New humanized model of ischemic stroke by endovascular approach in pigs:

Study of pathophysiological pathways in gyrencephalic brain and of new biochemical and imaging biomarkers with translational potential

Marc Melià Sorolla

09/01/2022

Thesis director: Teresa Gasull Dalmau

Background

Ischemic stroke (IS)

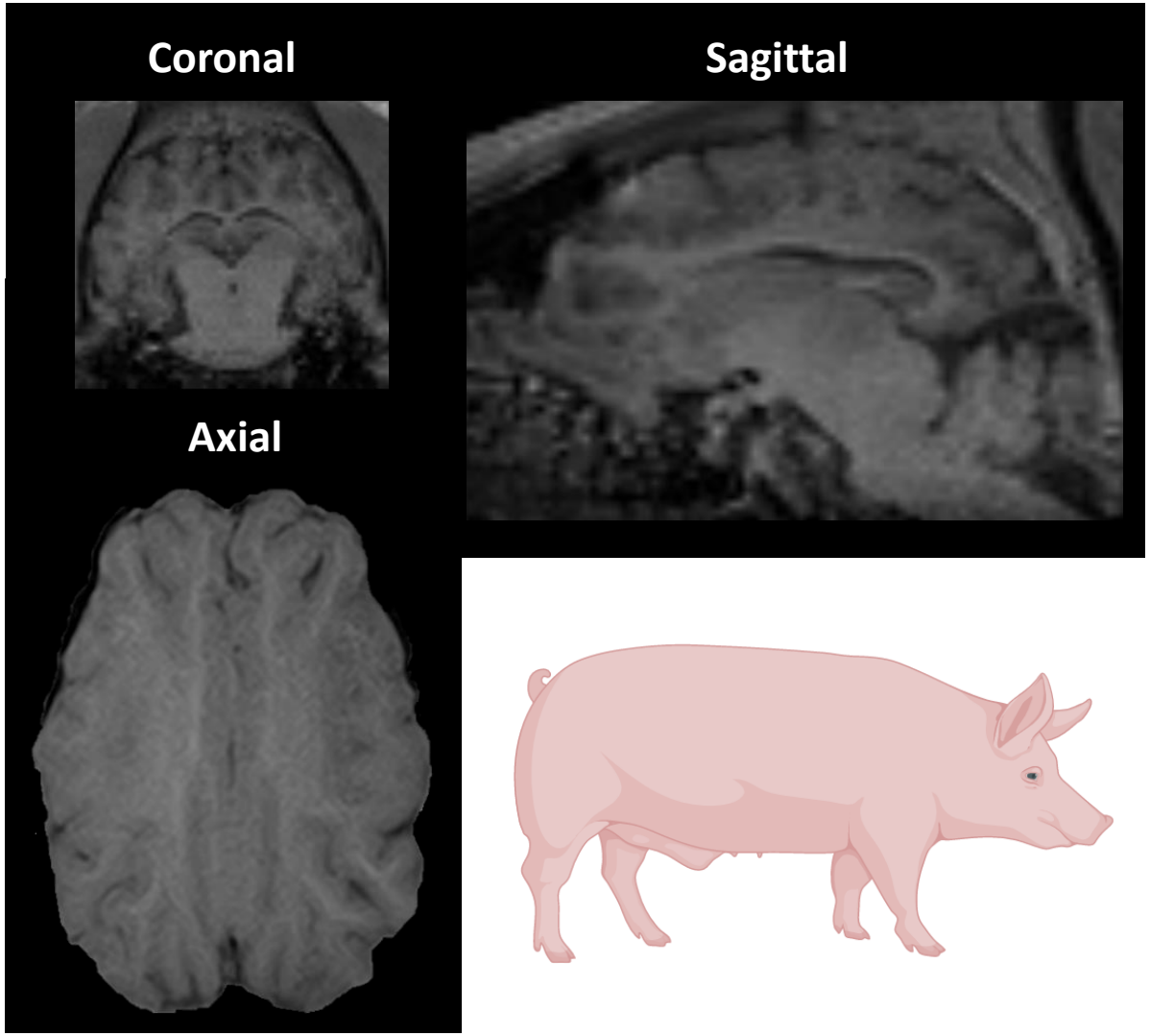
- >1000 neuroprotective proven in animal studies failed in clinical trials.
- Huge physiological and pathophysiological with small animals to humans.

Sorby-Adams AJ, *et al*. Large animal models of stroke and traumatic brain injury as translational tools. *Am J Physiol Regul Integr Comp Physiol*. 2018. 315: R165–R190

Porcine models

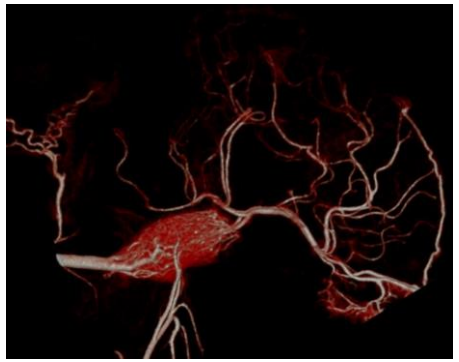
- Large mammals.
- Gyrencephalic brain.
- High white matter / gray matter ratio.
- Low ethical burden.

Melià-Sorolla M, *et al*. Relevance of porcine stroke models to bridge the gap from pre-clinical findings to clinical implementation. *Int J Mol Sci*. 2020 Sep 8; 21 (18): 6568

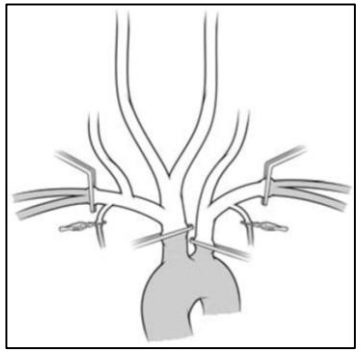


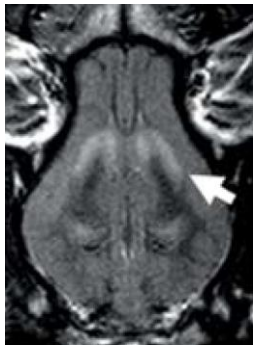
Background

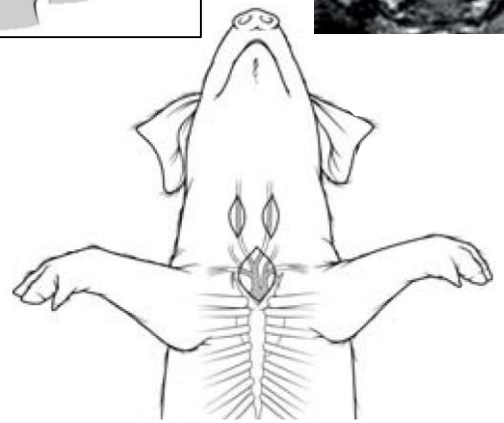
Porcine stroke models



Global ischemic stroke

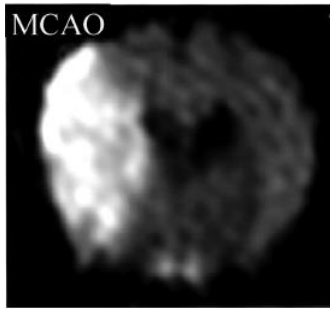


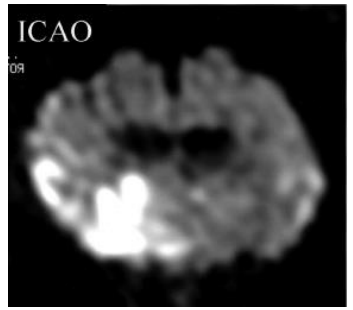




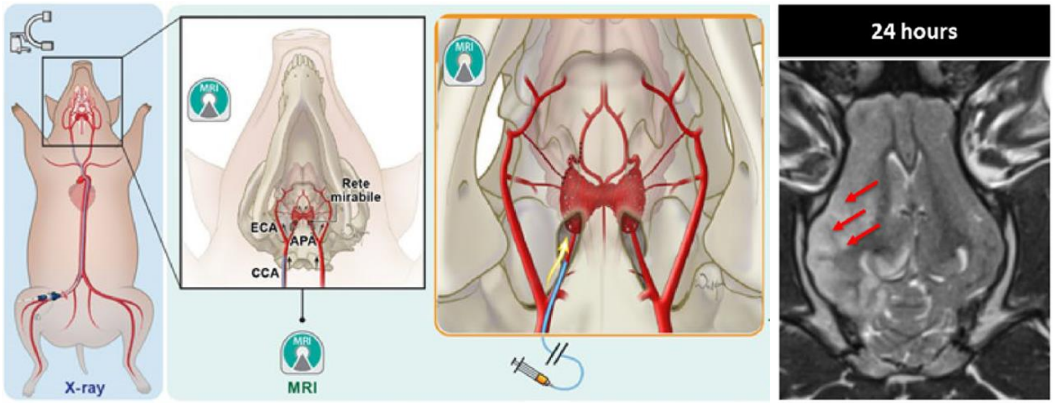
Allen BS, *et al*. Studies of isolated global brain ischaemia: I. A new large animal model of global brain ischaemia and its baseline perfusion studies. *Eur J Cardiothorac Surg*. 2012 May; 41 (5): 1138-46.

