IDENTIFICACIÓN DE POTENCIALES FÁRMACOS MEDIANTE UN ESTUDIO MULTIÓMICO EN EL PRONÓSTICO POST-ICTUS

Natalia Cullell Fornés
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Sesión científica RICORS
13/01/2025

Stroke Pharmacogenomics and Genetics

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Potential drugs (drug repositioning)

05

04

03

In vitro / in vivo models

Transcriptomics /Proteomics

02

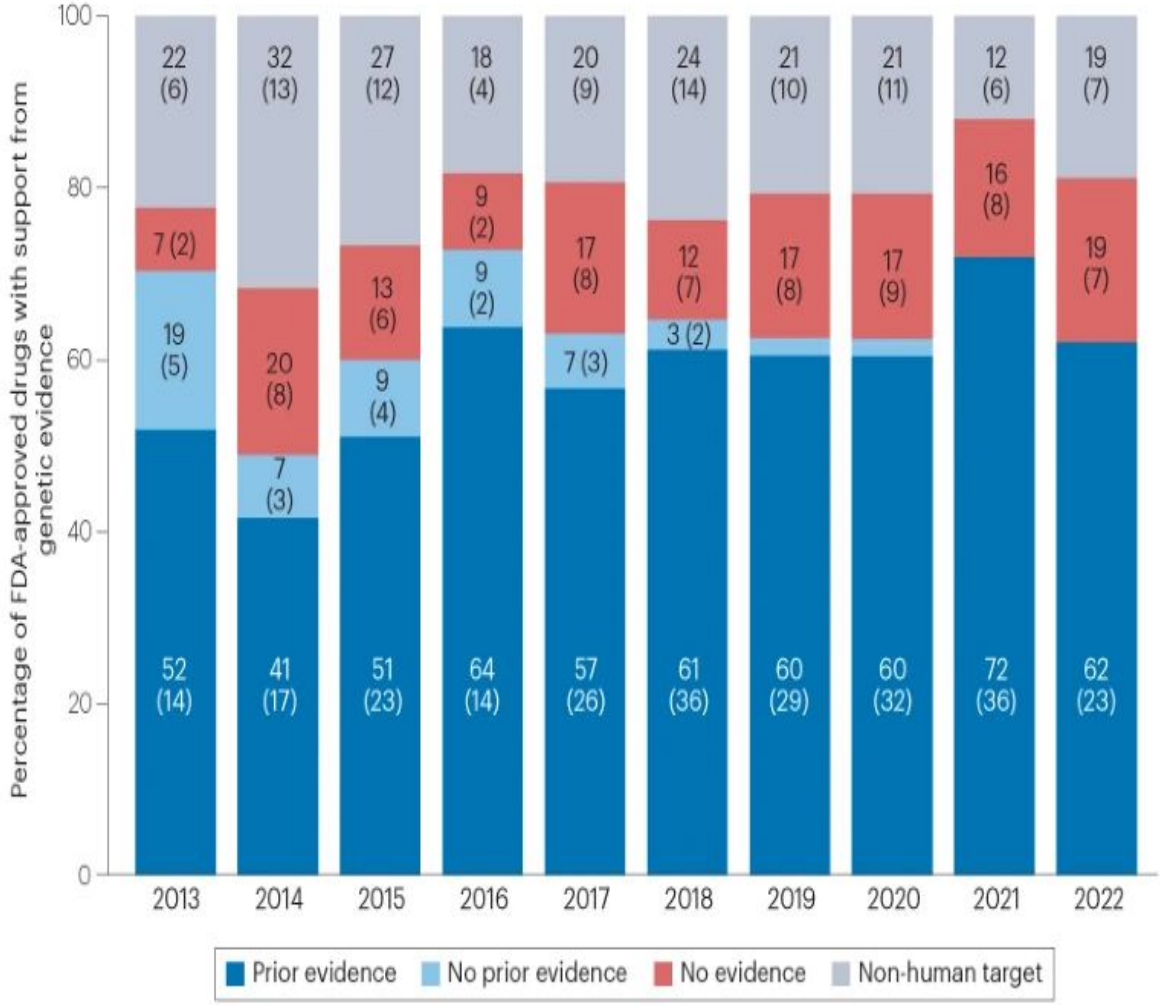
01

Clinical trial **(human)**

Animal models / *in vitro* studies

Post-stroke outcome (mRS 3 months)

Genomic studies to identify drugs



Two-thirds of the new drugs approved by the FDA in 2021 had evidence derived from human genetic studies.

Stroke Pharmacogenomics and Genetics

Rusina et al. Nat Rev Drug Discovery, 2023

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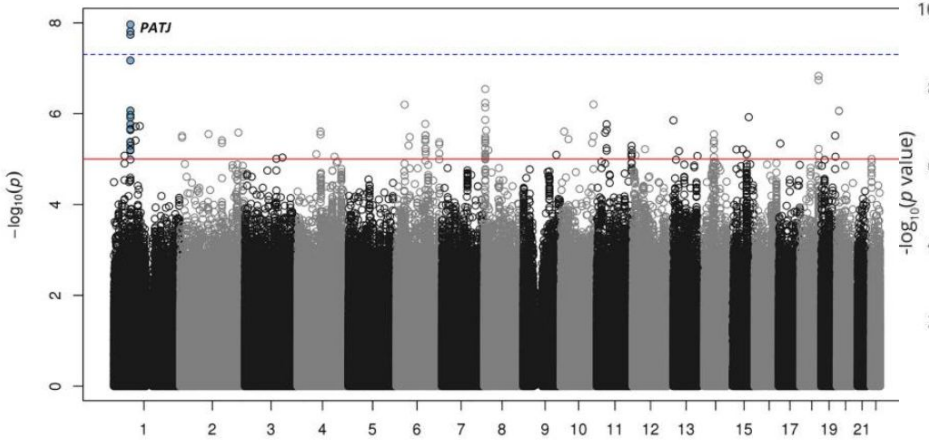
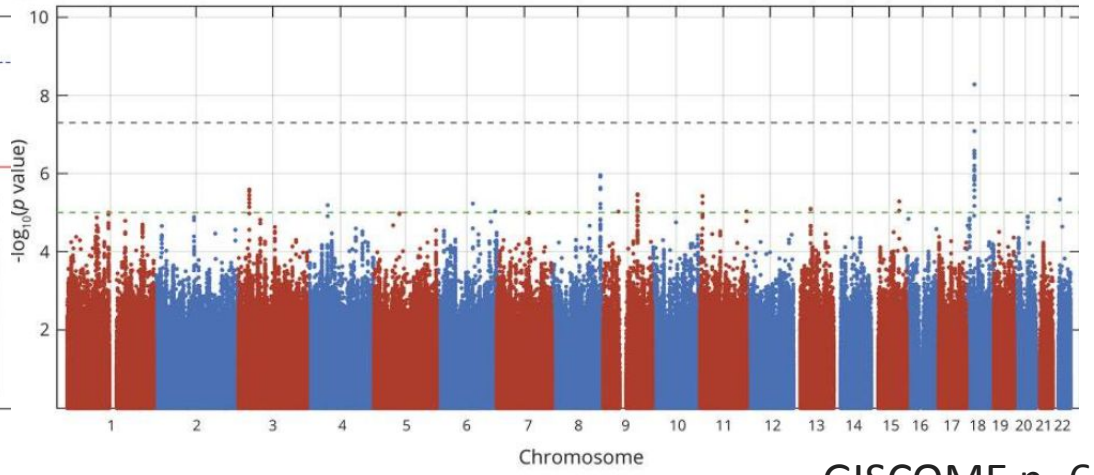
GWAS in the stroke outcome



