Breast Cancer Brain Metastases: Translational implications from preclinical models

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Brain Metastases

- Brain metastases are the most frequent CNS tumors in adults.
  - Most common primary tumor sites are lung (50-60%), breast (15-20%), melanoma (5-10%), kidney and GI tract (4-6%)

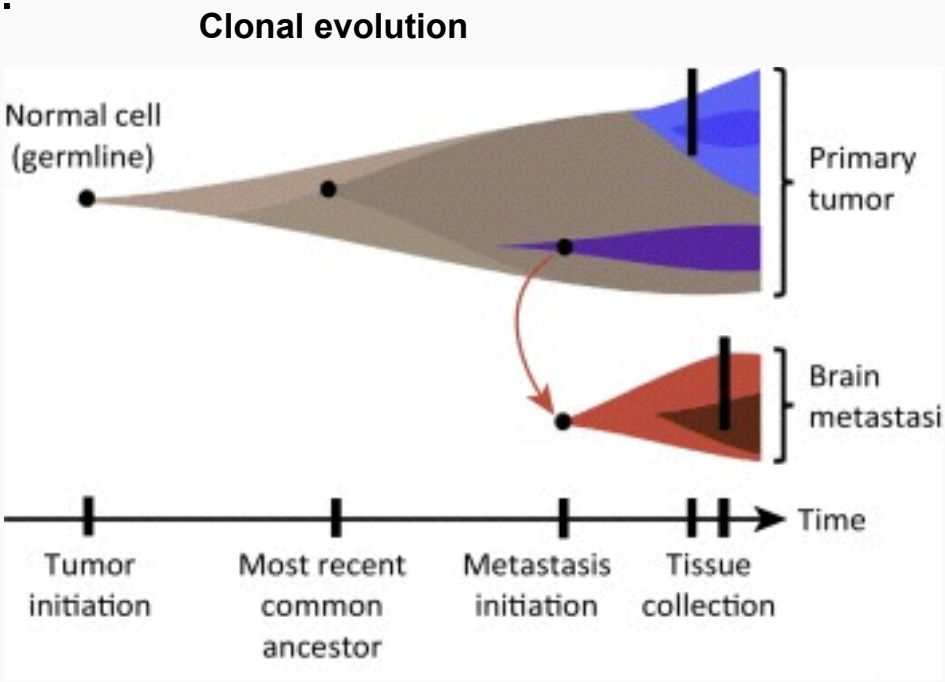
- Breast Cancer Subtype Incidence:

  - **HER2-positive:** 15-20% of all breast cancer patients; 25-40% of them will have brain metastases
  - **Triple-Negative:** 20% of all breast cancer patients; up to 50% of them may present brain metastases

- Traditional systemic therapies are ineffective

Challenges to treat brain metastases

- **Brain metastases have different oncogenic drivers** compared to the primary tumor.

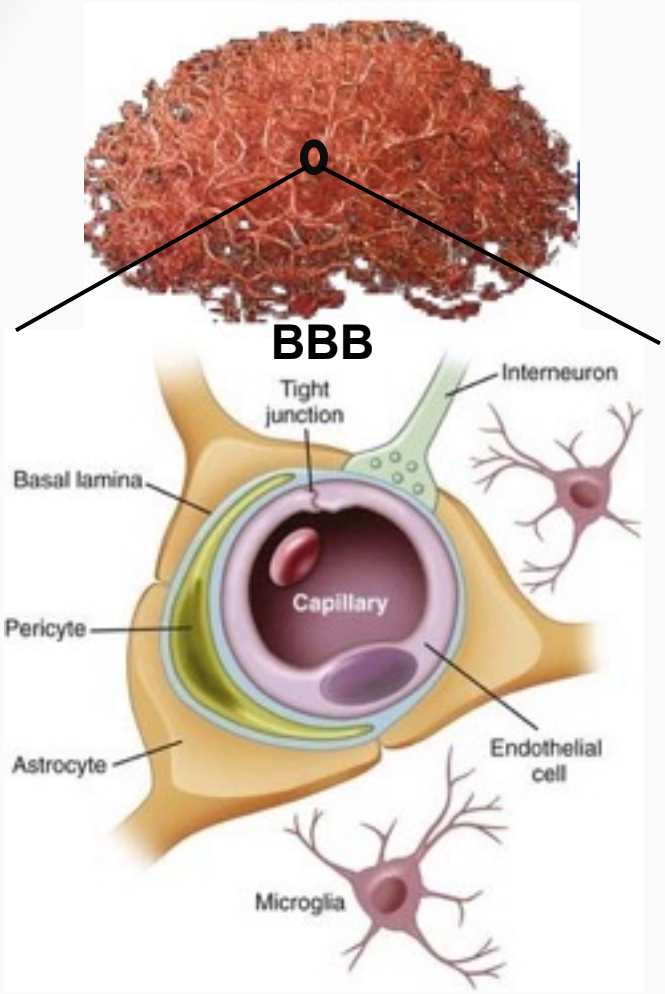


Dagogo-Jack et al. *Trends in Cancer* 2016

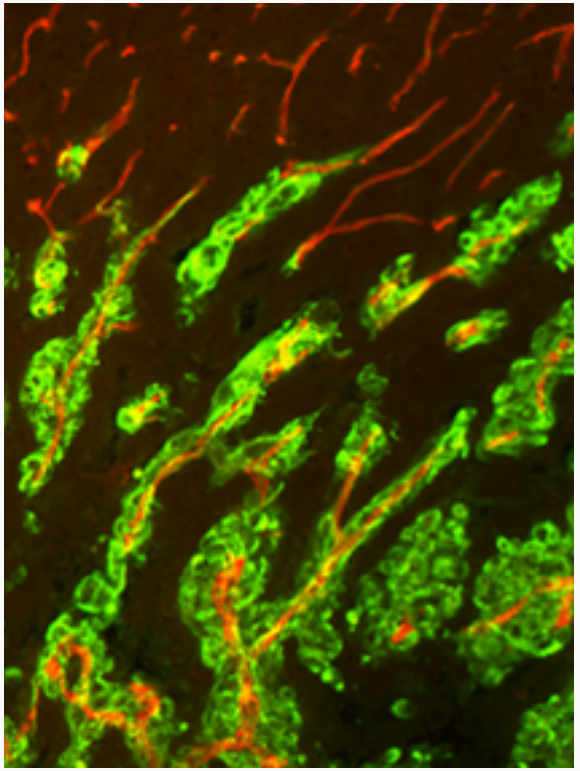
- **Singularity of the brain microenvironment:**
  - **the blood-brain barrier**

The Blood-Brain Barrier and the Blood-Tumor Barrier

Brain vasculature of the human brain.



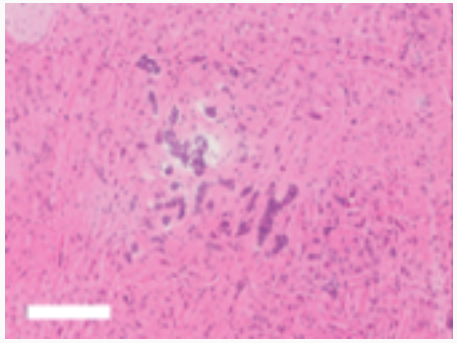
Shahid M. Nimjee et al. BBB chapter 2015



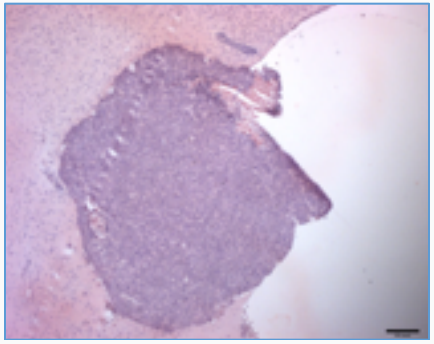
Metastatic cells growing alon
vasculature form the BTB

What is the Blood-Tumor Barrier (BTB), how is it related to treatment efficacy?