Focal ischemic stroke





Imai H, *et al*. A new model of focal cerebral ischemia in the miniature pig. *J Neurosurg*. 2006 Feb; 104 (2 Suppl): 123-32



Golubczyk D, *et al*. Endovascular model of ischemic stroke in swine guided by real-time MRI. *Sci Rep*. 2020 Oct 14;10(1):17318.

Objectives

Objective list

1) Study the feasibility of a new focal IS in pig by an endovascular approach, and determine its reproducibility.

2) Adapt multimodal imaging biomarkers from the clinics to study the ischemic lesion.

3) Validate the imaging results with *ex vivo* lesion characterization.

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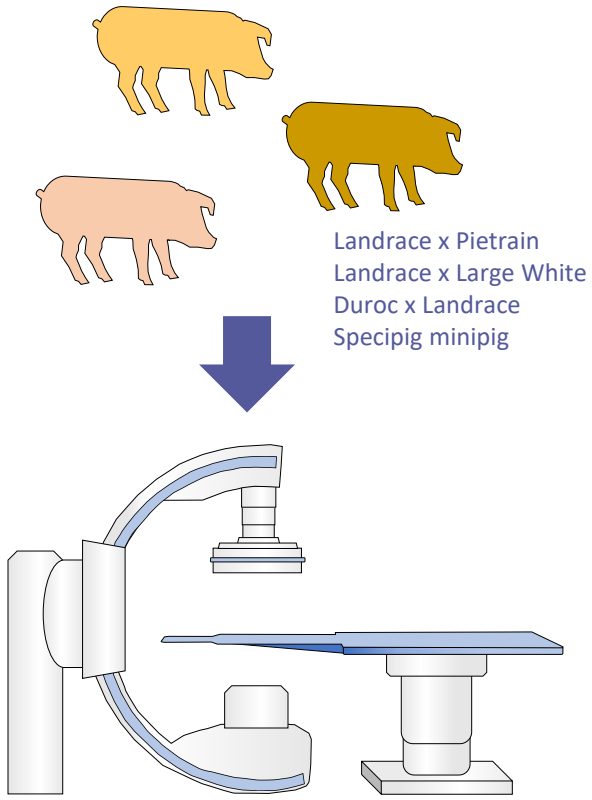
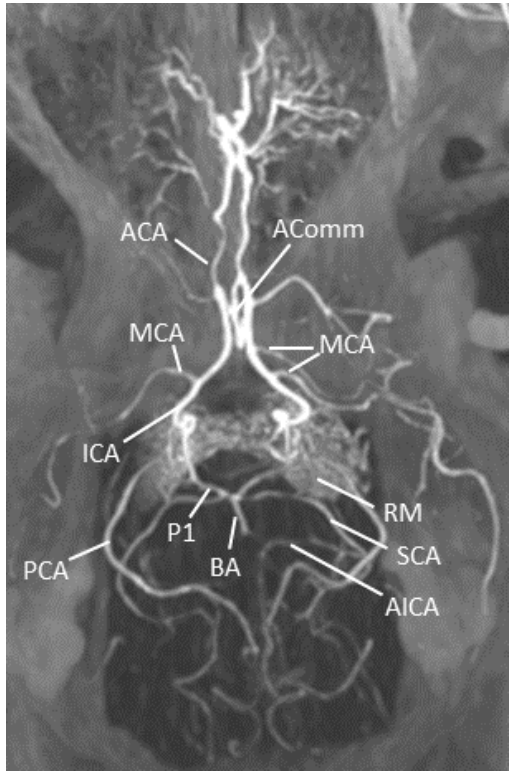
Results (I)

Endovascular access

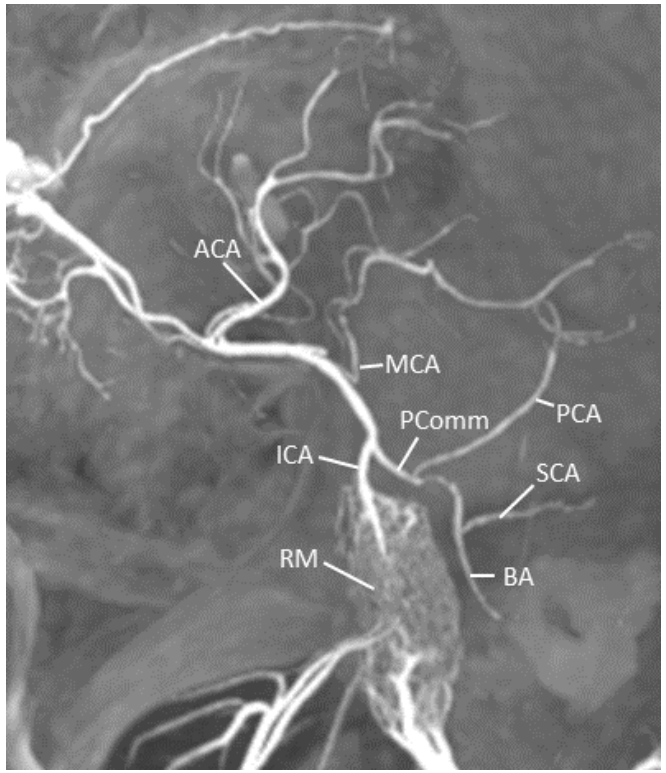


Landrace x Pietrain
Landrace x Large White
Duroc x Landrace
Specipig minipig

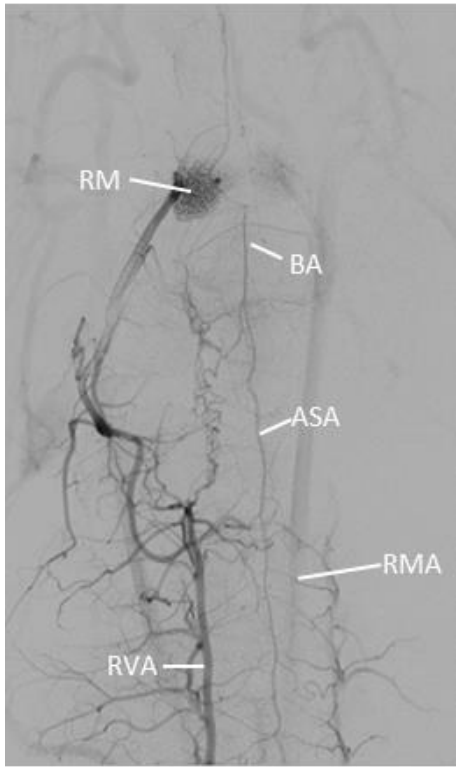
Anterior view



Sagittal view



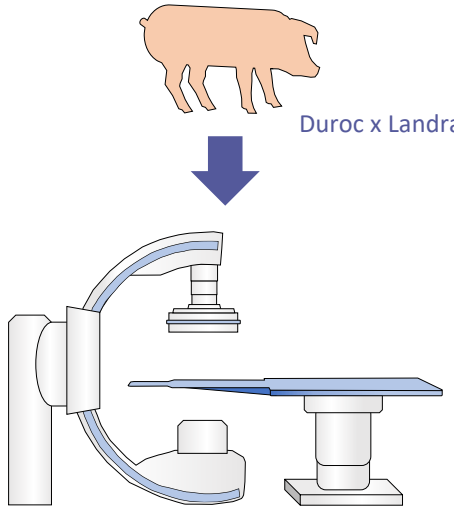
Anterior view



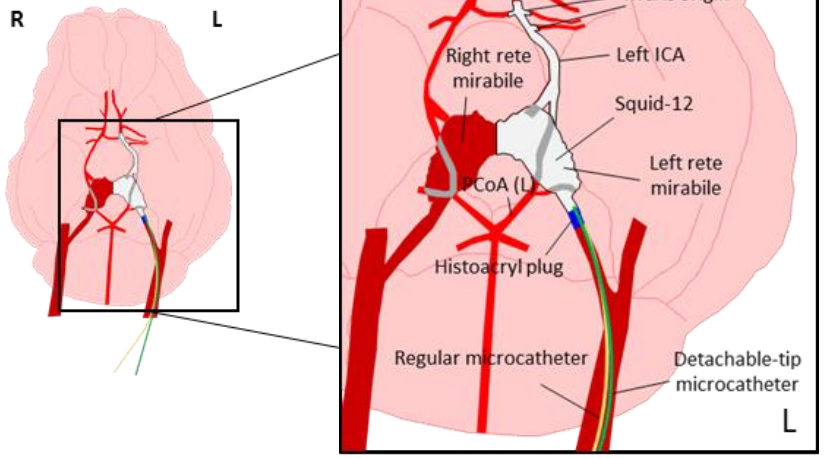
No endovascular access found to the circle of Willis

Results (I)

Porcine IS model (permanent occlusion)

