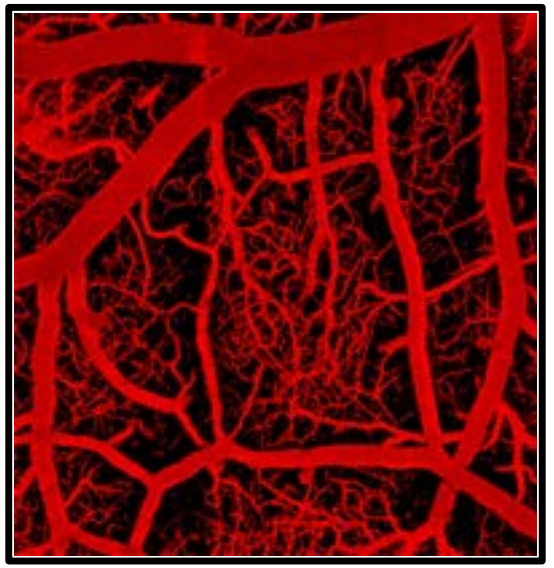
Laboratory of Experimental Stroke Research

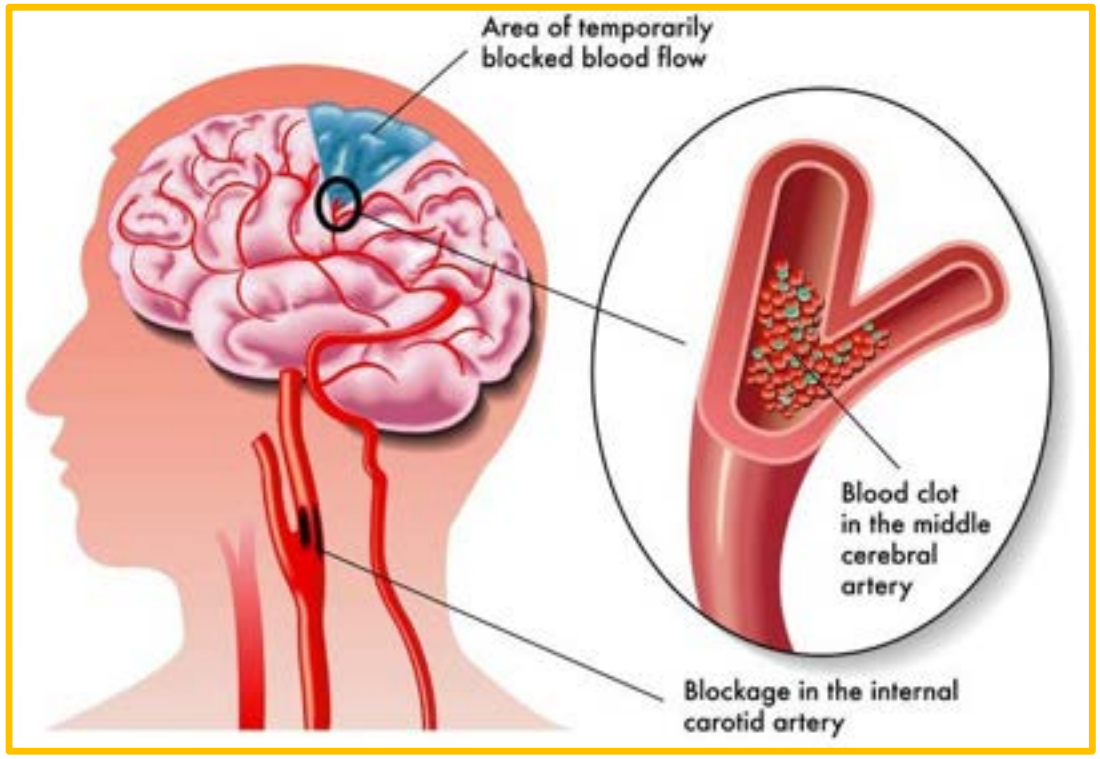


Simone Beretta, MD, PhD

Laboratory of Experimental Stroke Research
Department of Medicine and Surgery
University of Milano Bicocca
Department of Neurology and Stroke Unit
Fondazione IRCCS San Gerardo dei Tintori
Monza

STROKE

Definition (OMS):
Rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent cause other than vascular origin.



STROKE

Epidemiology:

- 2° leading cause of DEATH
- 2° leading cause of DEMENTIA
- 3° leading cause of DISABILITY
- 200.000 cases every years
- 80% new event
- Age related incidence

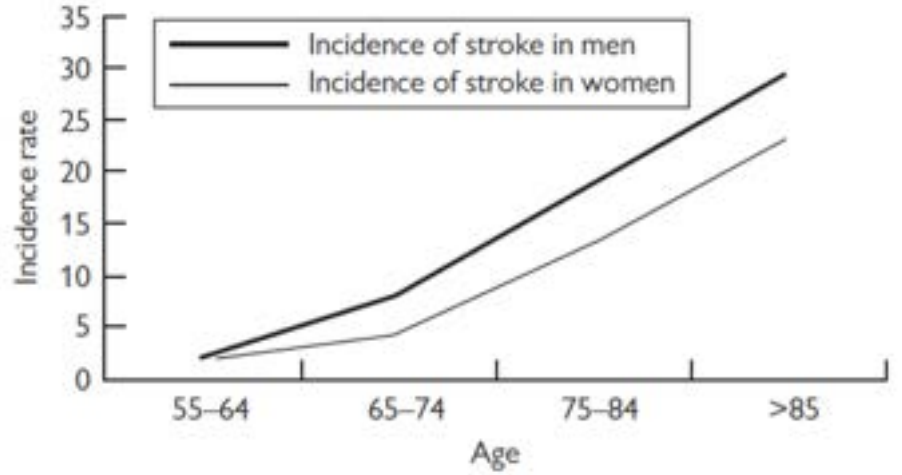


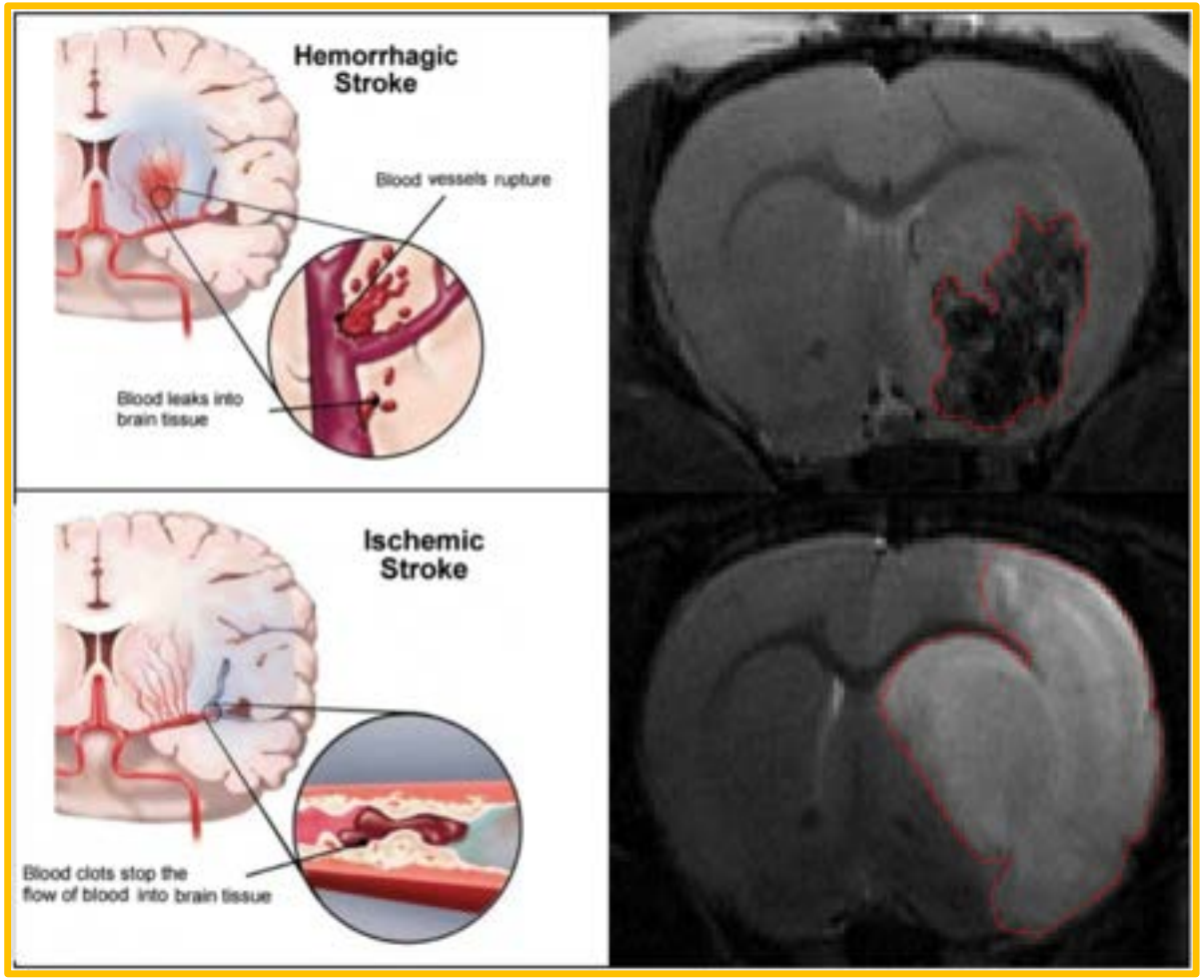
Fig. 1.2 Graph showing incidence rate per 1000 person-years for stroke in relation to age and gender.

Adapted from *J Neurol Neurosurg Psychiatry*, 74(3), Hollander M, Koudstaal P J, Bots M L, Grobbee D E, Hofman A, Breteler M M B, Incidence, risk, and case fatality of first ever stroke in the elderly population. The Rotterdam Study, pp. 317–21, Copyright (2003), with permission from BMJ Publishing Group Ltd.

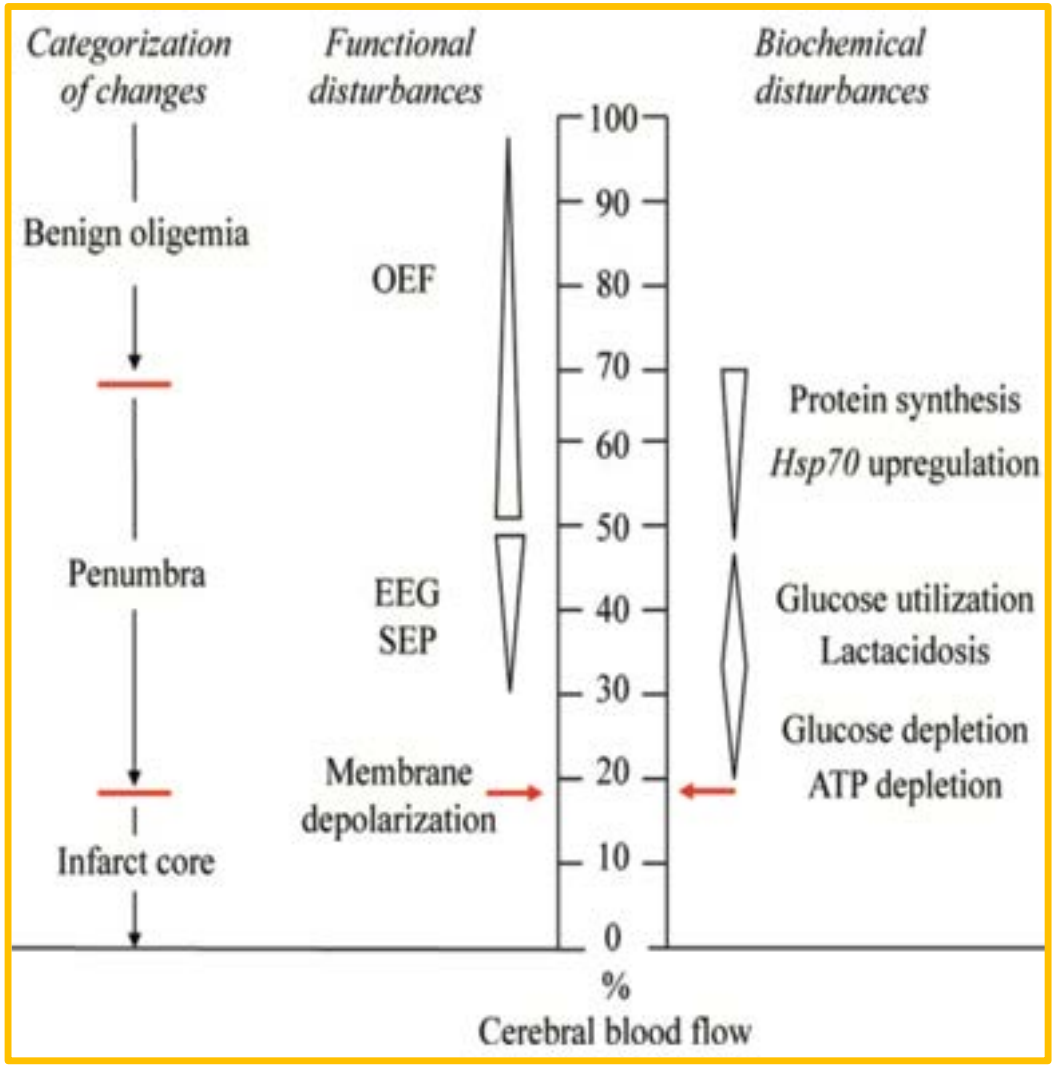
STROKE

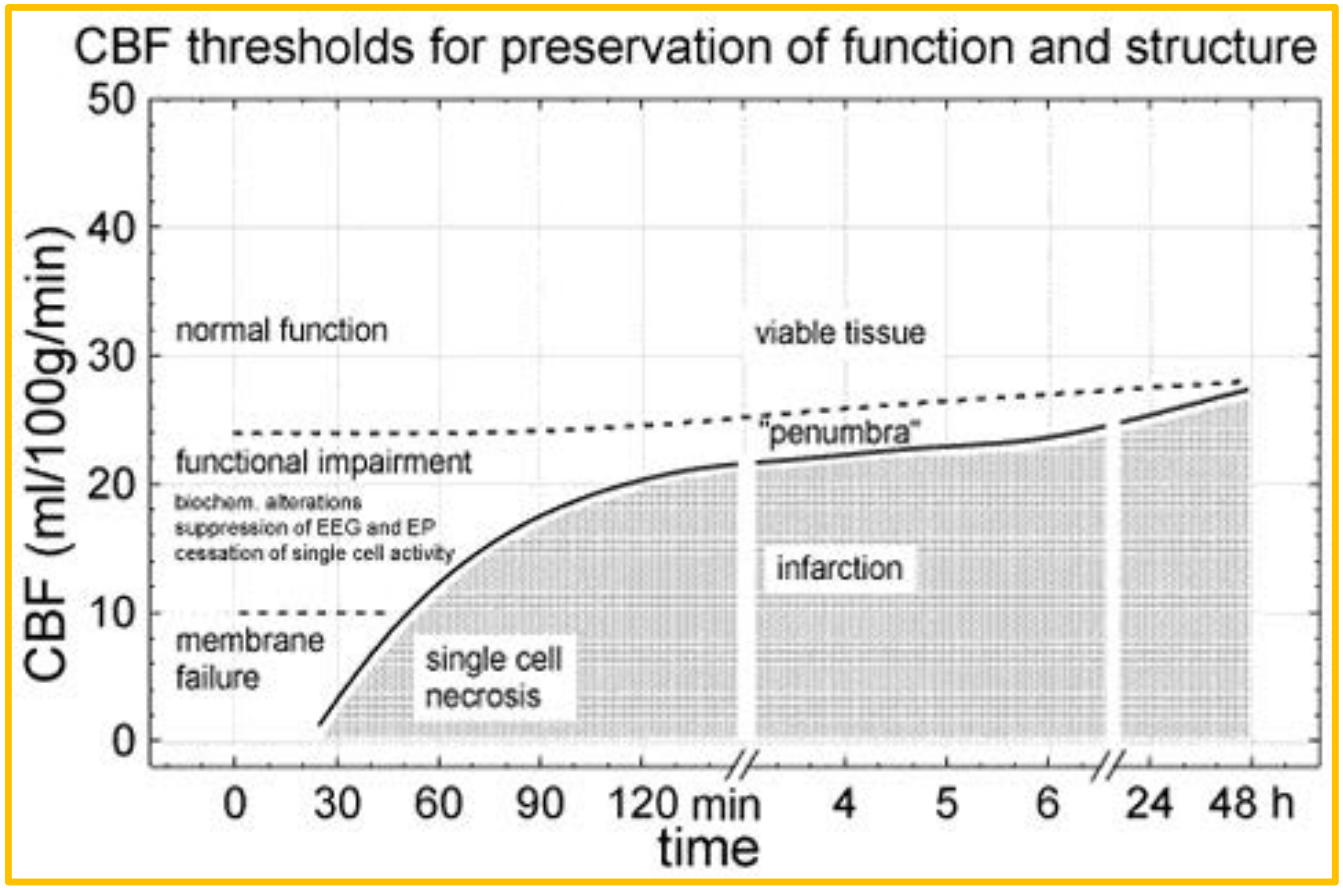
Eziology

- Ischemic stroke (80-85%)
  - Thrombotic stroke
  - Embolic stroke
- Hemorragic stroke (10%)
- Subaracnoid Hemorrage (5%)

