Preclinical Insights into Brain Metastasis of Breast Cancer

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Women's Malignancies Branch

Brain Metastases

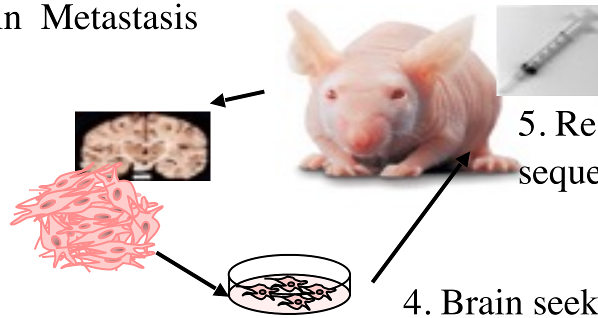
- Brain metastases outnumber primary brain tumors by 10 to 1.
  - Most common primary tumor sites are lung (50-60%), breast (15-20%), melanoma (5-10%) and GI tract (4-6%)
- Breast Cancer
  - Most prevalent in the triple negative and Her-2+ subpopulations
  - Increase being seen in Her2+ as a first site of metastatic therapy relapse
- Traditional drug therapies are ineffective for brain metastasis

Cancer 58:832, 1986
Cancer 69:972, 1992
Cancer 76:232, 1995
Int J Clin Onco 14:299, 2009

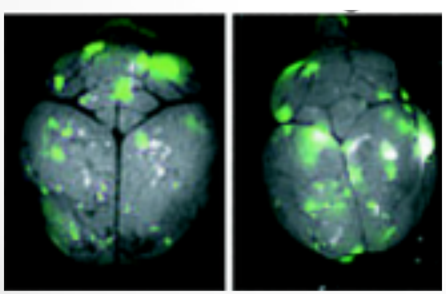
In Vivo, Quantifiable Brain Metastasis Assay

- Intracardiac injection of **231-BR** **eGFP** labeled, “brain seeking” variant of breast cancer cells (triple neg).

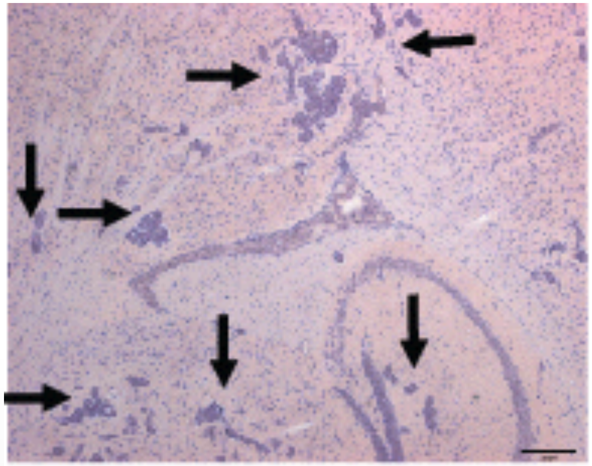
- 1-2 month



231-BR eGFP



H&E 231-BR metastases



Good Metastasis Research = Good Preclinical Models

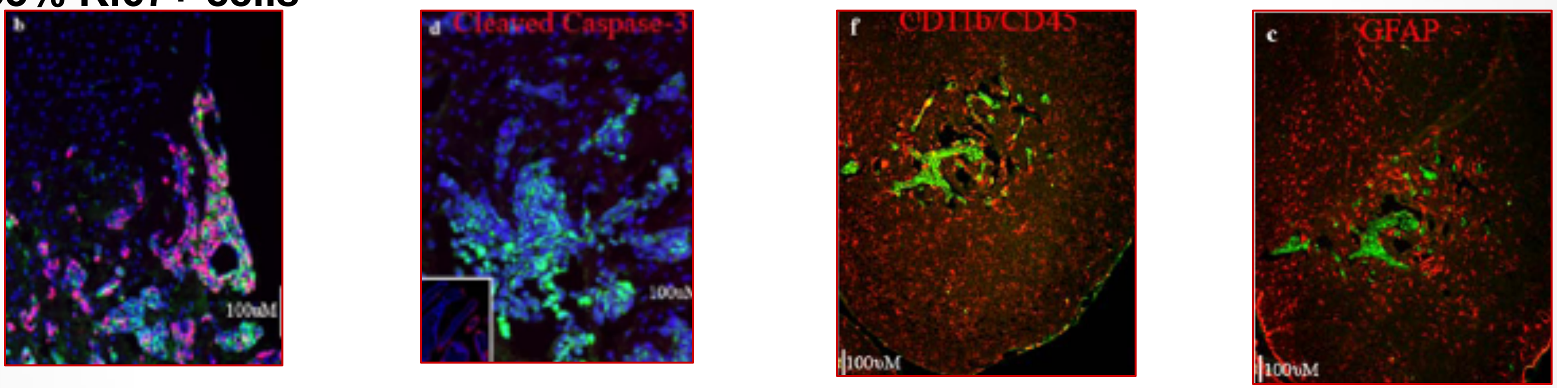
231-BR Model is Similar to Human Brain Metastases in Proliferation, Apoptosis and Neuro-inflammatory response.

