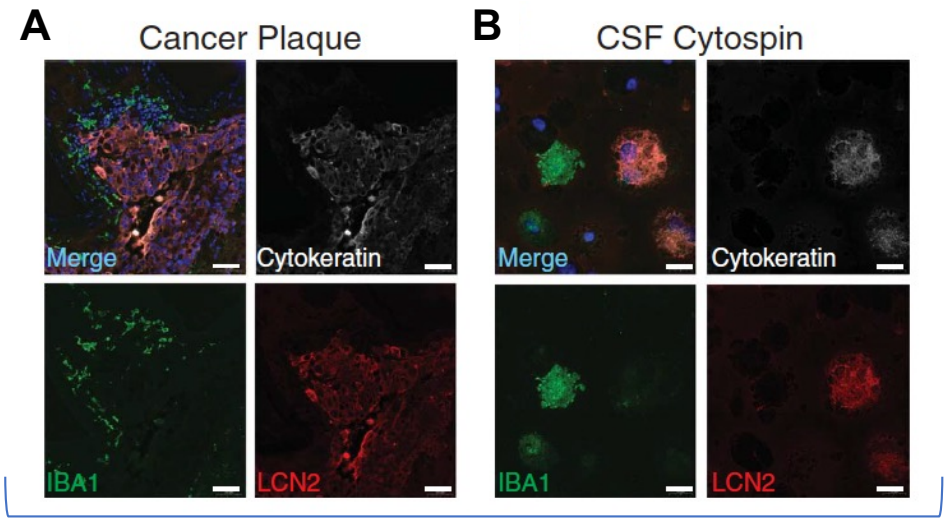
Carrier	Kd (M)
Lcn2 (+catechol)	10^{-45} 10^{-10}
Transferrin	10^{-23}
Lactoferrin	10^{-20}
Deferoxamine	10^{-34}

Memorial Sloan Kettering Cancer Center

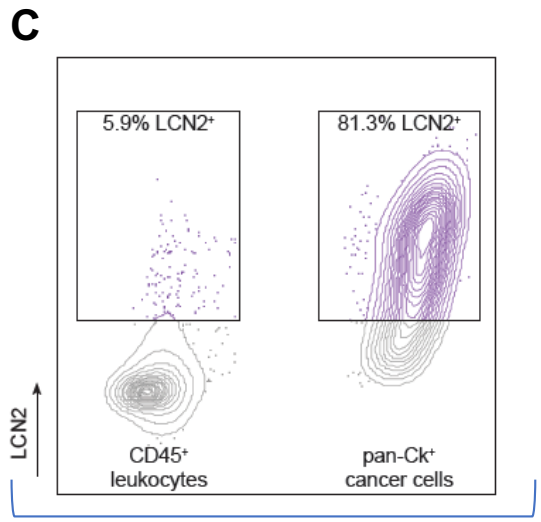
Christine Iacobuzio-Donahue

Jan Remsik

CSF cancer cells express LCN2; Macrophages do not



Autopsy Series

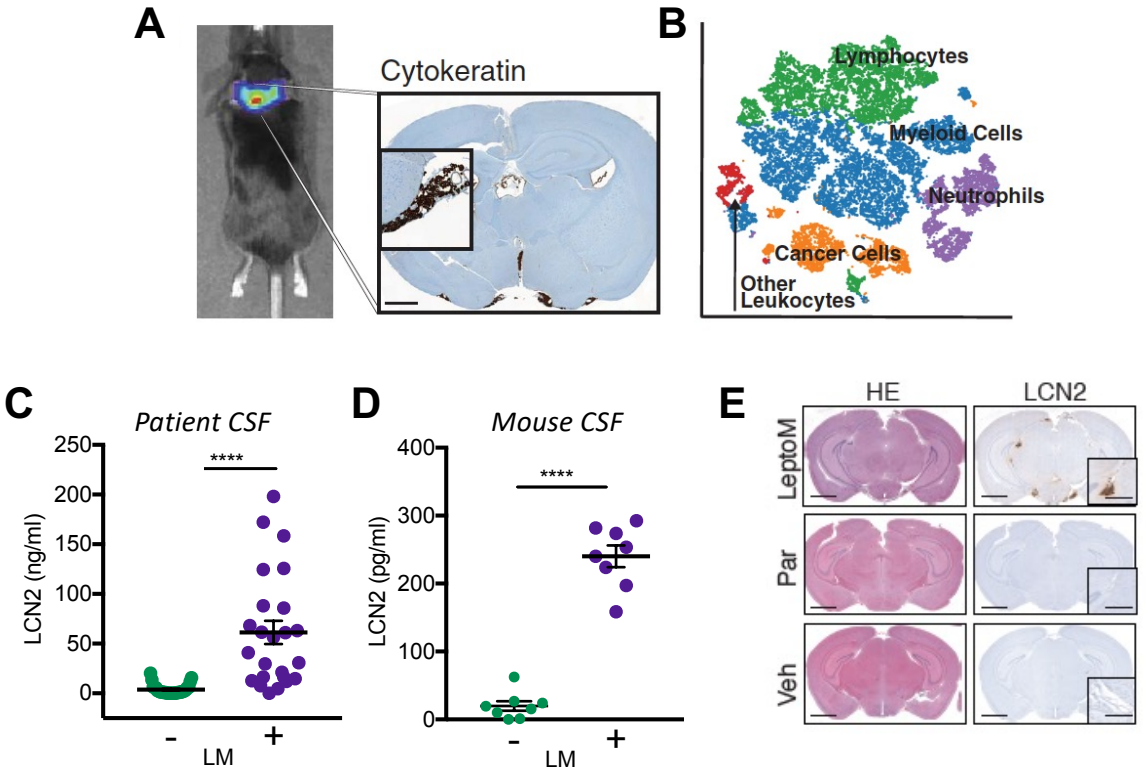


CSF Flow Cytometery

Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

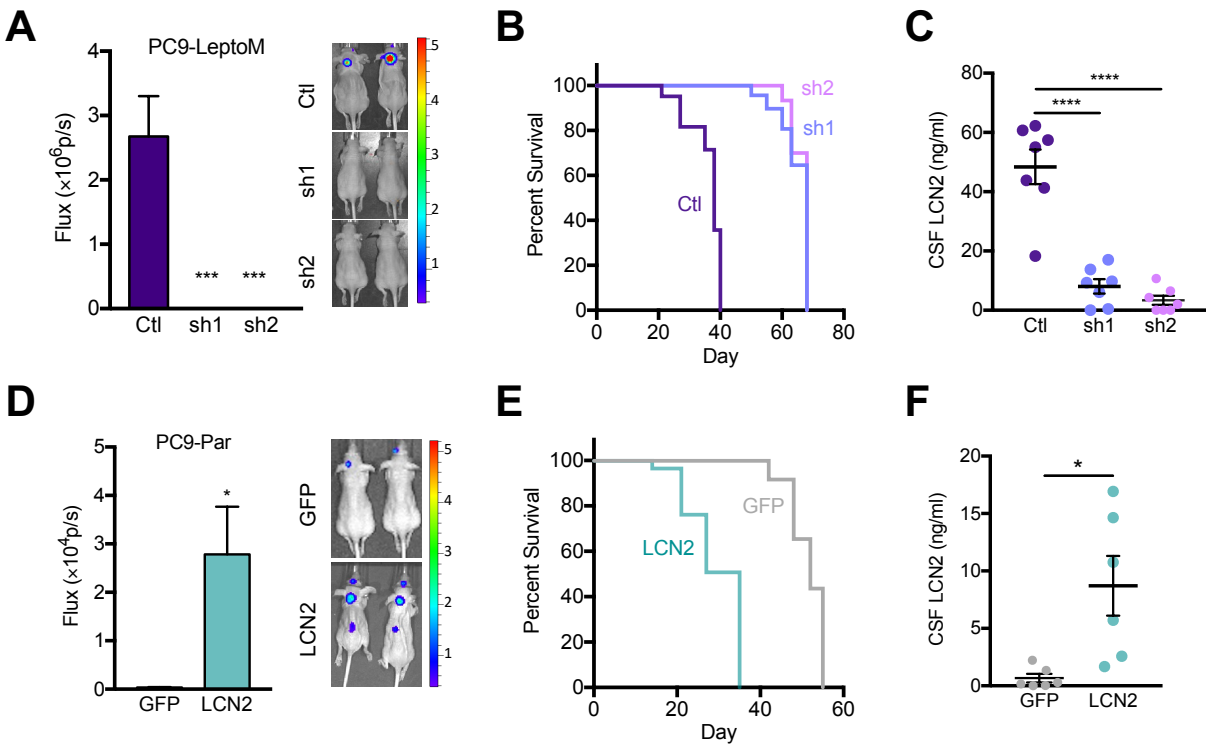
LCN2 and SLC22A17 is associated with LM in human disease *and* mouse models of LM



Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

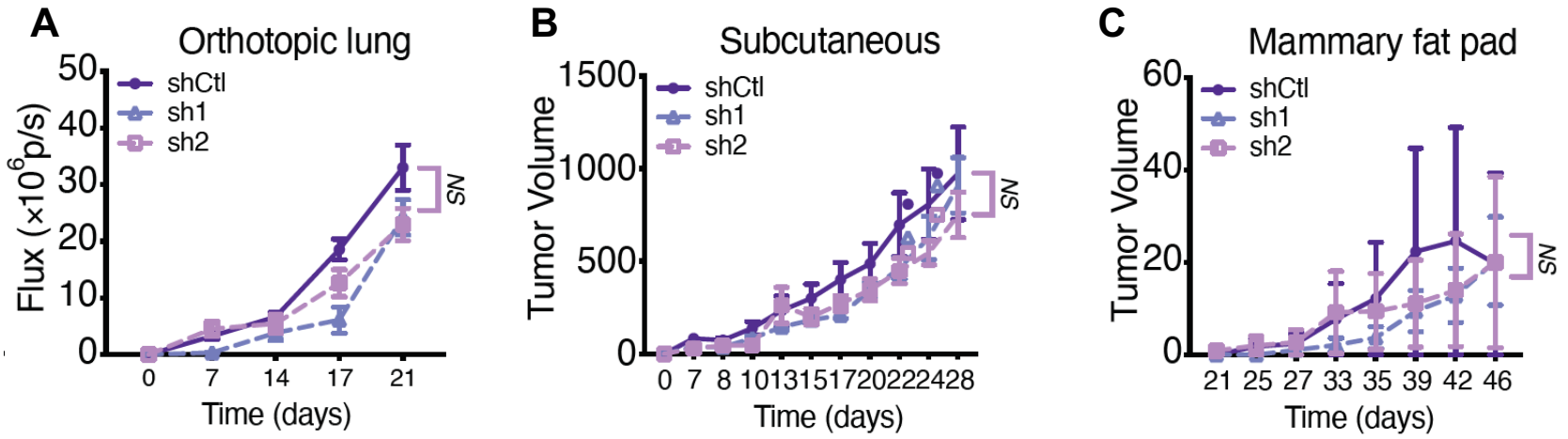
LCN2 supports cancer cell growth in CSF



Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

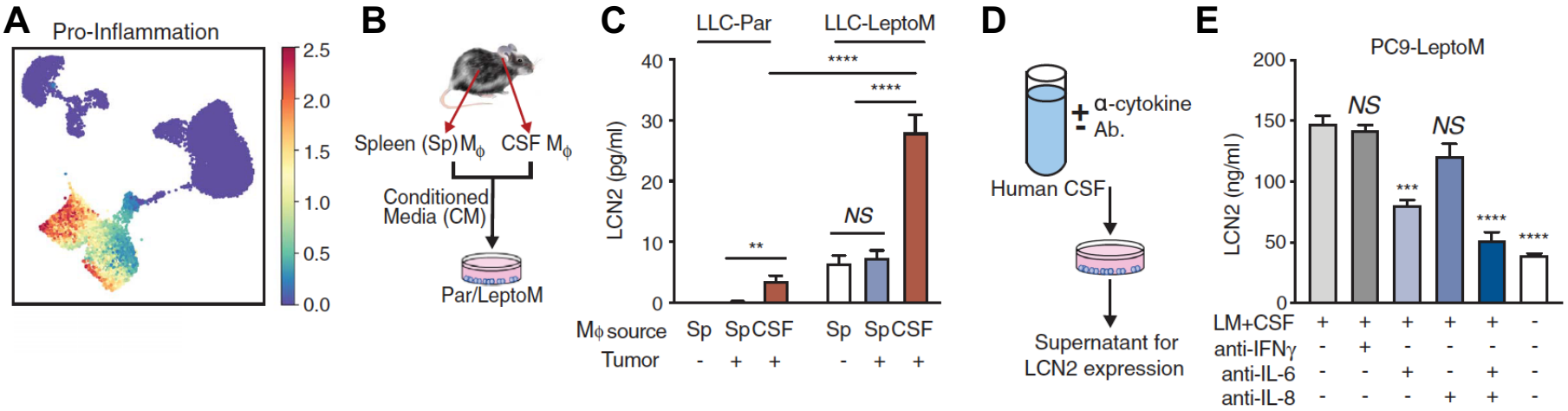
LCN2 does *not* support cancer cell growth in orthotopic sites



Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

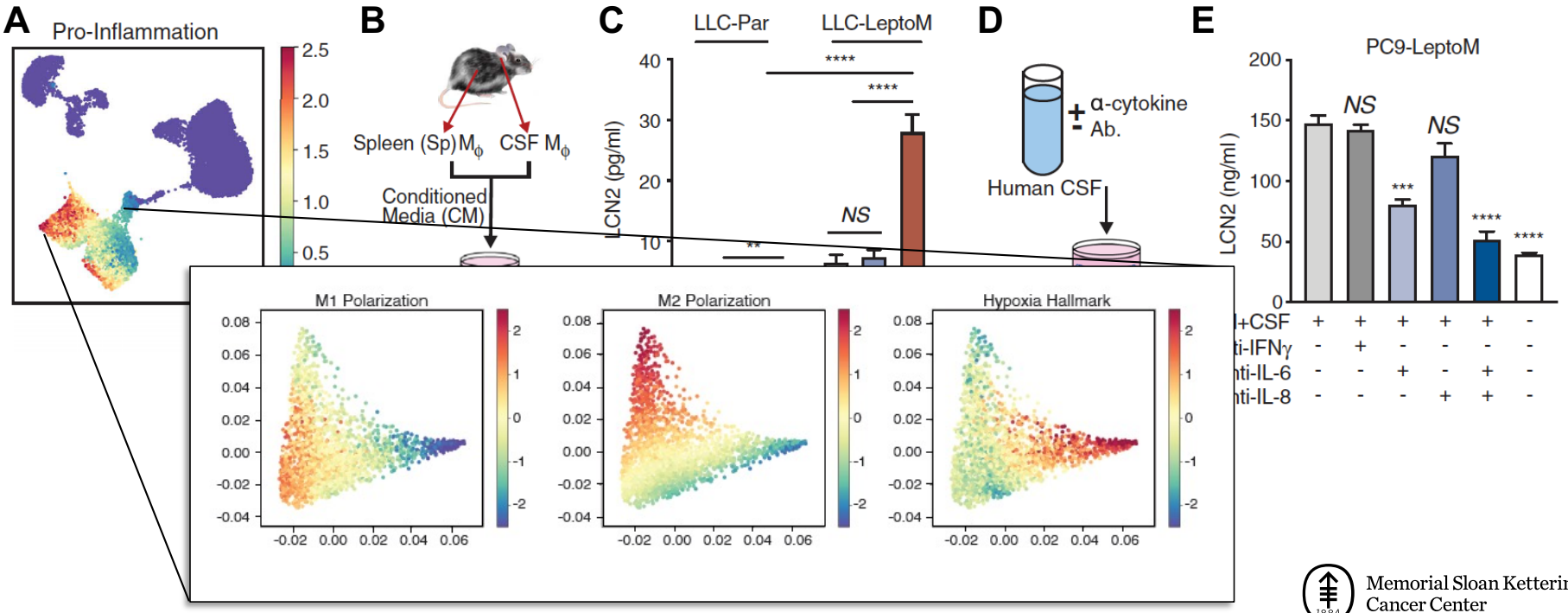
CSF Macrophage cytokines induce cancer cell LCN2 expression



Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

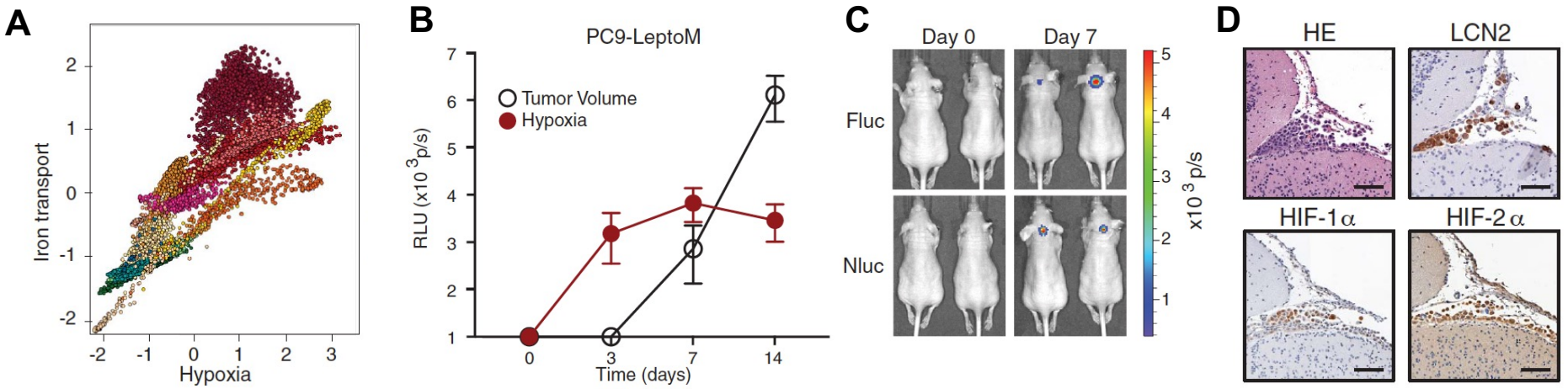
CSF Macrophage cytokines induce cancer cell LCN2 expression



Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

CSF Macrophage cytokines induce cancer cell LCN2 expression

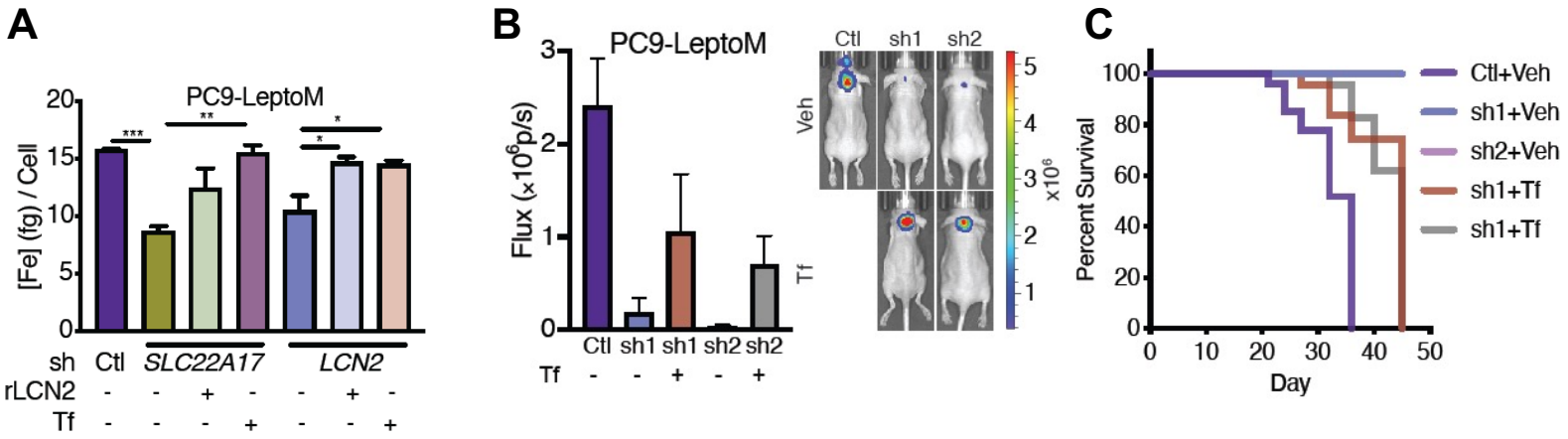


Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

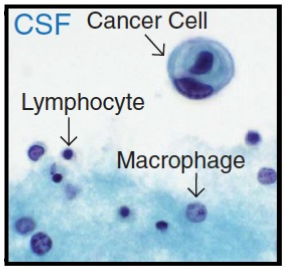
Tiffany Thomas (ICP-MS) CUMC

Cancer cells employ Lcn2 to collect extracellular iron

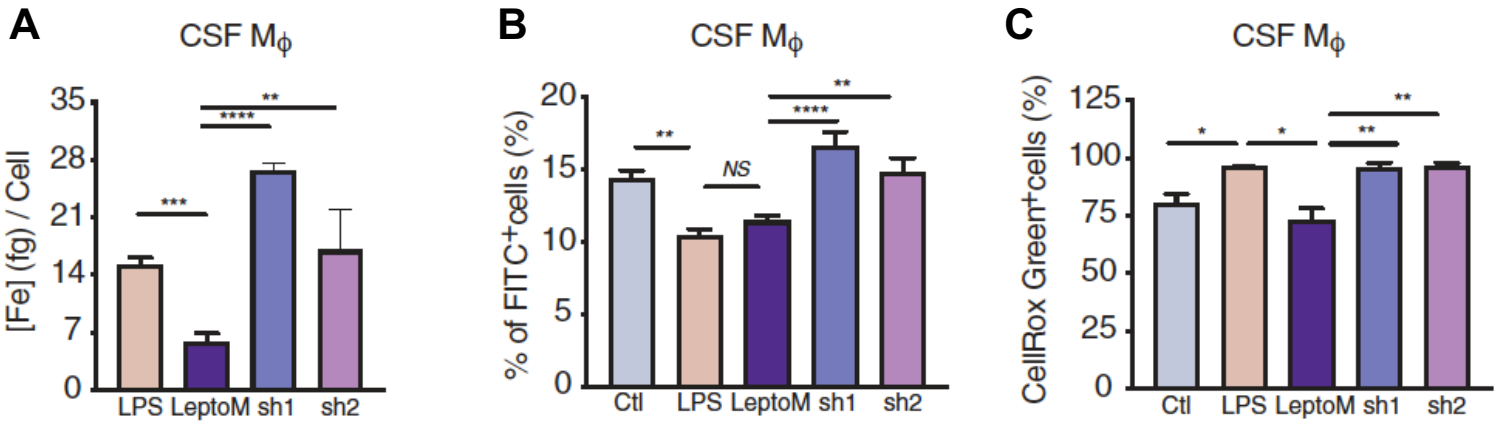


Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center



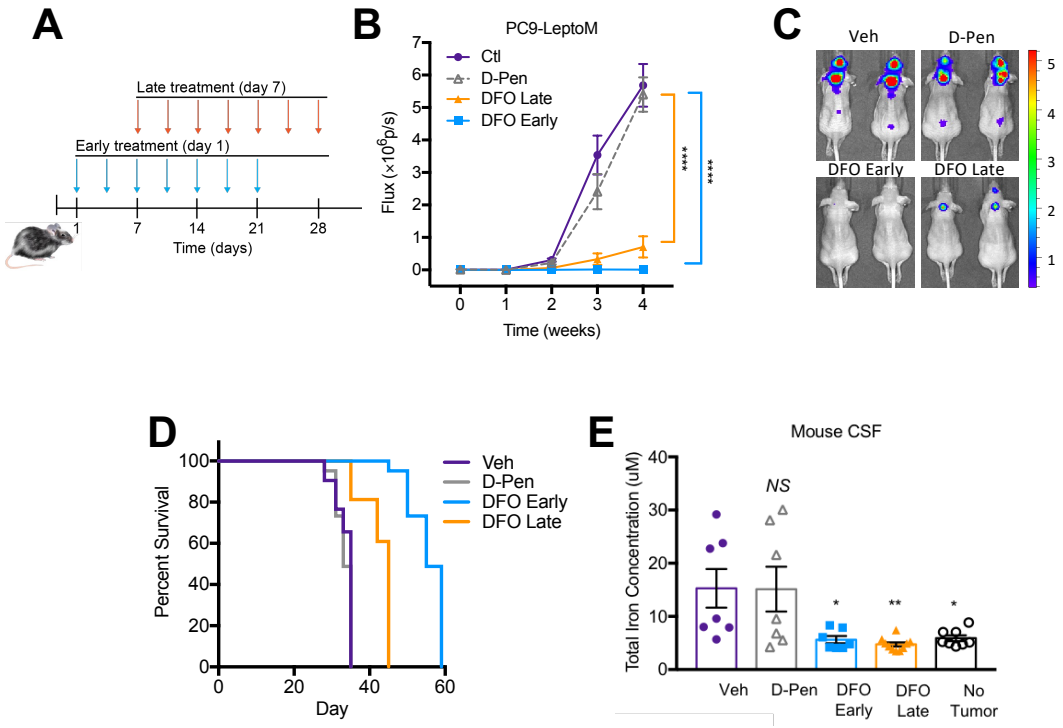
Cancer cells use LCN2 to outcompete macrophage for extracellular iron



Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

Iron chelation inhibits cancer cell growth in CSF



Chi Y et al Science 369(6501):276-282.

Memorial Sloan Kettering Cancer Center

Current concepts and questions in CNS metastasis

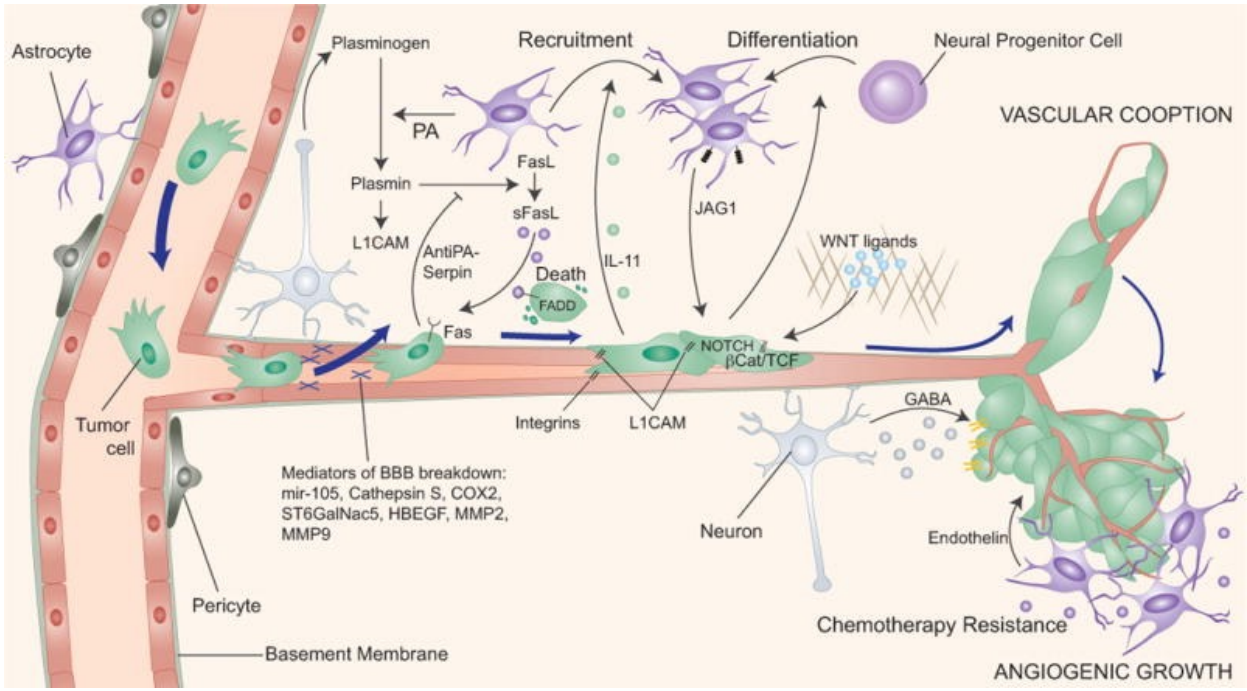
Modeling CNS metastasis

CNS barriers

Microenvironmental selective pressure

Interactions with glia: Journal Club

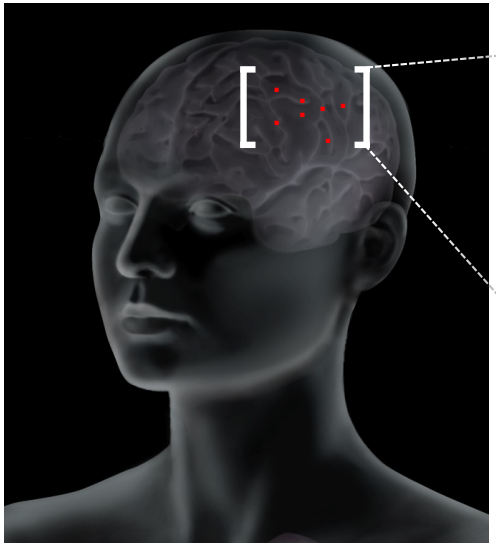
Genes that Mediate Parenchymal Brain Metastasis