GODS n=1.791

Mola-Caminal M et al.,Circ Res. 2019

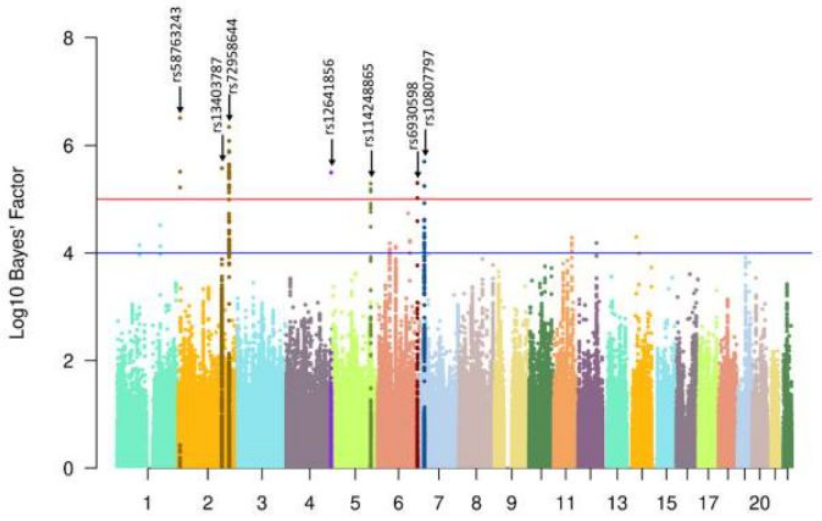


Söderholm M et al.,Neurology. 2019

GISCOME n=6.165

mRS3

GENISIS
n= 5.876



Ibanez L et al.,Brain. 2022

ΔNIHSS

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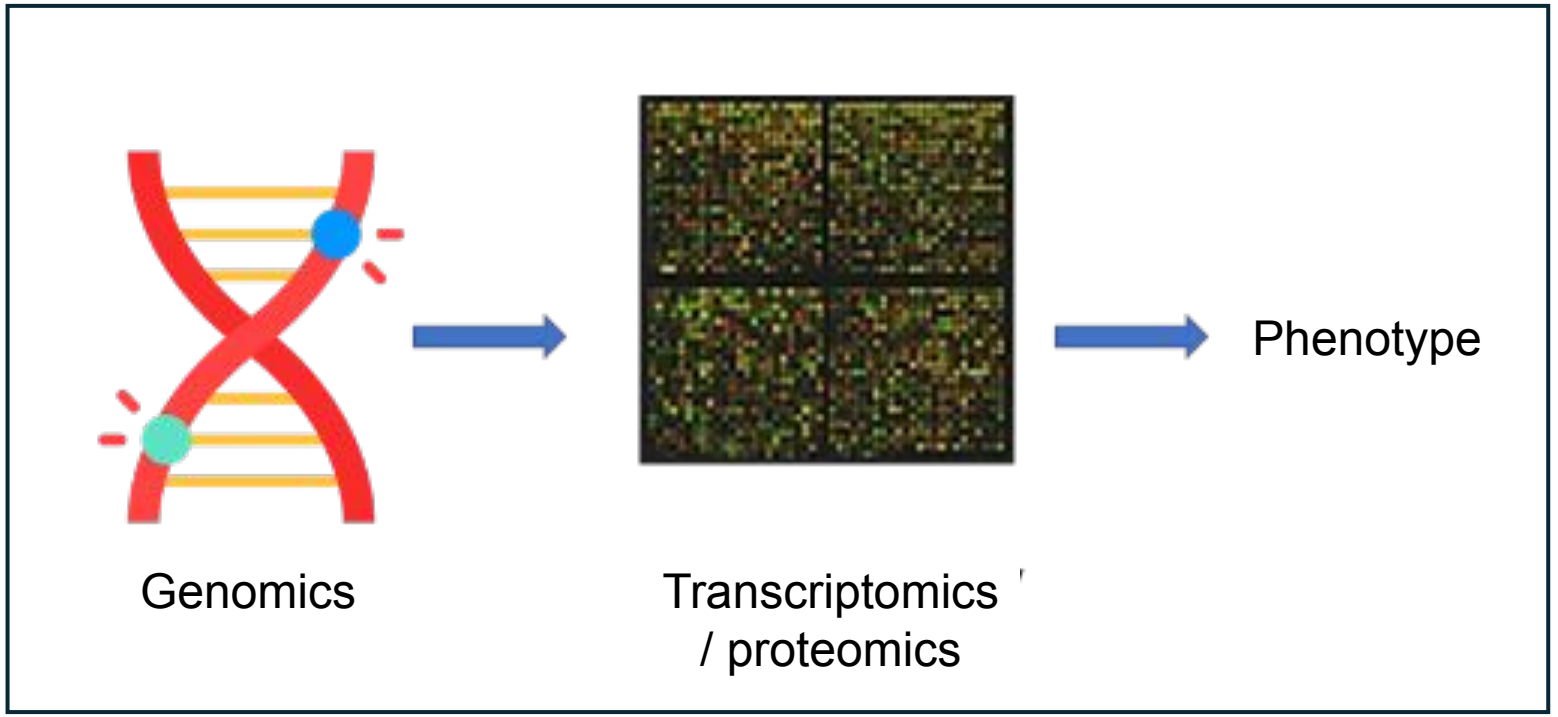
Proteome/Transcriptome Wide association study (PWAS / TWAS)

1) Identification of coding genes causing the phenotype.
2) Identification of new associations for a disease.



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Objectives

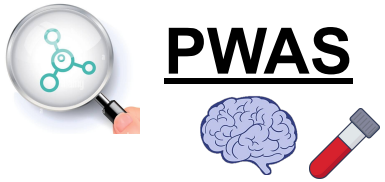
1. To identify proteins/genes associated with 3 months mRS using PWAS/TWAS.

2. To identify **potential neuroprotective drugs** for enhancing long-term stroke outcomes based on the results from PWAS and TWAS.

Methodology

Workflow

FUSION



PWAS

Discovery
Replication 1
Replication 2

Meta-analysis → **COLOC / SMR**

Sensitivity analyes

dorsolateral prefrontal cortex

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Methodology

Workflow

PWAS

Discovery
Replication 1
Replication 2

Brain proteomic dataset:

ROS/MAP cohort
376 subjects and 1.475 heritable proteins

Bennett. D.A. et al.. J. Alzheimer's Dis. 2018

GWAS:
GODS Study
n=1.791 ischemic stroke patients

Mola-Caminal et al.,Cir Res.2019

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Methodology

Workflow

PWAS

Discovery

Replication 1

Replication 2

new

Brain proteomic dataset:

Banner Sun Health Research Institute cohort
152 subjects and 1.139 heritable proteins

Beach. T.G et al..Neuropathology 2015

GWAS:
GODS Study
n=1.791 ischemic stroke patients

Mola-Caminal et al.,Cir Res.2019

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Methodology

Workflow

PWAS

Discovery
Replication 1
Replication 2

Brain proteomic dataset:

ROS/MAP cohort
376 subjects and 1.475 heritable proteins

Bennett. D.A. et al.. J. Alzheimer's Dis. 2018

new

GWAS GODS (replication)

n = 688 ischemic stroke patients

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Methodology

Workflow

PWAS

Discovery
Replication 1
Replication 2

Meta-analysis

adaptively weighted Fisher's method (AW-Fisher)

Nominal: p-value < 0.05
Bonferroni Adjusted p-value (q-value) < 0.05

Pathway enrichment analysis (Webgestalt)

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Methodology

Workflow

PWAS

Discovery
Replication 1
Replication 2

Meta-analysis

adaptively weighted Fisher's method (AW-Fisher)

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Stroke Pharmacogenomics and Genetics

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Methodology

Workflow

PWAS

Discovery
Replication 1
Replication 2

Meta-analysis

TWAS

Brain gene expression dataset:

Common mind consortium cohort
452 subjects and 5.420 heritable proteins

GWAS:
GODS Study
n=1.791 ischemic stroke patients

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Results

PWAS

coloc

	DISCOVERY					REPLICATION1				REPLICATION2				META-ANALYSIS	
ID	pQTL	pQTL Z	PWAS Z	PWAS P	COLOC PP4	pQTL	pQTL Z	PWAS Z	PWAS P	pQTL	pQTL Z	PWAS Z	PWAS P	P	Q-value
THEM4	rs16833668	-10.7	3.04	2.35E-03	0.5	rs16833668	-8.69	2.84	4.51E-03	rs16833668	-10.8	2.3	2.35E-02	1.51E-04	6.93E-03
GSTP1	rs7941648	-8.99	-2.56	1.04E-02	0.7	rs1695	-9.13	-2.06	3.93E-02	rs7941648	-9	-0.06	9.55E-01	1.40E-02	6.42E-01
APOL2	rs8136528	-6.19	-2.37	1.77E-02	0.8	rs9619597	-6.5	-3.31	9.32E-04	rs8136528	-6.2	0.6	5.28E-01	8.66E-04	3.98E-02

Gene	b_SMR	se_SMR	p_SMR
THEM4	0.62	0.21	2.85E-03
APOL2	-0.84	0.32	9.40E-03

SMR

+THEM4 –> + mRS3 –> worse outcome

Results not published

Results

Meta-analysis

PWAS

30 nominal associations and 5 significant proteins

Pathway enrichment analysis

Gene Set	Description	Size	Ratio	P-Value
hsa00062	Fatty acid elongation	30	121.18	8.24E-03
hsa04152	AMPK signaling pathway	120	30.296	3.27E-02

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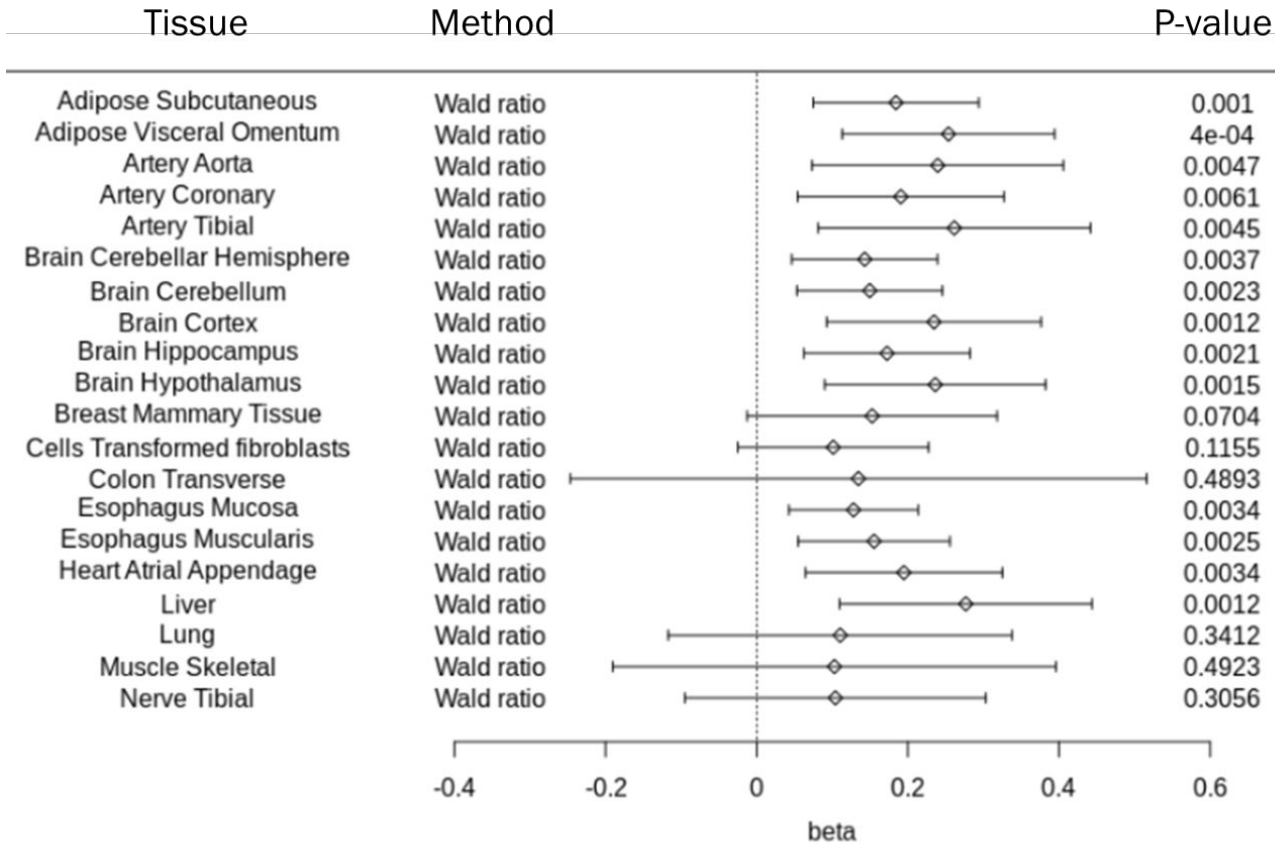
Results

TWAS

ID	EQTL.ID	EQTL.Z	TWAS.Z	TWAS.P	COLOC.PP4
THEM4	rs13320	-15,62	3,36	7,71E-04	0,80
APOL2	rs129607	-4,79	1,459	1,45E-01	NA
GSTP1	rs614080	9,94	-2,41	1,60E-02	0,21

GTEx



THEM4 expression is associated with post-stroke outcome in different tissues

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Results

PWAS/TWAS Summary

		DISCOVERY PWAS	REPLICATIO N 1 PWAS	REPLICATION 2 PWAS	EVIDENCE FOR CAUSALITY		TWAS (brain)
Protein/Gene	CHR				COLOC	SMR	
APOL2	22	Nominal	Replicated	No	Yes	Yes	No
GSTP1	11	Nominal	Replicated	No	Yes	No	Yes
THEM4	1	Nominal	Replicated	Replicated	Yes	Yes	Yes

