

7-8 Junio 2022

RD21/0006/0001

IMAS12.

Instituto de Investigación Biomédica Hospital 12 de Octubre.

Ignacio Lizasoain

7-8 Junio 2022

**MADRID (IMAS12)
RD21/0006/0001**




Fundación
Centro Nacional de
Investigaciones
Cardiovasculares
Carlos III



FACULTAD DE
MEDICINA
UNIVERSIDAD COMPLUTENSE DE MADRID


Instituto de Investigación
Hospital 12 de Octubre

 **Hospital Universitario
12 de Octubre**


Instituto de Salud Carlos III


Instituto de Salud Carlos III

7-8 Junio 2022



1. Dra. María Ángeles MORO (IP)
2. Dra. M Isabel Cuartero
3. Dra. Alicia García-Culebras
4. Cristina Torres
5. Enrique Fraga
6. Sandra Vázquez
7. F Javier de Castro
8. Marcos Galán
9. Tania Jareño
10. Sandra Sacristán
11. Jorge H Matarazzo

1. Dr. Jesús M PRADILLO (IP)
2. Dr. Manuel Navarro-Oviedo
3. Dr. Juan M García-Segura
4. Dra. Olivia Hurtado
5. Cristina Granados
6. Gaohong Di
7. Nuria Alfageme
8. Miguel Angel Anta
9. Lidia García

1. Dr. Ignacio LIZASOAIN (IP)
2. Dra. Patricia CALLEJA (Unidad de Ictus)
3. Dr. Antonio Martínez-Salio
4. Dra. Ana Moraga
5. Dra. Carolina Peña-Martínez
6. Blanca Díaz
7. Lluís Alzamora
8. Fernando Ostos
9. Paloma Martín
10. Lucas Roca
11. María Gutiérrez
12. Alvaro Ruíz



EXPERIMENTAL

DISTAL ISCHEMIC STROKE MODELS

- Fibrin rich-clot thrombosis (thrombin injection)
- Platelet-rich clot thrombosis (Rose bengal and FeCl³)
- Permanent and transient ischemia (ligature and filament)

HEMORRHAGIC STROKE MODELS

- Intracerebral hemorrhages (collagenase injection)

HEMORRHAGIC TRANSFORMATION STROKE MODELS

- Fibrin rich-clot thrombosis (delayed tPA administration)

VASCULAR COGNITIVE IMPAIRMENT MODELS

- Focal hypoperfusion
- Chronic hypoperfusion (BCCAS, microcoils)
- High-sodium diet

7-8 Junio 2022



EXPERIMENTAL

Microscopy Lab (<https://www.cnic.es/en/investigacion/2/1187/tecnología>)

- Standard microscopy (confocal)
- Higher resolution microscopy
- Image analysis

Imaging Lab (<https://www.cnic.es/en/investigacion/2/7249/tecnología>)

- MRI (1Tesla, 4,7T and 7T)
- High resolution PET/CT nanoPET
- Intravital microscope
- Echocardiography
- Doppler


<https://www.redib.net/bioimac>

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Genomic/Proteomic Labs

<https://www.cnic.es/en/investigacion/2/1189/tecnología>



Maria A Moro

Neurovascular Pathophysiology





Maria A Moro

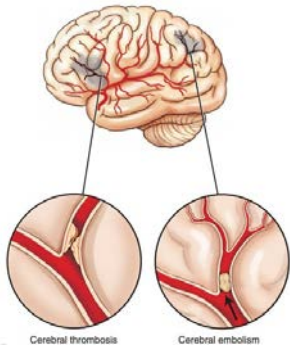
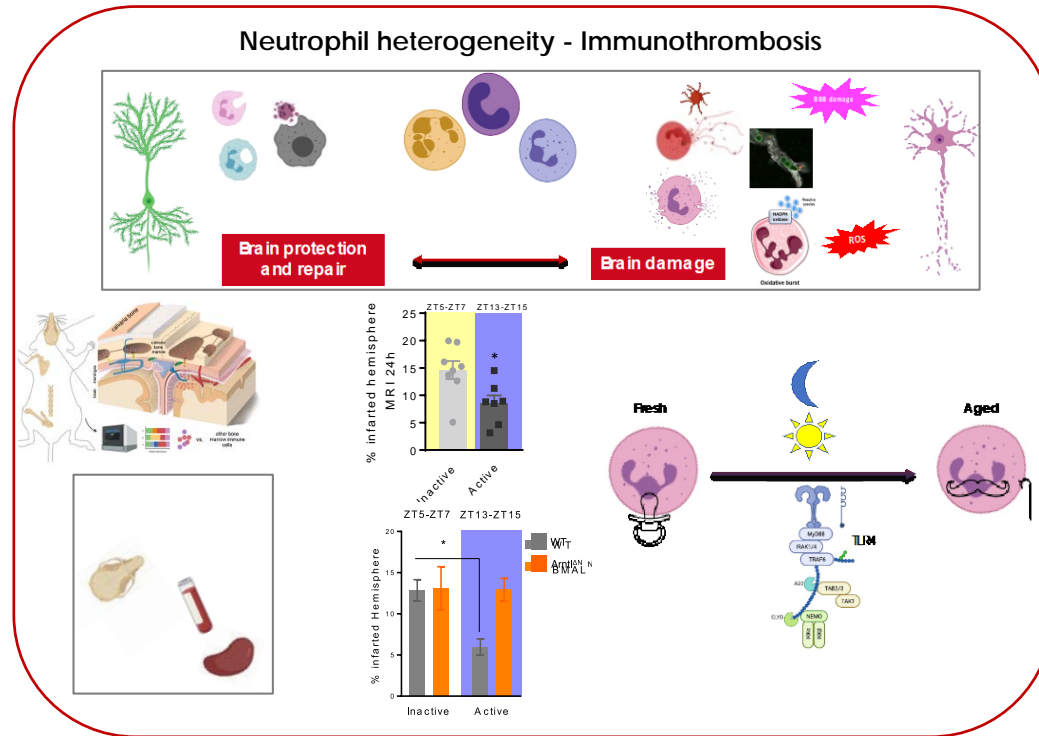
LINEAS INVESTIGACIÓN

