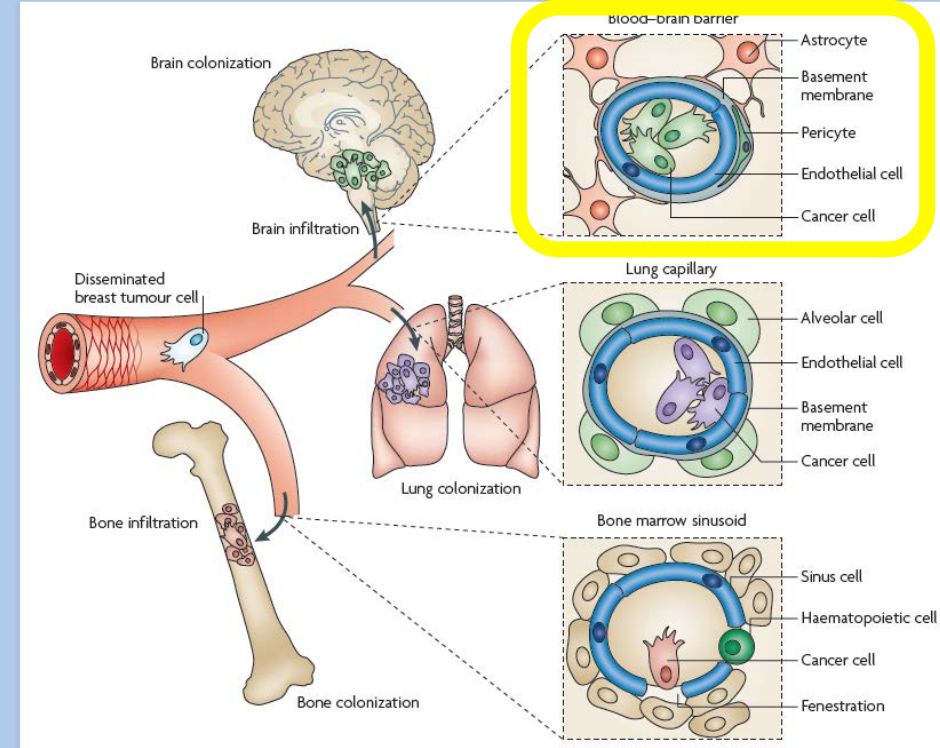
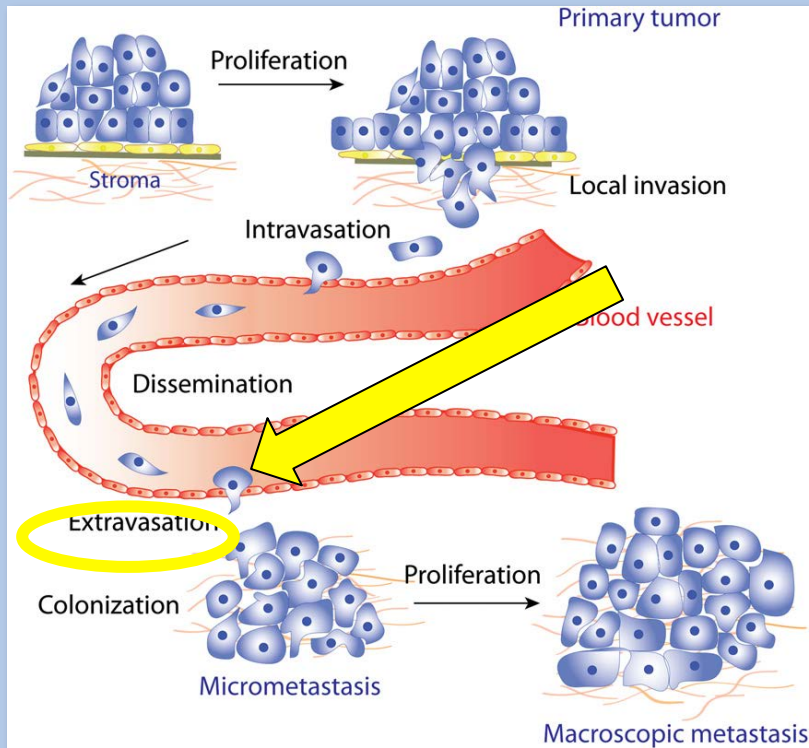


4th Annual Brain Metastases Research  
and Emerging Therapy Conference,  
September 20, 2014, Marseille, France

**MOLECULAR AND CELLULAR  
MECHANISMS OF METASTATIC CANCER  
CELL EXTRAVASATION ACROSS THE  
BLOOD BRAIN BARRIER**

Ruth Lyck (PhD)  
Theodor Kocher Institute  
University of Bern  
Bern, Switzerland

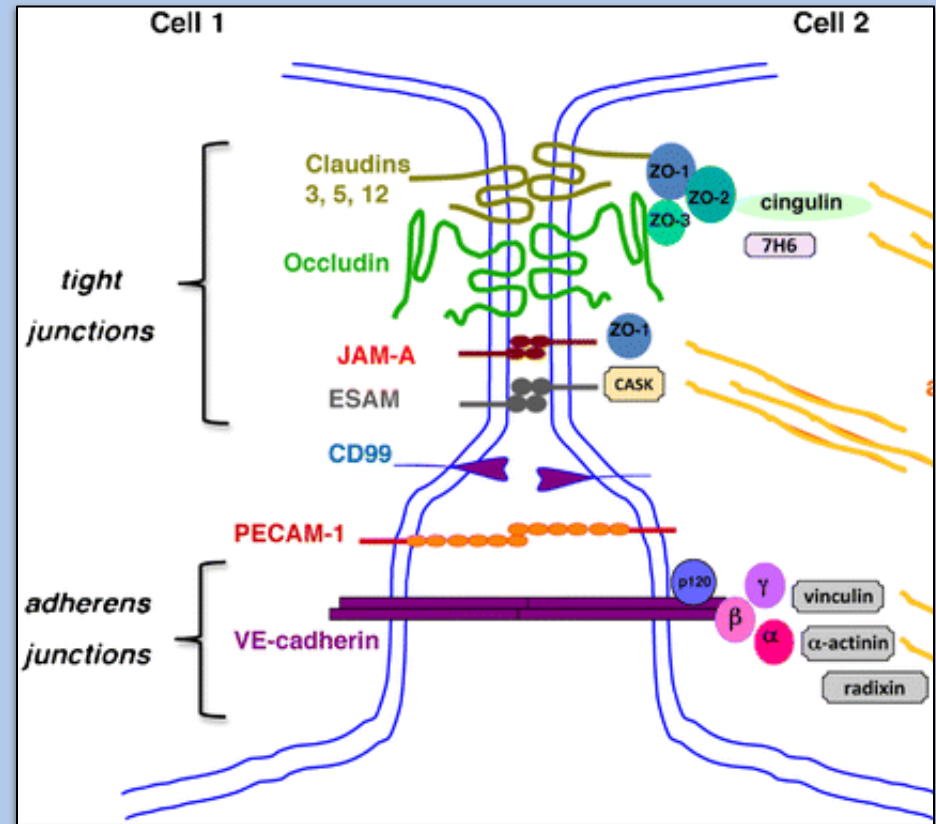
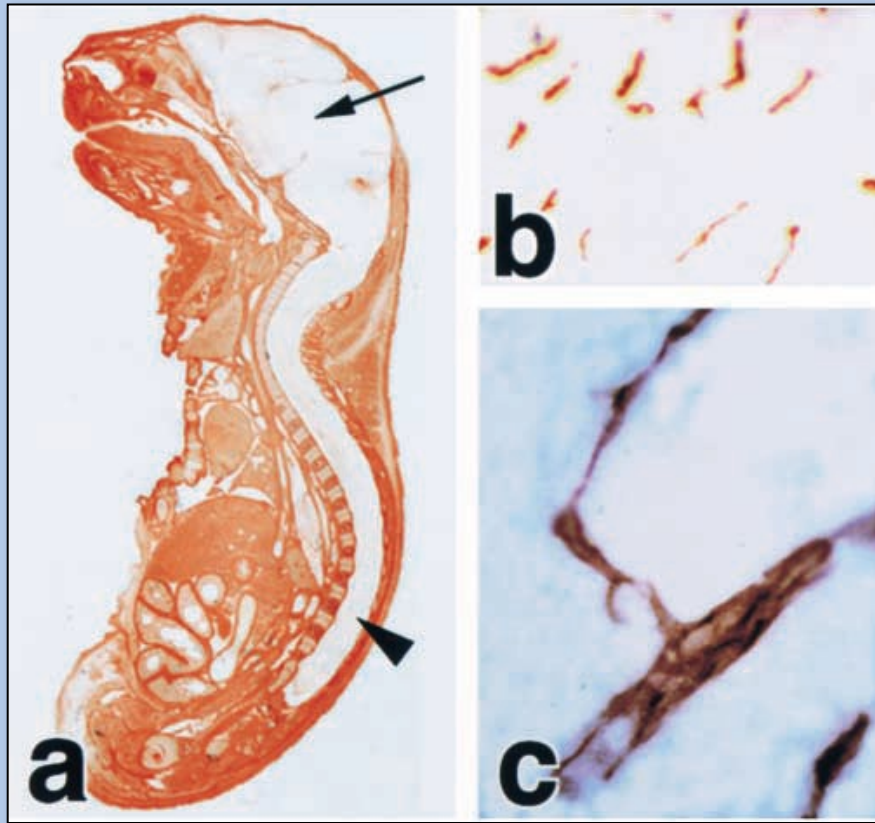
# Focus: Extravasation of MCCs across the BBB



Saxena and Christofori, Mol Oncology, 2013

Nguyen, Bos, Massagué, 2009

# The physical barrier is formed by tight junctions of the endothelial cells

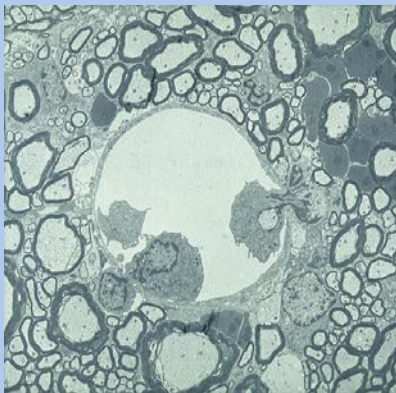
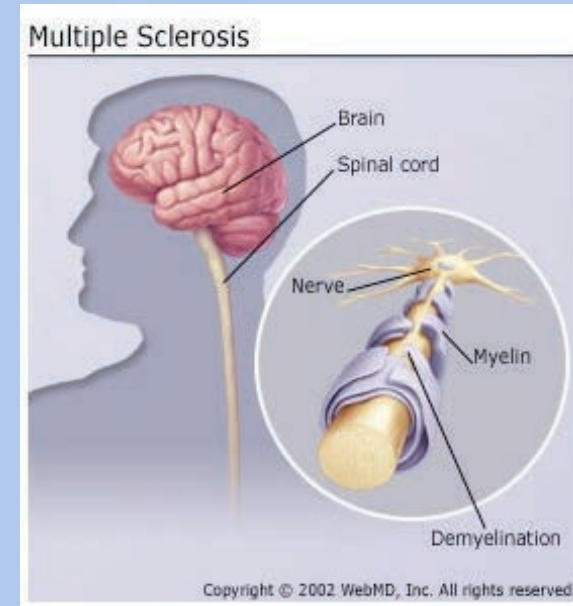