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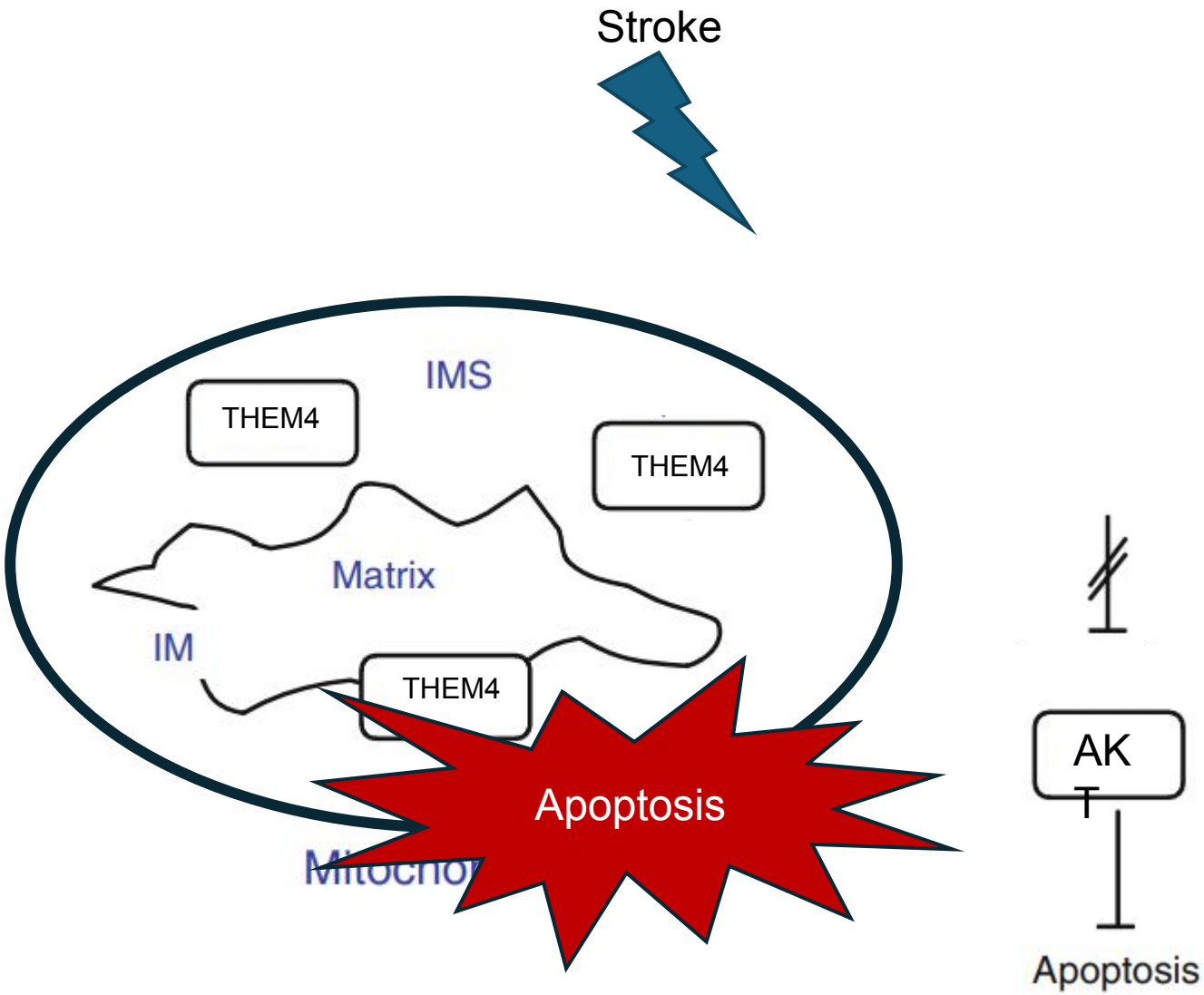
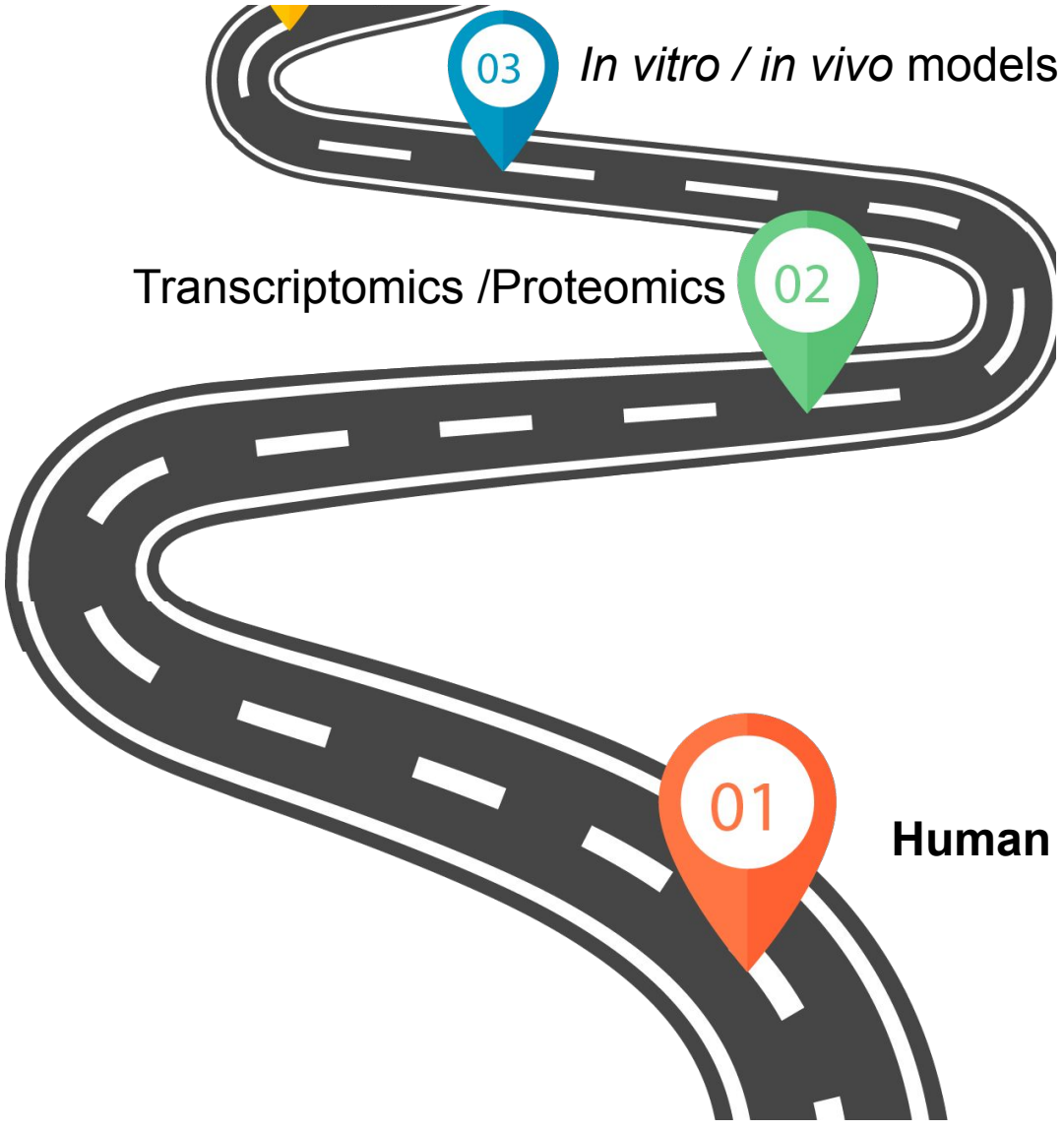
CTMP/THEM4

CTMP = THEM4



Curr Top Microbiol Immunol. 2010;346:31-56

Clinical trial **(human)**



In vitro / in vivo models

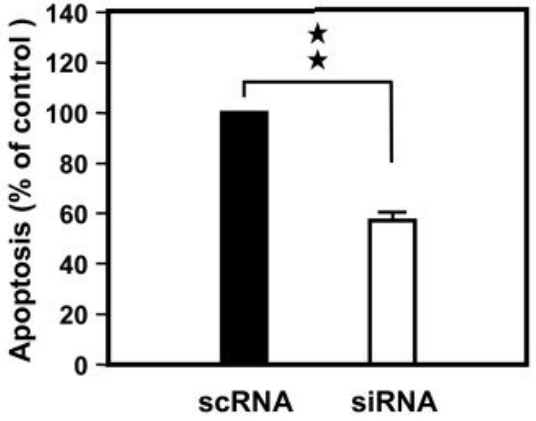
Transcriptomics /Proteomics

Human genomics

Post-stroke outcome
(mRS 3 months)

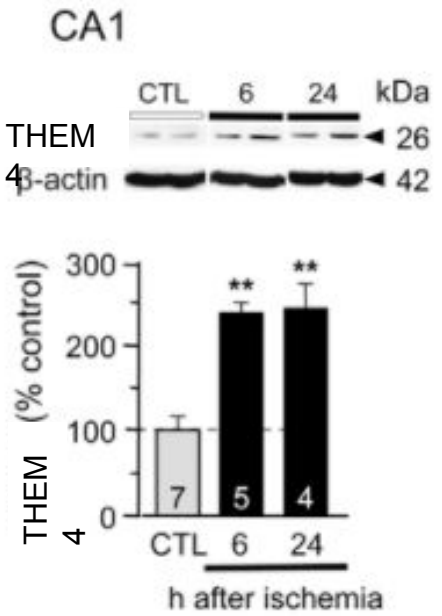
CTMP/THEM4 *in vitro/in vivo*

Mol Neurobiol. 2015 Apr;51(2):543-57



Reduction of Hypoxic Neuronal Apoptosis with THEM4 siRNA treatment

Nat Neurosci. 2009 May; 12(5): 618–626.