Neurovascular Pathophysiology



ISCHAEMIC STROKE

ACUTE STROKE

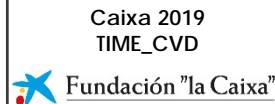


Stroke Unit



RICORS-ICTUS

- ✓ Distal or proximal middle cerebral artery occlusion (MCAO).
- ✓ MCAO by fibrin-rich thrombosis
- ✓ MCAO by platelet-rich thrombosis



TNE-19CVD01
Stroke-Impact



TNE-21CVD04
Circadian Effects on Stroke

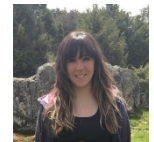
PID2019_STEMENTIA



Collaboration with
Andrés Hidalgo's lab



Maribel Cuartero



Alicia García-Culebras



Sandra Vázquez



Tania Jareño





Maria A Moro

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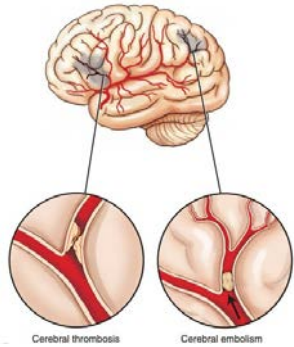
Neurovascular Pathophysiology



ISCHAEMIC STROKE

CHRONIC STROKE

POST-STROKE DEMENTIA



✓ Distal or proximal middle cerebral artery occlusion (MCAO).

✓ MCAO by fibrin-rich thrombosis

✓ MCAO by platelet-rich thrombosis

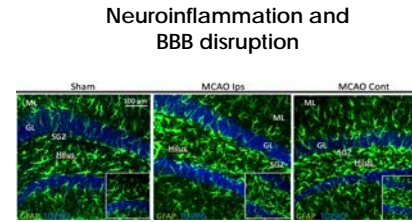
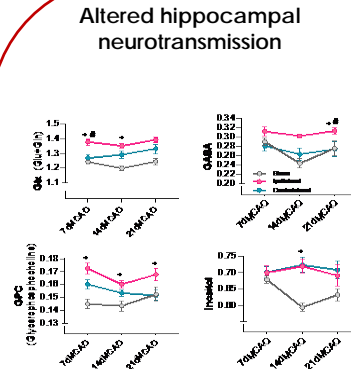


Stroke Unit

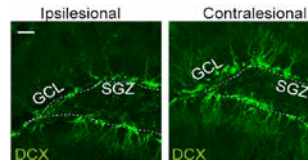


RICORS-ICTUS

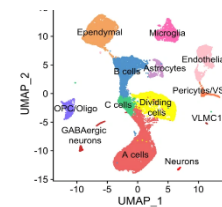
PID2019_STEMENTIA



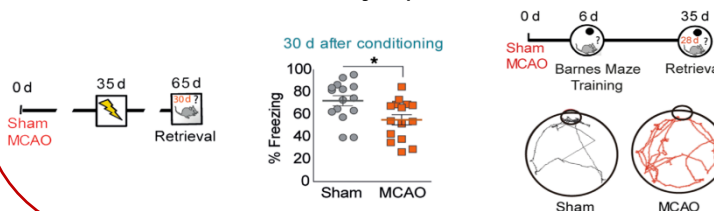
Maladaptive hippocampal neurogenesis and disrupted connectivity



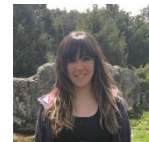
Transcriptional signatures of post-stroke hippocampal niche



Memory impairment



Maribel Cuartero



Alicia García-Culebras



Cristina Torres



Javier de Castro



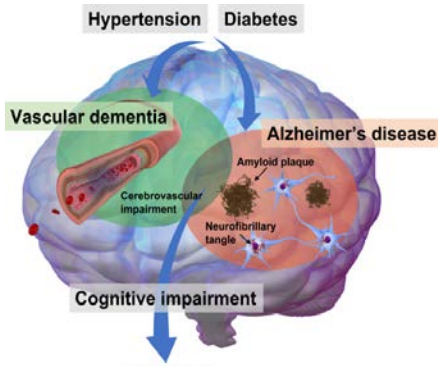
Jorge H. Matarazzo



Maria A Moro

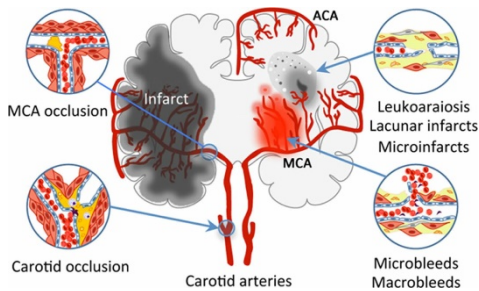
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Neurovascular Pathophysiology

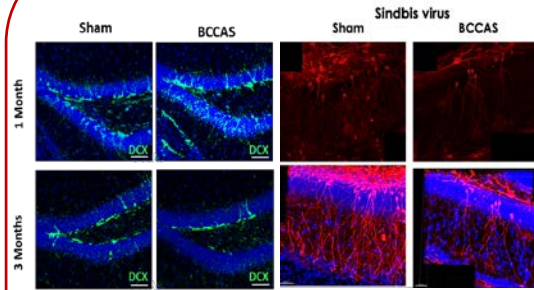


CVD-DRIVEN DEMENTIA

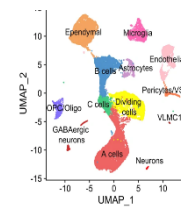
Post-stroke dementia
 Global cerebral hypoperfusion (BCCAS)
 High-sodium diet