Nitta et al 2003, JCB 161: 653

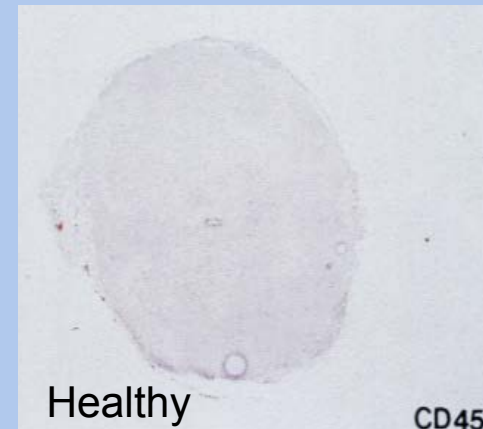
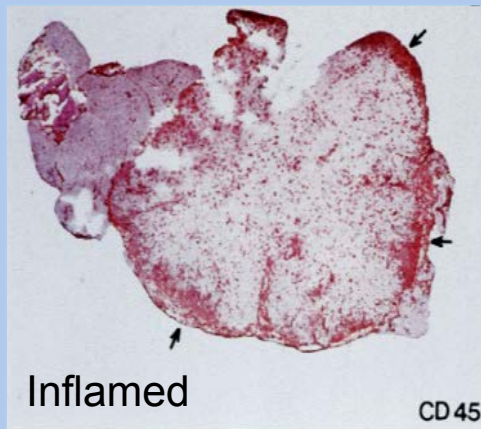
Engelhardt and Sorokin, 2009

# In Multiple Sclerosis (MS) encephalitogenic T cells breach the BBB

- > MS is a chronic and disabling human disease.
  - > In the course of MS
    - autoaggressive immune cells enter the central nervous system (CNS),
    - damage the myelin sheath of the nerve cells and
    - disrupt function of the nerve fibers.
- ➔ Effector cells, eg  $CD4^+$   $T_{EM}$  cells, migrate across the BBB



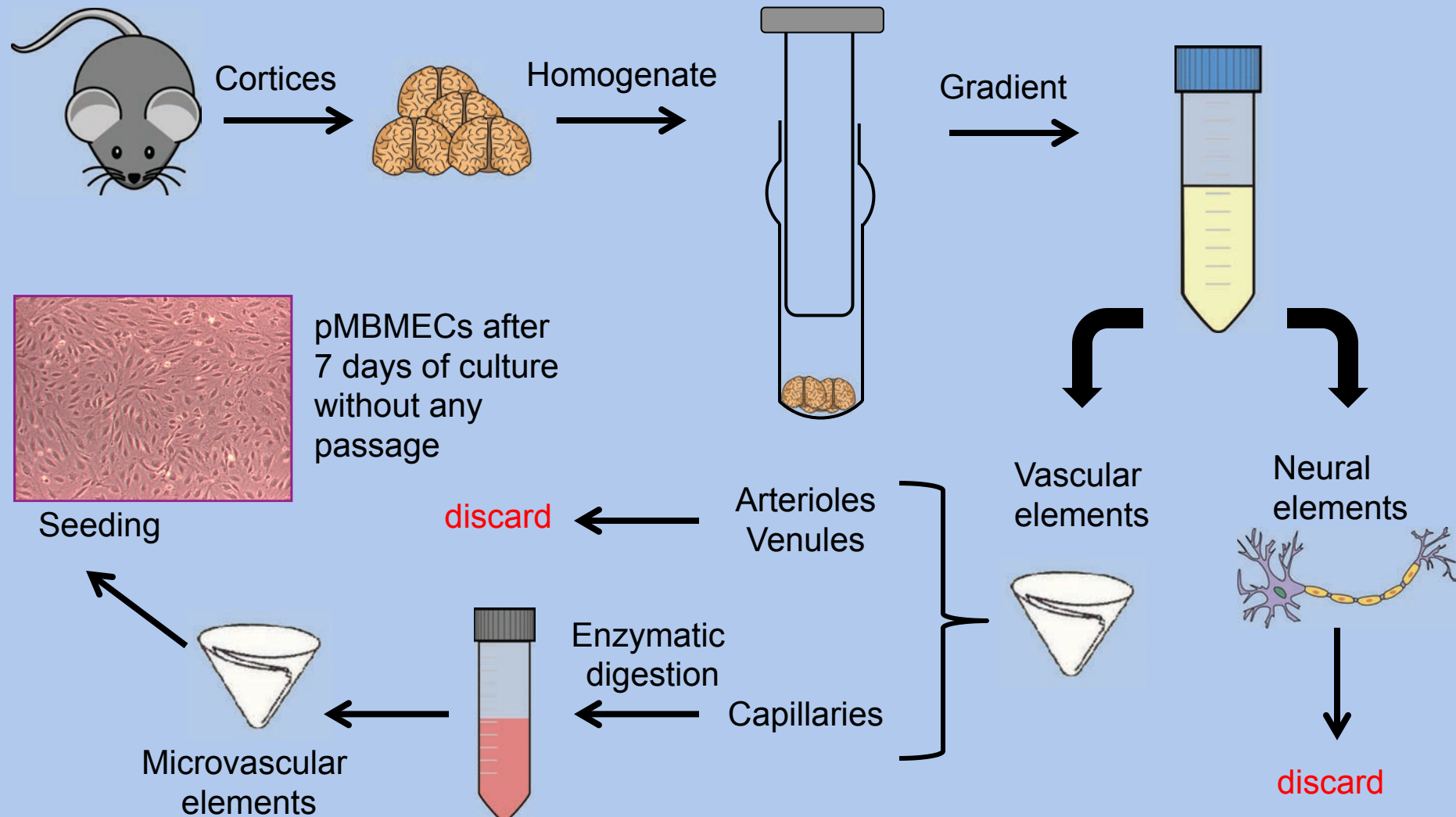
Meyermann, Wekerle 1986



***In vitro* model**  
**to study molecular events of**  
**T cell or cancer cell**  
**extravasation**  
**across the BBB**  
**under flow conditions**



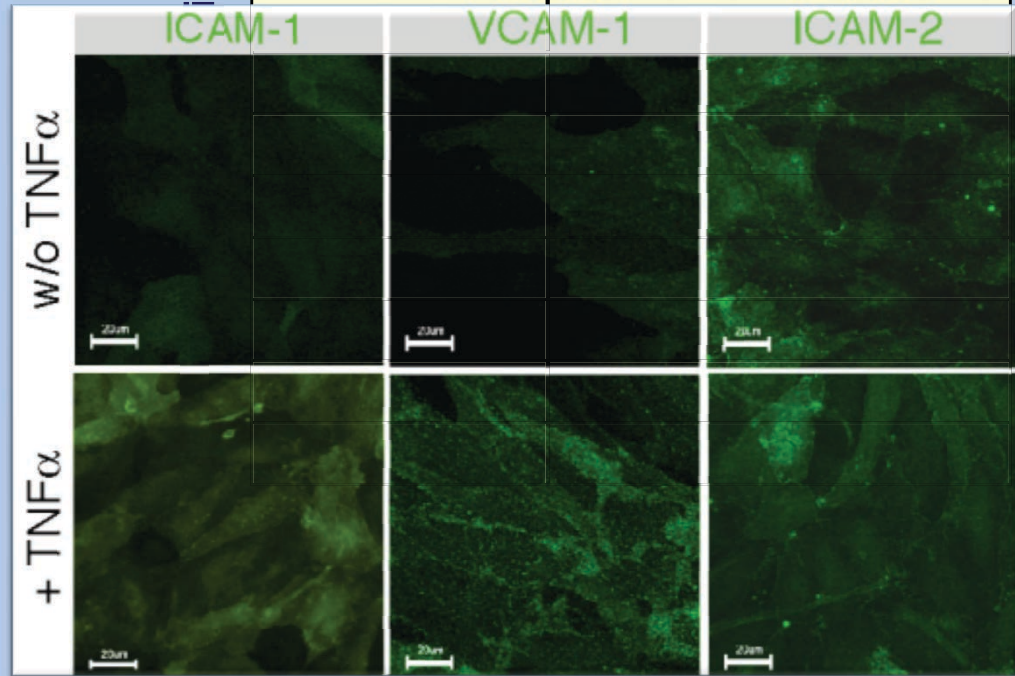
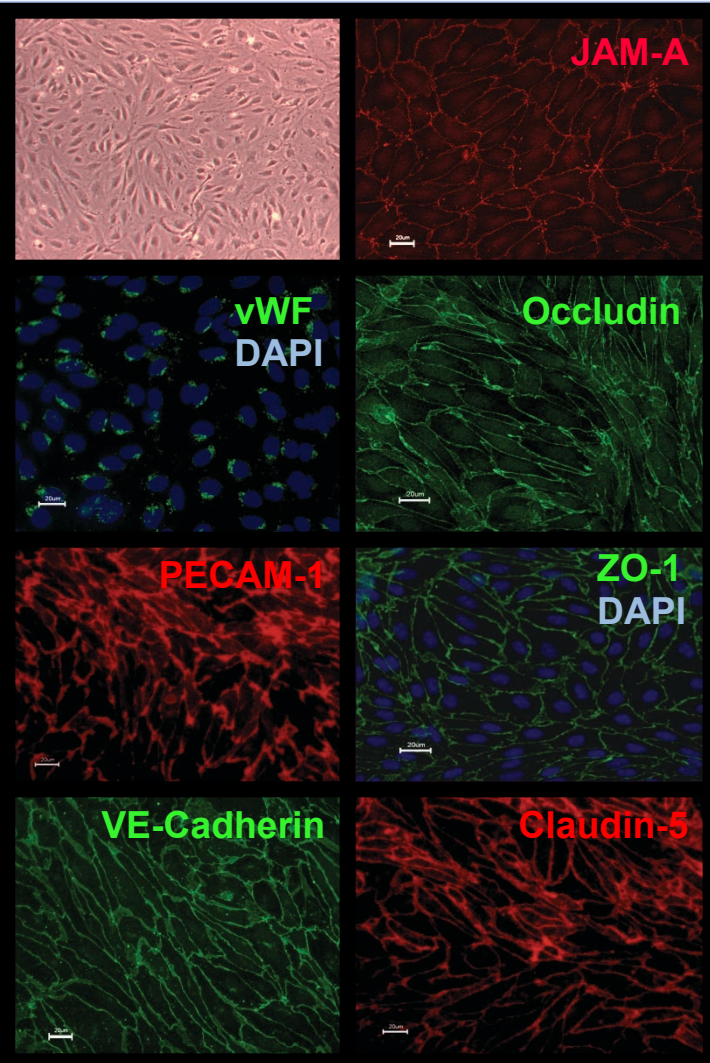
# Primary mouse brain microvascular endothelial cells (pMBMECs)