Tumor Cells with DAPI and immunoflourescent markers

Fitzgerald et al.
Clin. Exp. Met.., 2008

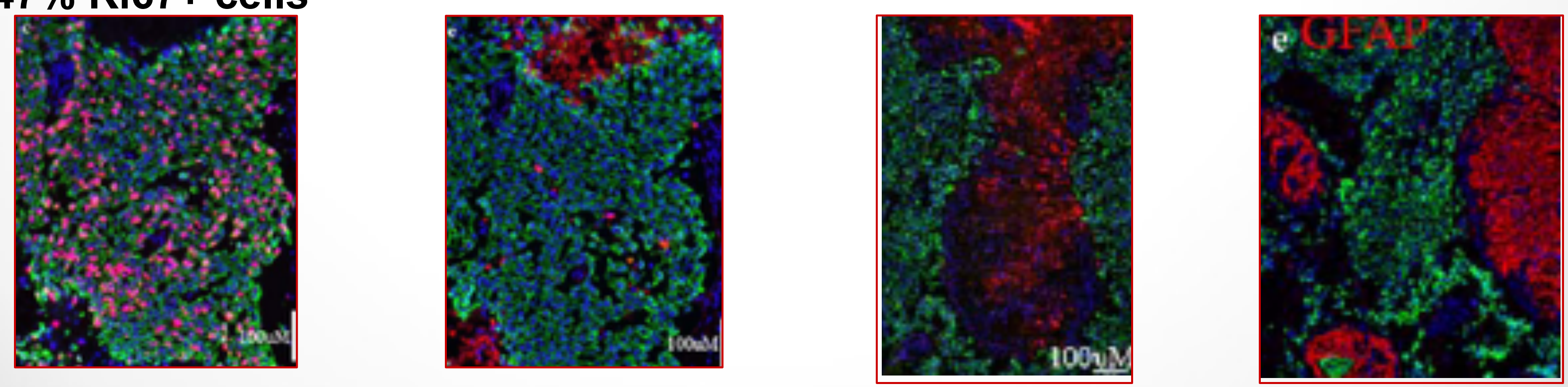
53% Ki67+ cells

M
O
U
S
E



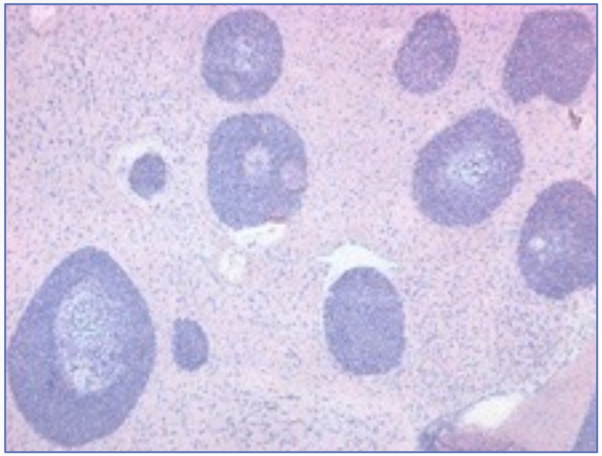
47% Ki67+ cells

H
U
M
A
N

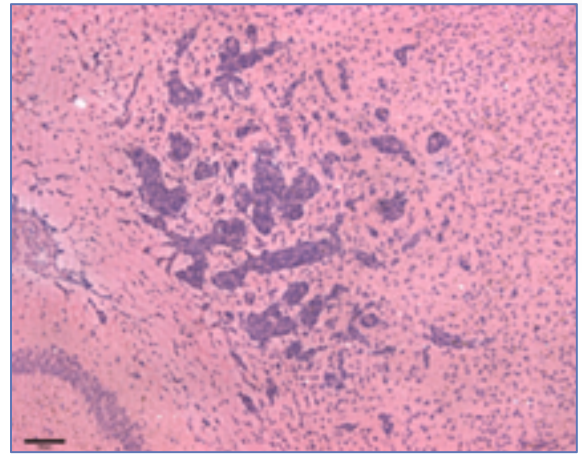


Ki 67 **Cleaved Cas 3** **Microglia** **Astrocytes**

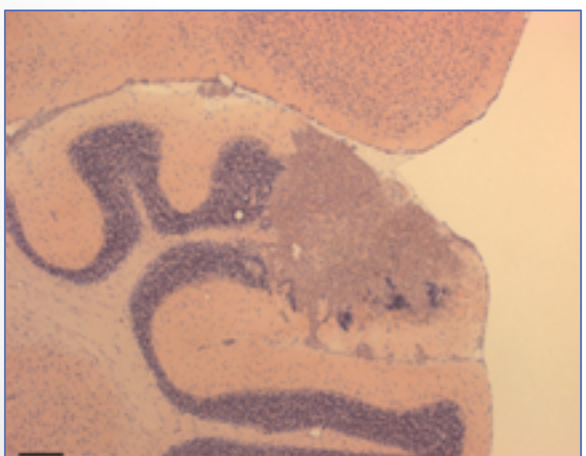
Additional Experimental Brain Metastasis Models Reflect the Heterogeneity of the Disease



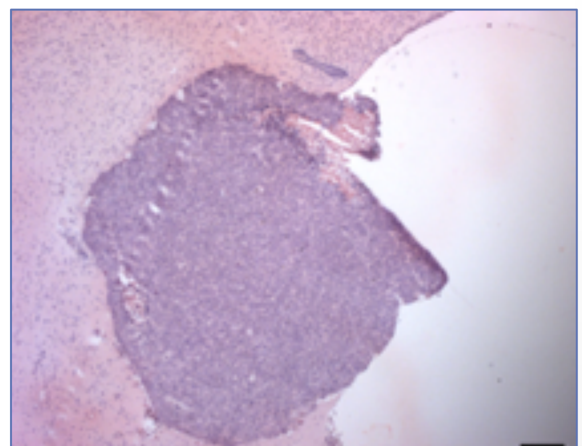
Sum190 BR3 (Her-2+, IBC)



4T1 BR5 (triple neg)



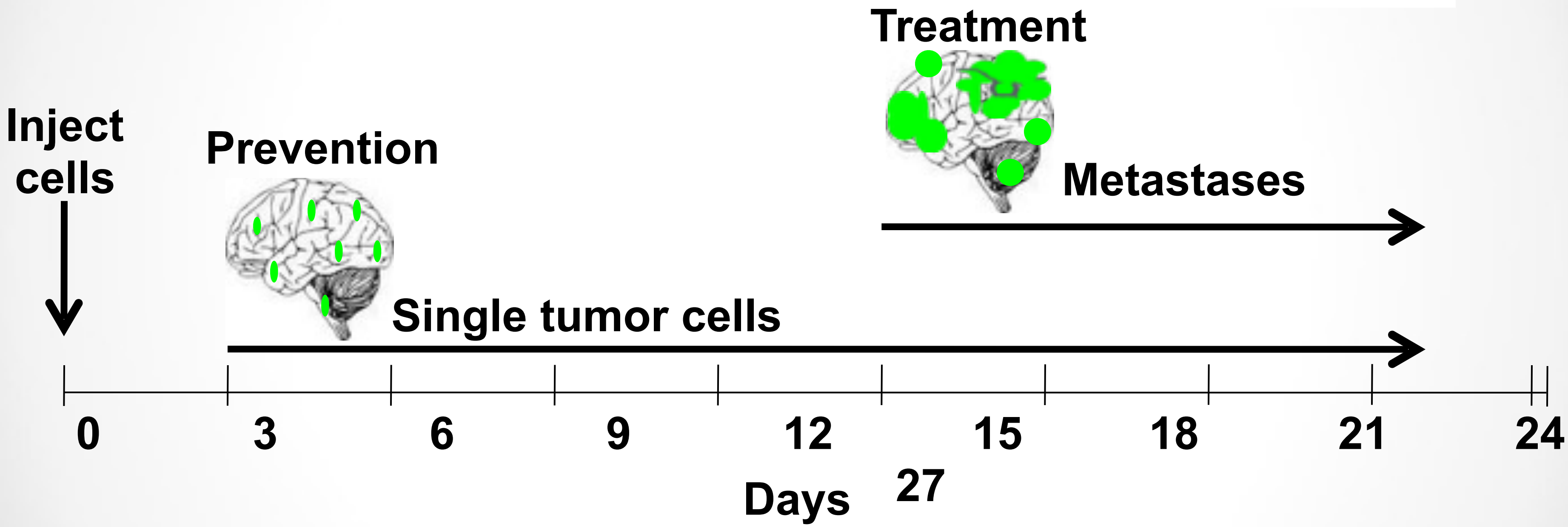
JIMT-1-BR3 (Her-2+)



MCF7 Her-2 BR3(ER+, Her-2+)

231-BR Brain Metastasis Models for Preclinical Drug Testing

- Intracardiac Injection: 4 week assay
- Count micrometastases and large metastases



Endpoints:
Prevention - Prevent development of brain metastases
Treatment - Shrink or stabilize size of already developed metastases
- Similar to most clinical trials

We tested 18 compounds
NONE of the Drugs Tested Had "Treatment" Activity in the 231-BR Model

Compound efficacy in preventive setting in mouse models:

•**Lapatinib**, 52% in HER2-transfected 231-BR cells. *JNCI* 100:1092, 2008

•**Saracatinib/Lapatinib**, ~66% in HER2-transfected 231-BR cells *Cancer Res*. 73:5764, 2013

•**Pazopanib**, 73% in HER2+ 231-Br; 55% in HER2-MCF-7-BR. *Clin. Cancer Res.* 17:142, 2011
Inhibition of neuroinflammatory astrocytes *Am. J. Pathol*. 182: 2368, 2013

•Low dose **Temozolomide,** 100% in MGMT- 231-BR. *Clin Cancer Res* 20:2727, 2014

Brain metastasis secondary prevention trial. Prevention of additional metastases in patients with limited brain metastases.
Endpoint: Time until a new brain metastasis

An Open Secondary Brain Metastasis Trial:
Dr. Minesh P. Mehta, University of Maryland
Clinical trials.gov NCT01924351, HER2-positive Breast Cancer With Brain Metastasis

Brain metastasis secondary prevention trial

HER2+ metastatic
Breast Cancer
with brain metastasis
recently treated with SRS

R

TDM-1 alone

TDM-1
plus Temozolomide

TDM-1
plus Pazopanib

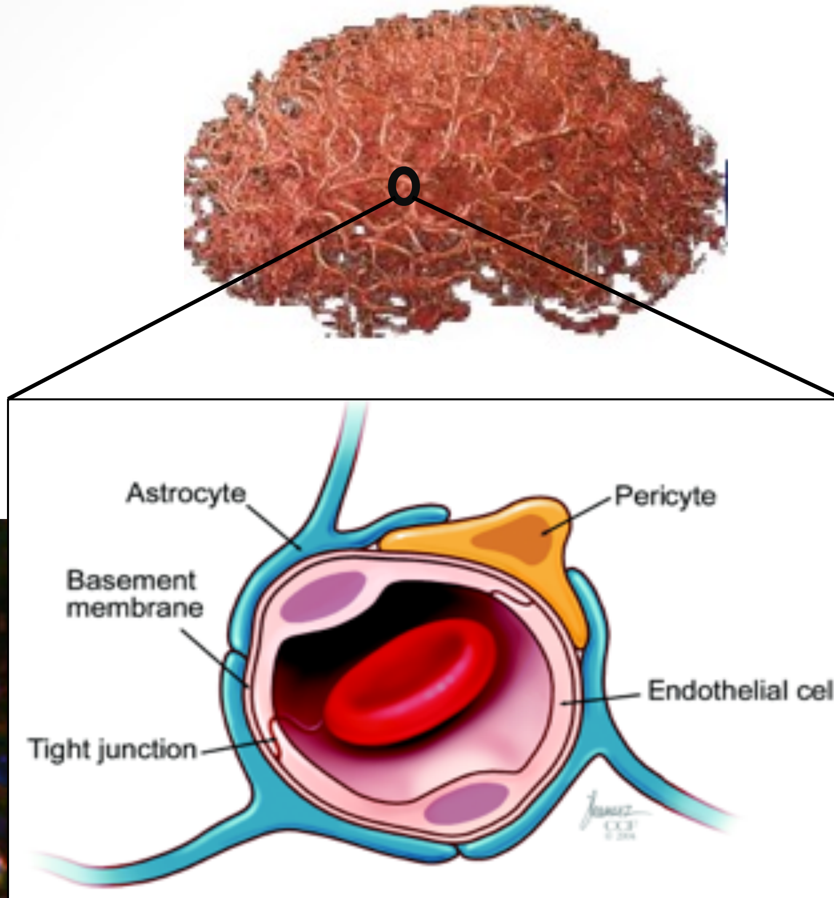
Primary endpoint: RFS brain in 6 months
Secondary endpoints: OS, toxicity, neurocognitive function

