Instituto de Investigación Hospital 12 de Octubre

Instituto de Salud Carlos III

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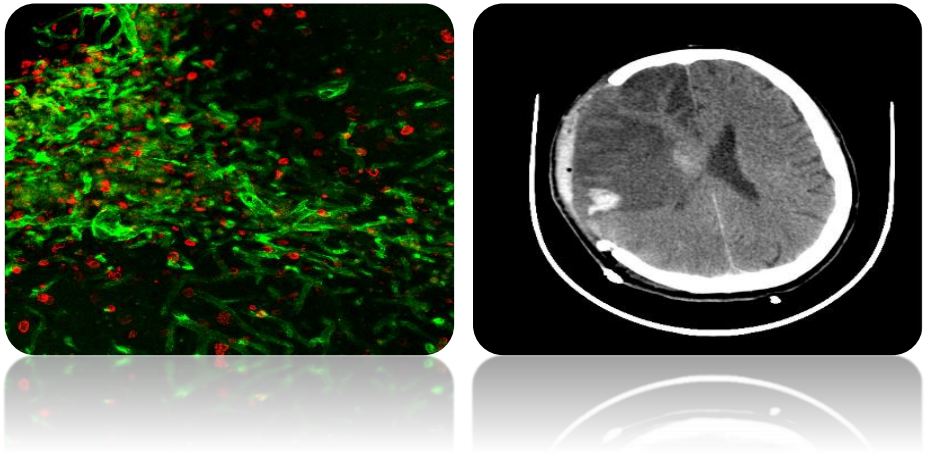
NextGenerationEU

Plan de Recuperación, Transformación y Resiliencia
Financiado por la Unión Europea NextGenerationEU

II Congreso Anual de Ictus
RICORS-ICTUS



Una historia de investigación traslacional en cerebroprotección: de los estudios preclínicos a los ensayos clínicos

IGNACIO LIZASOAIN
MACARENA HERNÁNDEZ

What do we need to develop a pharmacological treatment?

1. **Identify the problem: STROKE**
2. Study the problem in depth
3. Generate new ideas – hypothesis and results
4. Contrast my hypothesis - safety and efficacy studies
    - 4.1 Non-regulatory animal studies
    - 4.2 Funding and intellectual property
    - 4.3 Regulatory animal studies
    - 4.4 Clinical trials

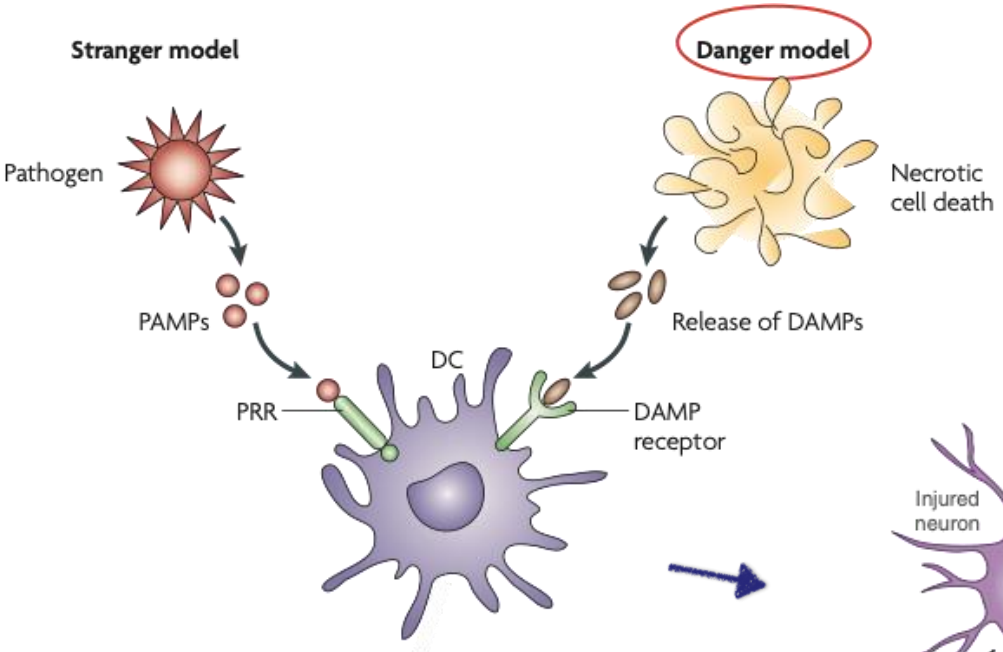
2. Study the problem in depth

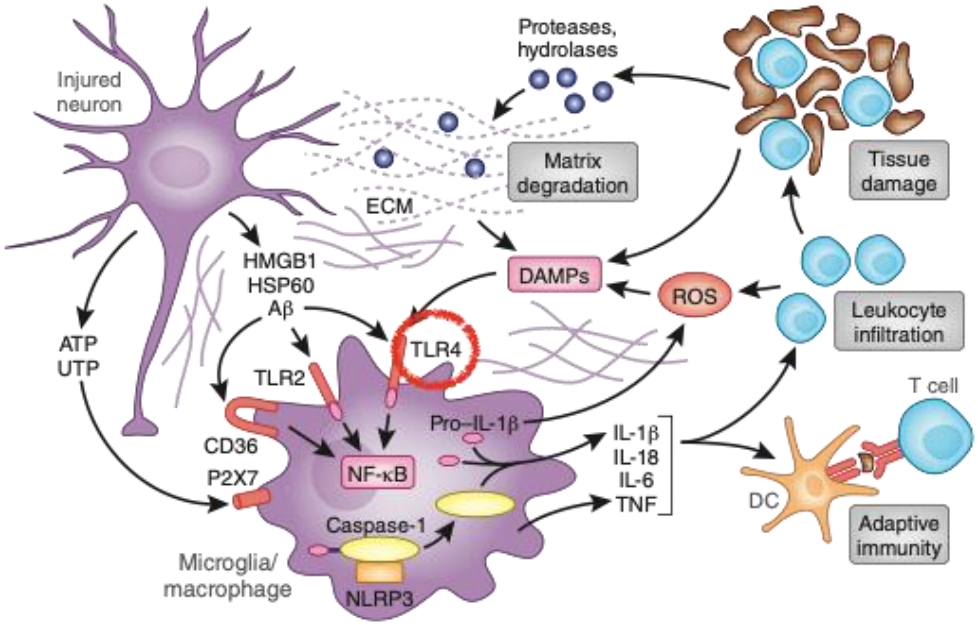
ISCHEMIC STROKE TREATMENT
Cerebral Perfusion

	Therapeutic window	Limitations
Thrombolysis (t-PA)	0 - 4.5 hours	• Hemorrhagic Transformation (HT) • Low recanalization rates (<50%)
Endovascular thrombectomy	0 – 24 hours	• Large vessel occlusion • Qualif interventional neuroradiologist • High cost infrastructure

(*Henderson, Weitz & Yim, 2018*)
(*Joung-Ho & Jeffrey, 2007*)