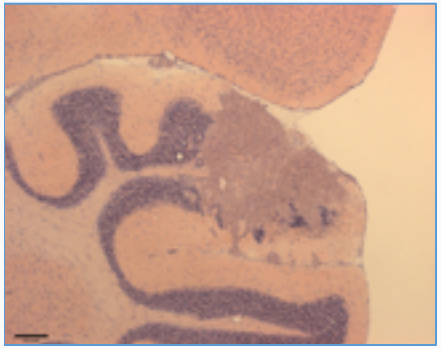
Experimental Hematogenous Brain Metastasis Models of Breast Cancer Produced or Optimized by the Steeg Lab



231 BR7
Triple negative

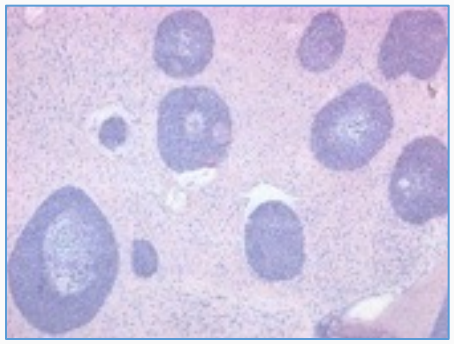


MCF7 Her-2 BR3
(ER+,Her-2+)

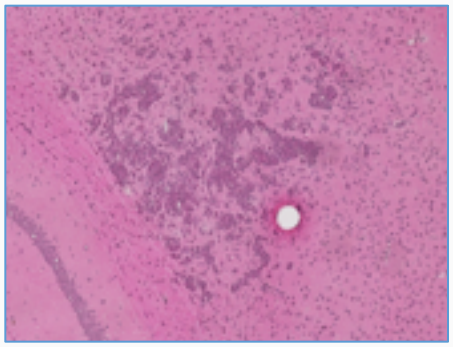


JIMT-1-BR3
Her-2+, Resistant

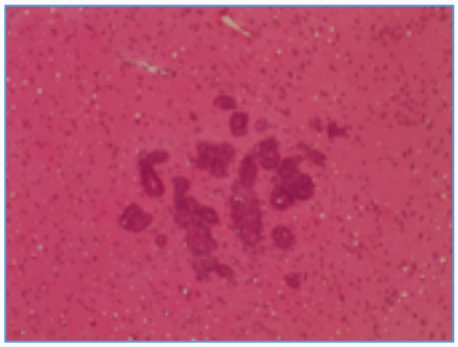
1. *Clin. Cancer Res*. 16: 5664, 2010;
2. *Clin. Cancer Res*. 10: 2727, 2014;
3. *Clin. Cancer Res*. 17: 142, 2011
4. *Clin. Cancer Res*. 22:5287, 2016



Sum190 BR3
Her-2+, IBC



New E0771-BR4 *
Triple-negative
Immunocompetent



New LN2-BR *
Luminal B
Immunocompetent

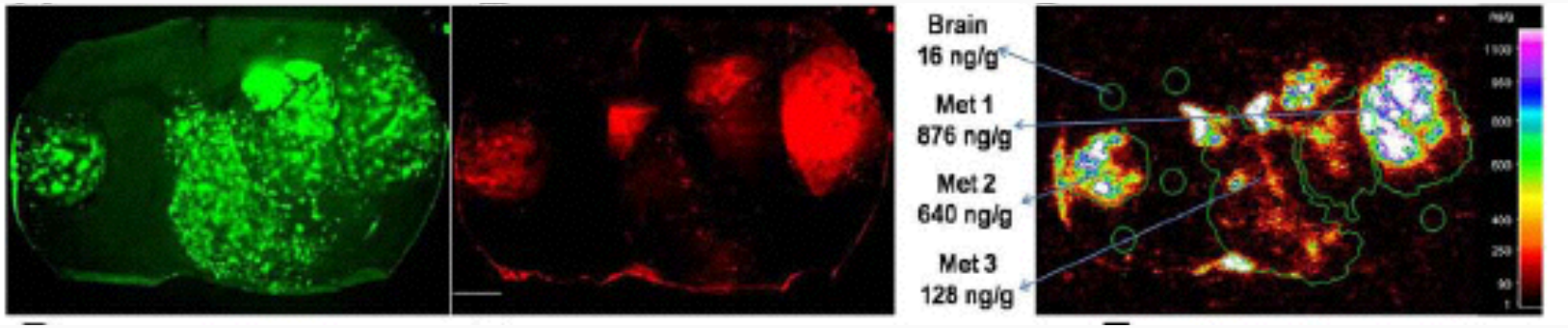
* Unpublished

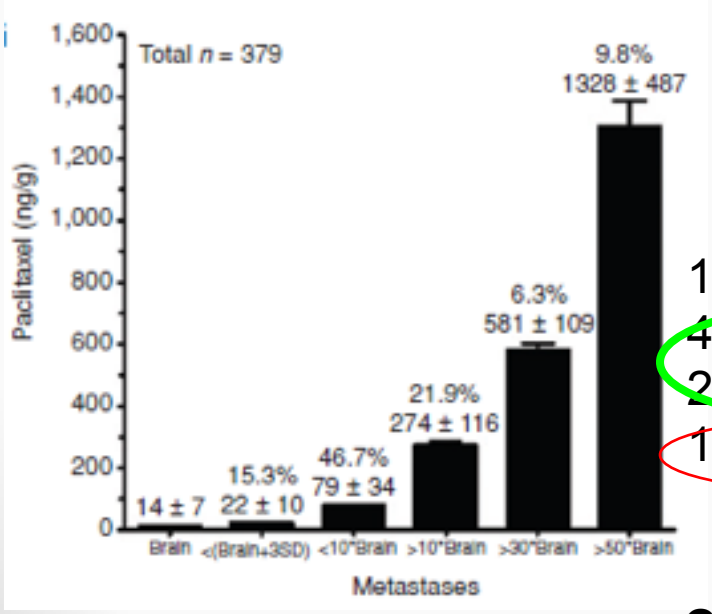
Heterogeneous Uptake of ^{14}C-Paclitaxel in Brain Metastases

Similar results with Lapatinib and doxorubicin

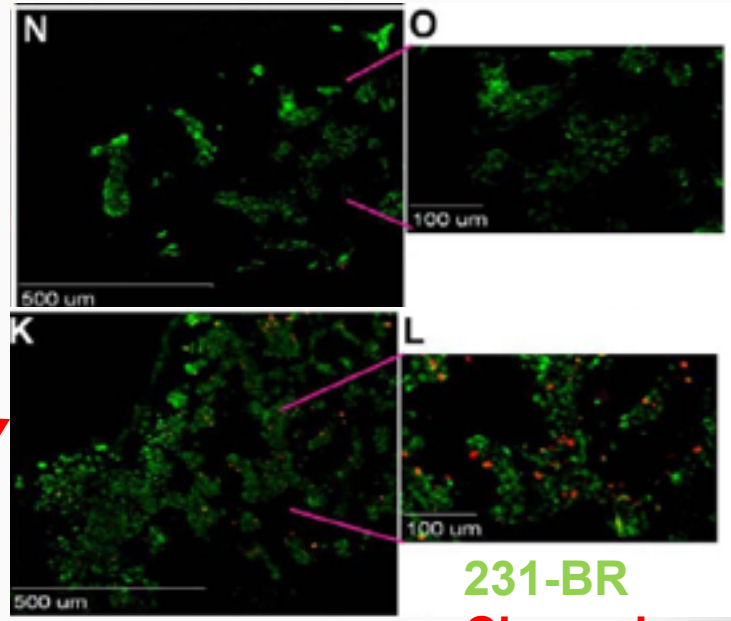
Metastasis | **3kDa TR Dextran** | **^{14}C- Paclitaxel**

231-BR
eGFP





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

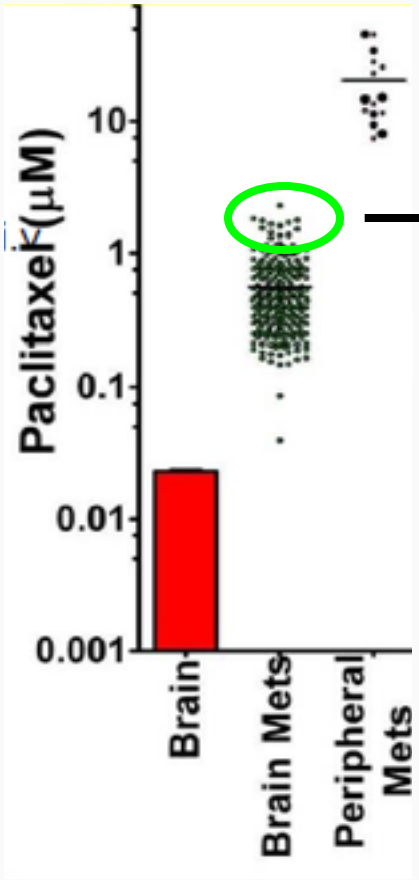


231-BR
Cleaved Caspase 3

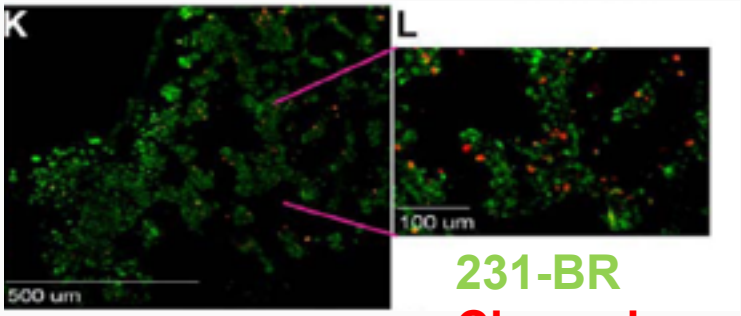
Quentin Smith, Texas Tech University
Paul Lockman, West Virginia University