Analysis stratification per number of brain lesions and extracranial disease (controlled or not)

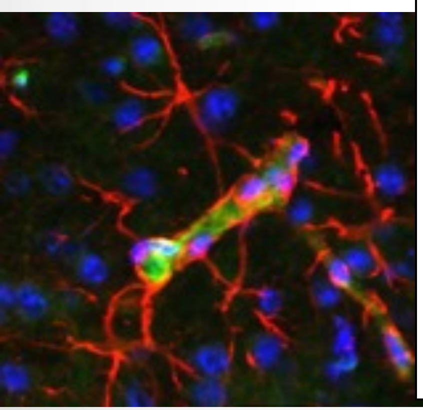
Alex Zimmer, NCI

The brain is *sanctuary site*, protected by the Blood-brain barrier (BBB)

Brain vasculature of the human brain.

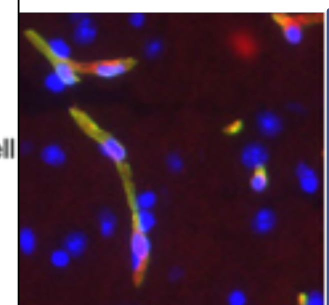


Weil et al., 2005. *AJP*. 176(4):913



Pericytes
Astrocytes
DAPI

- Capillary Endothelial Cells
  - Continuous tight junctions
  - Lack fenestrations

Blood-Brain Barrier	Blood-Tumor Barrier
	?

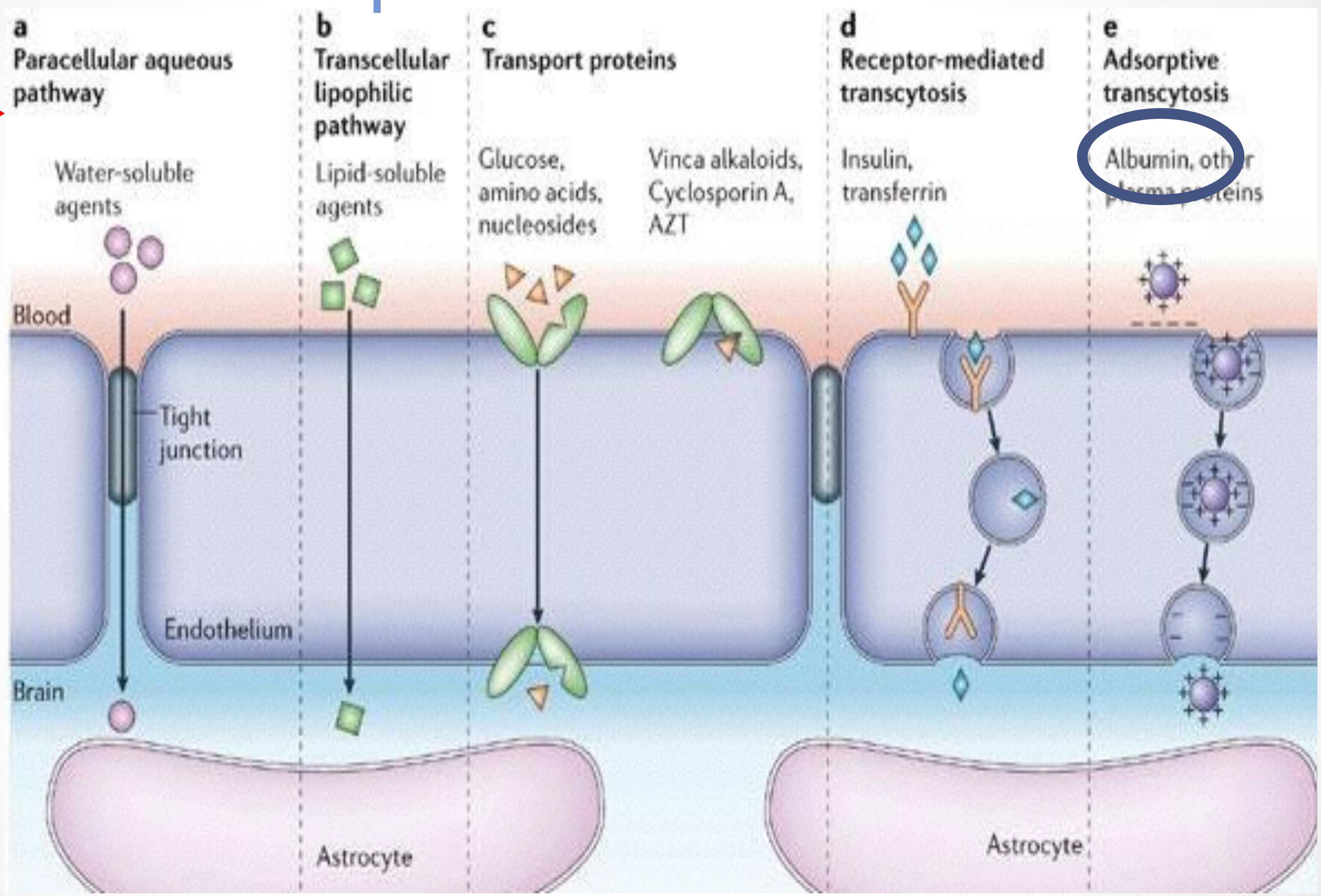
Basement membrane
Endothelial cells
DAPI (cluster = tumor)

Mechanisms of transport across the brain microvascular endothelial cells that form the lumen of brain capillaries.

Transcellular

Texas Red Dextran

Biocytin



Transcytosis is downregulated in healthy brain by pericyte coverage

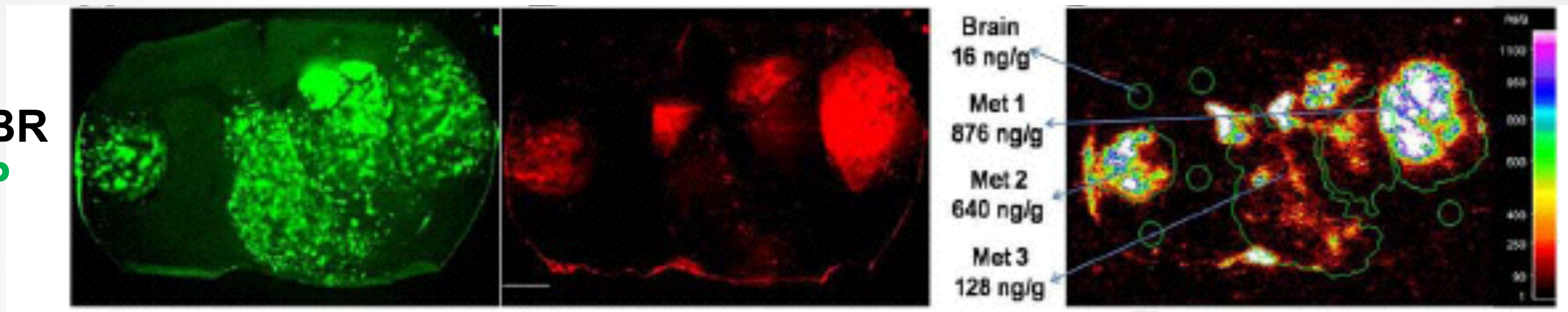
Abbott NJ, et al (2006 Nat Rev Neurosci 7: 41-53