3. Generate new ideas – HYPOTHESIS





Innate immunity (TLR4)
is involved in the evolution of stroke

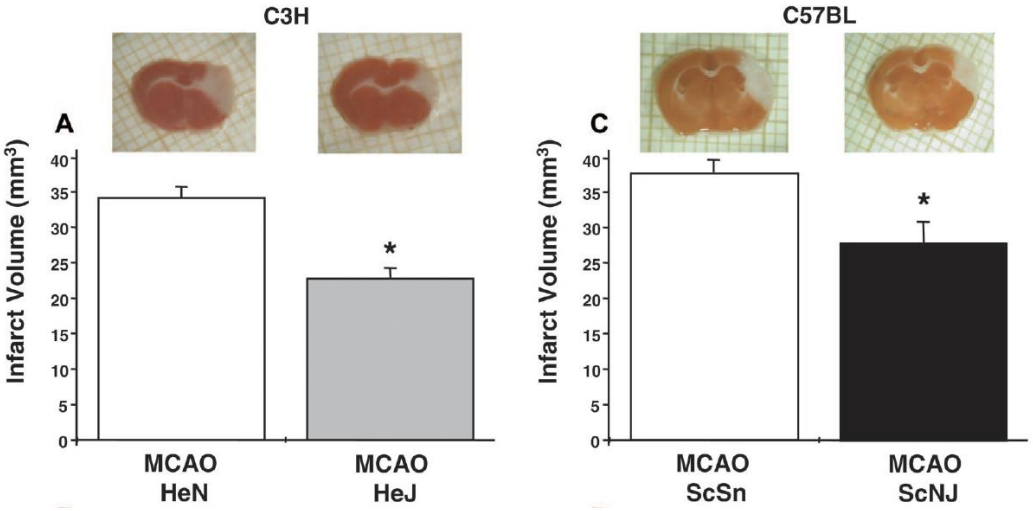
3. Generate new ideas – RESULTS

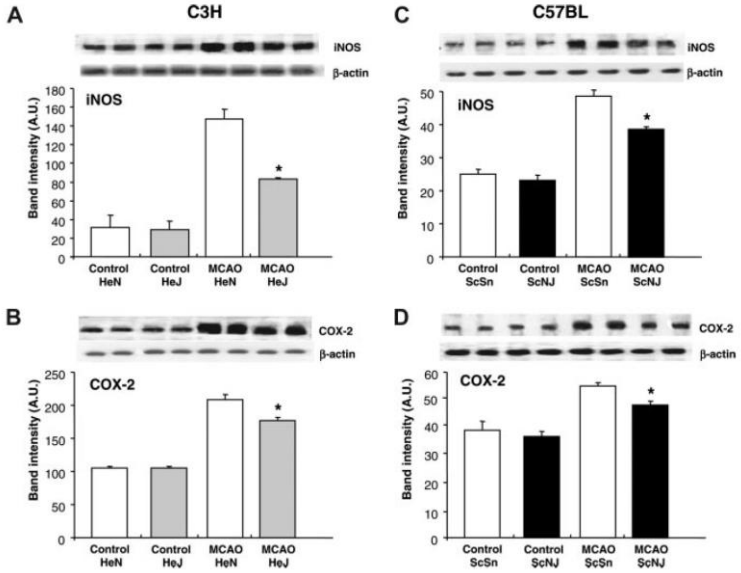
Toll-Like Receptor 4 Is Involved in Brain Damage and Inflammation After Experimental Stroke

Javier R. Caso, Jesús M. Pradillo, Olivia Hurtado, Pedro Lorenzo, María A. Moro and Ignacio Lizasoain

Circulation. 2007;115:1599-1608.

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION



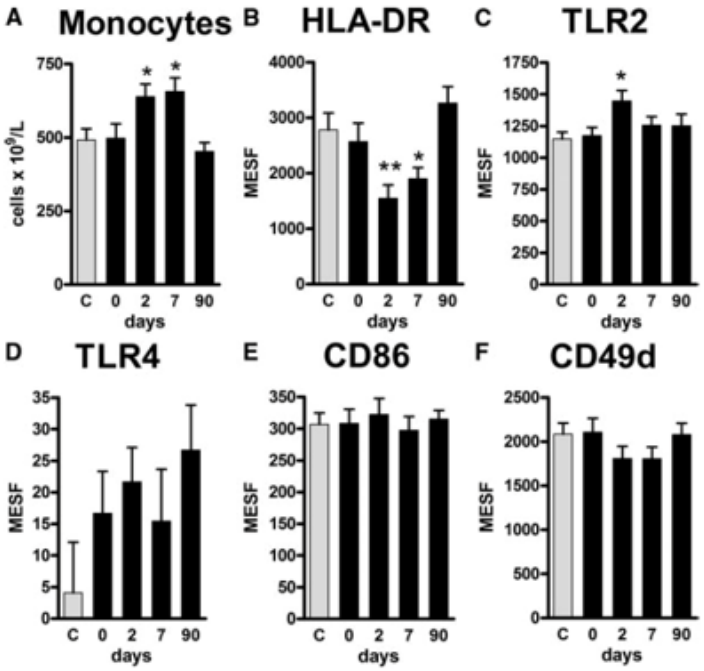


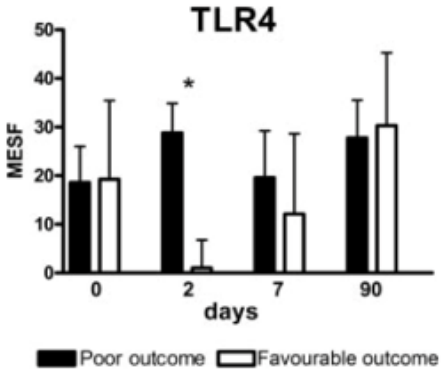
3. Generate new ideas – RESULTS

Monocytes Are Major Players in the Prognosis and Risk of Infection After Acute Stroke

Xabier Urra, Álvaro Cervera, Víctor Obach, Núria Climent, Anna M. Planas and Ángel Chamorro

Stroke 2009;40;1262-1268; originally published online Jan 22, 2009;





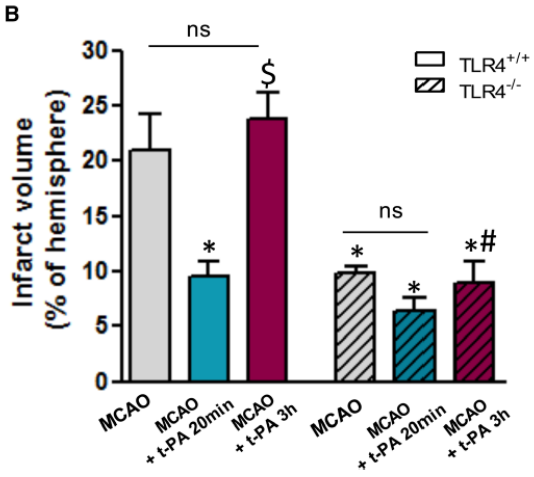
TLR4 in circulating monocytes participates in acute damage after stroke in patients

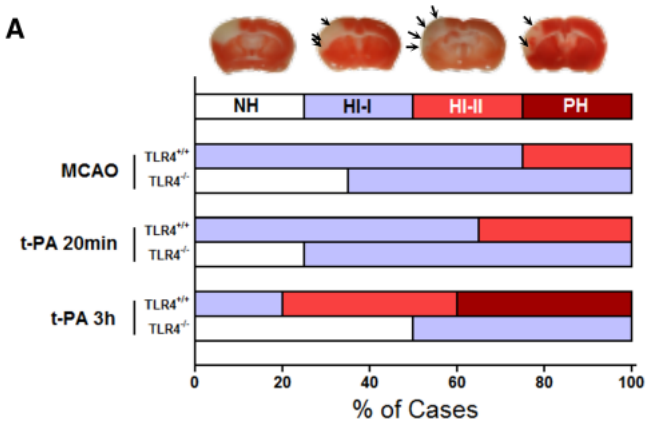
3. Generate new ideas – RESULTS

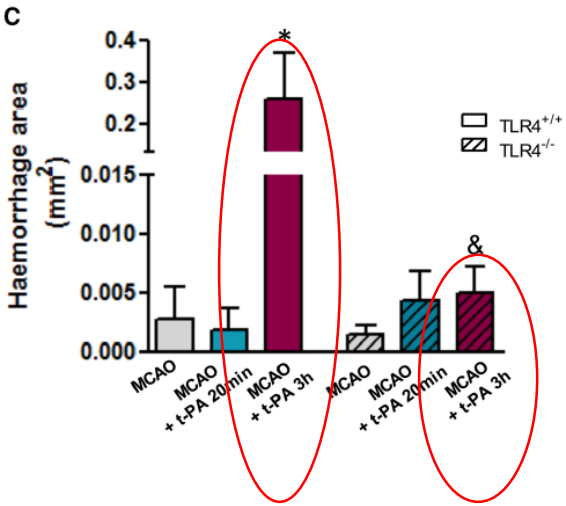
Toll-Like Receptor 4 Mediates Hemorrhagic Transformation After Delayed Tissue Plasminogen Activator Administration in In Situ Thromboembolic Stroke

Stroke. 2017;48:1695-1699.