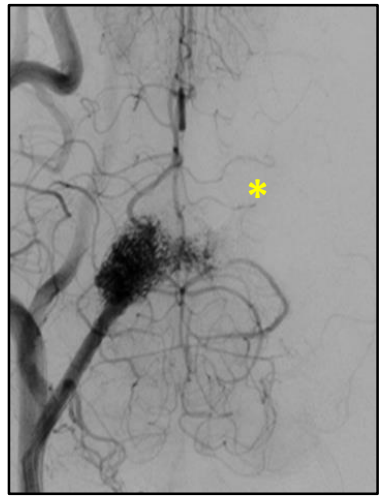


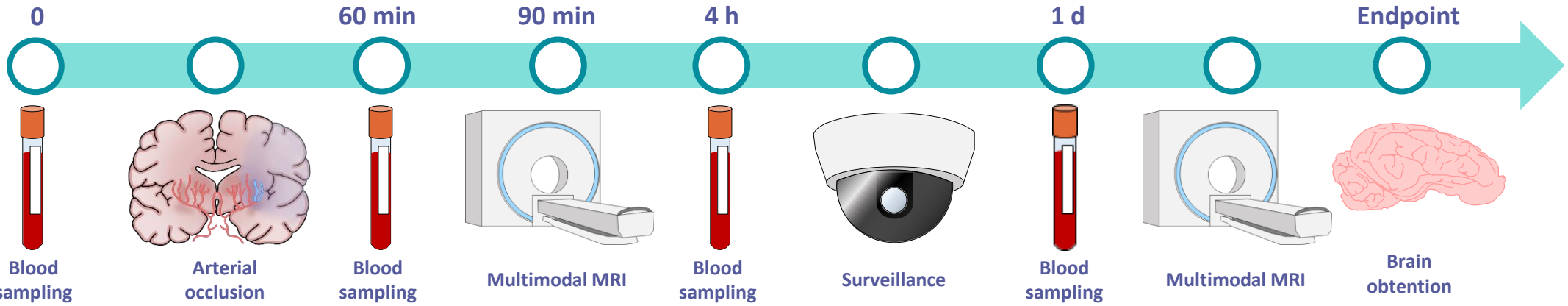
Duroc x Landrace



Pre-Squid

Post-Squid





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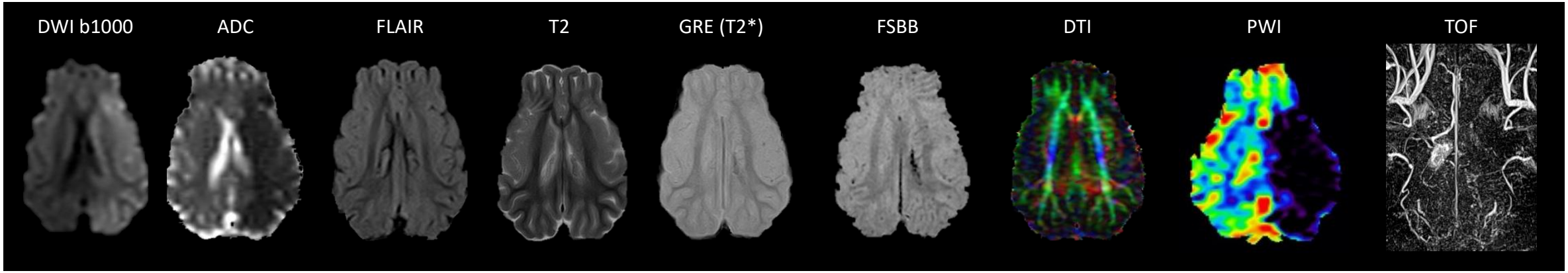
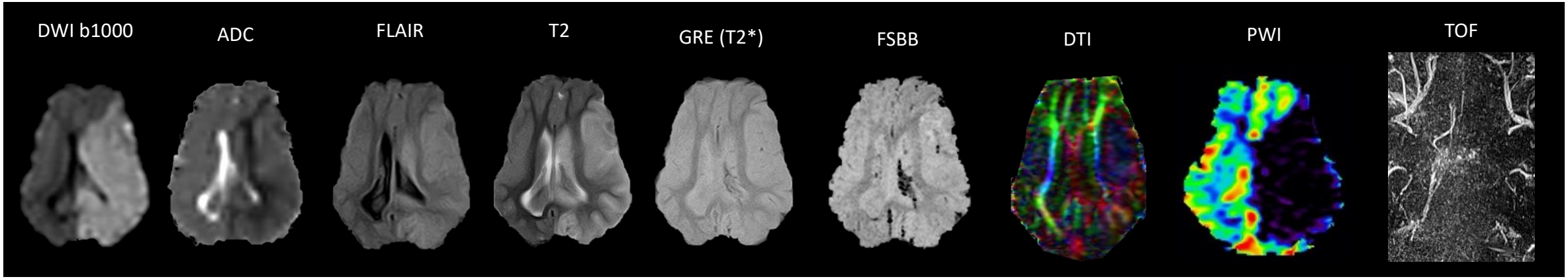
Results (II)

Multimodal MRI

90 min post-occlusion (Hyperacute)



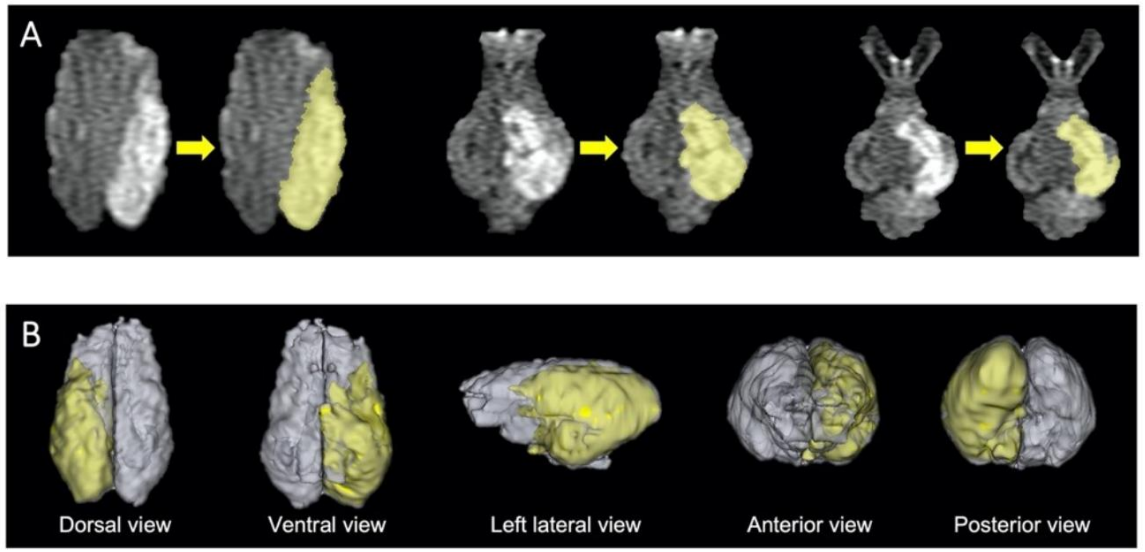
24 h post-occlusion (Acute)

