Azienda Ospedaliero Universitaria Careggi

CAMPO BASE NEUROVASCOLARE

Permeability and inflammation of ischemia

B.Piccardi

Campo Base NeuroVascolare

25 - 28 SETTEMBRE 2019

A.O.U. - Azienda Ospedaliero-Universitaria Careggi

Nuovo Ingresso Careggi - NIC

Padiglione 3 piano terra, Largo Brambilla 3, 50134

Firenze

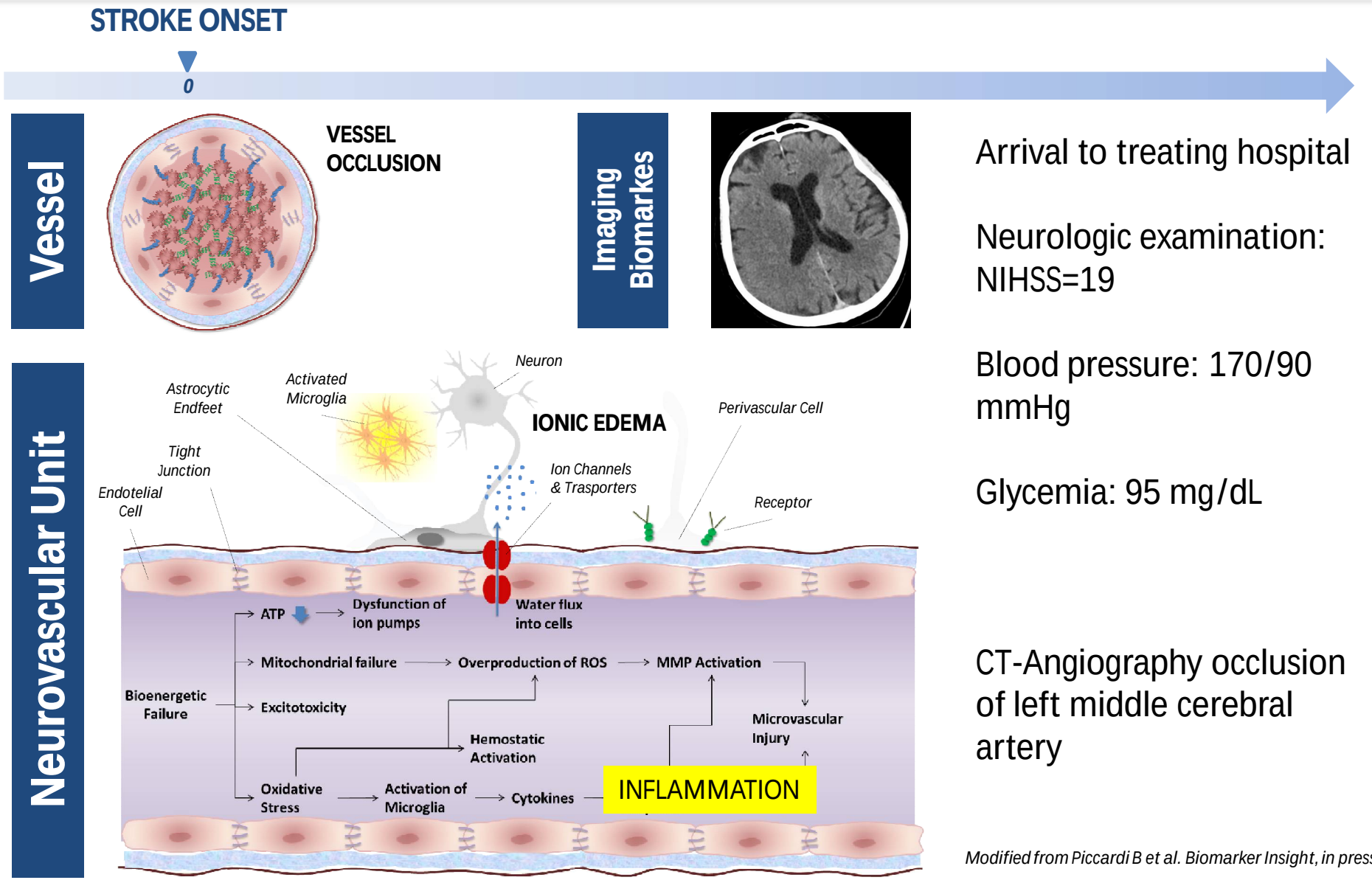
Overview

- From cellular level to bedside: a clinical case
- Blood Brain Barrier (BBB) structure and function
- Inflammation and BBB disruption
- Future directions

From cellular level to bedside: a clinical case

- **Mr. R**, 82-year-old right-handed hypertensive patient, presented with **sudden onset** of dense flaccid **left**-sided weakness

From cellular level to bedside



Arrival to treating hospital

Neurologic examination: NIHSS=19

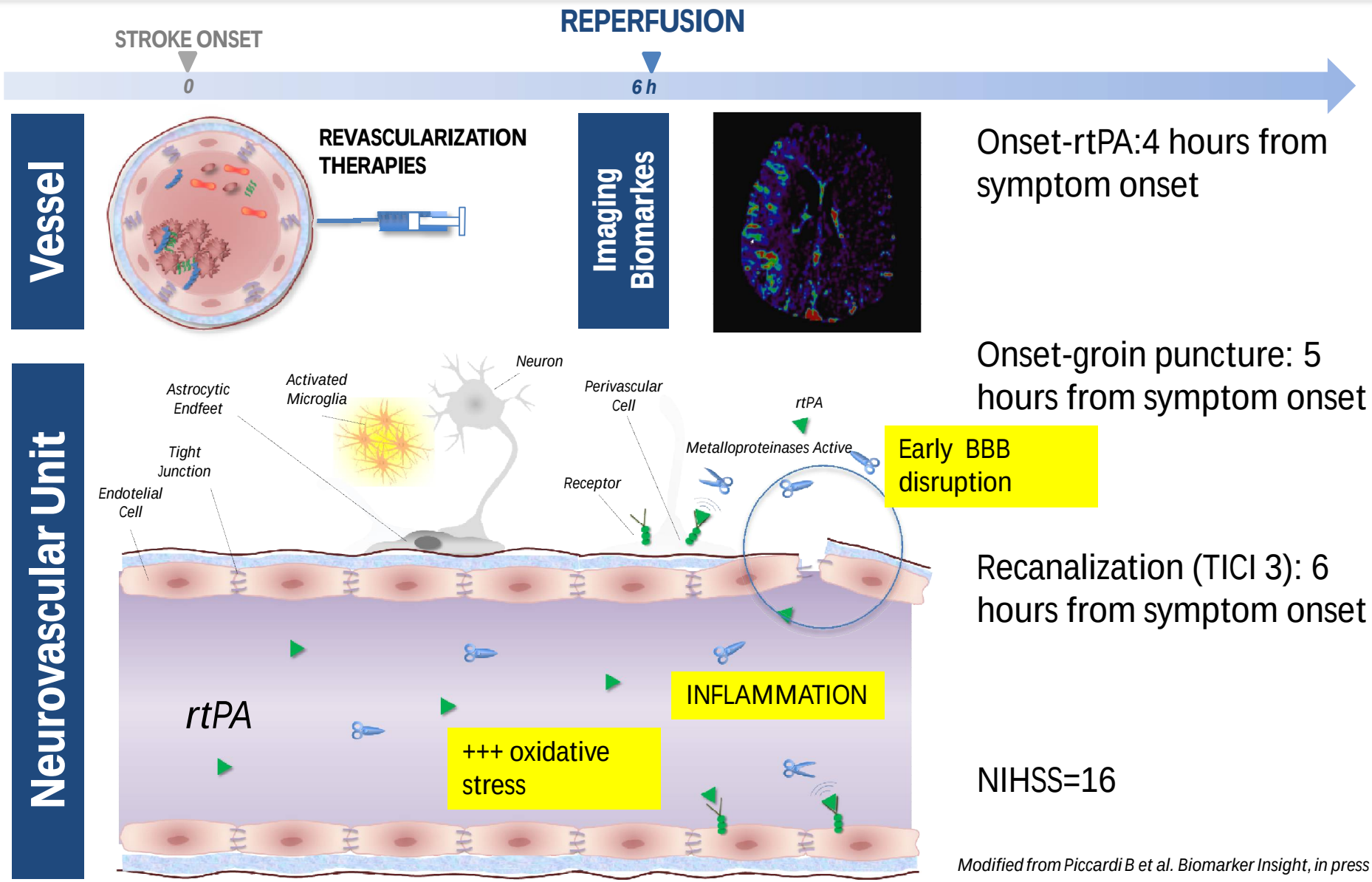
Blood pressure: 170/90 mmHg

Glycemia: 95 mg/dL

CT-Angiography occlusion of left middle cerebral artery

Modified from Piccardi B et al. Biomarker Insight, in press

From cellular level to bedside



Onset-rtPA:4 hours from symptom onset

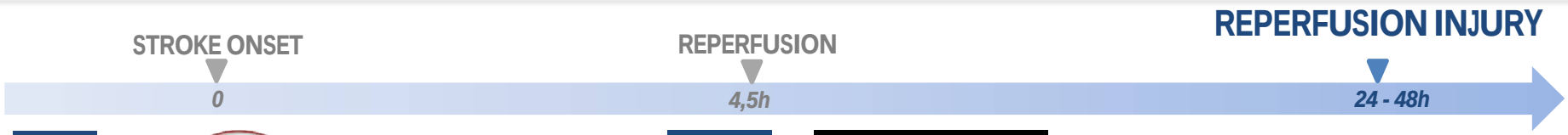
Onset-groin puncture: 5 hours from symptom onset

Recanalization (TICI 3): 6 hours from symptom onset

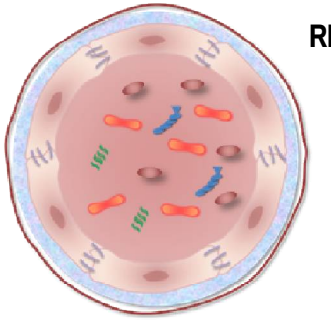
NIHSS=16

Modified from Piccardi B et al. Biomarker Insight, in press

From cellular level to bedside

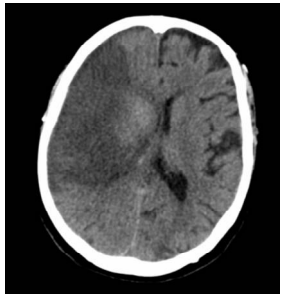


Vessel



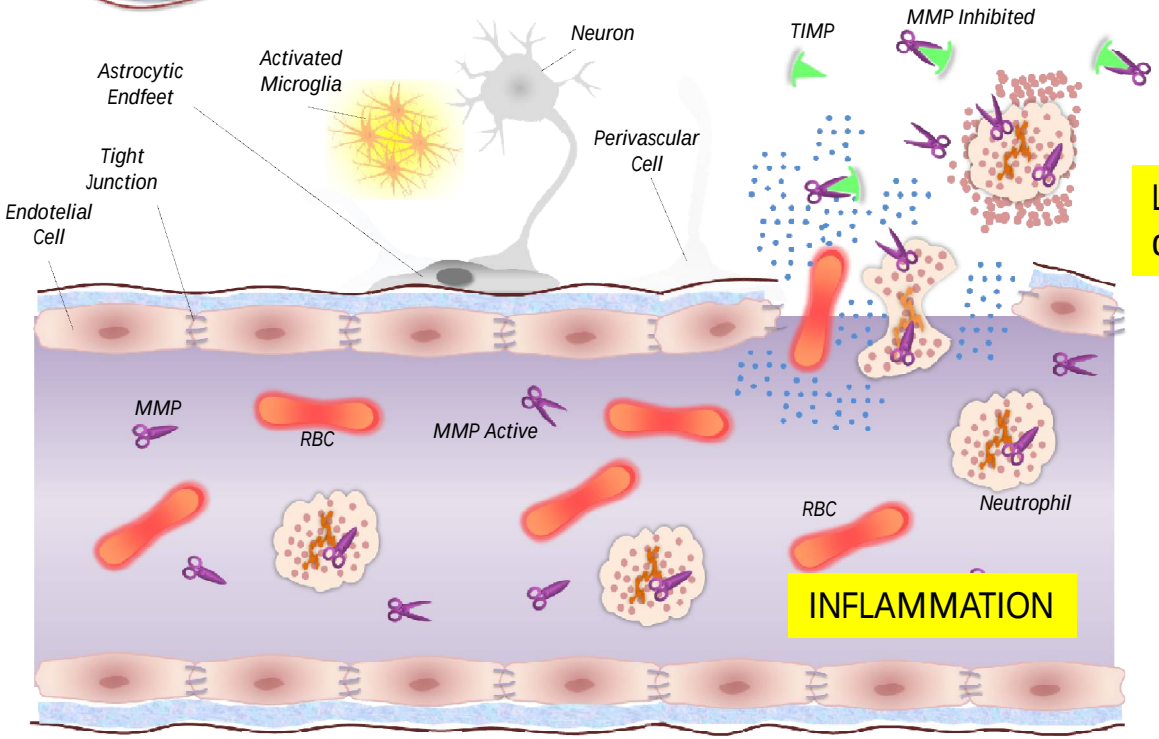
RECANALIZATION

Imaging Biomarkes



Clinical deterioration at 24 hours from symptom onset (NIHSS=23)