Lockman et al. Clin. Cancer Res. 2010

Heterogeneous and limited Paclitaxel Uptake in Brain Metastases



Top 10%



231-BR
Cleaved
Caspase 3

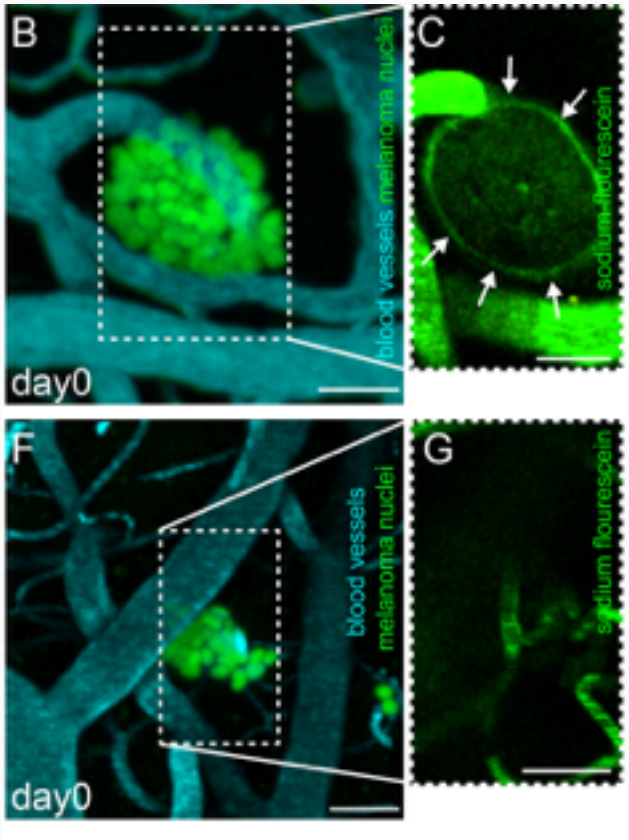
Lockman et al. Clin. Cancer Res. 2010

- Paclitaxel concentration in brain metastases is on average only 2-3 % of the concentration in peripheral metastases Smith, Q.R. Ph.D.

In vivo multiphoton laser-scanning microscopy for longitudinal measurements of brain metastasis models

Osswald et al. *Clin Cancer Res.* 2016

- Tumor growth
- Permeability (**sodium fluorescein**)