Maladaptive neurogenesis after BCAS

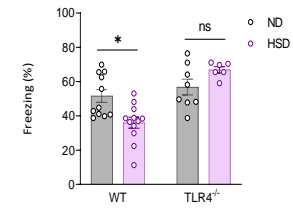


Transcriptional signatures of hippocampal neurogenic niche

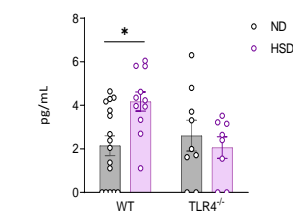


Neuroimmune activation in HSD

Contextual fear conditioning

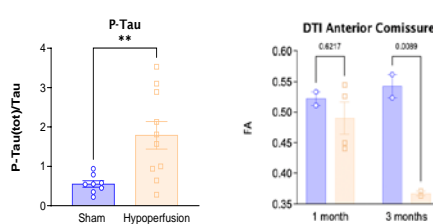


IL-17A

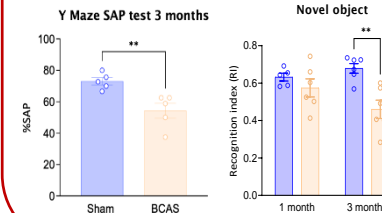
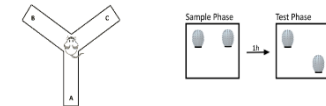


Tauopathy

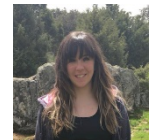
WM damage



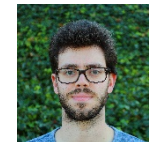
Memory impairment



Maribel Cuartero



Alicia García-Culebras



Enrique Fraga



Marcos Galán

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MINISTERIO DE SALUD



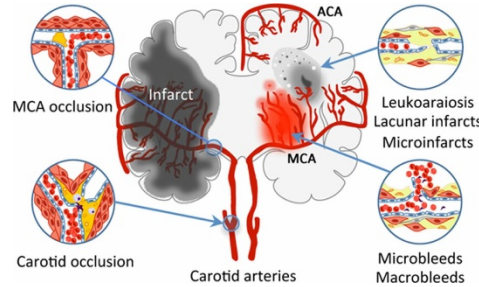
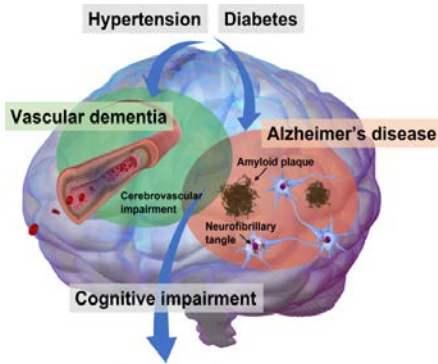
Maria A Moro

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Neurovascular Pathophysiology



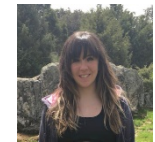
cnic Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III



CVD-DRIVEN DEMENTIA



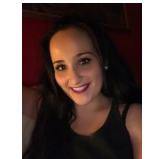
Maribel Cuartero



Alicia García-Culebras



Carmen Nieto



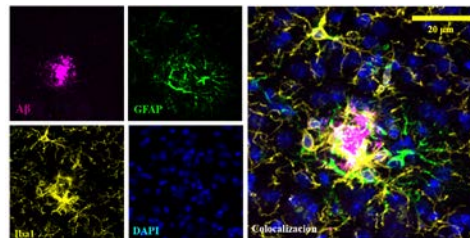
Sandra Sacristán



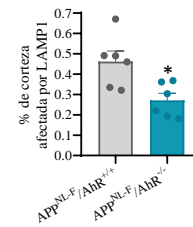
JM García-Segura

Mixed VaD/AD
APPki
APPki/AhR^{-/-}

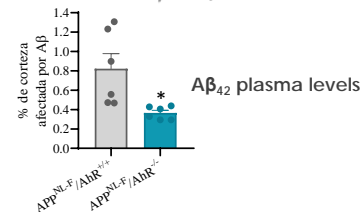
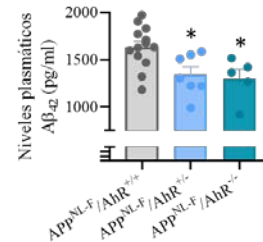
A β deposition is decreased in AhR^{-/-} mice



Amyloid plaque load



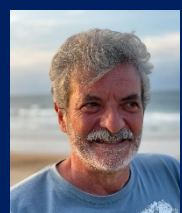
Synaptic dysfunction



PID2019_STEMENTIA



BIOIMAC
CENTRO DE BIOIMAGEN
COMPLUTENSE



Ignacio Lizasoain

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Neurovascular Research Unit



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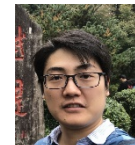
Carolina Peña