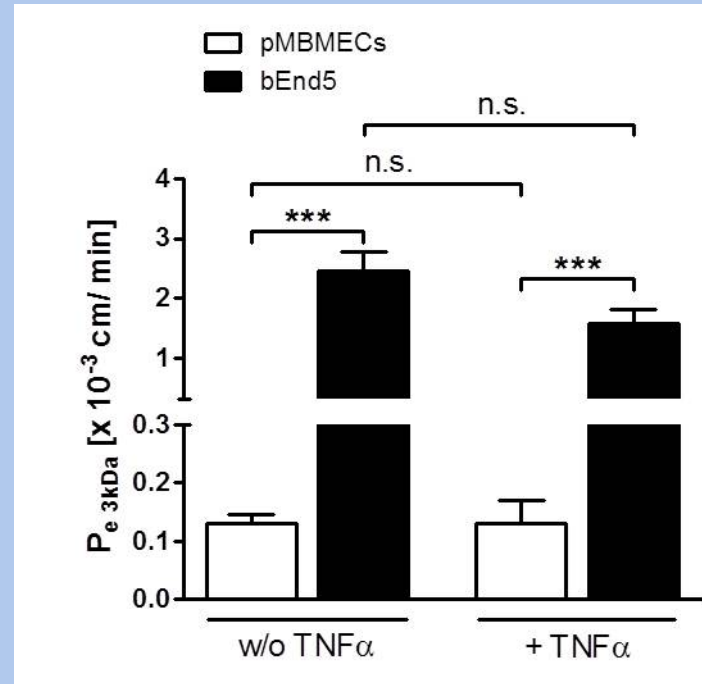
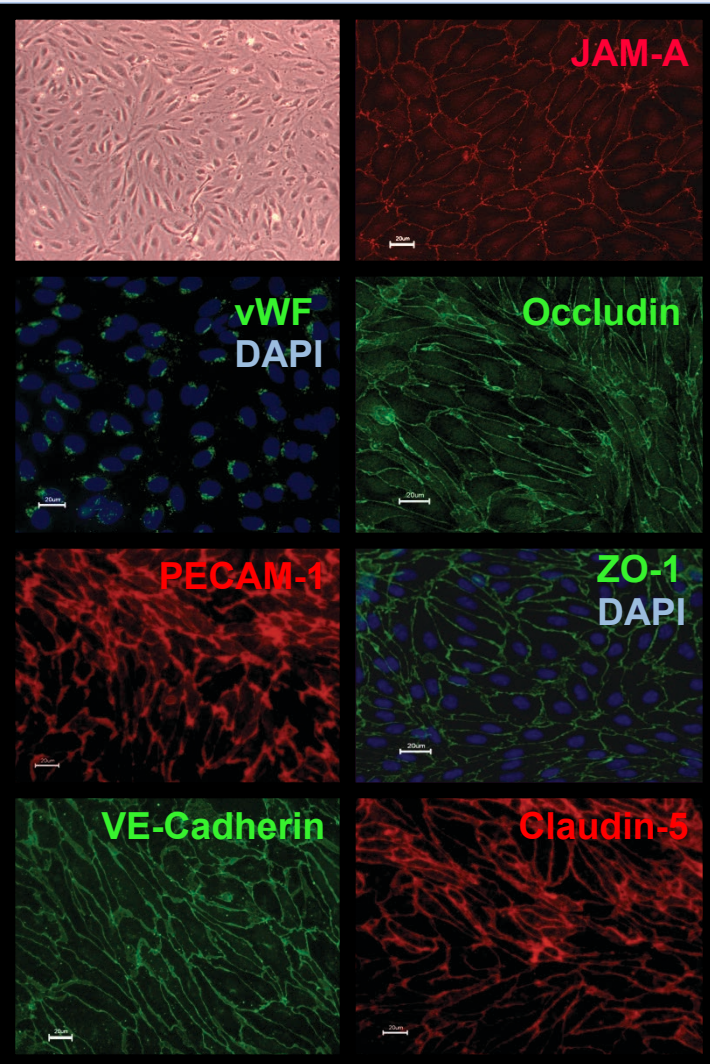
# pMBMECs model the BBB: Endothelial, tight junction and cell adhesion molecules are expressed and properly localized

tight junction

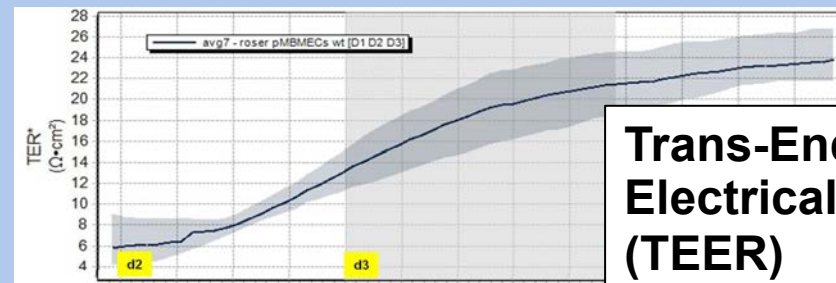
<i>Protein</i>	<i>Localization (Immunofluorescence)</i>
Occludin	junctional
ZO-1	junctional
ZO-2	junctional
Claudin-3	junctional



# pMBMECs model the BBB: Tight monolayer barrier properties are built up



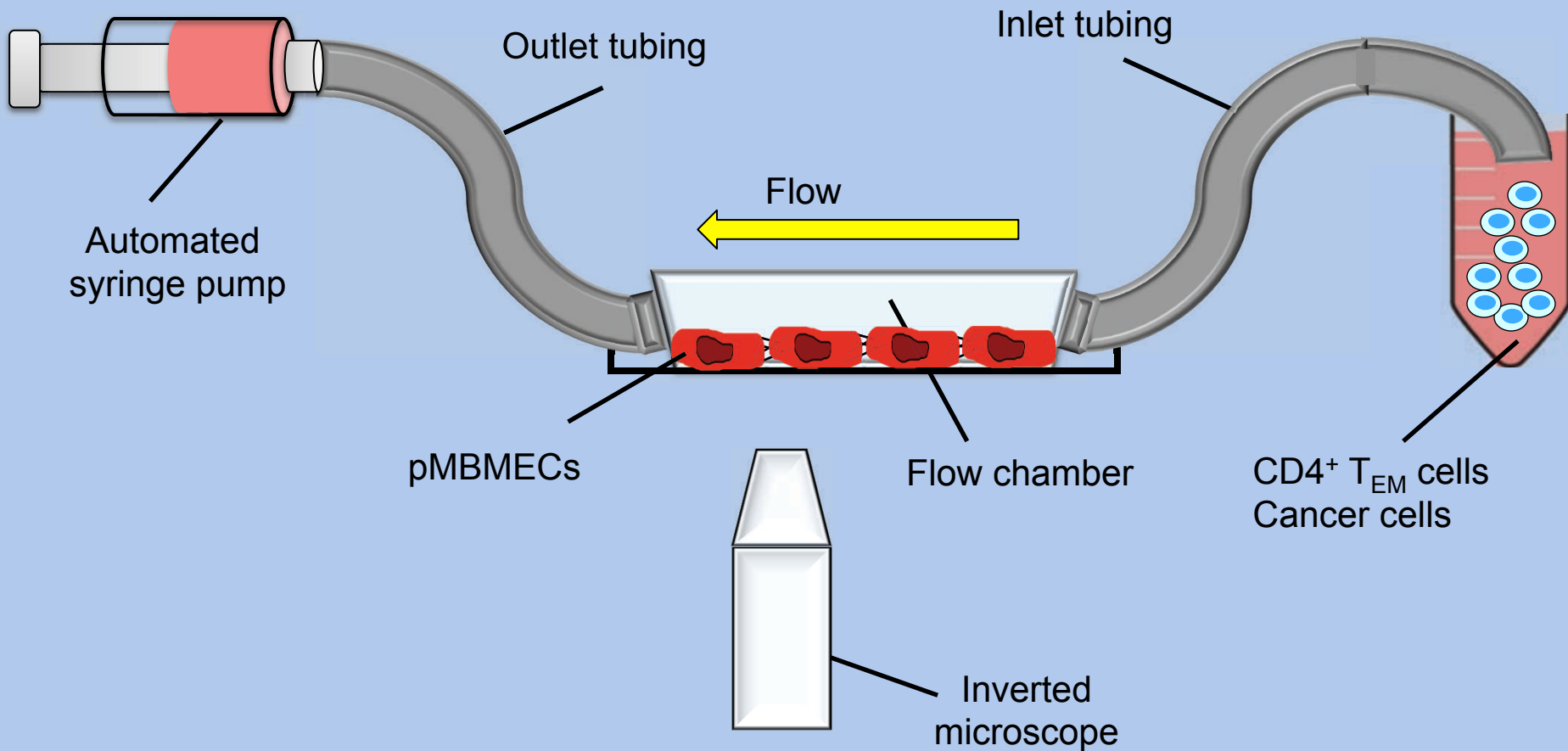
**Permeability**  
(3 kDa Dextran)