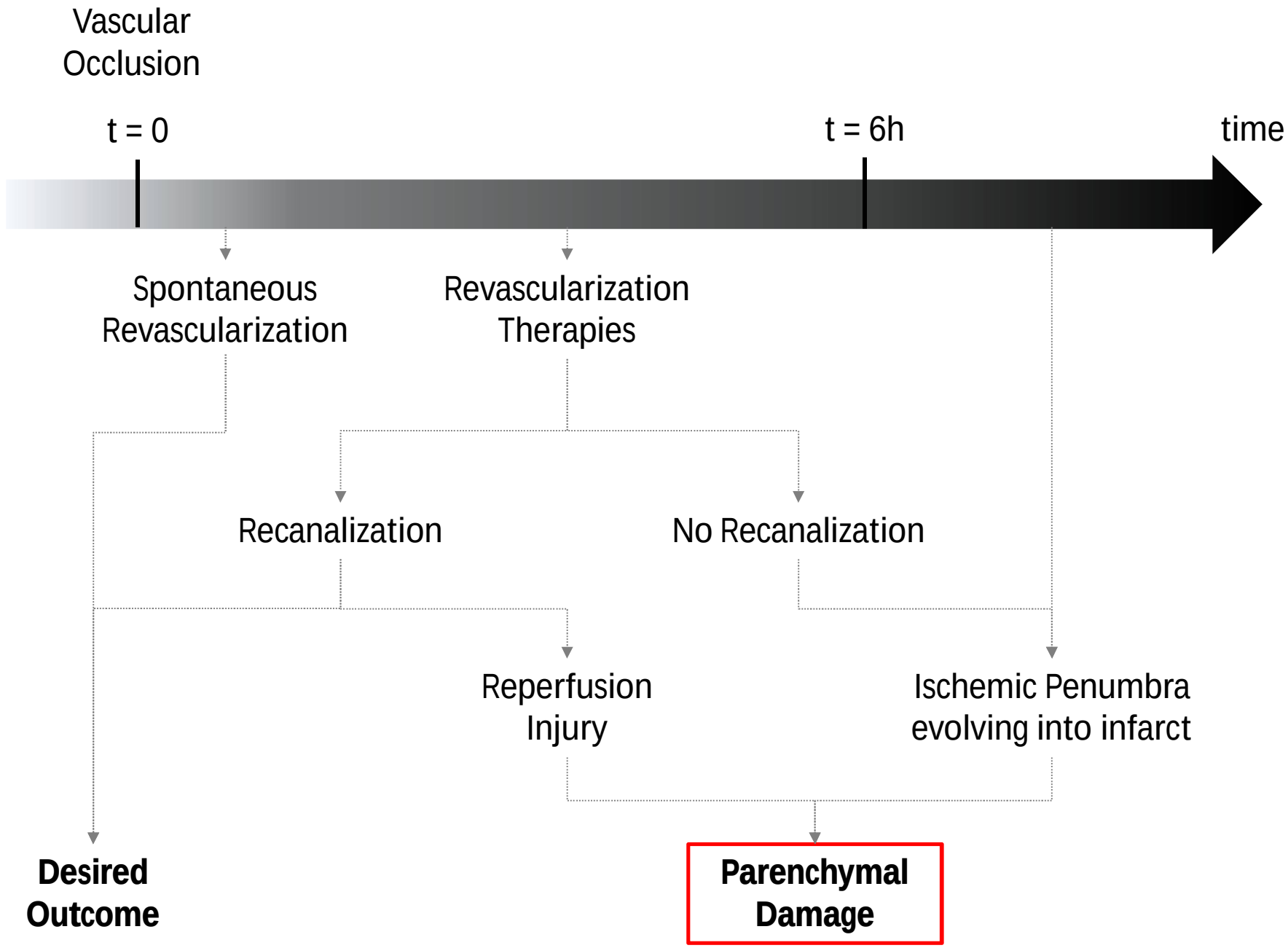
Neurovascular Unit



Late BBB disruption

3 month modified Rankin Scale=4

Modified from Piccardi B et al. Biomarker Insight, in press


Vascular Occlusion
t = 0
t = 6h
time
Spontaneous Revascularization
Revascularization Therapies
Recanalization
No Recanalization
Reperfusion Injury
Ischemic Penumbra evolving into infarct
Desired Outcome
Parenchymal Damage

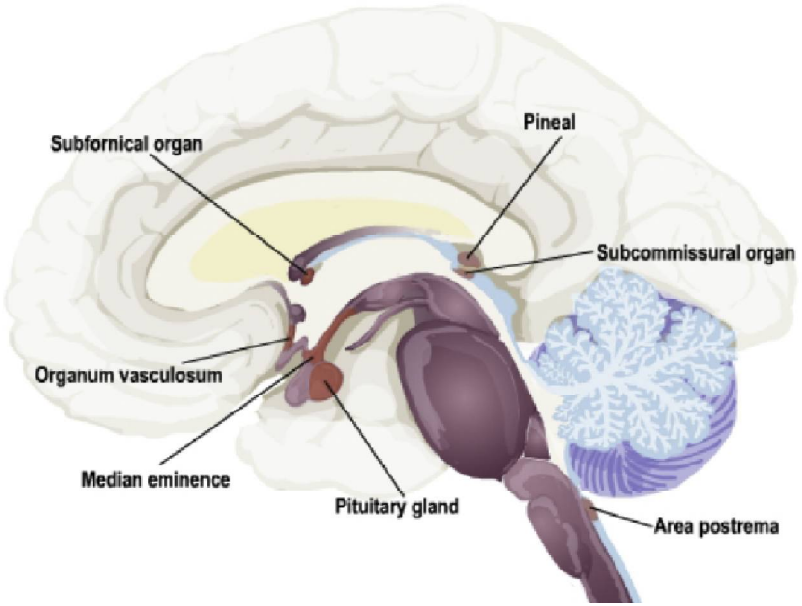
Reperfusion Injury

- *Adverse functional, metabolic or structural changes in ischemic tissues resulting from the restoration of blood flow (reperfusion) that may exacerbate ischemic damage and capillary dysfunction leading to brain edema, hemorrhagic transformation, necrosis and damage from free radicals with subsequent infarct growth*

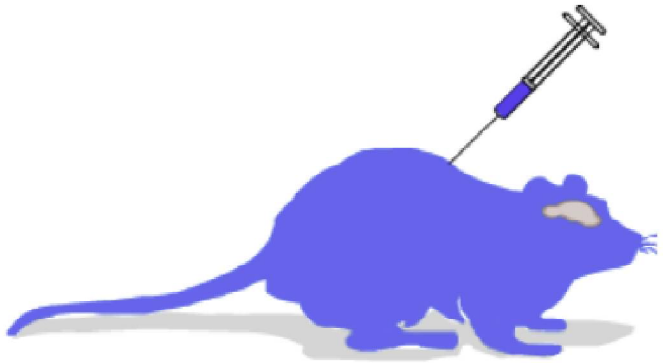
Blood–brain barrier (BBB)

Highly selective permeability barrier that separates the circulating blood from the brain extracellular fluid in the central nervous system

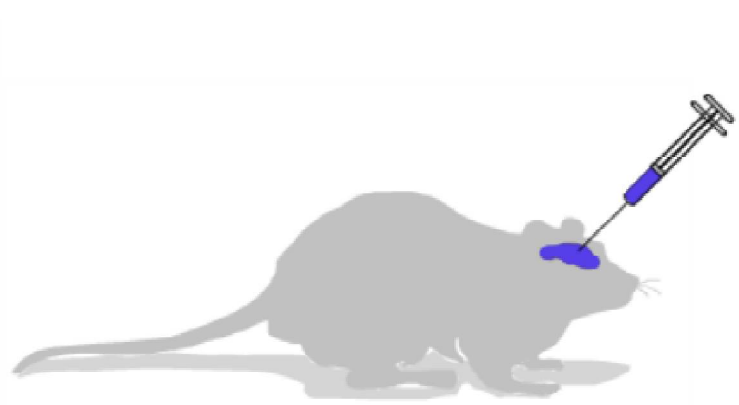
Exceptions: circumventricular organs



Initial Observations of the Blood-Brain Barrier

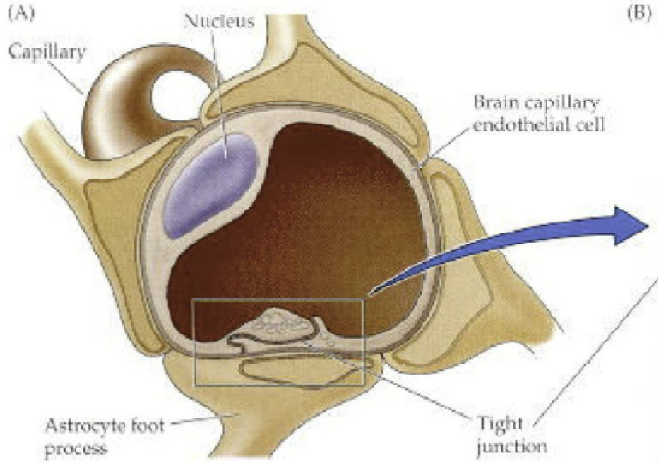


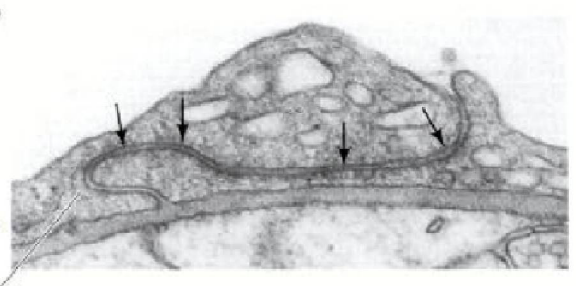
A: Systemic injection



B: Intraventricular injection

1885-1913
Ehrlich and Goldman experiments



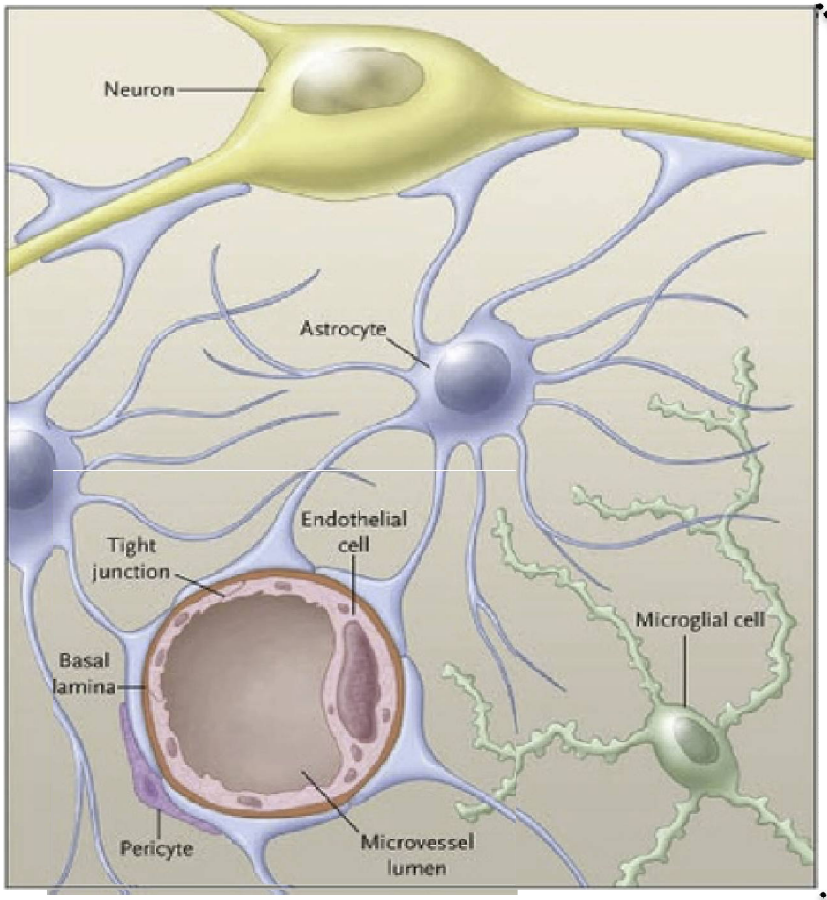


The cellular basis of the blood-brain barrier. (A) Diagram of a brain capillary in cross section and reconstructed views, showing endothelial tight junctions and the investment of the capillary by astrocytic end feet. (B) Electron micrograph of boxed area in (A), showing the appearance of tight junctions between neighboring endothelial cells (arrows). (A after Goldstein, Goldstein and Betz, 1986; B from Peters et al., 1991.)