THEM4 increases after ischemia

THEM4 increases contributes to worse neurological outcomes in older S-D rats

Objectives

1. To identify proteins/genes associated with 3 months mRS using PWAS/TWAS.
2. To identify **potential neuroprotective drugs** for enhancing long-term stroke outcomes based on the results from PWAS and TWAS.

Drugs targeting THEM4

THEM4

Drugs or compounds targeting this protein?

Drugs or compounds with previous evidence of stroke modulation in **clinical/preclinical trials**

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General anesthesia

Isoflurane

Sevoflurane

Stroke Pharmacogenomics and Genetics

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Anestesia general

Stroke Pharmacogenomics and Genetics

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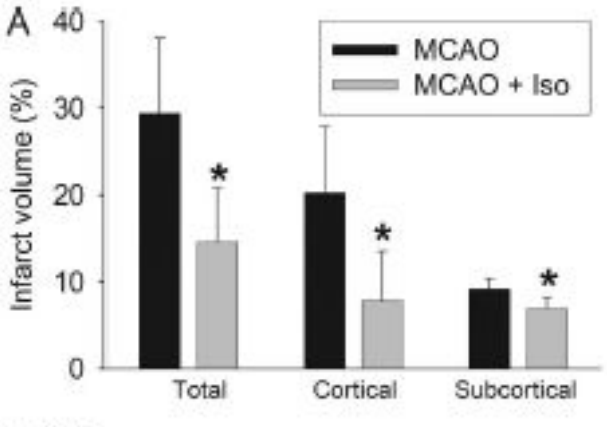
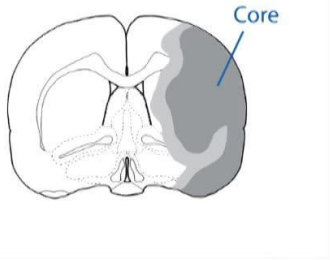
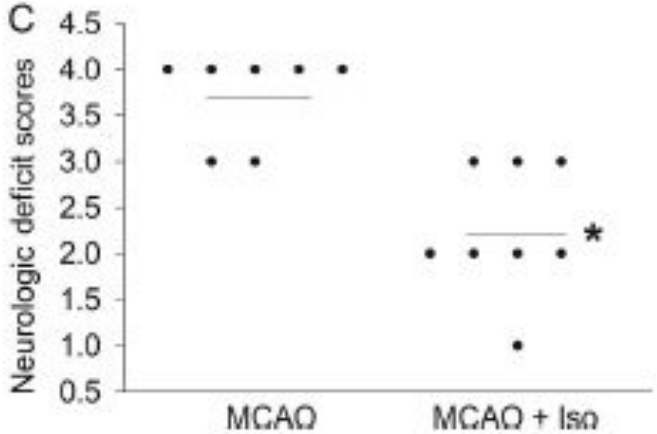
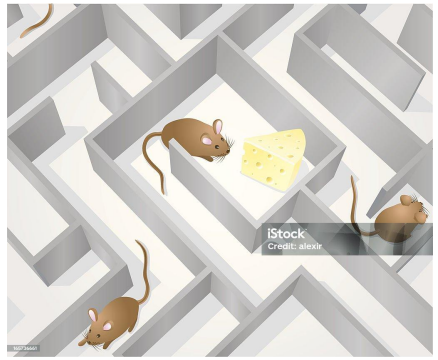
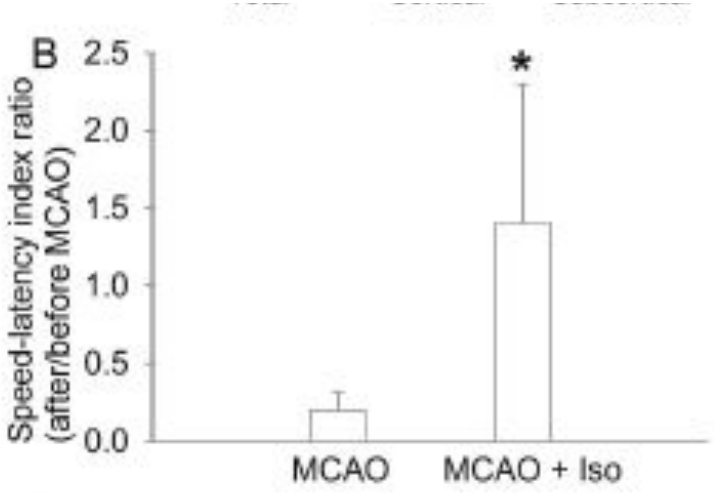
Isoflurane: post-conditioning











Neurological severity score	Points
RAISING RAT BY TAIL (MAXIMUM = 3)	
Flexion of forelimb	1
Flexion of hindlimb	1
Head moved >10° to vertical axis within 30 sec	1
SENSORIMOTOR TEST (MAXIMUM = 4) LESIONED SIDE	
Limb placing test: limb stays on the table 0	0
Delay before putting back limb on the Table 1	1
Limb falls down from table	2
BEAM BALANCE TESTS (MAXIMUM = 6)	
Balances with steady posture	0
Grasps side of beam	1
Hugs beam and 1 limb fall down from beam	2
Hugs beam and 2 limb fall down from beam, or spins on beam (>60s)	3

Stroke Pharmacogenomics and Genetics

Lee JJ. Anesthesiology 2008

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Isoflurane: post-conditioning

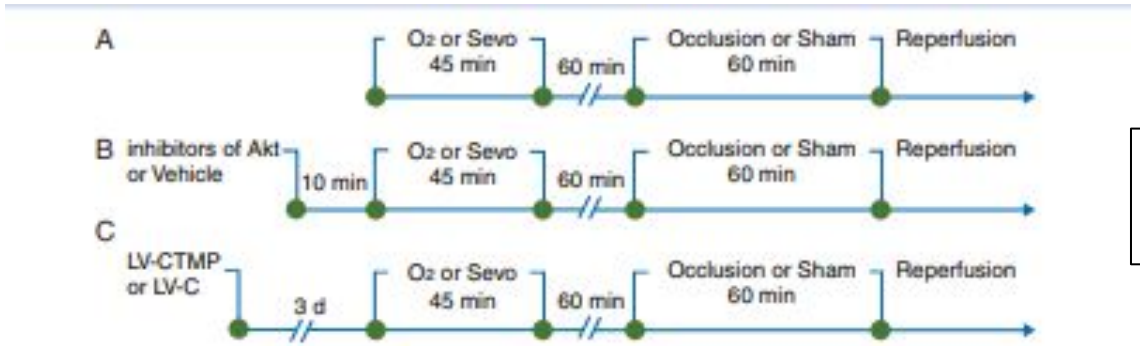
Table 1: The effect of ISO postconditioning

Animal	Model	Intervention	Conclusion
Rat	Focal cerebral I/R injury	ISO was delivered with the vehicle air (30% O_2 and 70% medical air)	ISO promotes angiogenesis, reduces brain cell death after cerebral I/R, and improves recovery.[21]
Rat	Cerebral I/R injury	ISO (1.5%) was administered for 1 h after immediate reperfusion	ISO reduces infarct volume after cerebral I/R injury and minimizes the cell death.[23]
Rat	MCAO	Rats in ISO groups inhaled different concentrations of ISO (1.5%, 3.0%, and 4.5%) for 60 min	The results showed that 1.5% isoflurane postconditioning significantly reduced the cerebral infarct volumes and improved the neurobehavioral deficit scores.[30]
Mouse	BCCAO	ISO preconditioning (98% O_2 and 1.2% ISO) at a rate of 1 h/d for 5 d	ISO preconditioning significantly improved the TMS and reduced neuronal degeneration after cerebral I/R.[31]