Lluís Alzamora



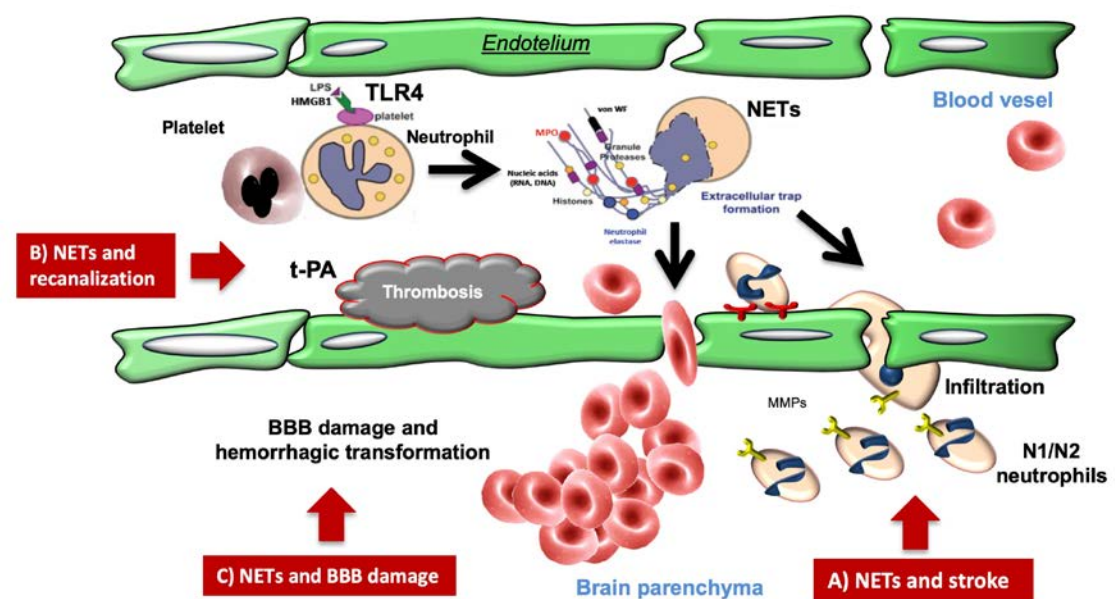
Blanca Diaz



Gaohong Di



JM García-Segura



A) NETs AND STROKE

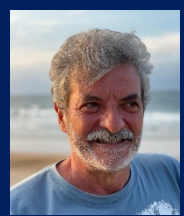
- A.1) To study the role of the neutrophil phenotype (N1 vs N2; fresh vs aged) in their inflammatory capacity and in NETosis development after stroke.
- A.2) To study the involvement of TLR4 in the neutrophil phenotype, as well as their ability to produce NETosis and cell damage after stroke.
- A.3) To determine the effect of blockers of TLR4, inhibitors of NET formation and NET lytic agents under the conditions described in A1/A2.
- A.4) To carry out a **pilot clinical study** to study the neutrophil phenotype in patients diagnosed with acute ischemic stroke depending on the time of day of the stroke onset.

B) NETs AND RECANALIZATION AFTER STROKE

- B.1) To study the role of the neutrophil phenotype in arteries recanalization after stroke.
- B.2) To study how basal levels of host DNases influence in the recanalization of the arteries after stroke.
- B.3) To determine the effect of blockers of TLR4, inhibitors of NET formation and NET lytic agents under the conditions described in B1/B2.
- B.4) To carry out a **pilot clinical study** to explore the basal levels of host DNases and its role in stroke outcome in patients diagnosed with acute ischemic stroke with/without spontaneous reperfusion and good/worse outcome respectively.

PI20/00535
NETs in STROKE

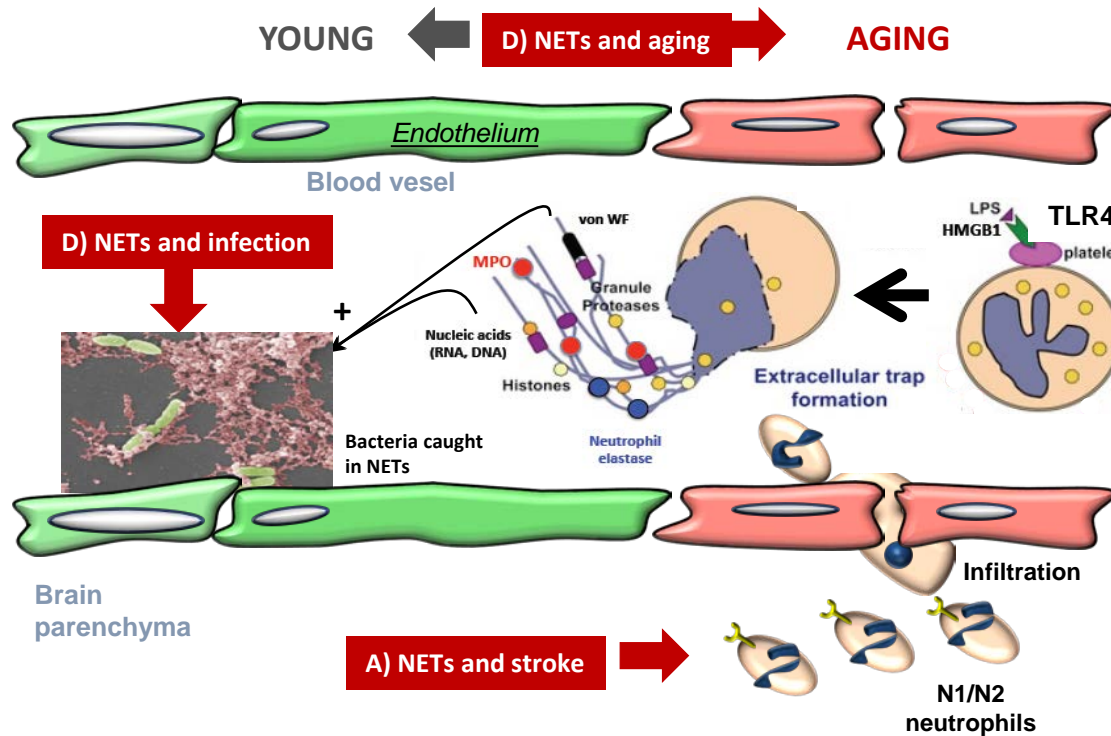




Ignacio Lizasoain

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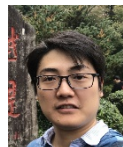
Neurovascular Research Unit



Carolina Peña



Jesus Pradillo



Gaohong Di



Cristina Granados



Miguel Angel Anta

D) NETs, AGING AND INFECTION AFTER STROKE

D.1) To study the role of aging in NET formation and subsequent brain damage after stroke using young vs old animals.