1950s
electron microscopy

Saunders NR, Front Neurosci. 2014

BBB within Neurovascular Unit (NVU)



Neurofunctional UNIT

1) Neuron

2) **Microvassels: a) endothelial cells; b) tight junctions; c) basal lamina; d) pericytes; e) astrocytes endfeet→BBB**

3) Microglia
4) Pericytes

Hallenbeck J et al, Stroke, 2006

BBB: barrier and carrier

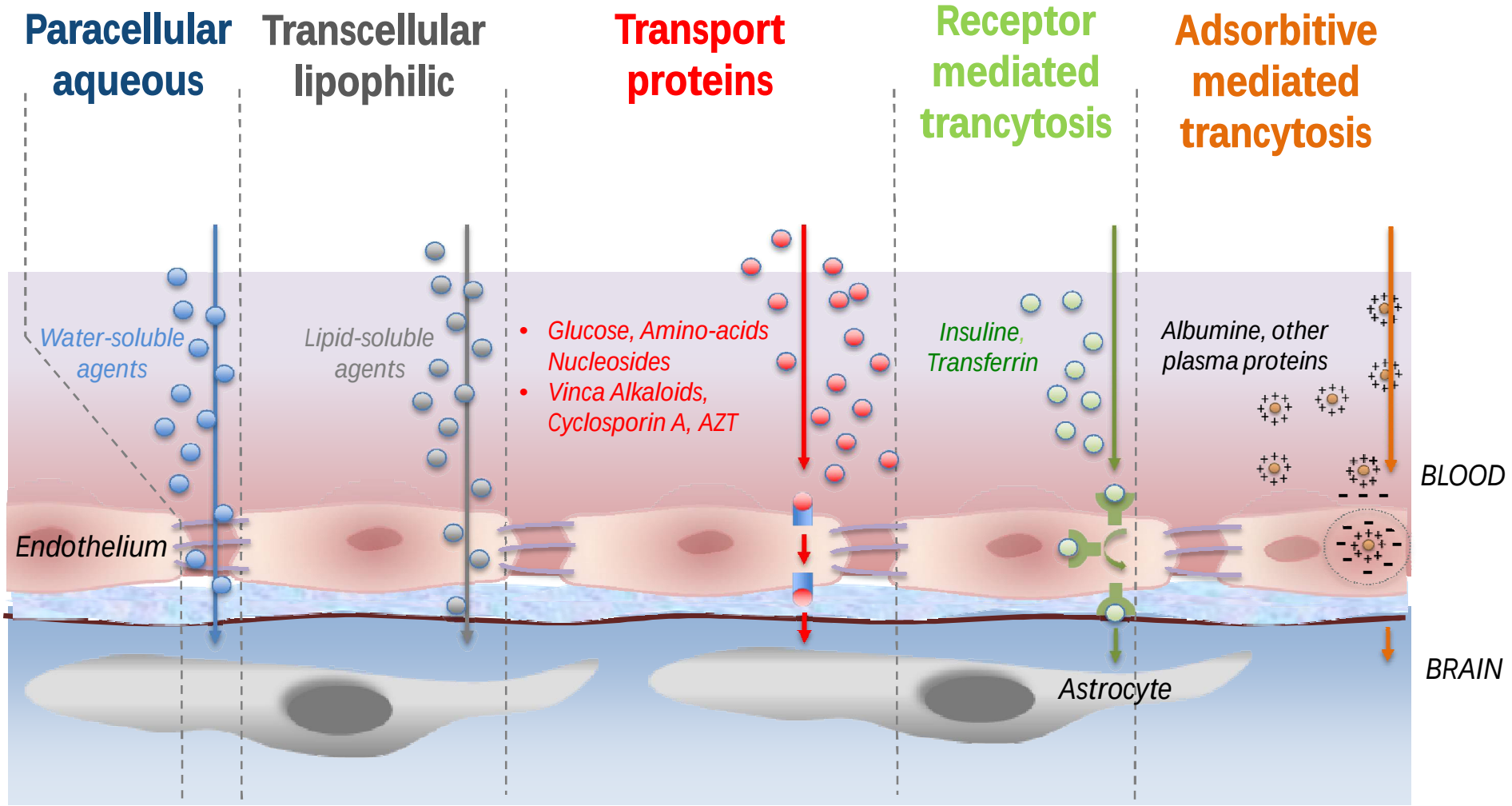
1) Separatation between the CNS from the blood and immune system

2) Selective permeability that prevents macromolecules from entering the brain

3) Omeostasis such as ionic composition

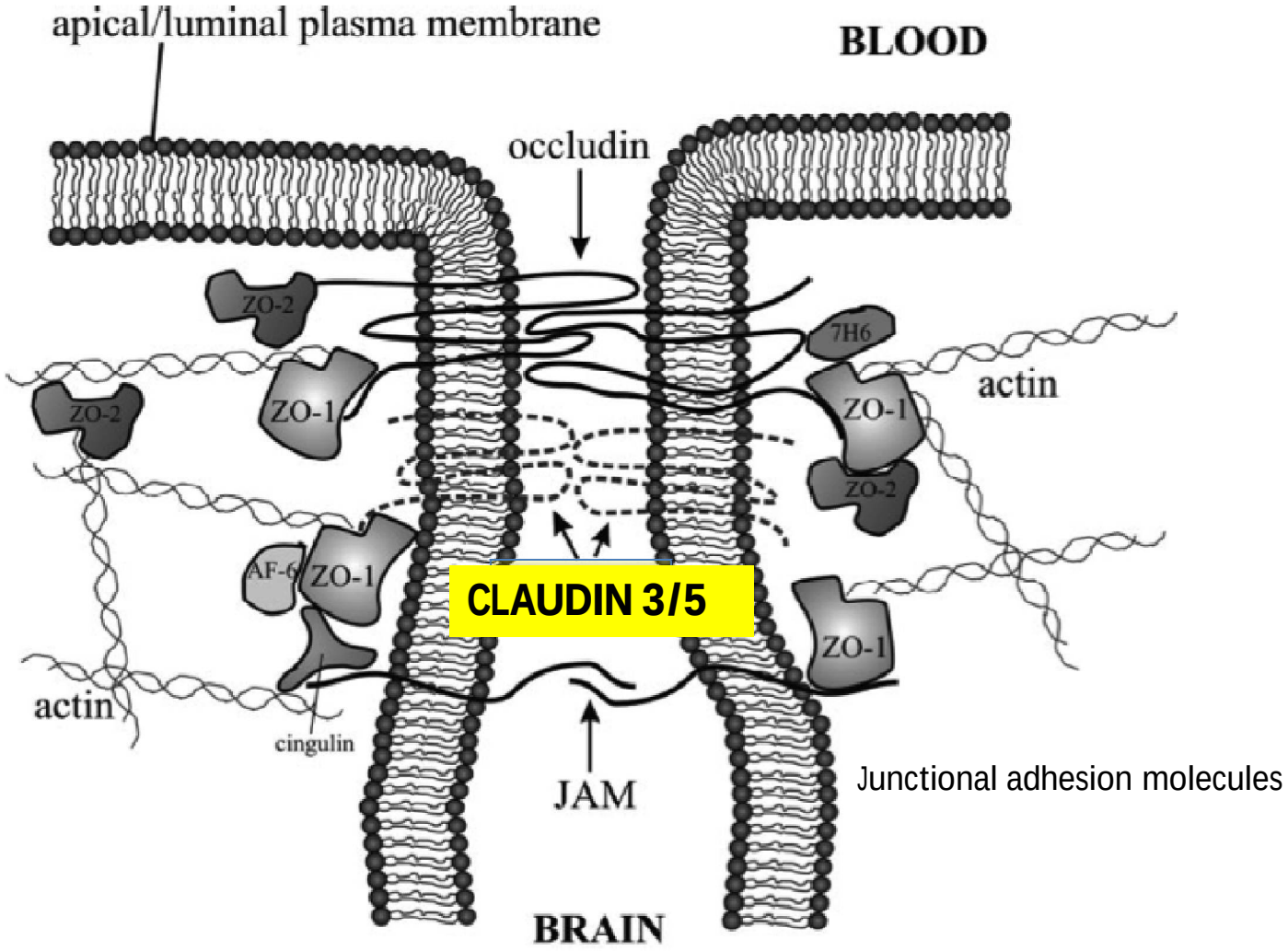
4) Material exchange and adequate brain nutrition supply

Pathways across BBB



Modified from Abbott NJ *et al.* (2006) Astrocyte–endothelial interactions at the blood–brain barrier
Nat. Rev. Neuro. **7:** 41–53 doi:10.1038/nrn1824

Basic molecular organization of BBB: tight junctions



Claudins

Extended family of transmembrane proteins, with 26 family members in humans

Claudin-5 is typical for endothelia such as BECs

Claudin-5 knockout does not result in a general breakdown of TJs but rather in a size-selective opening of the BBB for molecules just smaller than 800Da

Claudin-5 expression selectively decreases the paracellular permeability of BBB.

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Progress in Neurobiology

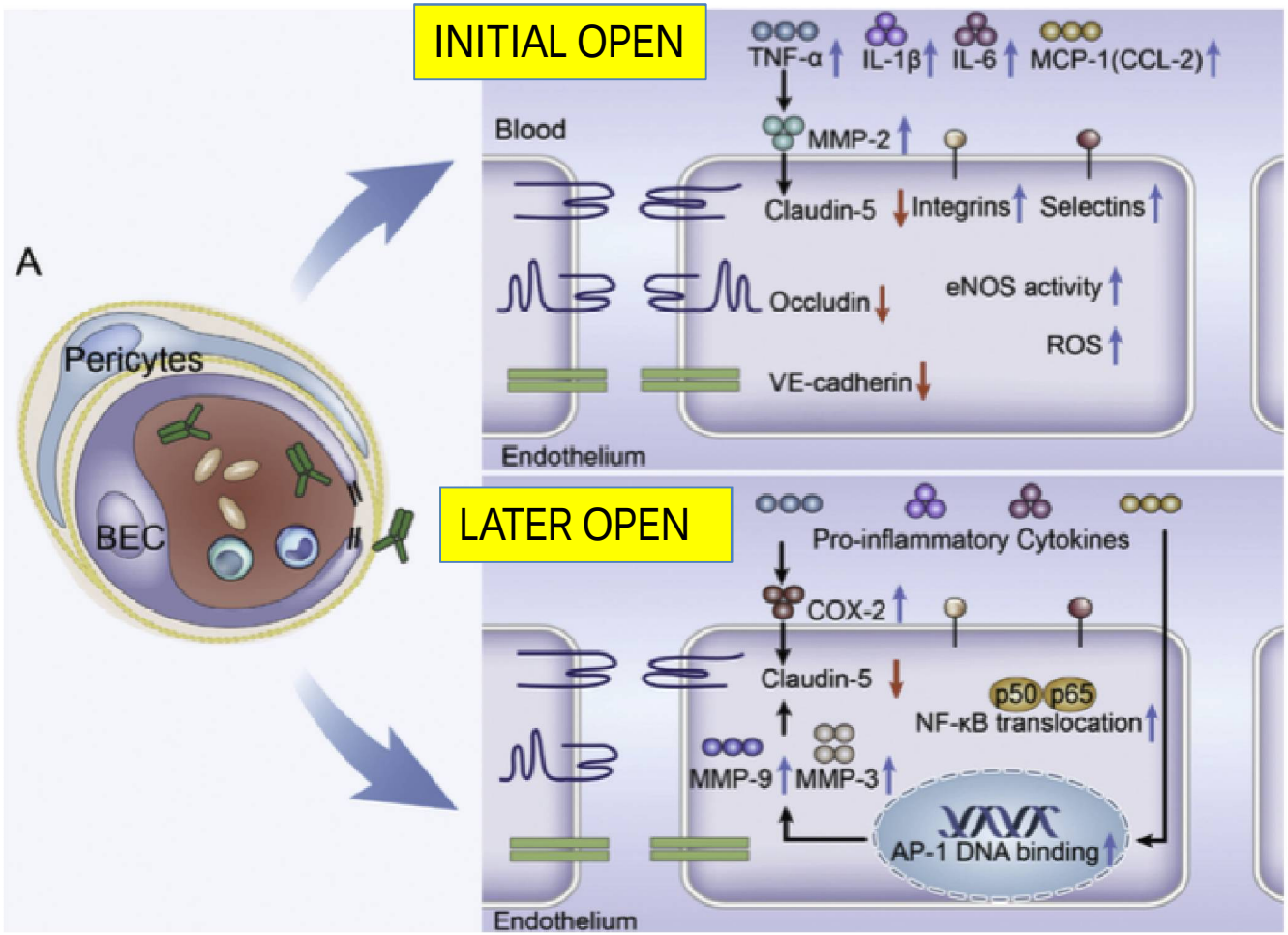
journal homepage: www.elsevier.com/locate/pneurobio

Review article

Focusing on claudin-5: A promising candidate in the regulation of BBB to treat ischemic stroke