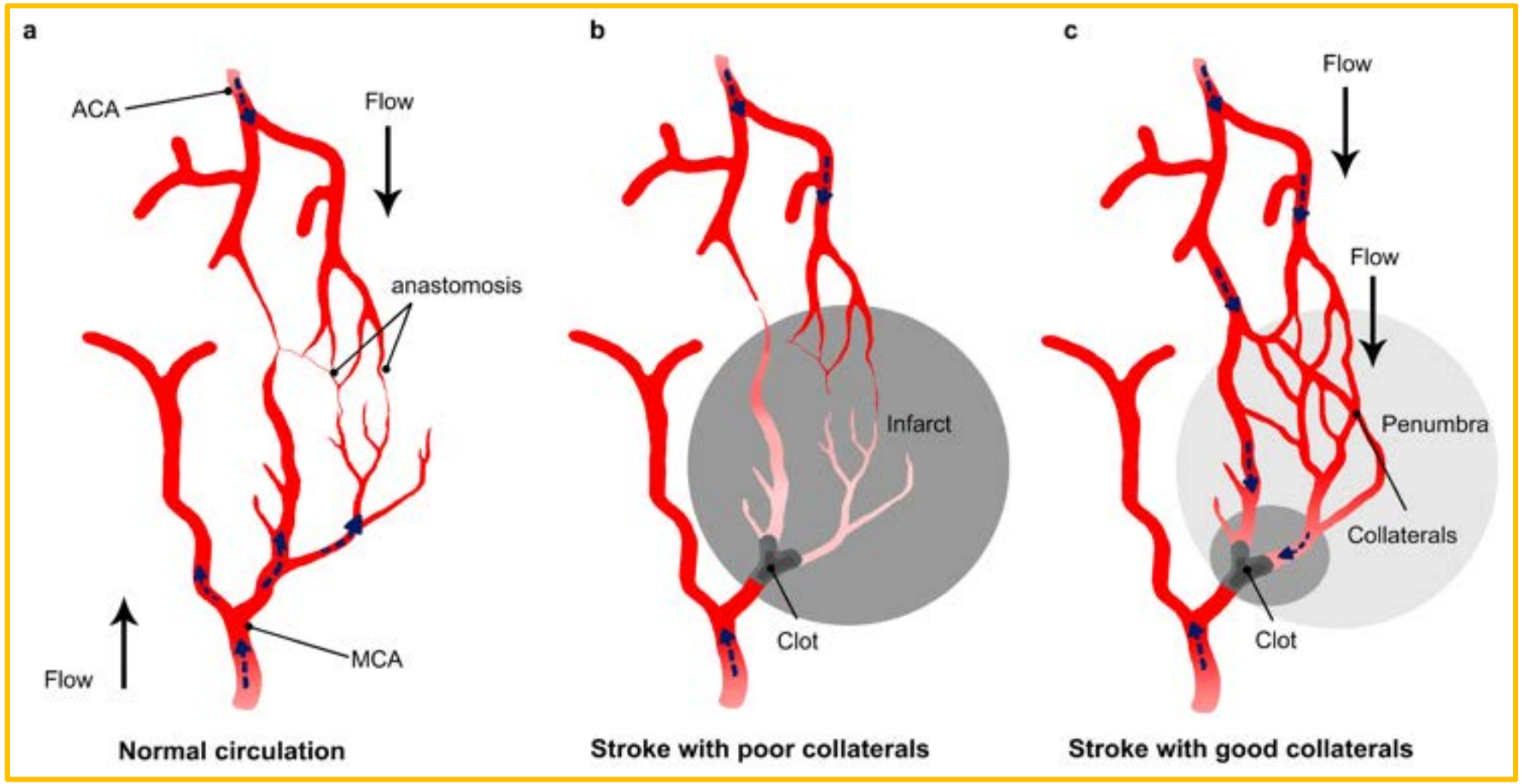


ISCHEMIC CORE AND PENUMBRA





CEREBRAL COLLATERALS

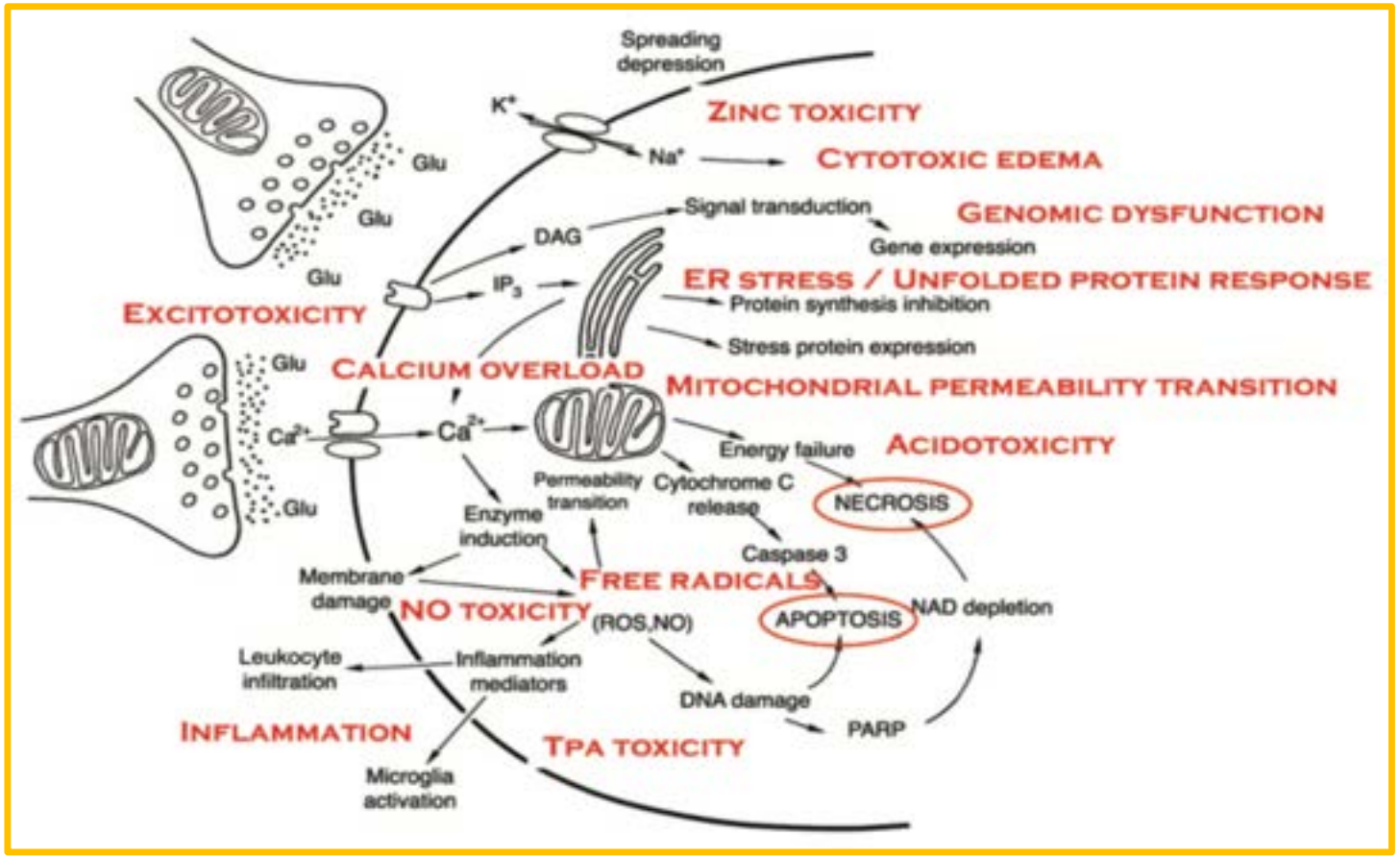


PATHWAYS INVOLVED

Loss of nutrients

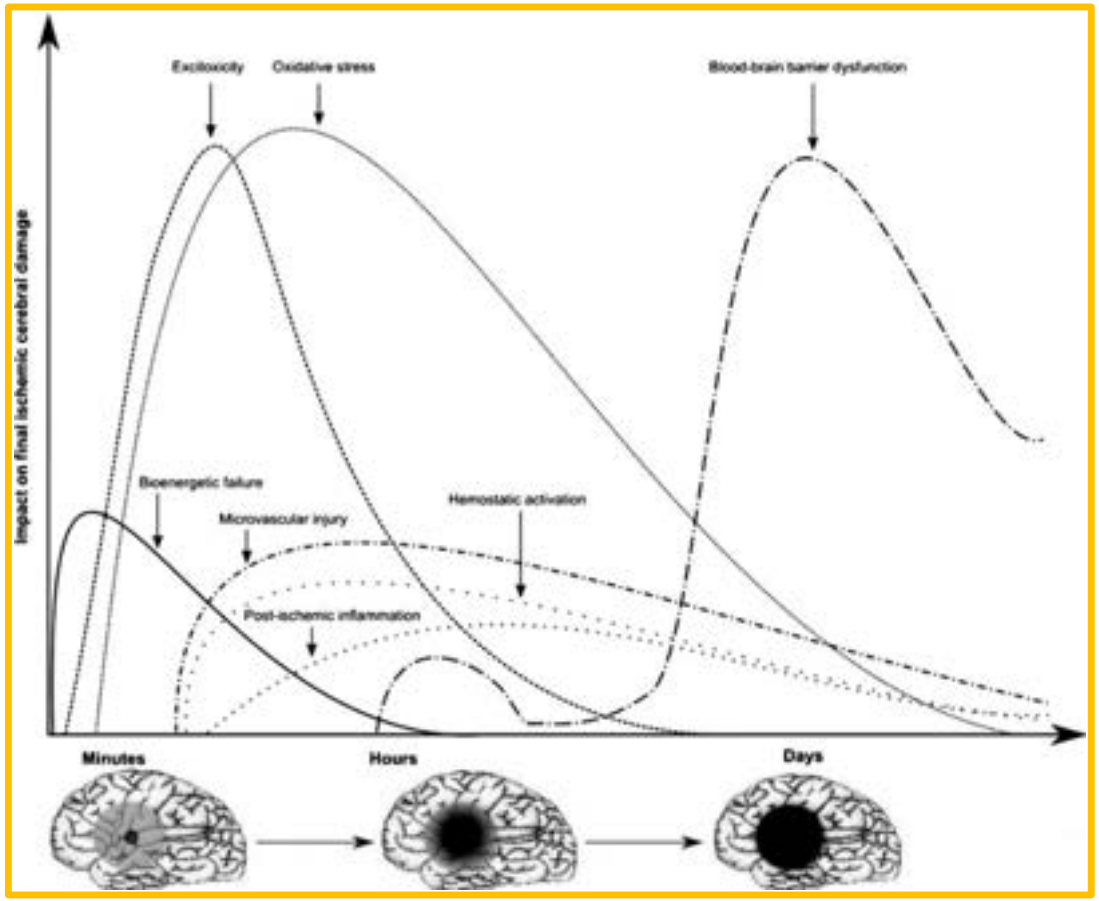
Energetic failure

Excitotoxicity

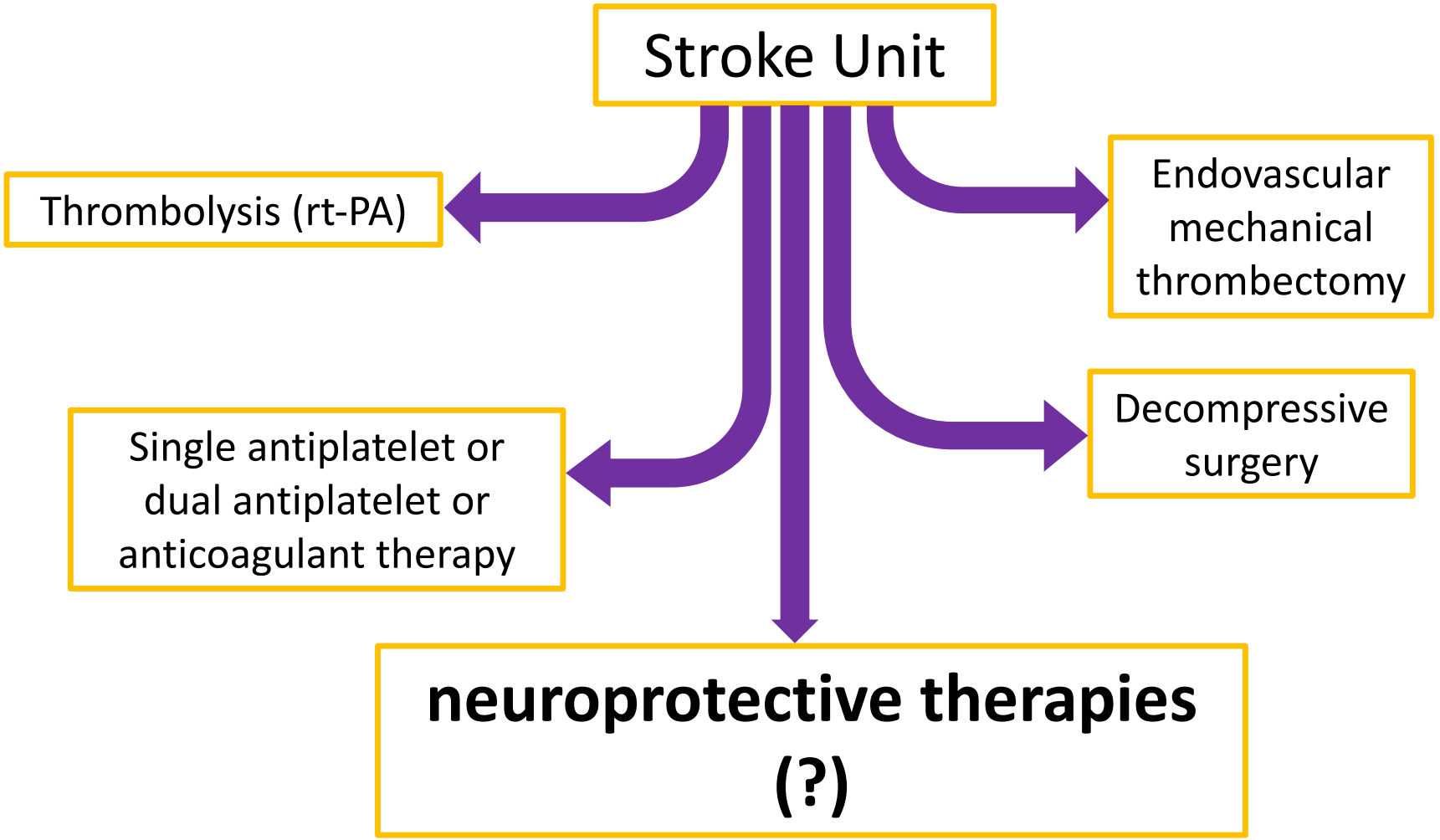


Edema → Stress OX → Post-ischemic inflammation → Cellular death

TIME IS BRAIN



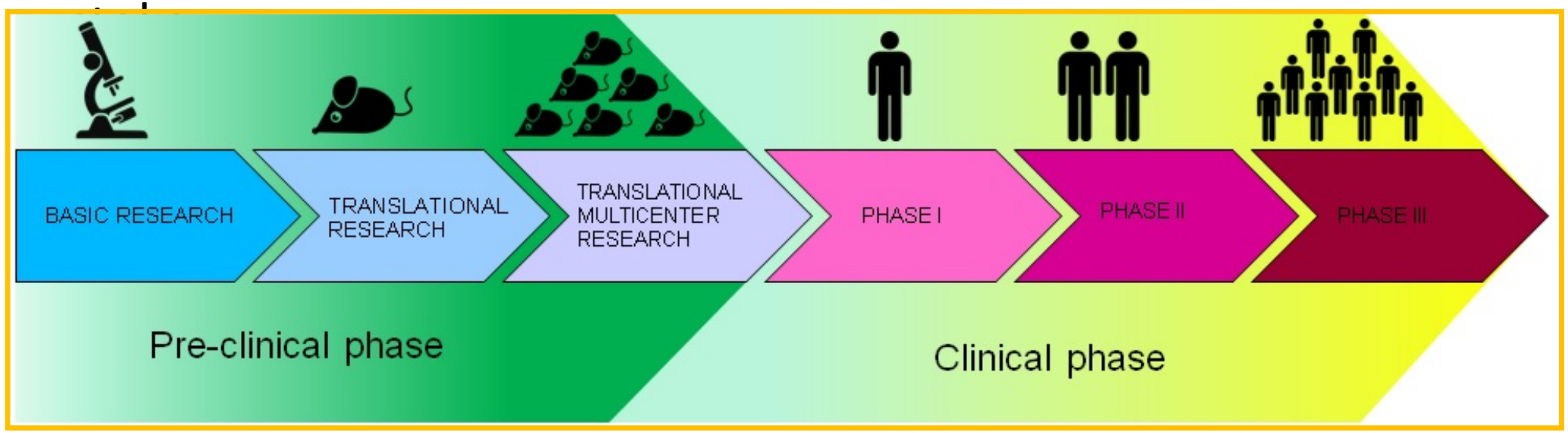
TREATMENT


Stroke Unit
Thrombolysis (rt-PA)
Endovascular mechanical thrombectomy
Single antiplatelet or dual antiplatelet or anticoagulant therapy
Decompressive surgery
neuroprotective therapies (?)

Our aim

Develop new therapeutics strategy to treat acute ischemic

Create new multicenter translational project.

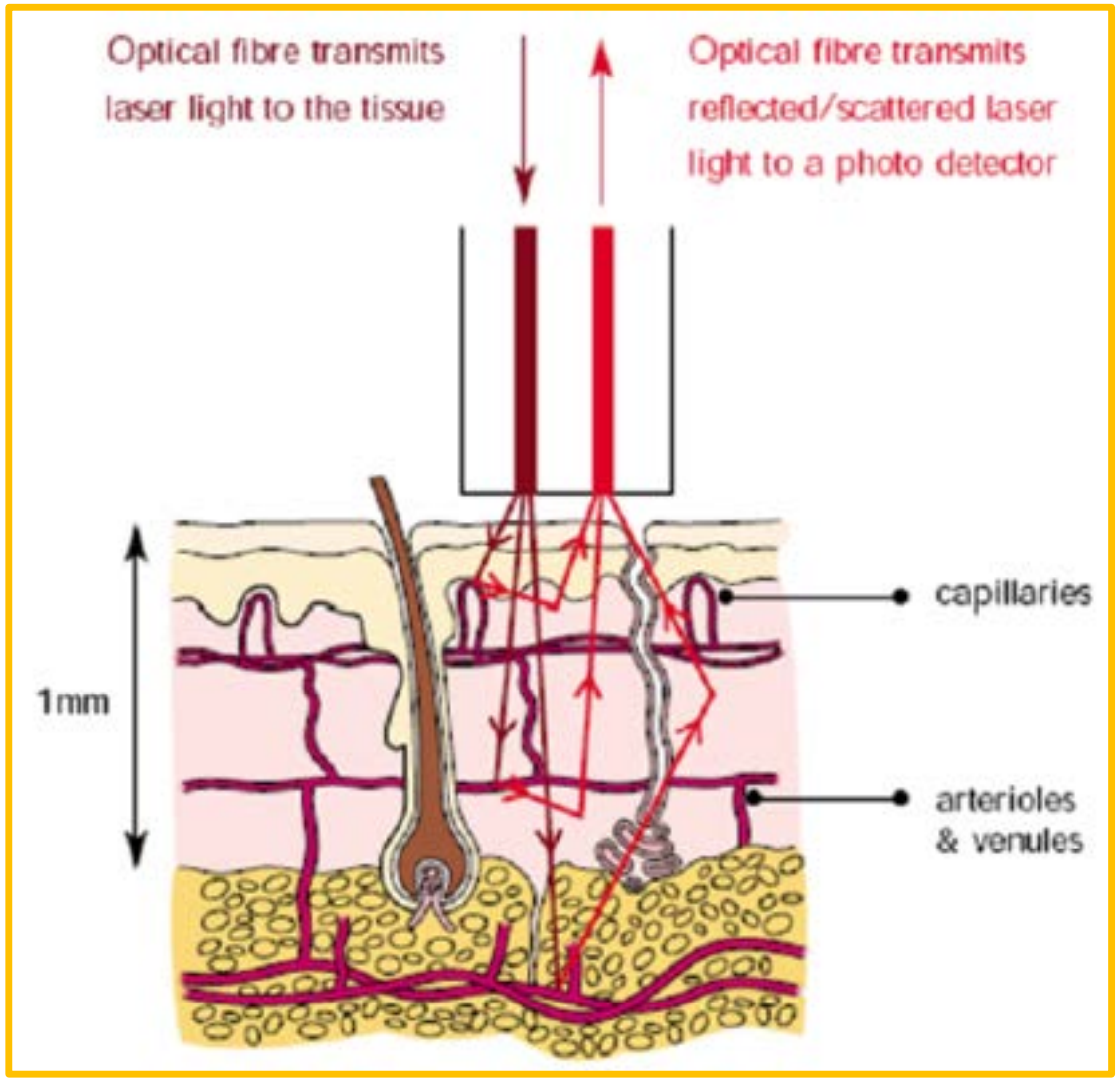


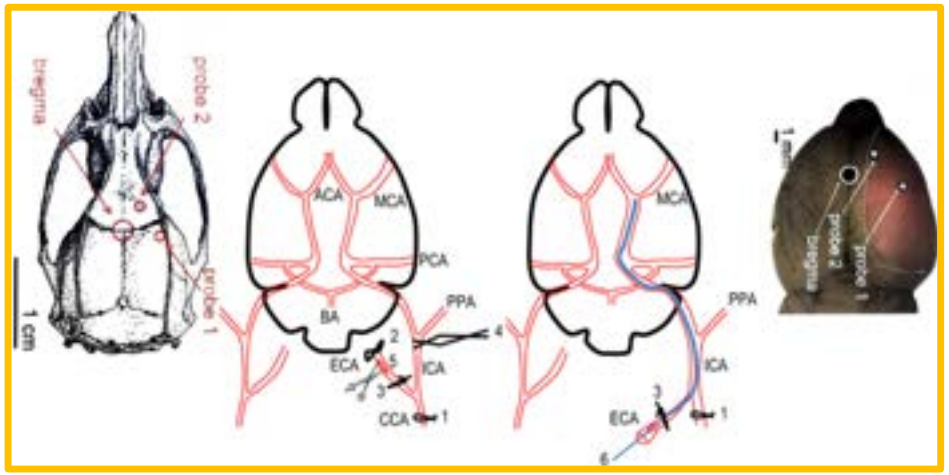
Transient endovascular middle cerebral artery occlusion in the rat

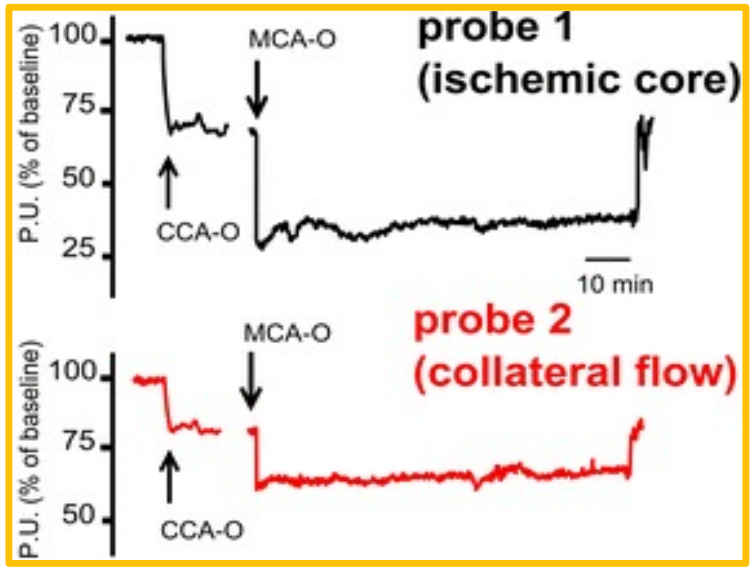
tMCAO

video of MCAO surgery

MOOR LASER DOPPLER







Neurobehavioural tests

Garcia Test

Corner Turning test

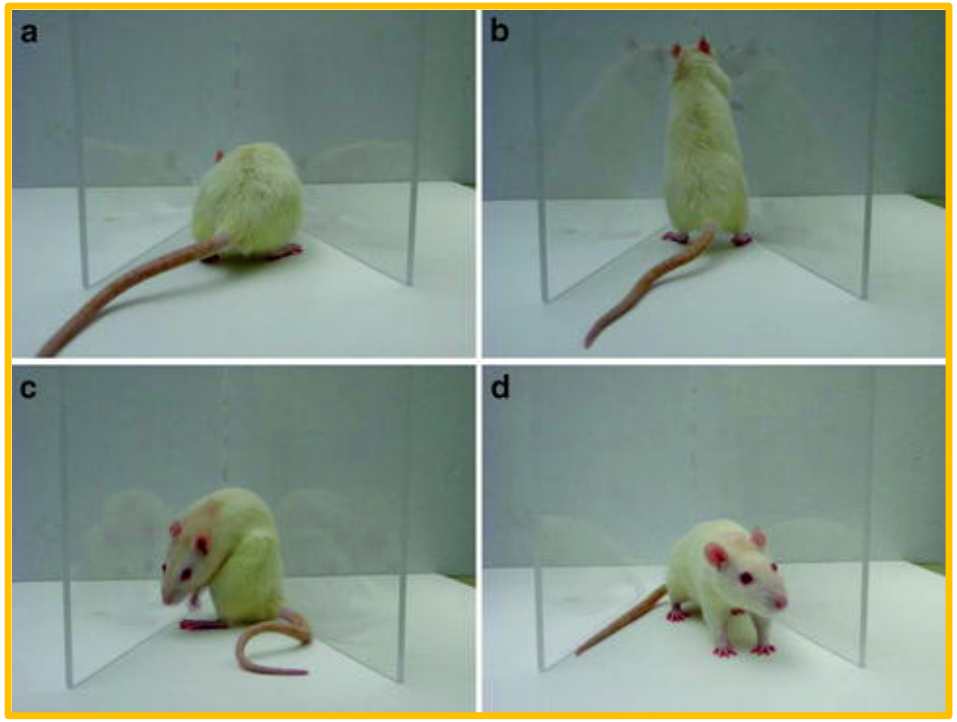
GARCIA NEUROSCORE

La somma dei punteggi determina il grado di deficit dell'animale sottoposto al test. Il valore 3 corrisponde ad un deficit severo, mentre il 18 a nessun deficit.

DATA: _____ **RATTO:** _____

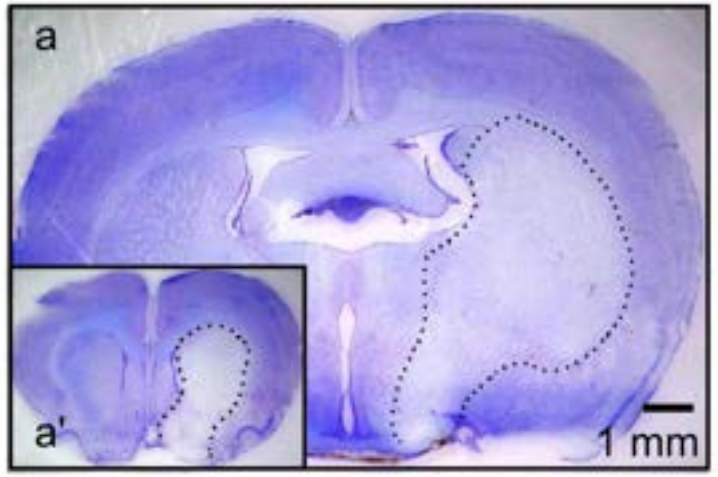
		0	1	2	3	SCORE
Attività Spontanea		*Nessun movimento*	*Movimento lieve*	*Si muove ma non approccia 3 lati*	*Si muove ed approccia 3 lati*	
Reazione al Contatto	**Vibrisse**		*Risposta assente*	*Risposta lieve*	*Risposta simmetrica*	
	Tronco		*Risposta assente*	*Risposta lieve*	*Risposta simmetrica*	
Simmetria Pattern di Movimento	**Zampe Anteriori**	*Pattern gravemente asimmetrico*	*Pattern moderatamente asimmetrico*	*Pattern lievemente simmetrico*	*Pattern simmetrico*	
	Sospensione da Coda	*Pattern gravemente asimmetrico*	*Pattern moderatamente asimmetrico*	*Pattern lievemente simmetrico*	*Pattern simmetrico*	
Grip			*Nulla*	*Lieve*	*Normale*	
					TOTALE	

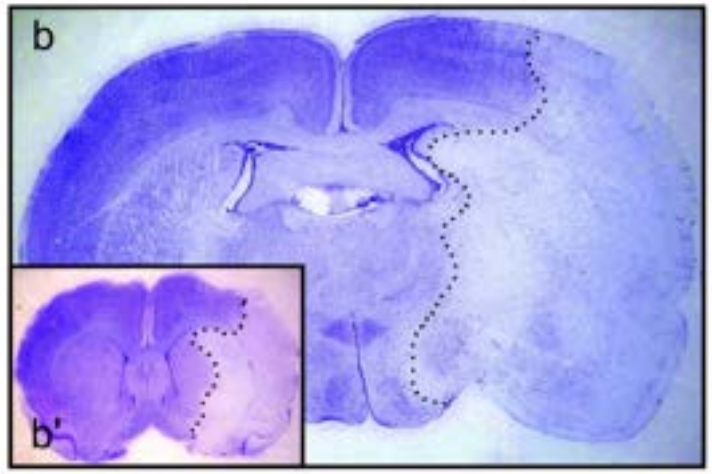
NOTE:

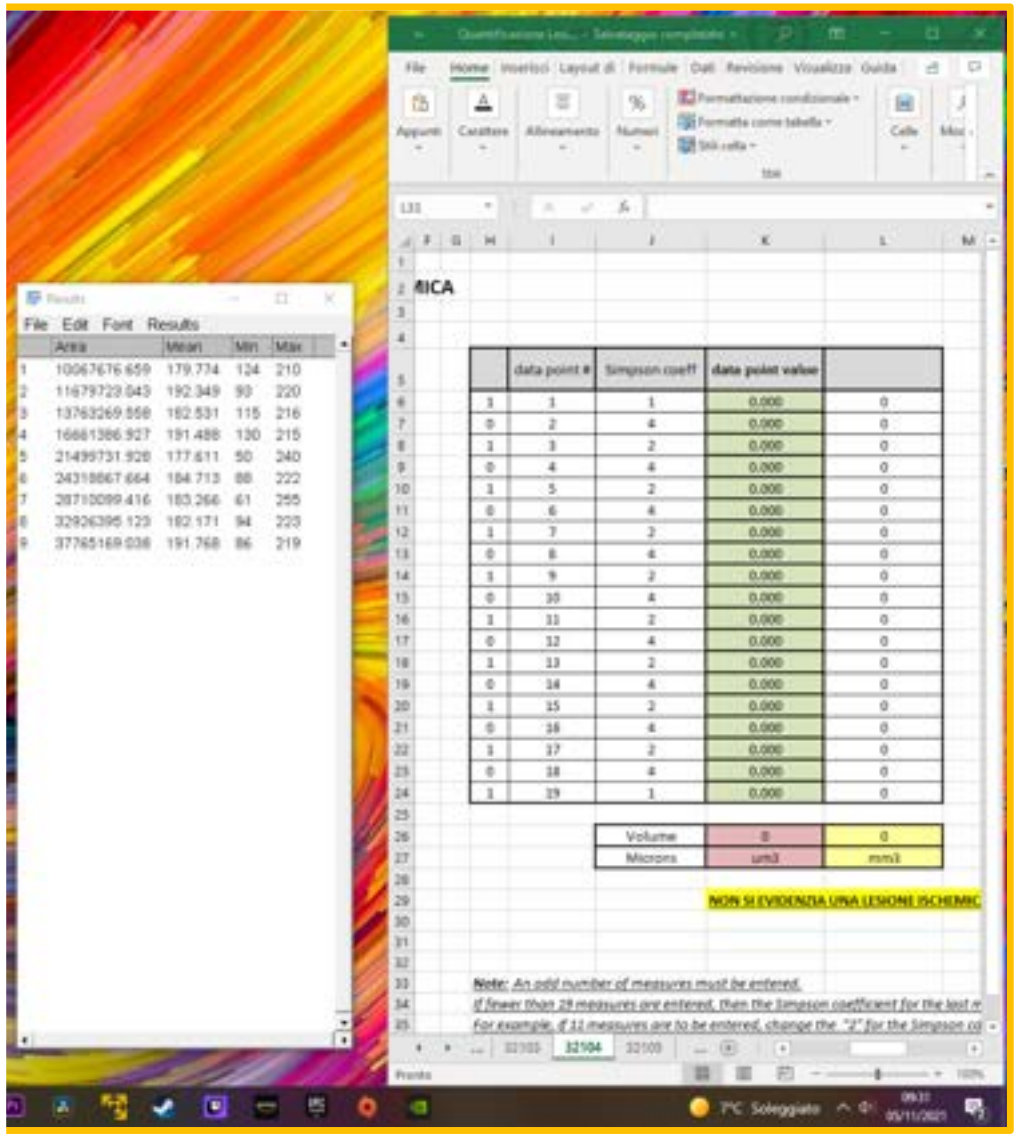


Hystology analisys

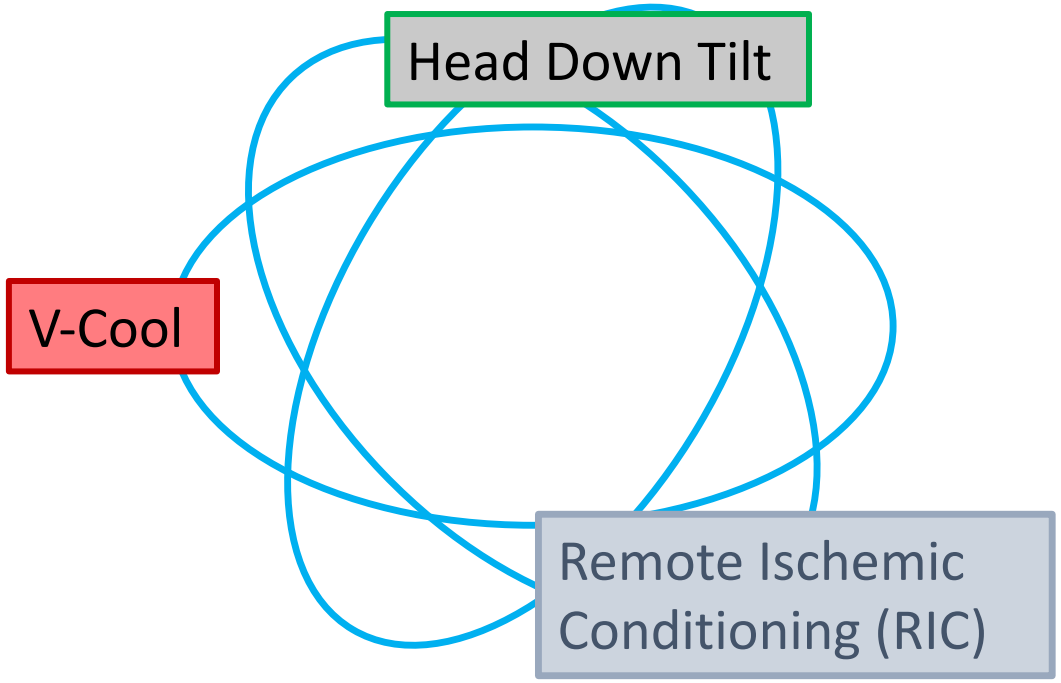
Quantification of ischemic lesion





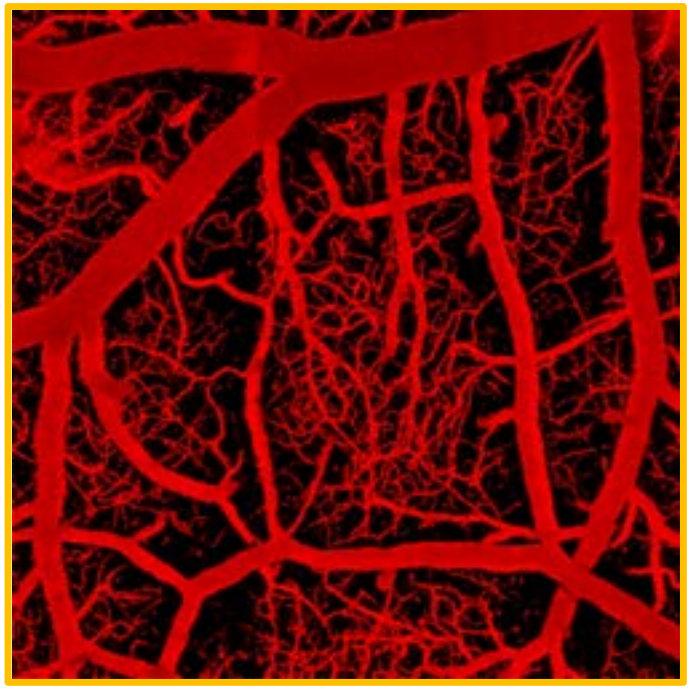


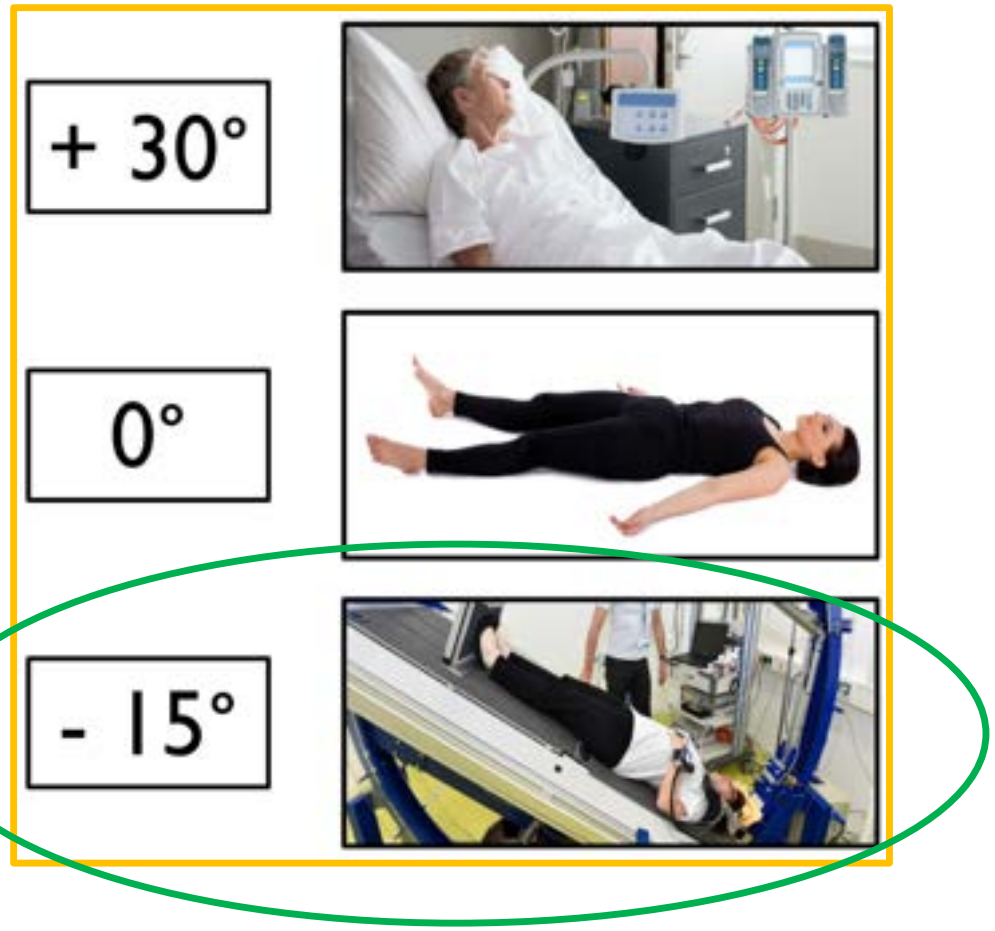
Research projects



HEAD DOWN TILT 15

HDT15° is simple, safe and non pharmacological treatment to increase collateral flow