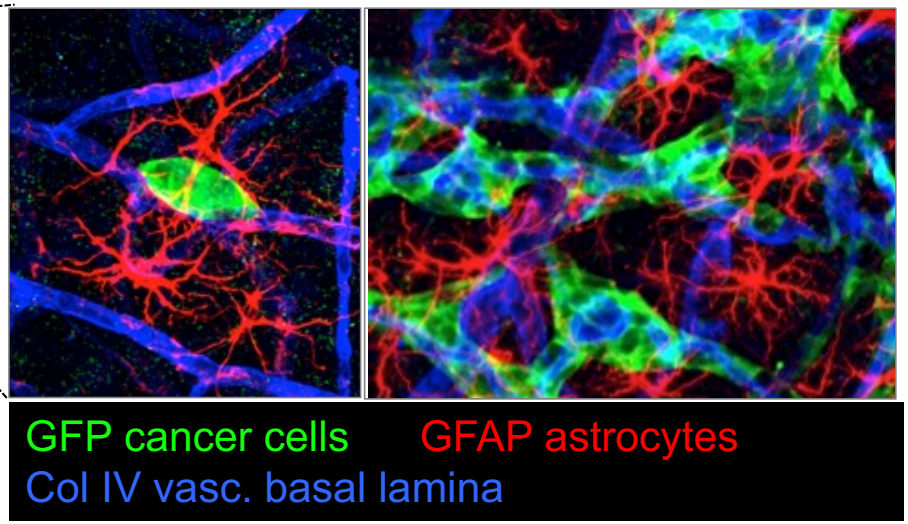


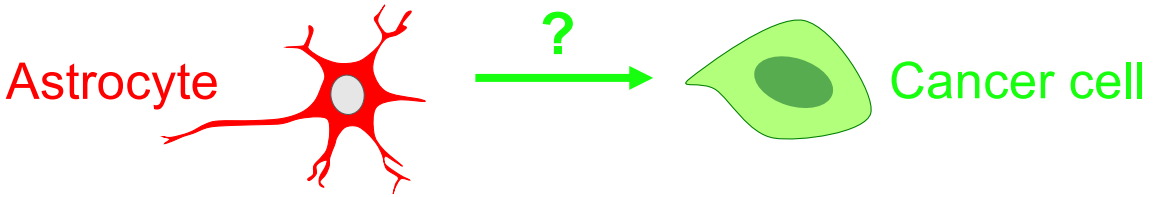
Memorial Sloan Kettering Cancer Center

Obenauf and Massagué 2015 Trends Cancer

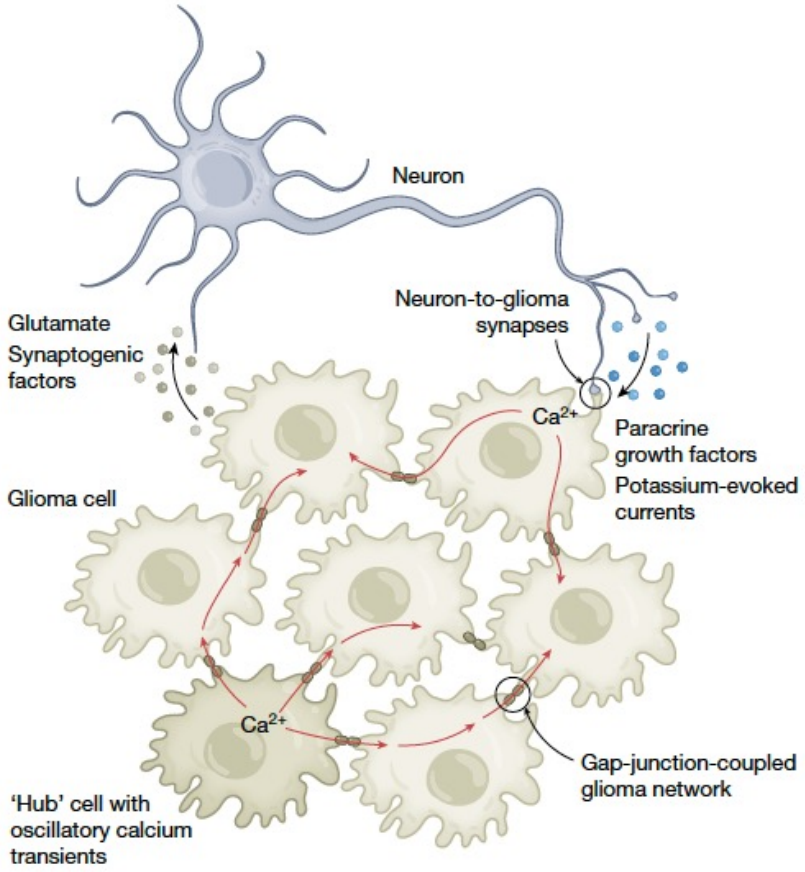
Reactive astrocytes: Journal Club!







Cancer Neuroscience: Metastasis Edition!



Mancusi R and M Monje 2023 Nature 618:468

Electrical and synaptic integration of glioma into neural circuits

High-grade gliomas are intimately tied to neuronal activity

- **High-grade gliomas** are the leading cause of CNS cancer-related death.
- Neuronal release of neuroligin-3 (**NLGN3**) is required for glioma progression.
- NLGN3 induces expression of **synaptic genes** in gliomas.
- Glioma cells resemble **OPCs**, which form synaptic connections to neurons.

Do gliomas also engage in synaptic communication, and is it essential for their progression?

Single-cell transcriptome shows expression of synaptic genes in OPC-like gliomas.

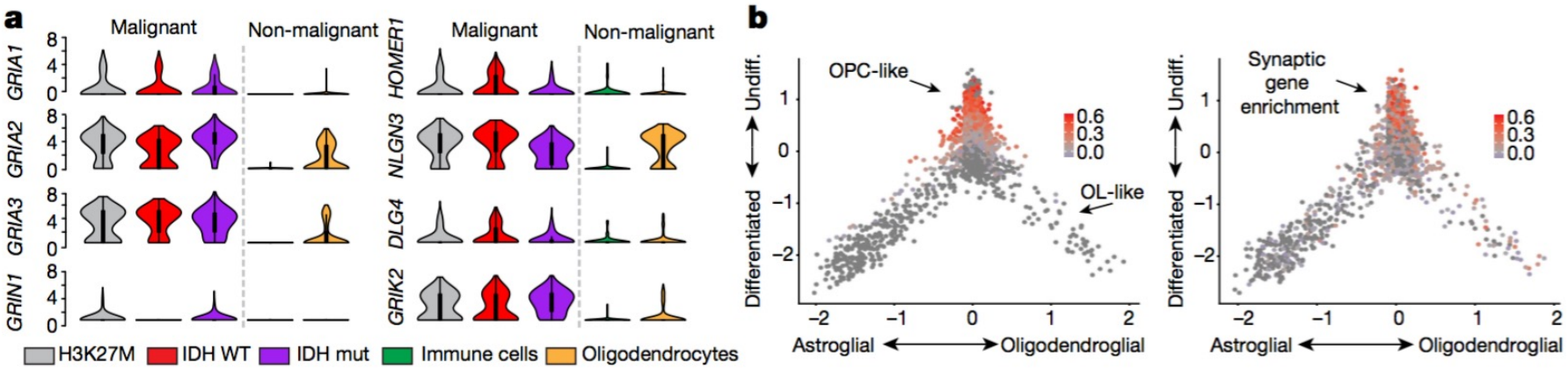
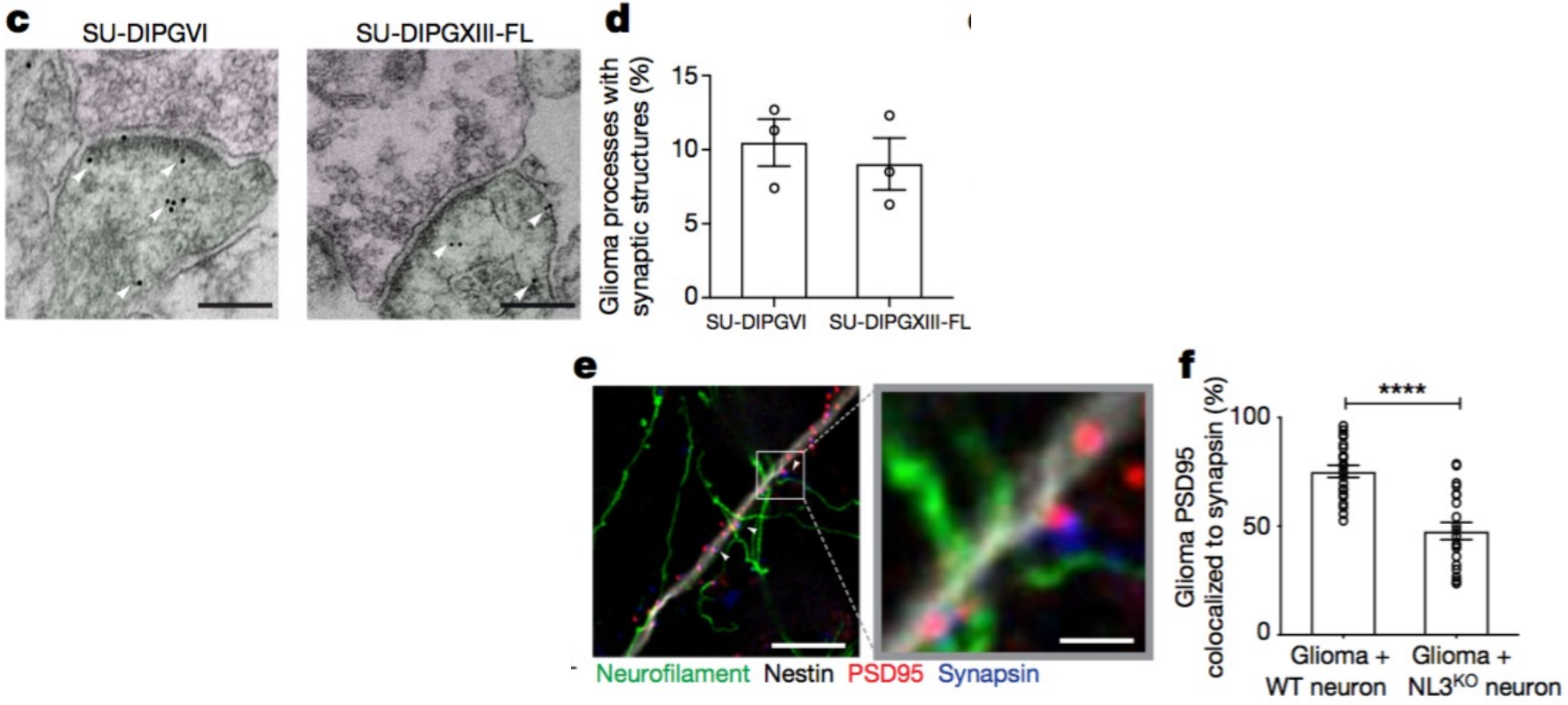


Fig. 1. a. Primary human biopsy from adult and pediatric high-grade gliomas. **b.** Principal component analysis of H3K27M+ DMG malignant single cells sorted from primary biopsies (n = 2,259 cells)

Gliomas cells indicate post-synaptic activity correlated with NLGN3 expression



Electrophysiological model for testing synaptic activity of gliomas

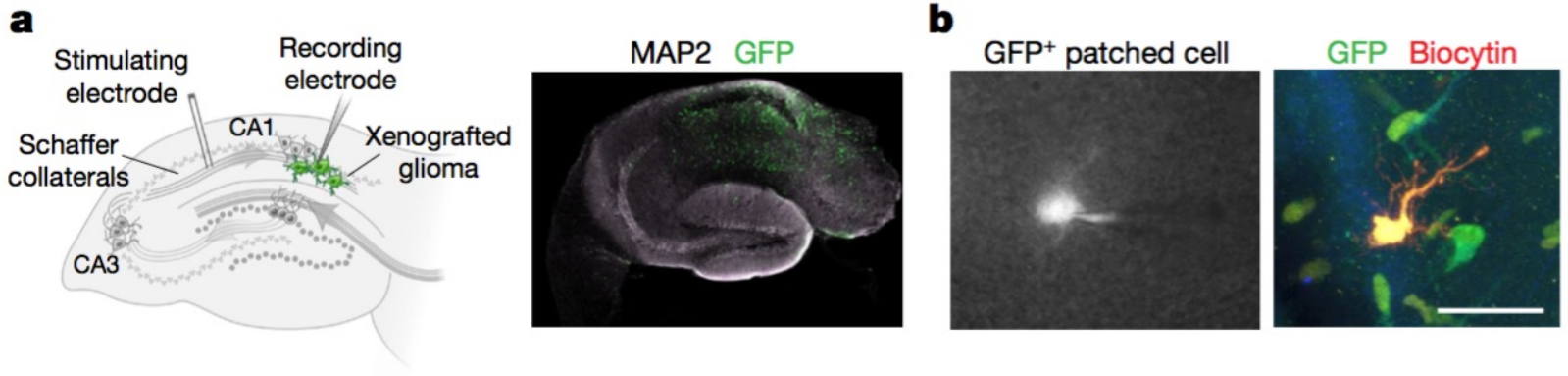


Fig. 2. a. b.