**Trans-Endothelial  
Electrical Resistance  
(TEER)**

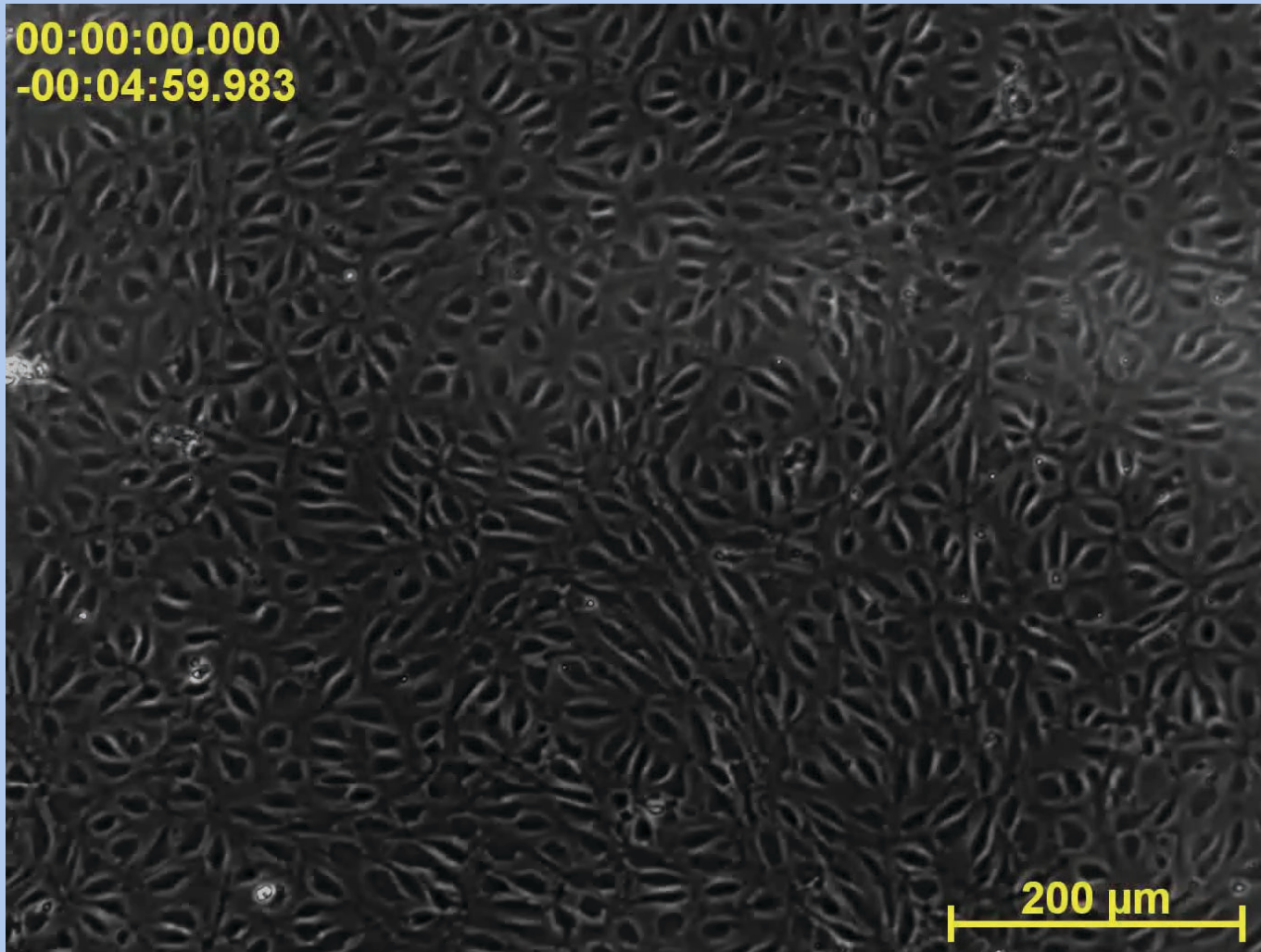


# *In vitro* live cell imaging under flow conditions

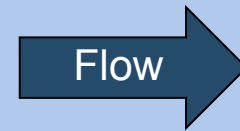




# CD4<sup>+</sup> T<sub>EM</sub> cells dynamically interact with inflamed pMBMECs



Total:  
19 min



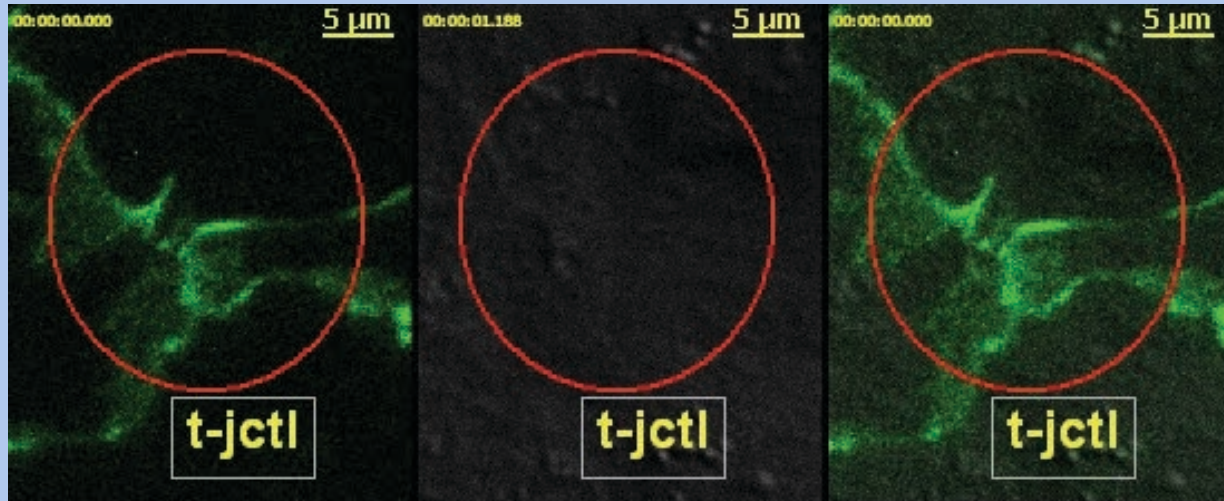
pMBMECs (TNF $\alpha$ ) (7 d)  
+ encephalitogenic CD4<sup>+</sup>  
T cells

Accumulation:  
0.15 dyn/cm<sup>2</sup>  
(initial arrest)

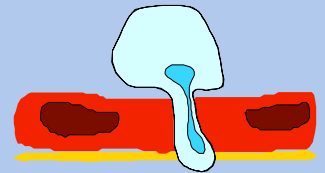
Physiological shear:  
1.5 dyn/cm<sup>2</sup>  
(dynamic interaction)



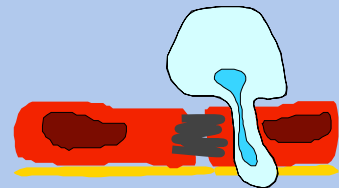
# CD4<sup>+</sup> T<sub>EM</sub> cells diapedese via the transcellular and via the paracellular pathways across pMBMECs



Paracellular



Transcellular





# CD4<sup>+</sup> T<sub>EM</sub> cells dynamically interact with inflamed pMBMECs

00:00:00.000  
-00:04:59.983

What about metastatic cancer cells?

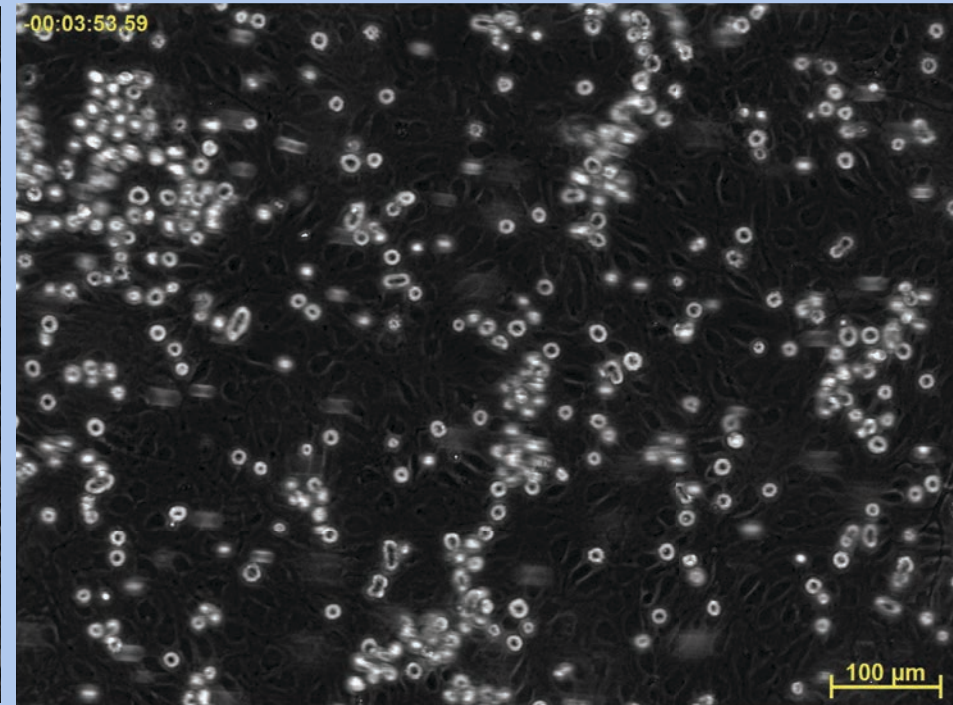
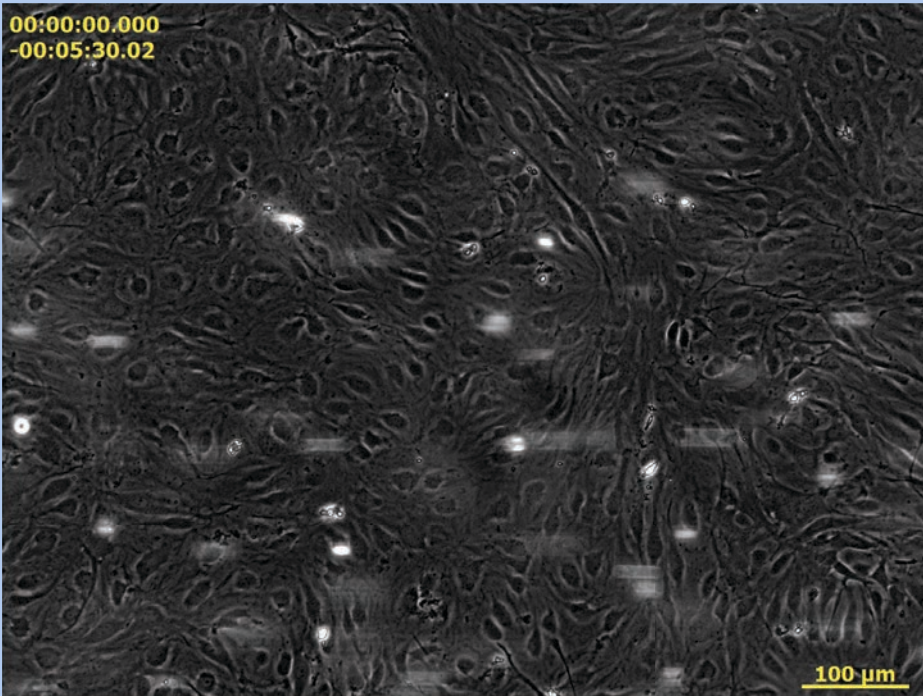
- do they follow a similar mechanism
- which is the role of  $\alpha$ v-integrin (Intetumumab = CNTO95, Wu et al. 2012)?