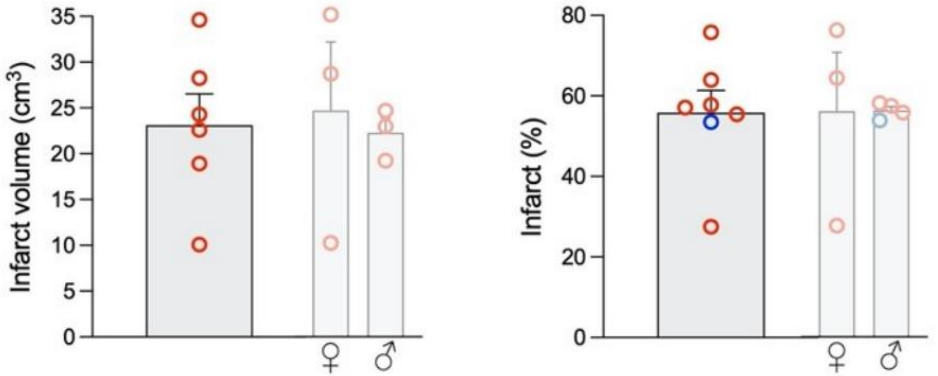


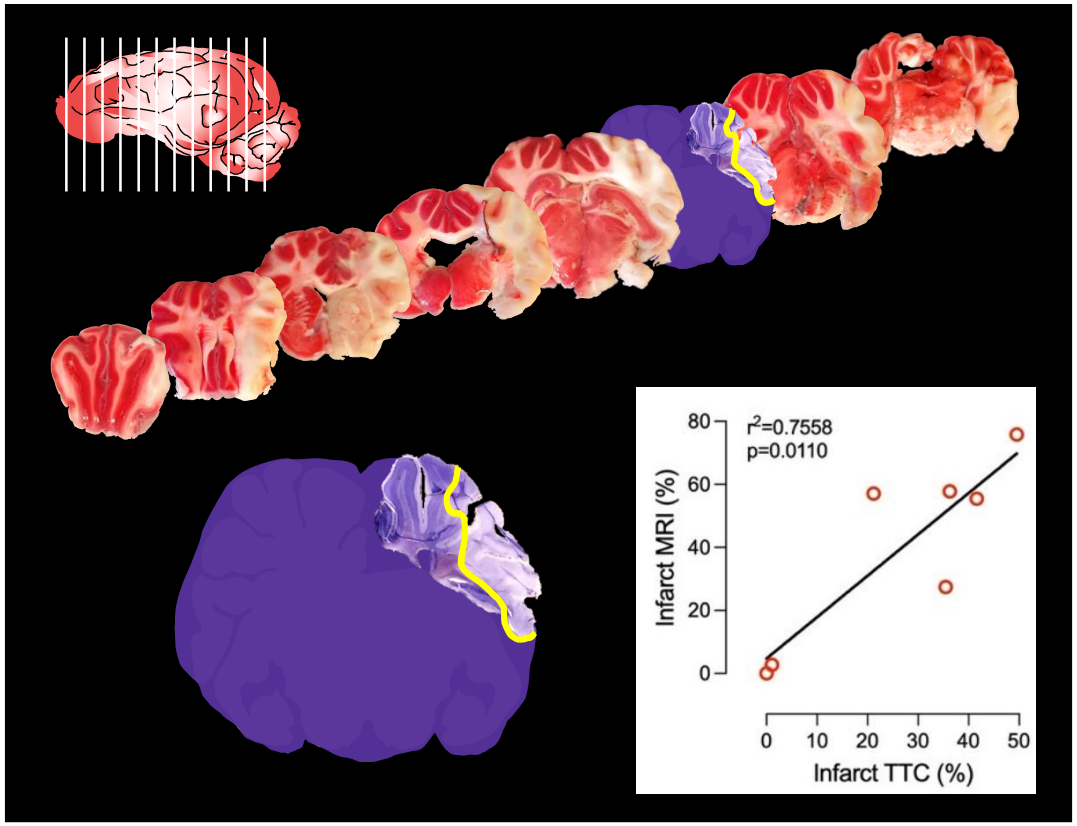
Results (II)

Induced infarct

DWI (b1000)







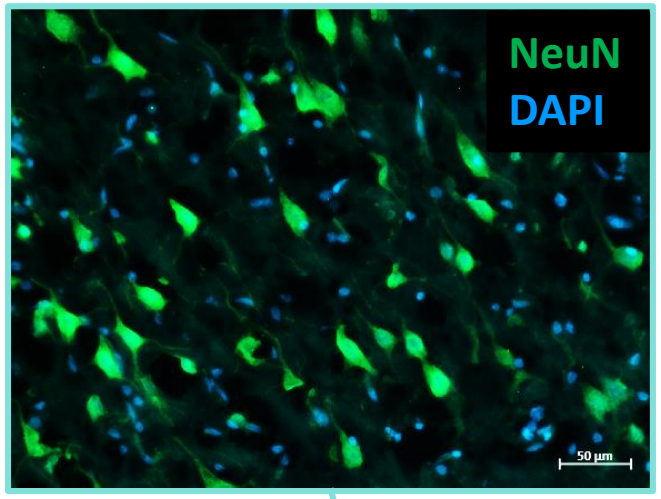
Reproducible infarcts in the pig model

Results (II)

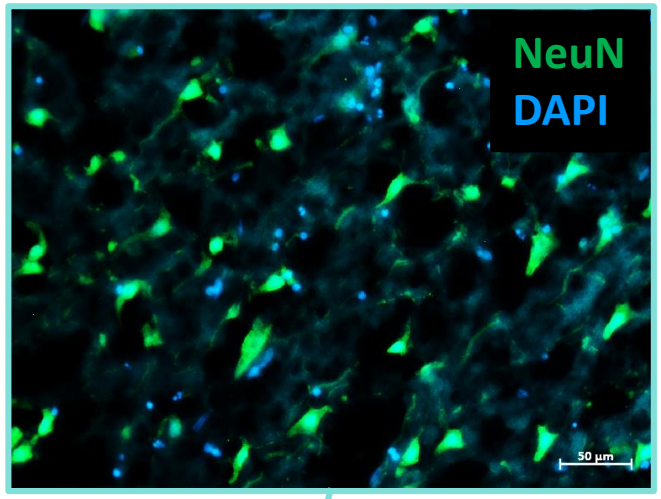
Cellular level affectation

Gray matter

Contralateral hemisphere (CL)



Ipsilateral hemisphere (IL)

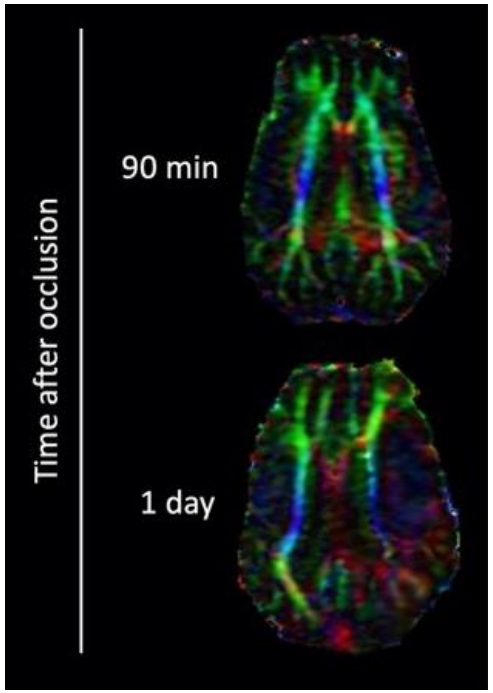


Infarcted region

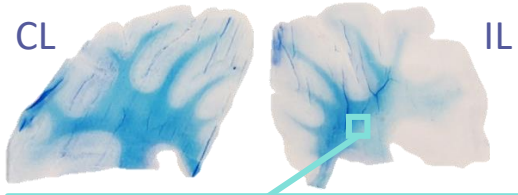
Cortical coronal slices

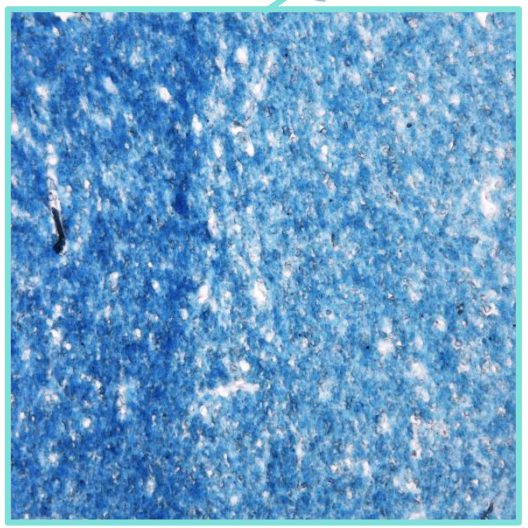
White matter

DTI



Coronal slice



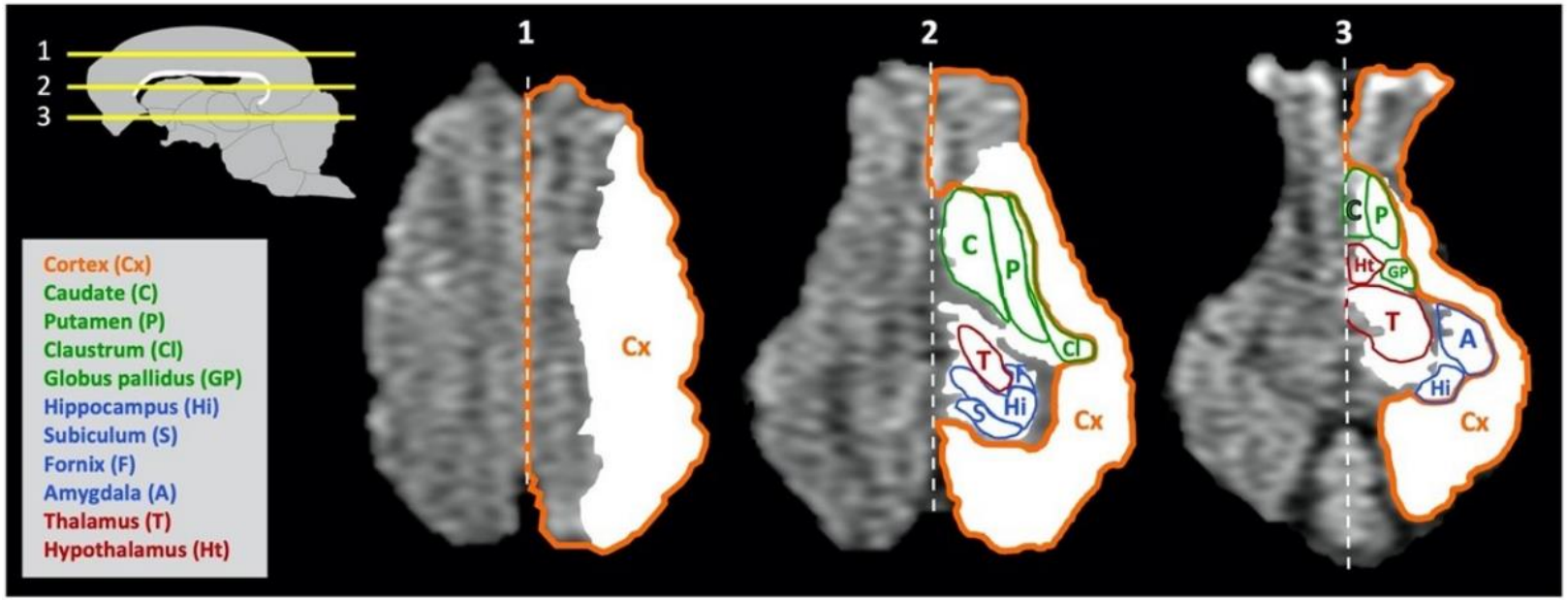


100x

Damage at the cellular level in gray and white matter

Results (II)