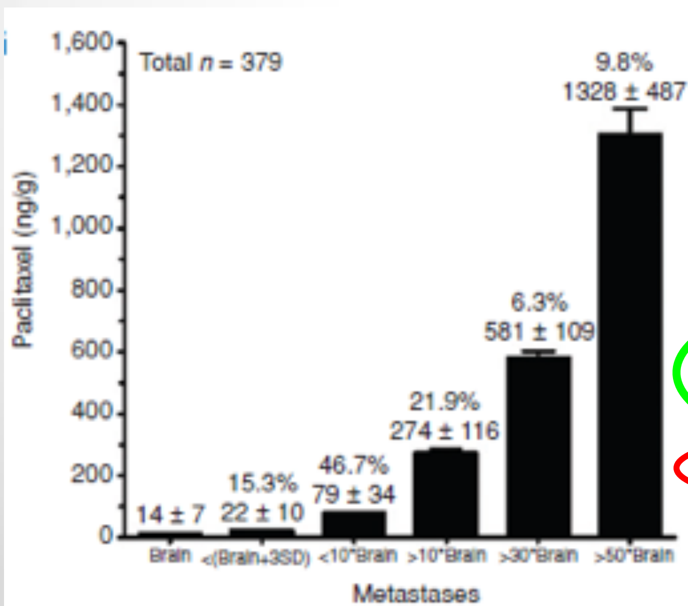
How does the Blood-Tumor Barrier (BTB) relate to chemotherapeutic efficacy?

Heterogeneous Uptake of ^{14}C-Paclitaxel in Brain Metastases

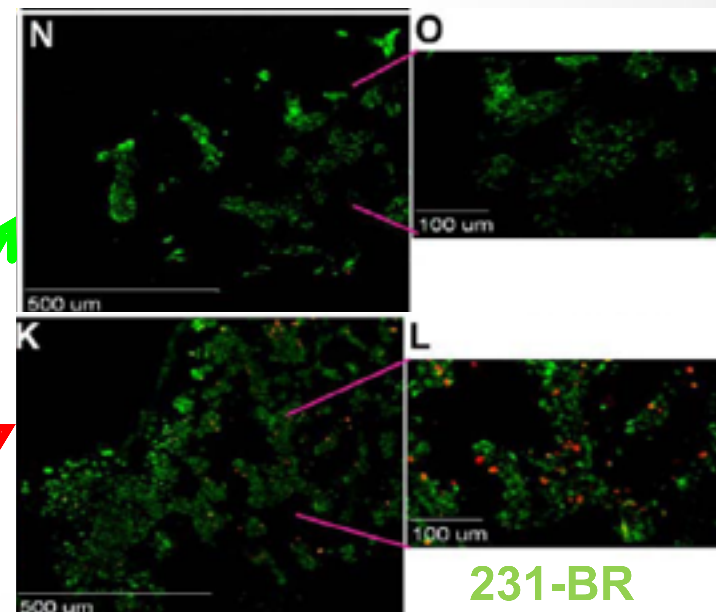
Metastasis | **3kDa TR Dextran** | **^{14}C- Paclitaxel**

231-BR
eGFP





15% = normal brain
47% : < 10-fold;
27% :10-50-fold;
10% : > 50-fold

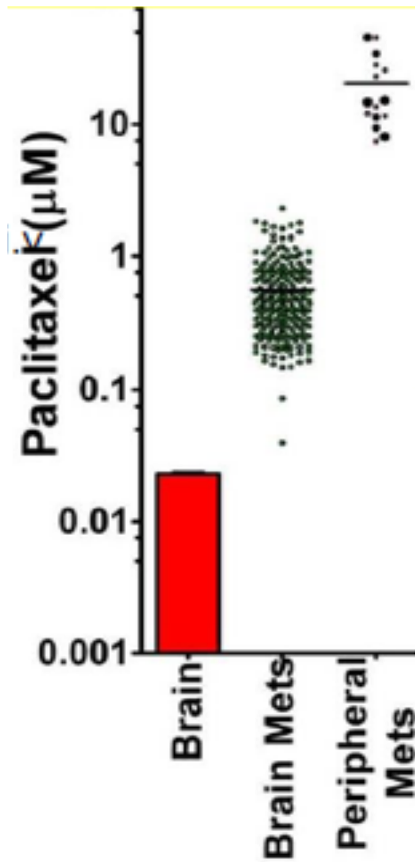


231-BR
Cleaved Caspase 3

Clin. Cancer Res. 16: 5664, 2010

Quentin Smith, Texas Tech University
Paul Lockman, West Virginia University

Brain Metastases Paclitaxel Uptake compared to other tissues



2h circulation
Mean ±SD
n= 4-6

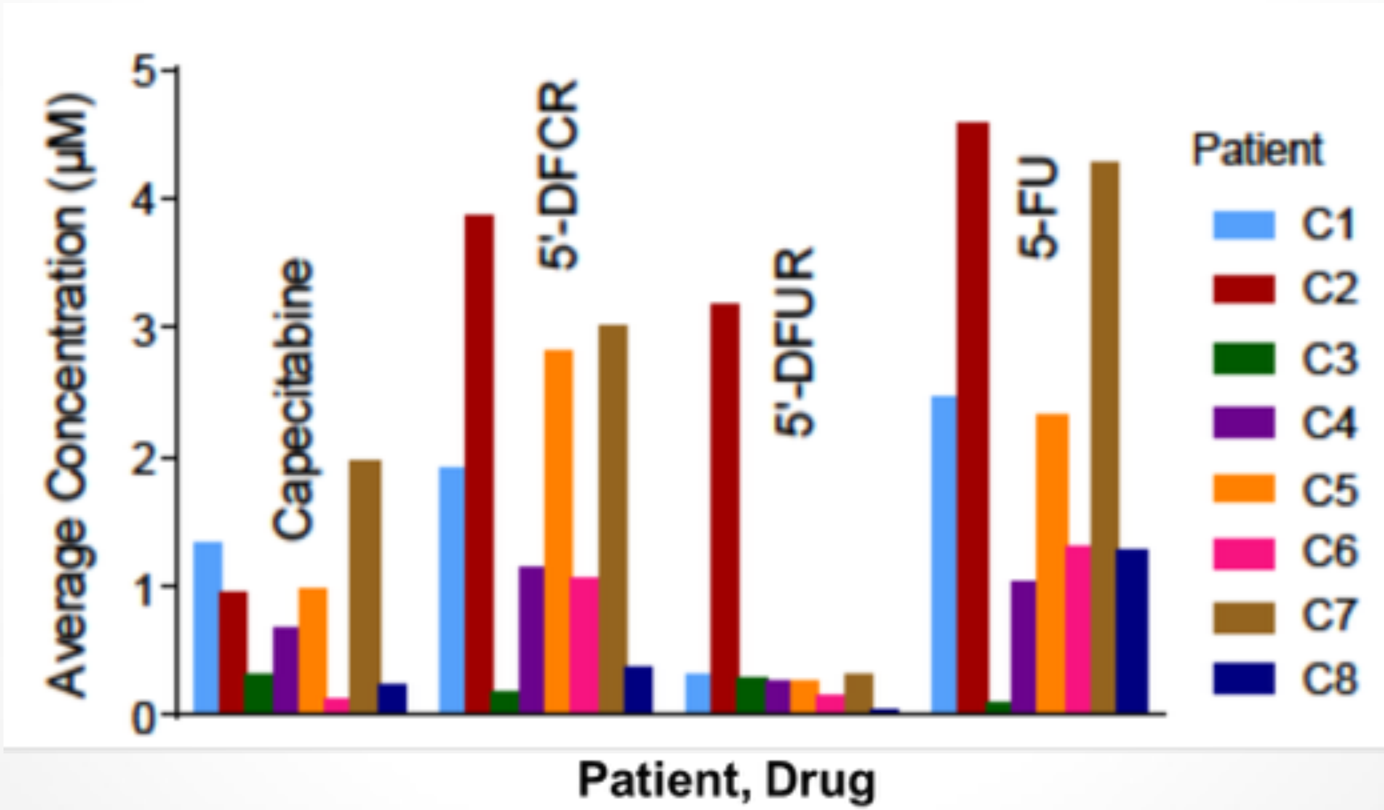
Impact of the BTB: paclitaxel concentration in brain metastases is on average only 2-3 % of the concentration in peripheral metastases.

Clin. Cancer Res. 16: 5664, 2010

Dr. Quentin R. Smith

Heterogeneous Capecitabine Uptake in Brain Metastases, between patients.