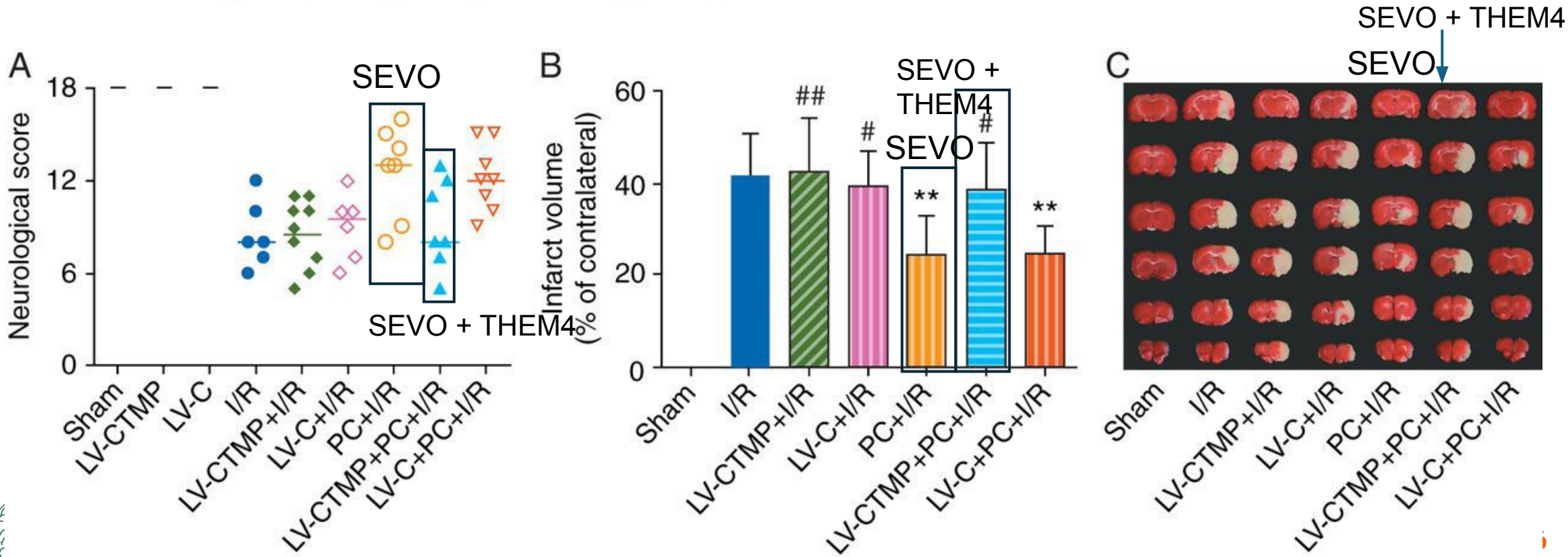
Note: BCCAO: Bilateral common carotid artery occlusion; I/R: ischemia reperfusion; ISO: isoflurane; MCAO: middle cerebral artery occlusion; TMS: total motor score.

Shu-Jun Chen. Med Gas Res. 2022;

Sevoflurane: pre-conditioning and THEM4



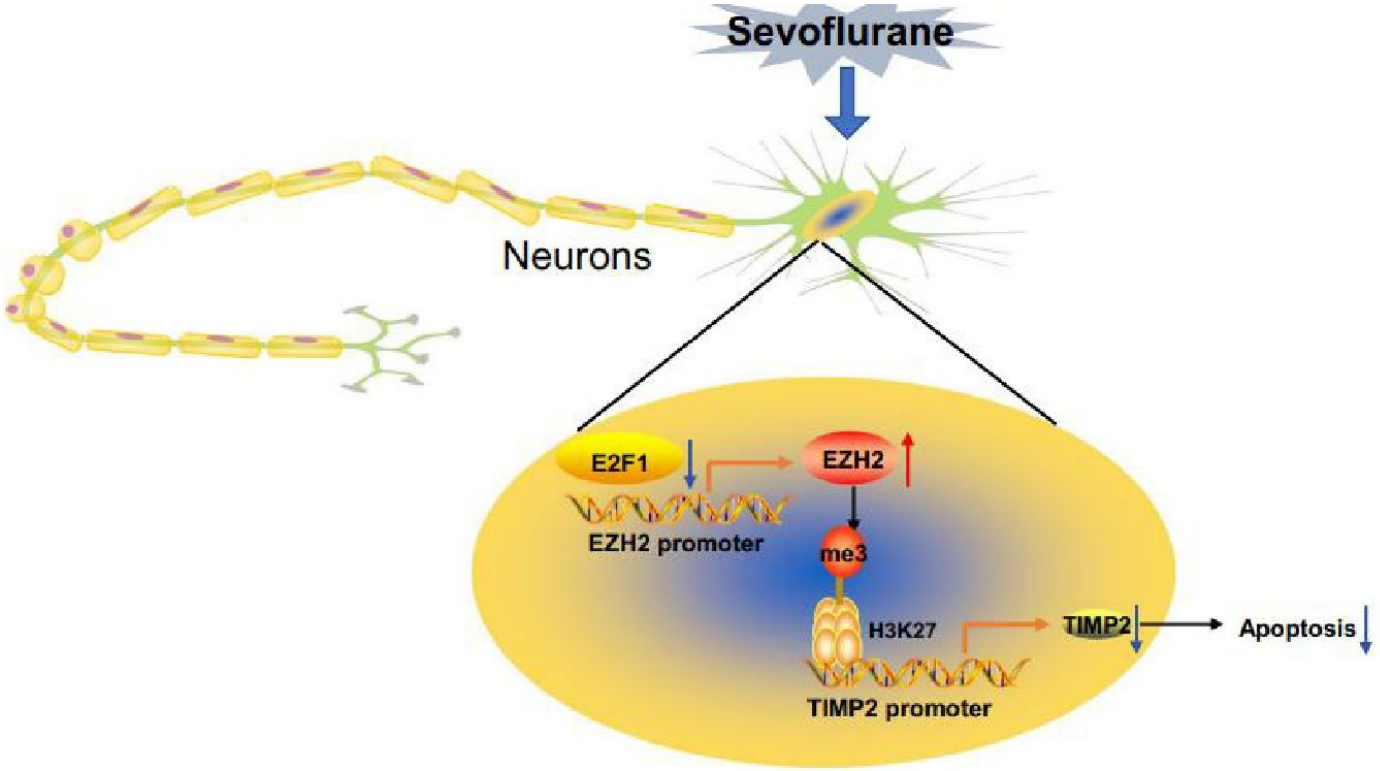
The administration of an AKT inhibitor or CTMP reduces the neuroprotective effects of sevoflurane.



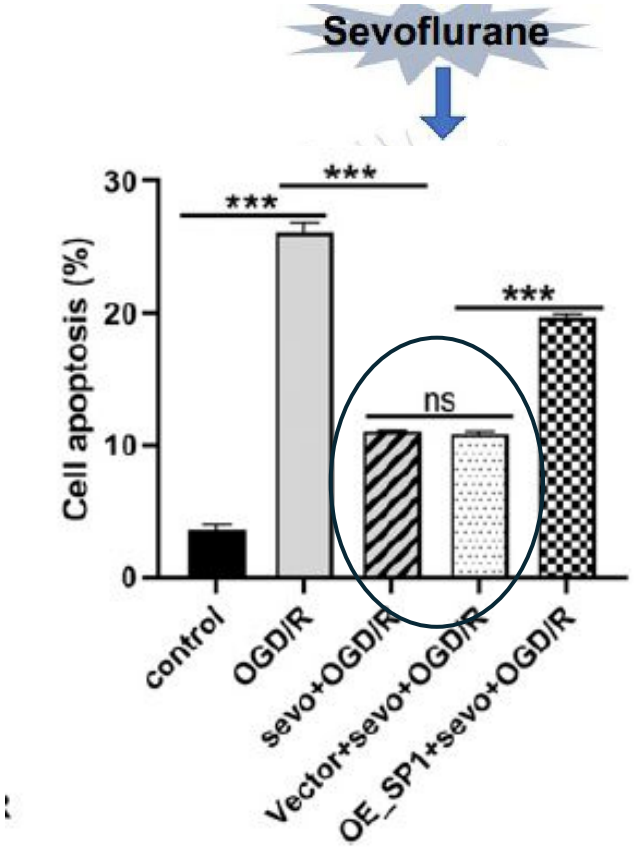
Chen et al., British Journal of Anaesthesia. 2015