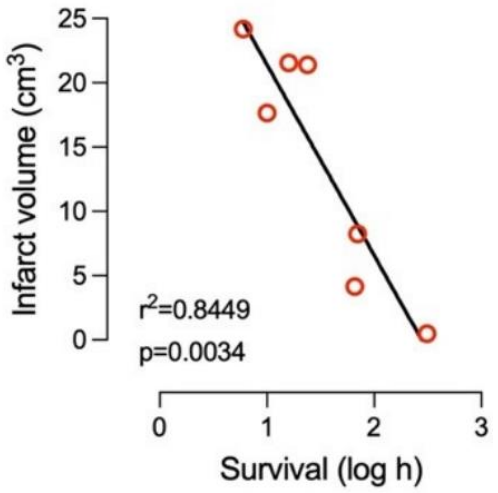
Affected areas



Structure	Cortex	Thalamus	Striatum	Limbic system	Epithalamus	Hypothalamus
Pigs affected at 90 min (%)	100	83	83	100	50	100
Pigs affected at 1-2 d (%)	100	83	100	100	67	100

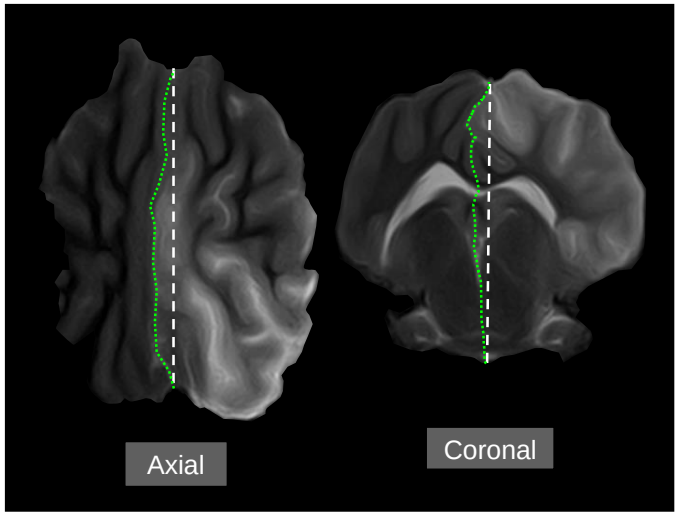
Results (II)

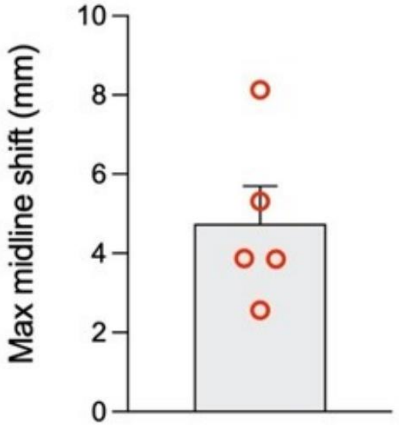
Lesion characterization



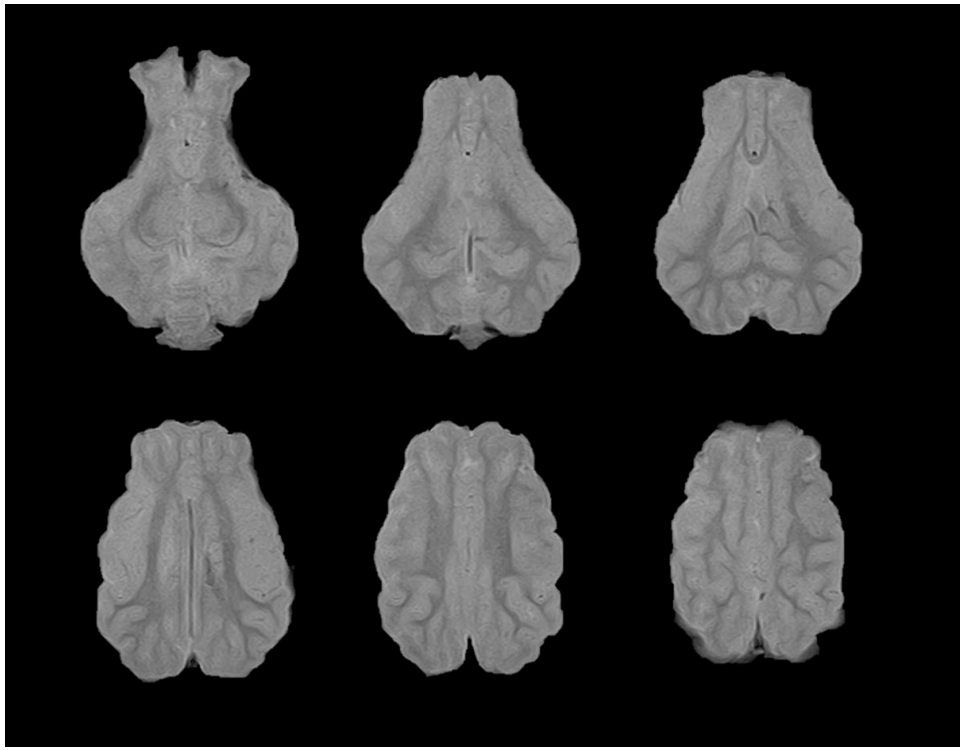
*Survival considering humanitarian endpoint application.

T2-weighted





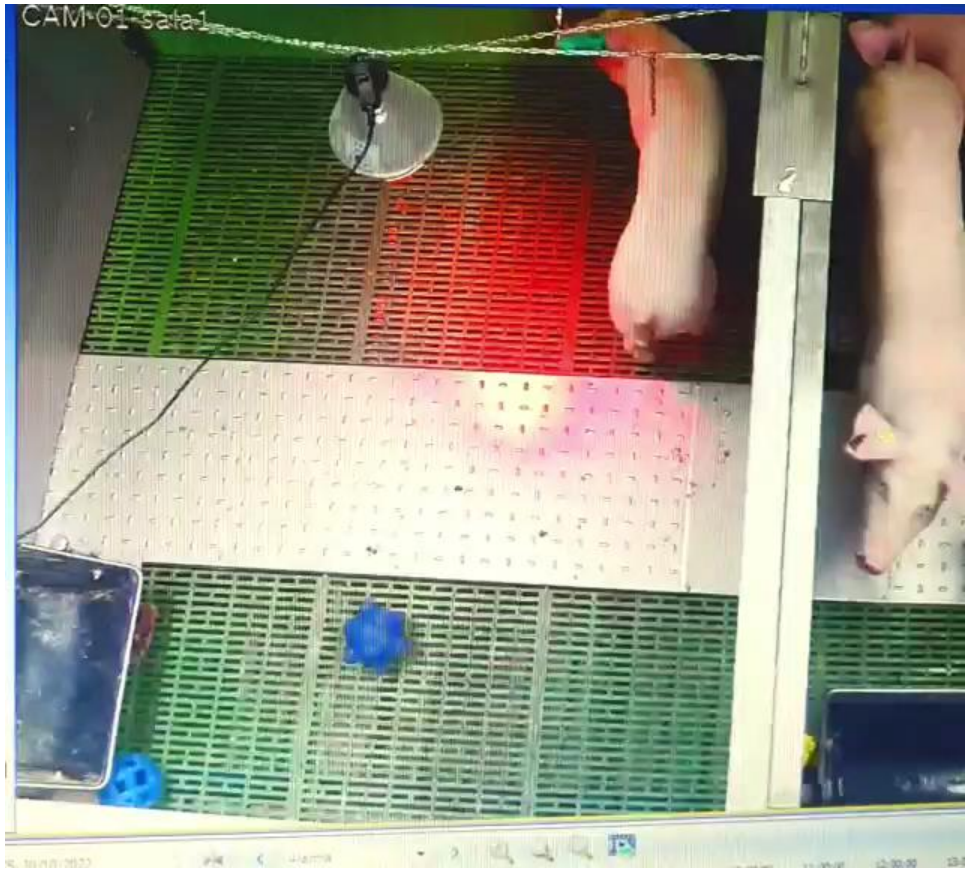
T2* (GRE)



Results (II)