David Peereboom, Robert Weil, Cleveland Clinic Foundation
Andrew Seidman, Aki Morikawa, MSKCC
Quentin Smith, Texas Tech University
Patricia Steeg, NCI



single preoperative oral dose of capecitabine (1250 mg/m2)
2–3 h before surgery.

Morikawa et al. *Neuro-Oncology*, 2014

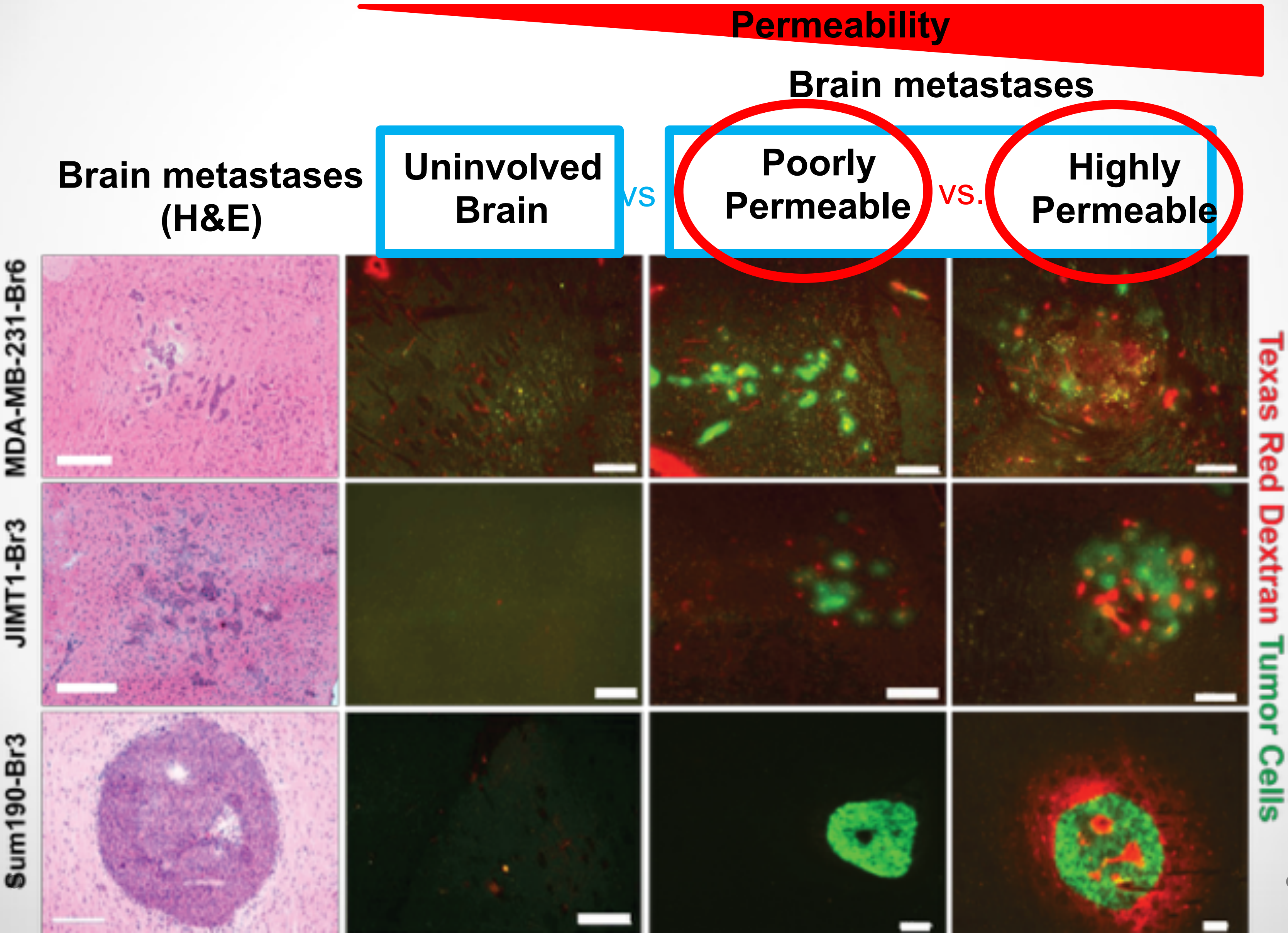
Hypothesis:

The structure of the BTB is different between permeable and less permeable metastases: identifying what component differ will guide us to improve drug delivery and develop treatment options.

Investigate the known identified components from the literature on brain injury or neurodegenerative disorders in the context of brain metastases.

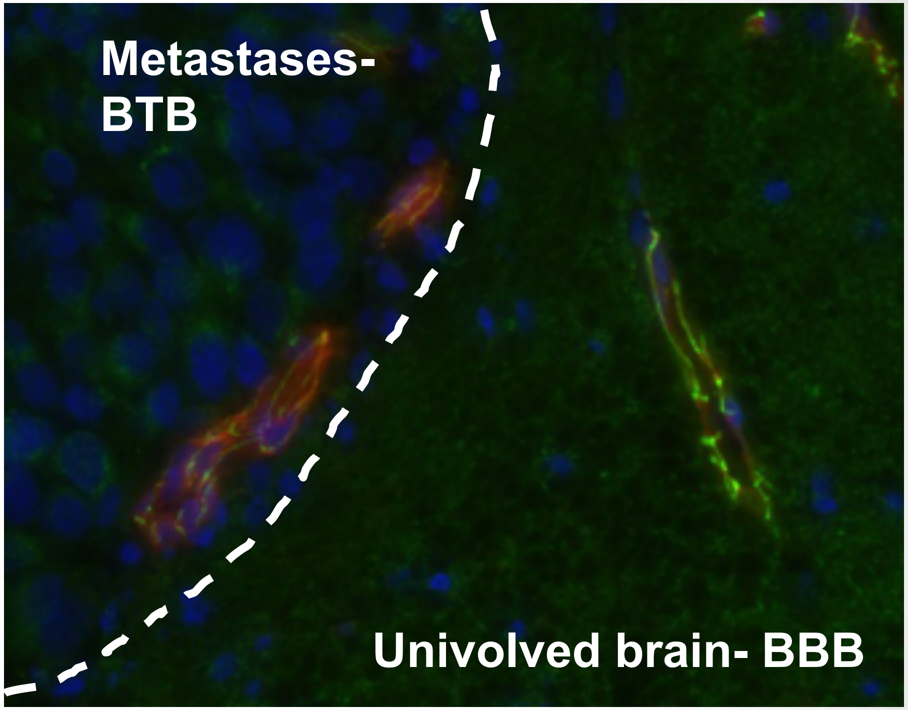
Candidates from Laser Capture Microdissection of brain metastases and micro-array analysis

Blood-Brain/Tumor Barrier Permeability in 3 Brain Metastasis Models



Most of the BBB Markers investigated showed a significant difference in expression between Brain Metastases and Uninvolved Brain.

GFAP overexpressing Astrocytes
Aquaporin 4 (polarity of astrocytes feet)
CD11b/CD45 Microglia
Zo-1 tight junction adaptor
Laminin α2 Astrocytic Basement membranes
CD13 + Pericytes
Desmin + pericytes
PDGFRbeta + pericytes
Vascular Endothelial Growth Factor



ZO-1/ CD31

The differences in permeability <u>among metastatic lesions</u> were only associated with <u>pericytes and laminin α2.</u>

Lyle et al., submitted

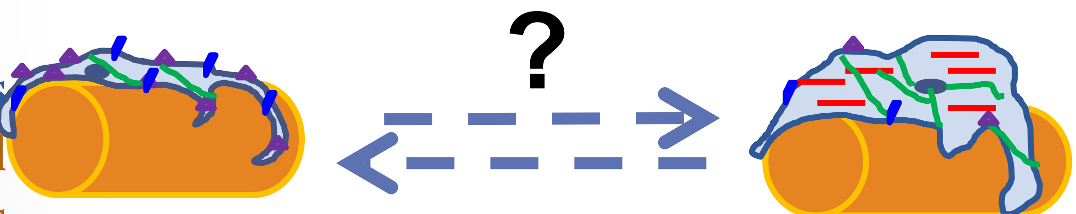
Pericyte heterogeneity and plasticity

Depending on the developmental stage and pathological context

PDGFRβ: pericyte recruitment during angiogenesis; brain pericytes
CD13: Type II membrane zinc dependent metalloprotease; brain pericytes
Desmin: type III intermediate filament; Structural protein
αSMA: Structural protein; no αSma in normal CNS