D.2) To study the role of NET formation in the stroke outcome of animals with stroke-associated infections.

D.3) To carry out a **pilot clinical study** to explore the influence of NETs formation in elderly vs young patients diagnosed with ischemic stroke.

D.4) To carry out a **pilot clinical study** to explore the influence of NETs formation in patients diagnosed with acute ischemic stroke-associated infections.

PI20/00535
NETs in STROKE





Jesús Pradillo

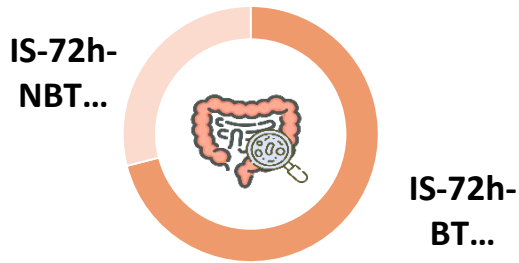
LINEAS INVESTIGACIÓN

Neurovascular Research Unit



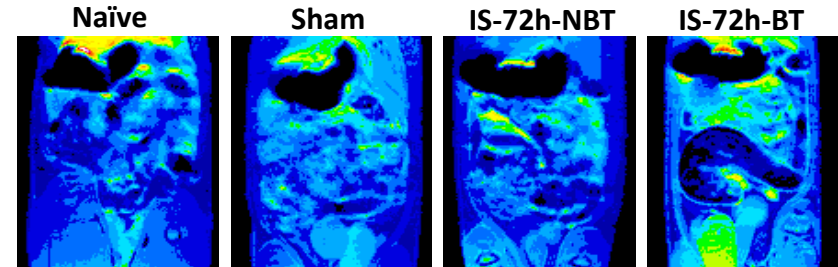
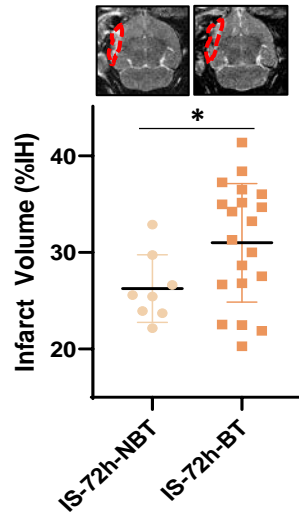
Gut barrier leakage after ischemic and hemorrhagic stroke: early detection by MRI and effect on inflammation, brain damage and outcome.

Resultados Preliminares

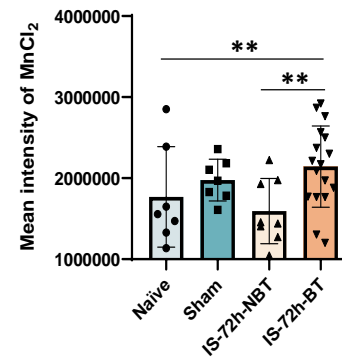


IS-NTB: Isquemia No Translocación Bacteriana

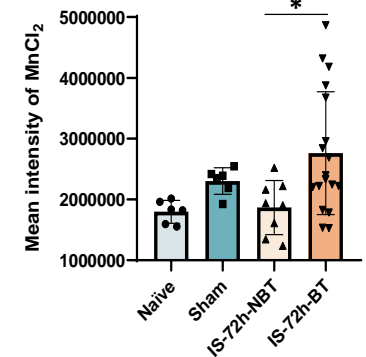
IS-TB: Isquemia Translocación Bacteriana



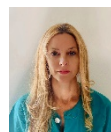
MESENTERIC NODES



SPLEEN



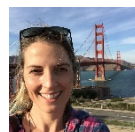
Olivia Hurtado



Patricia Calleja



Macarena Hernández



Ana Moraga



Cristina Granados



Nuria Alfageme



JM García-Segura





LINEAS INVESTIGACIÓN

Neurovascular Research Unit



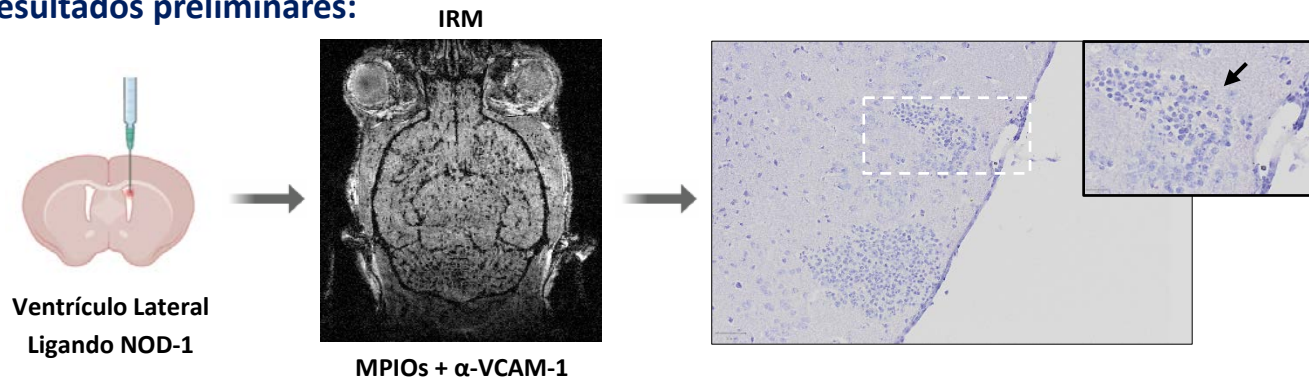
Manuel Navarro-Oviedo

Papel del receptor TOLL-LIKE 4 (TLR-4) de las células circulantes del sistema inmune en un modelo experimental de Vasculitis Cerebral Primaria