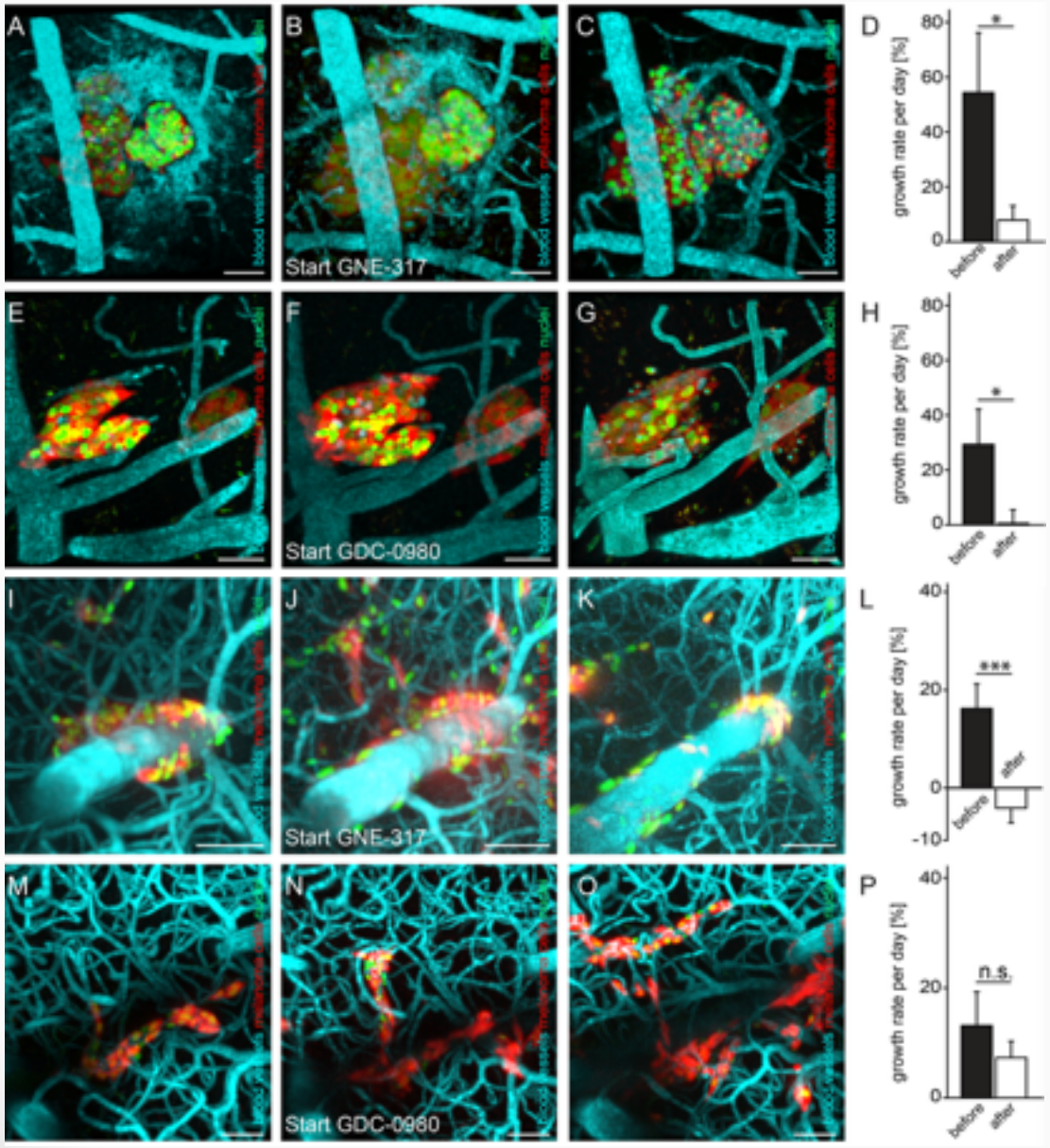
71.2% Non-permeable metastases

Brain permeable and non-permeable PI3K/mTOR inhibitors

-3days Start treatment +4 days

Permeable metastases

Non-permeable metastases



GNE-317, brain permeable

GDC-0980

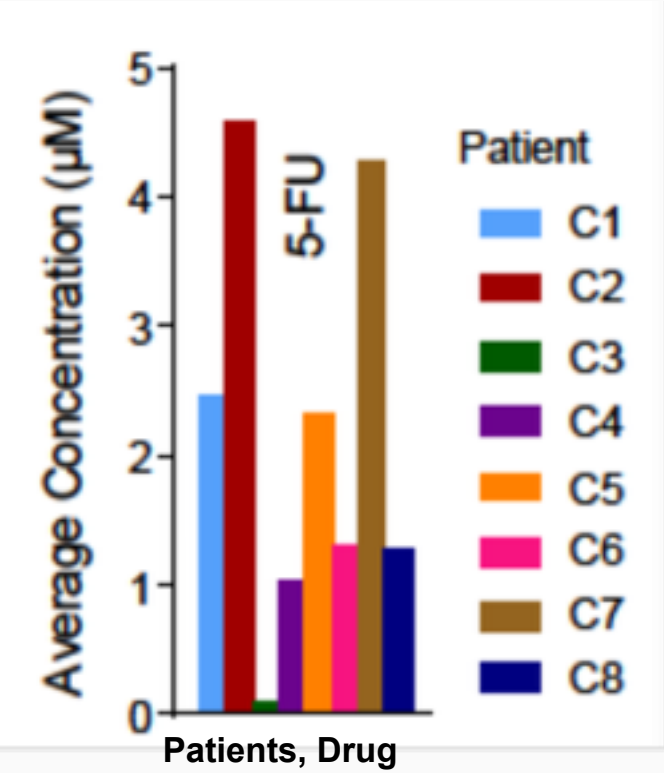
GNE-317, brain permeable

GDC-0980

Do not target non-permeable lesions

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

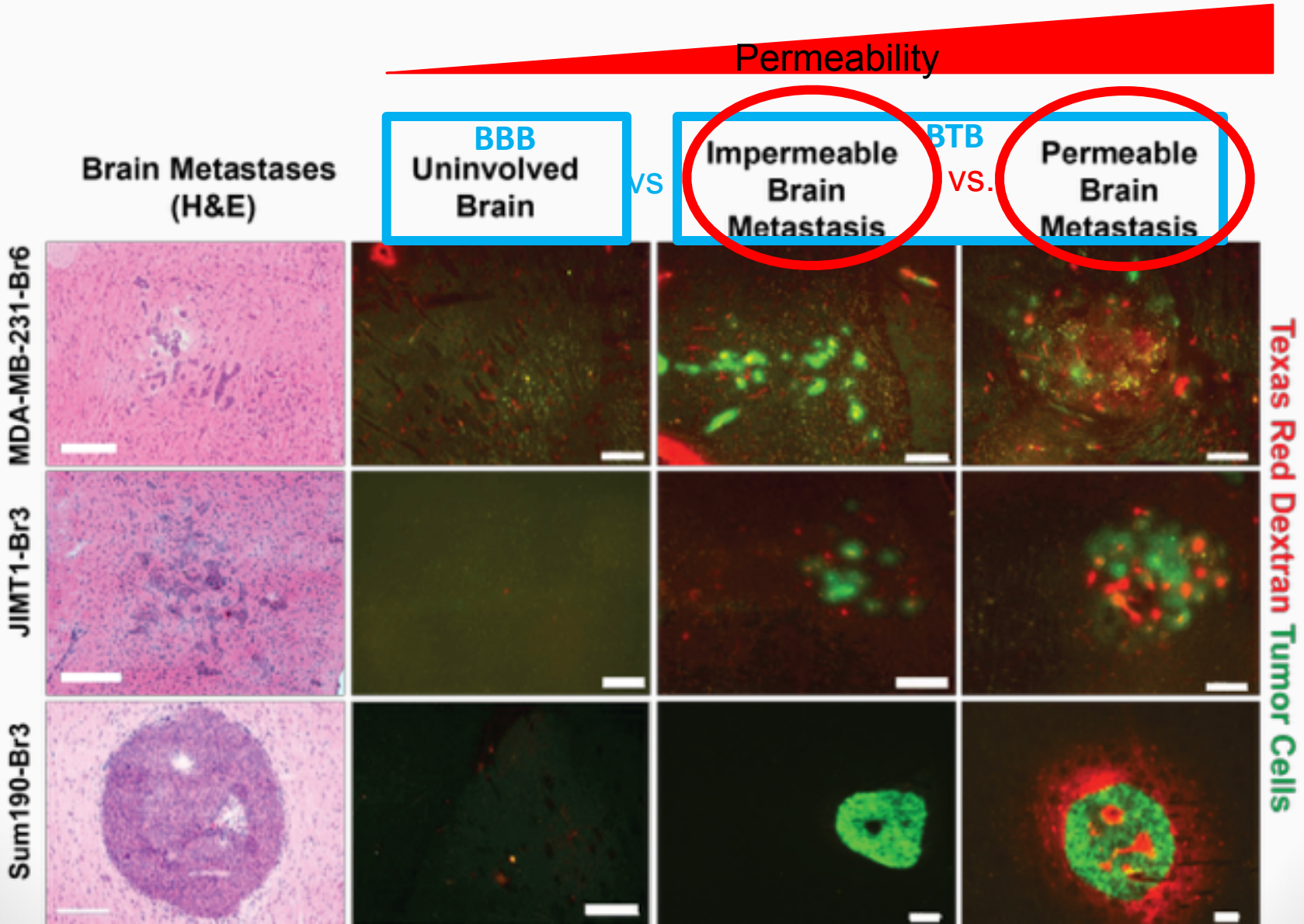
Hypothesis:

The BTB is not a chaotic and deteriorated version of the BBB, but have consistent changes, leading to a new structure.

- **BBB** vs. **BTB**,
- **highly** vs. **poorly permeable BTB**

Identifying the structural/molecular structure of the BTB will guide us to develop treatment options targeting specifically the BTB, avoiding the BBB.

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



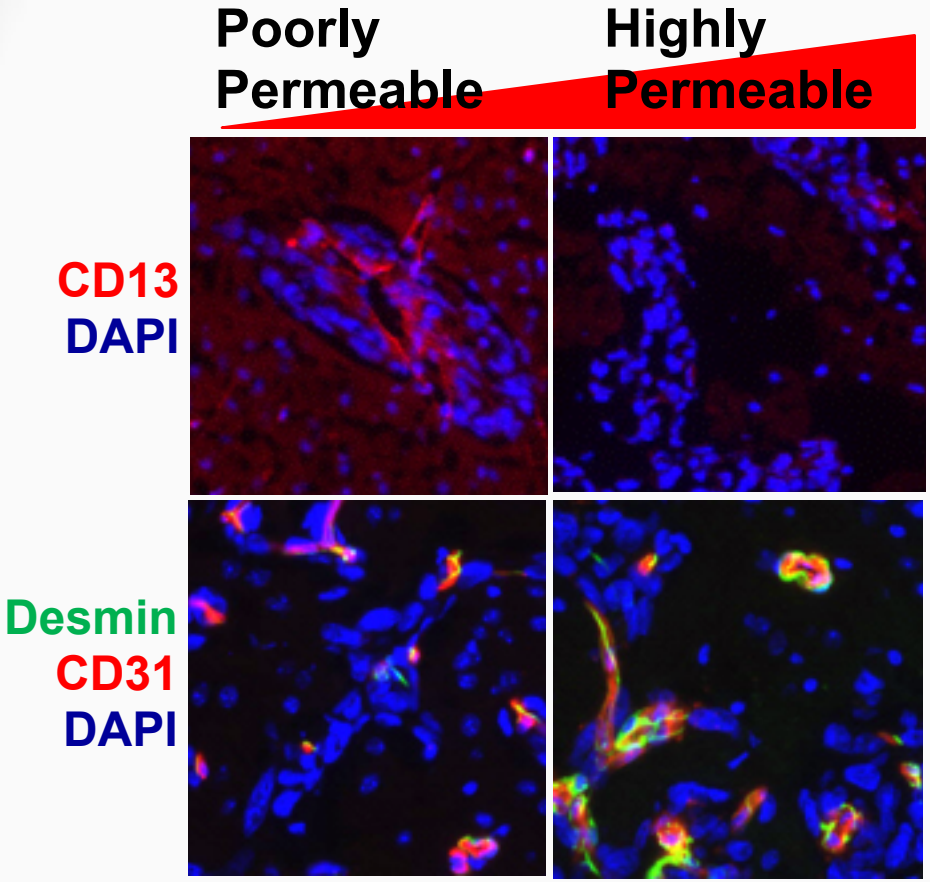
Analyzed Functional Components of the BBB-BTB

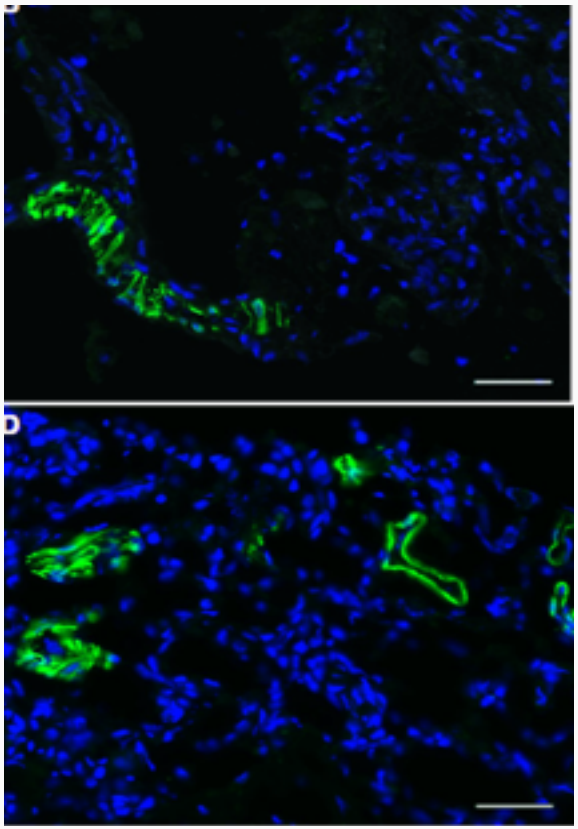
Lyle et al. *Clin. Cancer Res*. 2016

BBB Component	IF Marker	BTB vs. BBB	Highly vs. Poorly Permeable BTB
Endothelial Cells	CD31	Dilated vessels In Mets	
Tight junction adaptor	ZO-1	↓ BTB	
Vascular Endothelial Growth Factor	VEGF	↑ BTB	
Basement Membrane	COLIV	No trend	
Parenchymal Basement Membrane	LAMA2	↓ BTB	↓ in Highly permeable
Astrocytes	GFAP	↑ near Mets (astroglosis)	
Astrocyte polarization marker	Aqu4	↓ delocalization in BTB	
Microglia	CD11bCD45	↑ near Mets	
PDGFRβ+ pericytes	PDGFRβ	↓ In BTB	
CD13+ Pericytes	CD13	↓ In BTB	↓ in Highly permeable
Desmin+ Pericytes	Desmin	↑ In BTB	↑ in Highly permeable

Desmin+ Pericyte subpopulation correlated with BTB permeability

Lyle et al. *Clin. Cancer Res*. 2016





Dr. Brastianos
Dr. Duchnowska

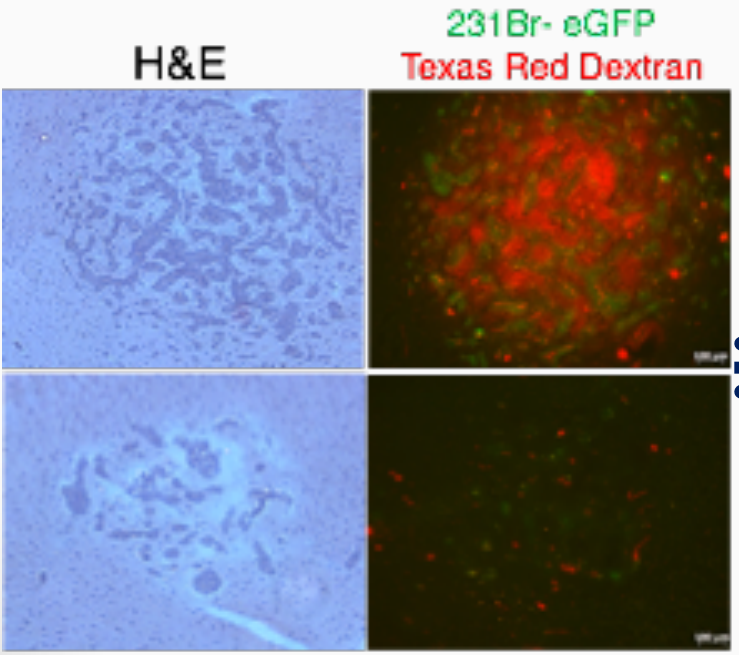
Desmin+ pericytes in 7/9 clinical specimens