200  $\mu$ m

# MDA-MB-231BR-HER2 do not arrest on pMBMECs

CD4+ Th1 eff cells

MDA-MB-231BR-HER2



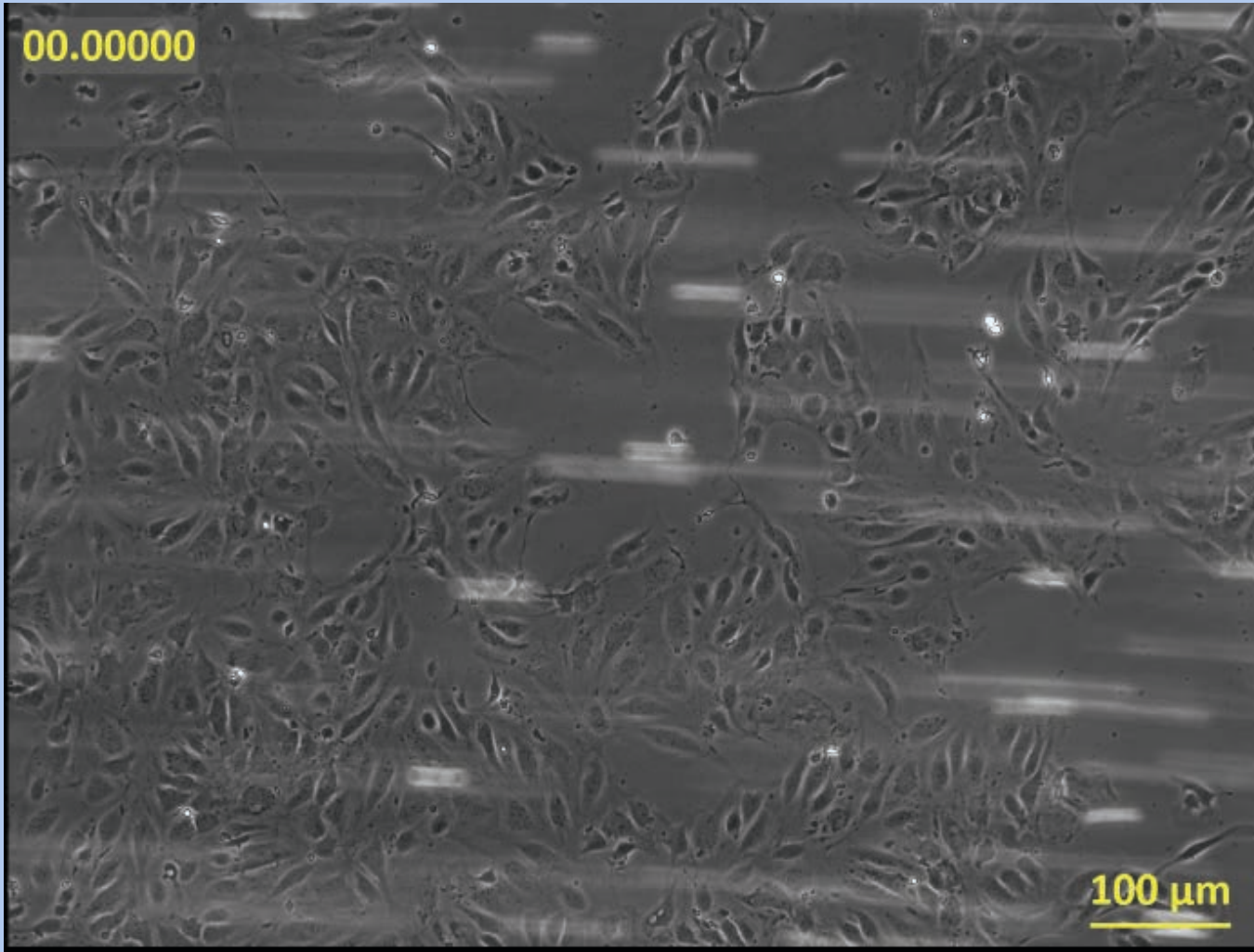
Human “brain seeking” breast cancer cell line

Yoneda et al. JBMR 2001 (Riko Nishimura)

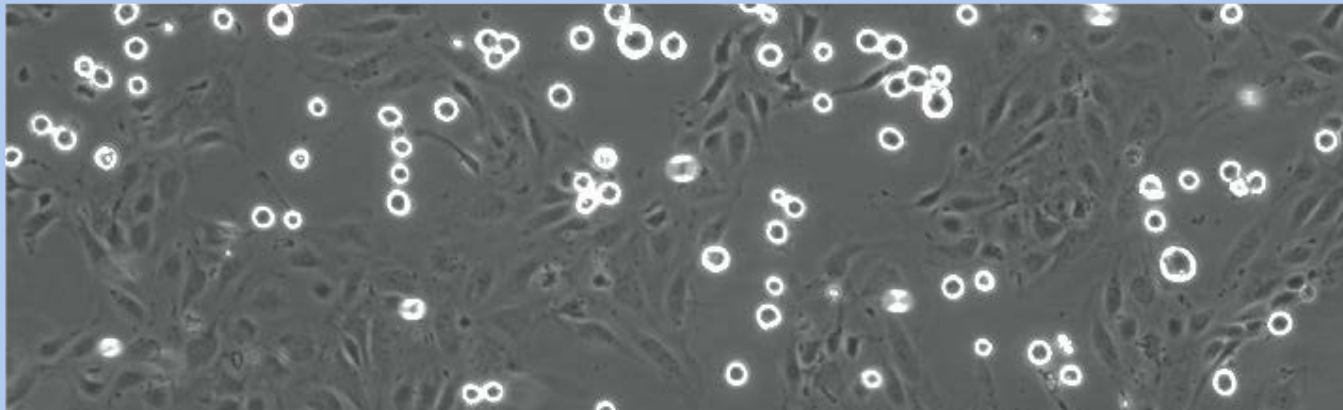
Palmieri et al. Cancer Res 2007 (**Patricia Steeg**)



# MDA-MB-231BR-HER2 arrest on basement membrane proteins (Matrigel)

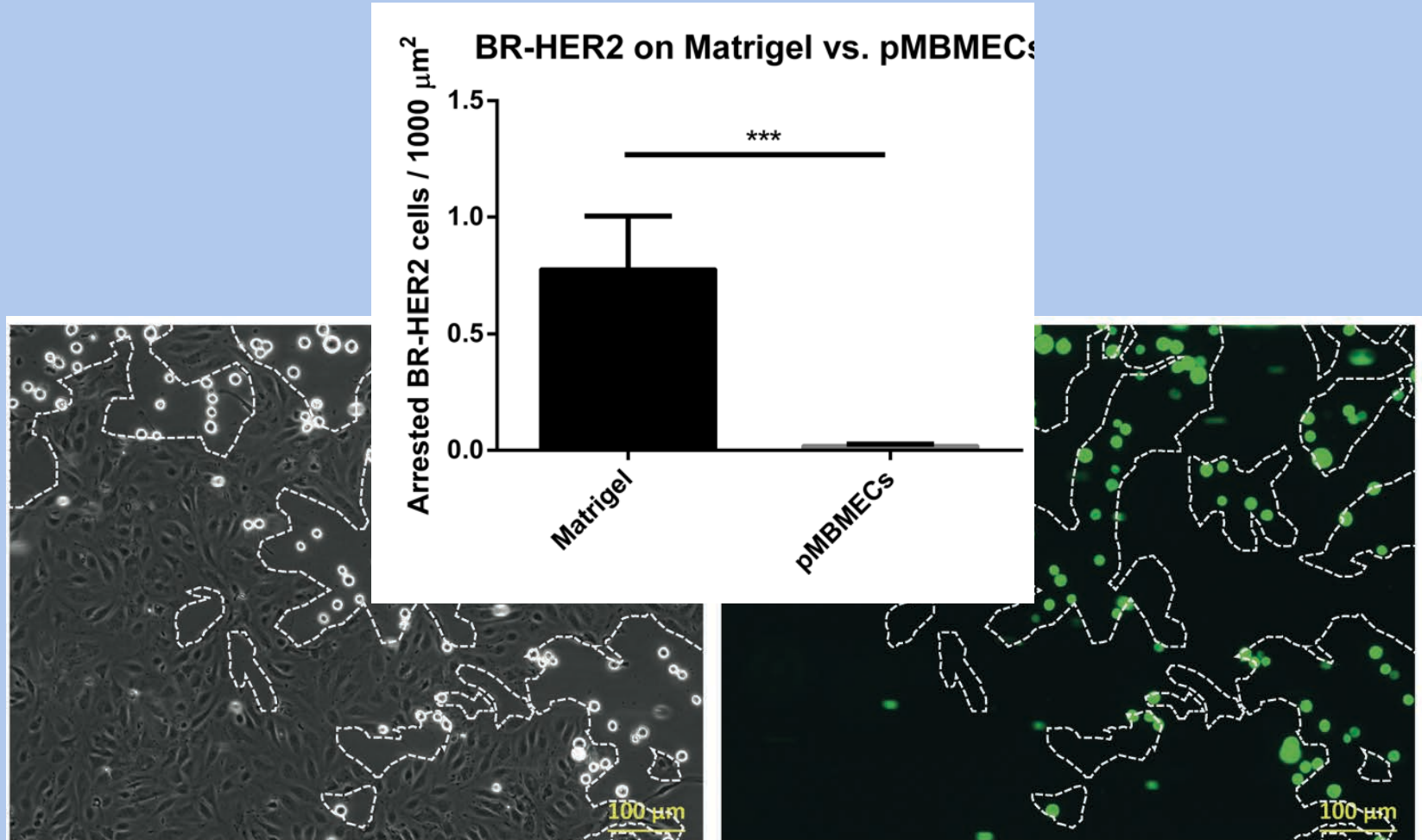


# MDA-MB-231BR-HER2 arrest on basement membrane proteins (Matrigel)





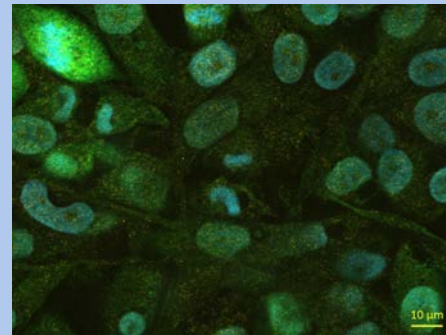
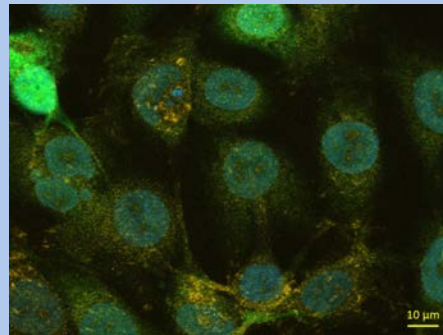
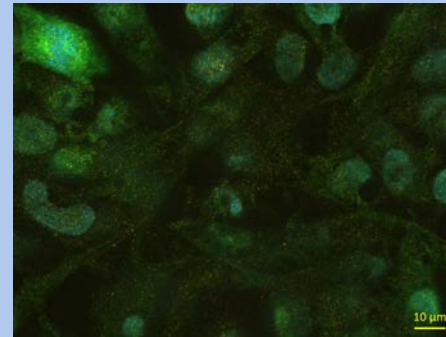
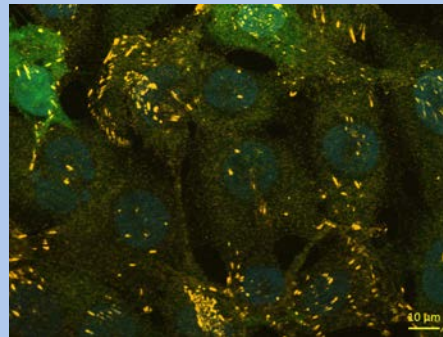
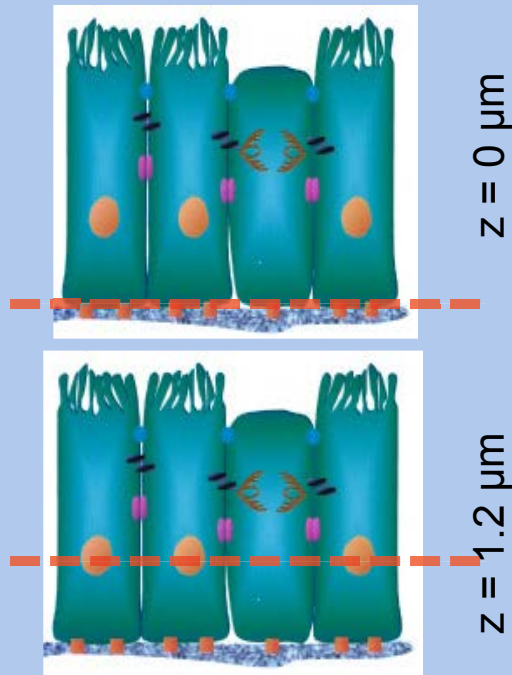
# MDA-MB-231BR-HER2 arrest on basement membrane proteins (Matrigel)