Acute Ischemic Stroke

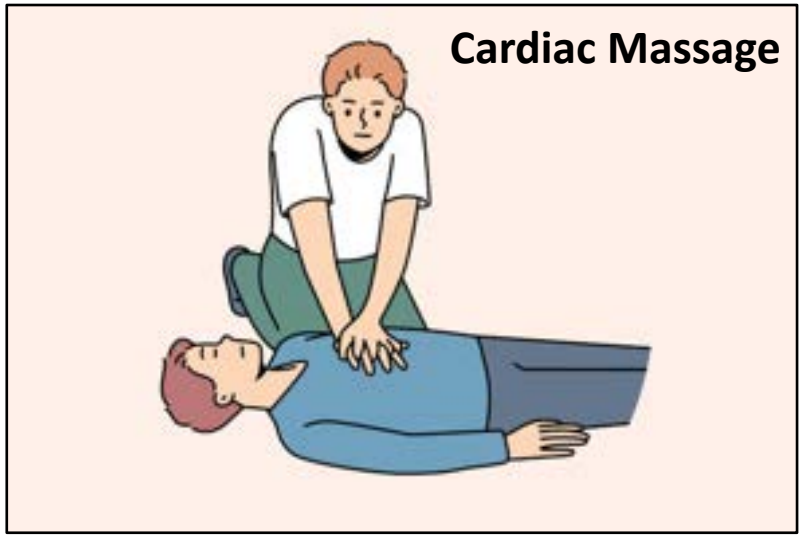
(suspected)

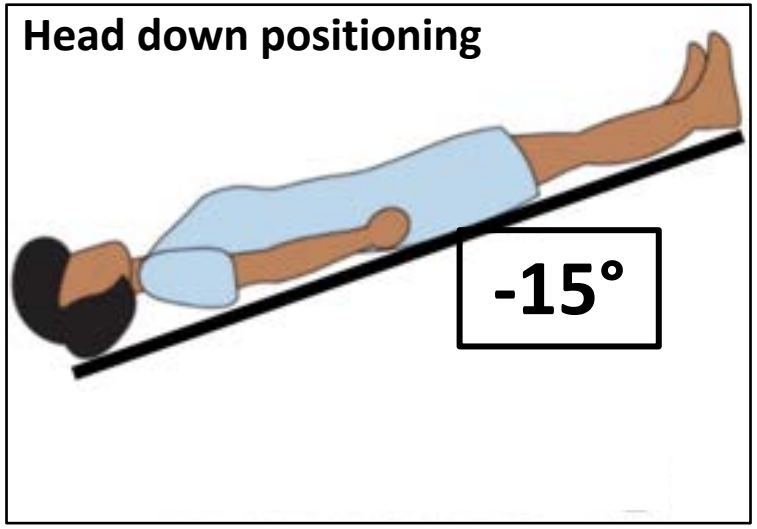
Collateral Therapeutics

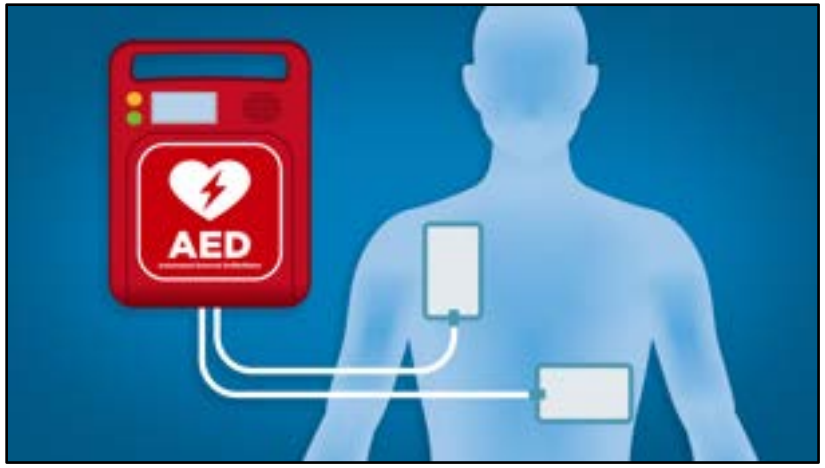
(HDT15)

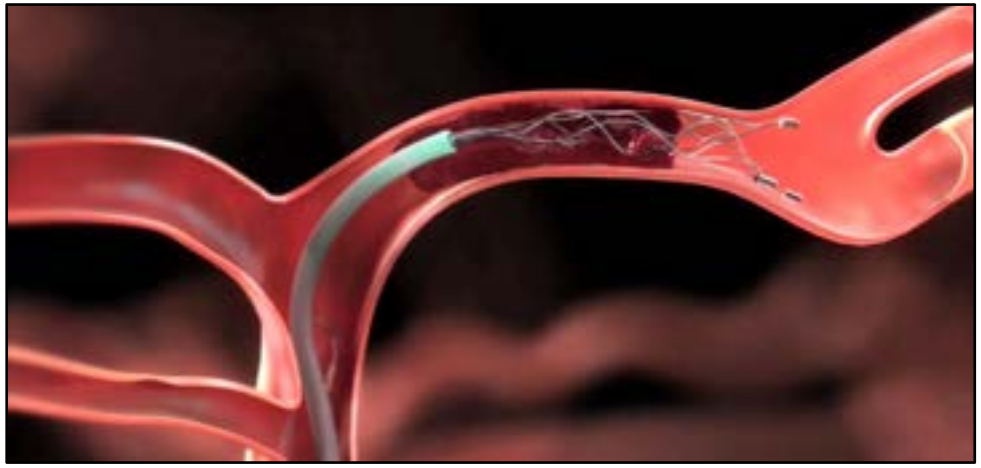
Recanalization Therapies

Inspired by analogies…

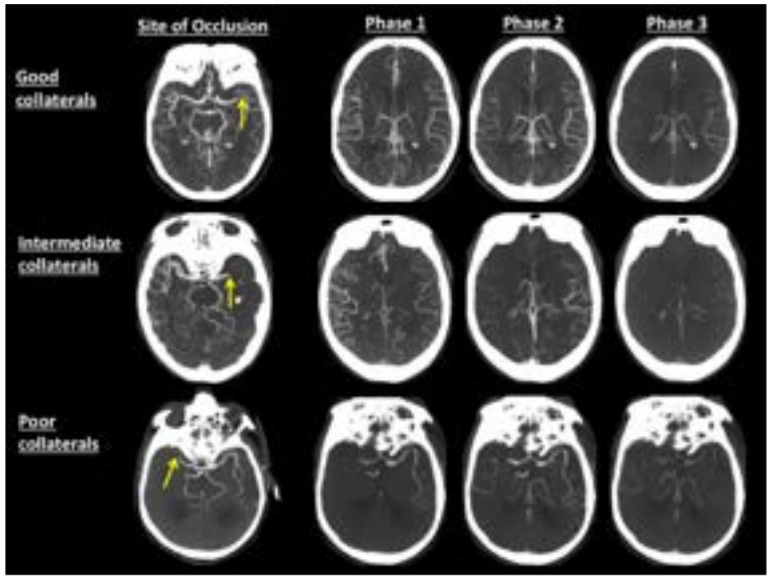


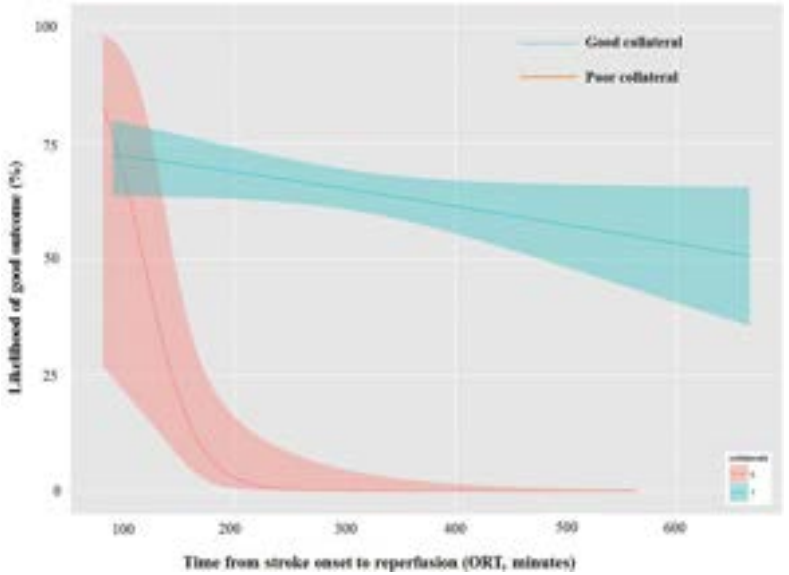


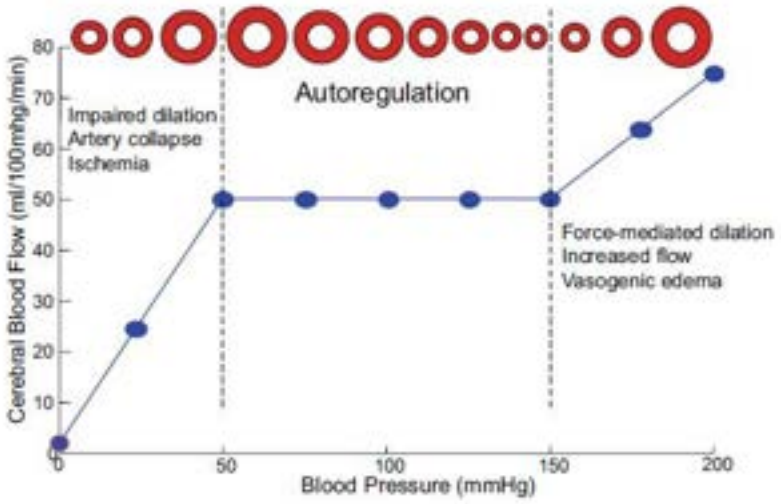




Head Positioning: enhancing collaterals?







Kim BM, *Journal of Neurology, Neurosurgery & Psychiatry* 2018

DOWN-PRIME study
pre-clinical
mechanism: effect on perfusion and tissue at risk

AIM
HDT efficacy in animal LVO stroke models (rats and macaques)

DOWN-SUITE trial
phase 2a
proof-of-concept: HDT increases collateral flow in human stroke

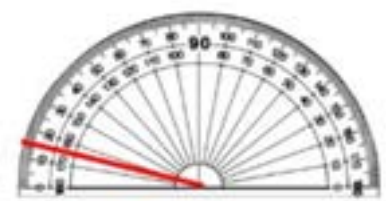
AIM
HDT feasibility, efficacy and safety in LVO patients (in-hospital)

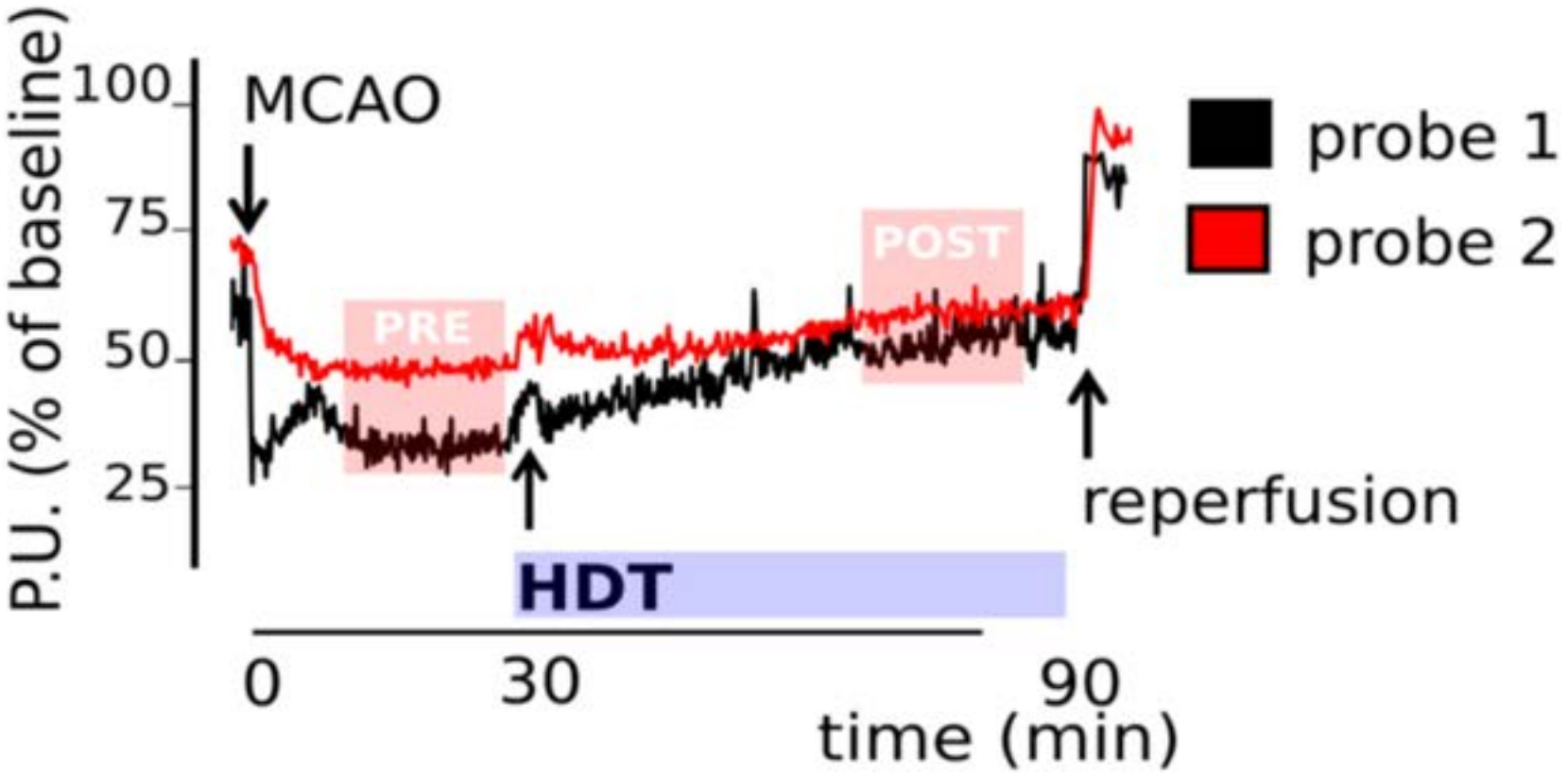
DOWN-SPOKE trial
phase 3
clinical benefit: HDT applied during transport from spoke centers

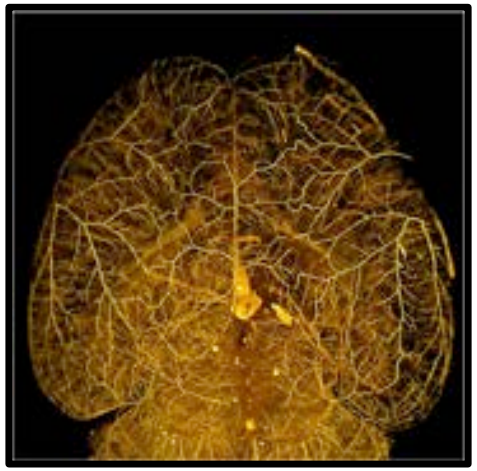
AIM
HDT feasibility, efficacy and safety in LVO stroke patients (spoke-to-hub)

DOWN-FAST trial
phase 3
clinical benefit: pre-hospital application to suspected acute stroke patients

AIM
HDT feasibility, efficacy and safety in unselected stroke patients (pre-hospital)







Original Article

JCBFM

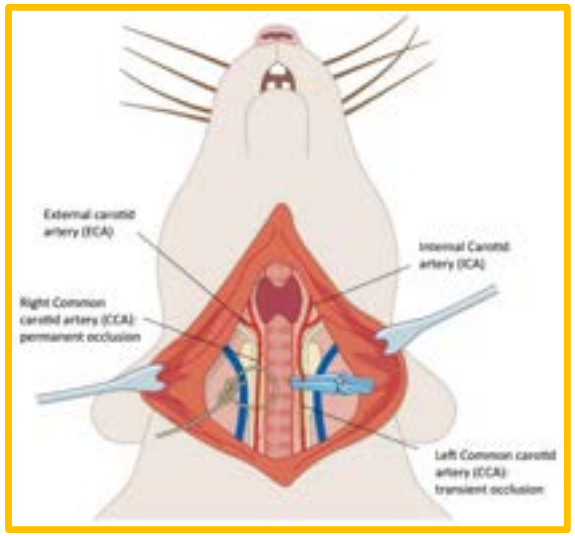
Journal of Cerebral Blood Flow & Metabolism
0(00) 1–11

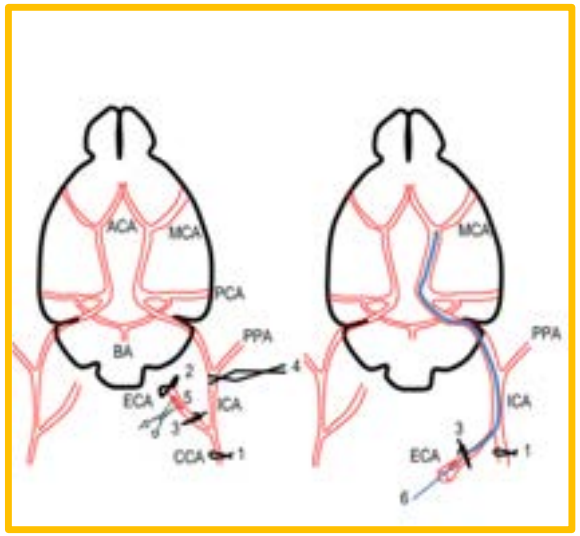
DOI: 10.1177/0271678X16688705
journals.sagepub.com/home/jcbfm
SAGE

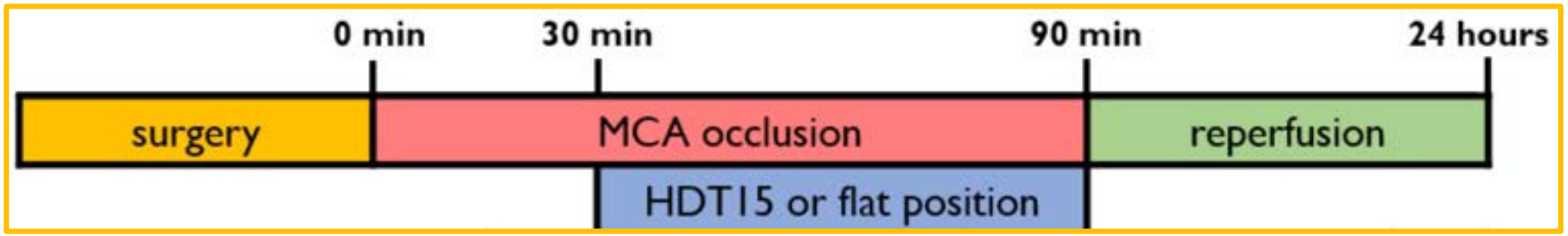
Cerebral collateral therapeutics in acute ischemic stroke: A randomized preclinical trial of four modulation strategies

Simone Beretta[1,2,3], Alessandro Versace[1], Davide Carone[1,2], Matteo Riva[1], Valentina Dell'Era[1], Elisa Cuccione[1], Ruiyao Cai[1], Laura Monza[1], Silvia Pirovano[1], Giada Padovano[1], Fabio Stiro[1], Luca Presotto[4,5], Giovanni Paternò[1], Emanuela Rossi[6], Carlo Giussani[1,2,3], Erik P Sganzerla[1,2,3] and Carlo Ferrarese[1,2,3]

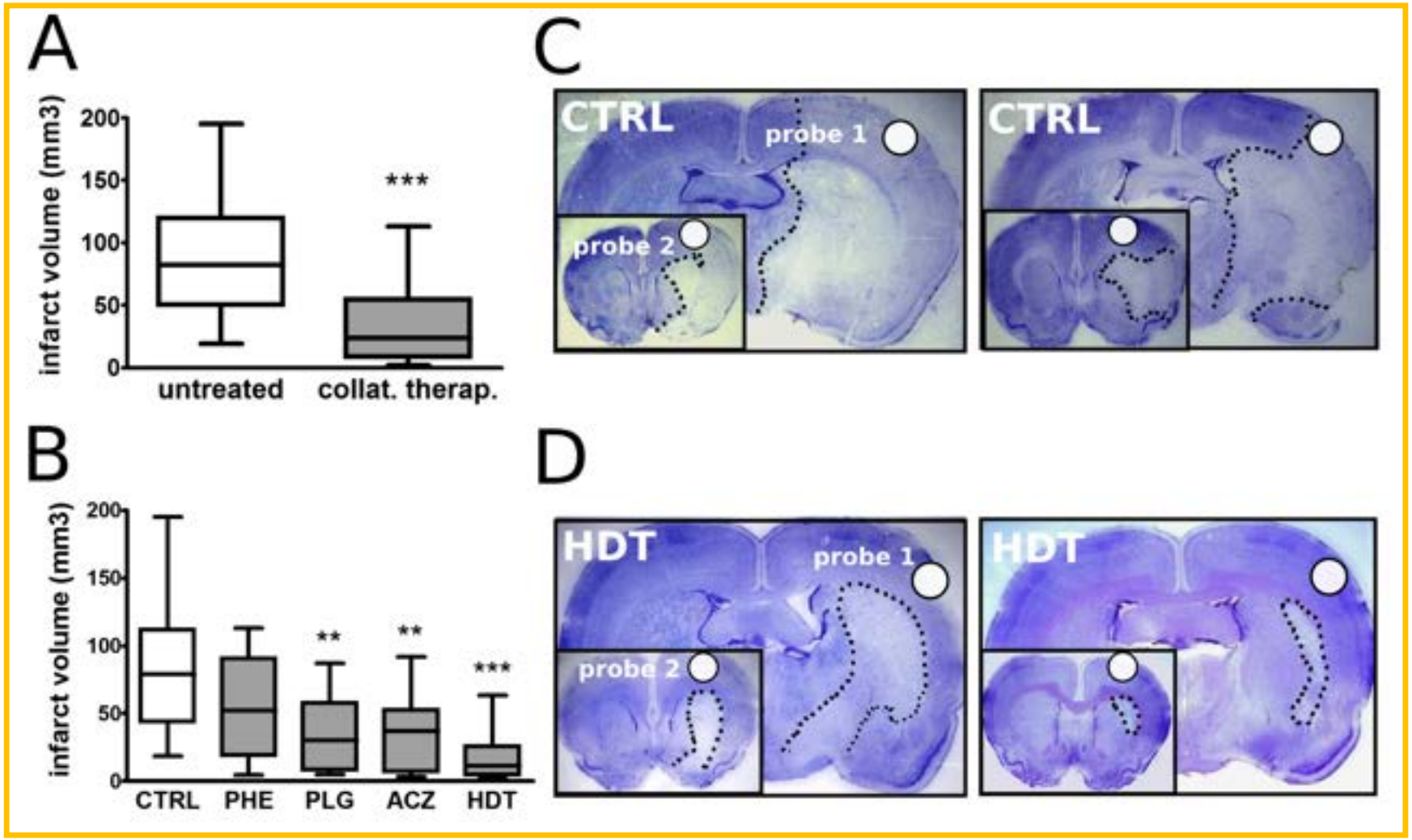
TIMING OF TREATMENT APPLICATION







HDT15 versus other COLLATERALS THERAPEUTICS



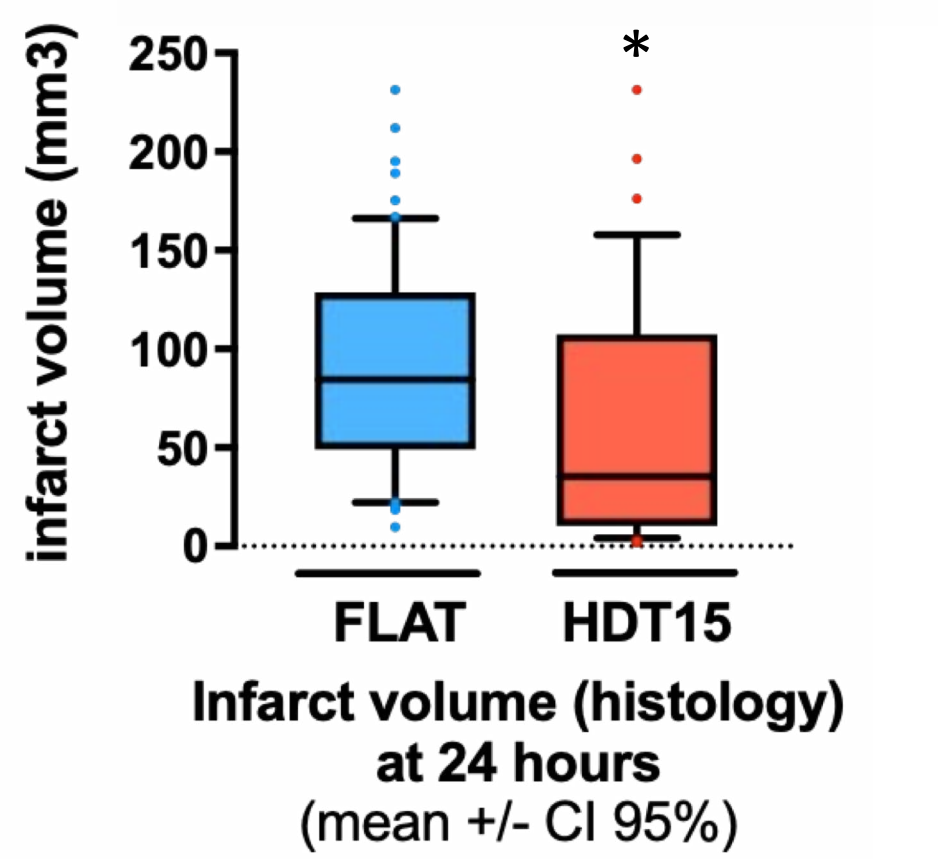
Beretta et al., *Journal of Cerebral Blood Flow & Metabolism* 2017