- On going investigation of the functional role of Desmin+ pericytes

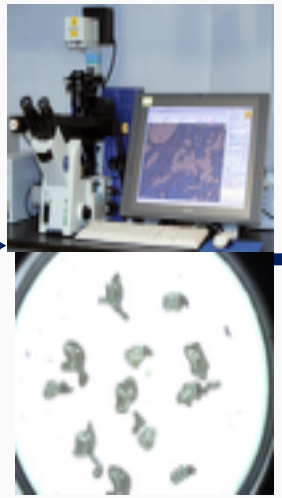
Zooming down from gross cellular changes to **gene expression changes** between highly and poorly permeable BTB

Laser Capture Micro Dissection

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



LCM



RNA extraction and amplification

Mouse Microenvironment: Mouse micro-array

Human metastatic cells: Human microarray

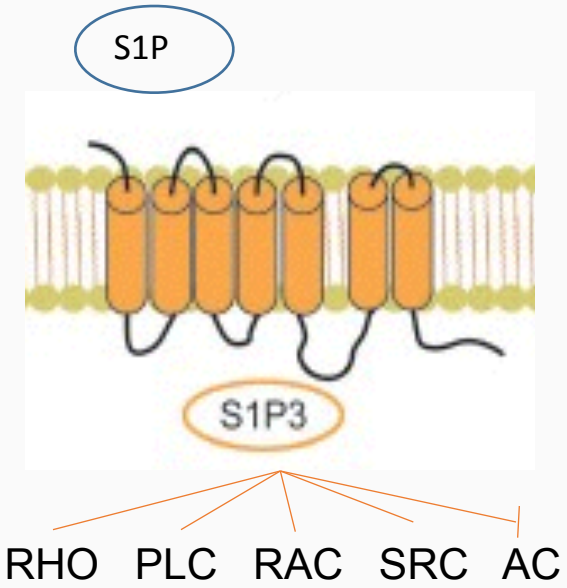
Gril, Paranjape *et al*. *Nat. Com.* 2018

Low and High Permeability Brain Metastases Differ in Micro Environmental Gene Expression.

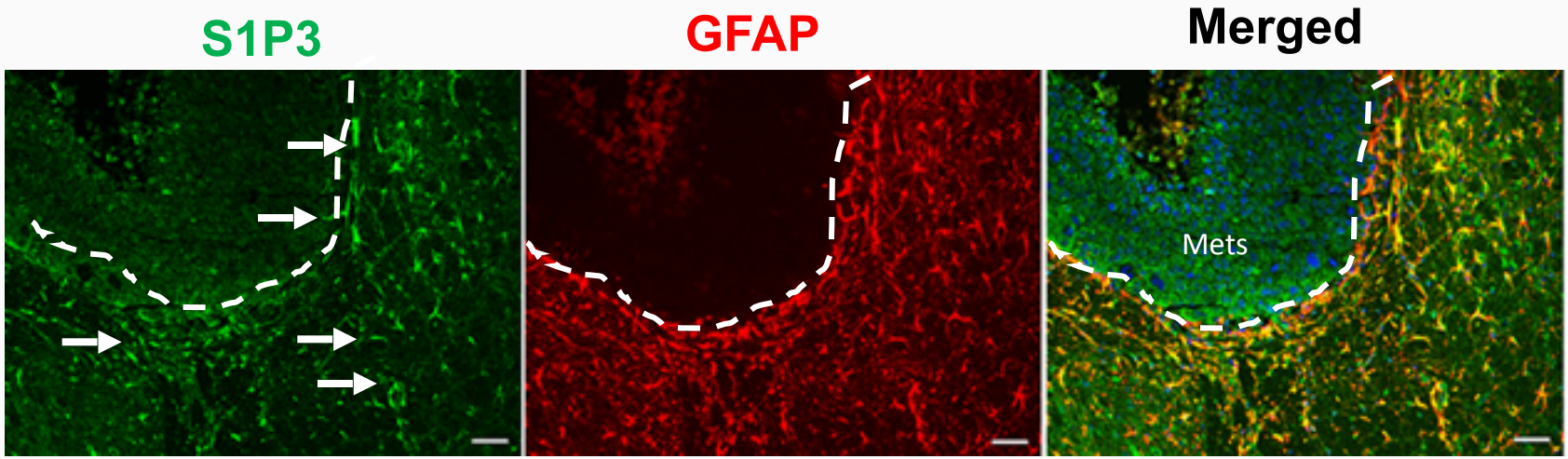
Gril *et al*. *Nat. Com.* 2018

Sphingosine-1-Phosphate Receptor 3

G protein-coupled receptor, binding the lipid mediator sphingosine-1−phosphate (S1P). There are 5 S1PRs