# Intetumumab: Anti human $\alpha v$ -integrin antibody stains focal adhesion like structures of adherent MDA-MB-231BR-HER2 cells

Intetumumab

Ctrl

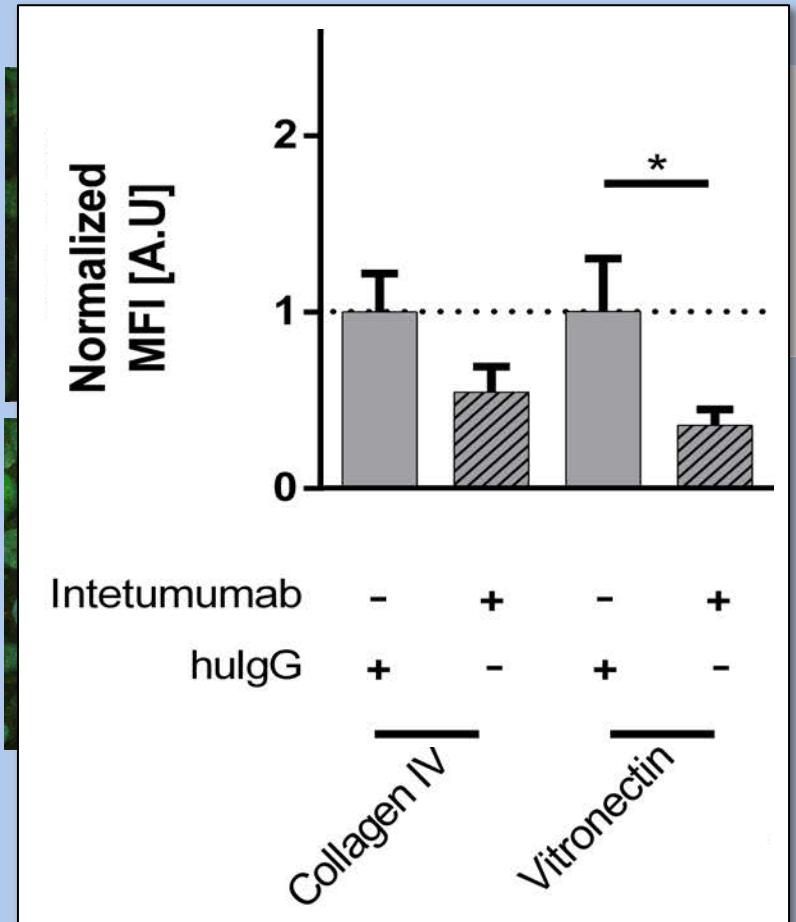
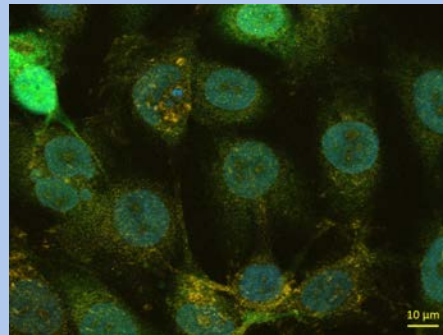
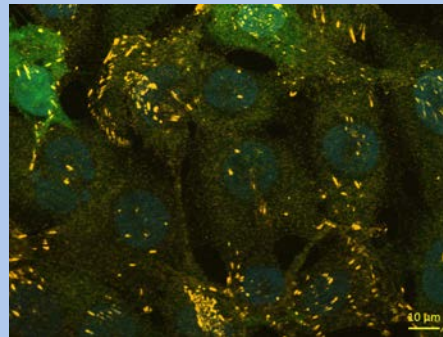
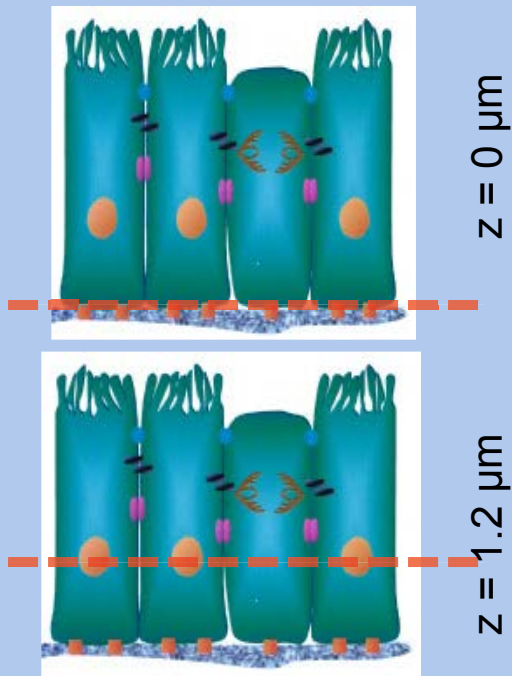


DAPI  
(nuclei)  
GFP  
(Cancer cells)  
Intetumumab  
( $\alpha v$ -integrin)

Does intetumumab block adhesion of MDA-MB-231BR-HER2 to BM proteins?

# Intetumumab blocks binding of MDA-MB-231BR-HER2 cells to Vitronectin

Intetumumab

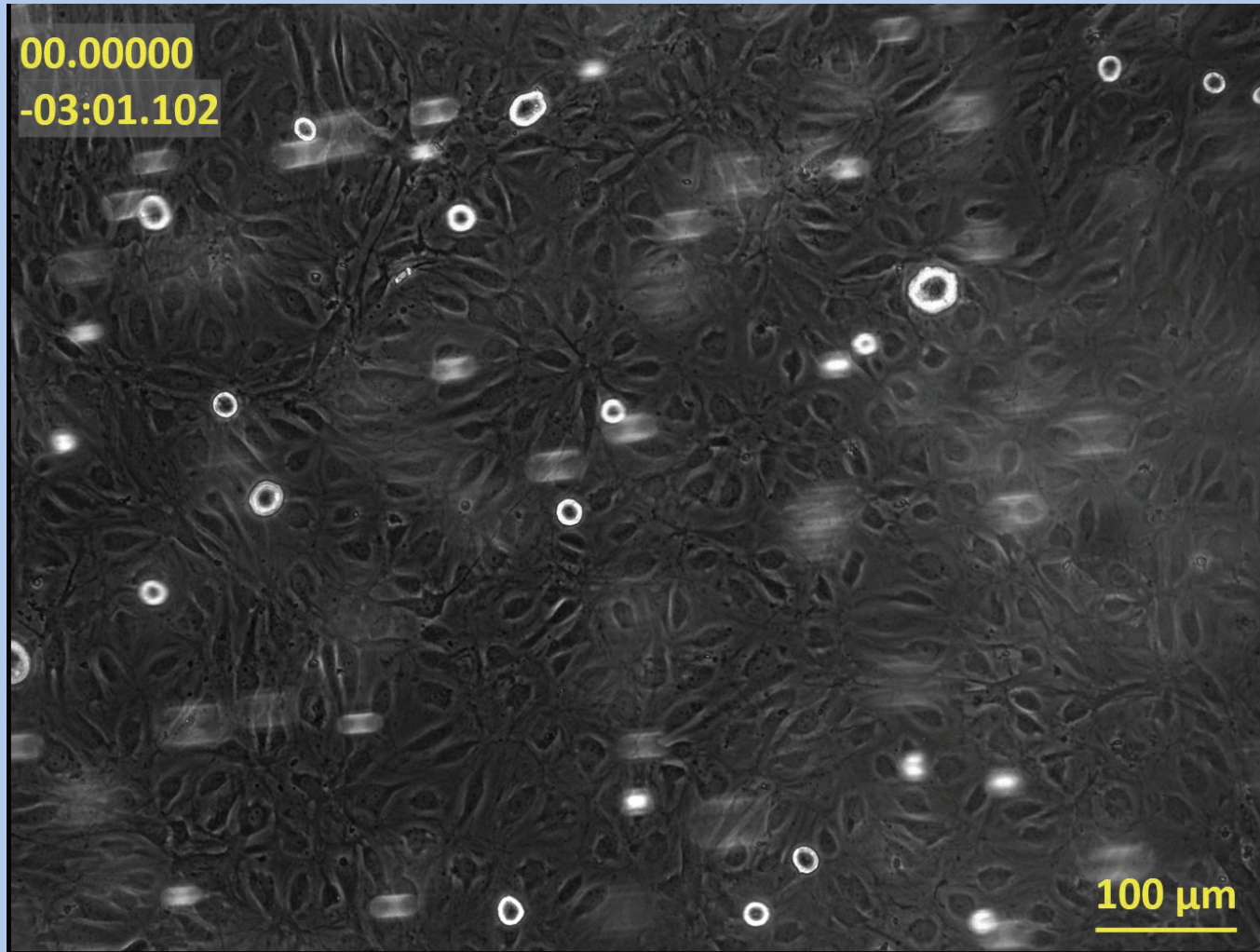


Does intetumumab block adhesion of MDA-MB-231BR-HER2 to BM proteins?