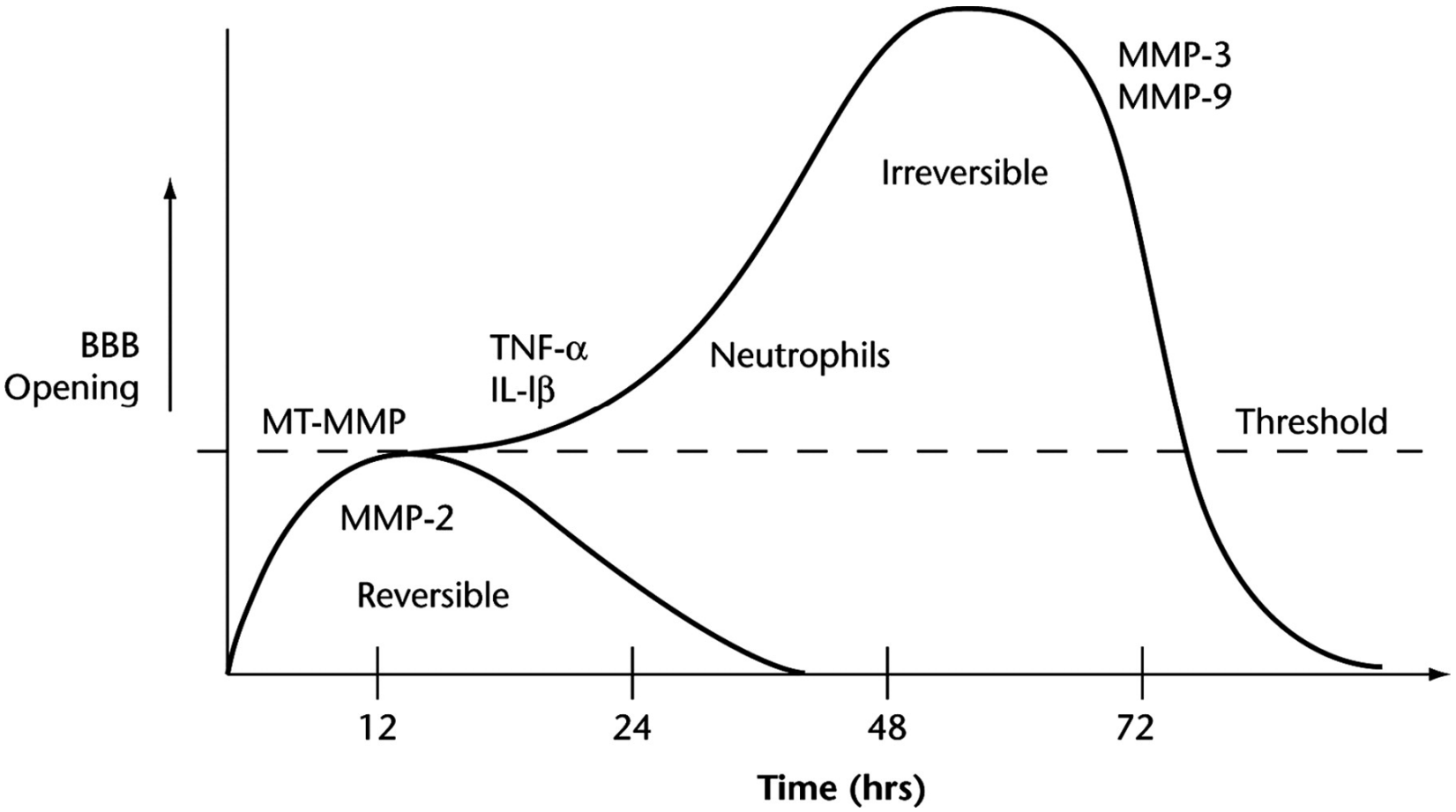
Jianjun Lv[a,b,1], Wei Hu[b,c,1], Zhi Yang[b], Tian Li[b], Shuai Jiang[d], Zhiqiang Ma[e], Fulin Chen[a], Yang Yang[a,b,*]

claudin-5 role in BBB permeability



Lv Jianjun, Progress in Neurobiology, 2017

Timing of BBB opening: biphasic theory

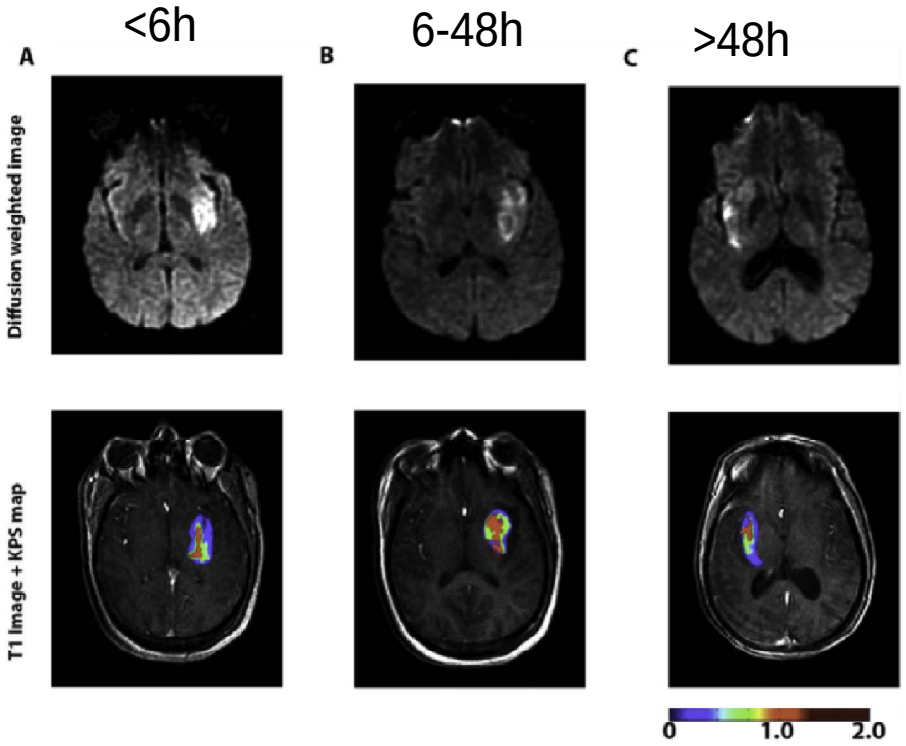


Yi Yang, Stroke, 2011

Timing of BBB opening: continually elevated permeability

Retrospective study including 42 acute stroke patients evaluated by single dynamic contrast-enhanced MRI sequence to measure BBB permeability during their initial workup.

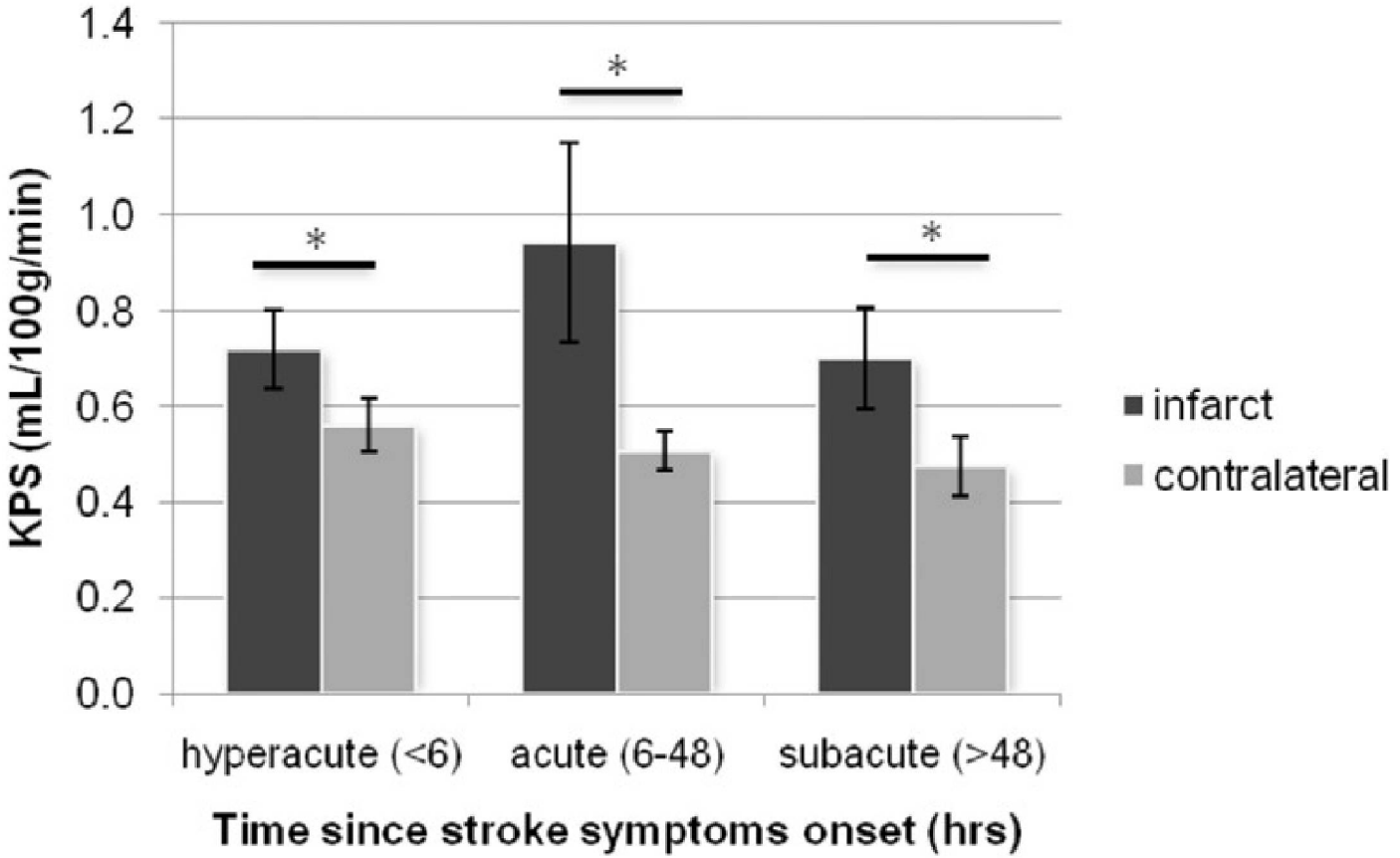
Patient sample underwent DCE-MRI at a mean time of 23.8hrs after the onset of AIS symptoms (range: 1.3– 90.7hrs).



Merali Z, Plos One 2017

Timing of BBB opening: continually elevated permeability

Blood-brain-barrier permeability within the infarct and a homologous region in the contralateral hemisphere stratified by time since stroke symptom onset.



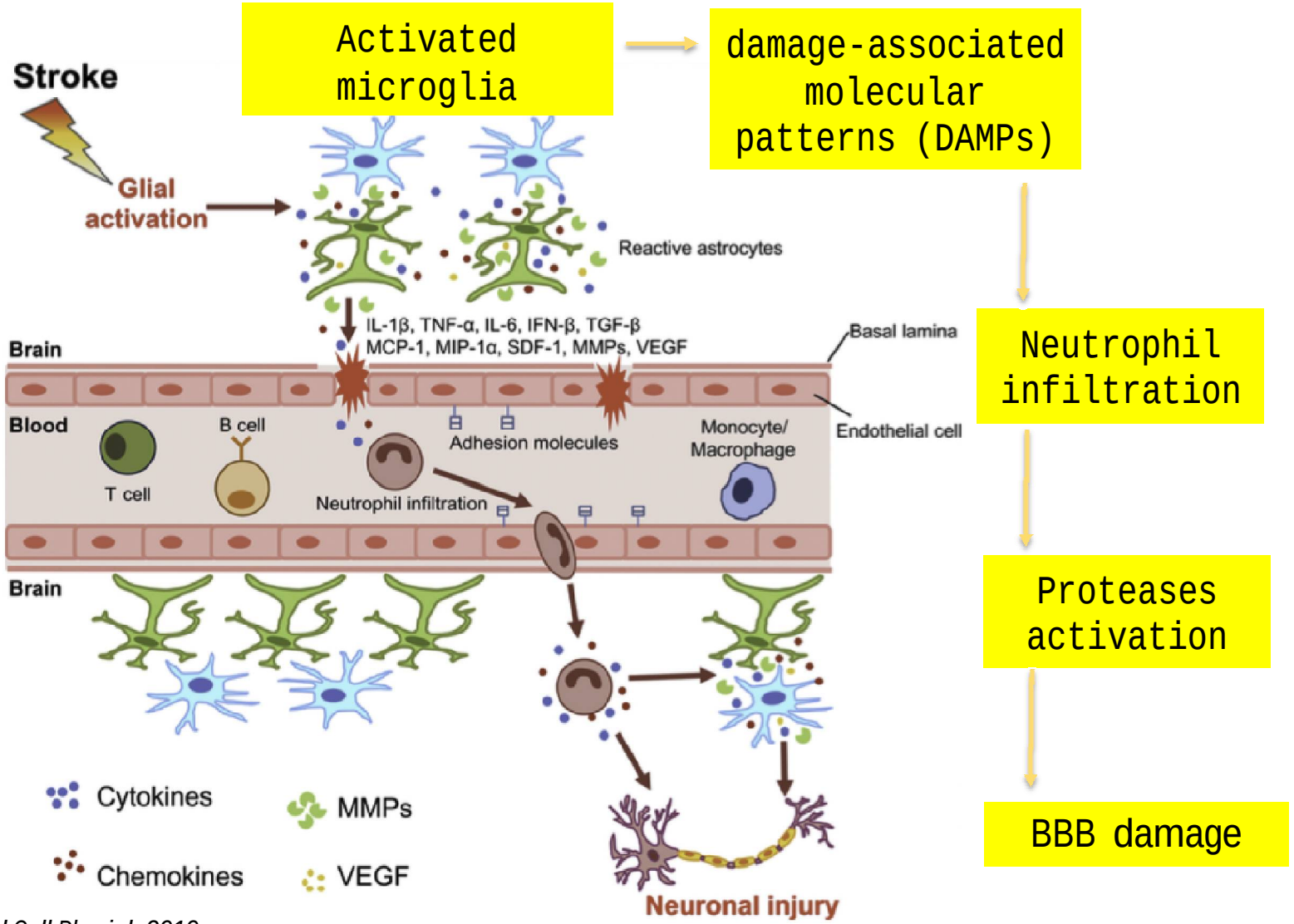
Merali Z, Plos One 2017

Inflammation and permeability

BBB is considered to be a true immunologic barrier, which blocks leukocyte migration under normal conditions