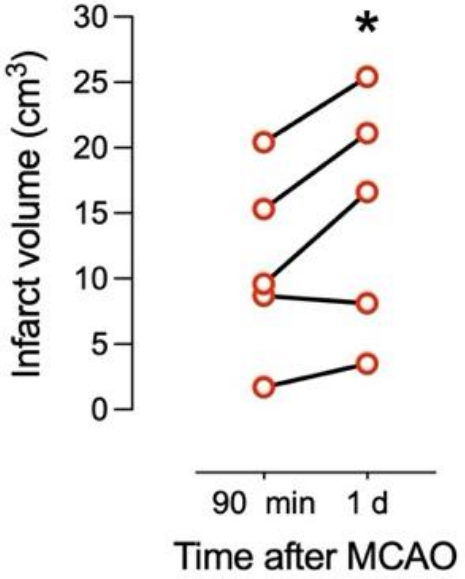
Neurological affectation

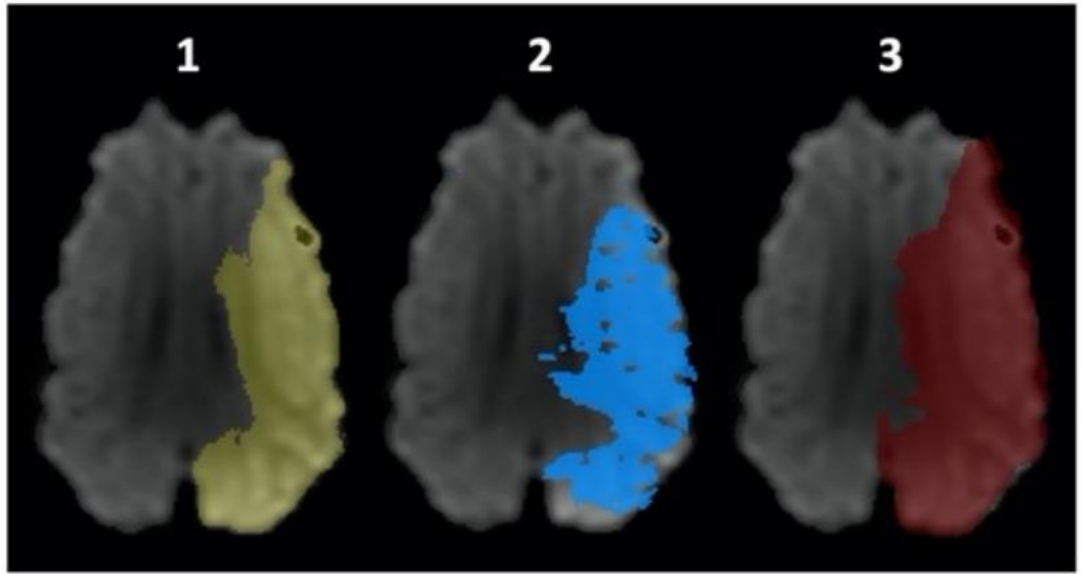
- Ataxia
- Lameness
- Nystagmus
- Tremors
- Repetitive head shakes
- Circling towards the ipsilateral damaged hemisphere
- Head pressing to the walls
- Occasional seizures of moderate or high intensity



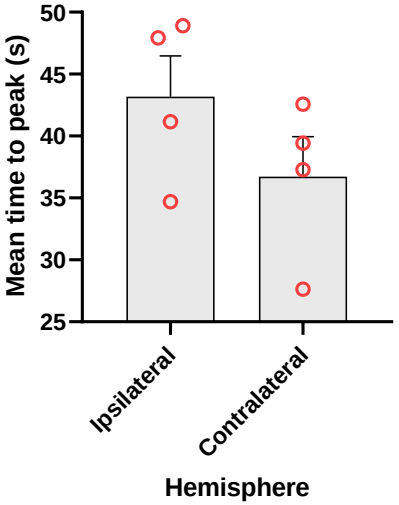
Results (II)

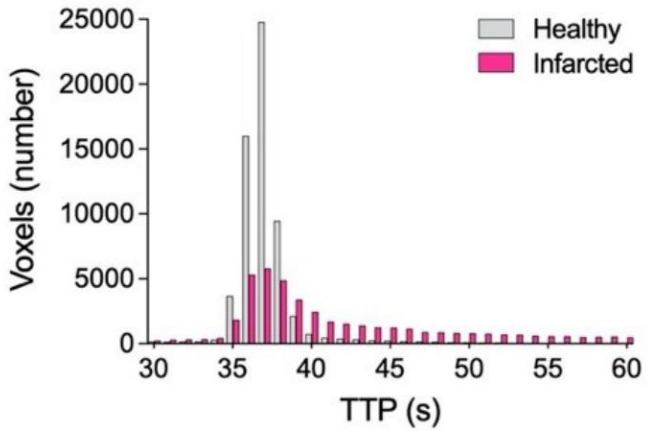
Salvageable brain





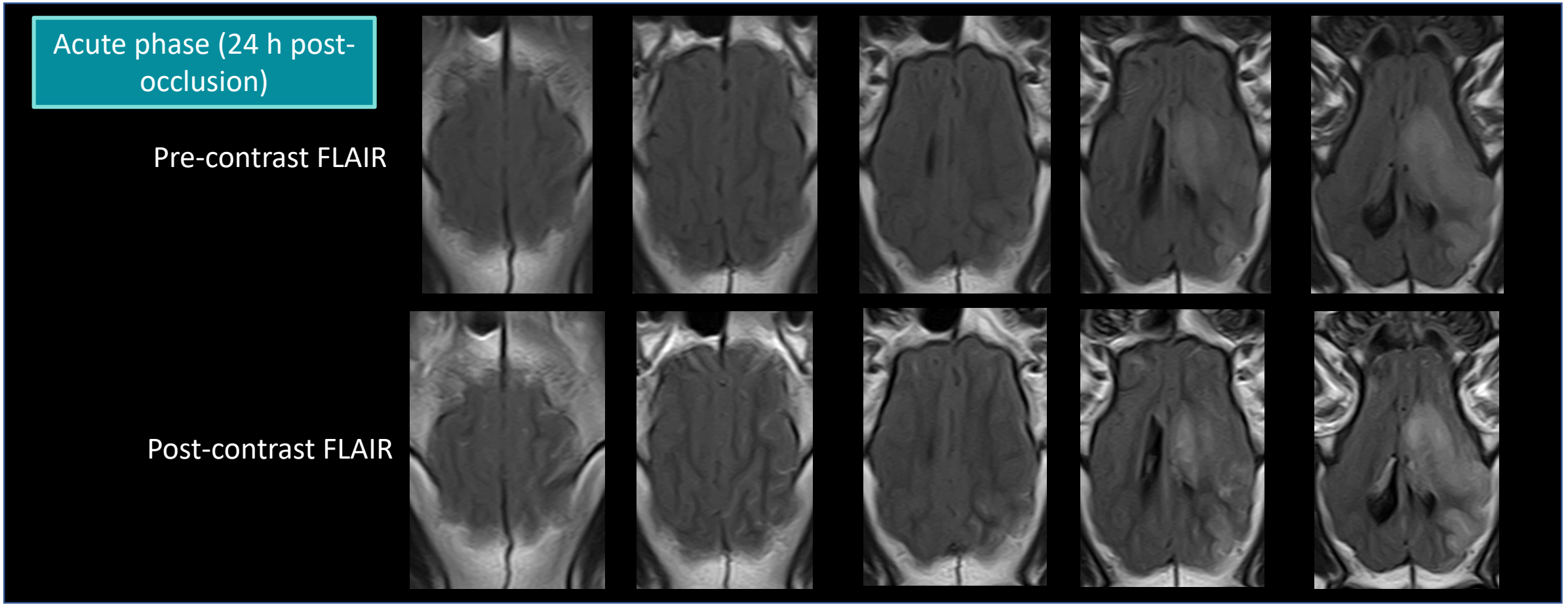
1. Infarct in DWI at 90 min post-occlusion.
2. TTP > 40 s at 90 min post-occlusion.
3. Infarct in DWI at 24 h post-occlusion.