Gliomas show excitatory post-synaptic currents (EPSCs) facilitated by AMPARs

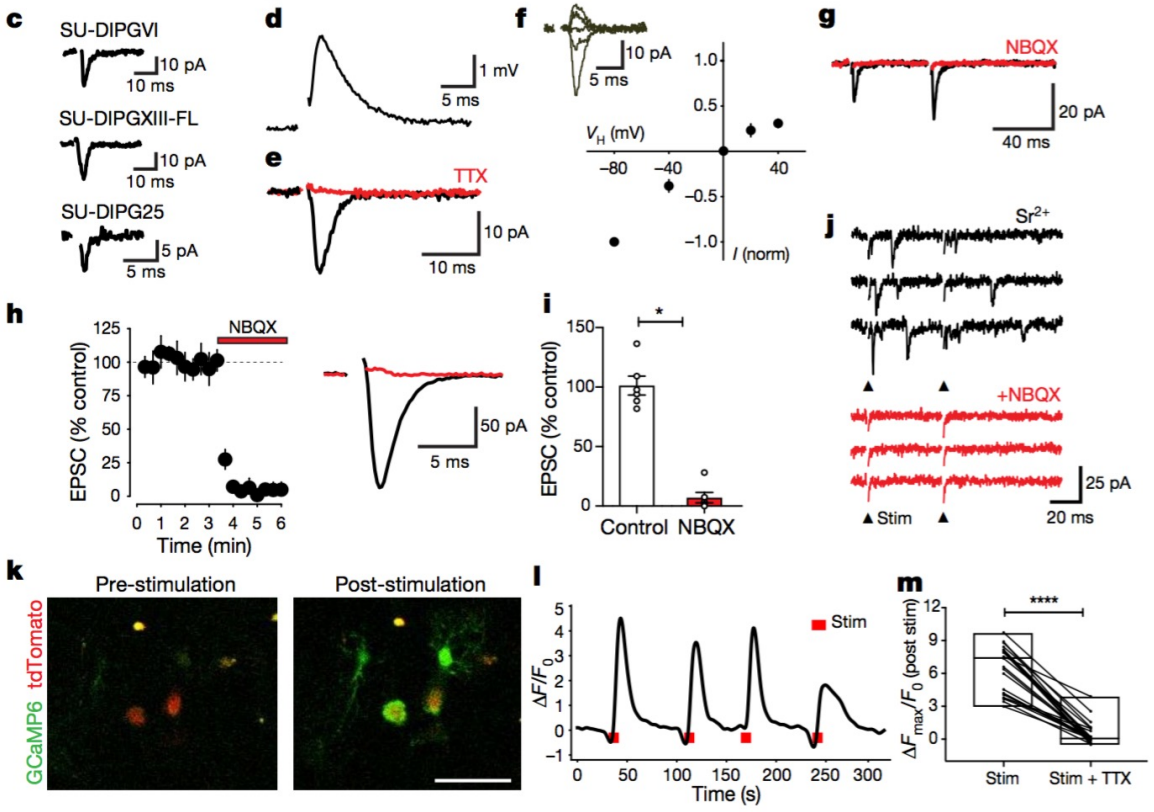


Fig. 2

Glioma also exhibit potassium-induced currents due to neuronal activity

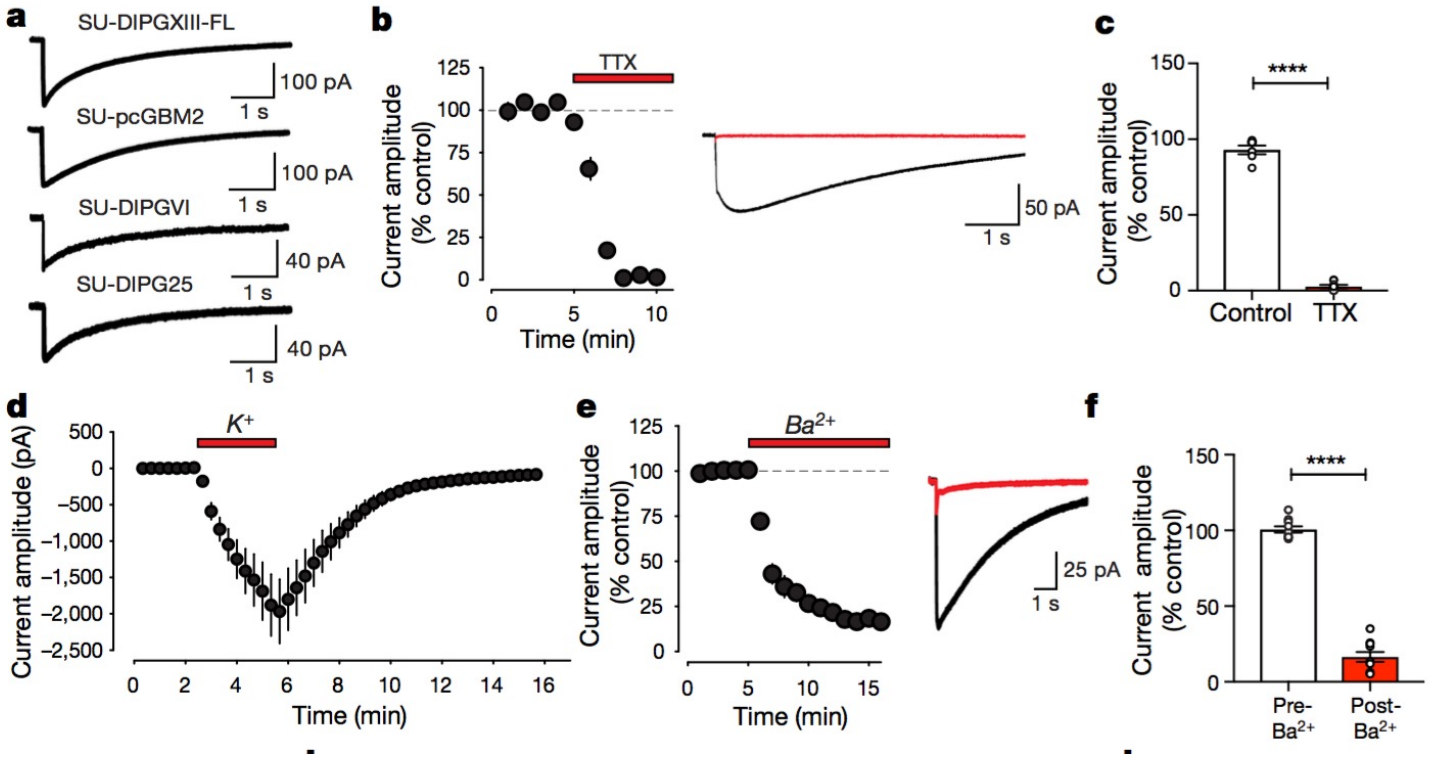


Fig 3.

Gap junction couplings can lead to longer potassium currents.

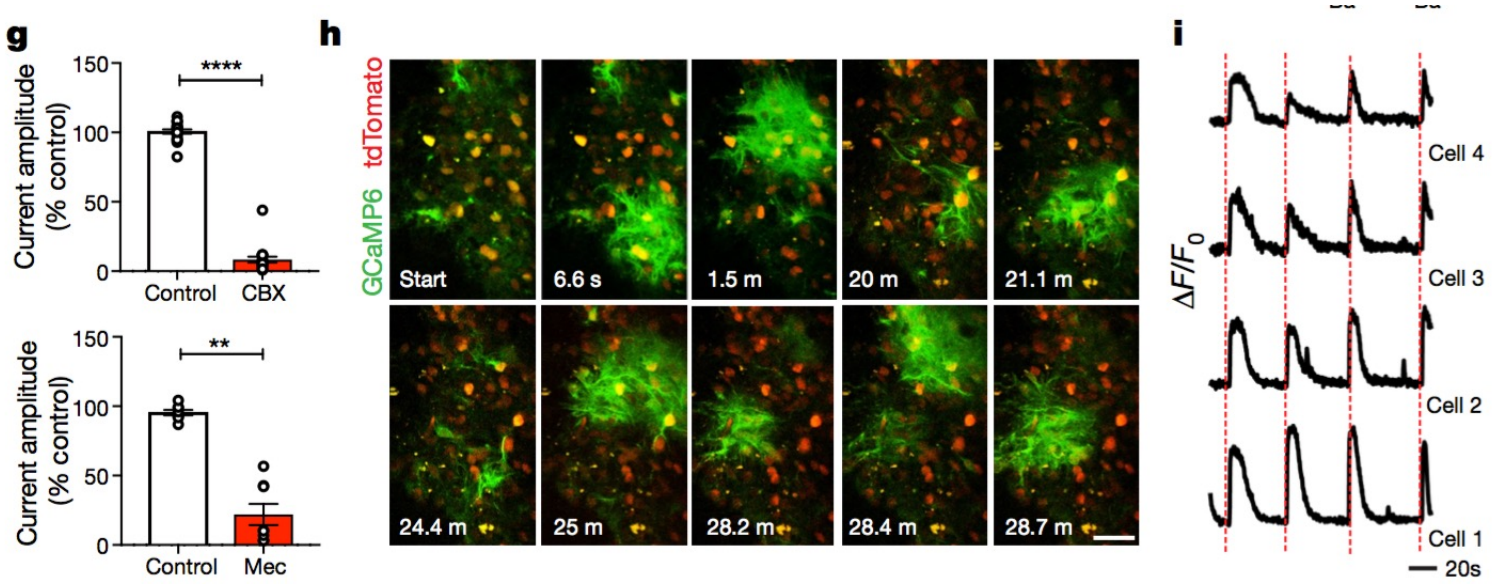


Fig 3.

Optogenetic depolarization of glioma induces proliferation.

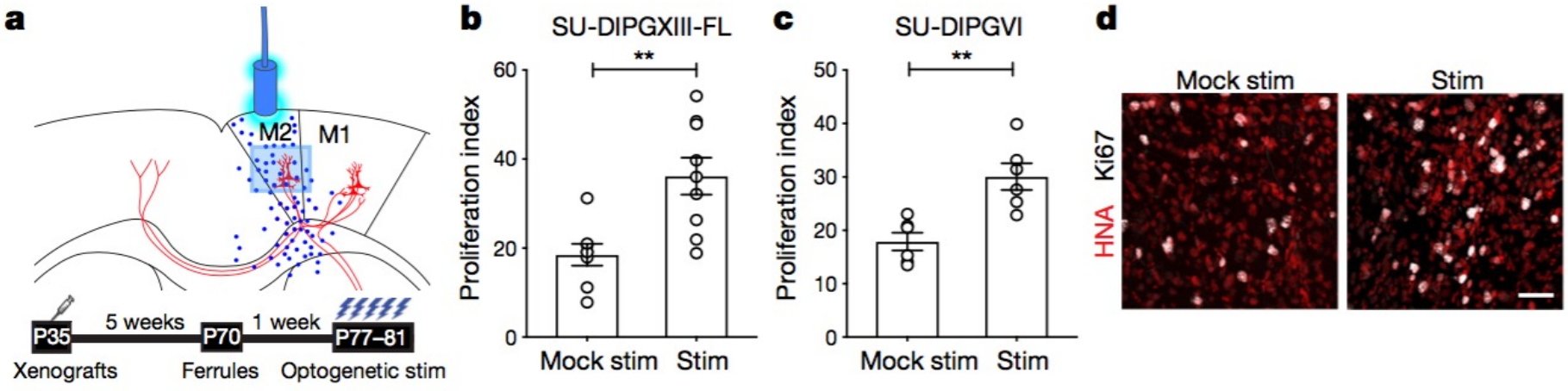


Fig 4.

GluA2 mediated activity regulates proliferation of glioma

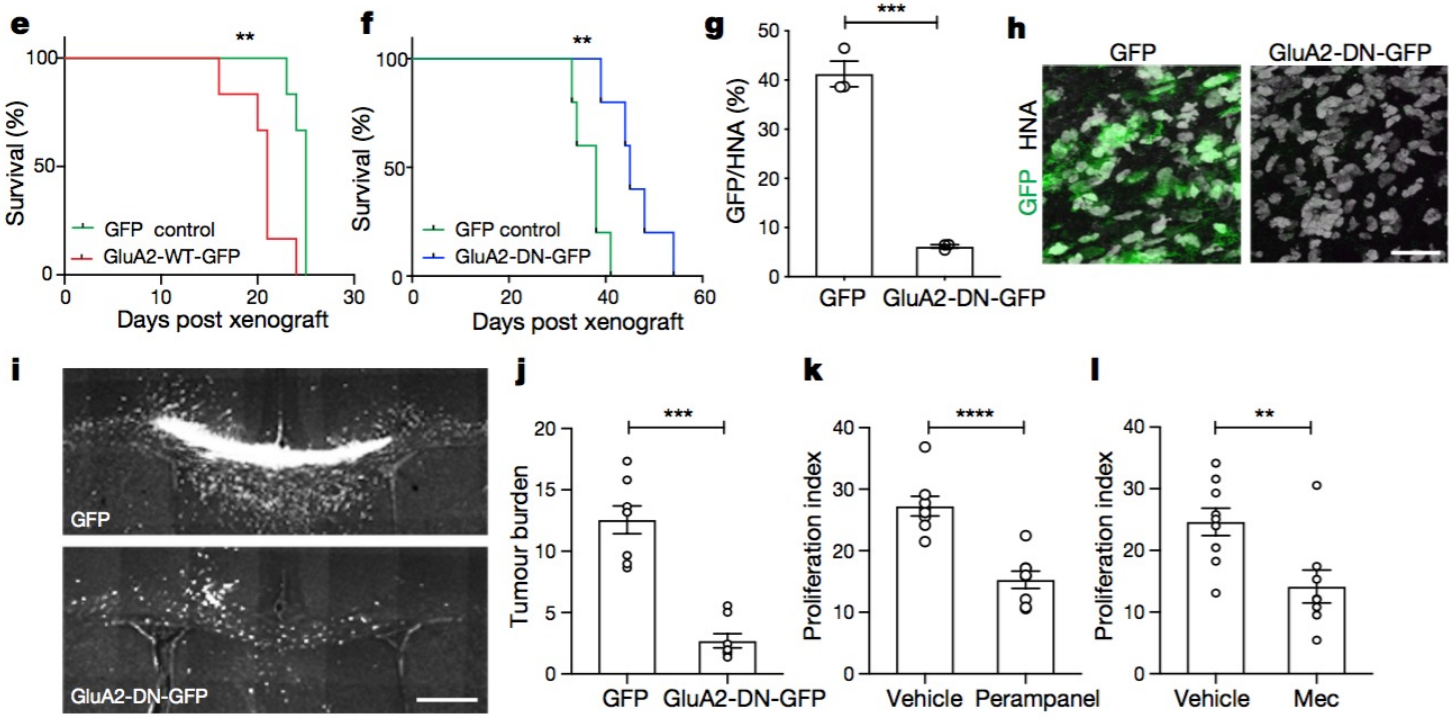
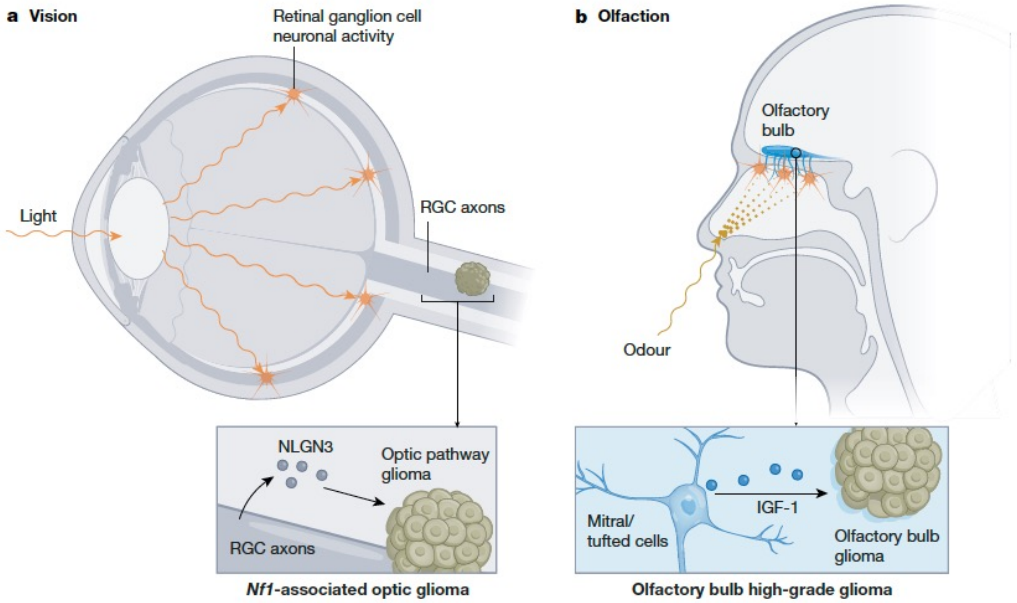


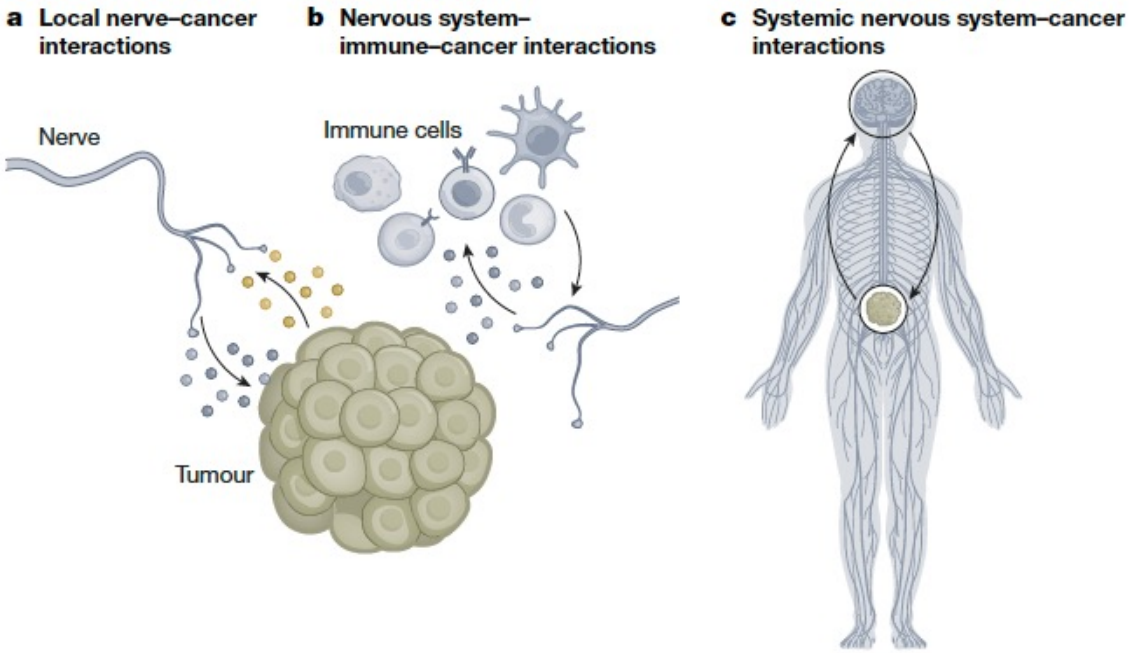
Fig 4.