Pooled analysis of stroke outcome

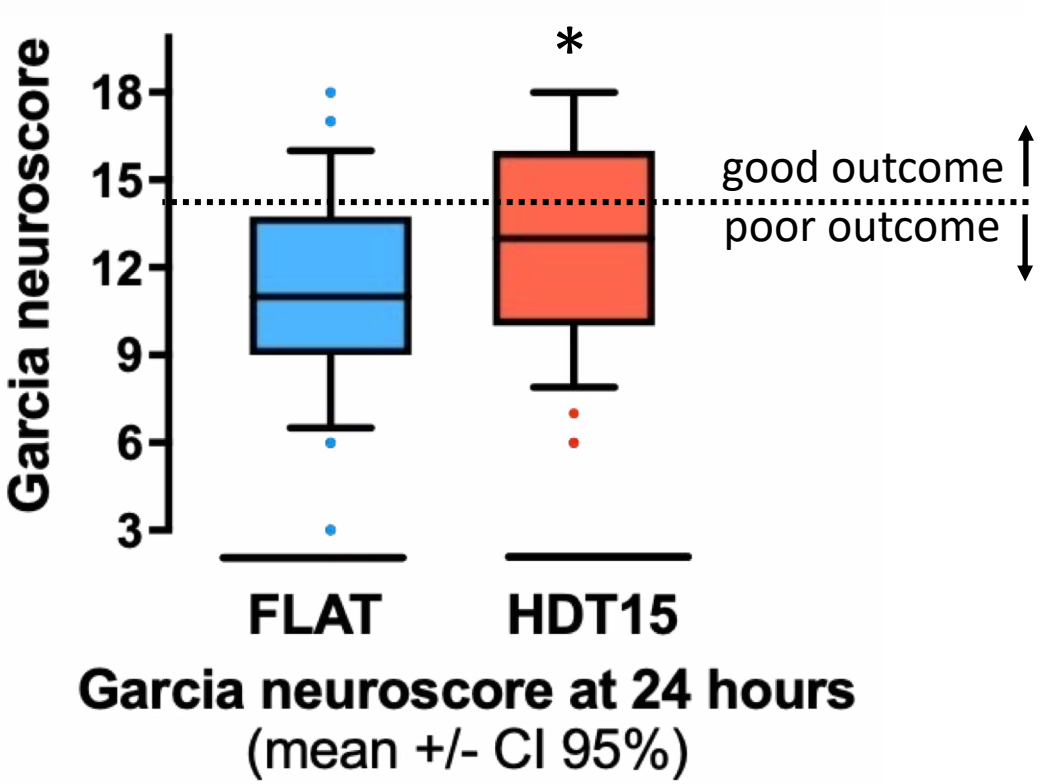
104 randomized rats (from 3 studies)

Diamanti et al., Eur J Neurosci 2022

34% reduction in infarct volume, p = 0.006

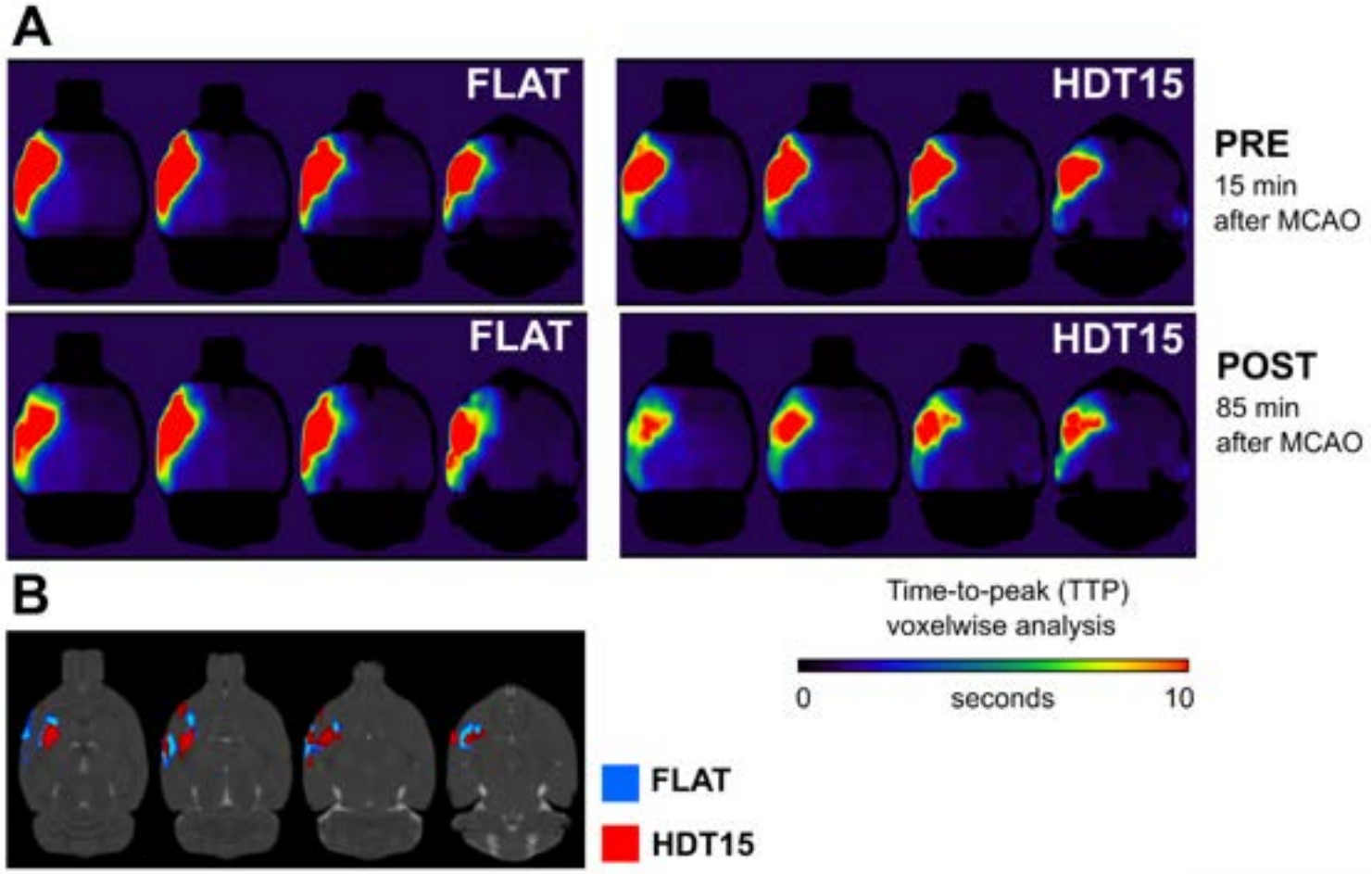


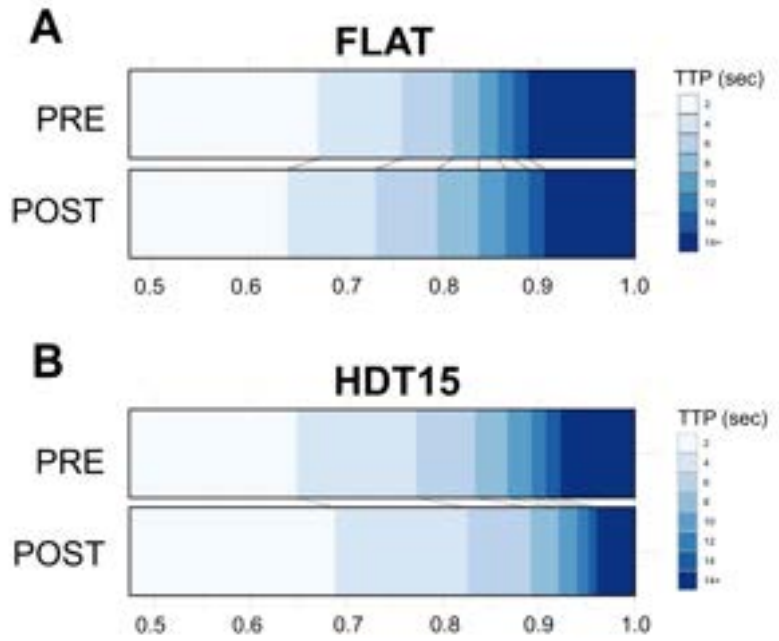
higher chance of good outcome OR 2.64, 96% CI 1.12-6.20, p = 0.015



Brain regional reduction of TTP after HDT15

voxelwise analysis and perfusion shift
POST versus PRE treatment (60 min)



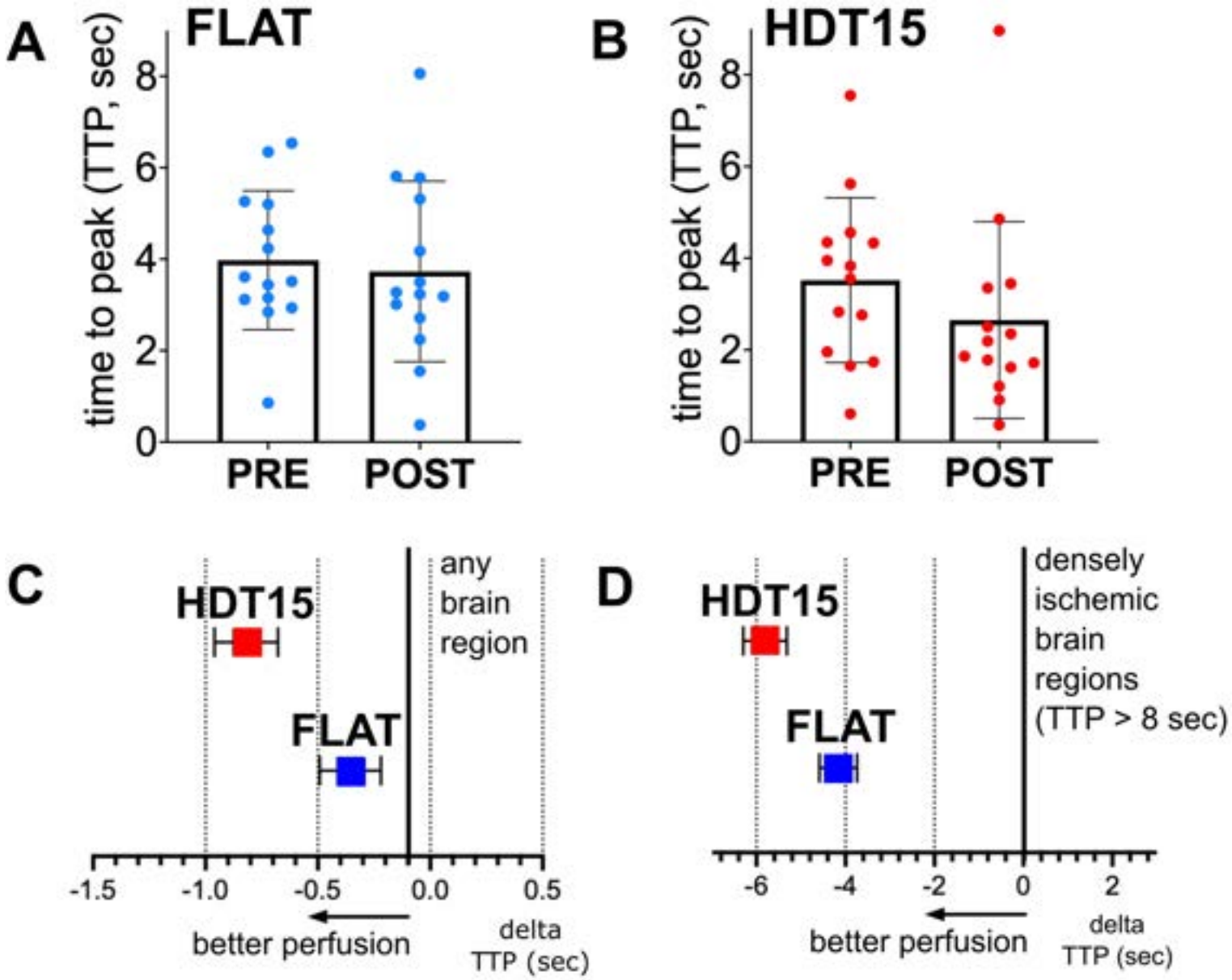


HDT15 versus FLAT
relative difference 56.8%; p < 0.0001

higher chance of better perfusion
OR 1.50, 96% CI 1.41-1.60, p < 0.0001

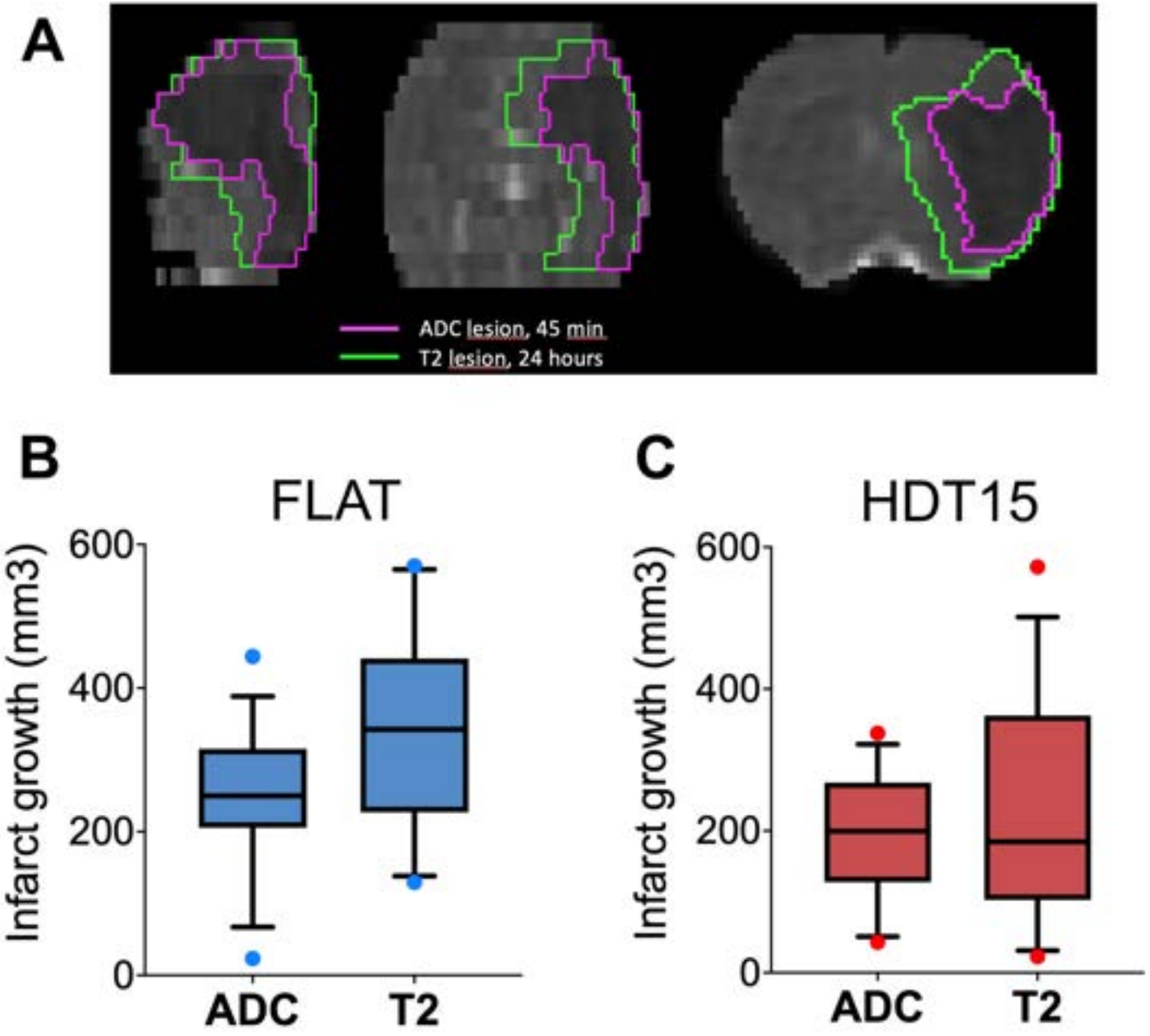
approximately 20%

absolute gain
in cerebral perfusion



Infarct growth

over the first 24 hours



DOWN-PRIME study

8 primates (macaques)

endovascular MCA occlusion/reperfusion

single arm, proof-of-concept, phase 1 trial

perfusion MRI (DSC)

perfusion/metabolism PET (^{15}O-water)

AAPG2021	CMRO2		PRCE
Coordinated by:	Tae-Hee CHO	Duration 48 months	ANR Requested Funding 950 k€
Scientific evaluation committee CE17 Axis 3.9 translational health research			

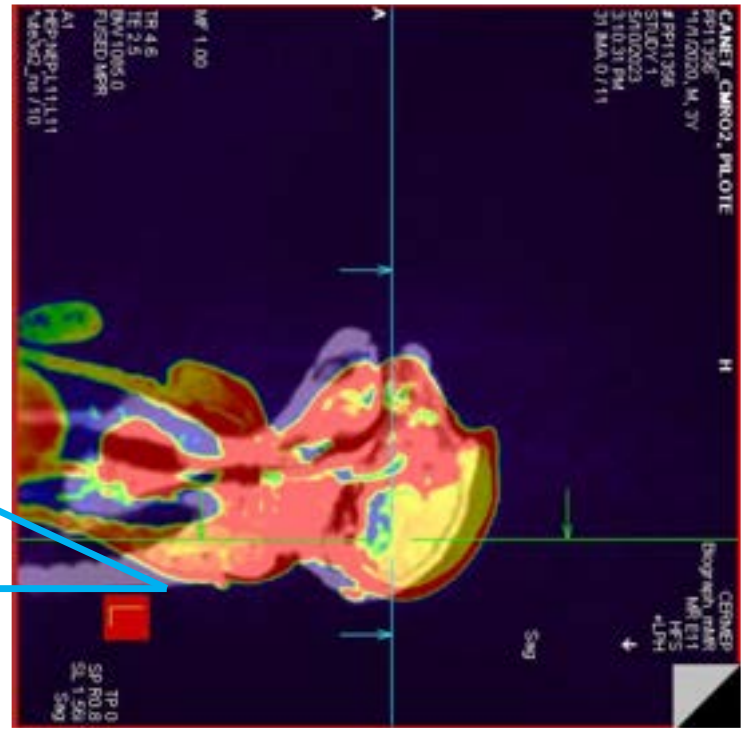
Oxygen metabolism by MRI in clinical stroke: Innovative biomarker in cerebrovascular diseases

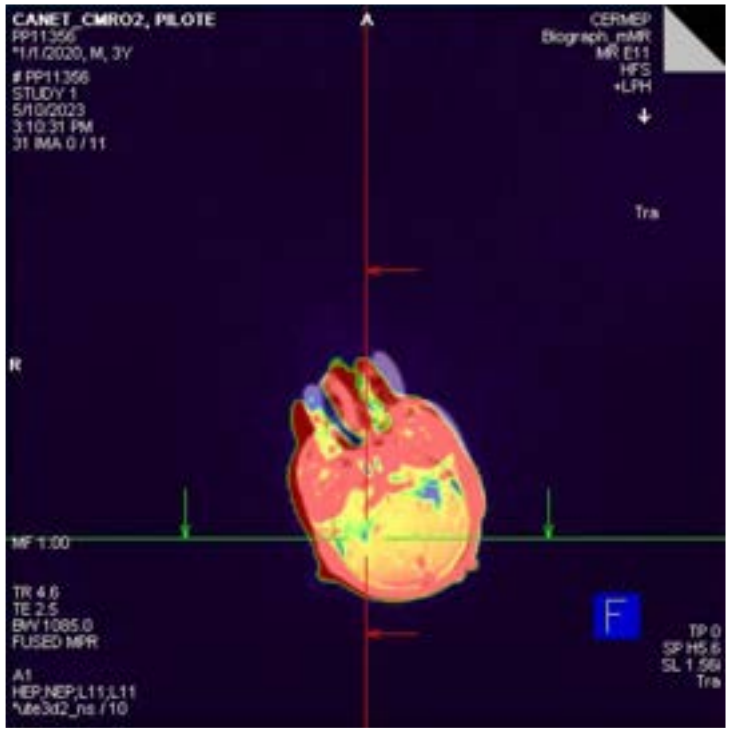
Substudy of CMRO2 project (embedded)

Lyon-Milan collaboration

First exp May 2023

15°





The V-COOL Project

Ministero della Salute Direzione Generale della Ricerca Sanitaria e Biomedica e della Vigilanza sugli Enti BANDO 2011-2012 PROGETTI DI RICERCA PROGETTO COMPLETO	Project Title: Selective cerebrospinal fluid hypothermia: bioengineering development and in vivo study of an intraventricular cooling device (V-Cool) for acute stroke therapy
Project Code: GR-2011-02347879	Principal Investigator: BERETTA SIMONE

Simone Beretta, MD, PhD
Laboratory of Experimental Stroke Research
Department of Medicine and Surgery
University of Milano Bicocca

doi:10.1093/brain/awm083 *Brain* (2007), **130**, 3063–3074

REVIEW ARTICLE

Hypothermia in animal models of acute ischaemic stroke: a systematic review and meta-analysis

H. Bart van der Worp,[1] Emily S. Sena,[2] Geoffrey A. Donnan,[3] David W. Howells[3] and Malcolm R. Macleod[2]

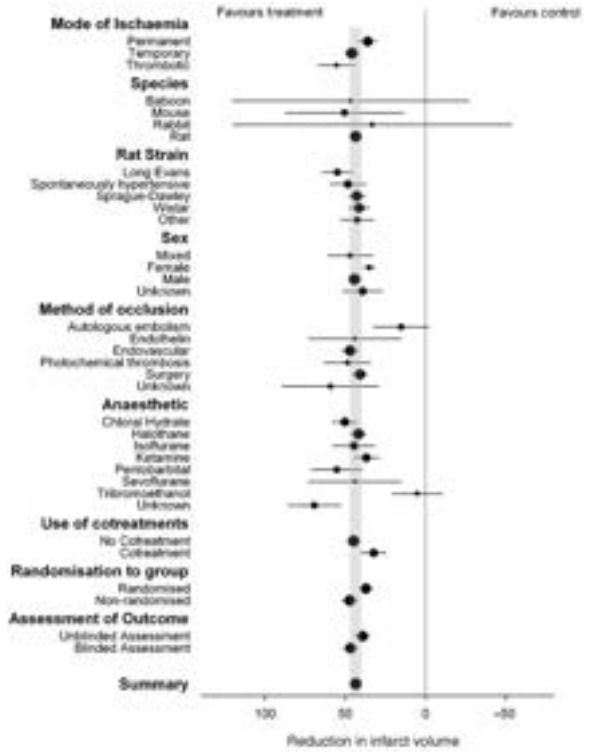


Fig. 2 Point estimate of effect on infarct size and 95% CI by mode of ischaemia, species, rat strain, sex, method of occlusion, anaesthetic, use of co-treatment, randomisation, and assessment of outcome. The grey band indicates the global estimate and its 95% CI.