Merali Z, Plos One 2017

Inflammation and permeability



Yang C, Am J Physiol Cell Physiol, 2019

Metalloproteinase (MMP): structure and activity

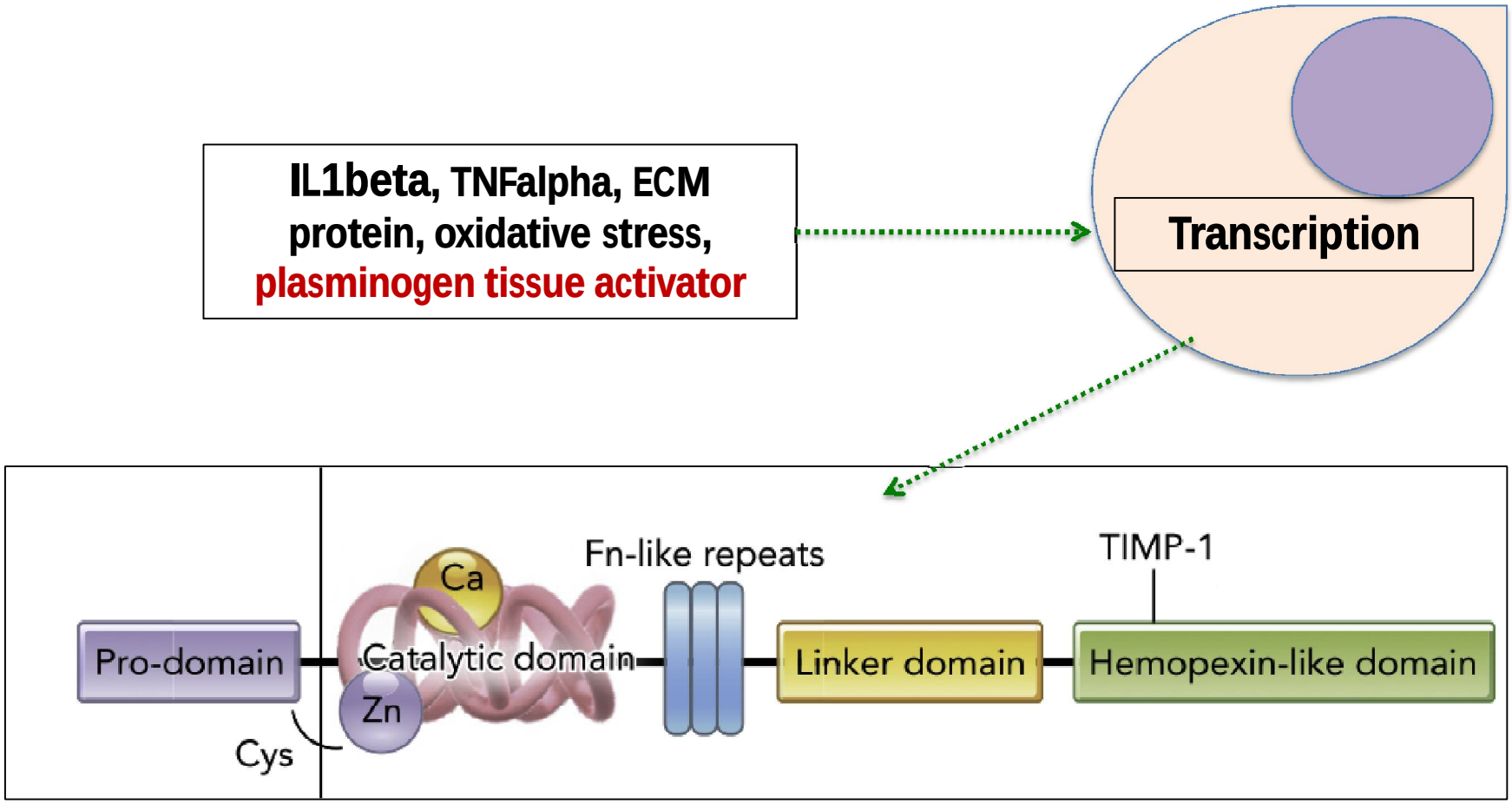
Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases

Collectively, these enzymes are capable of degrading all kinds of extracellular matrix proteins, but also can process a number of bioactive molecules

:

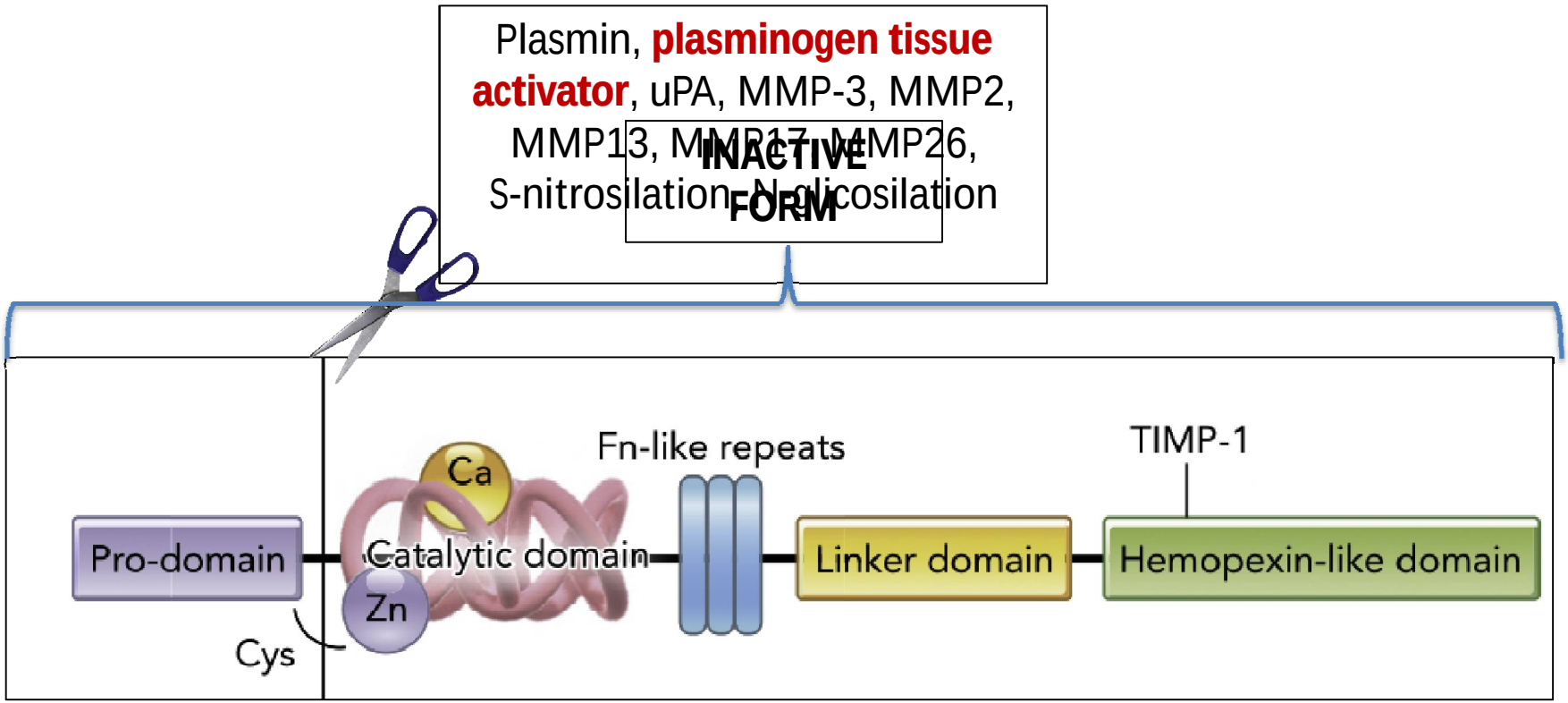
1) Cell proliferation
2) Angiogenesis
3) Migration
4) Apoptosis
5) Host defense

MMP9: expression



Modified from Yabluchanskiy A, Physiology, 2013

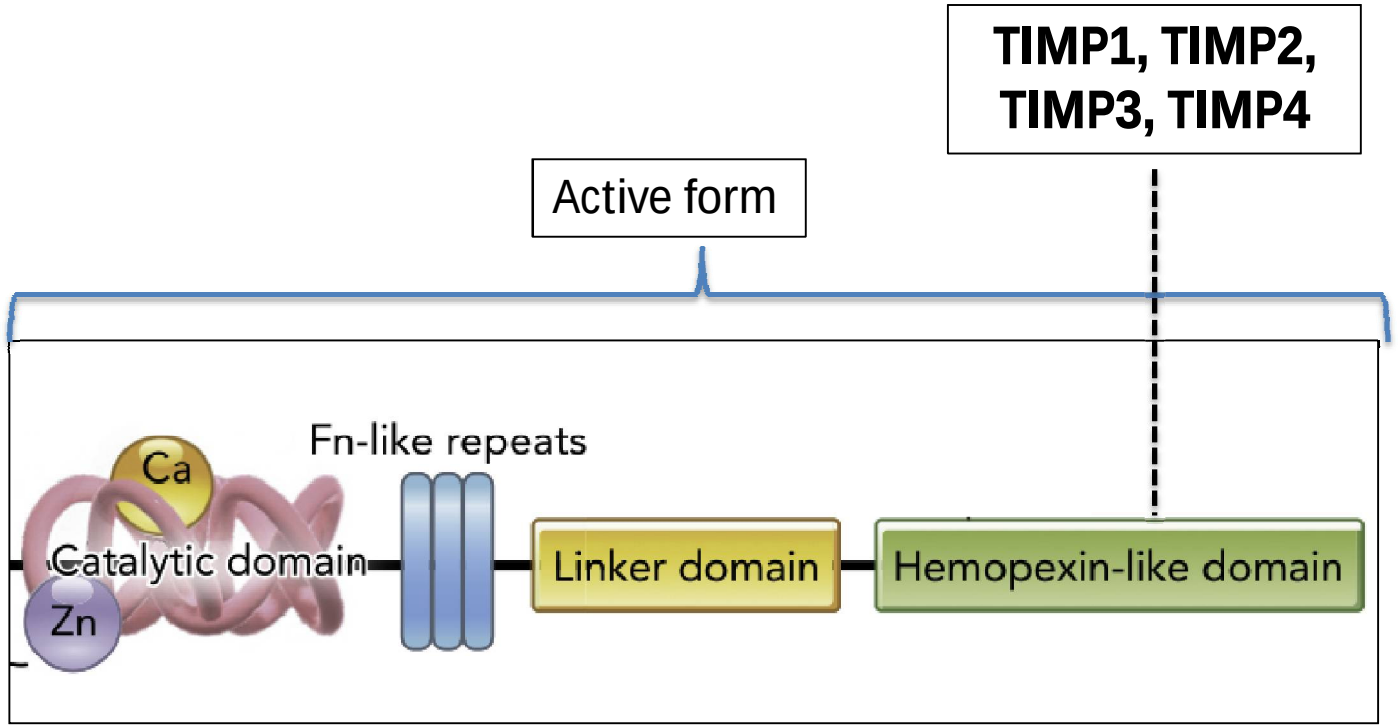
MMP9: activation



Modified from Yabluchanskiy A, Physiology, 2013

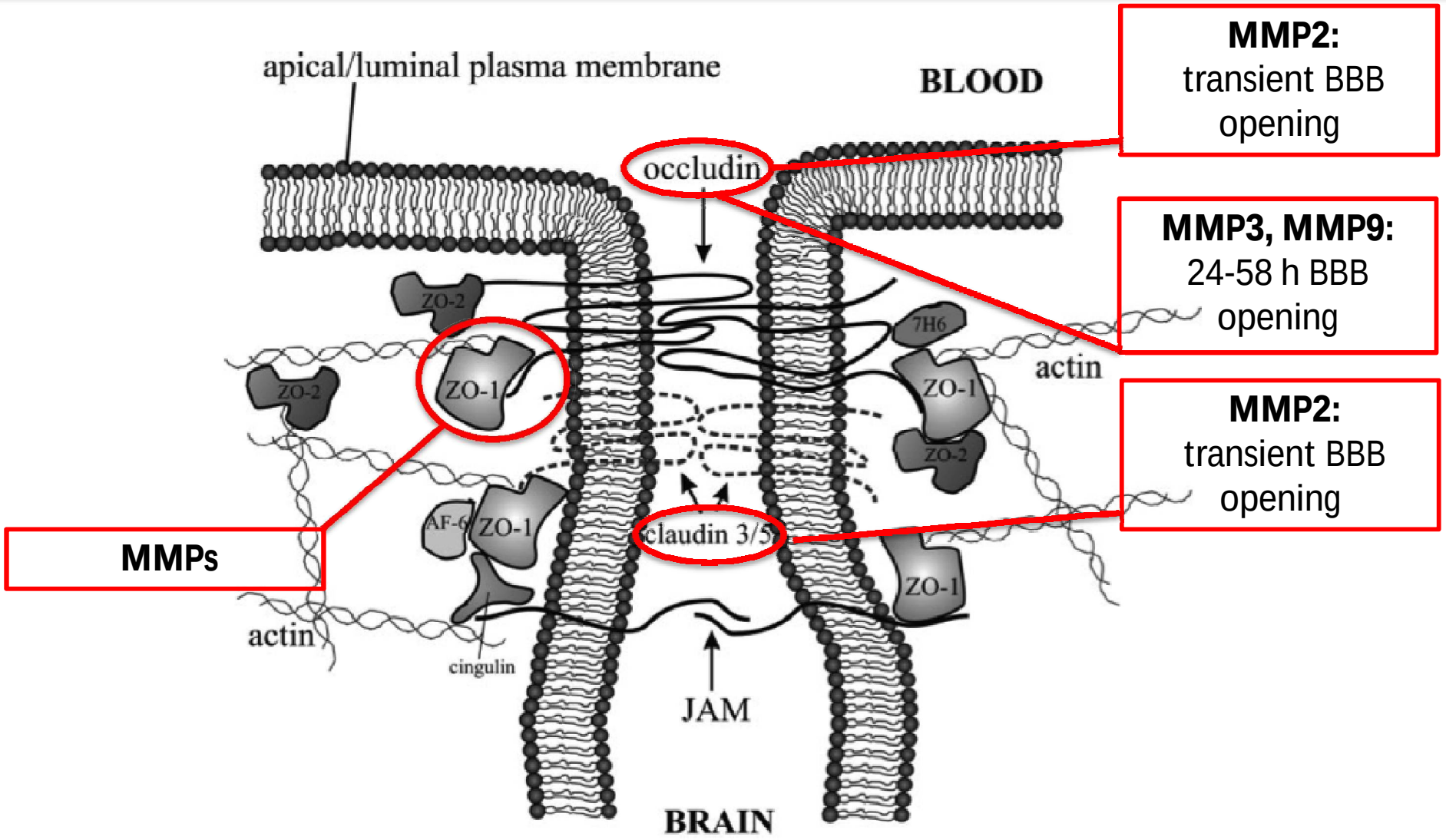
MMP9: regulation

The MMPs are inhibited by specific endogenous tissue inhibitor of metalloproteinases (TIMPs)