Alicia García-Culebras, MSc*; Sara Palma-Tortosa, MSc*; Ana Moraga, PhD; Isaac García-Yébenes, PhD; Violeta Durán-Laforet, MSc; Maria I. Cuartero, PhD; Juan de la Parra, MSc; Ana L. Barrios-Muñoz, MSc; Jaime Díaz-Guzmán, MD, PhD; Jesús M. Pradillo, PhD; María A. Moro, PhD†; Ignacio Lizasoain, MD, PhD†







HI: hemorrhagic infarction
PH: parenchymal hemorrhage

HAEMORRHAGIC TRANSFORMATION

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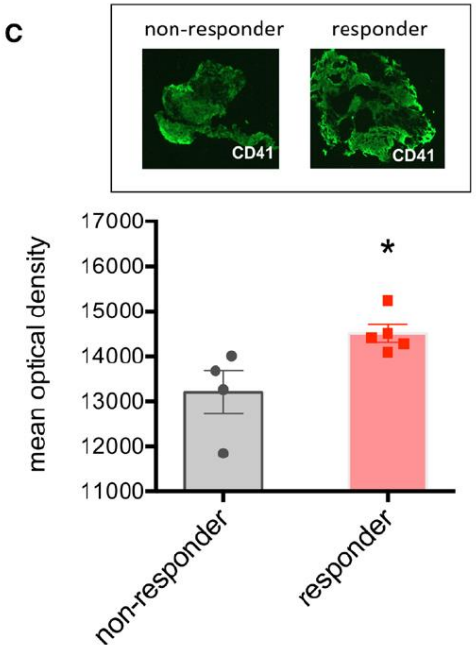
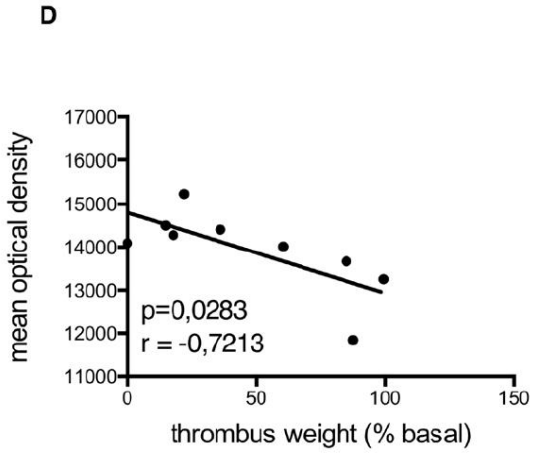
Pharmacological Modulation of Neutrophil Extracellular Traps Reverses Thrombotic Stroke tPA (Tissue-Type Plasminogen Activator) Resistance

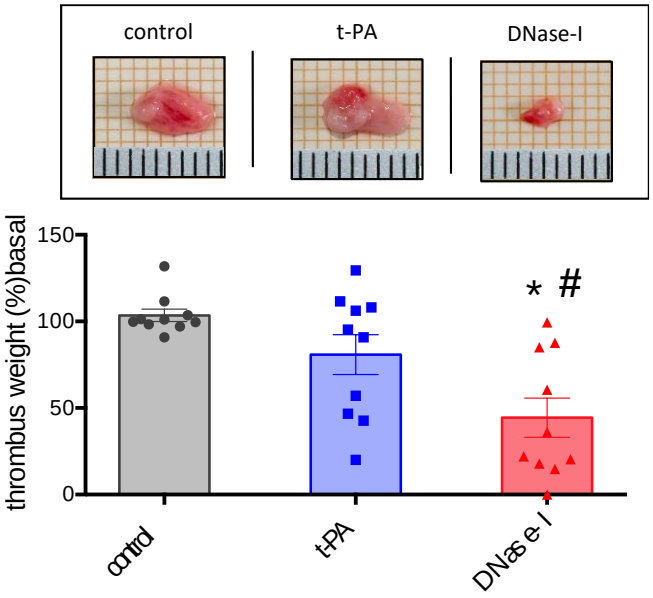
Carolina Peña-Martínez, MSc*; Violeta Durán-Laforet, MSc*; Alicia García-Culebras, PhD*; Fernando Ostos, MD; Macarena Hernández-Jiménez, PhD; Isabel Bravo-Ferrer, PhD; Alberto Pérez-Ruiz, MSc; Federico Ballenilla, MD; Jaime Díaz-Guzmán, MD, PhD; Jesús M. Pradillo, PhD; Ignacio Lizasoain, MD, PhD; María A. Moro, PhD

Stroke. 2019;50:3228-3237.

NETs are involved in thrombus formation in human stroke and may account for t-PA resistance



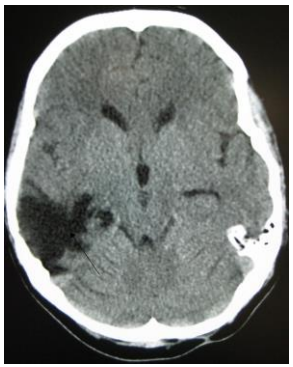




Response to DNAse depends on platelet (CD41) content

TLR4

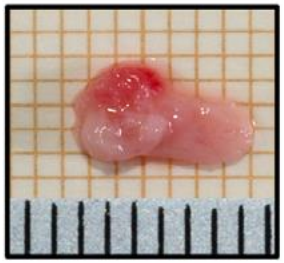
APTAMER



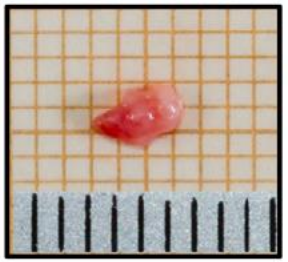
Brain damage

Hemorrhagic Transformation

t-PA



DNase-I



t-PA resistance and NETs

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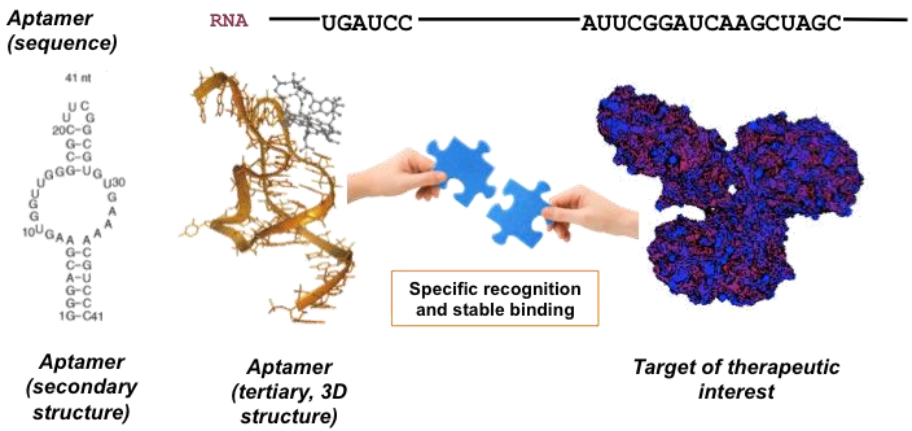
aptaTargets