Results (II)

Blood-brain barrier disruption



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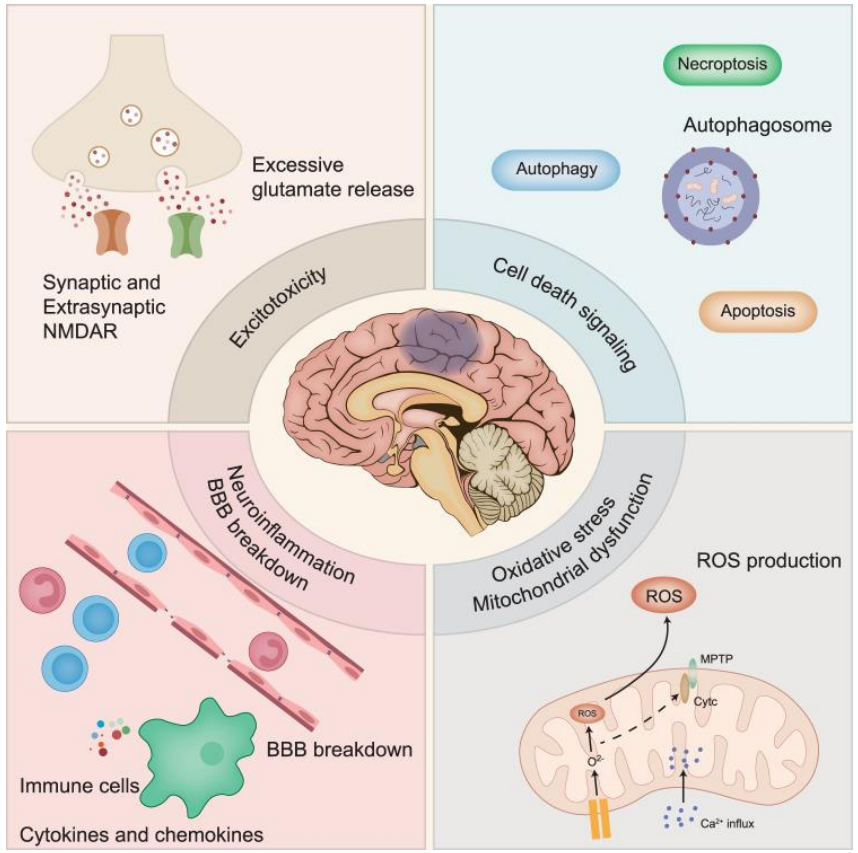
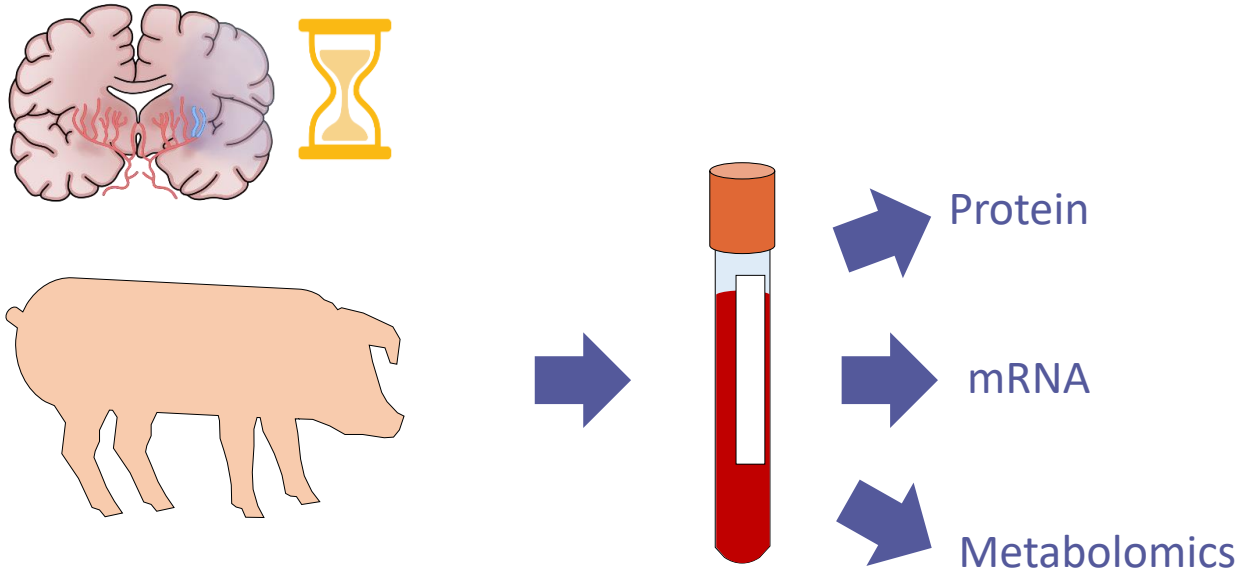
Results (III)

Blood Biomarkers

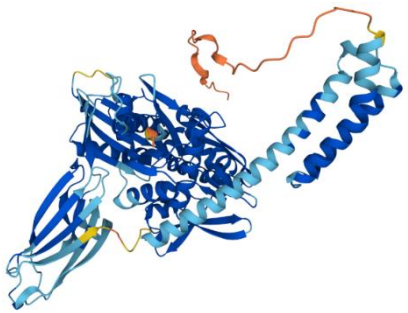


Qin C, *et al*. Signaling pathways involved in ischemic stroke: molecular mechanisms and therapeutic interventions. *Signal Transduct Target Ther*. 2022 Jul 6; 7 (1): 215



Results (III)

Protein Biomarkers



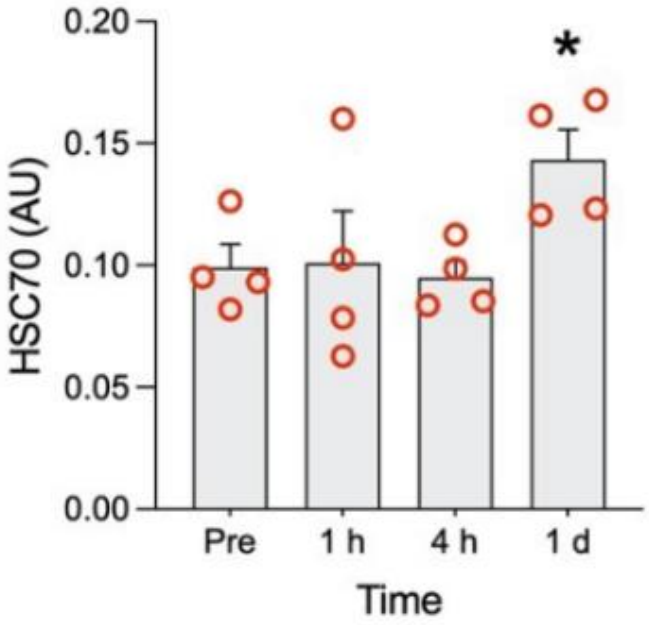
Hsc70

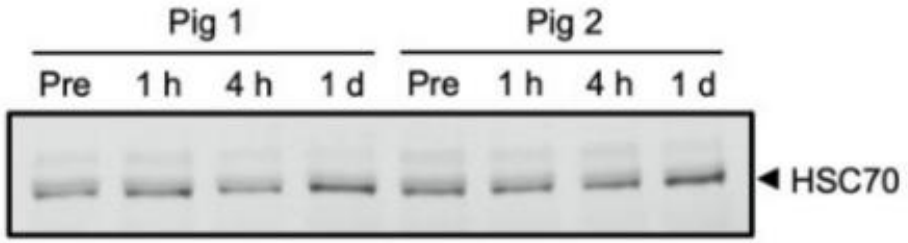
- **Blood biomarker** in a panel to differentiate IS vs HS

  Bustamante A, *et al*. Blood biomarkers for the early diagnosis of stroke: The Stroke-Chip Study. *Stroke*. 2017 Sep; 48 (9): 2419-2425.

- Predictive of seizure development

  Abraira L, *et al*. Blood biomarkers predictive of epilepsy after an acute stroke event. *Epilepsia*. 2020 Oct;61(10):2244-2253. doi: 10.1111/epi.16648.



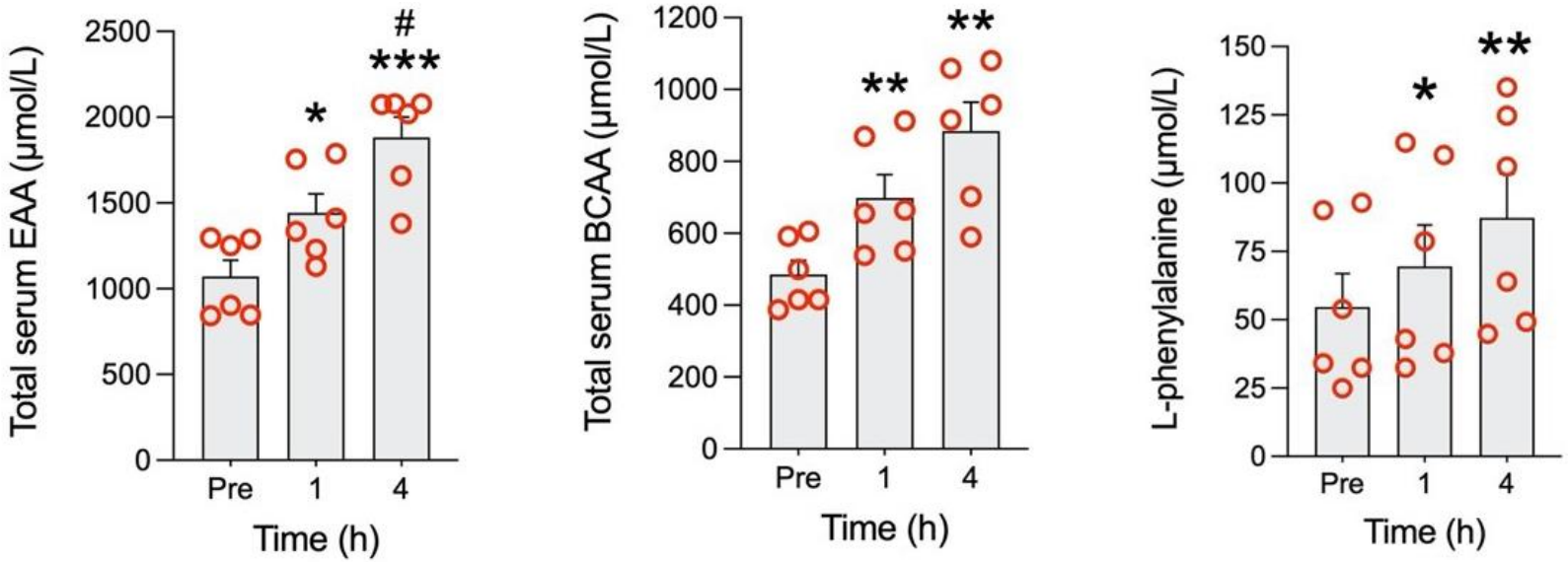


Results (III)

Metabolic Biomarkers

Essential amino acids (EAA)

- Arginine
- Histidine
- Isoleucine (Branched-chain amino acid, BCAA)
- Leucine (BCAA)
- Lysine
- Methionine
- Phenylalanine
- Threonine
- Tryptophan
- Valine (BCAA)



Phenylalanine increases to compensate glutamate increase.