Sevoflurane: other mechanisms

Sevoflurane □ decrease apoptosis



Jinhui Xu et al., Med Gas Res. 2024



Lyu et al., Human and Experimental Toxicology. 2023

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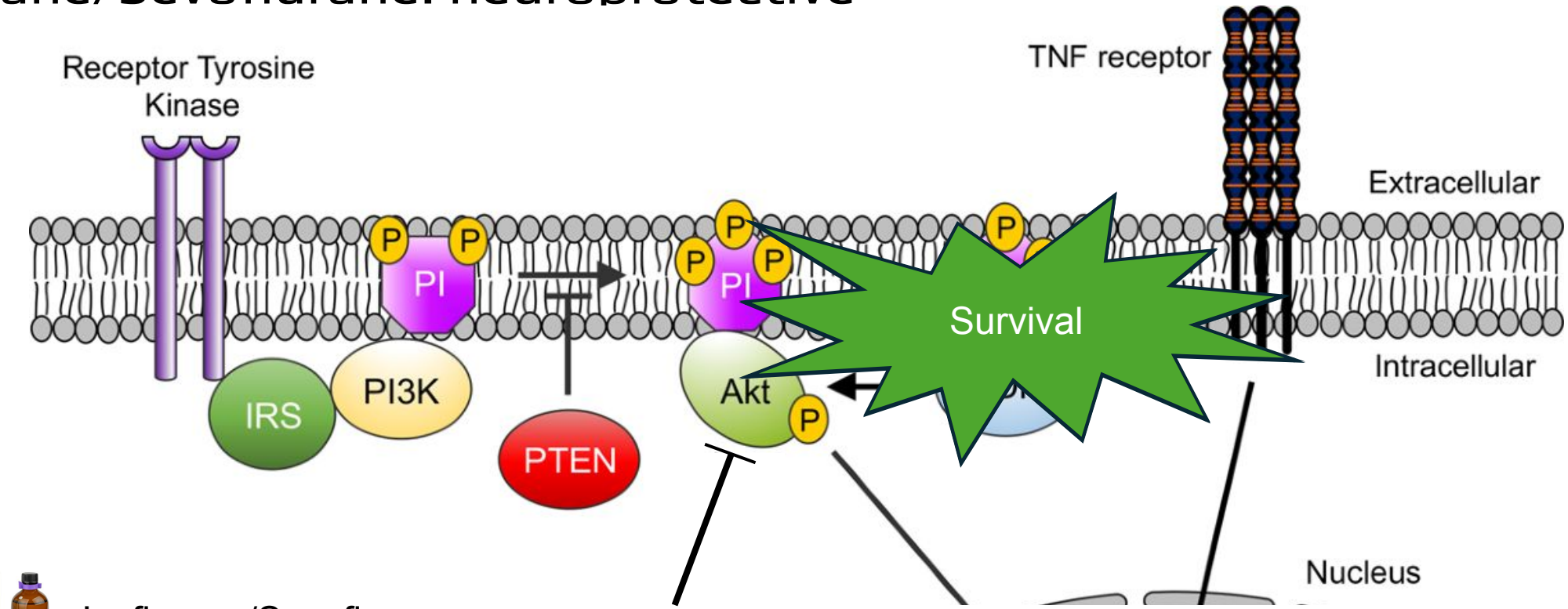
Isoflurane/Sevoflurane: neuroprotective



Isoflurane/Sevoflurane

ischemia → THEM4

Junmei Wang et al., Scientific Reports. 2019

Yu et al., Obesity. 2014

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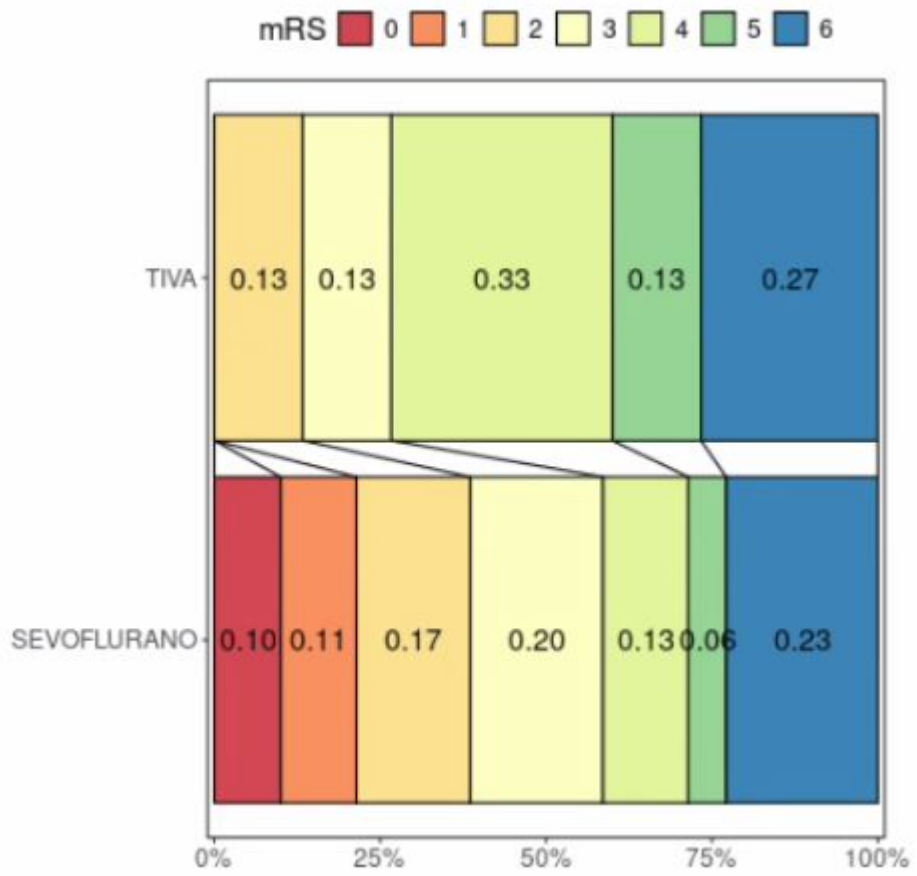
Retrospective study (pilot): HSCS

Sevoflurane: 73
TIVA (intravenous total anesthesia): 17



P-value = 0.05
P-value (NIHSS_B) = 0.16

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Sevoflurano vs. propofol para la Anestesia general en pacientes con ictus isquémico tratados mediante terapias endoVascularEs (SAVE trial)

Presentación del protocolo (ICI25/00082)

Pol Camps Renom
Unidad de Ictus
Hospital de la Santa Creu i Sant Pau
06/10/2025

Como se pueden mejorar los resultados de las TEV?