Stimulus-induced generation of cancer cell supportive factors



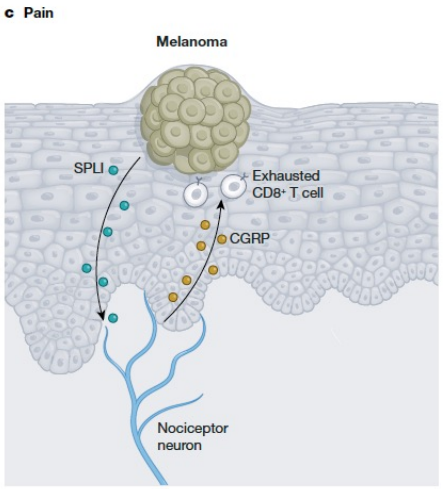
Mancusi R and M Monje 2023 Nature 618:468

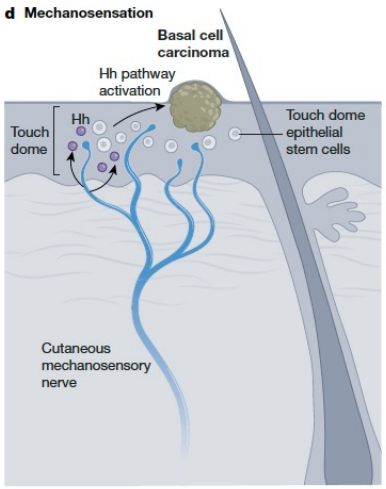
Might distant, extra-CNS interactions support CNS metastasis?

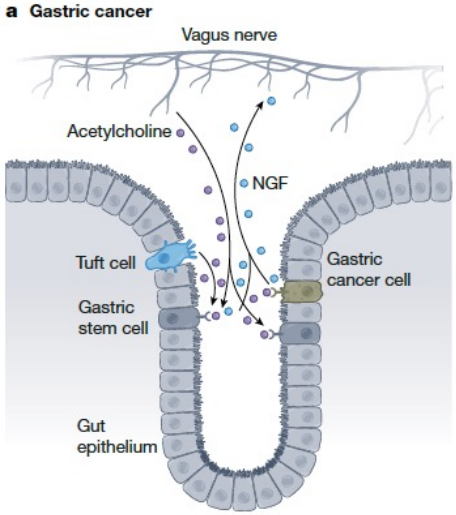


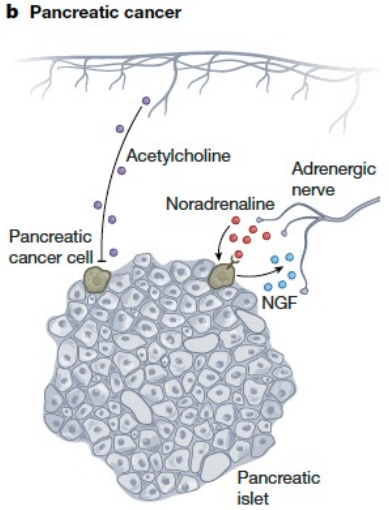
Mancusi R and M Monje 2023 Nature 618:468

Peripheral Nerves Support Cancer Cell Growth



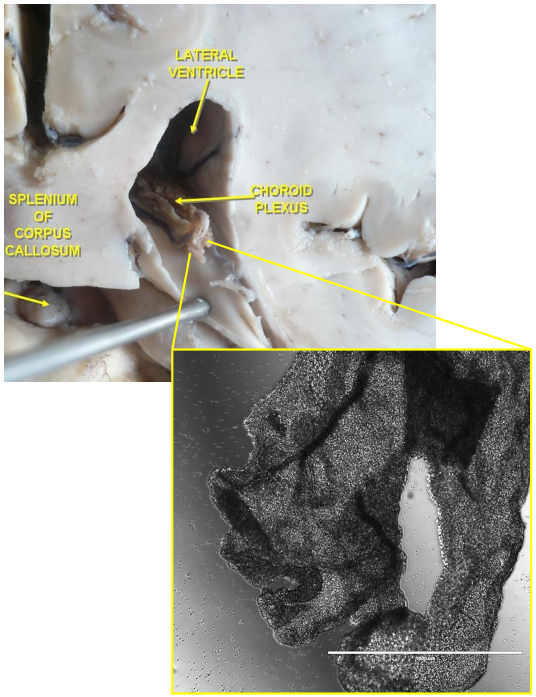




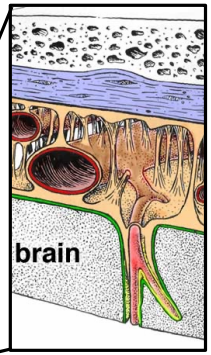


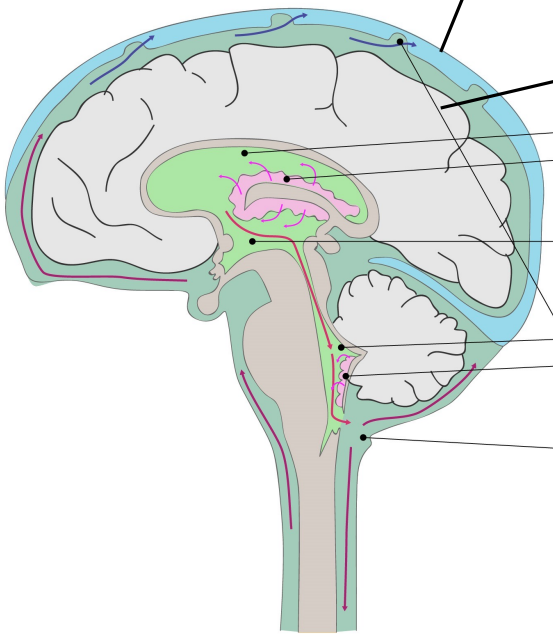
Mancusi R and M Monje 2023 Nature 618:468

CSF Circulation: Classical View



Virchow-Robin Spaces:
Potential spaces
Extending into cortex (but not beyond)
CSF-containing in pathologic contexts

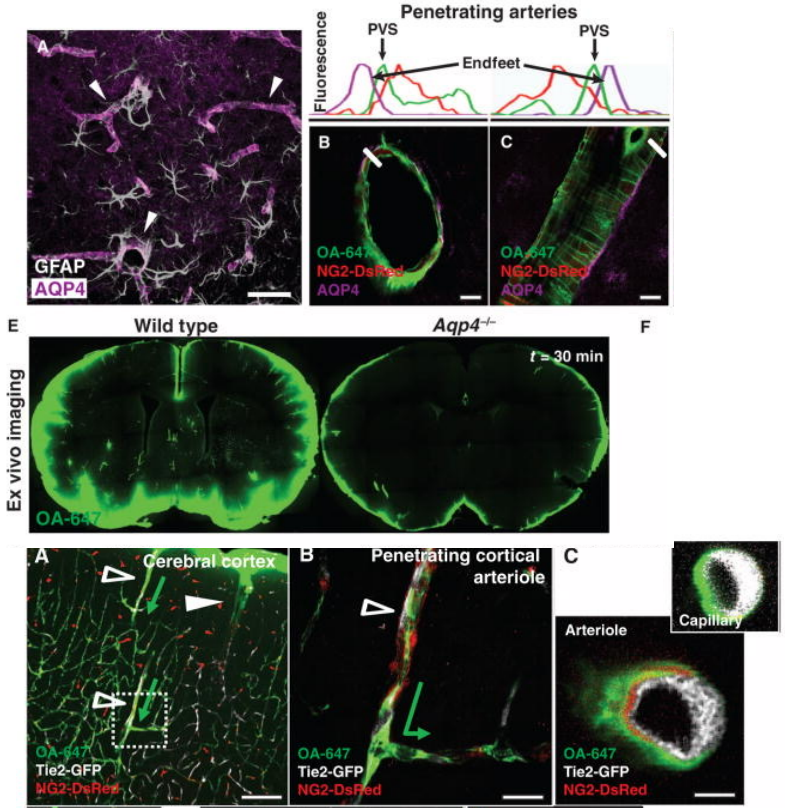




lateral ventricles
plexus choroideus
foramina Monroi
third ventricle
aquaeductus Sylvii
fourth ventricle
plexus choroideus
foramen Magendii
foramina Luschkae
subarachnoid space
arachnoid granulations
venous sinuses

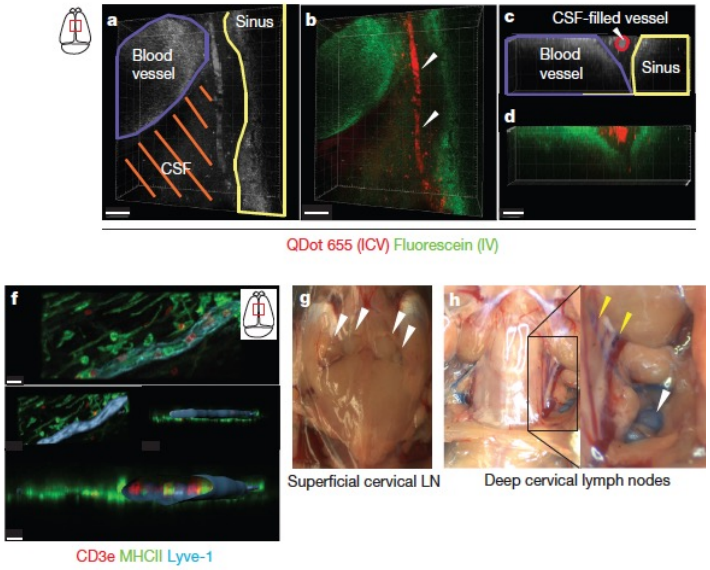
New Communications Uncovered Between CSF and Systemic Circulation

A. VRS Extend deep into the parenchyma



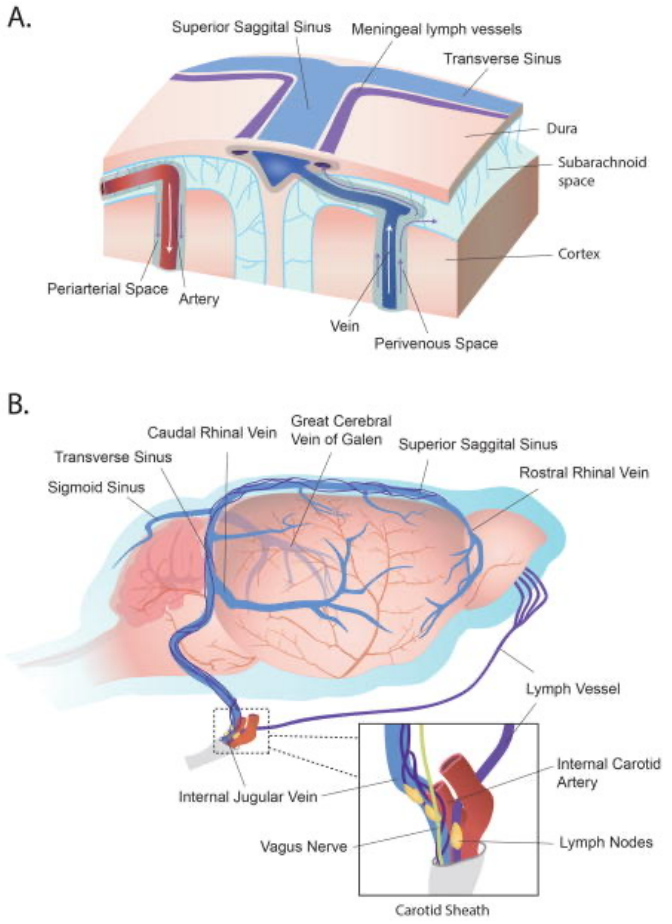
Iliff J et al 2012 STM 4(147):147

B. Communication between CSF and LN



Louveau A et al Nature 2015 523:337

New view of CSF circulation: Nedergaard & Kipnis



CSF is not a closed circulation

Communicates with systemic immune system through meningeal lymph vessels draining to LN

Primarily demonstrated through the use of small molecules (45 kDa or less).

Circulating immune cells? Macrophage?

Where are the meningeal vessels (really)- intradural or extradural?

VERY small, sparse vessels, is this vestigeal?

Plog BA and M Nedergaard 2018 Ann Rev Pathol 13:379

VEGF-C-driven lymphatic drainage enables immunosurveillance of brain tumours

Eric Song[1], Tianyang Mao[1], Huiping Dong[1], Ligia Simoes Braga Boisserand[2], Salli Antila[3], Marcus Bosenberg[1,4,5], Kari Alitalo[3], Jean-Leon Thomas[2,6,8*] & Akiko Iwasaki[1,3,7,8*]

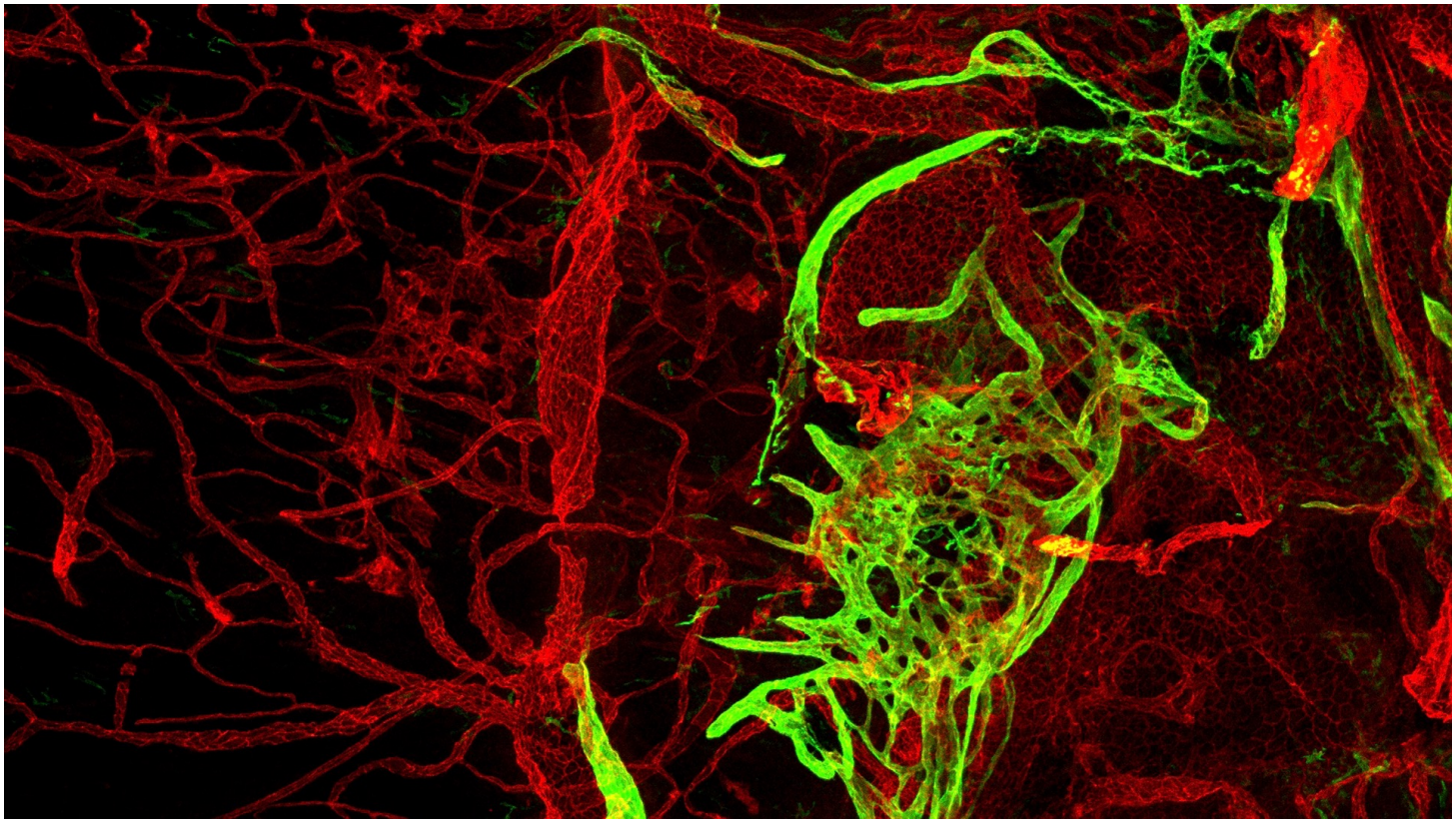
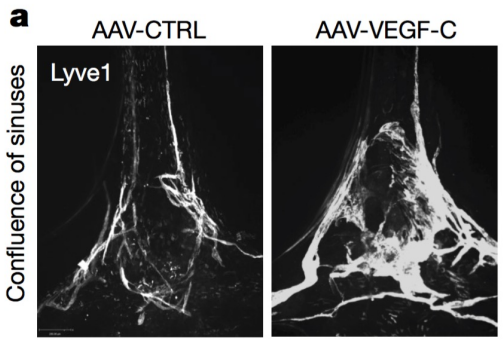
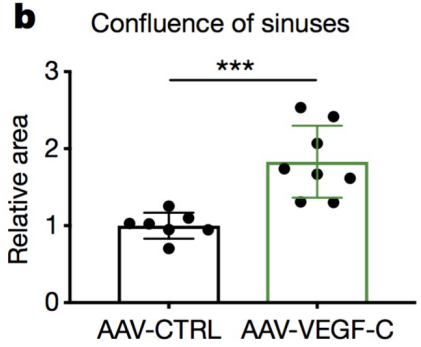