# What about metastatic melanoma cells?



# Metastatic melanoma cells (B16) do arrest on pMBMECs

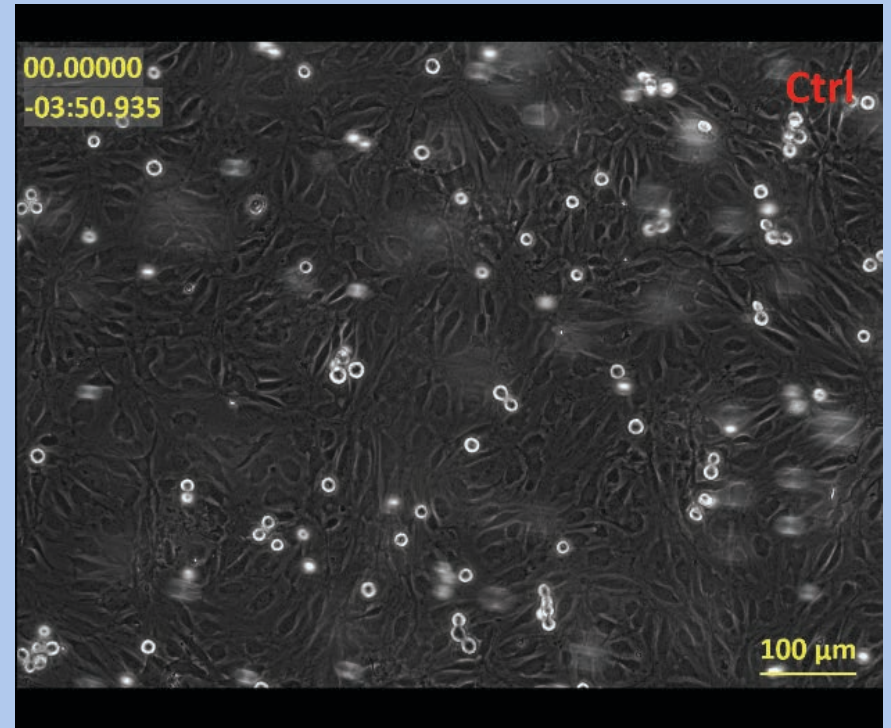
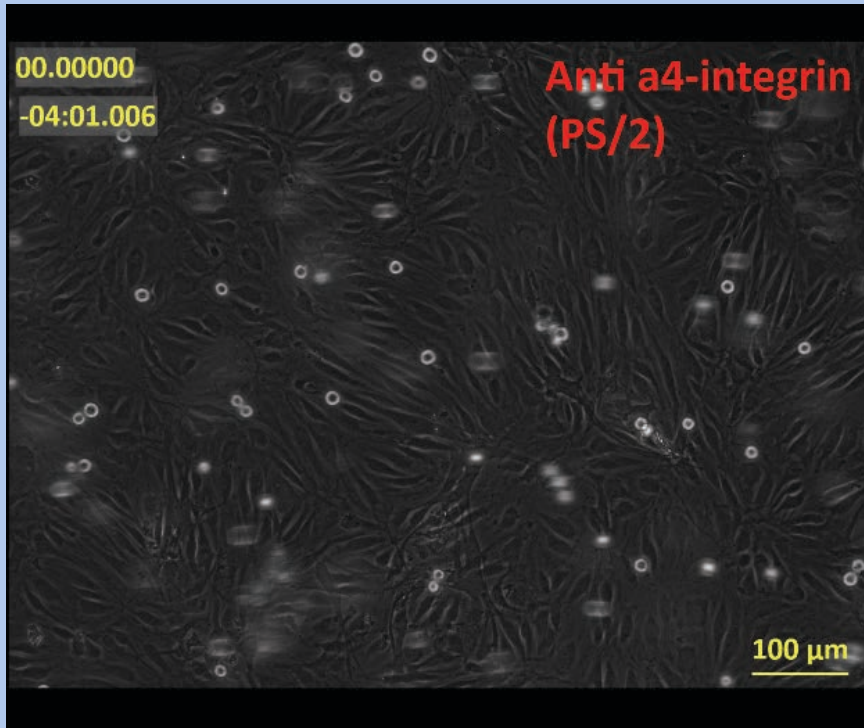


## B16 melanoma cells express $\alpha 4$ -integrin

	Antibody	B16-F1	B16-F10
$\alpha 4$ -Integrin	PS/2	+	+
$\alpha 4\beta 7$ -Integrin	DATK32	-	-
Mac-1 / $\alpha M$ -Integrin	M1/70	-	-
LFA-1 ( $\beta 2$ -Integrin)	FD441.8	-	-

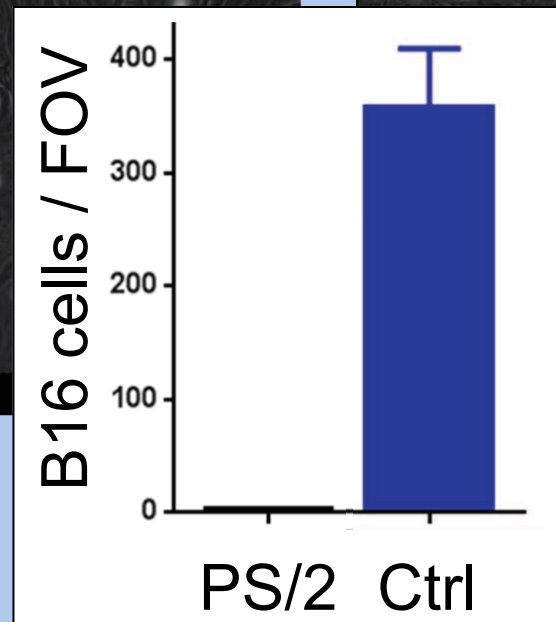
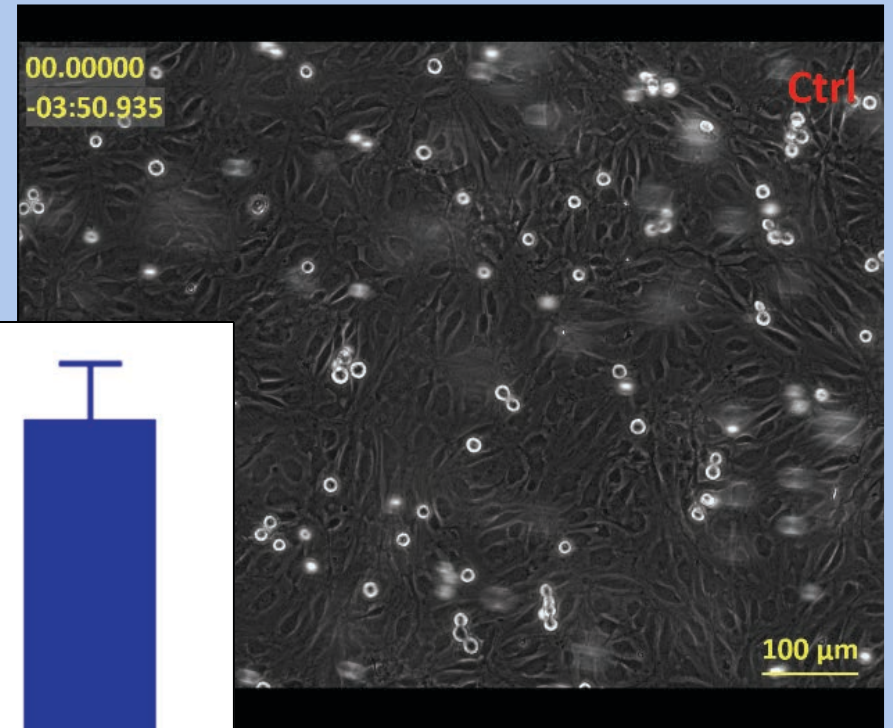
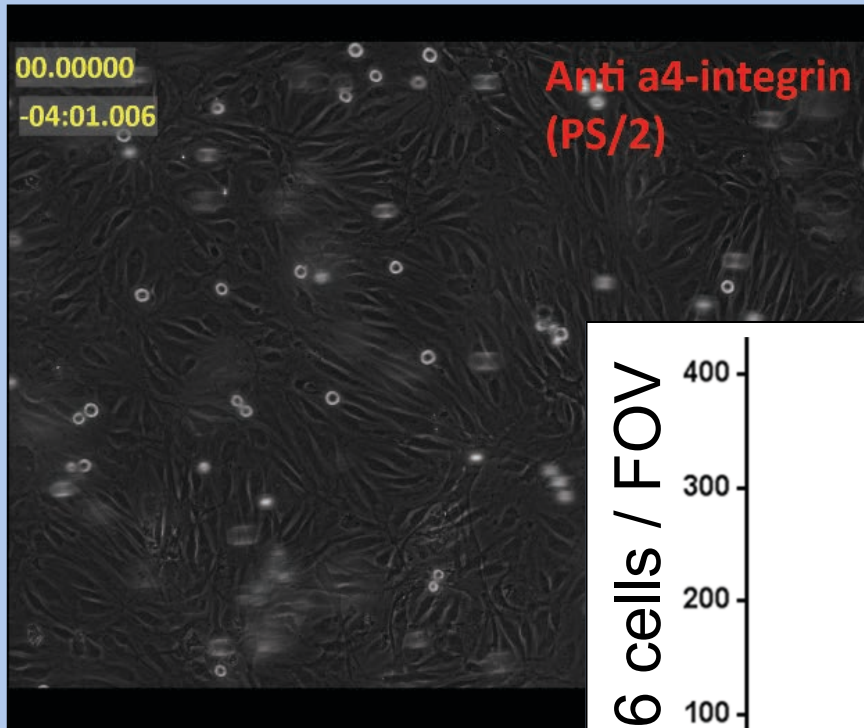
B16-F1 and B16-F10 express  $\alpha 4$ -integrin  
(presumably VLA-4)  
but not  $\alpha 4\beta 7$ -integrin or the  $\beta 2$ -integrins Mac-1 or LFA-1

# B16 melanoma arrest on pMBMECs is $\alpha 4$ -integrin dependent





# B16 melanoma arrest on pMBMECs is $\alpha 4$ -integrin dependent





## Conclusions

- > Metastatic human breast carcinoma cells MDA-MB-231BR-HER2
  - do **not** arrest on pMBMECs under flow.  
(= metastatic mouse breast carcinoma cells 4T1)
  - do efficiently arrest on ECM proteins – as accessible when the endothelial monolayer is injured.
- > Metastatic mouse melanoma cells B16 (B16F1, B16F10)
  - do arrest on pMBMECs under flow and withstand physiological shear (1.5 dyn/cm<sup>2</sup>).
- > Arrest of B16 is  $\alpha$ 4-integrin dependent.