Resting Pericytes | **Contractile Pericytes**

Pericytes
Endothelial cells



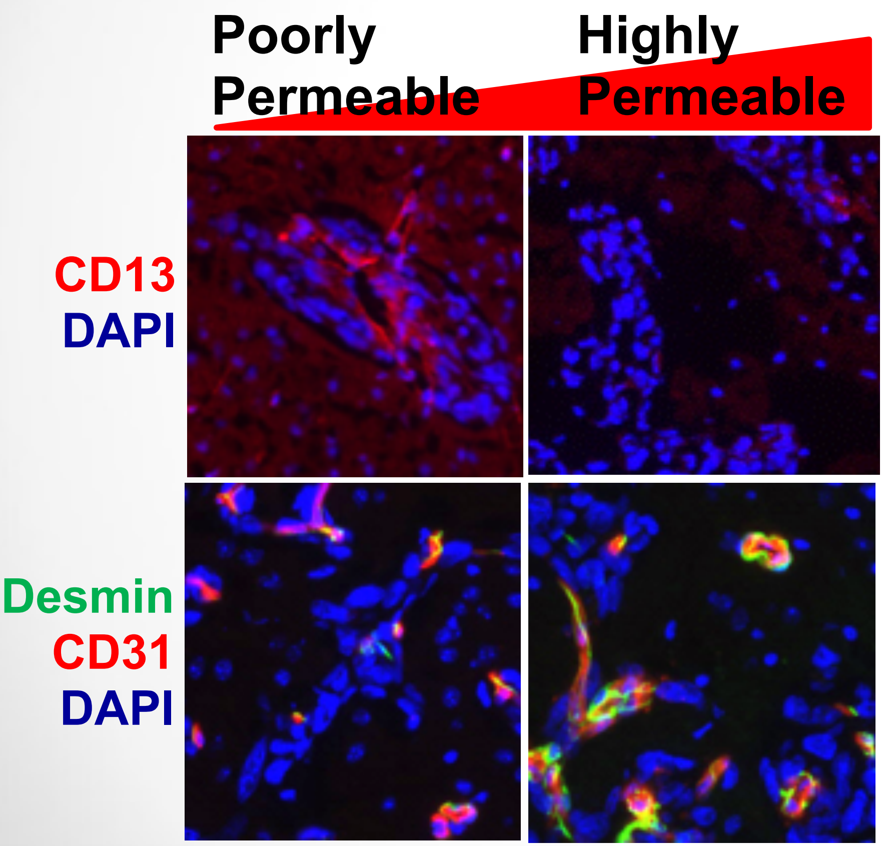
Pathological context:
- Fibrosis
- Inflammation
- Tumor

Resting Pericytes:
PDGFRb +++
CD13 +++
αSMA -
Desmin +

Contractile Pericytes:
PDGFRb +++
CD13 +++
αSMA +++
Desmin +++

Pericyte subpopulation alteration correlated with Blood-Tumor Barrier permeability

PDGFRβ (pan-pericyte marker) is significantly decreased in brain metastases compared to uninvolved brain, but no differences between permeable vs poorly permeable lesions.



Highly Permeable lesions:

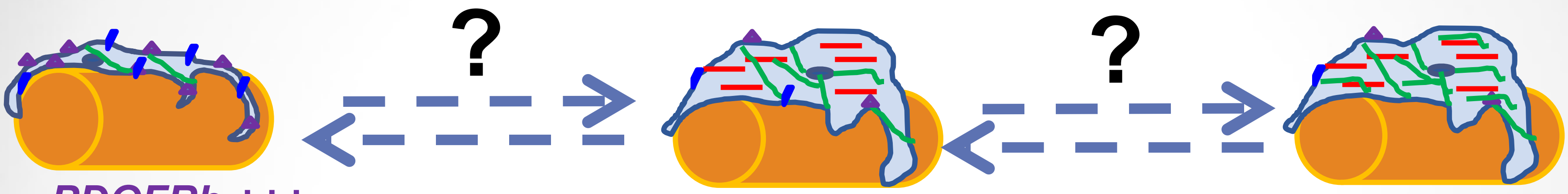
34% Decrease p= 0.0039- 231-BR
24% Decrease p=0.0001 JIMT-1-BR
No change - SUM190BR

37% Increase p = 0.0002 - 231-BR
99% Increase p< 0.05 - JIMT-1-BR
59% Increase p=0.002 - SUM190BR

Pericyte heterogeneity and plasticity in the brain

Our data:

BBB Uninvolved brain	**BTB** Poorly Permeable Mets	**BTB** Highly Permeable Mets



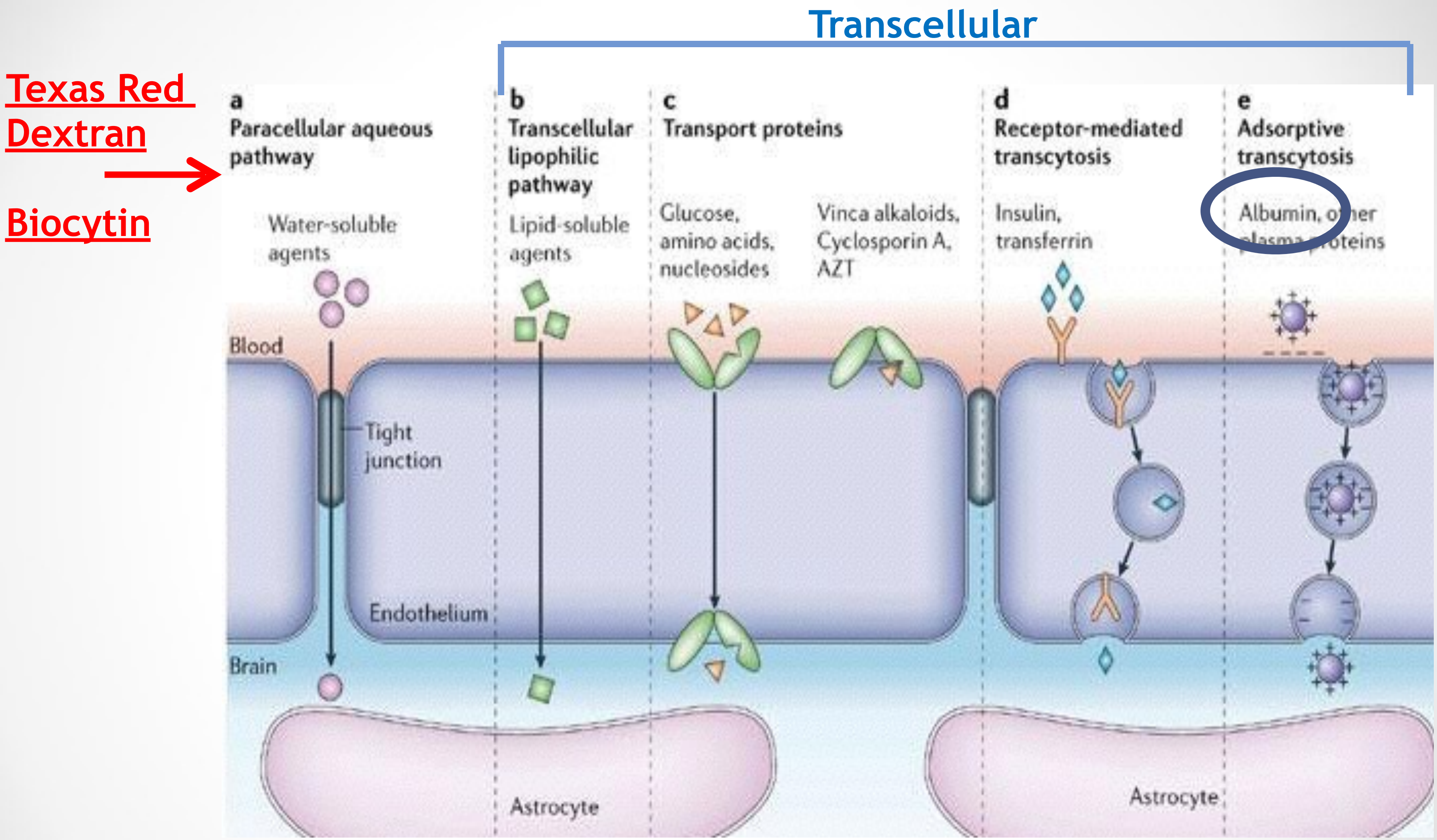
PDGFRb +++	*PDGFRb -/+*	*PDGFRb -/+*
CD13 +++	*CD13 +*	*CD13 -*
αSMA-	*αSMA++*	*αSMA++*
Desmin +	*Desmin ++*	*Desmin ++++*