Photo: Ahn JH and Koh GY/IBS Center for Vascular Research

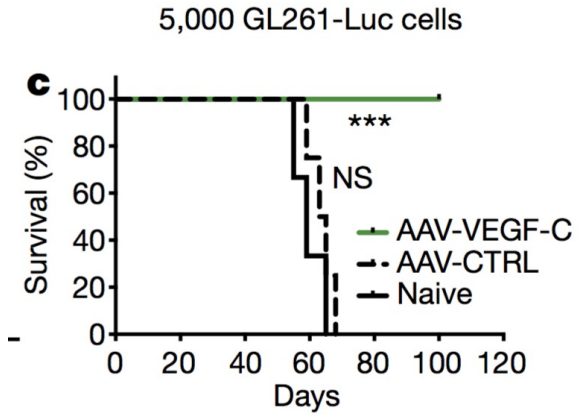
VEGF-C mediates protection against glioblastoma



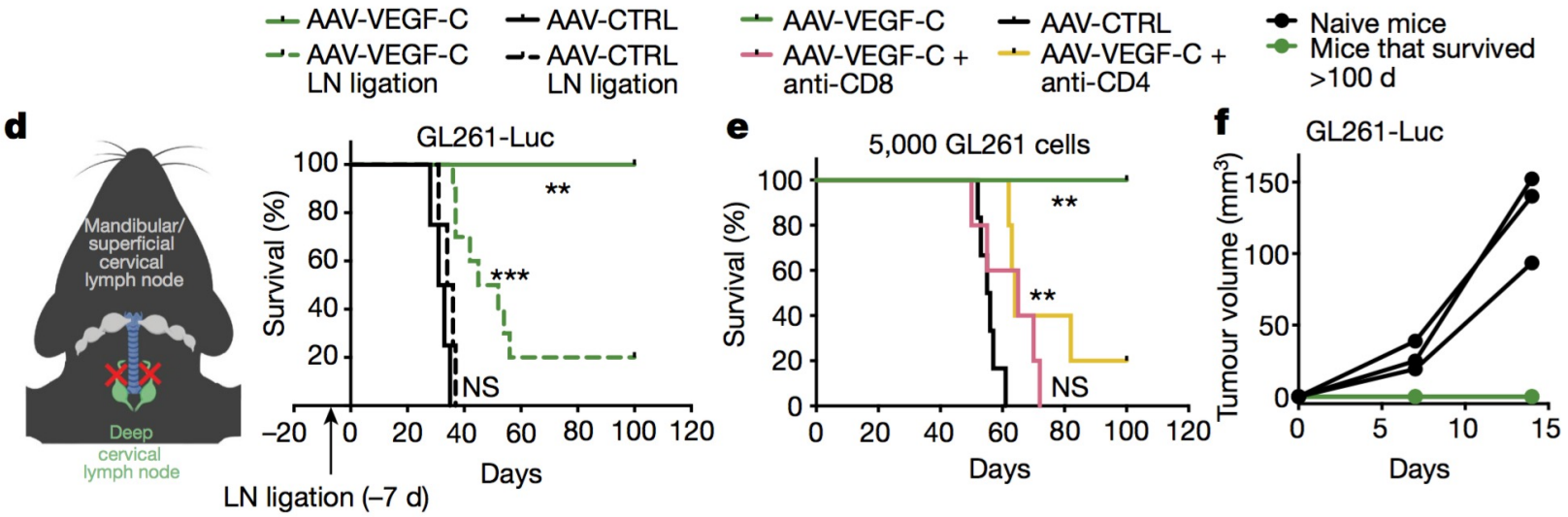


VEGF-C treatment remodels meningeal lymphatics by increasing dural confluence of sinuses.

Prophylatic treatment (2mo) with VEGF-C causes complete glioblastoma tumor rejection



VEGF-C protection depends on draining lymph nodes and T cells

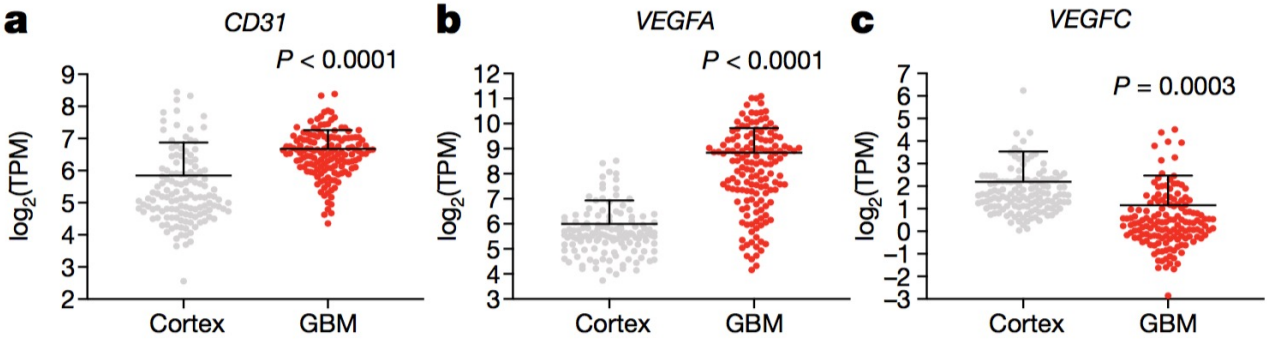


Ligation of deep cervical lymph nodes eliminates protection against tumor

Depletion of CD4+ and CD8+ T cells also negates VEGF-C mediated protection

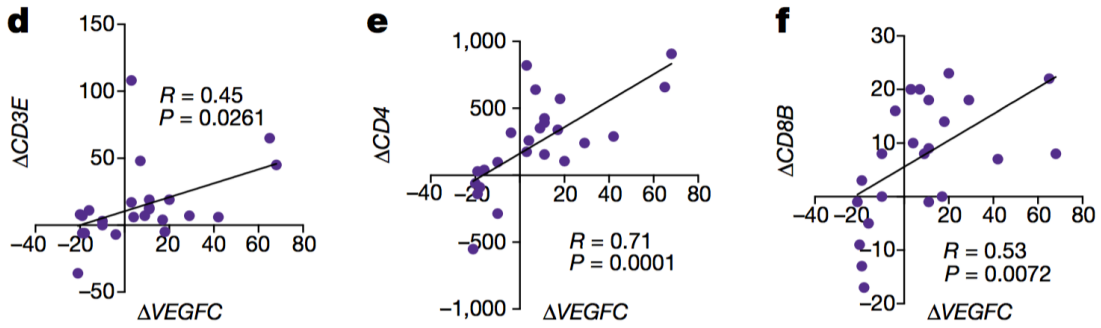
VEGF-C treated mice show systemic memory when rechallenged with tumor

Human glioblastoma is deprived of lymphangiogenic signals

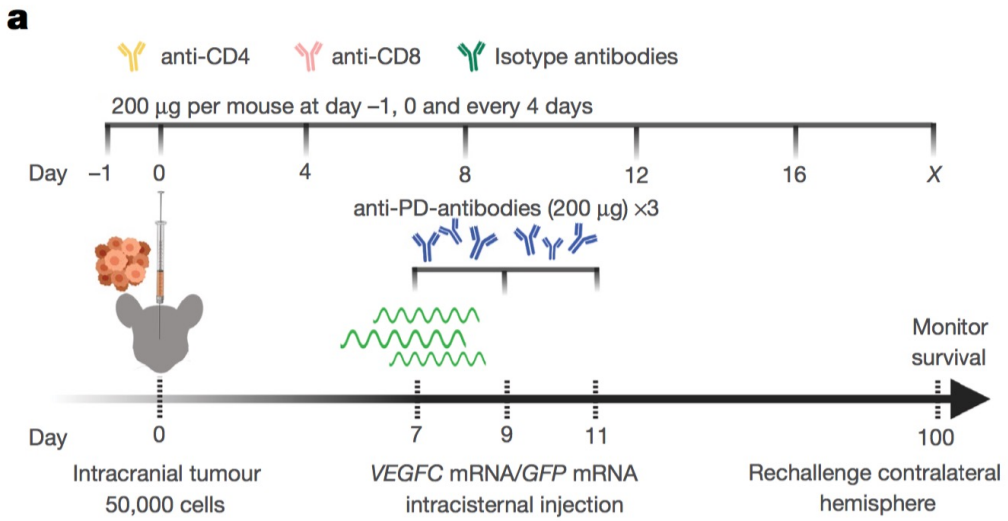


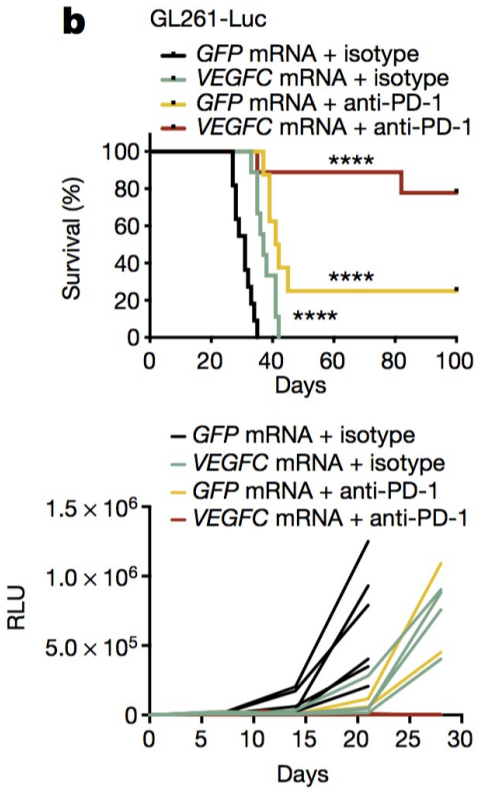
Human glioblastoma tumors show higher levels of CD31 and VEGFA, but **lower levels of VEGF-C**.

In patients treated with antiPD-1, expression of VEGF-C was correlated with increase of T-cell infiltration



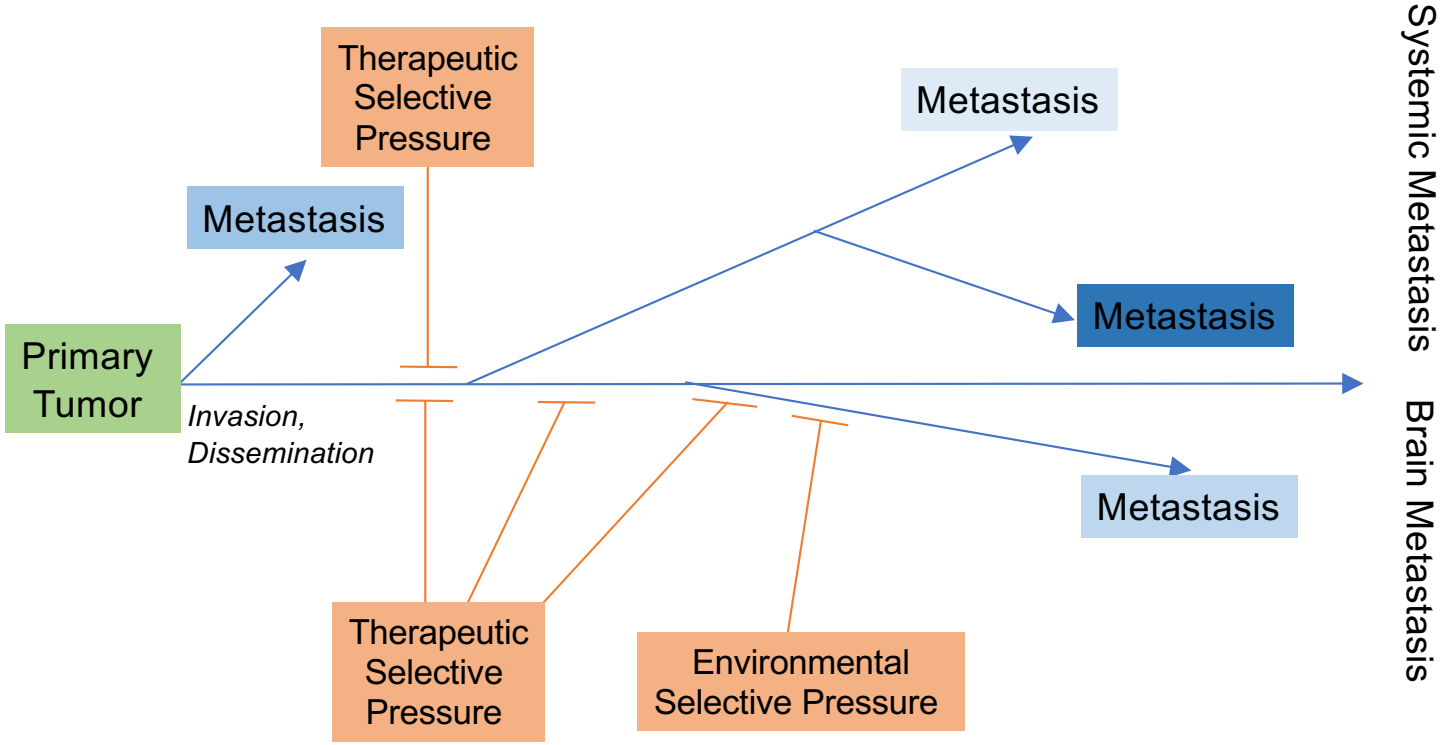
VEGF-C therapeutic treatment potentiates anti-PD-1 therapy