Objetivos:

- 1) **Desarrollar un modelo experimental de Vasculitis Cerebral Primaria**
 - Inyección Intraventricular de un ligando del receptor NOD-1
- 2) **Desarrollar una herramienta diagnóstica: IRM-molecular**
 - MPIOs (Micropartículas de Óxido de Hierro) + anti-VCAM-1
- 3) **Evaluar el papel del receptor TLR-4 (Inhibición genética y farmacológica)**

Resultados preliminares:



Ignacio Lizasoain



María A Moro



JM García-Segura



Proyecto Postdoctoral Margarita Salas – UCM

Proyecto en Colaboración con:

Physiopathology & Imaging of Neurological Disorders (PhIND)

- Denis Vivien
- Maxime Gauberti
- Sara Martínez de Lizarondo





Ignacio Lizasoain Patricia Calleja

LINEAS INVESTIGACIÓN

Neurovascular Diseases Group



Start
October 2018

Present
June 2022:
374 patients recruited
(428 candidates)



Antonio
Martinez-Salio



Ana Moraga



Blanca Díaz



Lluís Alzamora



Fernando Ostos



Paloma Martín



Lucas Roca



María Gutierrez

PI20/00535
NETs in STROKE





Ignacio Lizasoain Patricia Calleja

LINEAS INVESTIGACIÓN

Neurovascular Diseases Group

i+12
Instituto de Investigación
Hospital 12 de Octubre



Inclusion Criteria

- Age ≥ 18 years old
- Patients suffering ischemic stroke
- ≤ 9 hours since onset of symptoms / Wake-up stroke (established neurological symptoms assessed by NIHSS)
- Previously independent patients (previous modified Rankin Score ≤ 2)
- Patients admitted to the Stroke Unit

Exclusion Criteria

- Transitory Ischemic Attack or lacunar infarction.
- Bleeding secondary to underlying traumatic injury or subarachnoid hemorrhage
- Stroke, myocardial infarction, major surgery or systemic infection in the last 3 months
- Severe systemic disease: cancer, chronic kidney disease undergoing hemodialysis, liver failure
- Systemic inflammatory disease that is active or under treatment
- Pregnancy or puerperium
- Participation in other analytical research studies such as an acute phase clinical trial

PI20/00535
NETs in STROKE





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Neurovascular Diseases Group

i+12
Instituto de Investigación
Hospital 12 de Octubre



BASE DE DATOS

Episode Information

1. Date and time of onset / last time seen well
2. Known-onset / Wake-up Stroke
3. Treatment: tPA, thrombectomy
4. Infarct Volume (cc): MRI DWI (admission) or CT control scan (48h)
5. Occlusion territory
6. Etiology (atherothrombotic, cardioembolic, unknown, mixed causes)
7. ASPECTS (MCA territory)
8. TICI score (if thrombectomy)
9. Hemorrhagic transformation
10. Door-to-needle time
11. Door-to-puncture time
12. Time to reperfusion

Sample Processing information

1. Blood extraction time
2. Blood processing time

Patient Data and History

1. Age, Sex
2. CV Risk factors: smoking, alcohol use, hypertension,...
3. Previous medication: anticoagulant/antiplatelet agents
4. Mechanical valve prosthesis
5. History of prior hemorrhagic/ ischemic stroke
6. History of myocardial Infarction
7. Peripheral arterial disease
8. CKD

Neurological Status Scores

1. NIHSS (ER)
2. NIHSS (Stroke Unit)
3. Previous mRS
4. 3 month mRS

ER Constants and Blood Tests

1. Blood Pressure Readings (ER)
2. Blood test Biochemistry panel, cell counts and coagulation parameters (ER and at Stroke Unit)
 1. Creatinine
 2. Glucemia
 3. C Reactive Protein
 4. Leukocytes
 5. Neutrophils
 6. Lymphocytes
 7. Monocytes
 8. Platelets
 9. INR