Biopharmaceutical Focused on the development of therapeutic aptamers

Drugs targeting TLR4 based on aptamers

- **Nucleic acids** of single chain (ssDNA and RNA)
- Stable 3D structure in physiological conditions according to their nucleotide sequence
- Due to this 3D structure, they can specifically bind a particular target molecule (protein, small molecule, chemical, etc.) in a stable manner



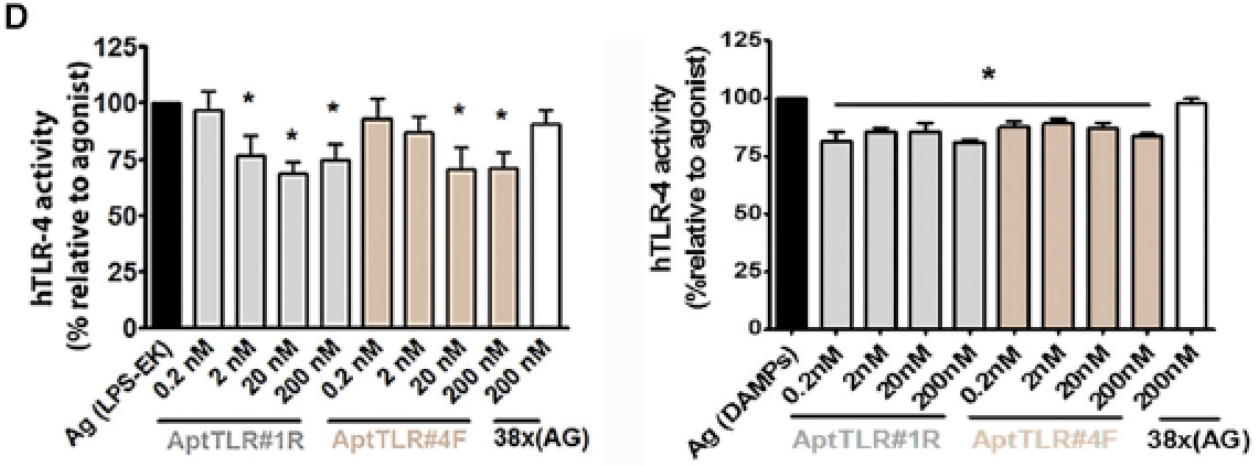
4.1 Non-regulatory animal studies

Identification of aptamers with highest hTLR4 binding affinity.

A

Aptamer	Sequence	nt	%A	%T	%G	%C
ApTLR#1R	gttgctcgtatttagggccaccggcacgggacaaggcgcgggacggcgtagatcaggtcgacaccagtcttcatccgc	78	19	18	33	29
ApTLR#1F	gcggatgaagactggtgtcgacctgatctacgccgtcccgcgccttgtcccgtgccggtggccctaaatacgagcaac	78	17	19	30	34
ApTLR#2R	gttgctcgtatttagggcacacacgcacgaagaccttggctgcccgttgtacaccagtcttcatccgc	68	19	25	24	32
ApTLR#2F	gcggatgaagactggtgtacaacgggcagccaaggtcttcgtgcgtgtgtgccctaaatacgagcaac	68	25	19	32	24
ApTLR#3R	gttgctcgtatttagggcaccgaggtcaccgaacttggtgtgcacagttgttggcgcgacaccagtcttcatccgc	76	17	26	29	28
ApTLR#3F	gcggatgaagactggtgtcgcgccaacaactgtgcacaccaagttcggtgacctcggtgccctaaatacgagcaac	76	26	17	28	29
ApTLR#4R	gttgctcgtatttagggccaaccgagtacatgctaacgcggcgatatggtttattggcacaccagtcttcatccgc	76	21	28	25	26
ApTLR#4F	gcggatgaagactggtgtgccaataaaccatatcgccgcgttagcatgtactcggttggccctaaatacgagcaac	76	27	21	26	25
ApTLR#5R	gttgctcgtatttagggccacatatgtgcacatcacaatccgcagagctgcacctacgacaccagtcttcatccgc	76	23	24	20	33
ApTLR#5F	gcggatgaagactggtgtcgtaggtgcagctctgcggattgtgatgtgcacatatgtggccctaaatacgagcaac	76	23	24	33	20
ApTLR#6R	gttgctcgtatttagggccaaggaaaaccccctggtcactggtactaatccgatccgtacaccagtcttcatccgc	76	22	25	21	32
ApTLR#6F	gcggatgaagactggtgtacggatcggattagtaccagtgaccaggggtttttccttggccctaaatacgagcaac	76	24	22	32	21
ApTLR#7R	gttgctcgtatttagggcgggtcaccacggaagagtgtagatacatagatacagtccgacaccagtcttcatccgc	76	24	24	26	25
ApTLR#7F	gcggatgaagactggtgtcggactgtatctatgtatctacactcttccgtggtgacccgccctaaatacgagcaac	76	23	25	25	26

D



TLR4-Binding DNA Aptamers Show a Protective Effect against Acute Stroke in Animal Models

Gerónimo Fernández,[1,7] Ana Moraga,[2,3,7] María I. Cuartero,[2,3,7] Alicia García-Culebras,[2,3] Carolina Peña-Martínez,[2,3] Jesús M. Pradillo,[2,3] Macarena Hernández-Jiménez,[4] Silvia Sacristán,[5] M. Irene Ayuso,[6] Rafael Gonzalo-Gobernado,[6] David Fernández-López,[2,3] M. Elena Martín,[5] María A. Moro,[2,3] Victor M. González,[5] and Ignacio Lizasoain[2,3]

Molecular Therapy Vol. 26 No 8 August 2018

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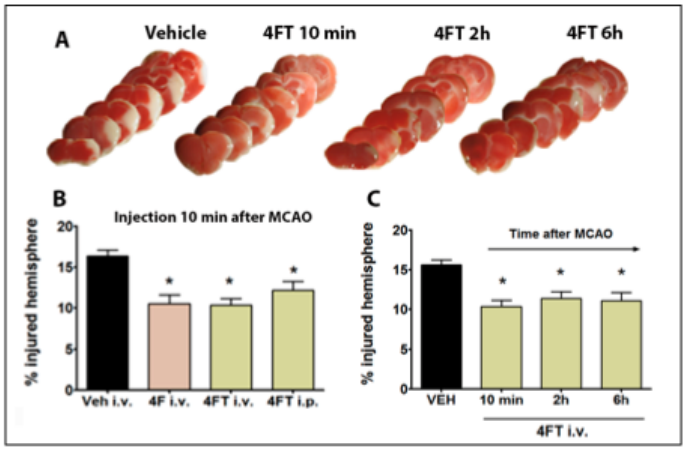
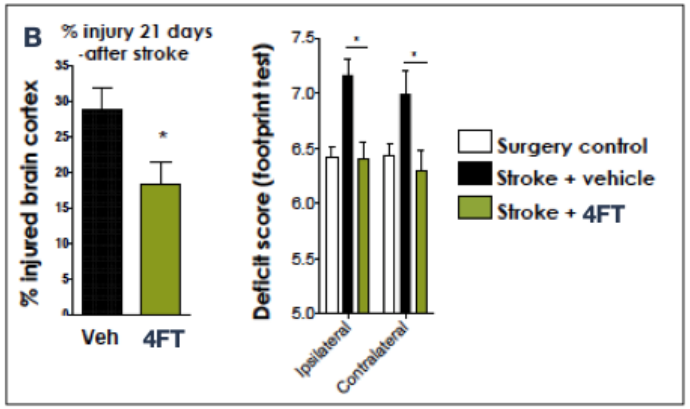
TLR4-Binding DNA Aptamers Show a Protective Effect against Acute Stroke in Animal Models