Modified from Yabluchanskiy A, Physiology, 2013

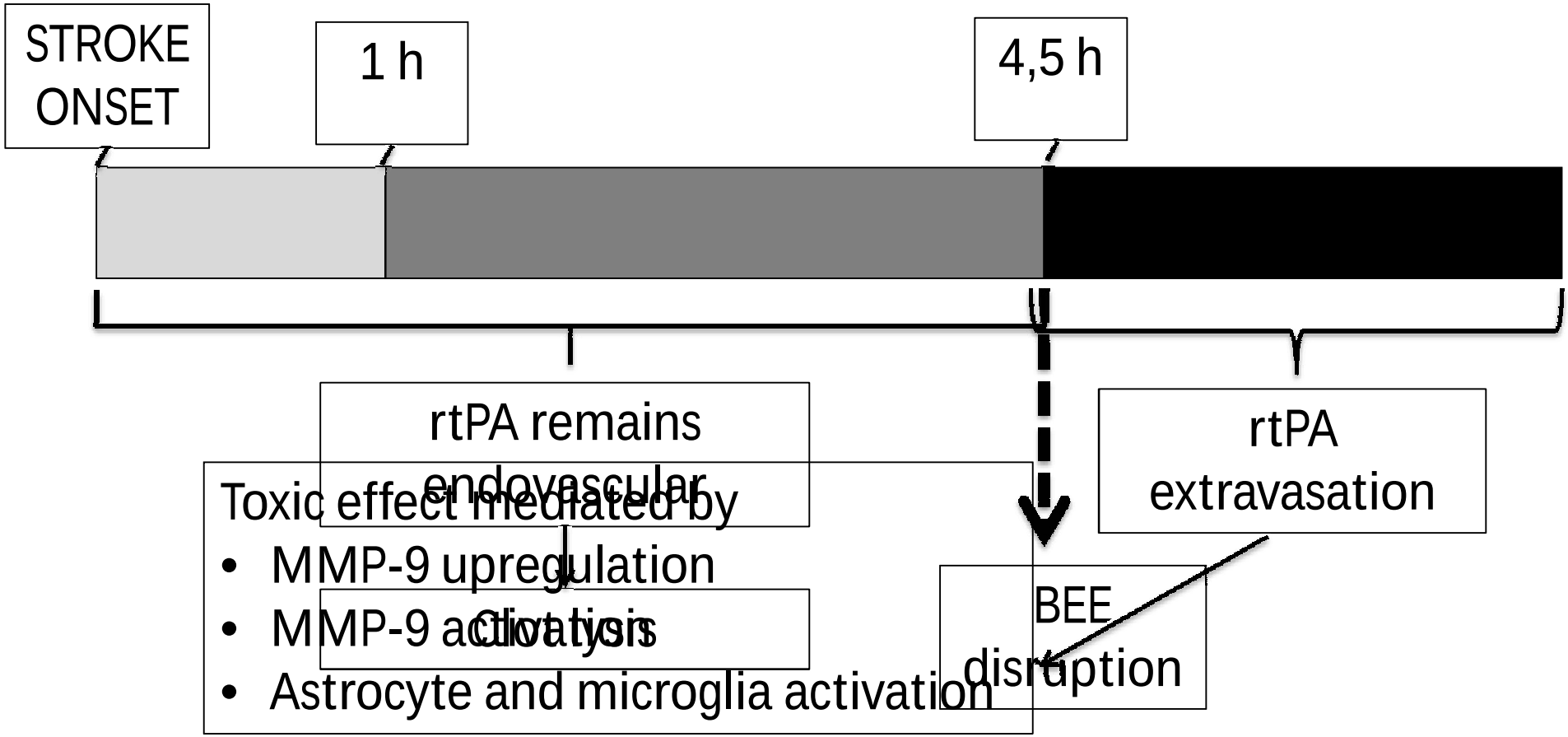
Proteases and permeability



Posada-Duque RA, Front Cell Neurosci. 2014

Hawkins BT, Pharmacol Rev. 2005

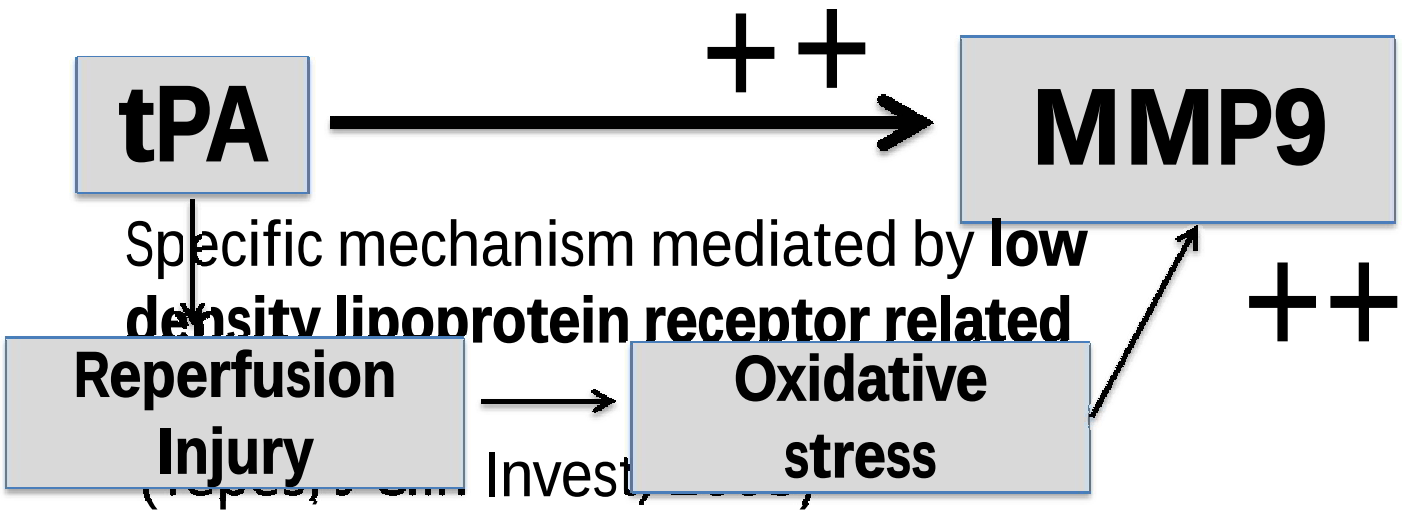
Pleiotropic effect of rtPA in acute ischemic stroke



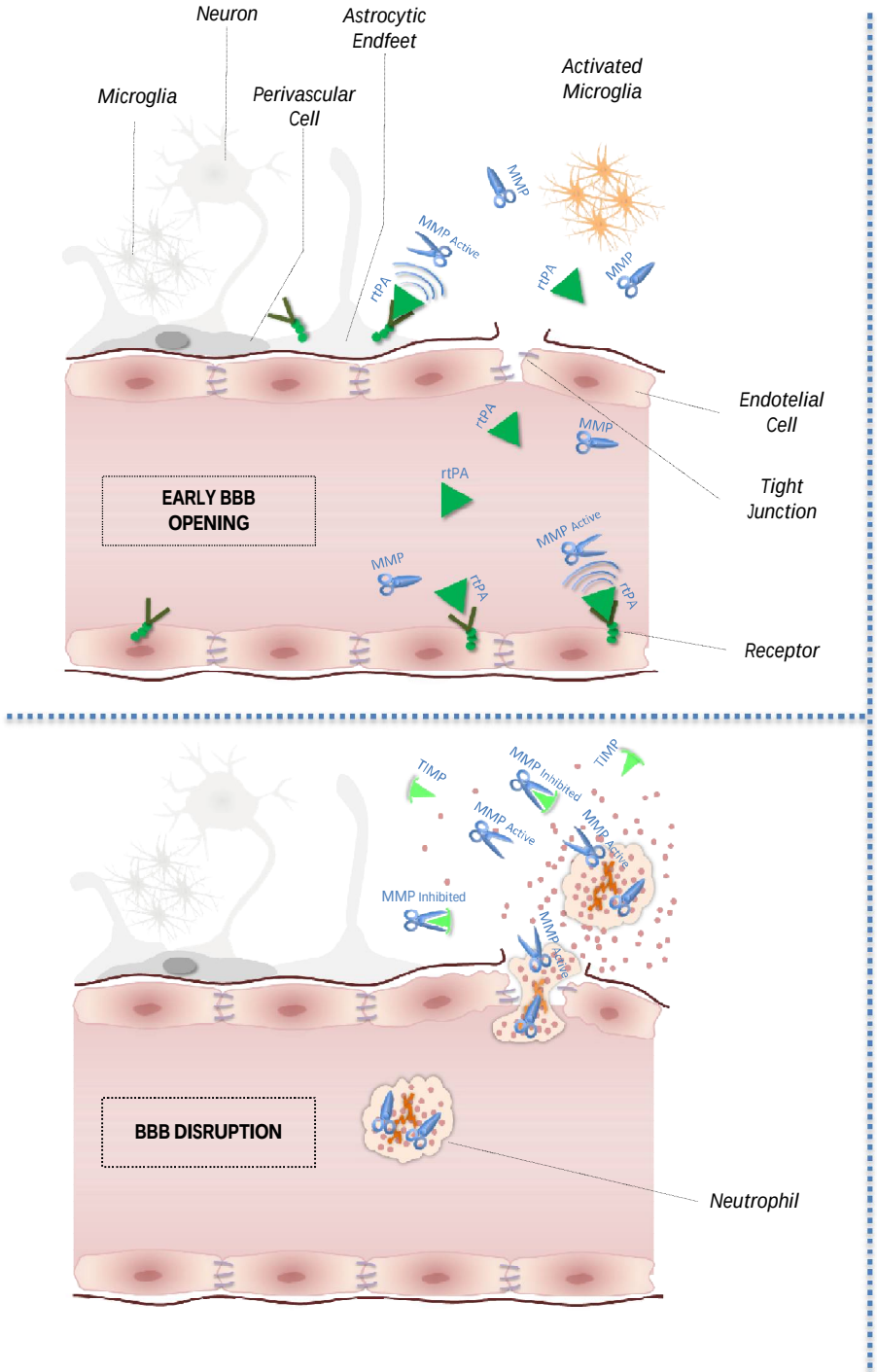
Kaur J, J Cereb Blood Flow Metab, 2004

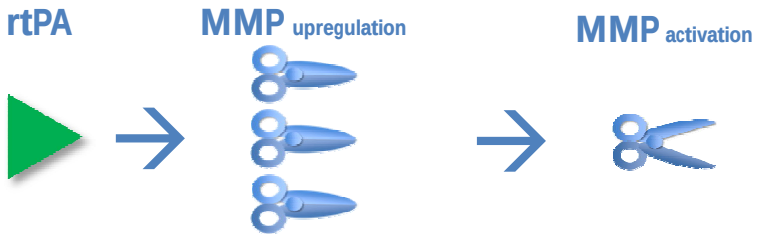
“tPA-induced MMP9 hypothesis”

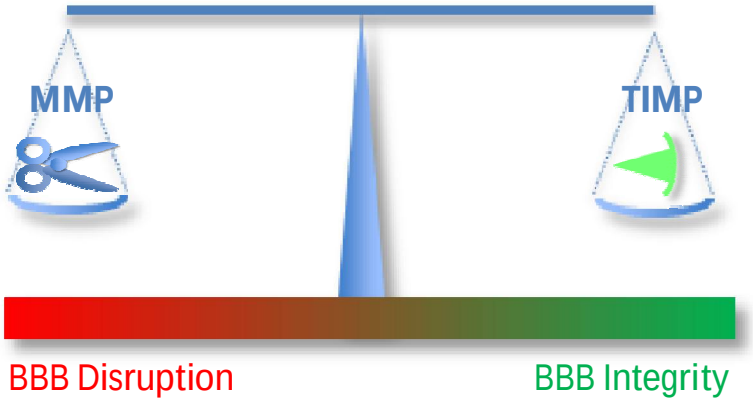
Lower MMP9 levels in tPA knockout mice compared with wild-type mice (Wang, Nat Med, 2003)