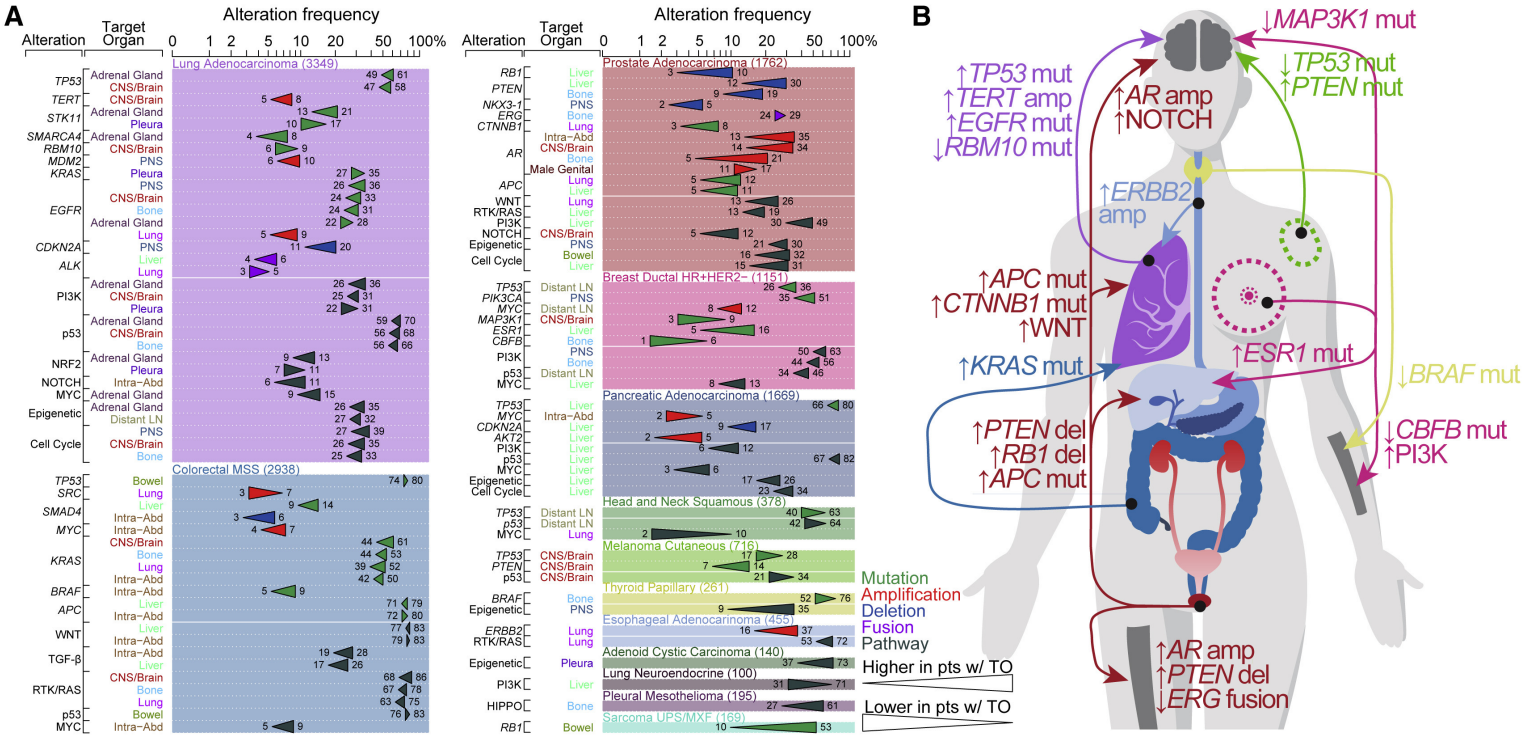
Treatment with VEGF-C mRNA paired with anti-PD-1 therapy increased survival as compared to anti-PD-1 therapy alone.

Metastasis: An Evolutionary Problem



Evidence of Divergent Evolution in Metastasis:

***Very** difficult to predict- must sequence!*



Nguyen B et al 2022 Cell 3(3):563

Memorial Sloan Kettering Cancer Center

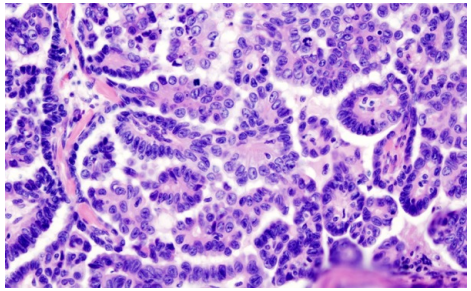
How to clinically cope with this dynamic genetic heterogenity?

Gold Standard:
Sequencing of Resected Tissue

- Not all lesions are accessible
- One metatasis at a time

- Invasive procedures require recovery prior to subsequent treatments

- Each procedure adds potential additional morbidity

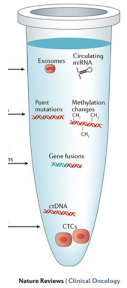


New Approach:
Liquid Biopsy

- No need for surgical approach
- Ensemble read of all metastases

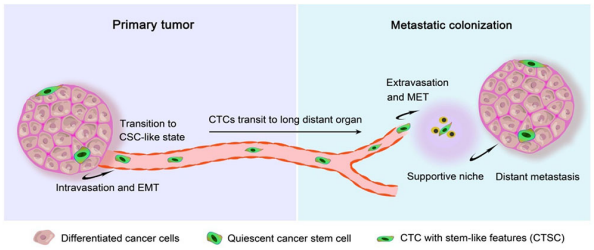
- No delay in treatment schedule
- Serial ”biopsies” possible

- Not a replacement for Pathologic diagnosis

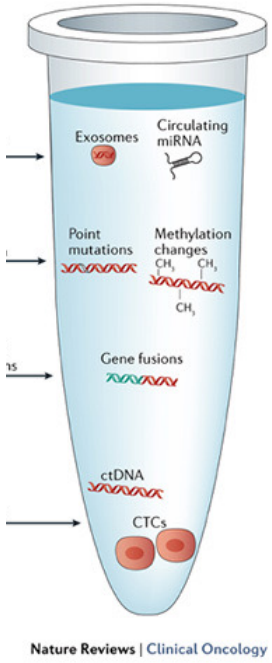


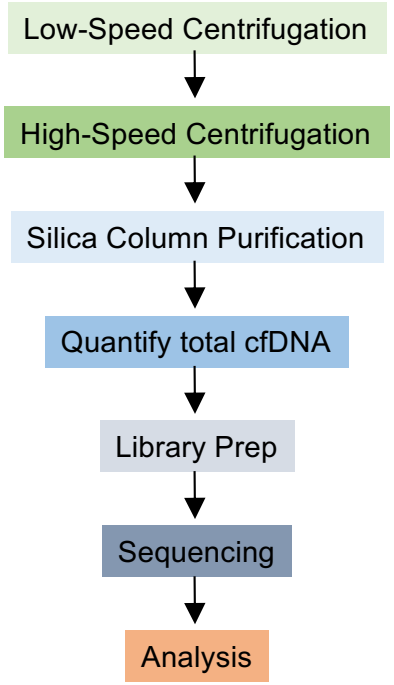
“Liquid Biopsy”

Analysis of a biological fluid to gain knowledge of cancer’s stage or molecular composition.

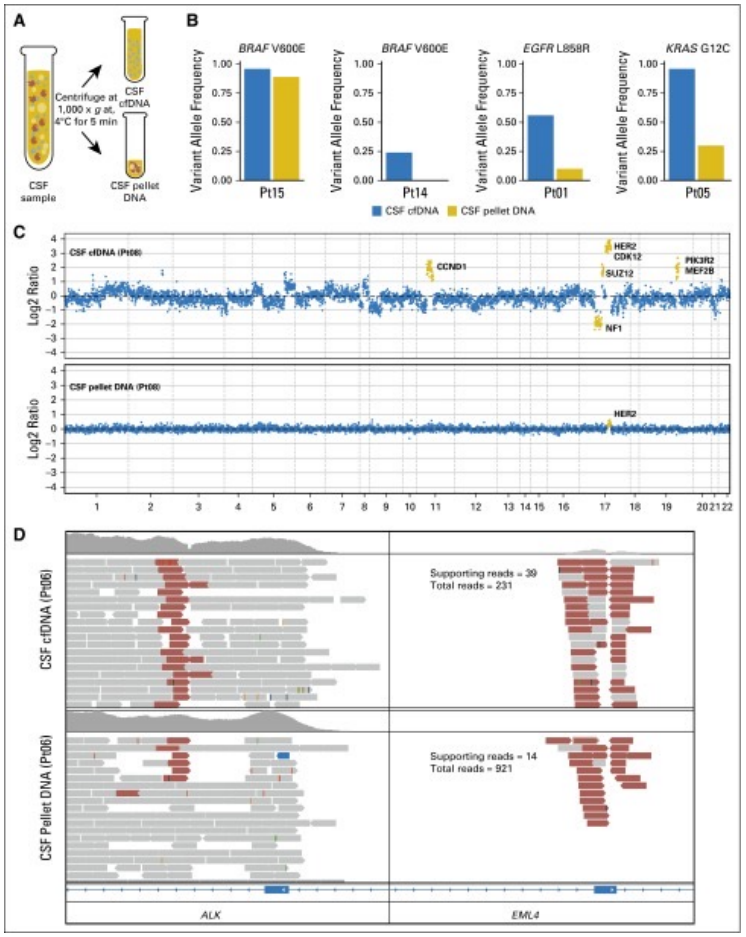


Front. Pharmacol., 01 February 2017

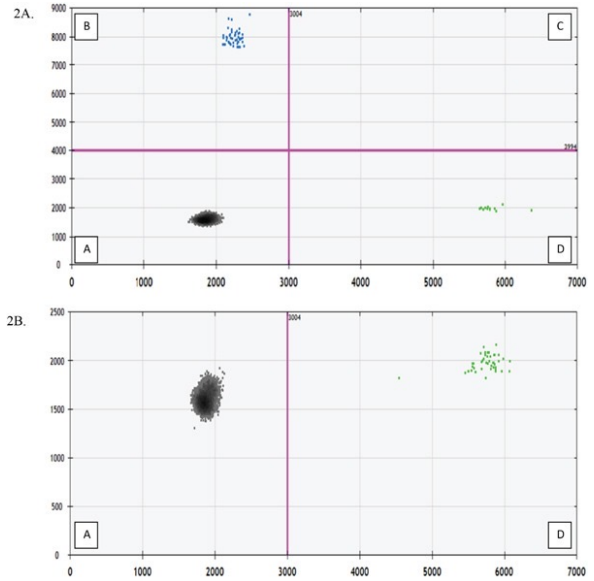




ctDNA Technology: Cells vs. cfDNA



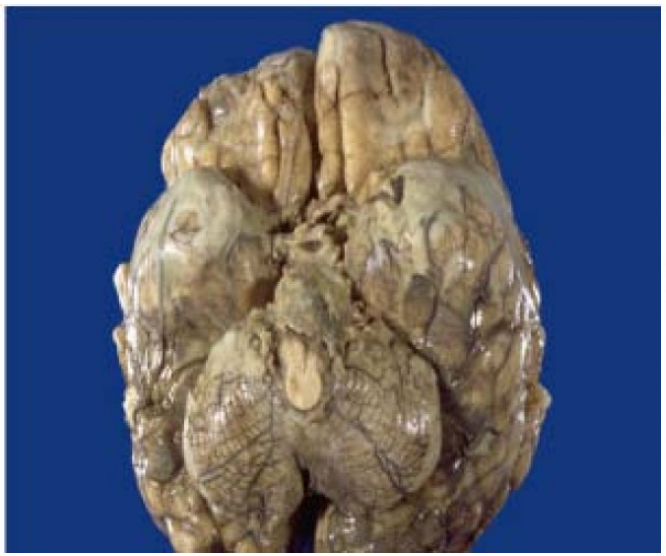
Pentsova EI et al 2016 JCO 34(20):2404

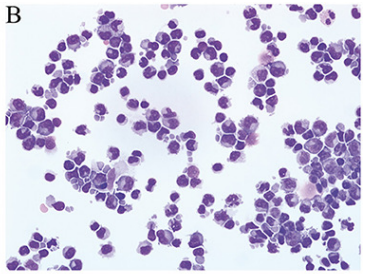


Momtaz P et al 2016 Oncotarget 7(51):85430

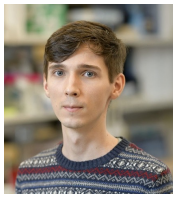
Immune System and CNS Metastais:
Leptomeningeal Metastasis AKA Carcinomatous Meningitis

Hermann Oppenheim





Oppenheim, H. 1888 “Ueber Hirnsymptome bei Carinomatose ohne nachweisbare Veränderungen im Gehirn” Charité Annalen, Vol XIII, p335
Schwarz und Bertels 1911 “Uber ’Meningitis’ carcinomatosa” Deutch. Zeitscher. F. Nervenheilk, Vol XLII, p85

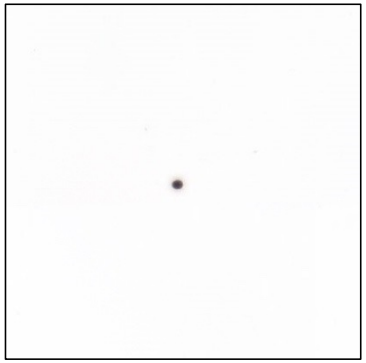


Jan Remsik

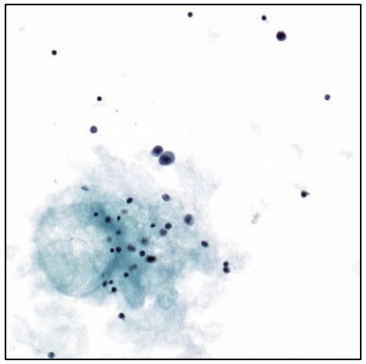
Pleocytosis is a hallmark of leptomeningeal metastasis (LM)