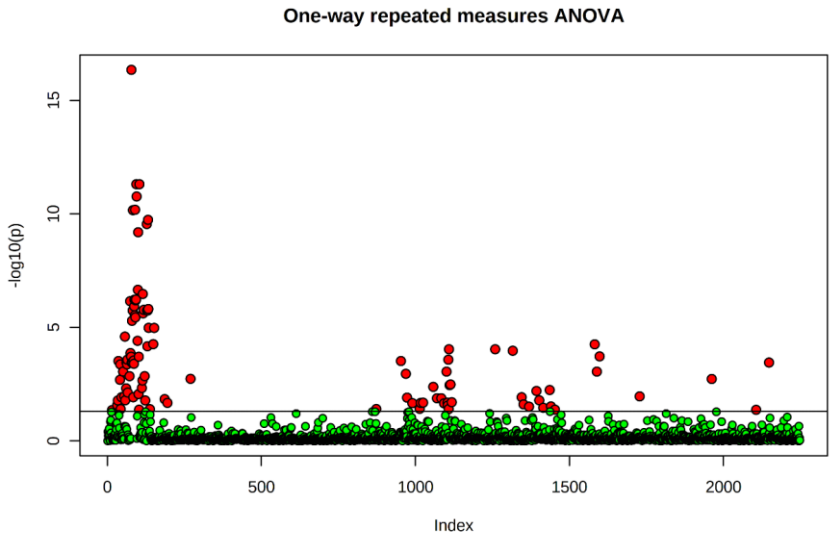
Jia J, *et al*. Application of metabolomics to the discovery of biomarkers for ischemic stroke in the murine model: A comparison with the clinical results. *Mol Neurobiol*. 2021 Dec;58(12):6415-6426.

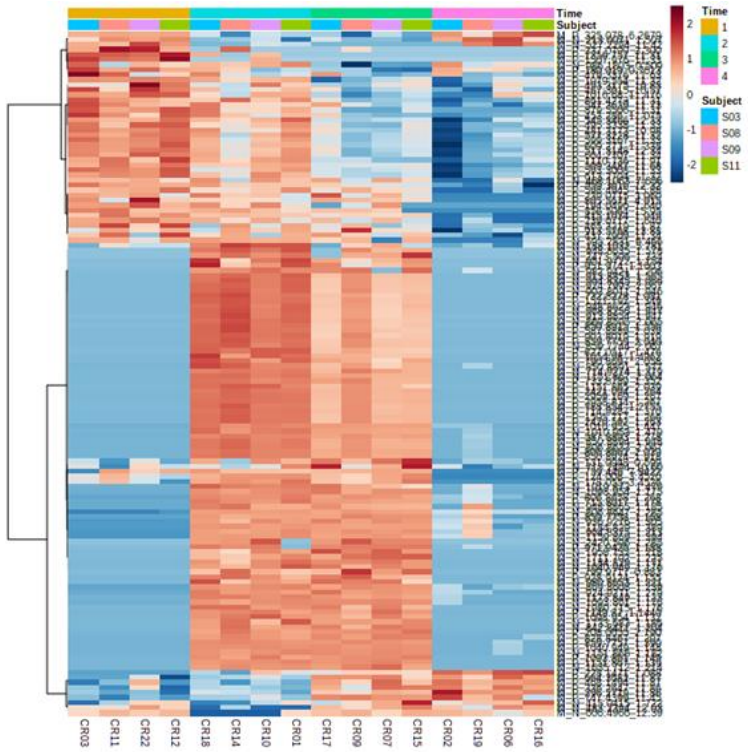
Detection of IS biomarkers in the plasma of the model

Ongoing

Metabolic Biomarkers

Untargeted metabolomics and lipidomics





IRB Lleida
Institut de Recerca Biomèdica

Collaboration with Dr. Gloria Arqué and Dr. Francisco Purroy's from **Clinical Neurosciences Research Group** (IRB Lleida)

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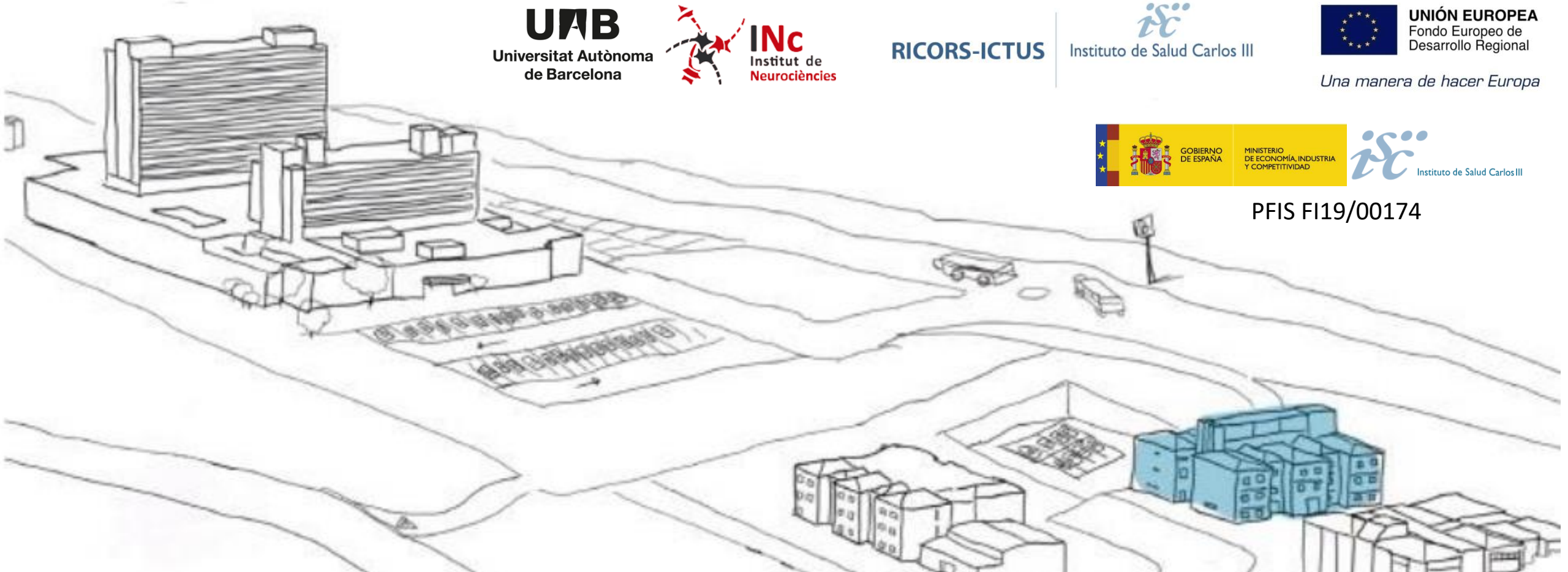
Alexia García-Serrán, PhD Student
Núria DeGregorio-Rocasolano, Postdoc
Octavi Martí-Sistac, Senior researcher
Teresa Gasull, PI

Brain Vascular Pathology Medical Group
Carlos Castaño, Neurointerventionist
Maria Rosa García-Sort, Nurse
María Hernández, Neurologist
Adrián Valls, Neurologist
Antoni Dávalos, Chief of Department

Comparative Medicine and Bioimage Centre of Catalonia (CMCiB)
Osvaldo Pino, Veterinarian
Jordi Grífols, Veterinarian
Gemma Cristina Monte, Bioimaging Manager
Josep Puig, Neuroradiologist

Canon Medical Systems
Alba Iruela
Alicia Palomar

PFIS FI19/00174



References

- Abraira L, Santamarina E, Cazorla S, *et al*. Blood biomarkers predictive of epilepsy after an acute stroke event. Epilepsia. 2020 Oct;61(10):2244-2253. doi: 10.1111/epi.16648.
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