Lo Eh, Stroke, 2004







Piccardi B et al. Frontiers Neurol

MMP9/TIMP1-2 Predicts Hemorrhagic Transformation of Lesion in Ischemic Stroke Patients Treated with Thrombolysis

	SICH* OR (95% CI)	p
Δ **MMP9/TIMP1**	1.67 (1.17-2.38)	0.005
Δ **MMP9/TIMP2**	1.74 (1.21-2.49)	0.003

*binary logistic regression analysis adjustment for age, sex, onset to treatment time, baseline blood glucose, baseline NIHSS, history of atrial fibrillation, history of congestive heart failure, center effect, history of recent infection / inflammation, statin use, aspirin use, antiplatelet use, antihypertensive use

sICH=symptomatic intracerebral hemorrhage

Piccardi B et al. Frontiers Neurol, in press

Circulating biomarkers and blood-brain-barrier leakage: results from the Reperfusion Injury in ischemic StroKe (RISK) study

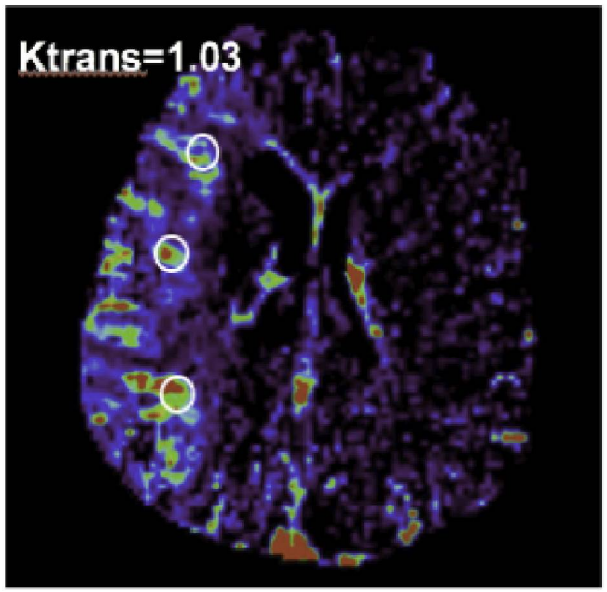
B. Piccardi[1], F. Arba[1], A.M. Gori[2], V. Palumbo[1], S. Biagini[1], F. Galmozzi[1], V. Iovene[1], M. nesi[1], M. Lamassa[1], F. Pescini[1], A. Poggesi[3], C. Sarti[3], S. Nannoni[1], B. Giusti[2], G. Pracucci[3], S. Mangiafico[4], N. Limbucci[4], S. Nappini[4], L. Renieri[4], E. Fainardi[5], D. Gadda[6], M. Moretti[6], S. Grifoni[7], D. Inzitari[3], P. Nencini[1]

[1]Careggi University Hospital, Stroke Unit, Florence, Italy; [2]Careggi University Hospital, Atherothrombotic Diseases Center-, Florence, Italy; [3]University of Florence, NEUROFARBA Department, Florence, Italy; [4]Careggi University Hospital, Neurovascular Interventional Unit, Florence, Italy; [5]University of Florence, Neuroradiology Department, Florence, Italy; [6]Careggi University Hospital, Neuroradiology Department, Florence, Italy; [7]Careggi University Hospital, Department of Emergency Medicine, Florence, Italy

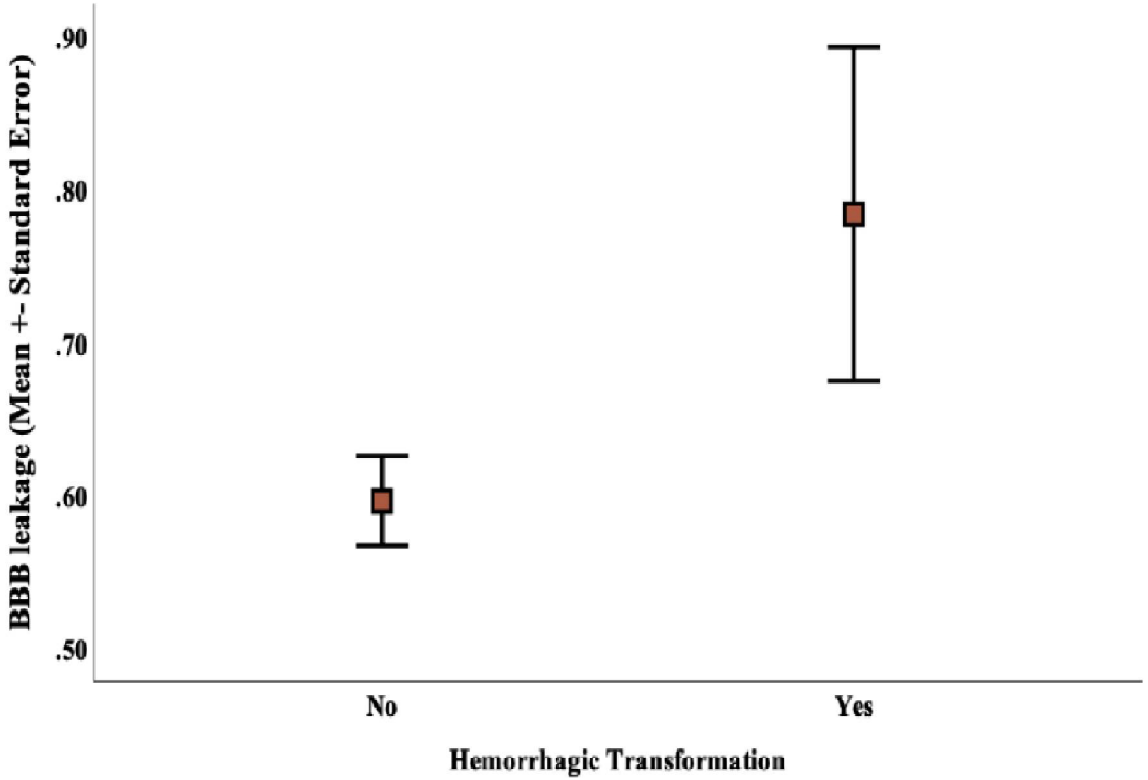
- 171 ischaemic stroke patients treated with revascularization treatments (18% only intravenous thrombolysis, 60% only endovascular treatment, 22% received both treatments).
- Mean (±SD) age 74.9 (±12.4) years, 84 (51%) males, median (IQR) National Institutes of Health Stroke Scale 18 (12-23).
- Circulating biomarkers taken before and after 24 hours from acute interventions.
- Pre-treatment BBB leakage assessed with CT perfusion by using Ktrans within the ischaemic area.

BBB leakage before reperfusion therapy associated with hemorrhagic transformation

Hemorrhagic transformation occurred in 29 (17%) patients (one patient treated only with rt-PA, 23 patients only with endovascular therapy, five patients with both treatments),

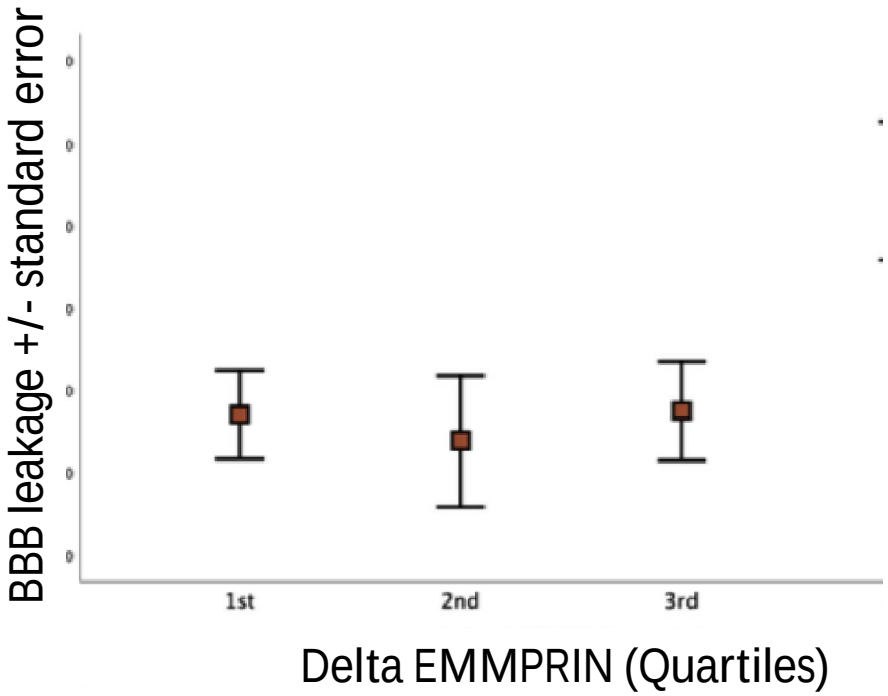


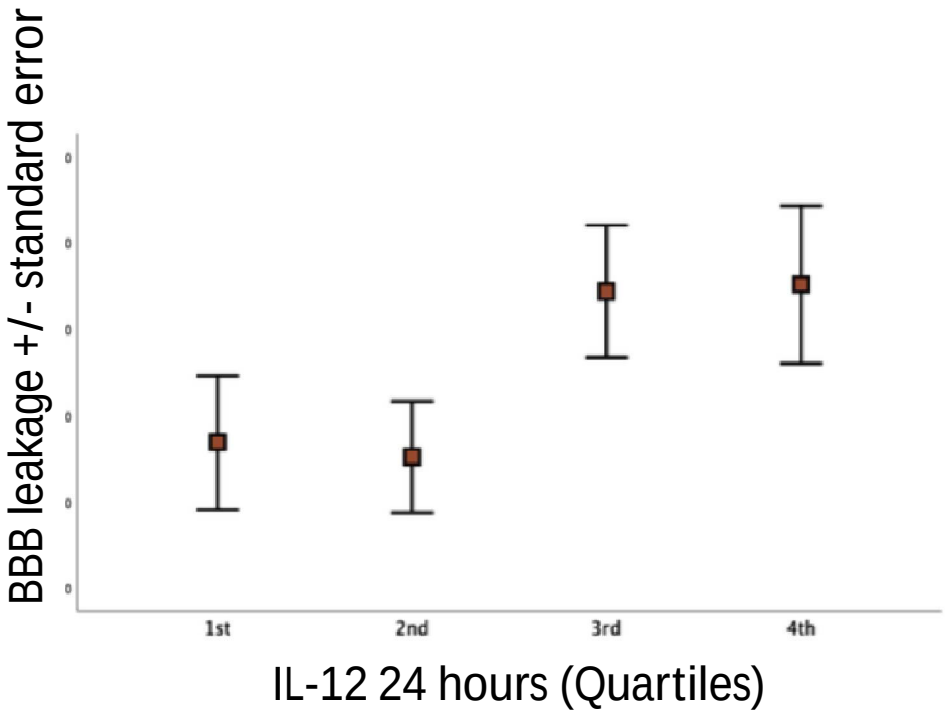
K trans= mean of the 3 ROIs



Arba et al. ESOC 2019 Poster presentation

BBB disruption and consequent inflammatory cascade in acute ischemic stroke





Piccardi et al. ESOC 2019 Poster presentation

Published in final edited form as:
Eur J Pharmacol. 2018 August 15; 833: 531–544. doi:10.1016/j.ejphar.2018.06.028.