Molecular Therapy Vol. 26 No 8 August 2018

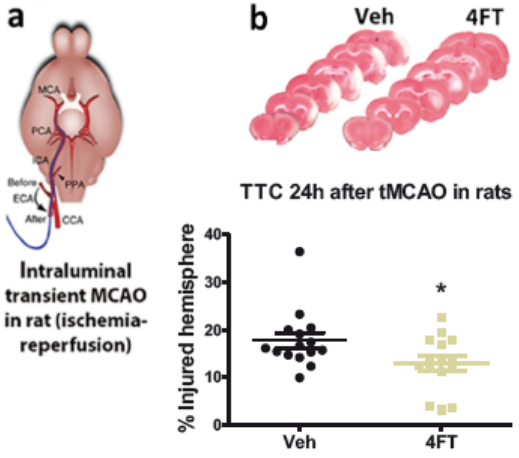
Gerónimo Fernández,[1,7] Ana Moraga,[2,3,7] María I. Cuartero,[2,3,7] Alicia García-Culebras,[2,3] Carolina Peña-Martínez,[2,3] Jesús M. Pradillo,[2,3] Macarena Hernández-Jiménez,[4] Silvia Sacristán,[5] M. Irene Ayuso,[6] Rafael Gonzalo-Gobernado,[6] David Fernández-López,[2,3] M. Elena Martín,[5] María A. Moro,[2,3] Victor M. González,[5] and Ignacio Lizasoain[2,3]

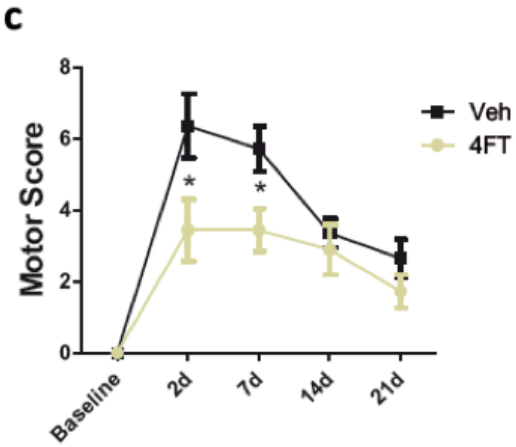
Permanent MCAO model (mice)





Transient MCAO model (rat





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EFFECT OF EARLY ADMINISTRATION OF THE APTAMER APTOLL IN A MODEL OF HEMORRHAGIC STROKE IN RATS

D. Quinto-Alemany[1], J.M. Pradillo[1], M. Hernández-Jiménez[2], D. Piñeiro[2], M.E. Fernández-Valle[1], D. Castejón[1], M. Moro[3] and I. Lizasoain[4,5]

European Stroke Conference 2021

Article

Targeting TLR4 with ApTOLL Improves Heart Function in Response to Coronary Ischemia Reperfusion in Pigs Undergoing Acute Myocardial Infarction

Rafael Ramirez-Carracedo [1,†], Laura Tesoro [1,†], Ignacio Hernandez [1], Javier Diez-Mata [1], David Piñeiro [2], Macarena Hernandez-Jimenez [2], Jose Luis Zamorano [3] and Carlos Zaragoza [1,*]

Biomolecules **2020**, *10*, 1167; doi:10.3390/biom10081167

Supporting central nervous system neuroprotection and remyelination by specific TLR4 antagonism

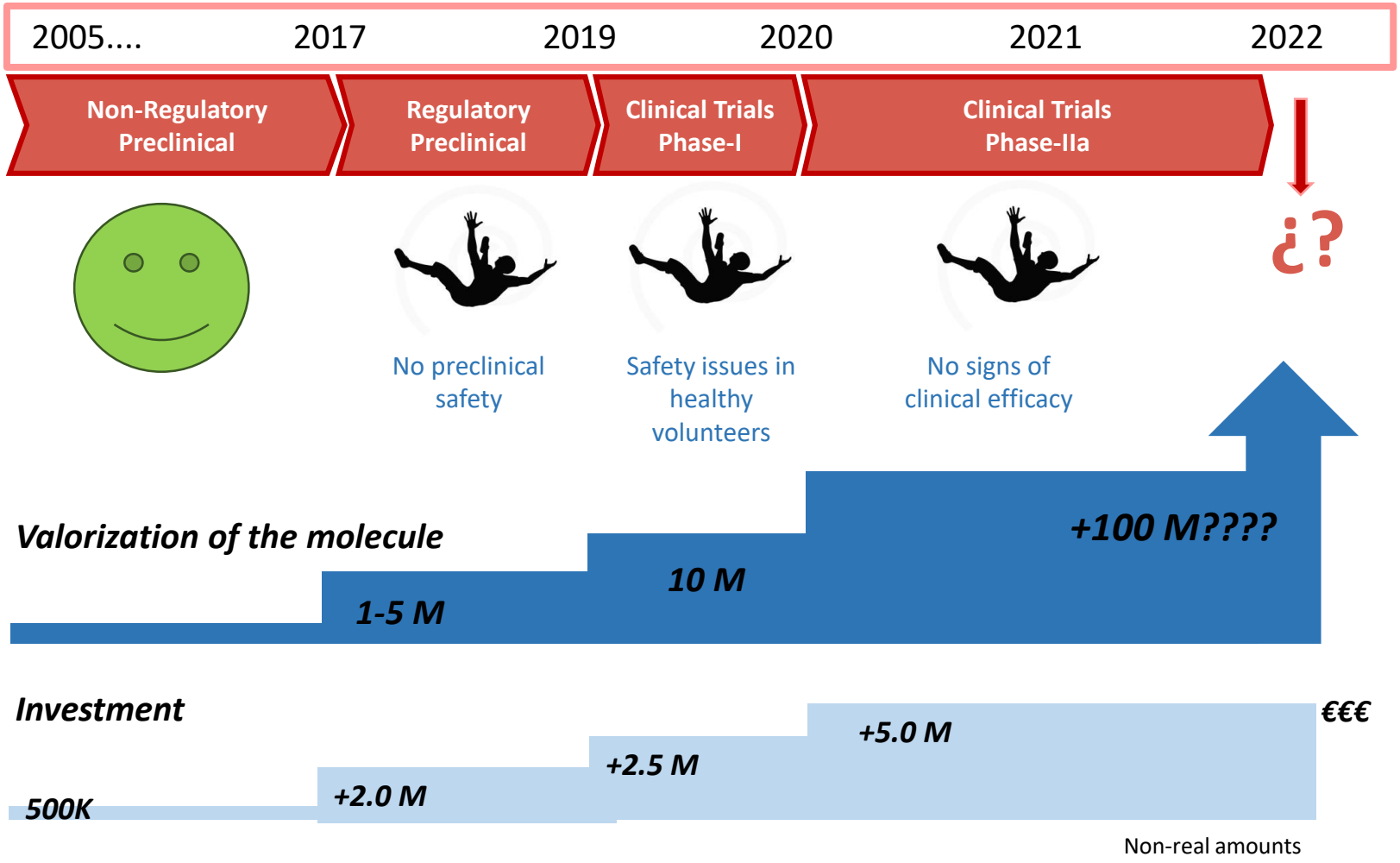
Beatriz Fernández-Gómez, Miguel A. Marchena, David Piñeiro, Yolanda Laó, Gloria Valencia, Sonia Nocera, Rocío Benítez-Fernández, Paula Gómez-Martín, Ana M. Castaño-León, Alfonso Lagares, Macarena Hernández-Jiménez, Fernando de Castro

doi: https://doi.org/10.1101/2023.01.22.524916

Beneficial effect of TLR4 blockade by a specific aptamer antagonist after acute myocardial infarction