NETs Studies

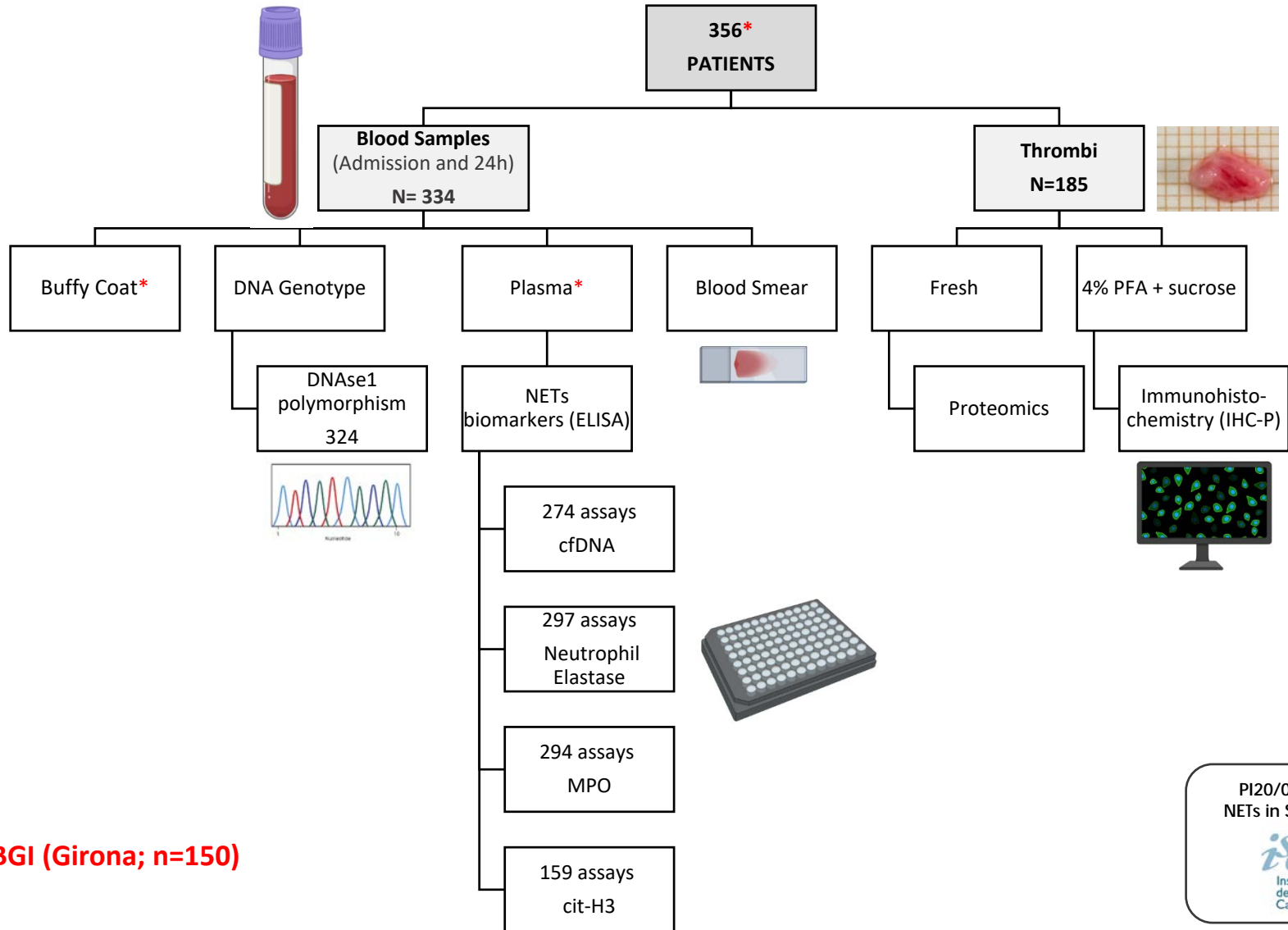
1. Plasma NETosis Biomarkers
 1. Cit-Histone 3
 2. Myeloperoxidase
 3. Neutrophil Elastase
 4. Cell-free dsDNA
2. DNase1 Polymorphism Phenotype
3. Thrombi Composition Determination: IHC-P and Proteomics



Ignacio Lizasoain Patricia Calleja

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Neurovascular Diseases Group



*IDIBGI (Girona; n=150)

PI20/00535
NETs in STROKE

Instituto de Salud Carlos III



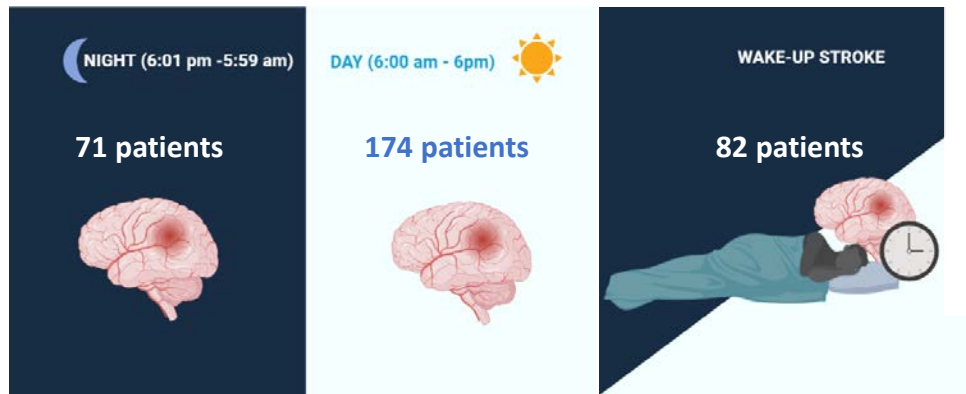
Ignacio Lizasoain Patricia Calleja

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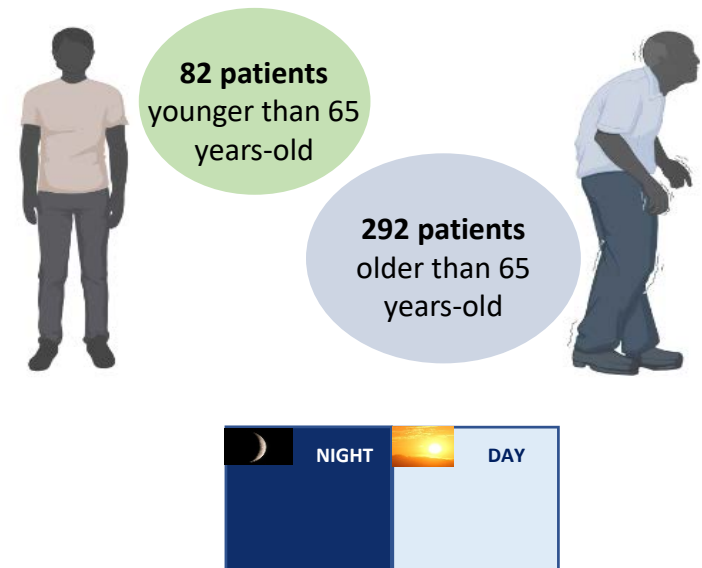
Neurovascular Diseases Group