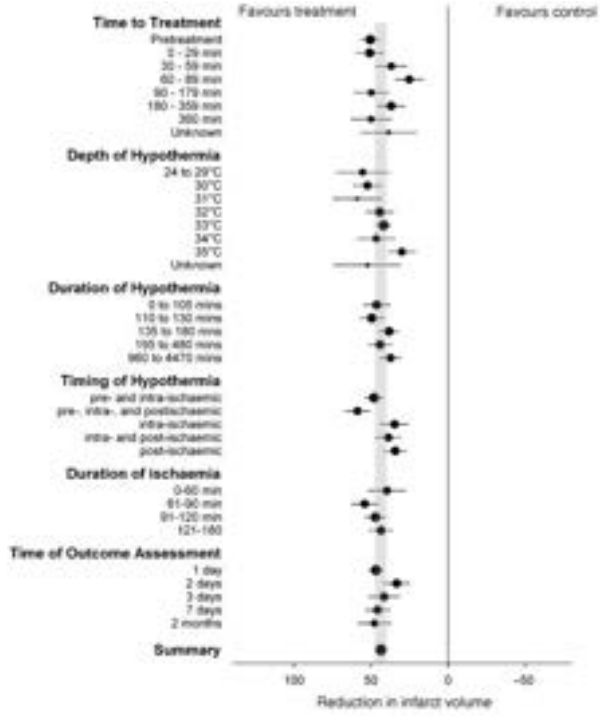


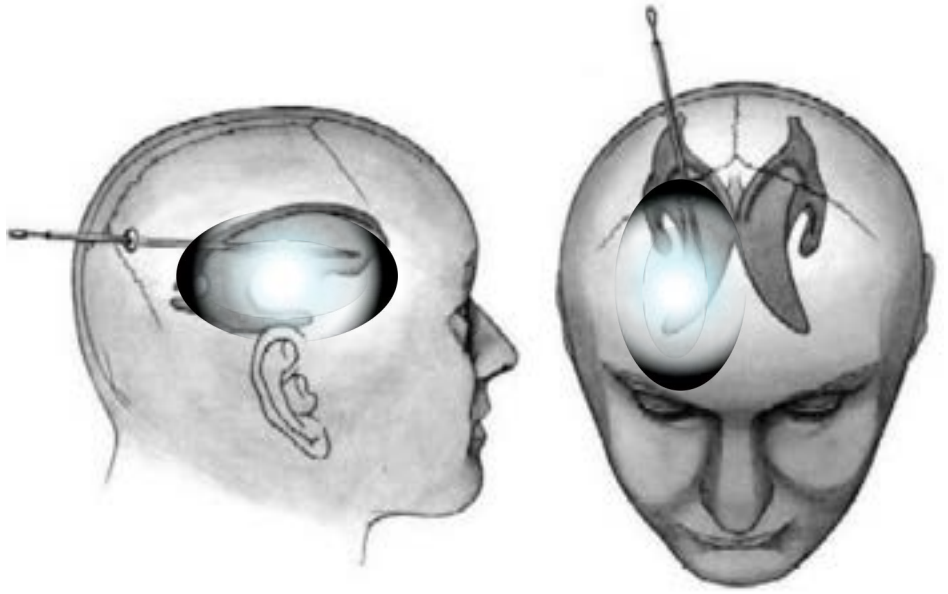
Fig. 4 Point estimate of effect on infarct size and 95% CI by duration of ischaemia in models of reperfusion, time to treatment, depth of hypothermia, duration of hypothermia, timing of hypothermia and time of outcome assessment. The grey band indicates the global estimate and its 95% CI.

Selective CSF hypothermia is a new unexplored concept.

Advantages compared to systemic hypothermia:

- targeted hypothermia
- deeper degree of hypothermia
- longer duration of hypothermia
- reduction of systemic side effects

shivering
infections (pneumonia)
cardiac arrhythmias
coagulopathy
sedation and intubation

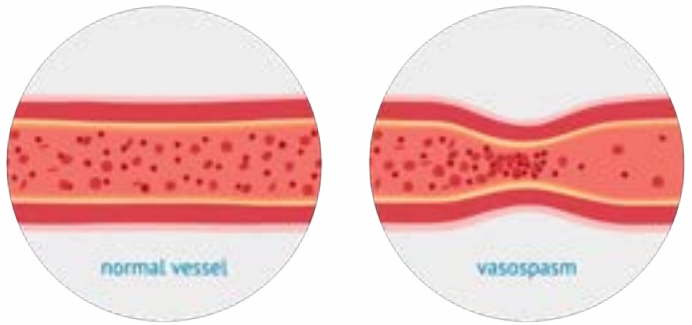


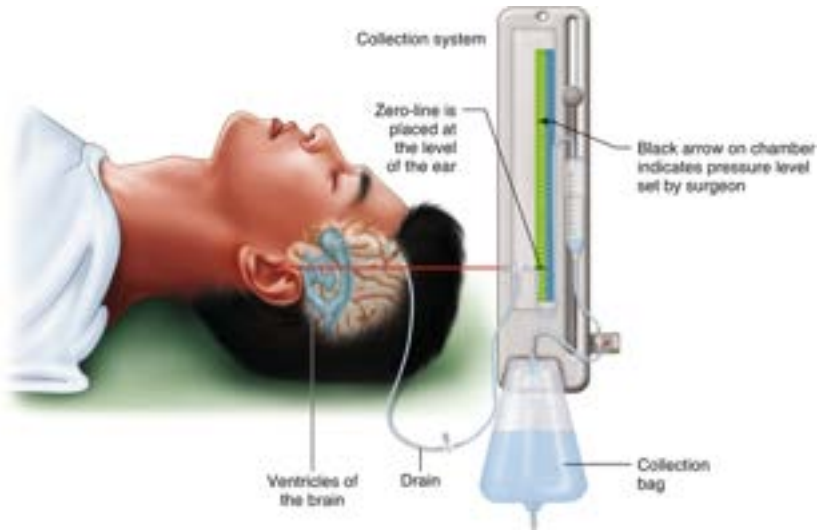
Selective CSF hypothermia is a new unexplored concept.

Clinical translation potential:

- **vasospasm after subarachnoid hemorrhage**
- acute ischemic stroke (severe)
- malignant cerebral edema
- traumatic brain injury
- super-refractory status epilepticus

Potential combination with external ventricular drains (EVD)





Neurotherapeutics
https://doi.org/10.1007/s13311-022-01302-y

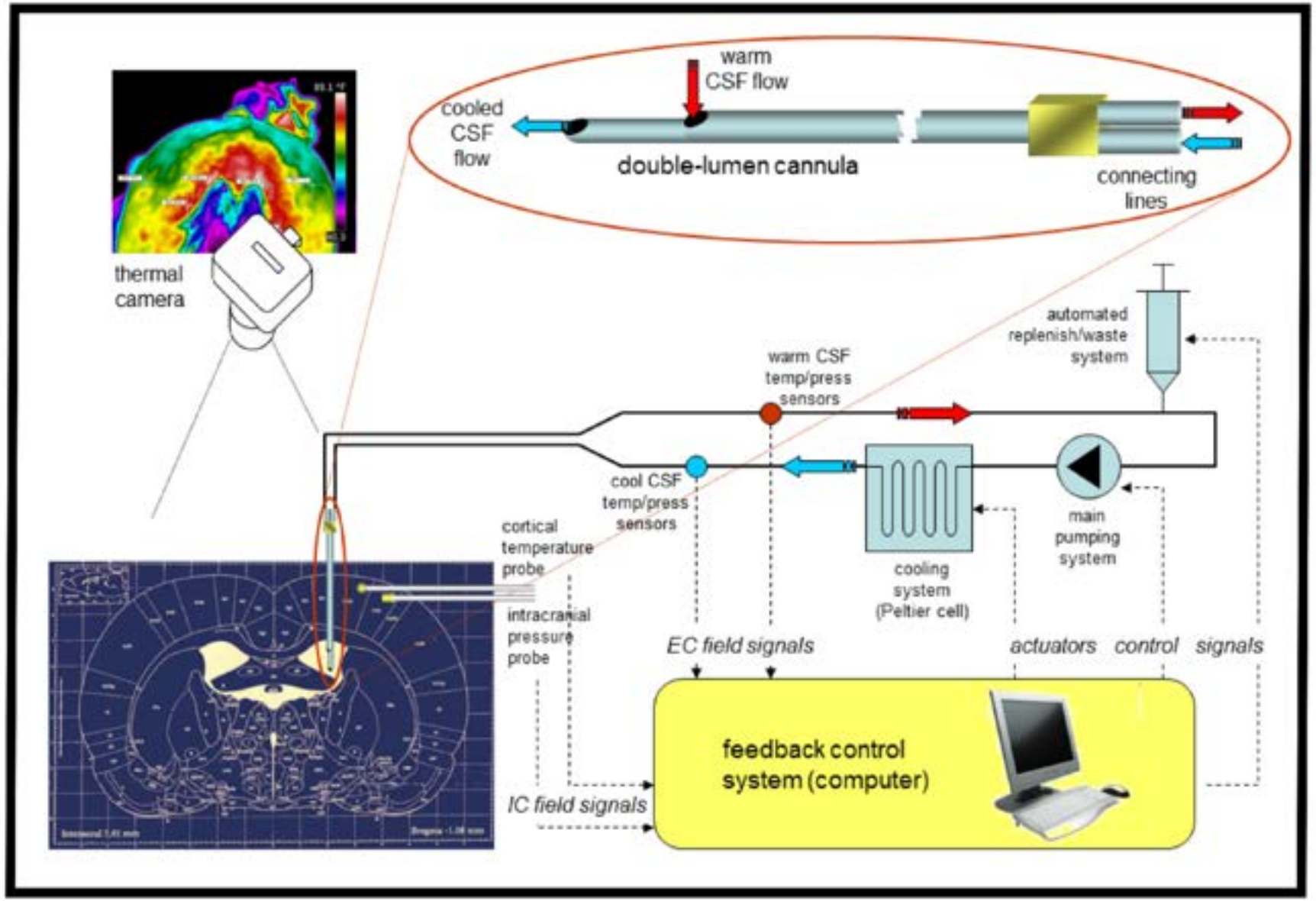
ORIGINAL ARTICLE

Selective Cerebrospinal Fluid Hypothermia: Bioengineering Development and In Vivo Study of an Intraventricular Cooling Device (V-COOL)

Simone Beretta[1,2] · Alessandro Versace[1] · Gianfranco Fiore[3] · Marco Piola[3] · Beatrice Martini[1] · Vittorio Bigiogera[1] · Lorenzo Coppadoro[2] · Jacopo Mariani[1] · Lorenzo Tinti[1] · Silvia Pirovano[1] · Laura Monza[1] · Davide Carone[1] · Matteo Riva[1] · Giada Padovano[1] · Gilda Galbiati[1] · Francesco Santangelo[1] · Marco Rasponi[3] · Francesco Padelli[5] · Isabella Giachetti[5] · Domenico Aquino[5] · Susanna Diamanti[1,2] · Laura Librizzi[4] · Maria Grazia Bruzzone[5] · Marco De Curtis[4] · Carlo Giussani[1,2] · Erik P. Sganzerla[1,2] · Carlo Ferrarese[1,2]

Accepted: 11 September 2022

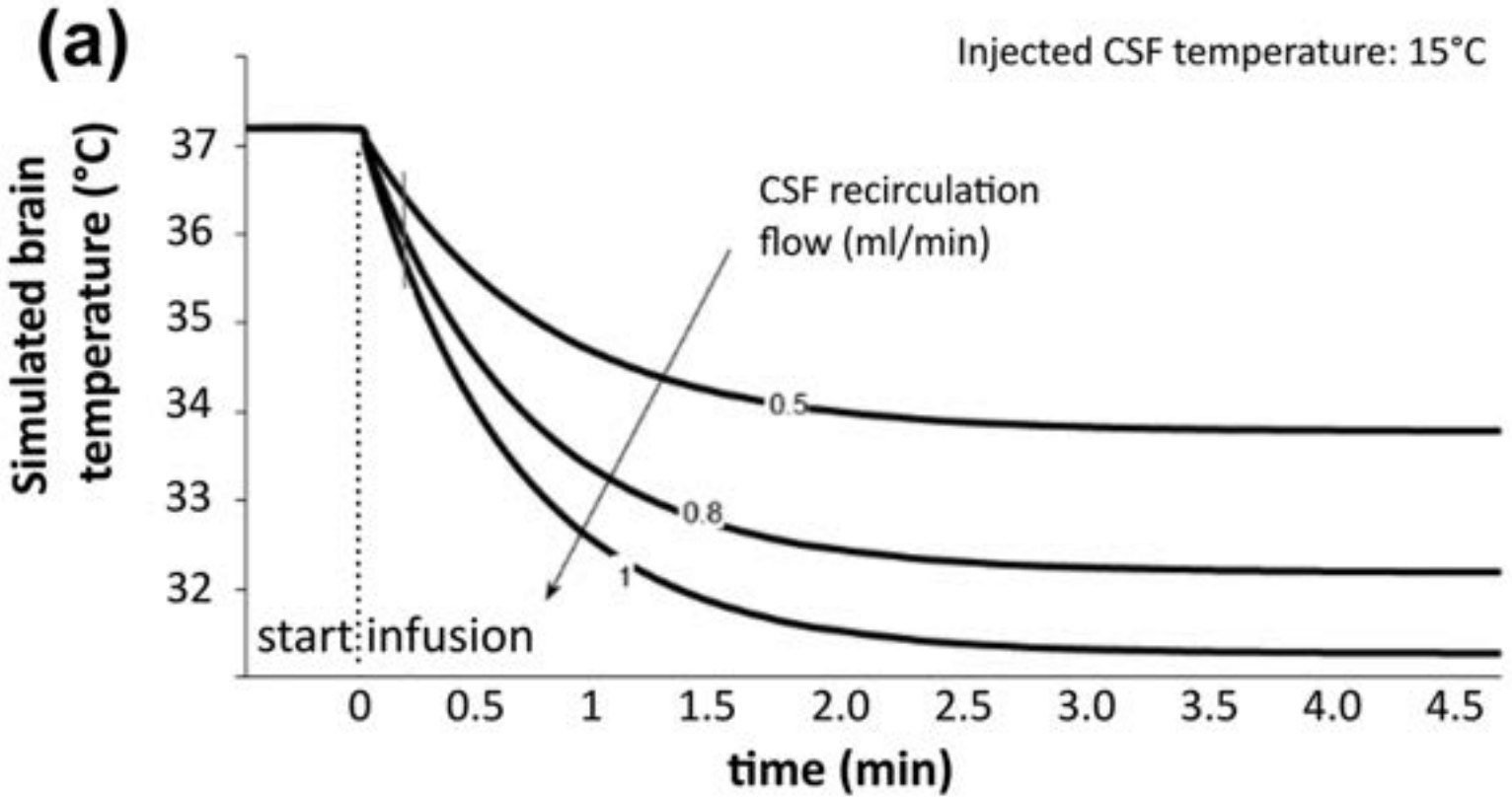
Concept design of the V-COOL device



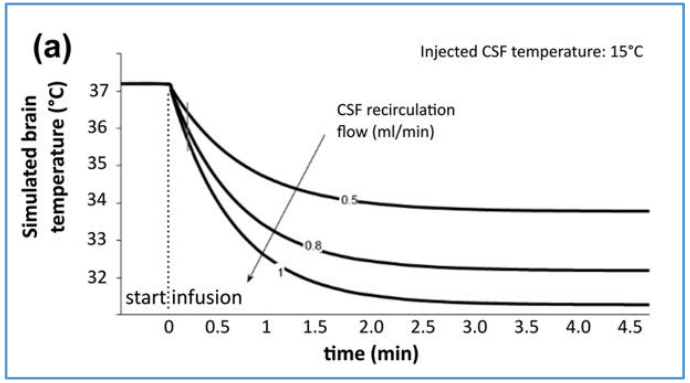
The **cooling mechanism** of the V-COOL device is based on **exchange of cool-versus-warm CSF**, similarly to hemodialysis or plasma exchange

In silico model of the V-COOL device

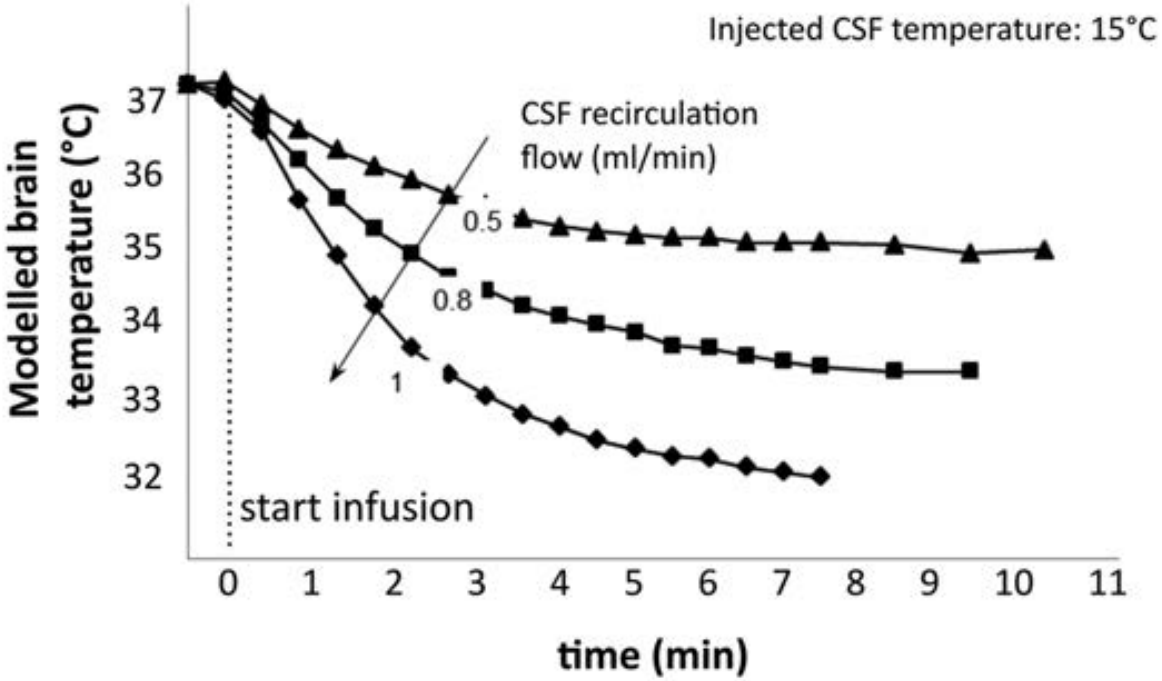
mono-compartmental



In vitro model of the V-COOL device



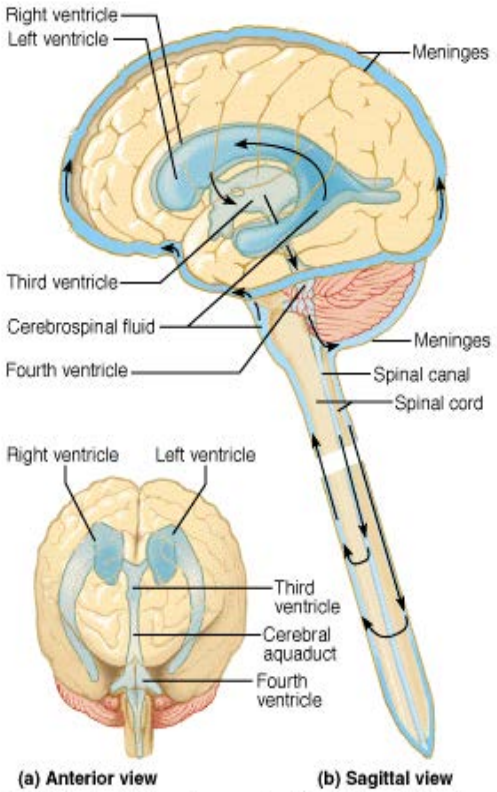
(b)



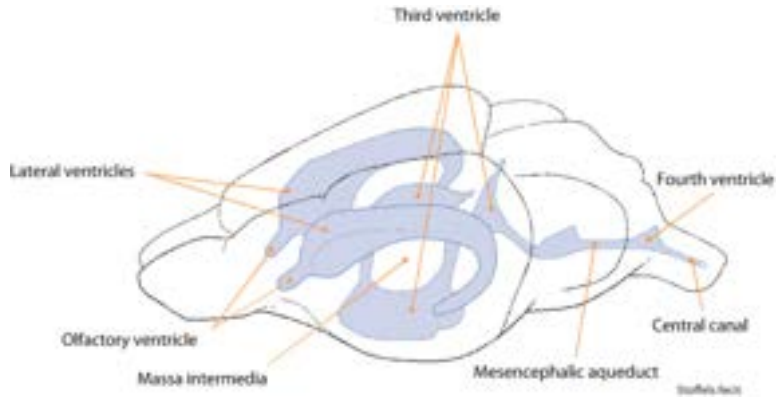
In vivo prototyping of the V-COOL device

CSF access

HUMAN

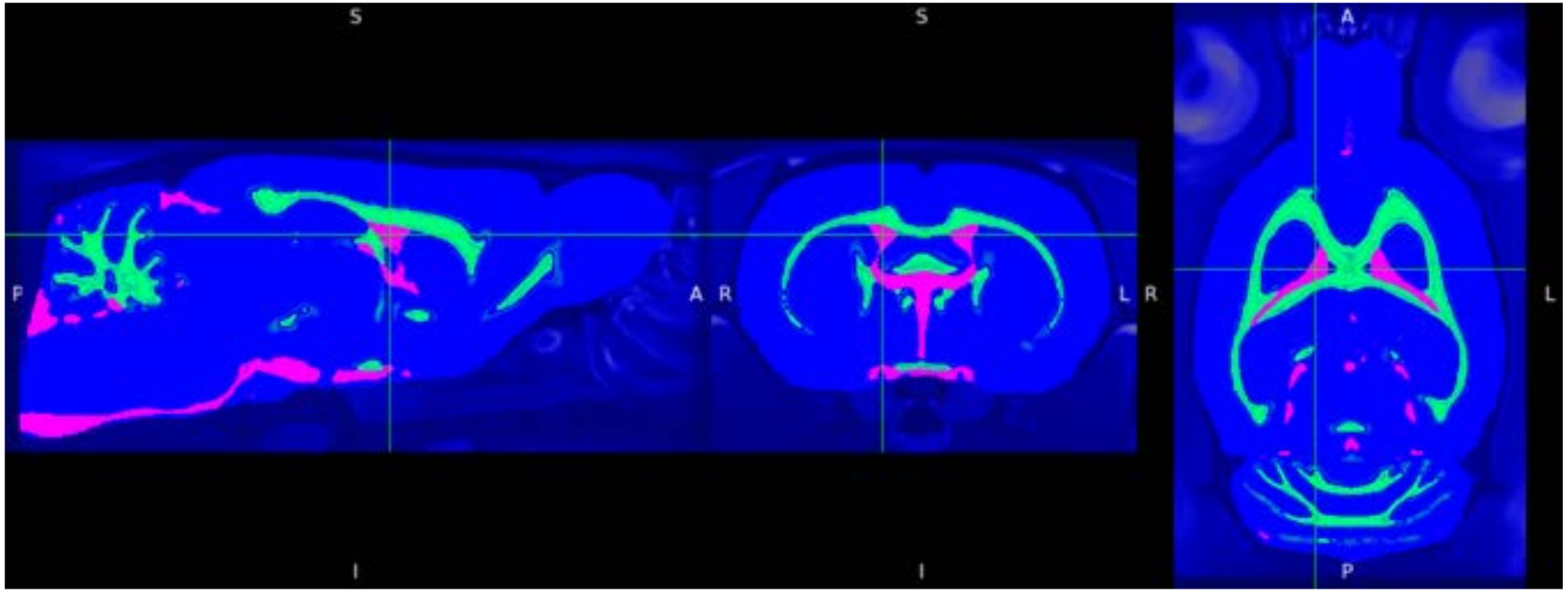


RAT

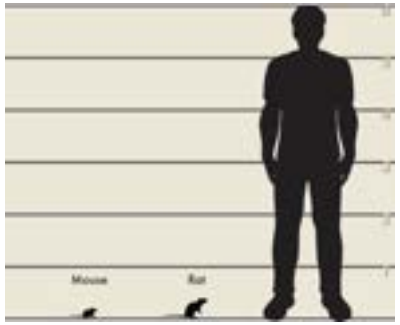


In vivo prototyping of the V-COOL device

CSF access

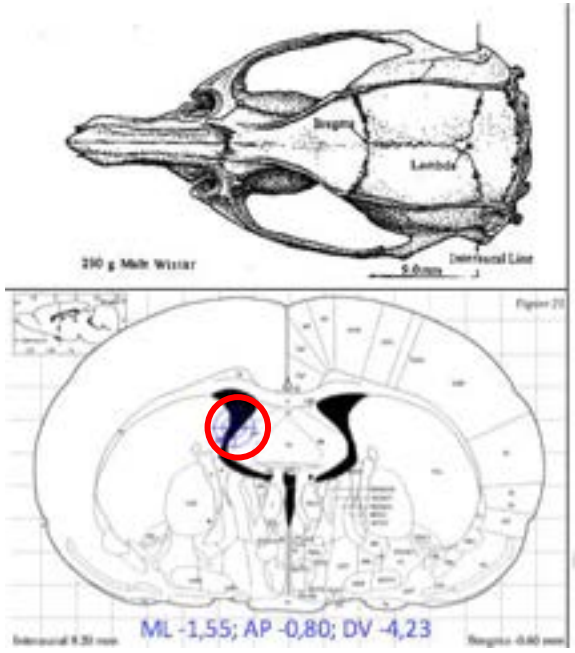


…size always matters!