Marta Paz-García [a], Adrián Povo-Retana [a], Rafael I. Jaén [a], Patricia Prieto [b], Diego A. Peraza [a], Carlos Zaragoza [c,d], Macarena Hernandez-Jimenez [e], David Pineiro [e], Javier Regadera [f], María L. García-Bermejo [g], E. Macarena Rodríguez-Serrano [g], Sergio Sánchez-García [a], María A. Moro [h], Ignacio Lizasoaín [i], Carmen Delgado [a,d], Carmen Valenzuela [a,d], Lisardo Boscá [a,d,j,*]

Biomedicine & Pharmacotherapy 158 (2023) 114214



Non-real amounts

4.2 FUNDING and intellectual property

Public and Private funding

aptaTargets

Local

GOBIERNO DE ESPAÑA · MINISTERIO DE CIENCIA, INNOVACIÓN Y UNIVERSIDADES

CDTI

Convocatoria de ayudas Proyectos de colaboración público-privada

International

- Venture capital
  - BioPharma
  - General
- Business angels
- FF

One-to-one partnering meetings (30-min): funds, venture capital, pharma industry, providers, CROs, consultants, recruiters, vendors, universities… everyone is there.

CROs: Contract Research Organization
CMOs: Contract Manufacturing Organization

Investor's roadshow takes time (>1-2 yrs!!!!)

4.2 Funding and intellectual property

Google Patents

← Back to results ✎ Inventor: **Macarena HERNÁNDEZ JIMÉNEZ;**

Treatment of TLR-4 mediated diseases and conditions with aptamers targeting TLR-4

Abstract

The present disclosure related to methods to treat, prevent (e.g., suppress, inhibit or delay), or ameliorate the symptoms of a TLR-4 mediated disease or condition comprising administering an aptamer of the present disclosure to a subject in need thereof, alone or combination with other pharmacological and/or surgical interventions. In a particular aspect, the aptamers of the present disclosure are administered before, during, or after pharmacological and/or surgical interventions (e.g., thrombolysis such as thrombectomy) or any combination thereof, for the treatment of ischemic (e.g., myocardial infarction or ischemic stroke), hemorrhagic (e.g., hemorrhagic stroke or hemorrhagic transformation), or neurodegenerative (e.g., multiple sclerosis) diseases or conditions. The disclosure also provides specific doses and dosage regimes.

Classifications

A61P21/00 Drugs for disorders of the muscular or neuromuscular system

View 3 more classifications

AU2020276703A1
Australia

Download PDF Find Prior Art Similar

Inventor: Lisardo BOSCÁ GOMAR, Fernando De Castro Soubriet, Beatriz FERNANDEZ GÓMEZ, Macarena HERNÁNDEZ JIMÉNEZ, Ignacio LIZASOAIN HERNÁNDEZ, María Ángeles MORO SÁNCHEZ, David PIÑEIRO DEL RIO, Diego Pérez Rodríguez, David SEGARRA DE LA PEÑA, María Eugenia ZARABOZO LEAL, Carlos ZARAGOZA

Current Assignee : Aptatargets SL

Worldwide applications

2020 • *WO* **CA** **AU** **CN** *WO*

Application AU2020276703A events

2019-05-16 • Priority to US201962849072P

2020-05-16 • Application filed by Aptatargets SL

2021-12-09 • Publication of AU2020276703A1

Show all events

Info: Patent citations (6), Similar documents, Priority and Related Applications

External links: Espacenet, Global Dossier, Discuss

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4.3 Regulatory animal studies

Non-Regulatory Preclinical → **Regulatory Preclinical** → Clinical Trials Phase-I → Clinical Trials Phase-IIa

Regulatory preclinical studies

- Pharmacokinetics
- Safety/toxicology: identification of safe starting dose and dose-escalation in humans
- Rodent and gyrencephalic species

Pharmacodynamics	***Pharmacokinetics***	***Toxicity***
In vitro pharmacodynamics: receptor binding, affinity, antagonistic activity, etc…	***In vitro*** approaches: half-life in presence of nucleases, half-life in plasma ex-vivo, etc…	***In vitro*** toxicology: cell toxicity assays
In vivo pharmacodynamics: efficacy in stroke animal models, dose-response, administration regimen, therapeutic window, long-term efficacy, etc…	***In vivo*** pharmacokinetics: distribution of drug in target organs (brain), half-life in plasma, tissue distribution, PK parameters, etc…	***In vivo*** toxicology: toxicokinetics, histopathology, immunotoxicity, CNS respiratory and cardiovascular toxicity, genotoxicity, etc…

4.4 Clinical trials

2005.... 2017 2019 2020 2021 2022

Non-Regulatory Preclinical → Regulatory Preclinical → Clinical Trials Phase-I → Clinical Trials Phase-IIa → ¿?

Safety issues in healthy volunteers

No signs of clinical efficacy

Regulatory Agencies: AEMPS, EMA, FDA

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First-in-human phase I clinical trial of a TLR4-binding DNA aptamer, ApTOLL: Safety and pharmacokinetics in healthy volunteers

Macarena Hernández-Jiménez,[1,10] Samuel Martín-Vílchez,[2,10] Dolores Ochoa,[2] Gina Mejía-Abril,[2] Manuel Romá Paola Camargo-Mamani,[2] Sergio Luquero-Bueno,[2] Bernd Jilma,[3] María A. Moro,[4,7,8] Gerónimo Fernández,[5] David Piñeiro,[1] Marc Ribó,[1] Víctor M. González,[5,6] Ignacio Lizasoain,[7,8] and Francisco Abad-Santos[2,9]

Molecular Therapy: Nucleic Acids Vol. 28 June 2022

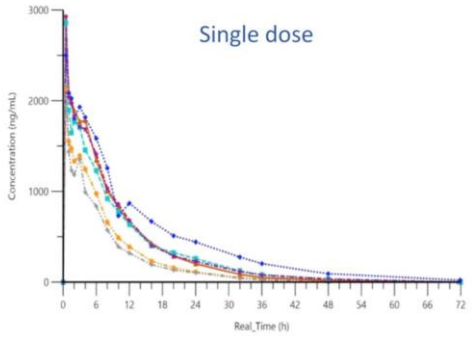
Population

SPAIN

46 Male healthy volunteers (18-55 years old)

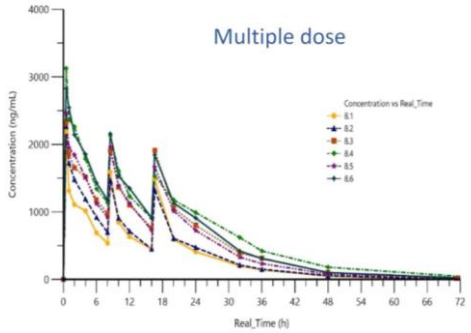
PHARMACOKINETICS

1. C_{max} increases with dose in the first levels but remains stable thereafter
2. Half-life = 9.3h
3. No accumulation after multiple doses



SAFETY

1. No AEs or SAEs attributable to ApTOLL administration
2. No clinically significant laboratory, vital signs or ECGs findings related to ApTOLL injection
3. Safety profile confirmed both in part A and in part B



4.4 Clinical trials

2005.... | 2017 | 2019 | 2020 | 2021 | 2022

Non-Regulatory Preclinical → Regulatory Preclinical → Clinical Trials Phase-I → Clinical Trials Phase-IIa → ¿?