From literature review on Blood-Brain Barrier:

Pericytes regulate BBB formation and maintenance reducing endothelial transcytosis
(Armulik et al., 2010; Daneman et al., *Nature* 2010)

In a mouse model of basement membrane KO, the differentiation from the resting to the contractile stage in the brain changes the functions of pericytes from barrier stabilization to barrier disruption (Yao et al *Nature* 2014)

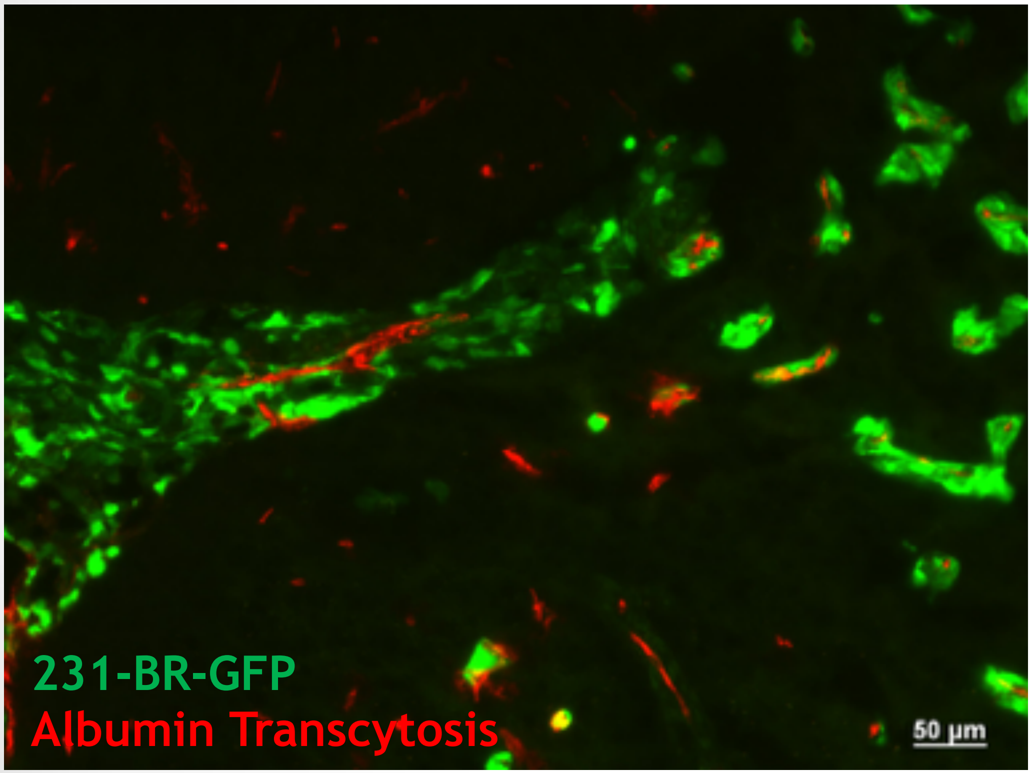
Mechanisms of transport across the brain microvascular endothelial cells that form the lumen of brain capillaries.



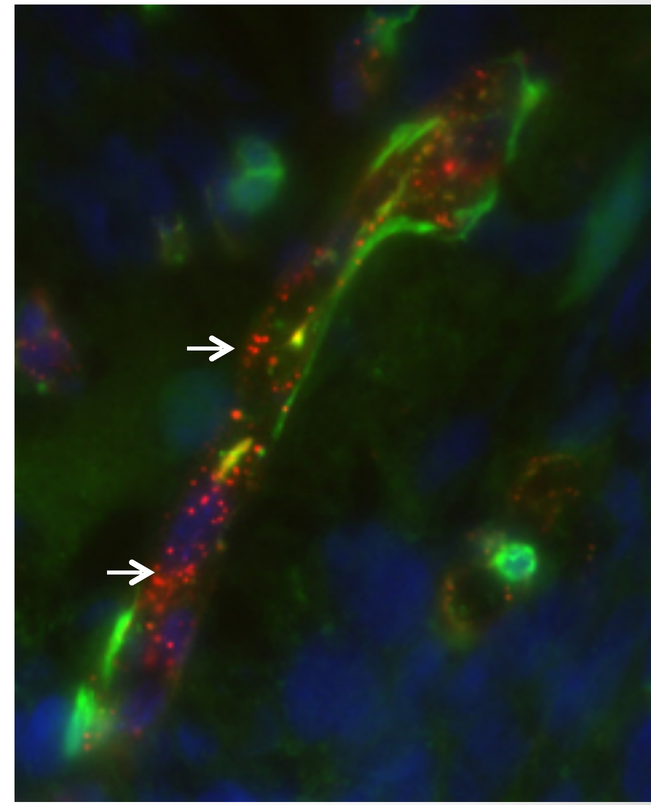
Abbott NJ, et al (2006 Nat Rev Neurosci 7: 41-53

Investigating Transcytosis in brain metastases

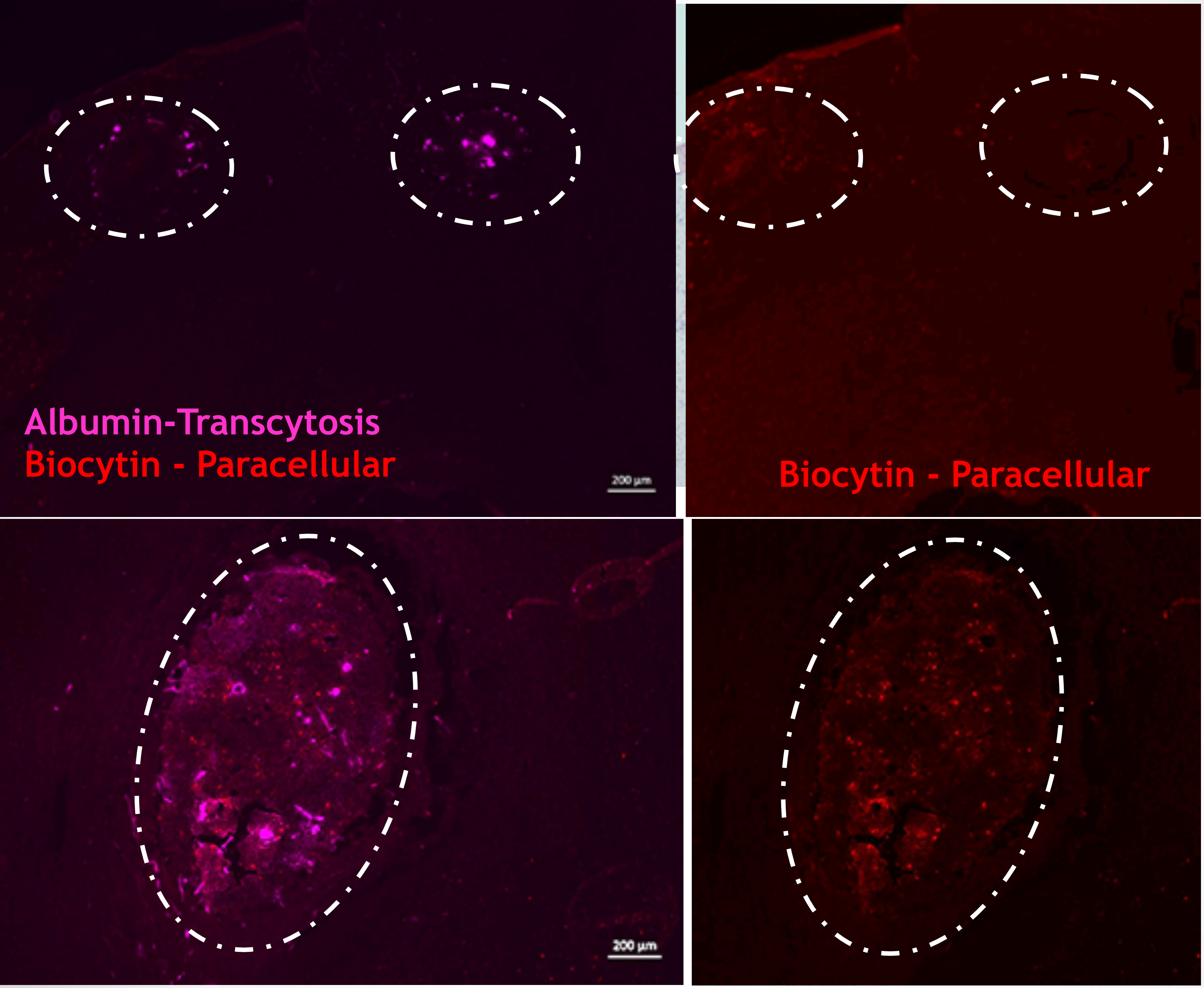
- **Transcytosis is downregulated in normal healthy brain by pan PDGFRβ +pericytes:** (Armulik et al., 2010; Daneman et al., *Nature* 2010)