In vivo prototyping of the V-COOL device

CSF access



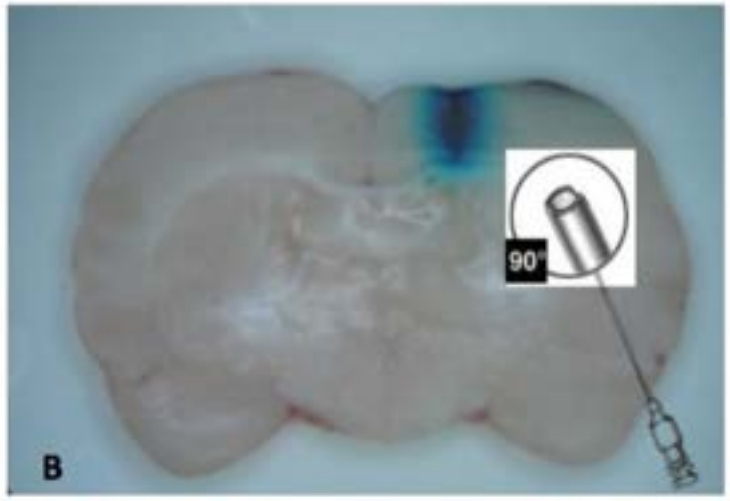
c

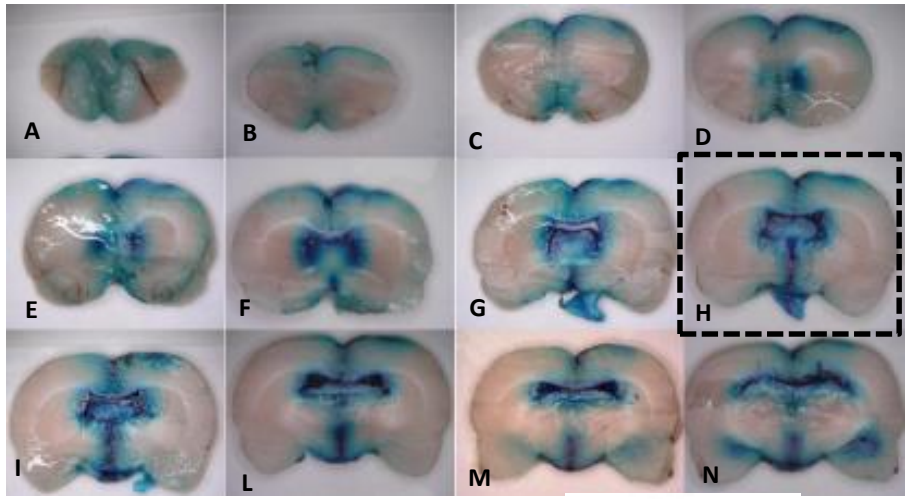
Target for **V-COOL device**

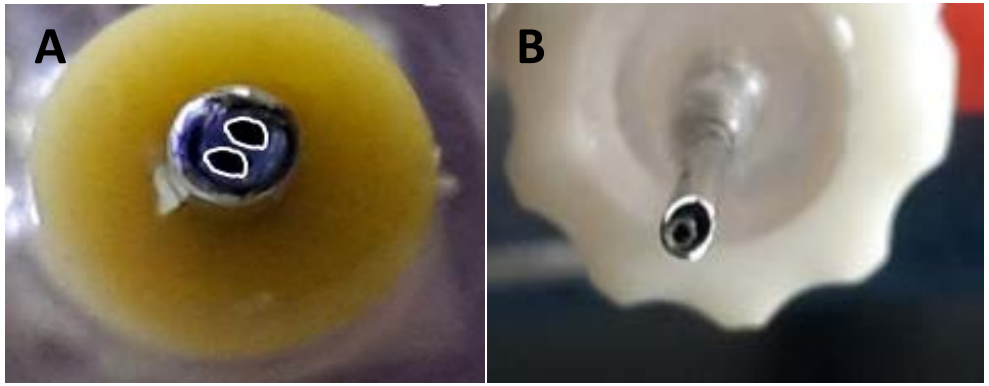
Target for **temperature (and pressure) probes**

In vivo prototyping of the V-COOL device

CSF access



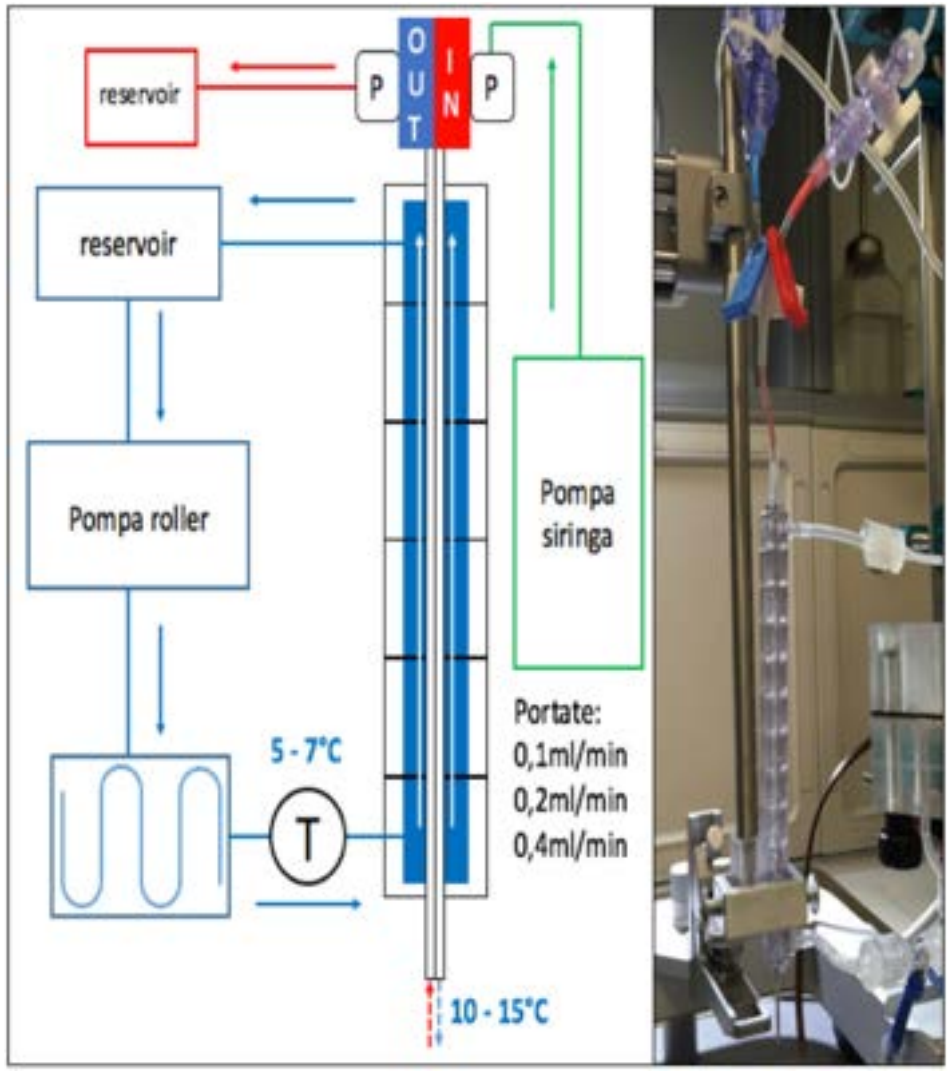




V-COOL 2.0 V-COOL 3.0

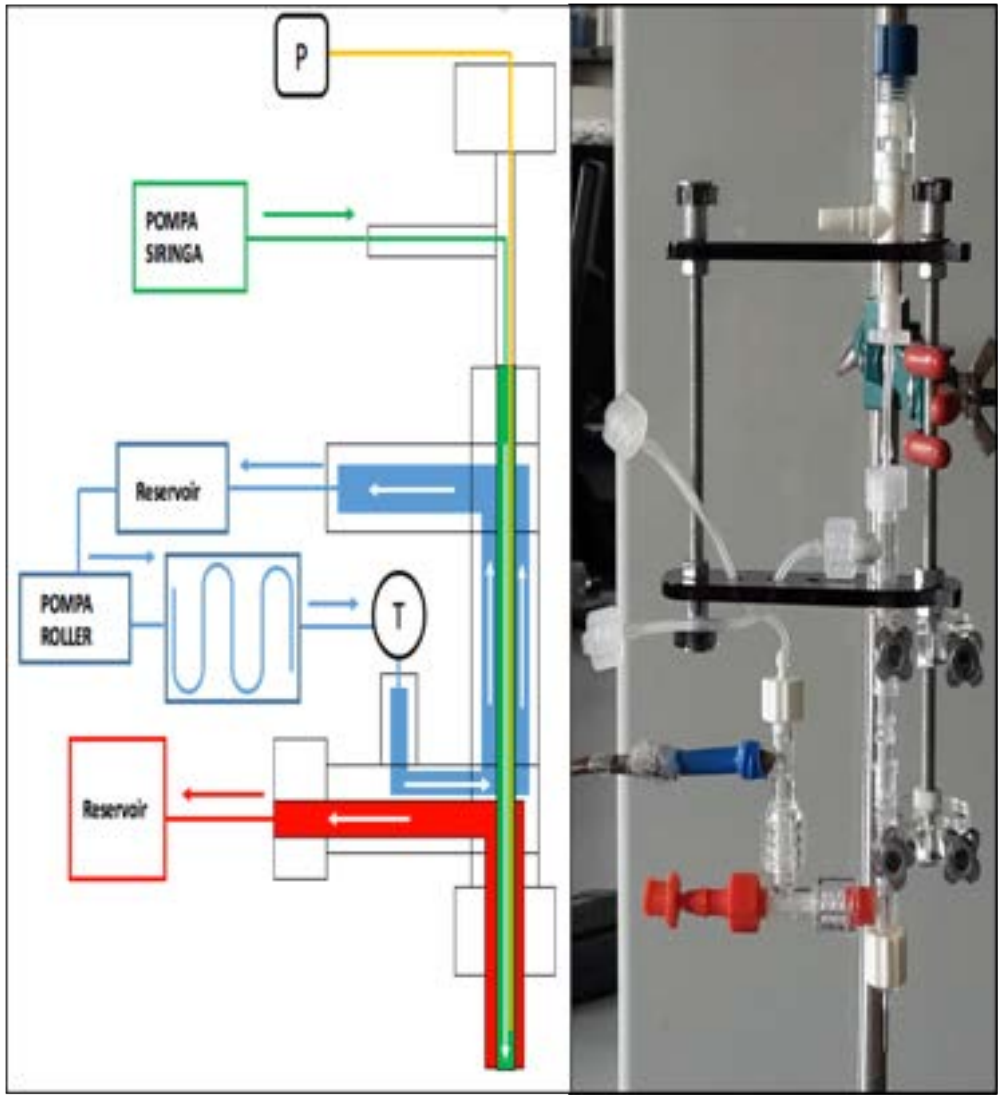
…tip always matters!

V-COOL 2.0 prototype



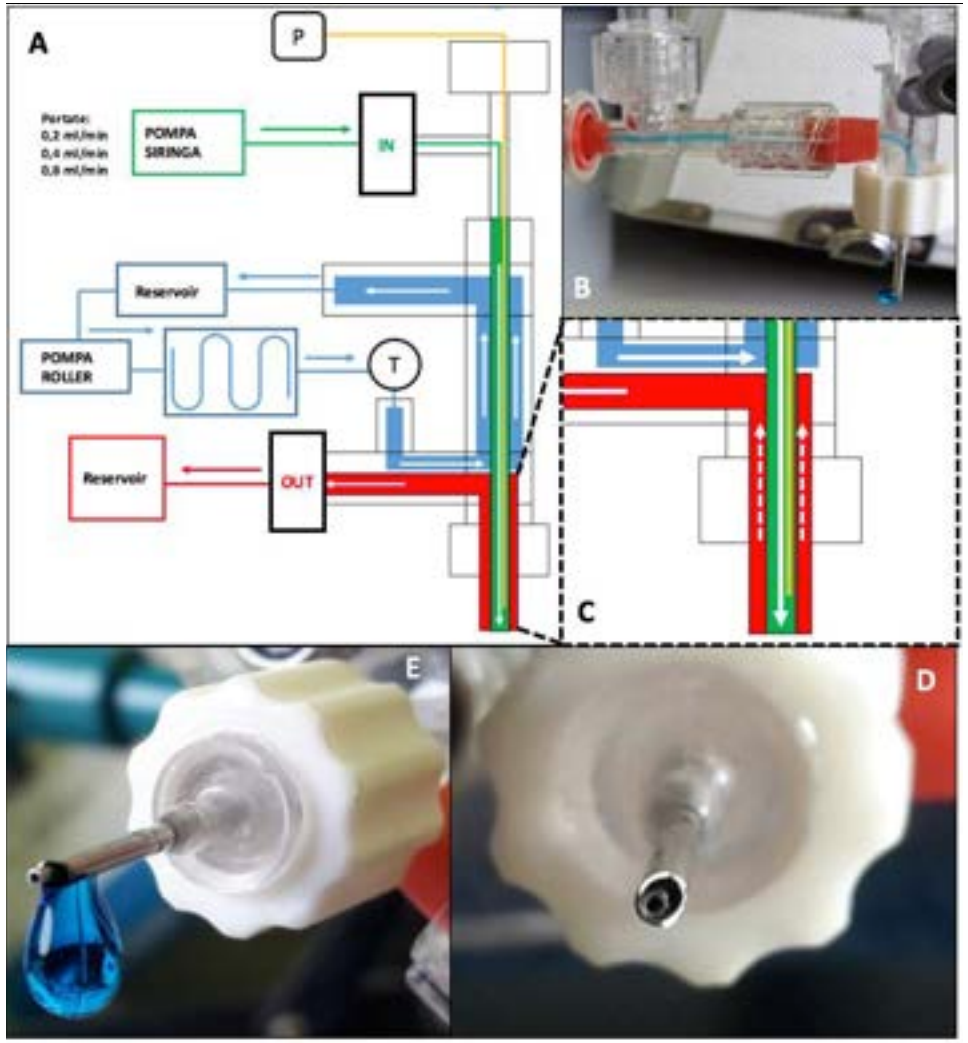
- Double parallel lumen, one for infusion (23GA) and one for drainage (22GA)
- Exoskeleton with internal cold water recirculation (5-7°C), cooled by an ice coil
- Peristaltic pump for infusion at 0.1 ml/min, 0.2 ml/min and 0.4 ml/min flow rates
- Double pressure transducer placed upstream of the infusion lumen and downstream of the drainage lumen
- Thermocouple for cooling circuit control

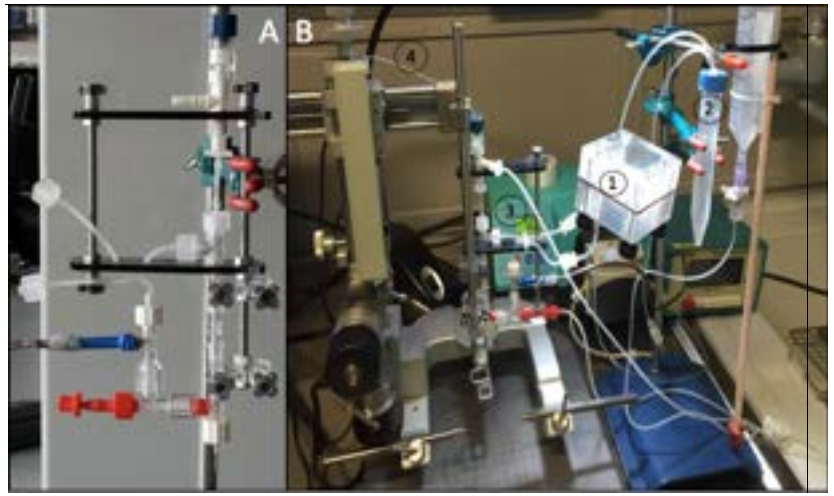
V-COOL 3.0 prototype

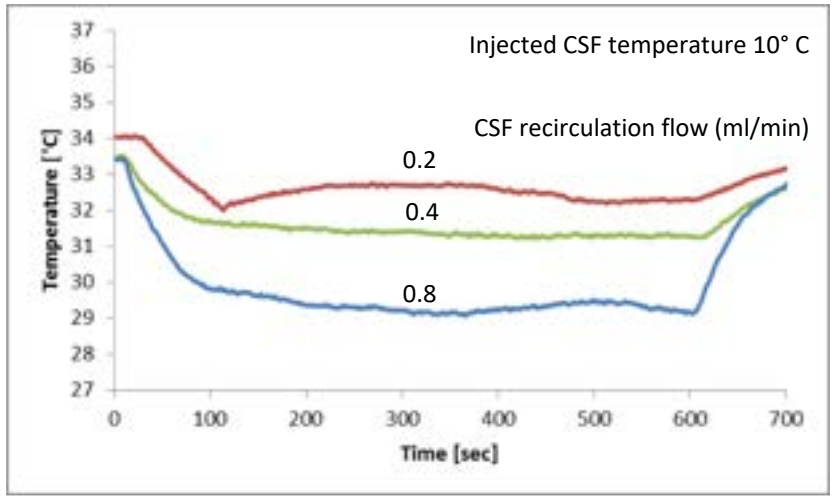


- Double concentric lumen, with larger and more efficient drain lumen (19GA) and 23.5GA infusion lumen
- Exoskeleton with internal cold water recirculation (5-7°C), cooled by Peltier module
- Syringe pump for infusion at flow rates of 0.2 ml/min, 0.4 ml/min and 0.8 ml/min
- Pressure transducer placed inside the drainage lumen
- Early detachment of the two lumens, to avoid heat exchange
- Thermocouple for cooling circuit control

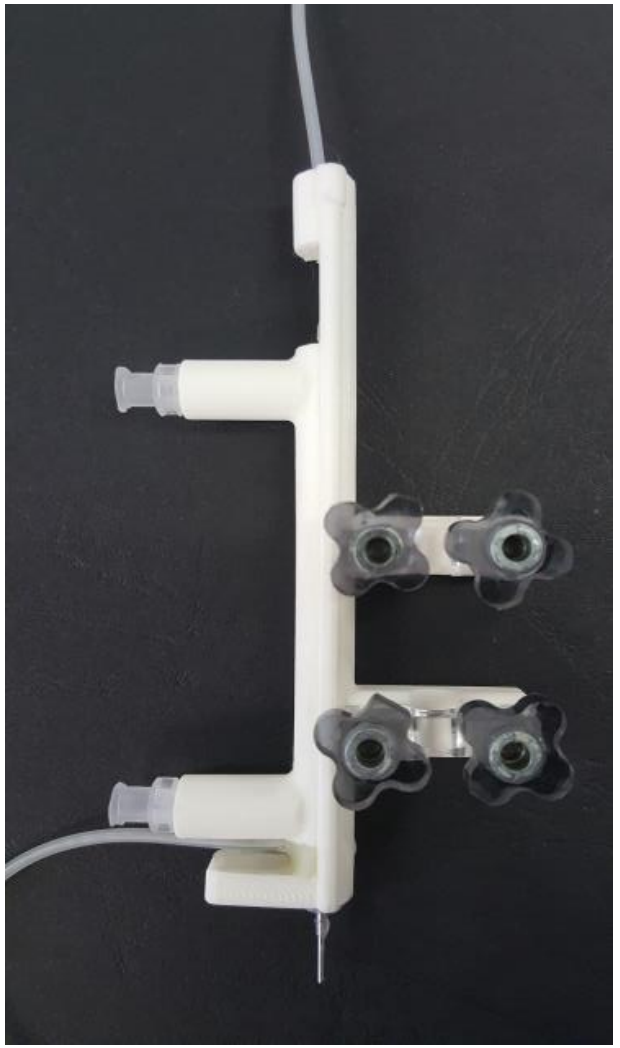
V-COOL 3.0 prototype







V-COOL 3.0 prototype



In vivo effect on brain and systemic temperature
of the V-COOL device

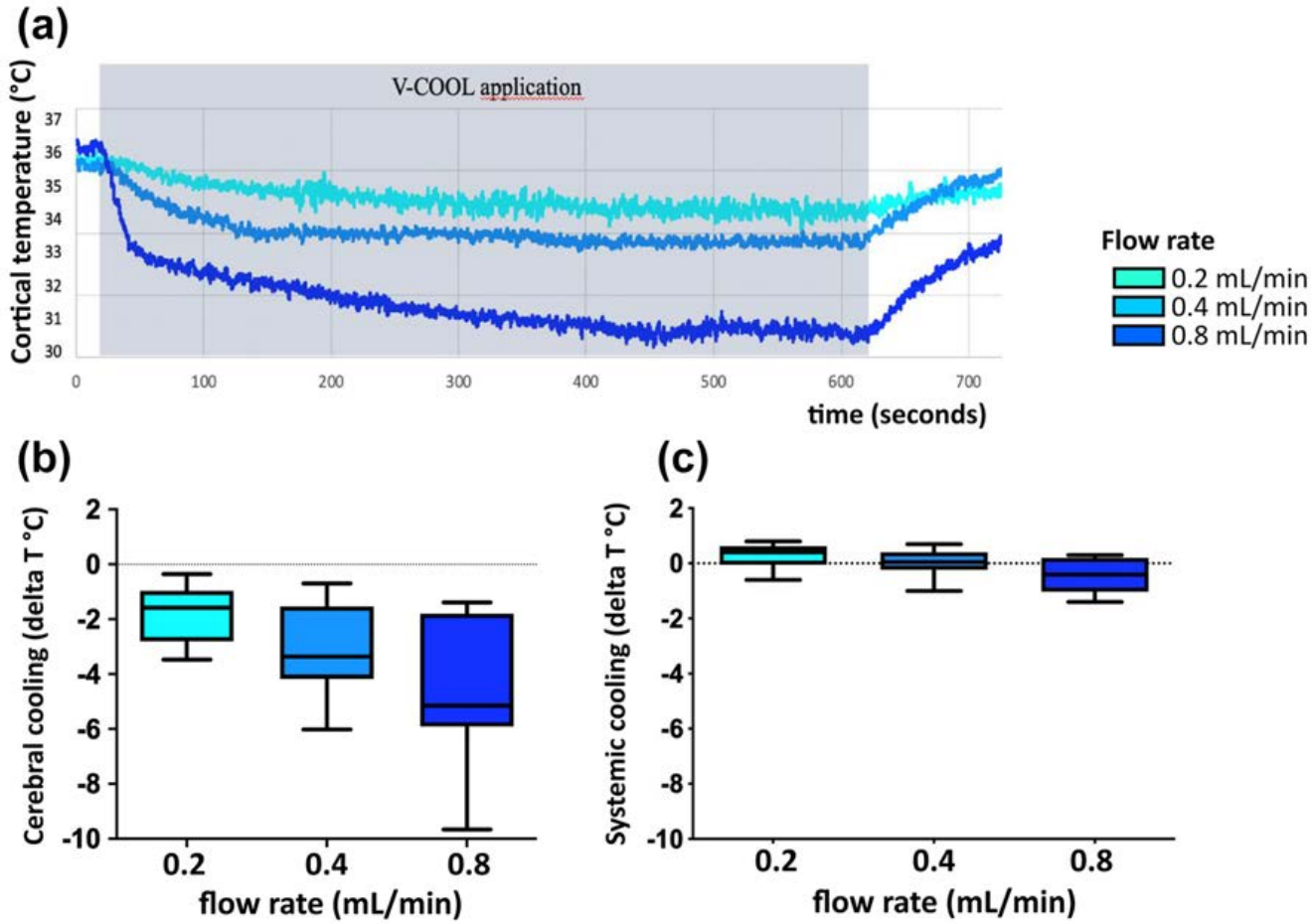


Fig. 3 Representative tracings showing the dynamics of cerebral cortical temperature in a rat during application of the V-COOL device (**a**). Mean cerebral cortical cooling ($n = 42$) during V-COOL application at increasing inflow rates (**b**). Mean systemic cooling (rectal temperature, $n = 42$) during V-COOL application at increasing inflow rates (**c**)

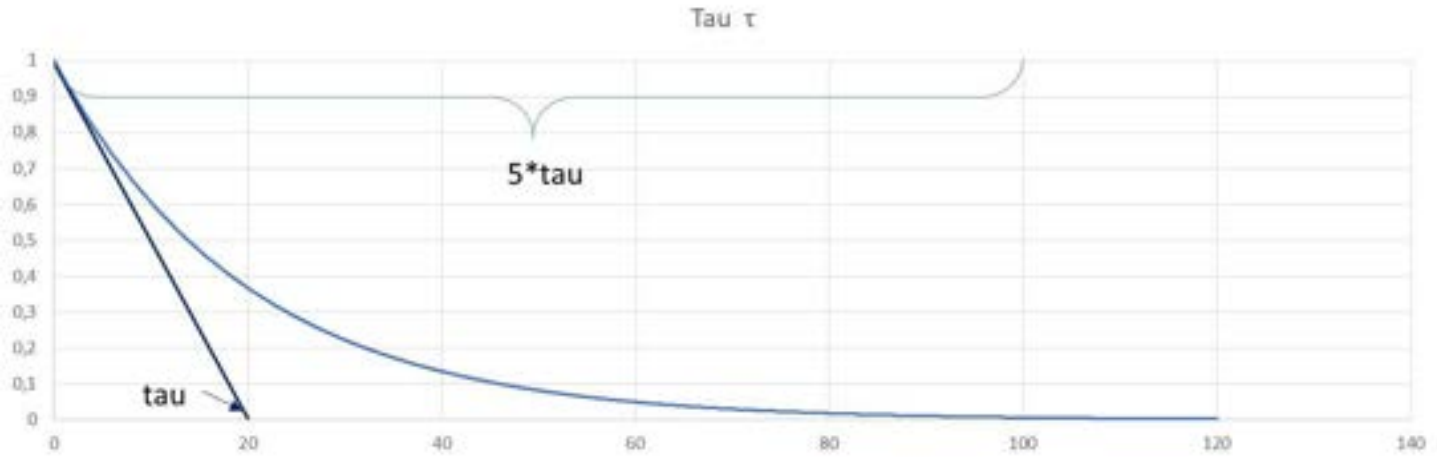


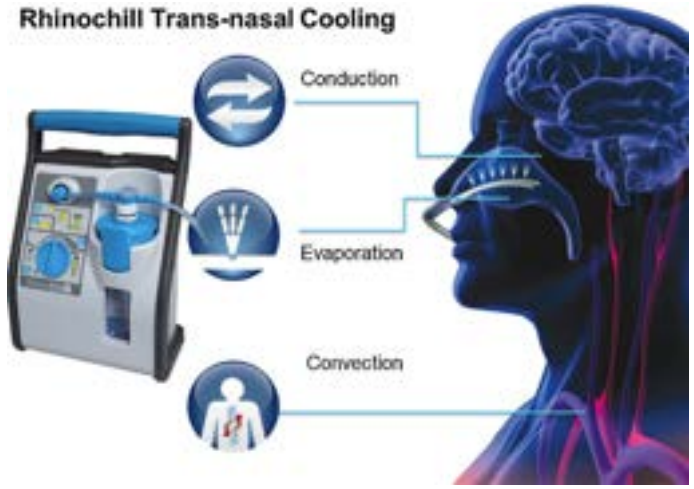
***Figura 56**.Esempio di curva iperbolica con rappresentazione di τ e 5τ.*

time to steady state 4.8 min

time to target temperature (5T; 5 times the time constant)

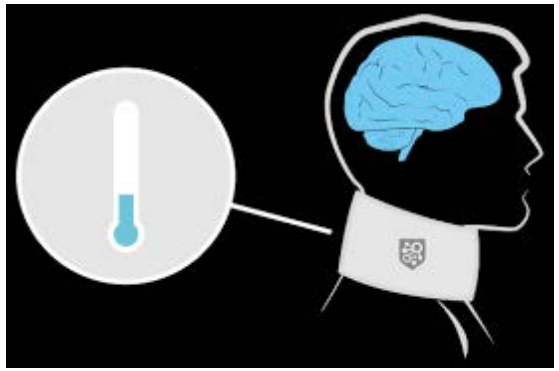
(0.4 mL/min)

Net cooling effect



Rhinochill
− 1.7°C in 60 min
target tympanic temperature of 34°C in 1.3 hours
(coupled with surface cooling)

Cooling helmet
− 1.8°C in 60 min
target cerebral temperature of 34°C in 3.4 hours