No signs of clinical efficacy

Number of doses

tPA or not tPA? Thrombectomy?

Biomarkers?

Time window?

Age?

Male? Female?

Hospitals?

Endpoints?

Staff

National or international?

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Plan de Recuperación, Transformación y Resiliencia

APRIL TRIAL design

CLINICAL AND POPULATION SCIENCES

Defining a Target Population to Effectively Test a Neuroprotective Drug

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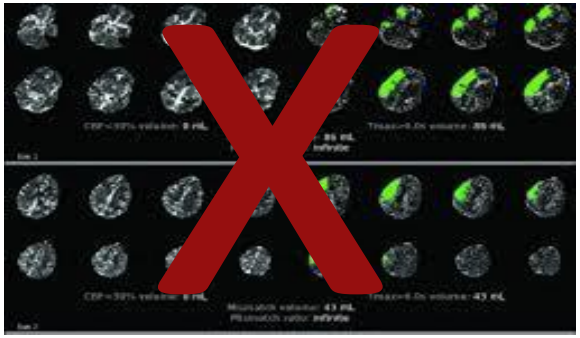
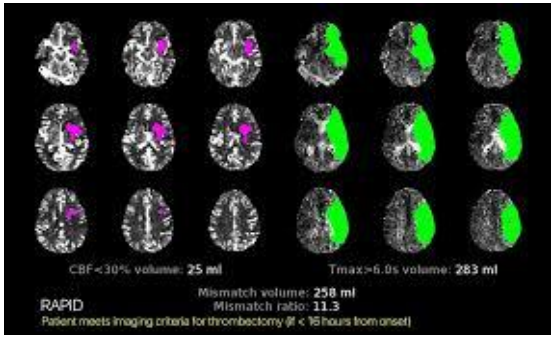
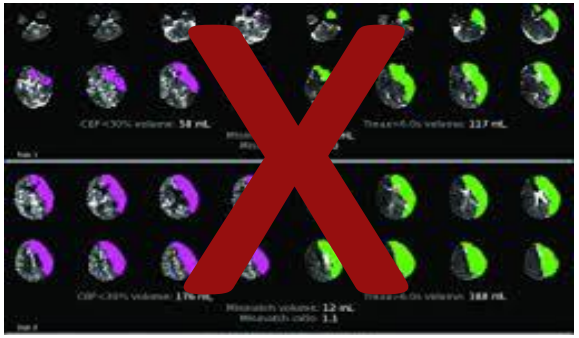
Stroke. 2021;52:505–510.

Imaging selection criteria

Predicted final infarct according to admission CBF<30% 5-70 ml







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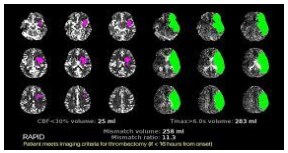
APRIL trial

LVO (TICA, M1, M2)
within 6h of onset
Baseline mRS 0-2
Presentation NIHSS ≥ 8 and ≤ 25

Receive CT/CTA/CTP or MRI
CT (ASPECTS 6 – 10)
Perfusion Imaging (Infarct core volume 5-70cc (CBF<30% or DWI))



Excluded:
ASPECS < 6 or Infarct core volume ≤ 5 and ≥ 70cc

Candidate to thrombectomy

Randomized to

Endovascular Thrombectomy + ApTOLL

Endovascular Thrombectomy + Placebo

Study drug administered as 30 min iv. infusion, initiated before groin puncture.

Enrollment period:
July 2020 to April 2022

Study conducted in 14 centers
11 centers in Spain
3 centers in France

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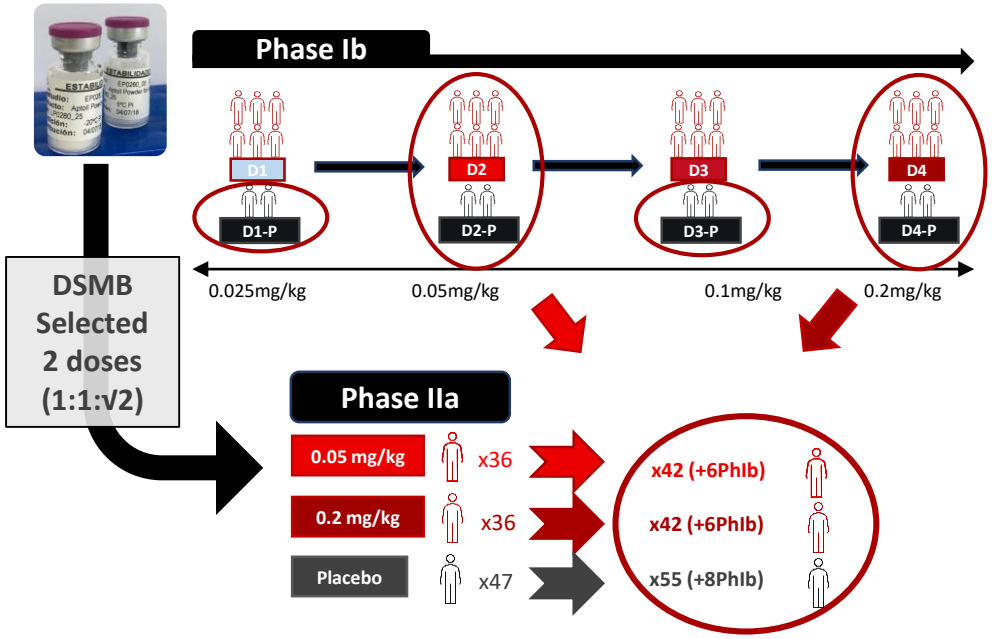
A Double Blind, Placebo-Controlled, Randomized, Phase Ib/IIa Clinical Study of ApTOLL for the Treatment of Acute Ischemic Stroke (NCT04734548)

Primary objective: evaluate **safety** and pharmacokinetics of ApTOLL based on:

- Death
- Symptomatic intracranial hemorrhage
- Malignant stroke
- Recurrent stroke

Secondary efficacy objectives:

- Final infarct volume (MRI at 72 hours)
- National Institutes of Health Stroke Scale Score (NIHSS) at 72 hours
- Disability at 90 days (modified Rankin Score [mRS])