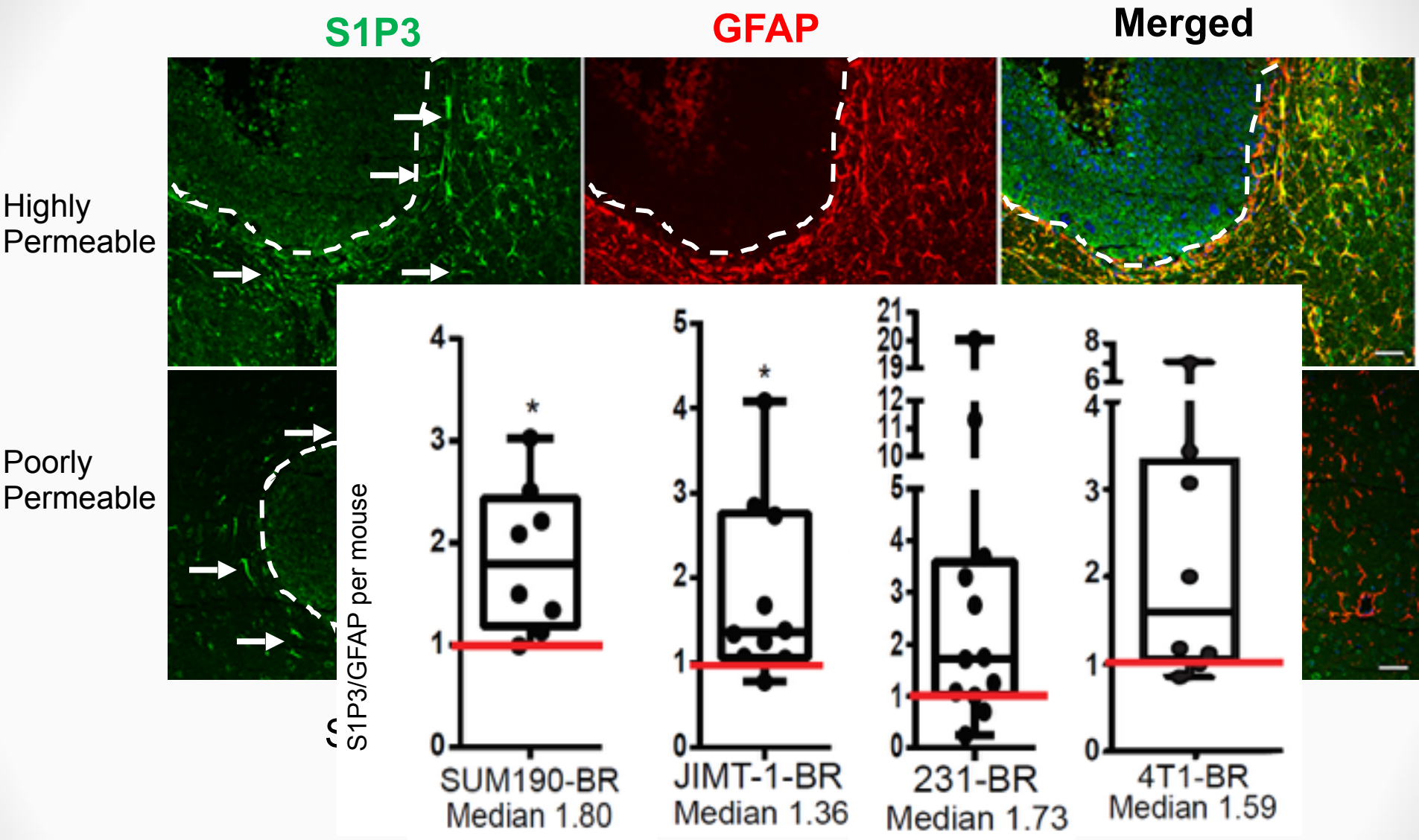


S1P3 is expressed by activated astrocytes surrounding the metastases



SUM190-BR metastatic lesions

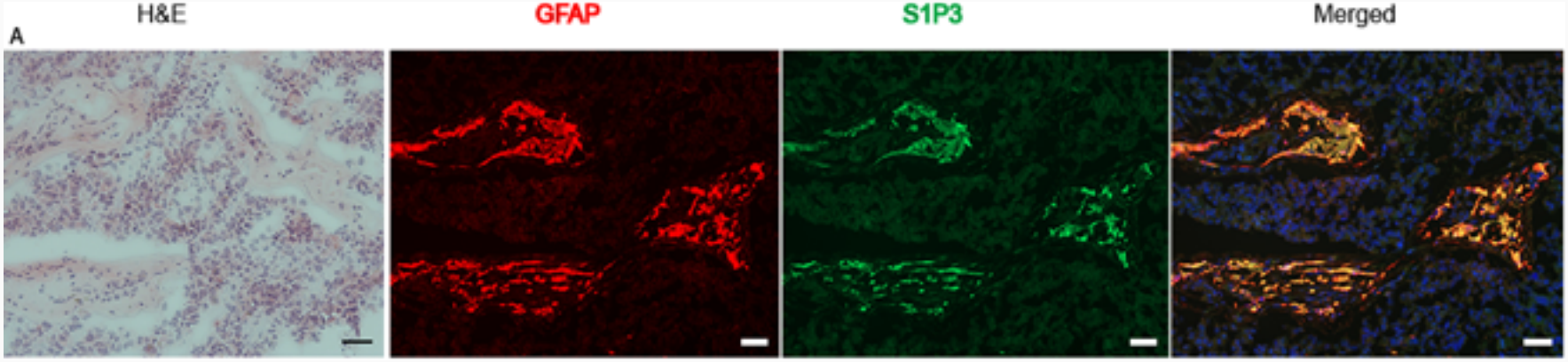
S1P3 Overexpression in Highly Permeable Lesions



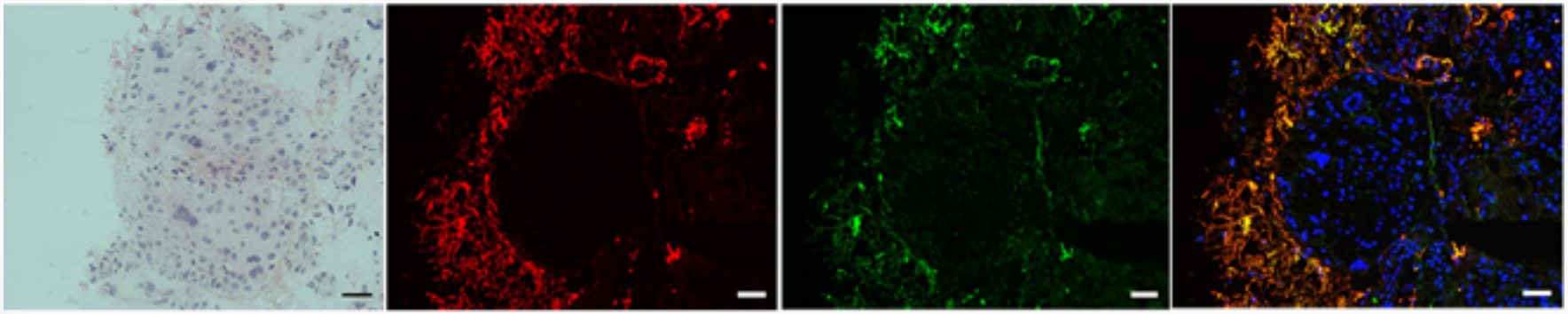
Clinical Relevance

17/19 human craniotomy specimens expressed S1P3 in GFAP+ astrocytes

Patient #1



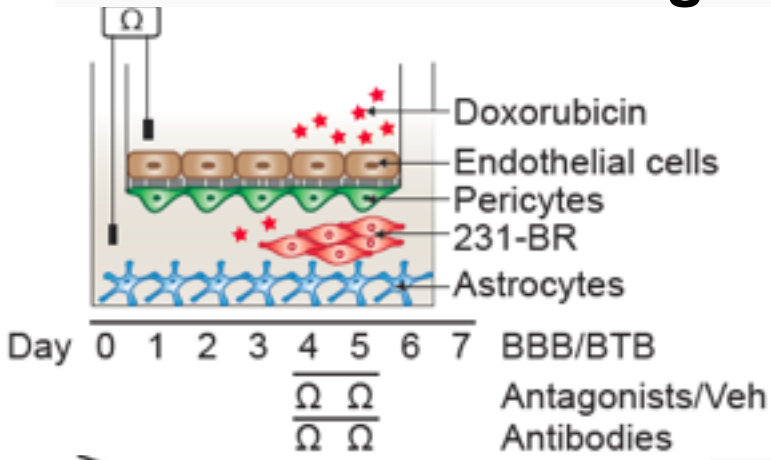
Patient #2



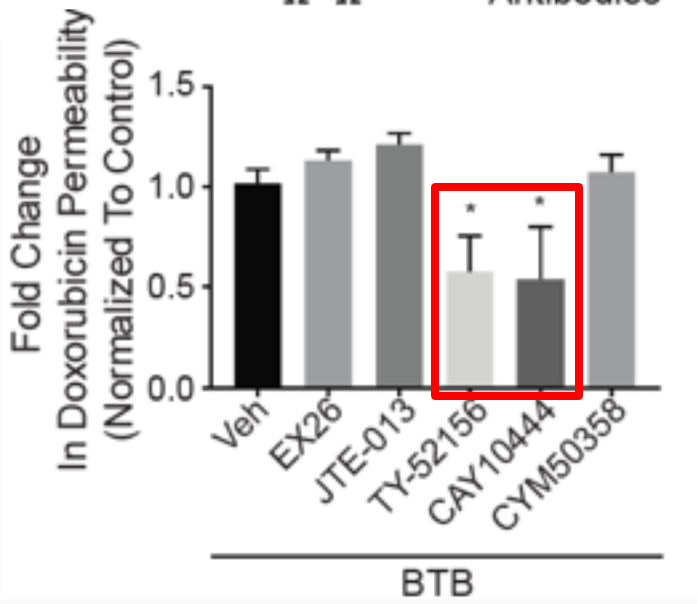
Specimens of brain metastases of breast and lung cancer: Dr. Priscilla K. Brastianos, Massachusetts General Hospital, Dr. Renata Duchnowska et al., Military Institute of Medicine, Poland and Dr. Philippe Metellus, Hopital Clairval, France

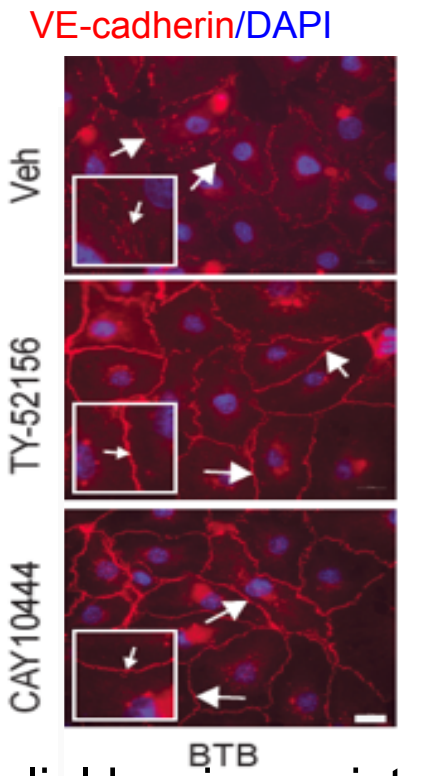
Hypothesis:
Astrocytic S1P3 pathway induces blood-tumor barrier permeability

Functional investigation of S1P3 *in vitro*



EX26	S1P1 antagonist
JTE-013	S1P2 antagonist
TY-52156	S1P3 antagonist
CAY10444	S1P3 antagonist
CYM50358	S1P4 antagonist



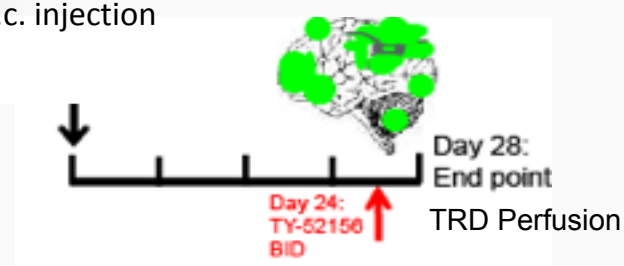


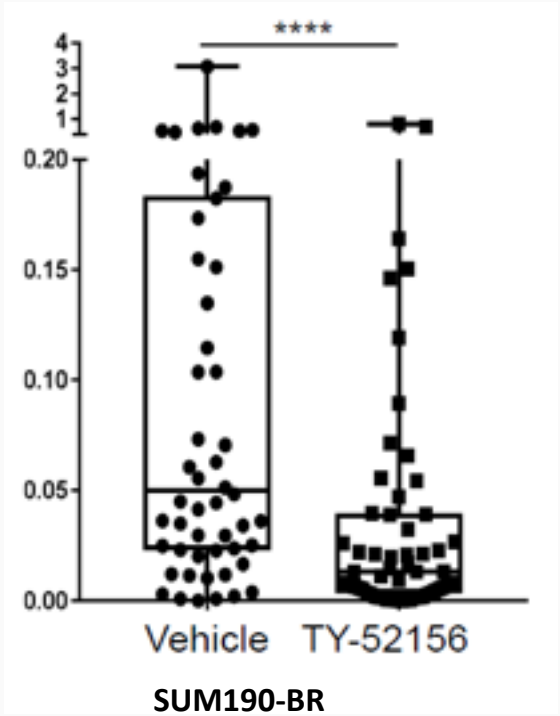
- S1P3 antagonist increased endothelial barrier resistance
- No effect when we removed astrocytes
- Same results with S1P3 knocked down in the astrocytes
- Astrocytic S1P3 acts through CCL2 and IL-6 secretion