# Acknowledgement

## Lyck Lab members

- > Michael Abadier
- > **Daniel Hauser**
- > Christoph Matti
- > **Ana Belén García Martin**
- > Mark Liebi

## Funding

- Swiss National Foundation (SNF)
- Suisse Multiple Sclerosis Society
- Foundation for clinical-experimental cancer research Bern

## Theodor Kocher Institute (TKI)

- > Prof. Britta Engelhardt
- > Workshop of the TKI  
— Adrian Bigler, Ulrich Kindler
- > All colleagues of the TKI

## Collaborations

- > Prof. Curzio Rüegg, Fribourg, CH  
— Christof Wyss
- > Prof. Dietmar Vestweber, Münster, D

## MDA-MB-231BR HER2

- > Prof. Patricia Steeg

## Intetumumab

- > Dr. Curt Kuder, Janssen  
Pharmaceutical Companies of Johnson  
and Johnson, PA, USA

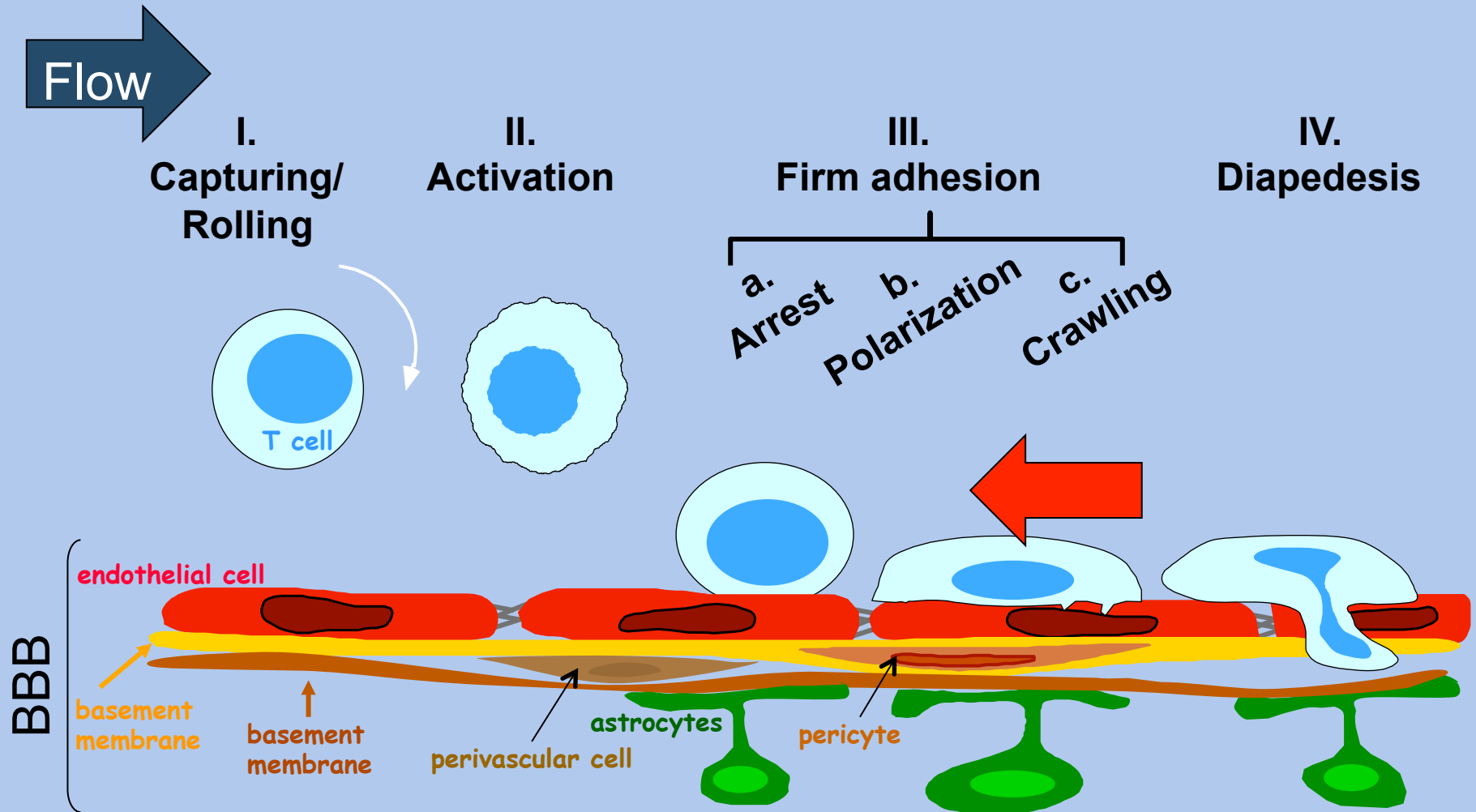


# ***In vivo*: Effector T cells dynamically interact with the BBB prior to diapedesis**

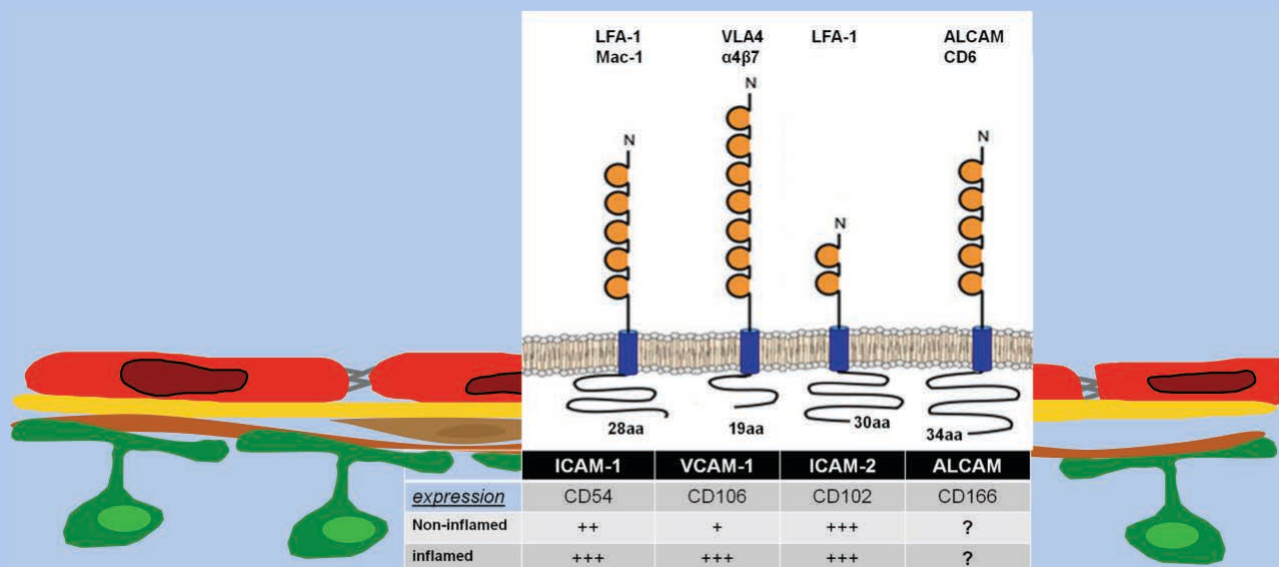
Crawling of Effector T Cells  
within Pial Vessels



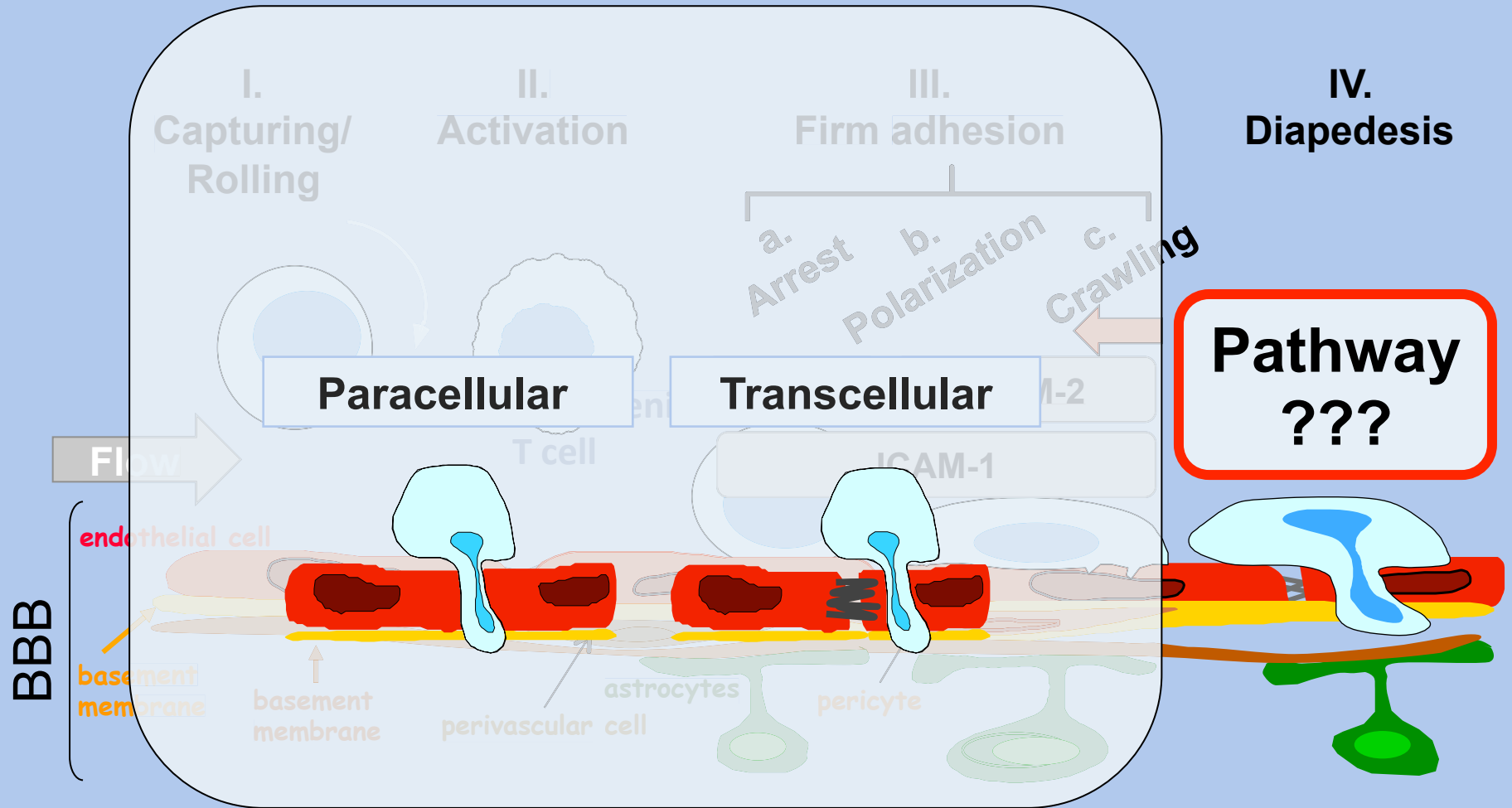
# CD4<sup>+</sup> T<sub>EM</sub> cells dynamically interact with inflamed pMBMECs



# Roles of endothelial ICAM-1, ICAM-2, VCAM-1 and ALCAM for CD4<sup>+</sup> T<sub>EM</sub> cell dynamic interaction with the BBB



# Pathway of diapedesis?



A diagram showing a cross-section of a tooth. The crown is filled with a red material, and the root is embedded in a yellow layer representing the jawbone. The root canal is filled with a blue material, and the crown is filled with a red material. The root is embedded in a yellow layer representing the jawbone.