Desmin + pericytes and
Albumin - 231-Br metastases



Transcytosis strongly increases in brain metastases while barely detectable in uninvolved brain area, **highlighting a potential relevant route for drug delivery.**

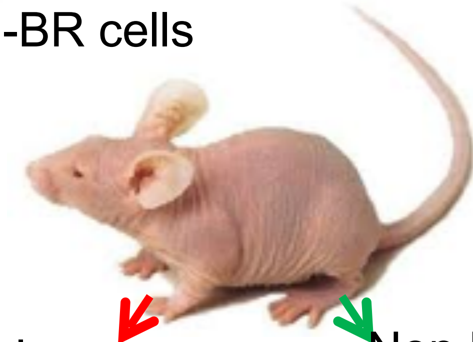


SUM190-BR
Brain mets
model

Transcytosis permeability is an earlier event than paracellular permeability

Laser Capture Micro dissection

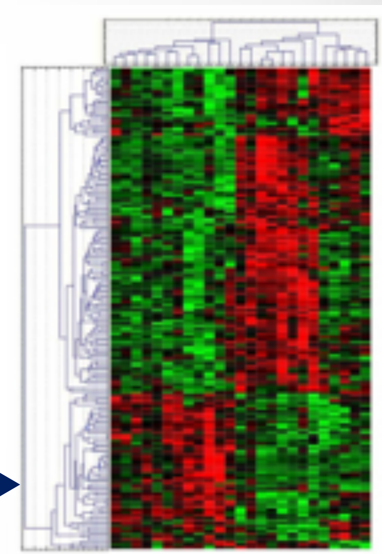
- Intracardiac Injection of 231-BR cells
- 4 week assay
- Inject Texas red dextran



Permeable Metastases

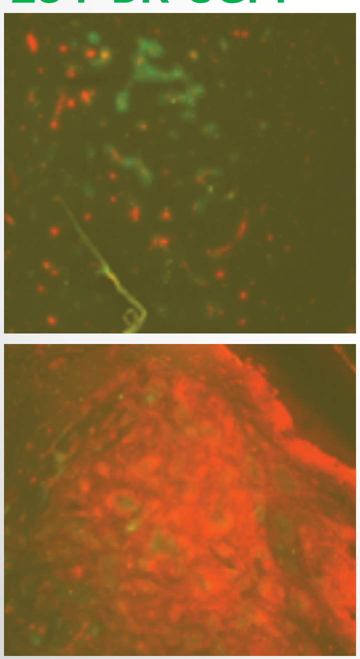
Non Permeable Metastases

Micro Array to compare gene expression

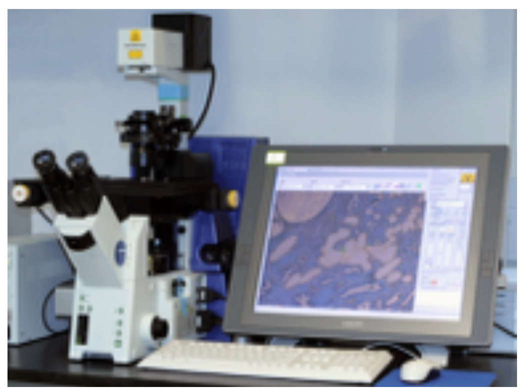


Localize Metastases

Texas Red Dextran
231-BR-eGFP



LCM

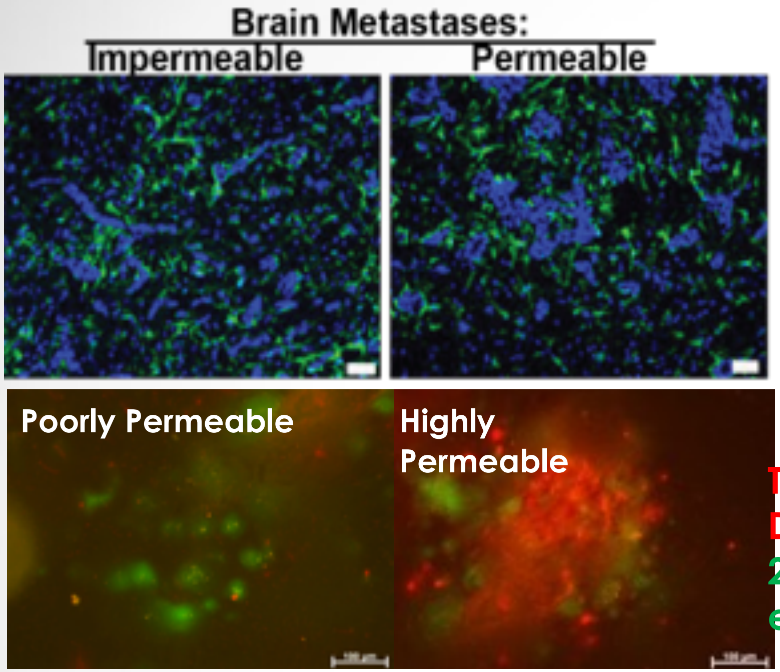


RNA extraction and amplification

Human Cancer Cells:
Human micro-array

Activated Astrocytes

Activated Astrocytes and Blood-Tumor Barrier Permeability



GFAP

Texas Red Dextran
231-BR-eGFP

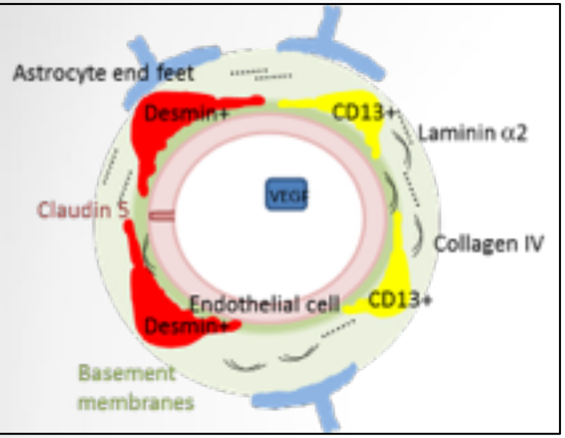
All metastases induces a strong astrogliosis regardless the level of permeability.

However molecular signaling pathways are differentially expressed in activated astrocytes, in permeable vs. less permeable metastases:

Ephrin receptor and GABA A receptor signaling lower in permeable lesions

Sphingosine-1-phosphate signaling higher in permeable lesions

Blood-Brain Barrier



Blood-Tumor Barrier
Poorly permeable

↓Pericytes
↓Tight Junction ZO-1
↓ Laminin Astrocytic BM
↓Astrocyte polarity

↑ Activated astrocytes
↑ VEGF

↑ Transcytosis

Blood-Tumor Barrier
Highly permeable

↓CD13 Resting Pericytes

↑ Desmin
Contractile Pericytes

↓EphR and GABAR
Activated Astrocytes

↑ S1PR
Activated Astrocytes

Understanding the role of pericyte plasticity, activated astrocytes and transcytosis in BTB permeability will lead to strategy to improve drug uptake.

Conclusion
Future directions

1- Only preventive approaches show efficacy against outgrowth of experimental brain metastases of breast cancer in mouse models.

→ Randomized Secondary Brain Metastasis Prevention Trials

2- Investigation of the molecular characteristics of Blood-Tumor Barrier permeability:

→ In BTB, transcytosis permeability is significantly increased.

→ Highly permeable mets displayed specific features: increase in contractile Desmin+ CD13- pericytes, and specific activated astrocytes: S1P signaling increase and Ephrin and GABA signaling decrease.

BrainMetsBC.org



Understanding brain metastases, available treatments, and emerging research.
A Website for Patients and Families . . .

Musa Mayer
Helen Schiff

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