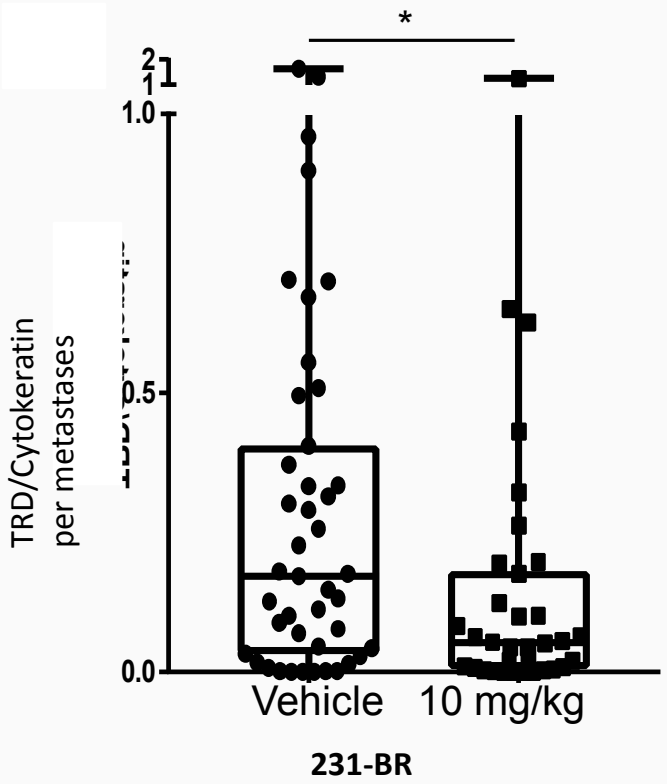
S1P3 antagonist tightened the BTB *in vivo*

Gril, Paranjape *et al*. *Nat. Com.* 2018

SUM190-BR
231-BR
i.c. injection



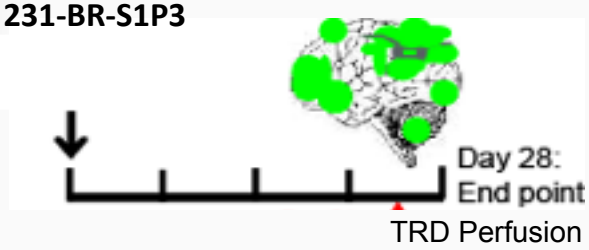


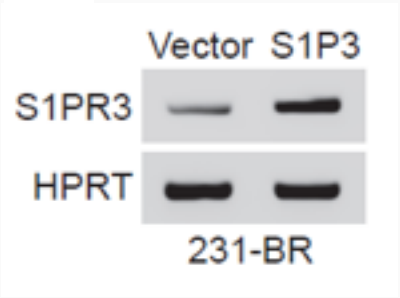


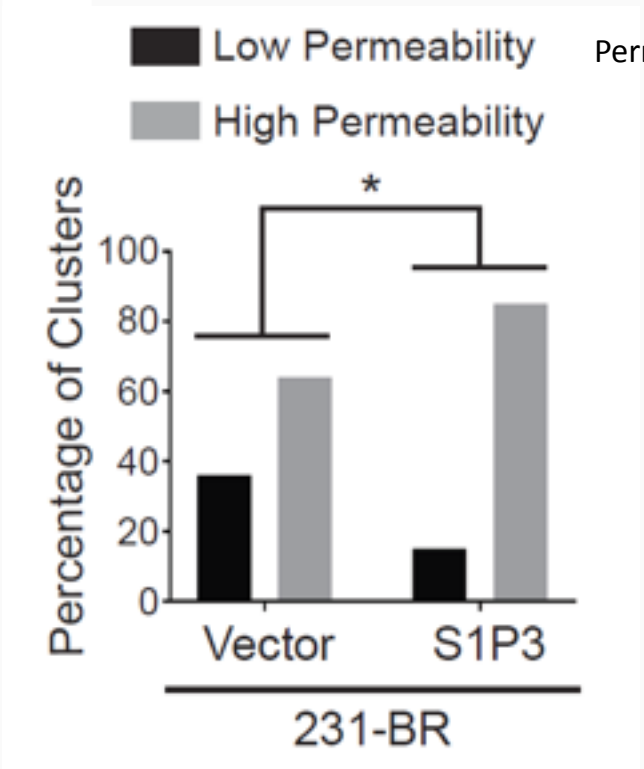
S1P3 antagonist decreased TRD diffusion by 3 fold

S1P3 overexpressing 231-BR increased BTB permeability *in vivo*

231-BR-vector
231-BR-S1P3







Permeability: TRD/Cytokeratin

Conclusion - 1

♦ The BTB is hetergogenously permeable to passive markers and drug compounds and limits drug accessibility.

♦ A Desmin+ pericyte subpopulation correlates with highly permeable metastases

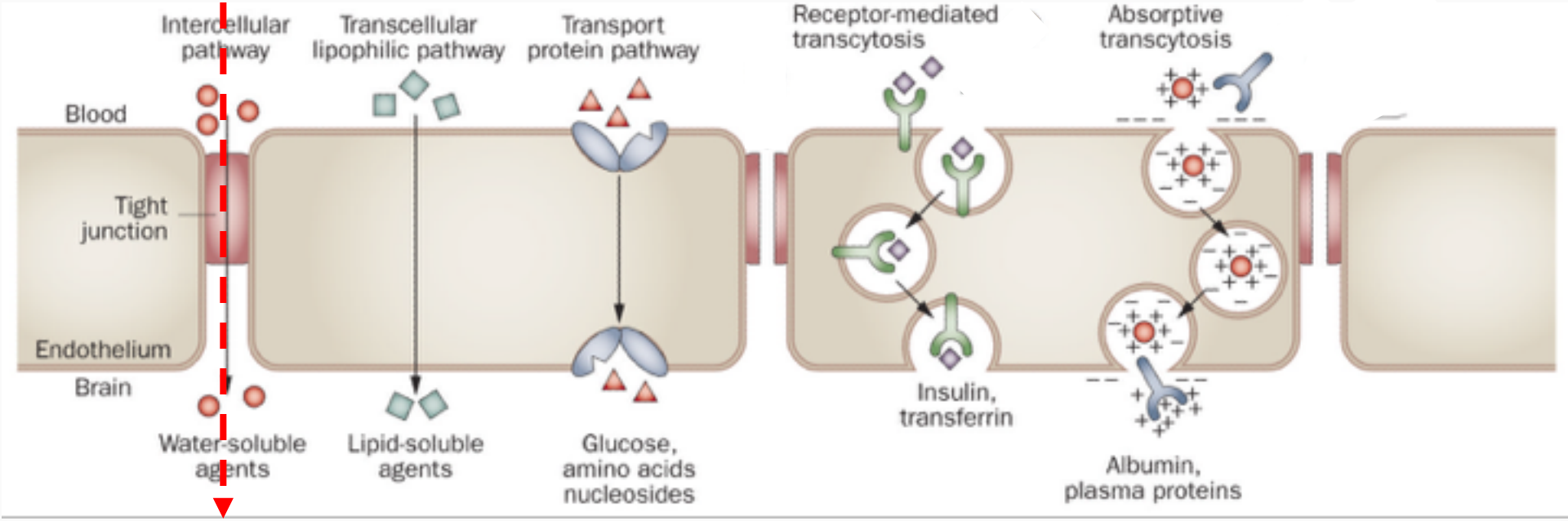
♦ Blocking S1P3 signaling in activated astrocytes restore the blood-tumor barrier integrity.

♦ Proof of concept to show that modulating astrocytes affects the BTB permeability.

Routes to cross the endothelium

Texas Red Dextran 3 kDa
Paracellular route

Transcytosis Route



Adapted from Eichler et al. *Nat Rev Clin Oncol.* 2011

Transcytosis in the brain

- Transcytosis is down regulated in healthy brain, i.e. BBB.

- In PDGFR β-/- (pericytes) mice, there is an increase in transcytosis-involved gene (Plvap, Caveolin-1). (Armulik et al., 2010; Daneman et al., 2010)

- PDGFRβ + pericytes are downregulated in the BTB.