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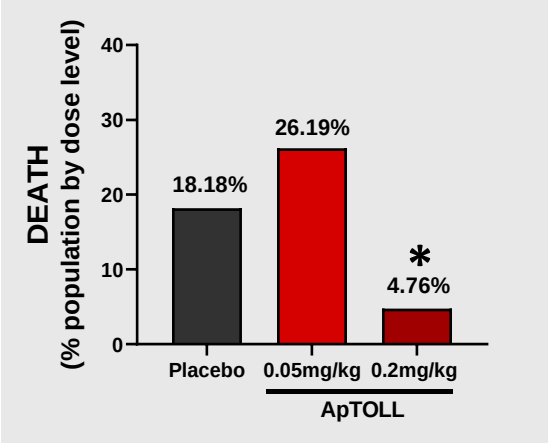
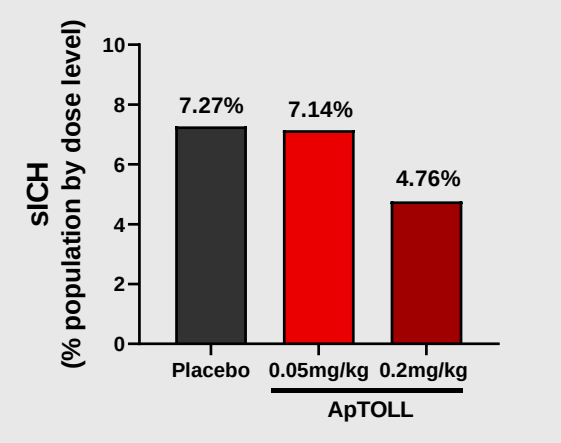
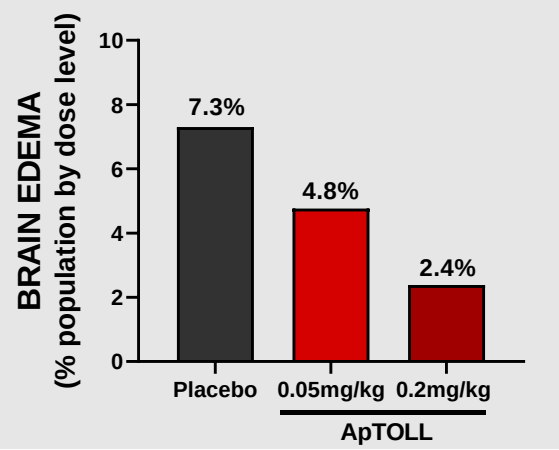
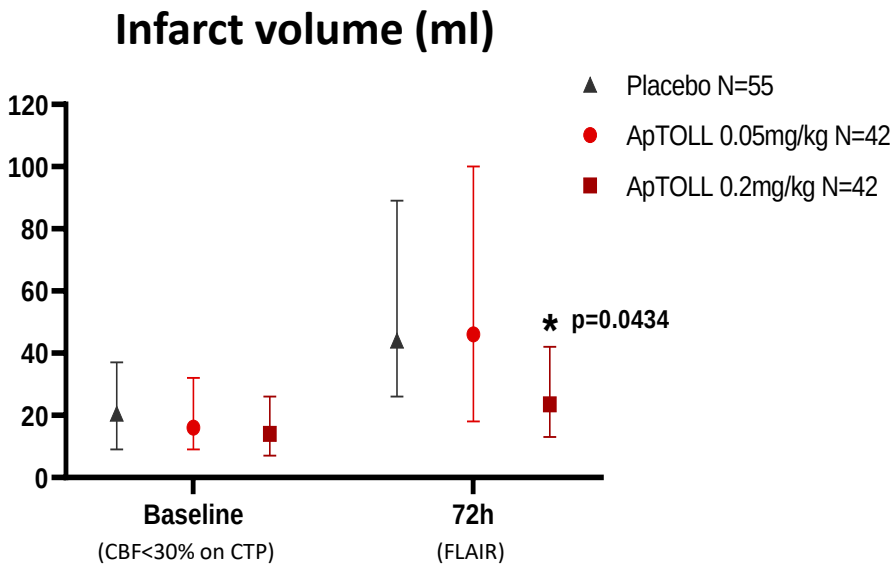
Primary endpoints: safety and pharmacokinetics

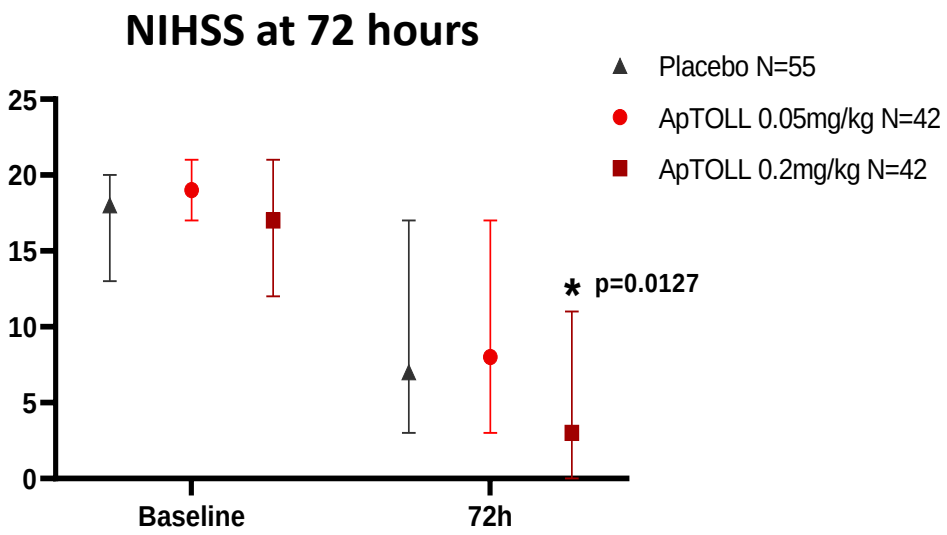




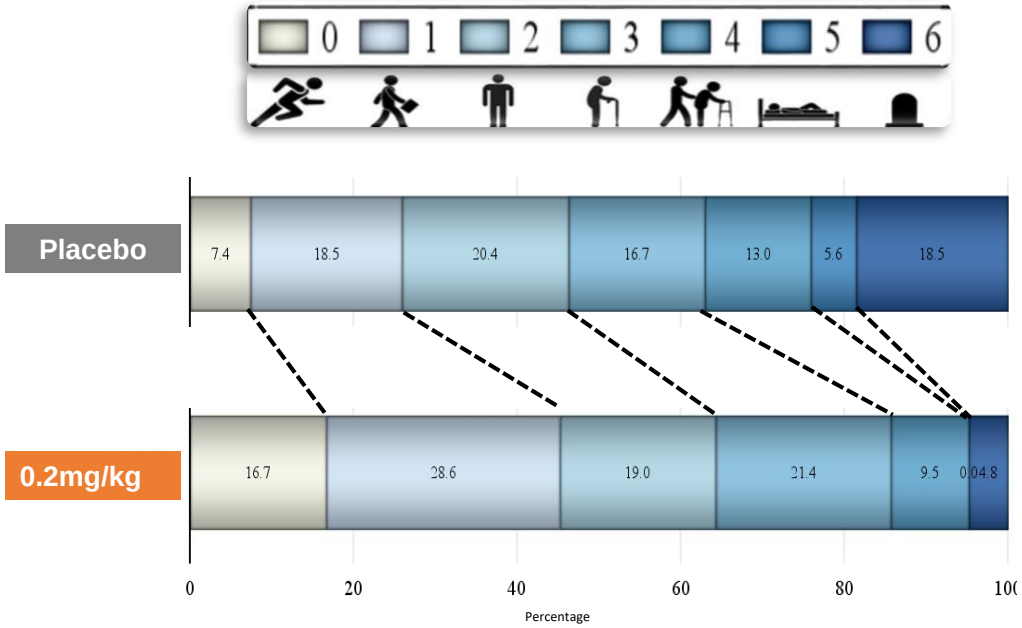


Secondary endpoints





Secondary endpoints



mRS shift analysis: Common OR: 2.5

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CONCLUSION

In acute ischemic stroke, 0.2mg/kg of ApTOLL administered within 6h of onset, in combination with EVT, was safe and associated with a potential meaningful clinical effect reducing mortality and disability at 90 days as compared to placebo

These preliminary findings await confirmation from a larger pivotal trial

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THANK YOU FOR YOUR ATTENTION

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