Targeting vascular inflammation in ischemic stroke: Recent developments on novel immunomodulatory approaches

INIZIATION (hours) → **INNATE IMMUNITY**

PROPAGATION (days) → **ADAPTATIVE IMMUNITY**

RESOLUTION (weeks)

CAVEAT: some degree of inflammation is predicted to be necessary to remove injured or necrotic tissue and contribute to wound healing

Recent clinical trials using biologics to treat ischemic stroke

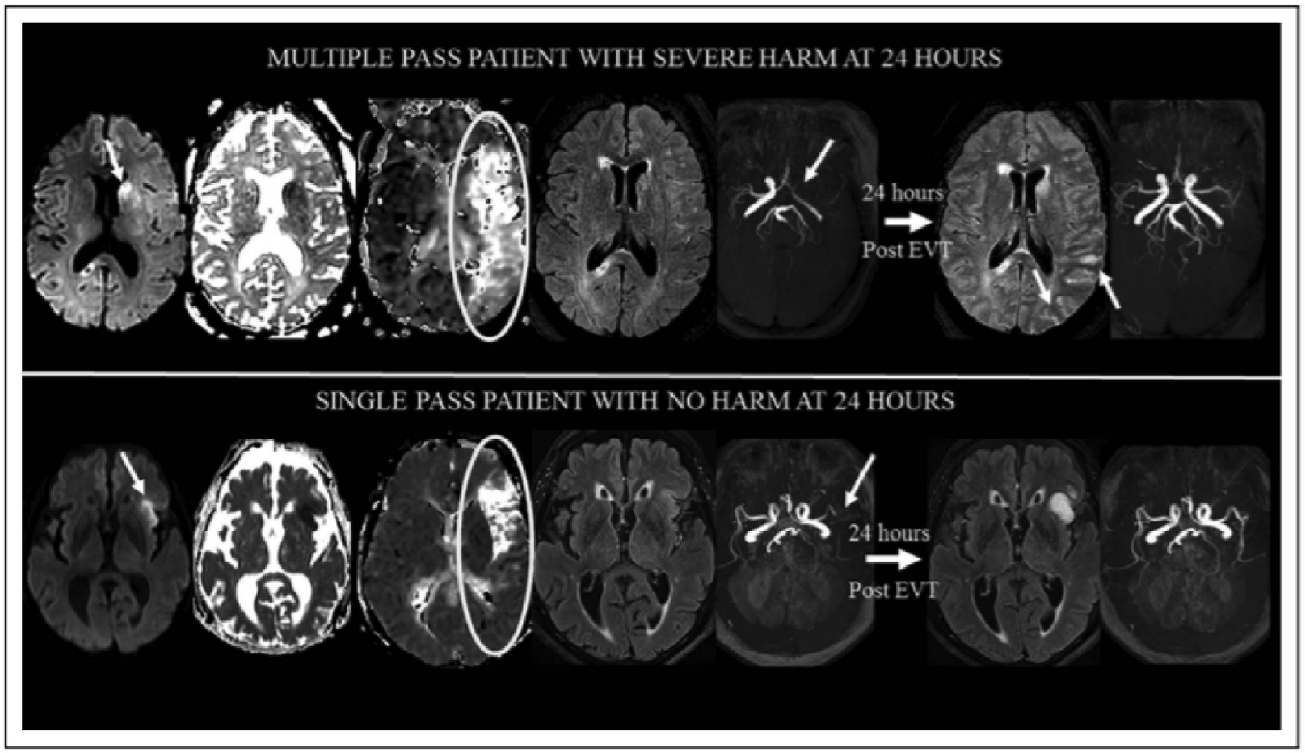
Drug	Target/MOA	Trial	Outcomes/Notes
Abciximab (Fab fragment of the human-murine monoclonal antibody	Glycoprotein IIb/IIIa receptor of platelets/ Inhibits platelet activation and aggregation	NCT00073372	Terminated due to intracerebral hemorrhage.
Canakinumab (Fully human mAb)	IL-1β	NCT01327846 (CANTOS)	Lower rate of recurrent cardiovascular events
Enlimomab (murine IgG2a mAb)	ICAM-1 / Reduces leukocyte adhesion to EC	Phase III	Terminated due to worse outcome and immune side effects associated with murine antibody
E-Selectin (recombinant protein)	Ligands for EC selectins on leukocytes / Mucosal tolerance	NCT00012454 NCT00069069	Terminated/suspended; results not available
Natalizumab (humanized monoclonal antibody)	Cell adhesion molecule α4-integrin / Blocks T-cell interaction with EC VCAM-1	NCT01955707	Treatment up to 9 h after stroke did not reduce infarct size; Associated benefits on functional outcomes warrant further investigation
rhIL-1ra/Anakinra	Receptor for IL-1α or IL-1β/Antagonist of M1 microglia/Mφ inflammatory signaling	(a) small (n = 34) phase II randomized controlled trial (b) ISRCTN74236229	(a) Lower blood neutrophil &WBC counts, CRP, and IL-6. Improved functional outcome (b) Lower plasma IL-6 and CRP. No improved favorable outcome on modified Rankin Scale

CANTOS, Canakinumab Anti-inflammatory Thrombosis Outcome Study; CRP, C-reactive protein; EC, endothelial cell; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; mAB, monoclonal antibody; MOA, mechanism of action; Mφ, macrophage; VCAM-1, Vascular cell adhesion protein 1; WBC, white blood cell.

Frequency of Blood-Brain Barrier Disruption Post- Endovascular Therapy and Multiple Thrombectomy Passes in Acute Ischemic Stroke Patients

-80 ischemic stroke patients, median age was 65 years, 64% female, 51% black/African American, median NIHSS=19, 56% treated with IV tPA, and 84% TICI 2b/3.

- Multiple-pass patients had signicantly higher rates of severe HARM at 24 hours (67% versus 29%; *P*=0.001), any hemorrhagic transformation (60% versus 36%; *P*=0.04) and poor clinical outcome (67% versus 36%; *P*=0.008).



Luby M, *Stroke* 2019

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Short communication

Neuroprotection for ischemic stroke in the endovascular era: A brief report on the future of intra-arterial therapy ☆

Julius Griauzde [a], Vijay M. Ravindra [b], Neeraj Chaudhary [a,c], Joseph J. Gemmete [a,c], Aditya S. Pandey [c,*]

[a] Department of Radiology, University of Michigan, Ann Arbor, MI, United States
[b] Department of Neurosurgery, Texas Children's Hospital, Houston, TX, United States
[c] Department of Neurosurgery, University of Michigan, Ann Arbor, MI, United States

- Potential to maximize local benefit while minimizing systemic effects
- Strategies under investigation include IA infusion of neuroprotective agents, IA administration of stem cells, and selective IA hypothermia.

Journal of Clinical Neuroscience, 2019

Global brain inflammation in stroke

Kaibin Shi, De-Cai Tian, Zhi-Guo Li, Andrew F Ducruet, Michael T Lawton, Fu-Dong Shi

Lancet Neurol., 2019

In addition to inflammation localised to the injured brain region, a growing body of evidence suggests that inflammatory responses after a stroke occur and persist throughout the entire brain. Global brain inflammation might continuously shape the evolving pathology after a stroke and affect the patients' long-term neurological outcome.

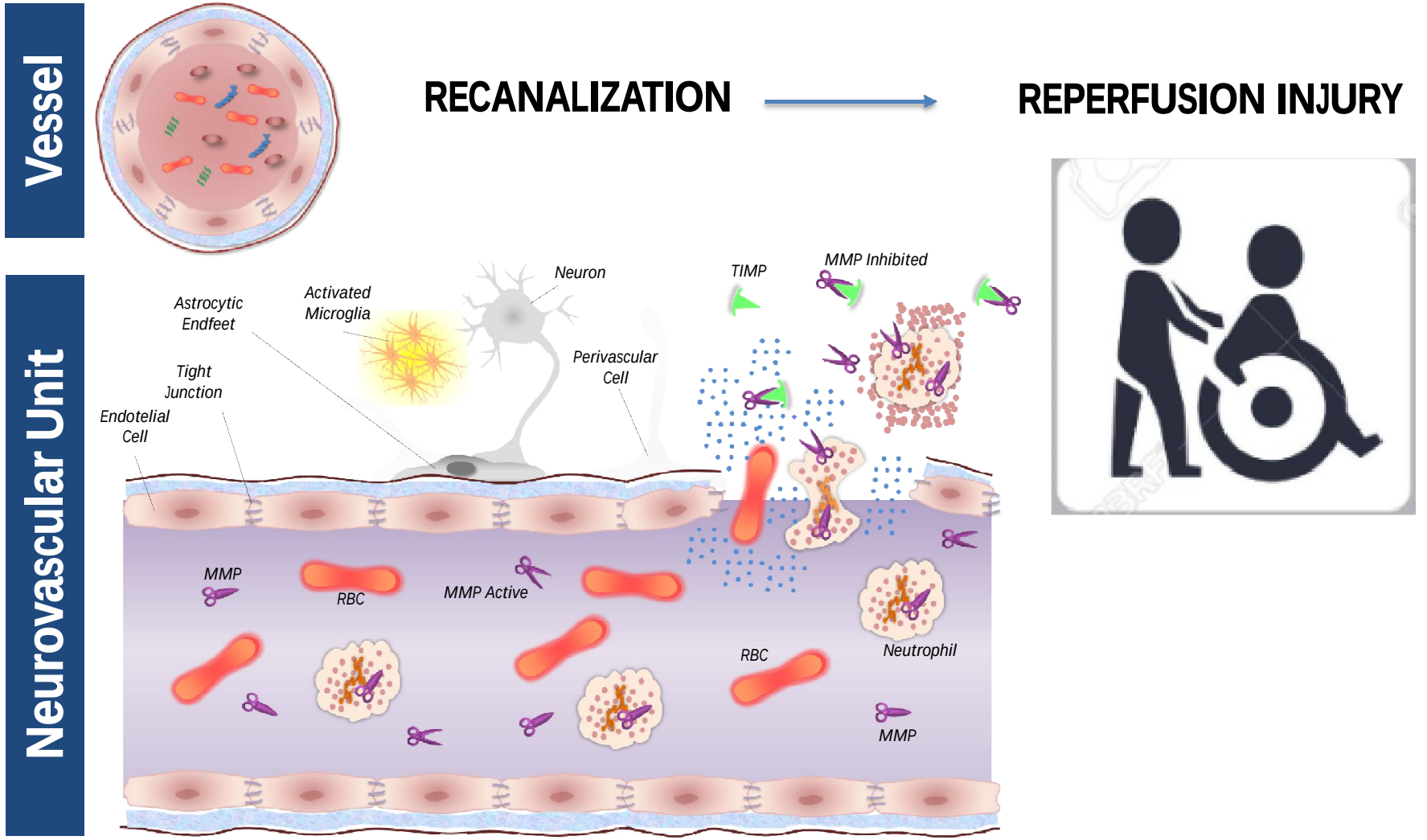
Blood–brain barrier permeability was increased even in vessel territories remote from the index infarct (Villringer K, Neurology 2017)

Future research directions

- Clarify the effect of focal and global brain inflammation on short/long-term brain structure changes and neurorepair and their associations to clinical outcome

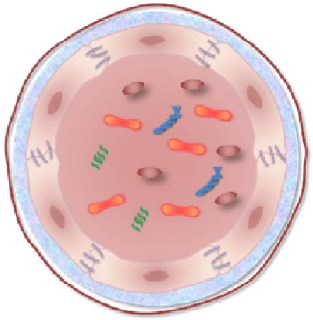
- Search for strategies that can modulate focal and global brain inflammation and BBB disruption after a stroke, and test whether these treatments could improve the disease outcome

....back to **Mr.R** bed

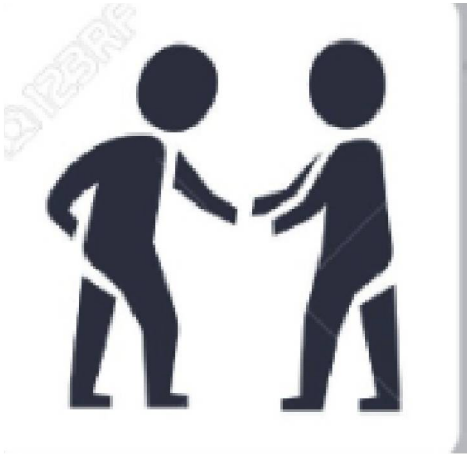


....back to **Mr.R** bed

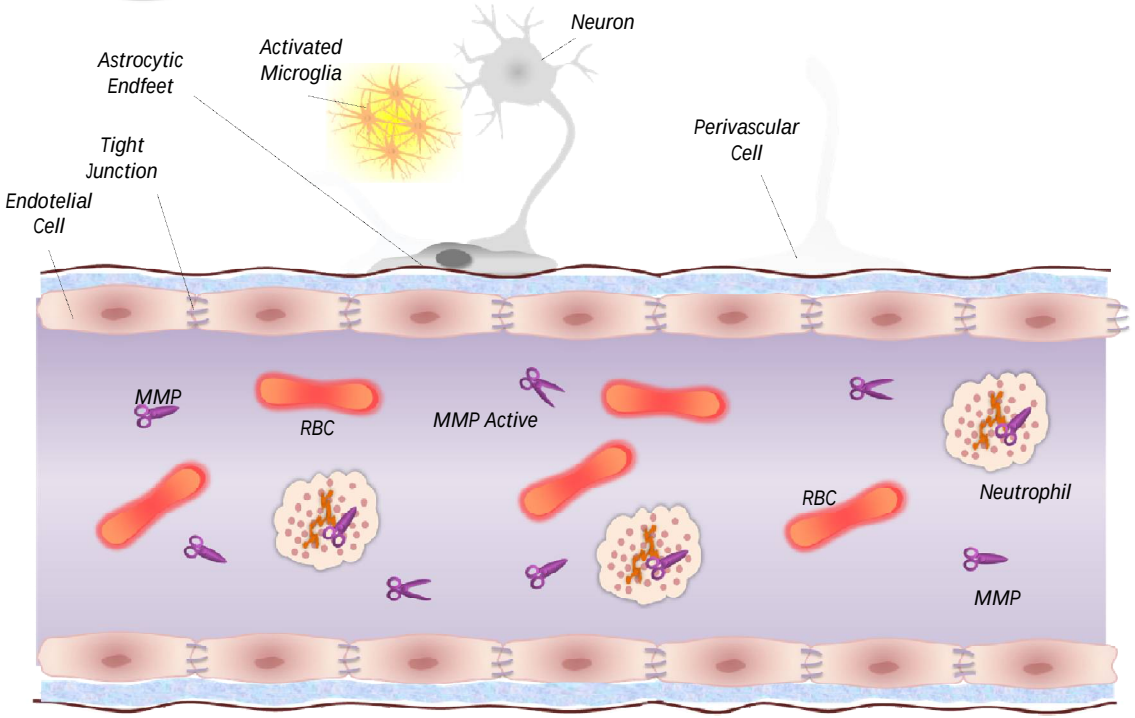
Vessel



RECANALIZATION → **NO REPERFUSION INJURY**



Neurovascular Unit



Grazie per l'attenzione!