Hypothesis:

The BTB and the BBB express a different transcytosis machinery. Identifying BTB transcytosis is necessary to develop compounds targeting brain metastases.

Investigating the Transcytosis Route

Collaboration with Dr. Rolf Swenson
Imaging Probe Development Center

Transcytosis Ligands :

- Transferrin-Cy5
- LDL receptor peptide-Cy5
- Albumin -Cy5
- Docosahexaenoic acid -Cy5
- lysophosphotidyl choline acid-Cy5
- Cationized albumin -Cy5
- Glucose transporter -Cy5
- folic acid transporter -Cy5
- vitamin B12 transporter -Cy5

Cancer Cells
i.c. injection

- **Ligand-Cy5** i.v. Inj.
-1 or 6h circulation
-Perfusion

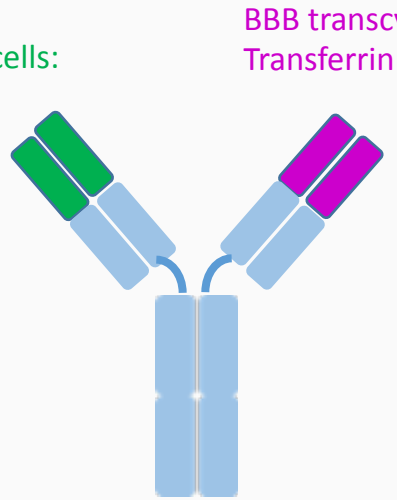
Endpoints:

-Transcytosis in metastases (BTB)
-Transcytosis in healthy part of the brain (BBB)
-Uptake in vital organs: Heart, Lung, Liver, kidney
-Potential symptoms of toxicity

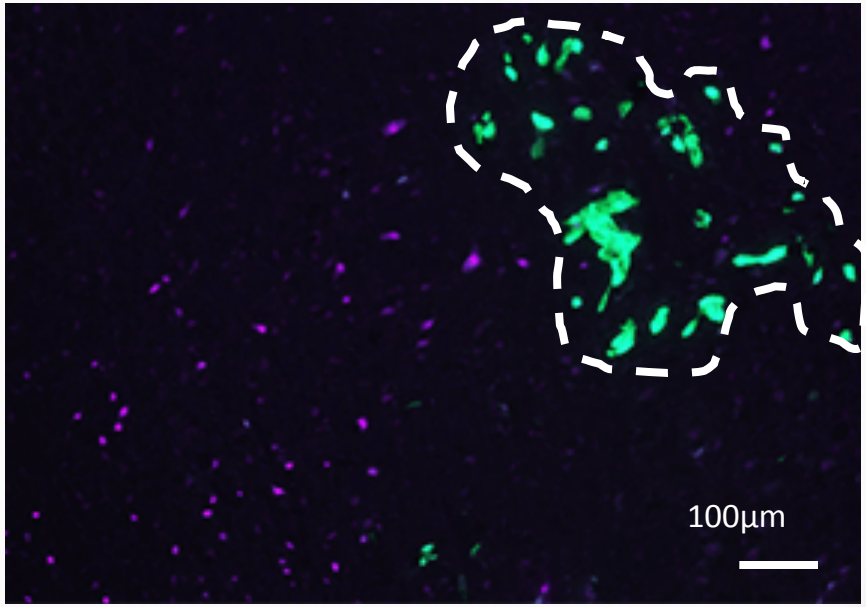
-Check receptor's expression in clinical samples of brain metastases.

Using Receptor-Mediated Transcytosis (RMT)

- Bi-specific antibodies to target BBB and cancer cells



- **JIMT-1-BR eGFP** cell i.c. injection
- Endpoint:
  - **Transferrin-Cy5** 1h circulation
  - Perfusion



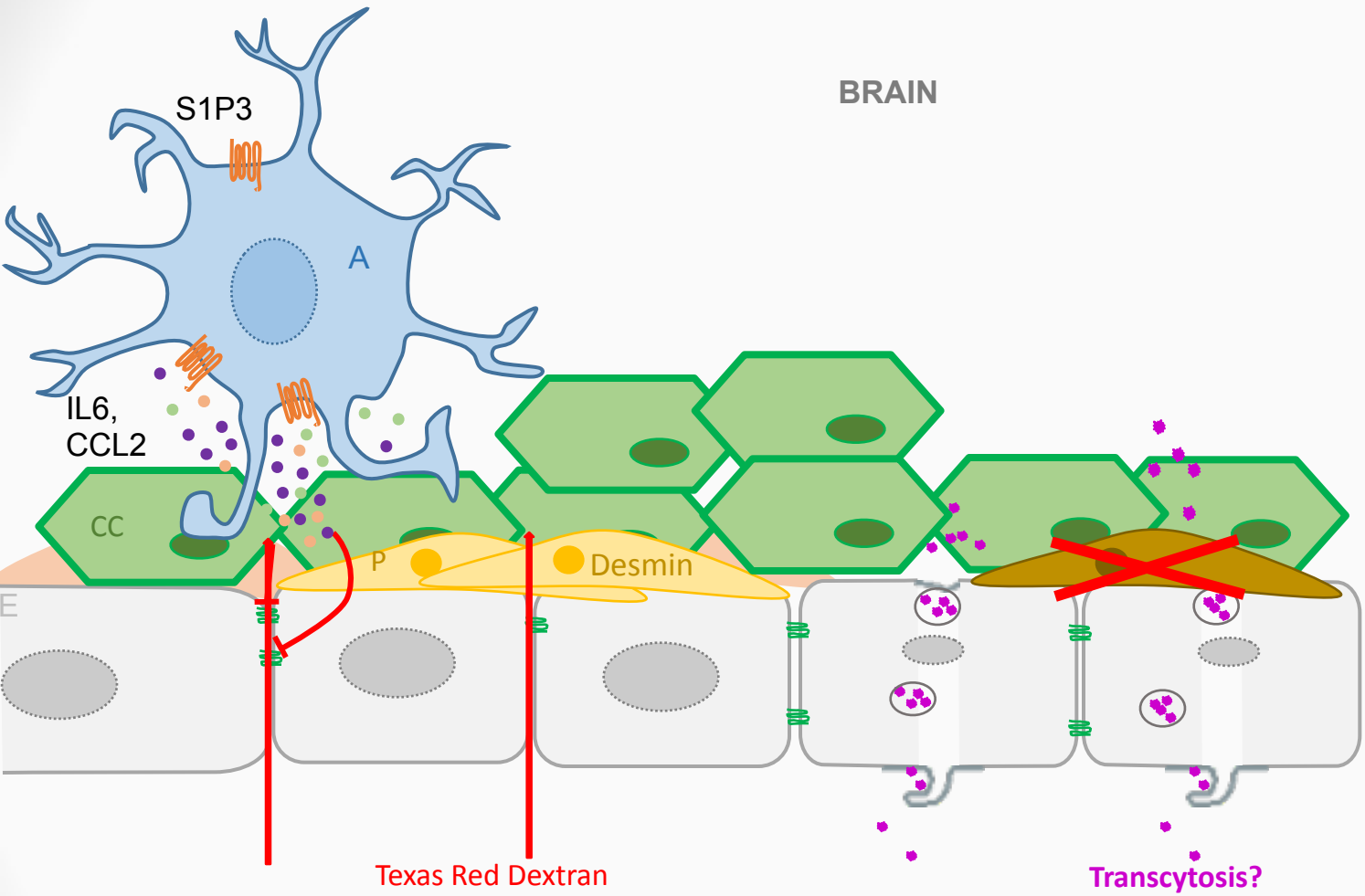
Targeting transferrin allows BBB transcytosis , but not BTB transcytosis

Conclusion - 2

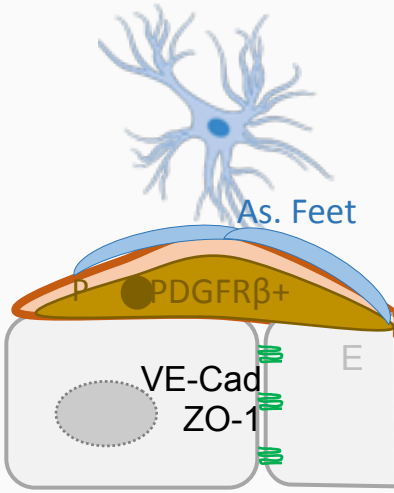
The endothelial cells in the BTB and the BBB express distinct patterns of receptors and transcytosis molecules.

Identifying BTB transcytosis routes will guide us to design drugs crossing specifically the BTB, avoiding healthy part of the brain.

The BTB



The BBB



Endothelium
Pericytes
Astrocytes
Cancer cell

Conclusion

The BBB evolved as a BTB, through systematic cellular and molecular changes, which differentiate:
BBB vs. **BTB** and **highly** vs. **poorly permeable BTB**

A **Desmin+ pericyte** subpopulation correlates with highly permeable metastases

Astrocytic S1P3 induces BTB permeability through IL-6 and CCL2 secretion, affecting adhesion and tight junction proteins in endothelial cells

The BTB and the BBB express a different **transcytosis** machinery

These molecular and cellular components of the BTB will guide us to design the best treatment option for brain metastases.

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