A

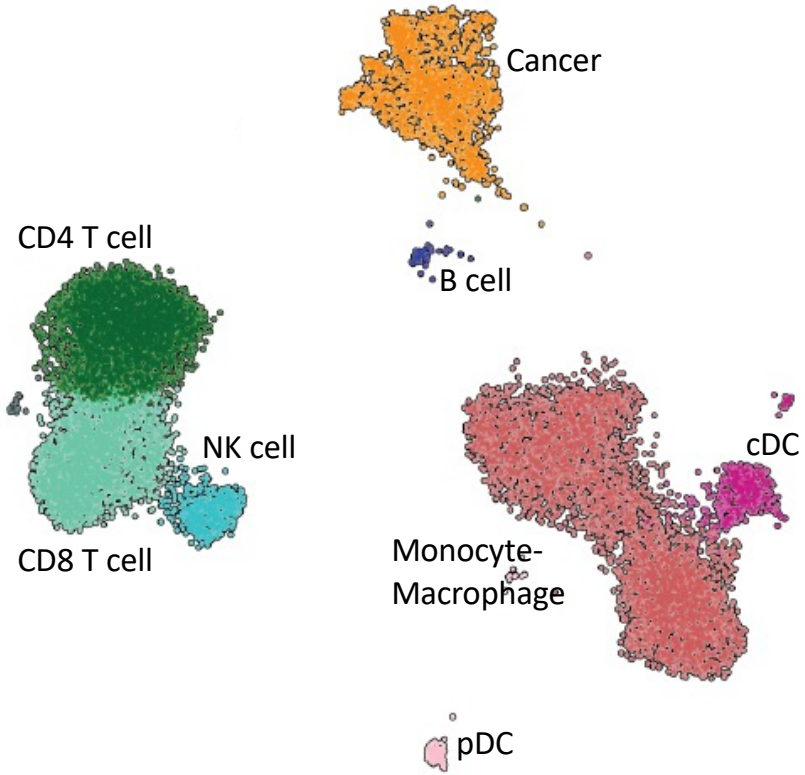
LM-



LM+

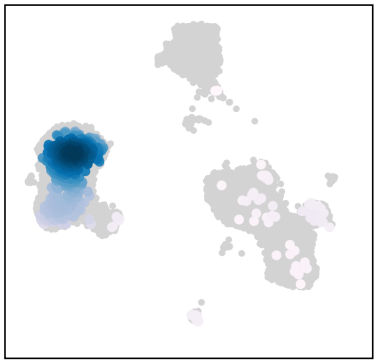


B

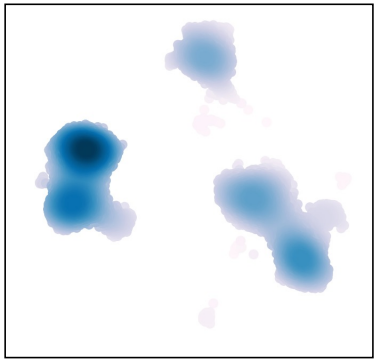


C

LM-



LM+

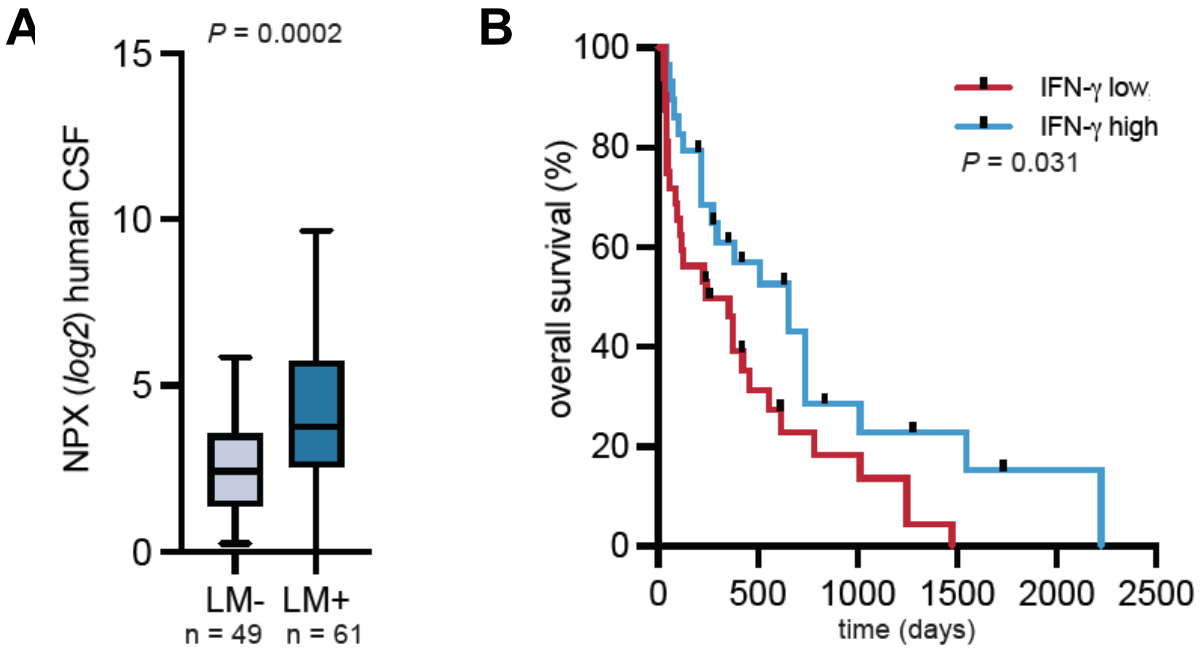


Memorial Sloan Kettering Cancer Center

Remsik *et al*., bioRxiv 2023

N = 10 patients; 6 LM+, 4 LM-; 5 Breast Ca, 5 NSCLC

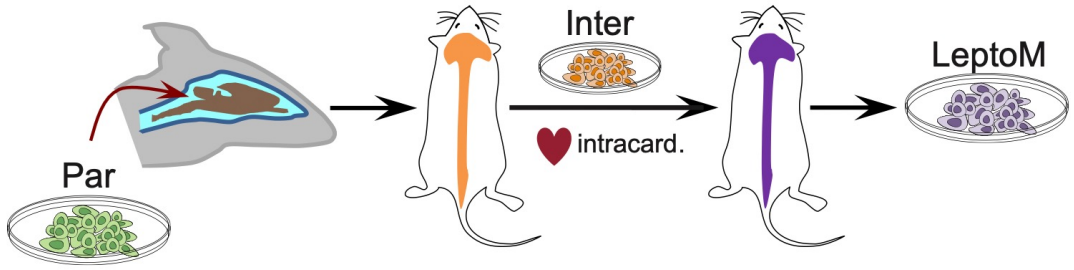
CSF Interferon-ɣ at diagnosis correlates with overall survival



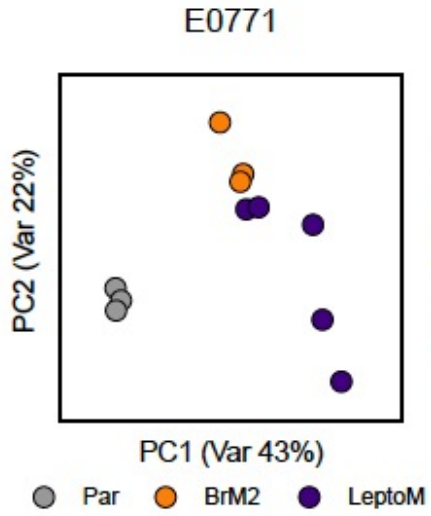
Memorial Sloan Kettering Cancer Center

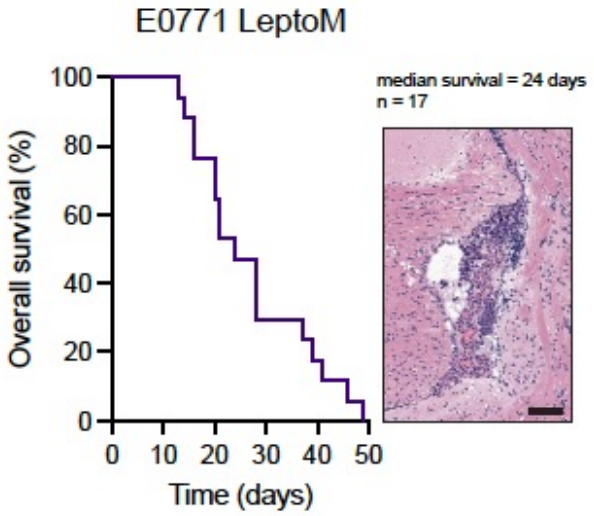
Remsik *et al*., bioRxiv 2023

Six new syngeneic mouse LM models



lung Ca	LLC	C57Bl/6
melanoma	B16-F10	
	Yumm5.2	
breast Ca	E0771	
	4T1	Balb/C
	EMT6	





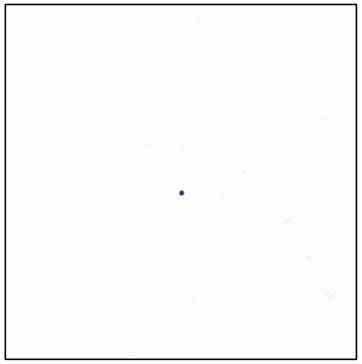
Remsik *et al*., bioRxiv 2023

Russell Kunes

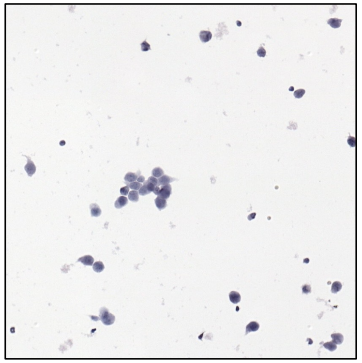
Mouse models of LM accurately mimic human disease

A

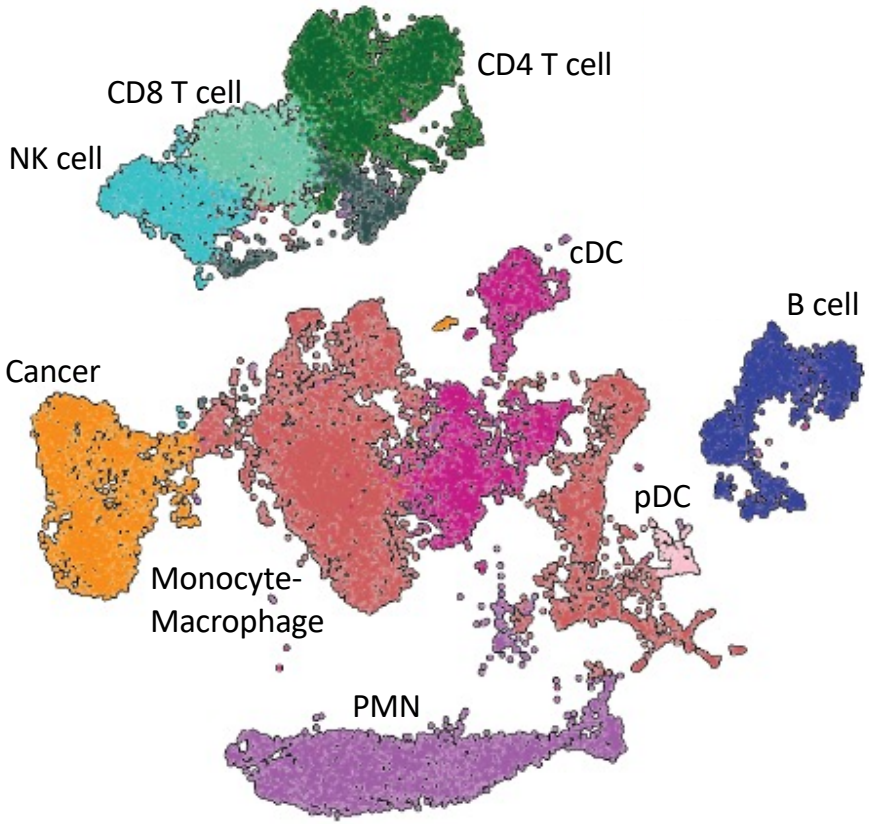
LM-



LM+



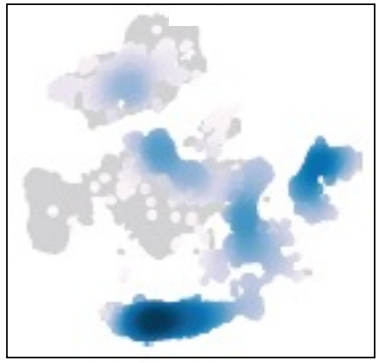
B



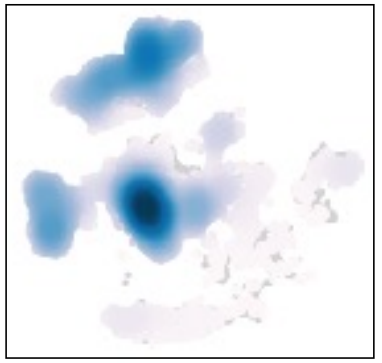
LLC LeptoM in C57/Bl6

C

LM-



LM+

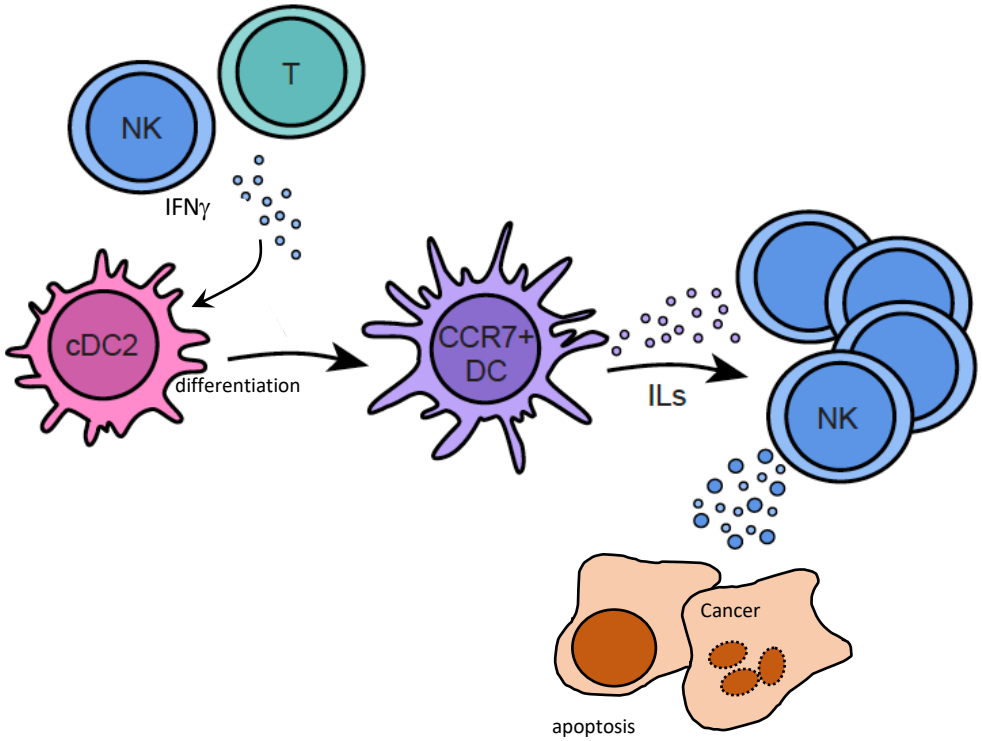


Memorial Sloan Kettering Cancer Center

Remsik *et al*., bioRxiv 2023

NK cells are effectors of cDC-orchestrated killing

Anti-cancer inflammatory signals function differently in the leptomeninges

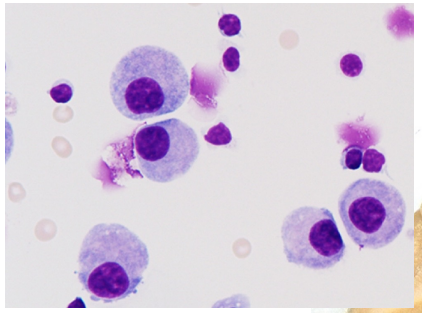


Remsik *et al*., bioRxiv 2023

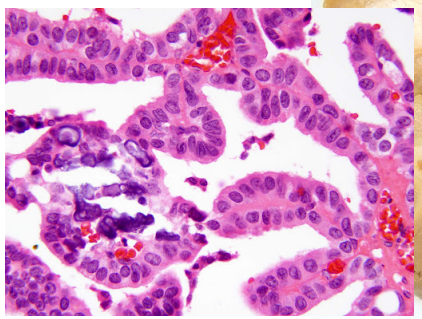
Memorial Sloan Kettering Cancer Center

CNS Metastasis exemplifies cancer as whole-person disease

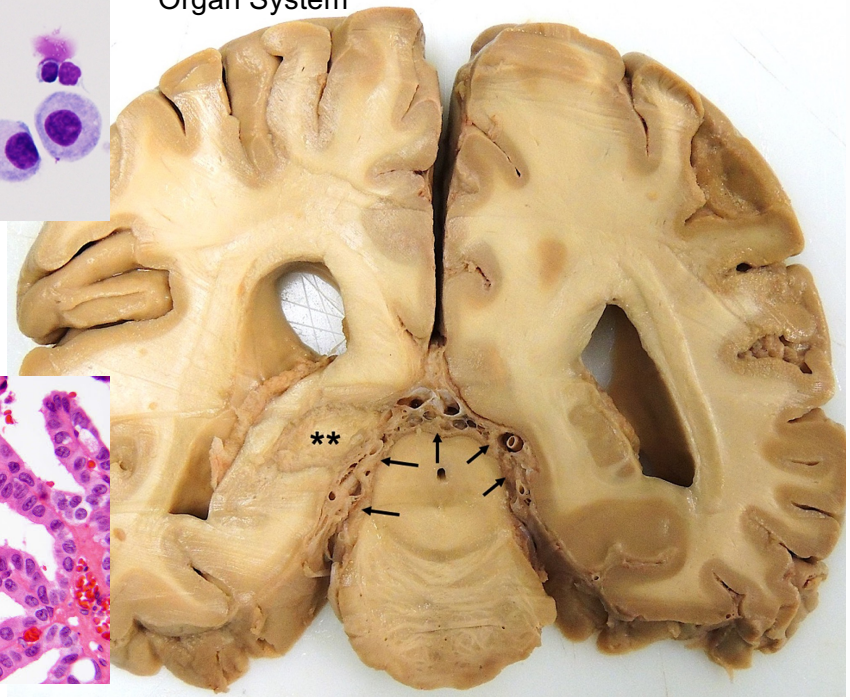
Cancer Cell



Microenvironment



Organ System



Organism