Neuron Guard

Neuron Guard
− 0.6°C in 60 min
Steady stated -1.2°C at 2 hours

In vivo effect on intracranial pressure
of the V-COOL device

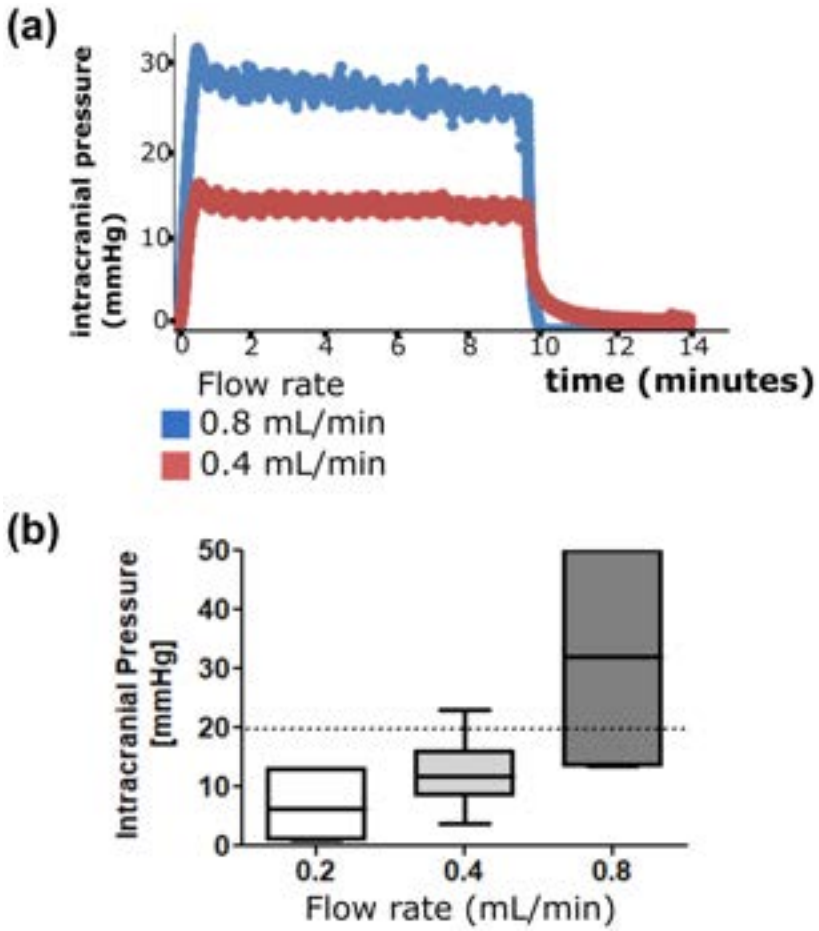
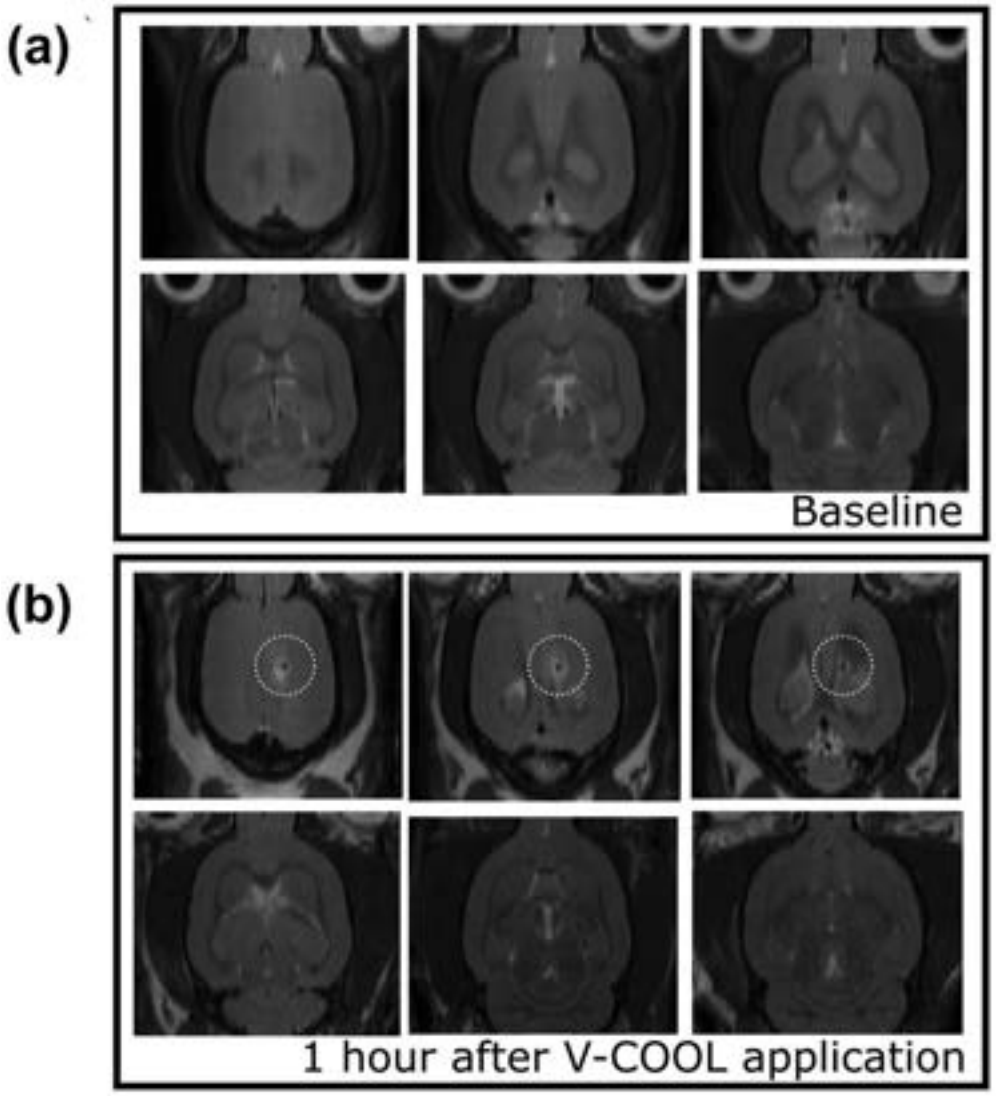


Fig. 4 Representative tracings showing intracranial pressure changes in a rat during application of the V-COOL device (**a**). Mean intracranial pressure ($n = 15$) during V-COOL application at increasing inflow rates (**b**)

In vivo effect on ventricular volume
of the V-COOL device



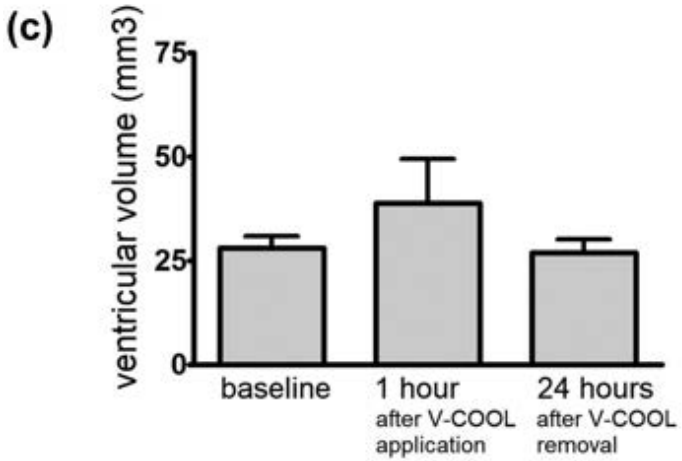


Fig. 5 Representative brain MRI images (axial view) of a rat before (**a**) and after 60-min application of V-COOL (**b**). The site of V-COOL access is highlighted (dotted circles). Mean ventricular volume ($n=5$) was calculated before, immediately after 60-min application of V-COOL, and 24 h after V-COOL removal (**c**)

Remote ischemic conditioning in ischemic stroke

Simone Beretta, MD, PhD
Department of Neurology and Stroke Unit
Fondazione IRCCS San Gerardo dei Tintori Monza
University of Milano Bicocca, ITALY

UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA

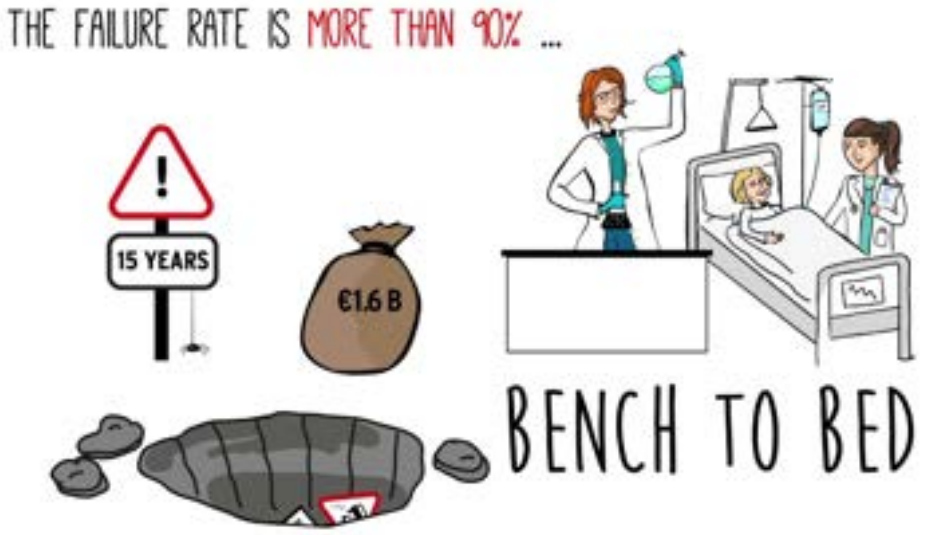
Sistema Socio Sanitario

Fondazione IRCCS San Gerardo dei Tintori

Quocumque modo faciemus

Regione Lombardia

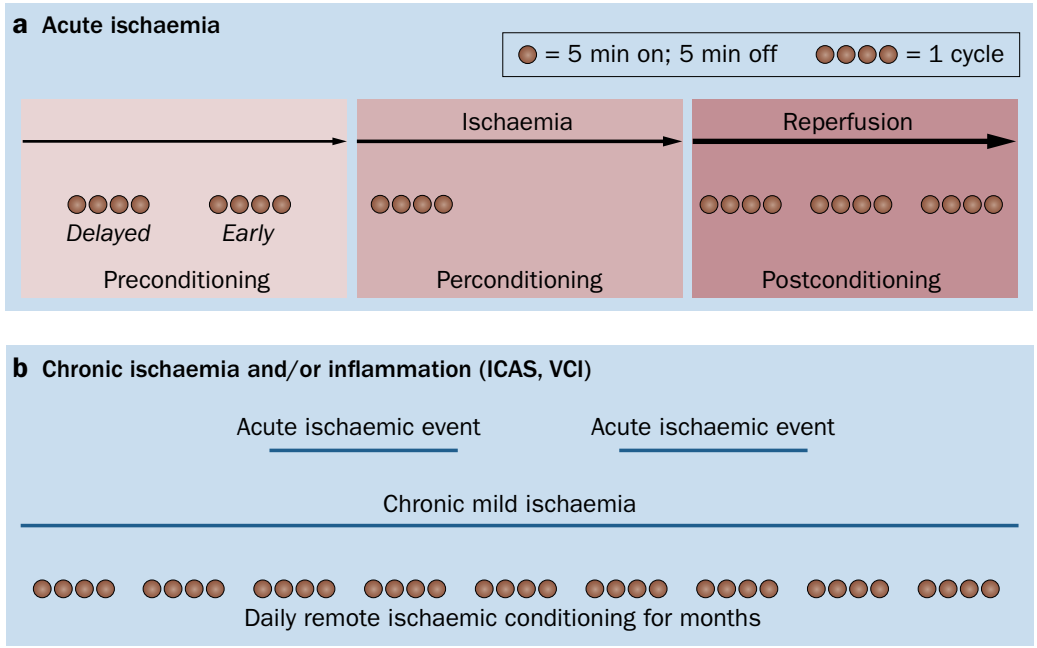
A translational story…

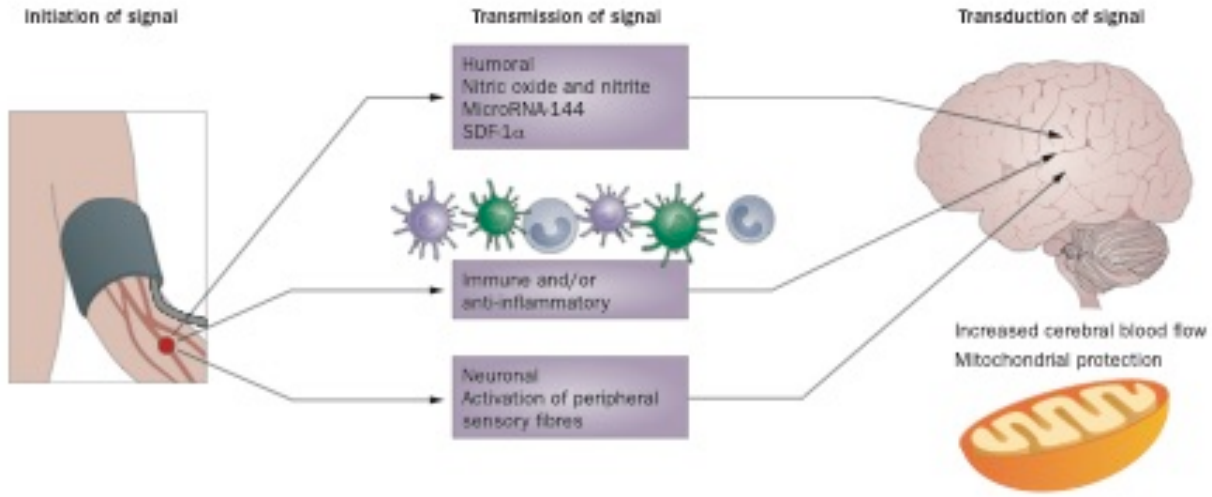


Remote ischaemic conditioning—a new paradigm of self-protection in the brain

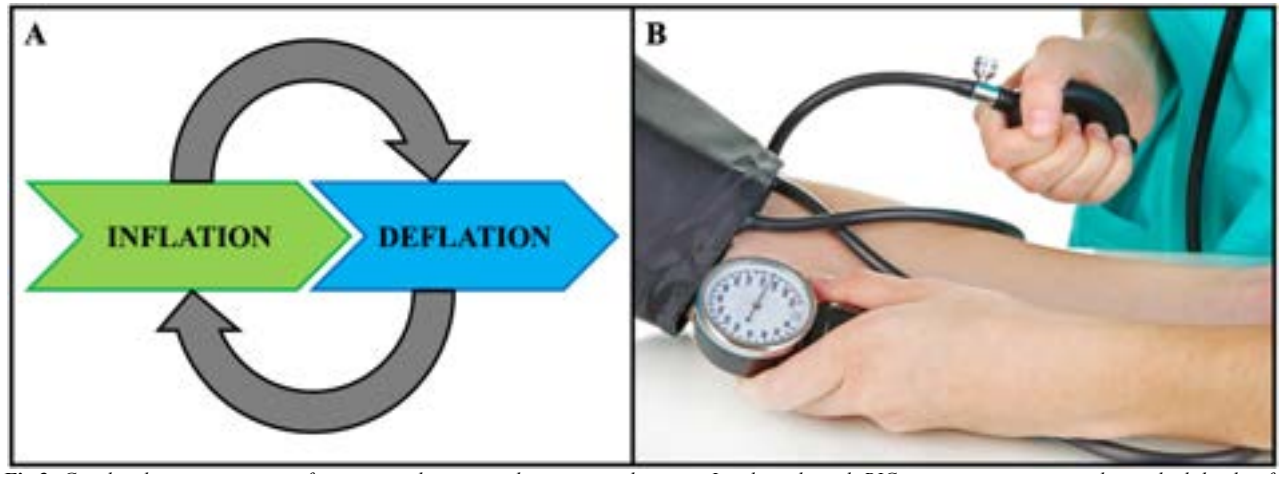
David C. Hess, Rolf A. Blauenfeldt, Grethe Andersen, Kristina D. Hougaard, Md Nasrul Hoda, Yuchuan Ding and Xunming Ji

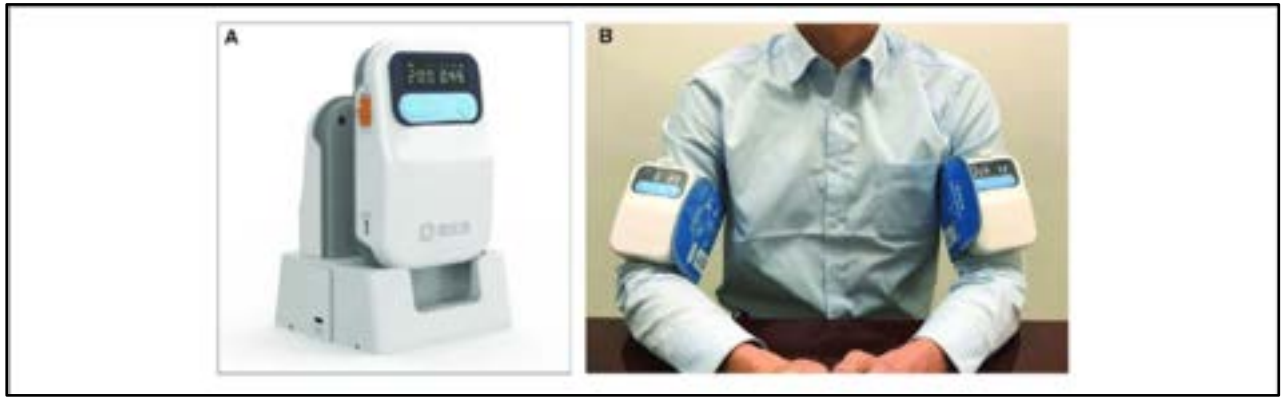
Hess, D. C. *et al. Nat. Rev. Neurol.* **11**, 698–710 (2015);

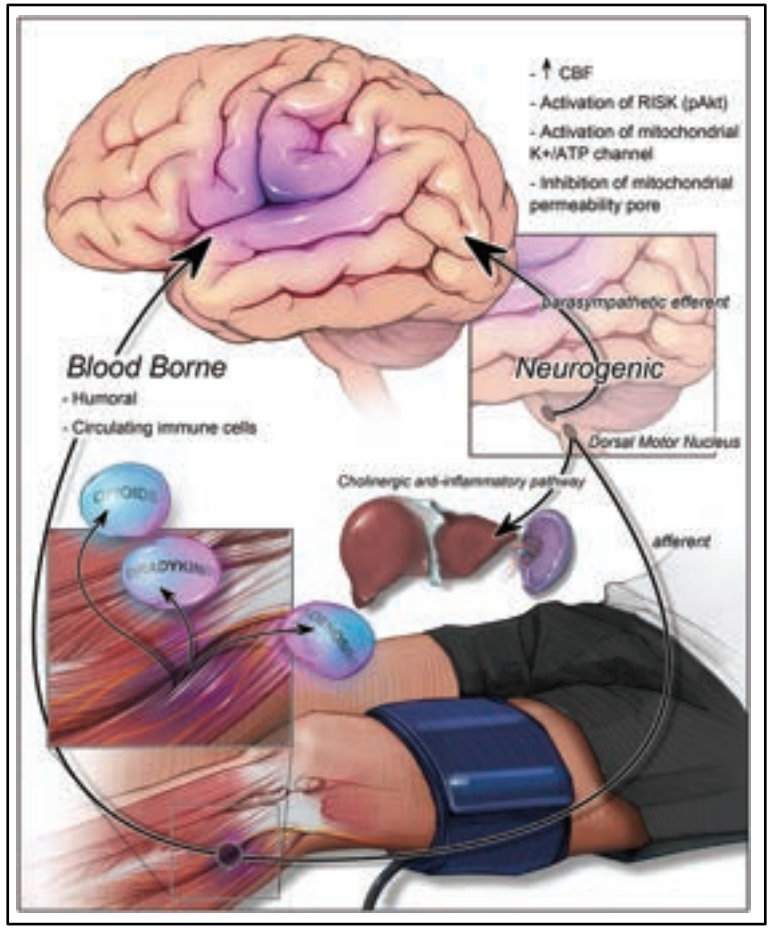




REMOTE ISCHEMIC CONDITIONING: HOW-TO







REMOTE ISCHEMIC CONDITIONING IN ACUTE ISCHEMIC STROKE

Evidence from basic science research

Review Article

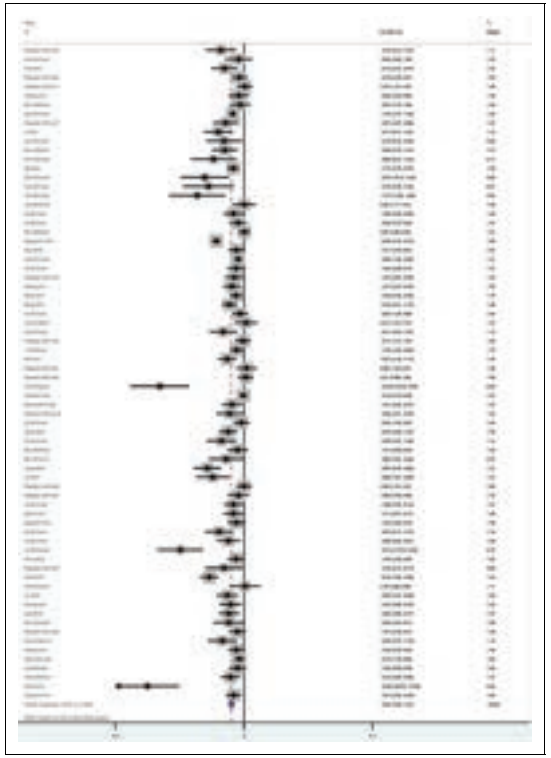
JCBFM

Journal of Cerebral Blood Flow & Metabolism
2021, Vol. 41(1) 3–13

DOI: 10.1177/0271678X20924077
journals.sagepub.com/home/jcbfm
SAGE

A meta-analysis of remote ischaemic conditioning in experimental stroke

Philippa Weir[1], Ryan Maguire[1], Saoirse E O'Sullivan[1] and Timothy J England[1,2]



	No. of experiments	No. of animals	SMD [95% CI]	*P* value
Remote ischaemic per/post-conditioning	72	1160	−2.00 [−2.38, −1.61]	<0.00001

Medium-to-large effect size on infarct volume

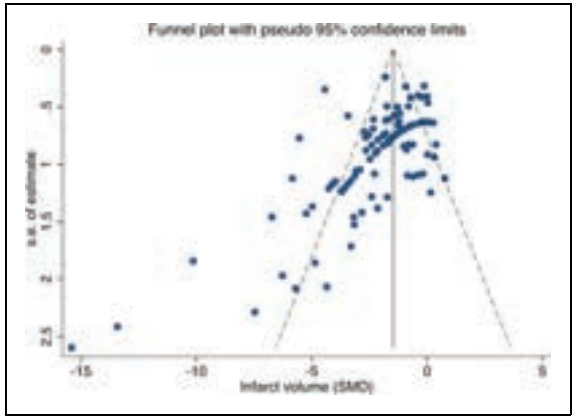
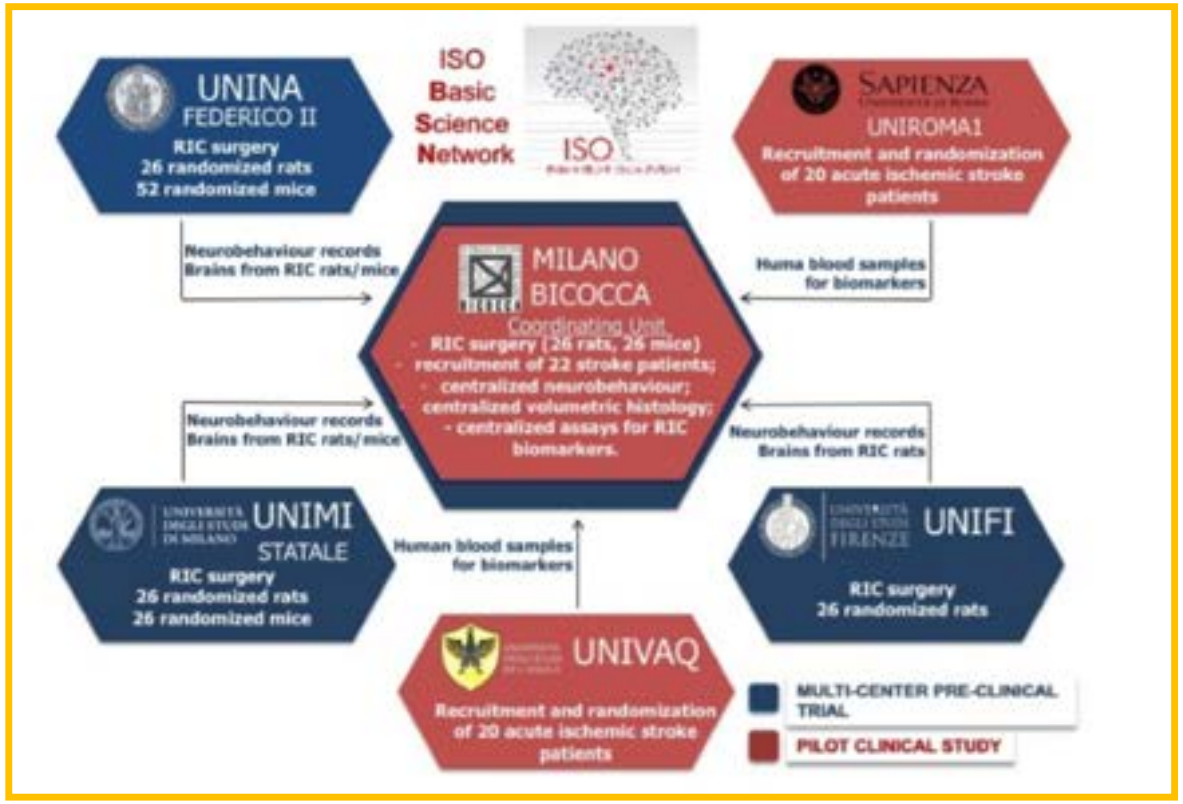


Figure 5. Begg's funnel plot. An asymmetric funnel indicates a relationship between treatment effect estimate and study precision. Egger's test suggested significant publication bias ($p < 0.001$).