A) NETs and STROKE



B) NETs and AGING



PI20/00535
NETs in STROKE





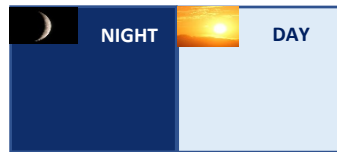
Ignacio Lizasoain Patricia Calleja

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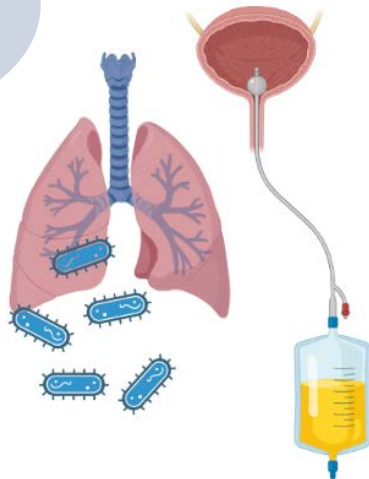
Neurovascular Diseases Group



C) NETs and INFECTION



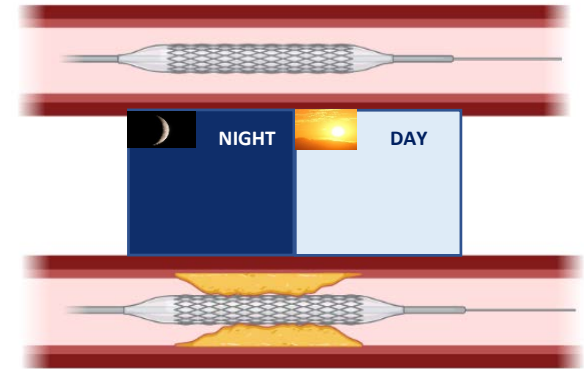
75 patients
with development of
infections
during their in-
hospital stay



D) NETs and REPERFUSION

175 patients
with complete
reperfusion (TICI 2c/3)

46 patients
with incomplete
reperfusion (TICI 0-2b)



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NETs in STROKE

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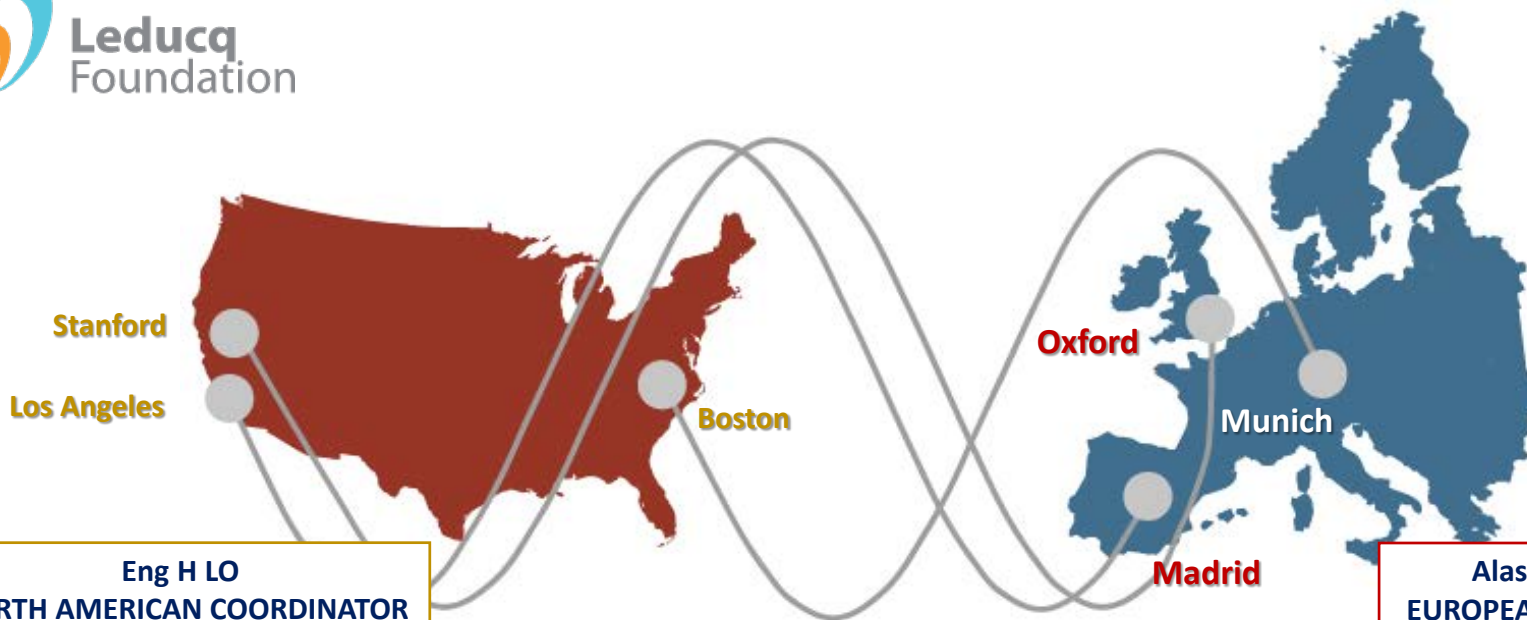
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