MULTICENTER PRE-CLINICAL AND CLINICAL TRIAL



ISO
ITALIAN STROKE ORGANIZATION

Pre-Clinical Trial

Pilot Clinical Trial

TRICS BASIC

210 randomized animals

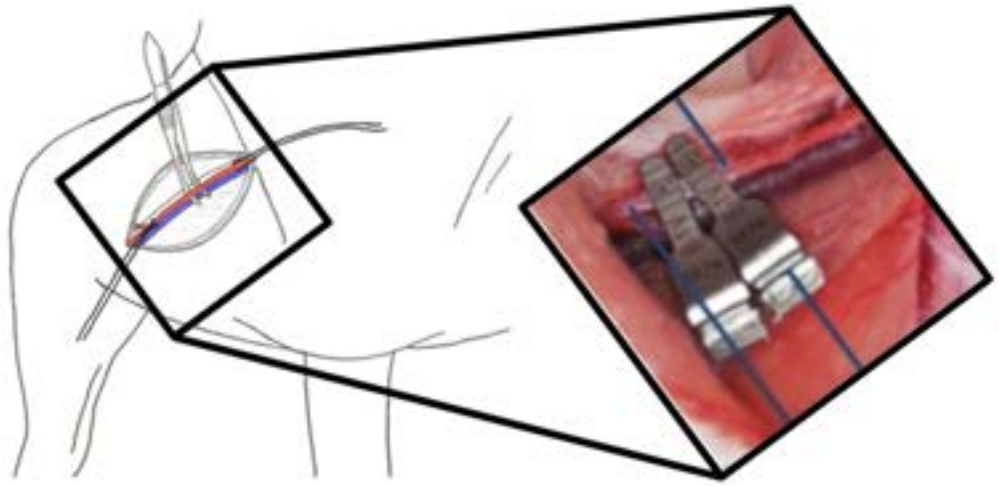
Multi-centric (7 laboratories in Italy)

Mice and Rats

Males and Females

Central randomization

Blinded surgeons, outcome assessment and centralized histology



Surgical RIC

(5-10 min femoral artery occlusion)

Single RIC application

Outcome assessed at 48 hours

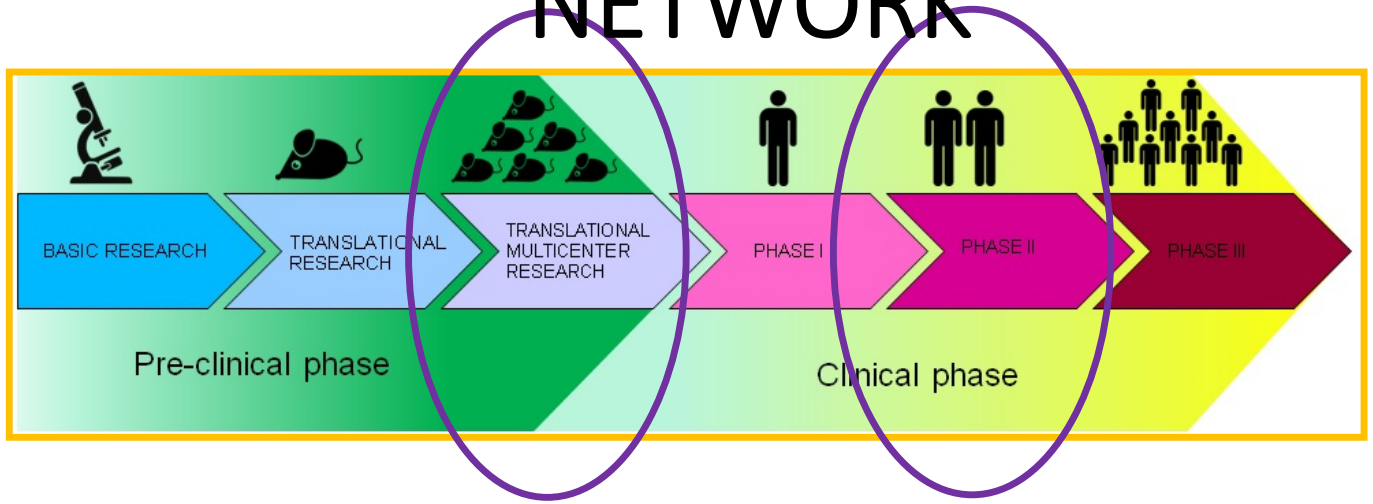
Open access Protocol

BMJ Open Science

Multicentre translational Trial of Remote Ischaemic Conditioning in Acute Ischaemic Stroke (TRICS): protocol of multicentre, parallel group, randomised, preclinical trial in female and male rat and mouse from the Italian Stroke Organization (ISO) Basic Science network

Mauro Tettamanti,[1] Simone Beretta ,[2] Giuseppe Pignataro,[3] Stefano Fumagalli,[1] Carlo Perego,[1] Luigi Sironi,[4] Felicita Pedata,[5] Diana Amantea ,[6] Marco Bacigaluppi,[7] Antonio Vinciguerra,[3] Alessia Valente,[1] Susanna Diamanti,[2] Jacopo Mariani,[2] Martina Viganò,[2] Francesco Santangelo,[2] Chiara Paola Zoia,[2] Virginia Rogriguez-Menendez,[2] Laura Castiglioni,[4] Joanna Rzemieniec,[4] Ilaria Dettori,[5] Irene Bulli,[5] Elisabetta Coppi,[5] Giorgia Serena Gullotta,[7] Giacinto Bagetta,[6] Gianvito Martino,[7] Carlo Ferrarese,[2] Maria Grazia De Simoni[1]

NATIONWIDE TRANSLATIONAL RESEARCH PROGRAM FROM THE ISO BASIC SCIENCE NETWORK



TRICS BASIC: Translational Trial of Remote Ischaemic Conditioning in Acute Ischaemic Stroke: multicentre, parallel group, randomised, preclinical trial in female and male rat and mouse from the Italian Stroke Organization (ISO) Basic Science network

TRICS-9: Multi-center randomized pilot clinical Trial on Remote Ischemic Conditioning in acute ischemic Stroke within 9 hours of onset in patients ineligible to recanalization therapies

INTRA-ISCHEMIC CLINICal ASSESSMENT

Intra-ischemic clinical assessment of successful MCA occlusion		
	no	yes
one or both palpebral fissures have an ellipsoidal shape	○	○
one or both ears extend laterally	○	○
asymmetric body bending on the ischemic side	○	○
limbs extend laterally and do not align to the body	○	○
Calcolo valore occlusione	_____	
Death during MCA surgery, before RIC	○ no	○ yes

Remote Ischemic post-Conditioning

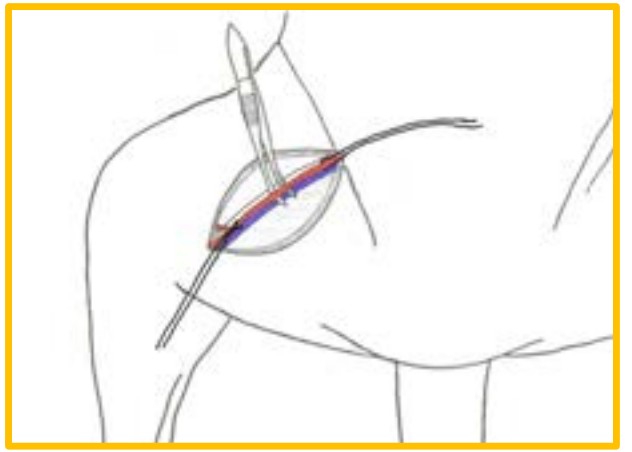
Surgery to expose Femoral Artery starts atfer riperfusion

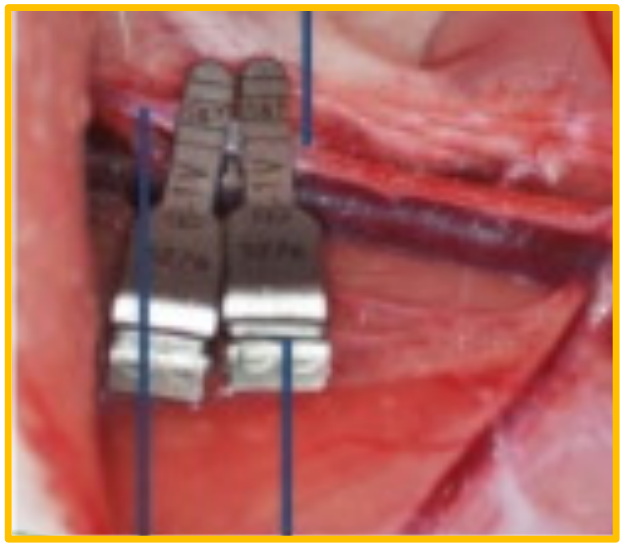
Waiting time before treatment application:

- Rats: 20min
- Mice: 10min

Femoral Artery clamping:

- Rats: 20min
- Mice: 10min





PRIMARY OUTCOME

De Simoni Composite Neuroscore

GENERAL DEFICITS

I. Hair (score 0-2)
0 - Hair neat and clean.
1 - Localized piloerection and dirty hair in 2 body parts (typically nose and eyes).
2 - Piloerection and dirty hair in more than 2 body parts.

II. Ears (score 0-2)
Mouse on OBT. Observation at the beginning with no interference, then stimulating by snapping fingers.
0 - Normal. Ears are stretched laterally and behind. They react to noise.
1 - Stretched laterally but not behind (one or both). They react to noise.
2 - Same as 1 but they do not react to noise.

III. Eyes (score 0-4)
Mouse on OBT. Observation with no interference or stimulation.
0 - Open, clean and quickly follow the surrounding environment.
1 - Open and characterized by aqueous mucus. Slowly follow the surrounding environment.
2 - Open and characterized by dark mucus.
3 - Ellipsodal shaped and characterized by dark mucus.
4 - Closed.

IV. Posture (score 0-4)
Place the mouse on the palm and swing gently.
0 - The mouse stands in the upright position with the back parallel to the palm (during the swing, it stands rapidly).
1 - The mouse stands humpbacked. During the swing, it flattens the body to gain stability.
2 - The head or part of the trunk lies on the palm.
3 - The mouse lies on one side, barely able to recover the upright position.
4 - The mouse lies in a prone position, not able to recover the upright position.

V. Spontaneous activity (score 0-4)
Mouse on OBT. Observation with no interference or stimulation.
0 - The mouse is alert and explores actively.
1 - The mouse seems alert, but it is calm and sluggish.
2 - The mouse explores intermittently and sluggishly.
3 - The mouse is somnolent and numb, few movements on-the-spot.
4 - No spontaneous movements.

VI. Epileptic behavior (score 0-12)
Mouse on OBT. The worse epileptic behavior detected during the whole observational period should be recorded and reported according to the following score.
0 - None.
3 - The mouse is reluctant to handling, shows hyperactivity.
6 - The mouse is aggressive, stressed and stares.
9 - The mouse shows hyperexcitability, chaotic movements and presence of convulsion following handling.
12 - Generalized seizures associated with wheezing and unconsciousness.

FOCAL DEFICITS

VII. Body symmetry (score 0-4)
Mouse on OBT, observation of undisturbed resting behavior and description of the virtual nosetail line.
0 - Normal.
a. Body: normal posture, trunk elevated from the bench, with fore and hindlimbs leaning beneath the body.
b. Tail: straight.
1 - Slight asymmetry.
a. Body: leans on one side with fore and hindlimbs leaning beneath the body.
b. Tail: slightly bent.
2 - Moderate asymmetry.
a. Body: leans on one side with fore and hindlimbs stretched out.
b. Tail: slightly bent.
3 - Prominent asymmetry.
a. Body: bent, on one side lies on the OBT.
b. Tail: bent.
4 - Extreme asymmetry.
a. Body: highly bent, on one side constantly lies on the OBT.
b. Tail: highly bent.

VIII. Gait (score 0-4)
Mouse on OBT. Observation of undisturbed movements.
0 - Normal. Gait is flexible, symmetric and quick.
1 - Stiff, inflexible. The mouse walks humpbacked, slower than normal mice.
2 - Limping with asymmetric movements.
3 - Trembling, drifting, falling.
4 - Does not walk spontaneously. When stimulated, the mouse walks no longer than three steps

IX. Climbing (score 0-4)
Mouse on a gripping surface 45° to OBT. Place the mouse in the centre of the gripping surface.
0 - Normal. The mouse climbs quickly.
1 - Climbs with strain, limb weakness present.
2 - Holds onto slope, does not slip or climb
3 - Slides down slope, unsuccessful effort to prevent fall.
4 - Slides immediately, no effort to prevent fall.

X. Circling behavior (score 0-4)
Mouse on OBT. Observation of the mouse walking undisturbed on the OBT.
0 - Absent. The mouse equally turns left or right.
1 - Predominantly one-sided turns.
2 - Circles to one side, although not constantly.
3 - Circles constantly to one side.
4 - Pivoting, swaying, or no movement.

XI. Forelimb symmetry (score 0-4)
Mouse suspended by the tail. Movements and position of forelimbs are observed.
0 - Normal. Both forelimbs are extended towards the bench and move actively.
1 - Light asymmetry. Contralateral forelimb does not extend entirely.
2 - Marked asymmetry. Contralateral forelimb bends towards the trunk. The body slightly bends on the ipsilateral side.
3 - Prominent asymmetry. Contralateral forelimb adheres to the trunk.
4 - Slight asymmetry, no body/limb movement.

XII. Compulsory circling (score 0-4)
Forelimbs on bench, hindlimbs suspended by the tail. This position reveals the presence of the contralateral limb palsy.
0 - Absent. Normal extension of both forelimbs.
1 - Tendency to turn to one side. The mouse extends both forelimbs but starts to turn preferably to one side.
2 - Circles to one side. The mouse turns towards one side with a slower movement compared to healthy mice.
3 - Pivots to one side sluggishly. The mouse turns towards one side failing to perform a complete circle.
4 - Does not advance. The front part of the trunk lies on the bench. Slow and brief movements.

XIII. Whisker response (score 0-4)
Mouse on the bench. Using a pen, touch gently the whiskers and the tip of the ears from behind, first one the lesioned and then on the contralateral side.
0 - Normal symmetrical response. The mouse turns the head towards the stimulated side and withdraws from the stimulus.
1 - Light asymmetry.
a. The mouse withdraws slowly when stimulated on the ischemic side.
b. Normal response on the contralateral side.
2 - Prominent asymmetry.
a. No response when stimulated on the ischemic side.
b. Normal response on the contralateral side.
3 - Absent response ipsilaterally, slow response when stimulated on the contralateral side.
4 - Absent response bilaterally.

Dichotomised neuroscore
(13 items, range 0-56 points)

- 0-20 Good outcome
- 21-56 Bad outcome

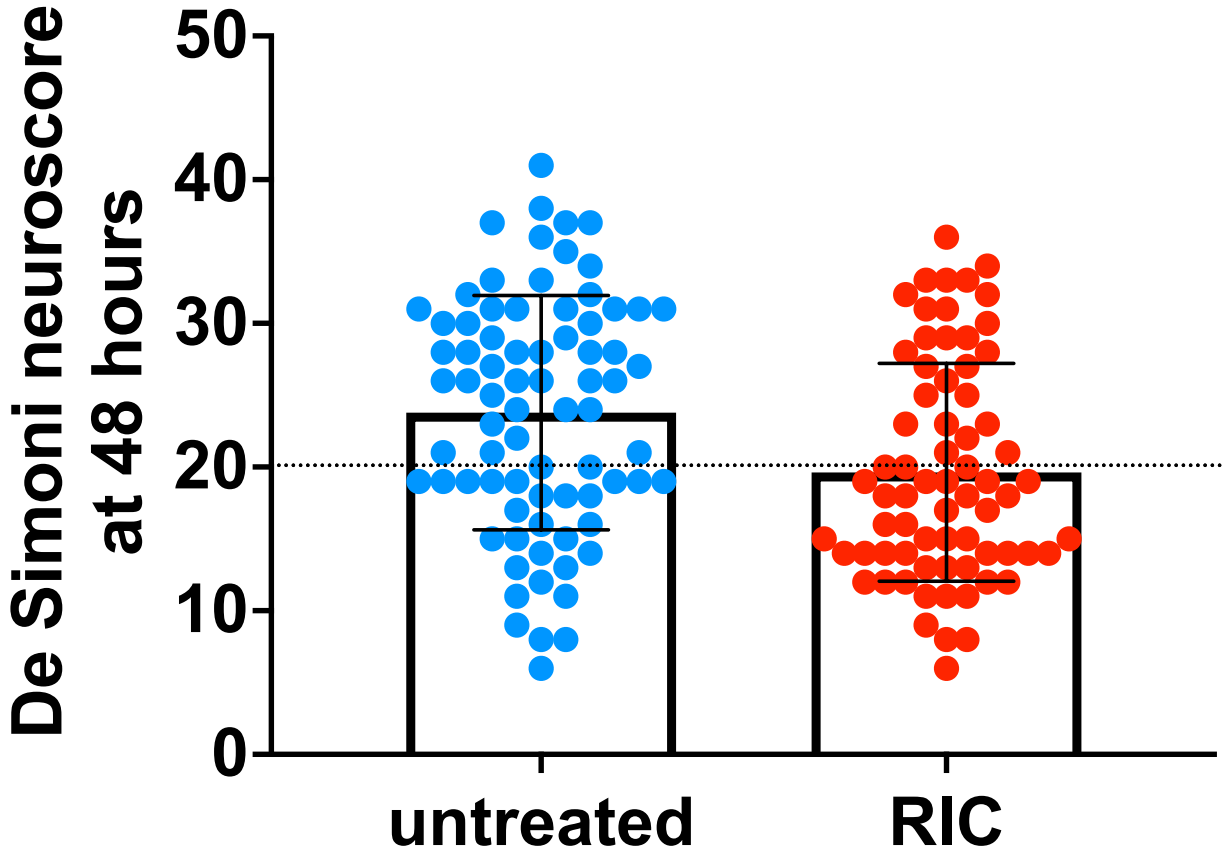
https:// figshare. com, DOI: 10.6084/m9.figshare.13031861)

HEALTH REPORT AT 24/48h

Low distress		
Reduced food and water intake	○ no	○ yes
Abnormal behaviour upon handling (increased or decreased reaction to being handled)	○ no	○ yes
Lethargy and reduced motility	○ no	○ yes
Piloerection / staring coat	○ no	○ yes
Discharge from the eyes and nose	○ no	○ yes

Moderate distress		
Animal not drinking	○ no	○ yes
Animal not eating (including wet mash)	○ no	○ yes
Severe surgical wound complication (infection, bleeding, opening)	○ no	○ yes
Absence of faeces	○ no	○ yes
Audible respiratory noises (rasping, wheezing), intermittent, without respiratory effort	○ no	○ yes
Weight loss exceeding 10%		
		(0 = no, 1 = yes)

High distress		
Presence of barrel rolling	○ no	○ yes
Presence of tonic clonic seizures	○ no	○ yes
Continuous laboured respiration with increased respiratory effort	○ no	○ yes
Animal not moving, unresponsive to stimulation, or in a lateral recumbent position	○ no	○ yes



Open access Protocol

BMJ Open Science

Multicentre translational Trial of Remote Ischaemic Conditioning in Acute Ischaemic Stroke (TRICS): protocol of multicentre, parallel group, randomised, preclinical trial in female and male rat and mouse from the Italian Stroke Organization (ISO) Basic Science network

Mauro Tettamanti,[1] Simone Beretta ,[2] Giuseppe Pignataro,[3] Stefano Fumagalli,[1] Carlo Perego,[1] Luigi Sironi,[4] Felicita Pedata,[5] Diana Amantea ,[6] Marco Bacigaluppi,[7] Antonio Vinciguerra,[3] Alessia Valente,[1] Susanna Diamanti,[2] Jacopo Mariani,[2] Martina Viganò,[2] Francesco Santangelo,[2] Chiara Paola Zoia,[2] Virginia Rogriguez-Menendez,[2] Laura Castiglioni,[4] Joanna Rzemieniec,[4] Ilaria Dettori,[5] Irene Bulli,[5] Elisabetta Coppi,[5] Giorgia Serena Gullotta,[7] Giacinto Bagetta,[6] Gianvito Martino,[7] Carlo Ferrarese,[2] Maria Grazia De Simoni[1]

Good outcome at 48 hours	Risk difference	Odds Ratio	95% CI	p value
RIC versus untreated	0.1993	2.33	1.23-4.42	0.009

JAMA | Original Investigation

Effect of Remote Ischemic Conditioning vs Usual Care on Neurologic Function in Patients With Acute Moderate Ischemic Stroke
The RICAMIS Randomized Clinical Trial

Hui-Sheng Chen, MD; Yu Cui, PhD; Xiao-Qiu Li, MD; Xin-Hong Wang, MD; Yu-Tong Ma, MM; Yong Zhao, BSM; Jing Han, MM; Chang-Qing Deng, MM; Mei Hong, BSM; Ying Bao, MM; Li-Hong Zhao, MM; Ting-Guang Yan, BSM; Ren-Lin Zou, BSM; Hui Wang, MM; Zhuo Li, MM; Li-Shu Wan, MM; Li Zhang, BSM; Lian-Qiang Wang, BSM; Li-Yan Guo, MM; Ming-Nan Li, BSM; Dong-Qing Wang, MM; Qiang Zhang, MM; Da-Wei Chang, MM; Hong-Li Zhang, BSM; Jing Sun, BSM; Chong Meng, BSM; Zai-Hui Zhang, BSM; Li-Ying Shen, BSM; Li Ma, MM; Gui-Chun Wang, BSM; Run-Hui Li, MM; Ling Zhang, BSM; Cheng Bi, MM; Li-Yun Wang, BSM; Duo-Lao Wang, PhD; for the RICAMIS Investigators

JAMA August 16, 2022 Volume 328, Number 7

Table 2. Primary and Secondary Outcomes in the Full Analysis Set

	Group, No. (%)			Unadjusted		Adjusted[b]	
	Remote ischemic conditioning (n = 863)	Control (n = 913)	Treatment effect metric[a]	Treatment difference (95% CI)	*P* value	Treatment difference (95% CI)	*P* value
Primary outcome							
mRS score of 0 to 1 within 90 d[c]	582 (67.4)	566 (62.0)	RR[d]	1.17 (1.03 to 1.32)	.02	1.18 (1.04 to 1.34)	.007
			RD, %[d]	5.4 (1.0 to 9.9)	.02	6.2 (2.0 to 10.4)	.004
Secondary outcomes							
mRS score of 0 to 2 within 90 d[c]	687 (79.6)	689 (75.5)	RR[d]	1.20 (1.01 to 1.43)	.04	1.22 (1.03 to 1.45)	.02
			RD, %[d]	4.1 (0.3 to 8.0)	.04	4.3 (0.9 to 7.8)	.01
Change in NIHSS score at day 12 from baseline, median (IQR)[g,d]	4 (2 to 6)	4 (2 to 5)	GMR	1.02 (0.99 to 1.05)	.32	1.02 (0.99 to 1.05)	.30
Death within 90 d[h]	7 (0.8)	10 (1.1)	HR	0.74 (0.28 to 1.94)	.54	0.63 (0.24 to 1.70)	.37

1893 randomized patients

within 48 hours of onset (median 25 hours)

NIHSS 6 to 16 (median 7)

ineligible to recanalization therapies

pre-stroke mRS <=1

Excluded

uncontrolled hypertension

cardioembolic etiology

Anterior circulation 60%

Posterior circulation 35%

RIC application

Bilateral arm, automated

5 minutes on/off, **5 cycles**

Twice a day for **10-14 days**

JAMA | Original Investigation

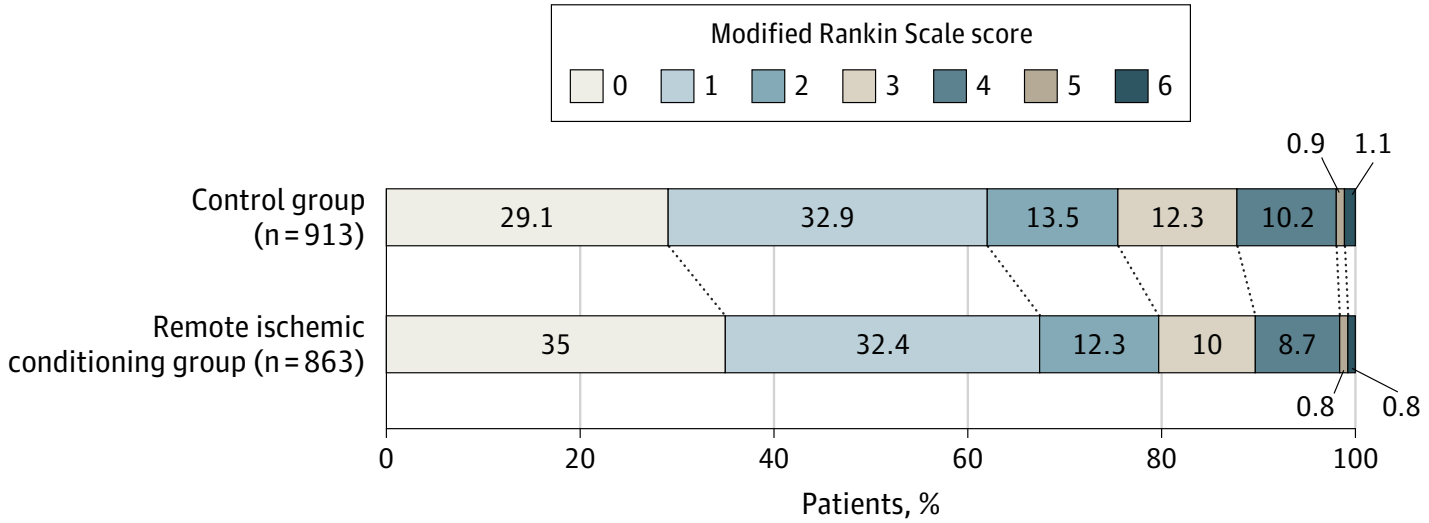
Effect of Remote Ischemic Conditioning vs Usual Care on Neurologic Function in Patients With Acute Moderate Ischemic Stroke

The RICAMIS Randomized Clinical Trial

Hui-Sheng Chen, MD; Yu Cui, PhD; Xiao-Qiu Li, MD; Xin-Hong Wang, MD; Yu-Tong Ma, MM; Yong Zhao, BSM; Jing Han, MM; Chang-Qing Deng, MM; Mei Hong, BSM; Ying Bao, MM; Li-Hong Zhao, MM; Ting-Guang Yan, BSM; Ren-Lin Zou, BSM; Hui Wang, MM; Zhuo Li, MM; Li-Shu Wan, MM; Li Zhang, BSM; Lian-Qiang Wang, BSM; Li-Yan Guo, MM; Ming-Nan Li, BSM; Dong-Qing Wang, MM; Qiang Zhang, MM; Da-Wei Chang, MM; Hong-Li Zhang, BSM; Jing Sun, BSM; Chong Meng, BSM; Zai-Hui Zhang, BSM; Li-Ying Shen, BSM; Li Ma, MM; Gui-Chun Wang, BSM; Run-Hui Li, MM; Ling Zhang, BSM; Cheng Bi, MM; Li-Yun Wang, BSM; Duo-Lao Wang, PhD; for the RICAMIS Investigators

JAMA August 16, 2022 Volume 328, Number 7

Figure 2. Distribution of Modified Rankin Scale Scores at 90 Days in the Full Analysis Set



The raw distribution of scores is shown. Scores range from 0 to 6 (0 = no symptoms, 1 = symptoms without clinically significant disability, 2 = slight disability, 3 = moderate disability, 4 = moderately severe disability, 5 = severe disability, and 6 = death). The odds ratio was 1.29 (95% CI, 1.09-1.52), and the *P* value was .003; the adjusted odds ratio was 1.37 (95% CI, 1.16-1.63), and the adjusted *P* value was <.001.

Mentimeter

www.menti.com