PREPROCEDIMIENTO
- Mejorar flujos de pacientes
- Bundle of care en ambulancia (TA, glicèmia, posició, rtpa,...)
- Neuroprotección

PERIPROCEDIMIENTO
- Manejo de la TA
- Incrementar el «first-pass effect»
- Reducir tiempo
- Reducir complicaciones
- Neuroprotección

POSTPROCEDIMIENTO
- Manejo de la TA (HOPE)
- Mejorar reperfusión/neuroprotección (CHOICE)
- Reducir complicaciones

Potenciales neuroprotectores

- Poca replicación
- GAP modelos animales-humanos
- Pocos ensayos

1. **Agentes antioxidantes** (Edaravona, N-acetilcisteína, Ácido úrico)
2. **Inhibidores de excitotoxicidad** (Memantina, Magnesio)
3. **Agentes antiinflamatorios** (Minociclina, Glucocorticoides)
4. **Moduladores de la apoptosis (**nerinetida, eritropoyetina)
5. **Reguladores del flujo sanguíneo cerebral (**Citicolina, Estatinas**)**
6. **Agentes vasodilatadores (**Nitroglicerina, nitroprusiato, Agonistas beta-2 adrenérgicos**)**
7. **Inhibidores del estrés oxidativo mitocondrial** (MitoQ, Idebenona)
8. **Reguladores del metabolismo energético (**Piruvato, Creatina)

THEM4 COMO DIANA

Sevoflurano vs. Propofol

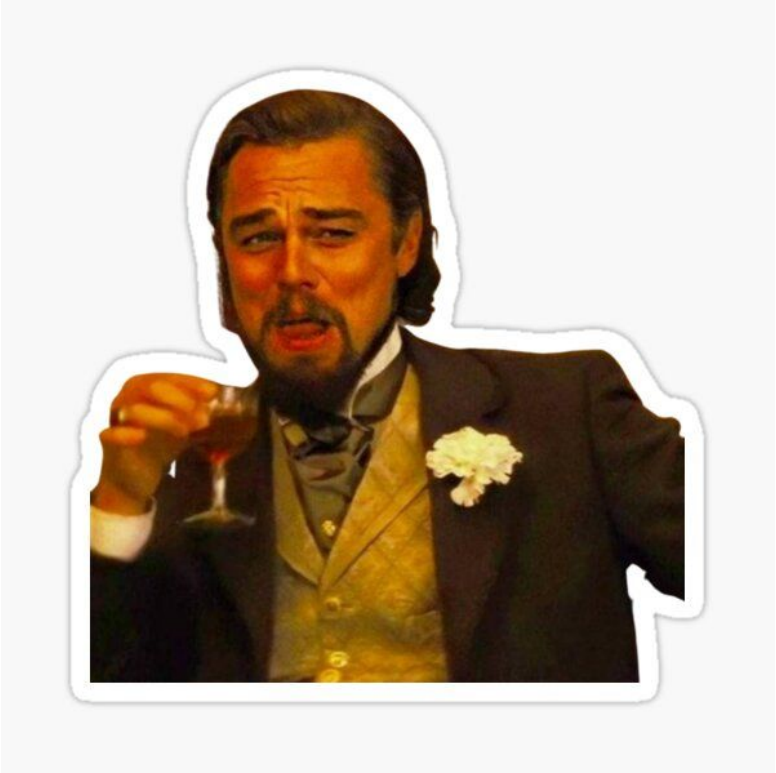
SEVOFLURANO

- Efecto rápido
- Incrementa el FSC
- Disminuye umbral epileptógeno
- La sala d'arterios tiene que estar preparada para gases halogenados

PROPOFOL

- Altamente disponible y económico
- - Antiepiléptico
- - Vida media muy corta
- Puede producir hipotensiones

Cuando utilizamos AG en una TEV?



Anestesia general vs. Sedación consciente o VAM

Meta-análisi HERMES (Campbell BC. Lancet. 2018)

- 797 pacientes en el brazo TEV
- AG se asocia a peores resultados clínicos

SIESTA, GOLIATH, CANVAS Pilot, AnStroke,…

Revisión Cochrane 2022

- 982 pacientes (en 6 ensayos clínicos)
- AG se asocia a mayor tasa de recanalizaciones y menos complicaciones
- No impacto sobre resultados clínicos

Cuando utilitzamos AG en una TEV?

1. Inestabilidad hemodinámica/insuficiencia respiratoria
2. Falta de colaboración/agitación
3. Características del procedimiento
4. Preferencias del equipo

02. Hipótesis y objetivos

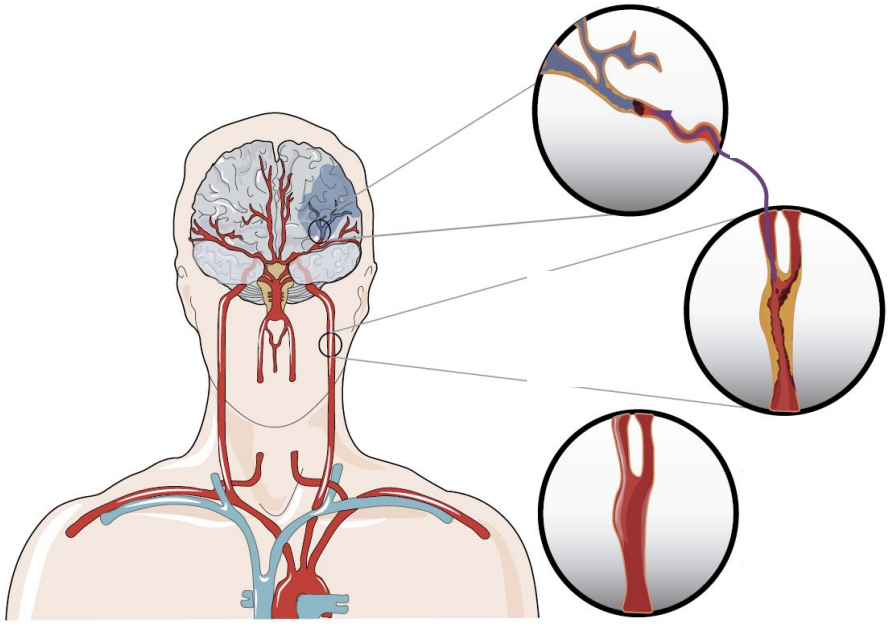
HIPÓTESIS PRINCIPAL

En pacientes con ictus isquémico agudo sometidos a TEV y que requieren anestesia general, el uso de Sevoflurano se asocia a volúmenes de infartos finales más pequeños.

OBJETIVO PRINCIPAL

Llevar a cabo un ensayo clínico multicéntrico con diseño PROBE con aletorización 1:1 a recibir Sevoflurano vs. Propofol durante el mantenimiento anestésico de pacientes cometidos. aTEV que requieren una anestesia general durante el procedimiento

03. Diseño del estudio



SAVE Trial

Diseño PROBE (Prospective, Randomized,

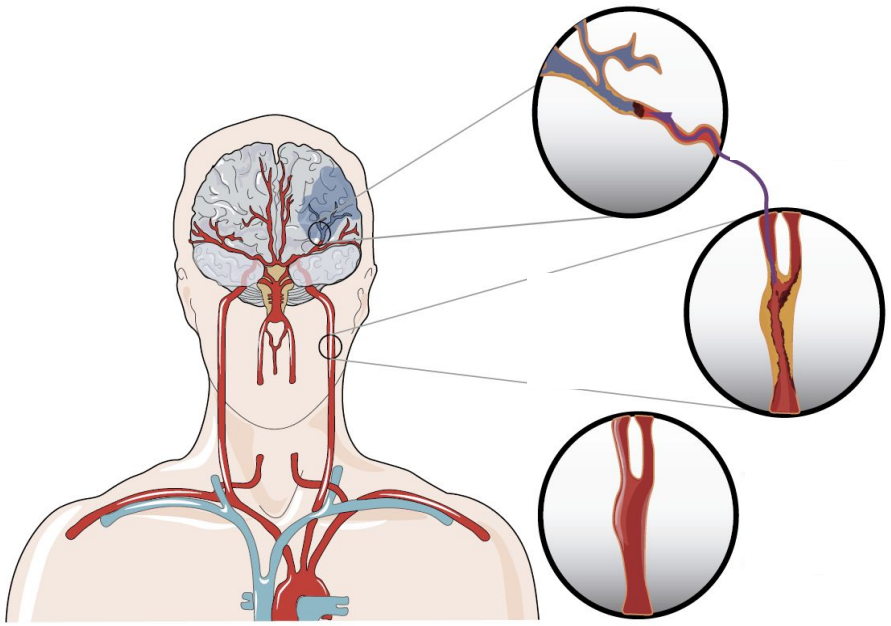
Open-label, Blinded for the end-point Evaluators)

Ensayo multicéntrico y multidisciplinario

Planteamiento “Proof-of-concept”

Fuente de financiación: ISC III (ICI25/00082)

03. Población de estudio



SAVE Trial

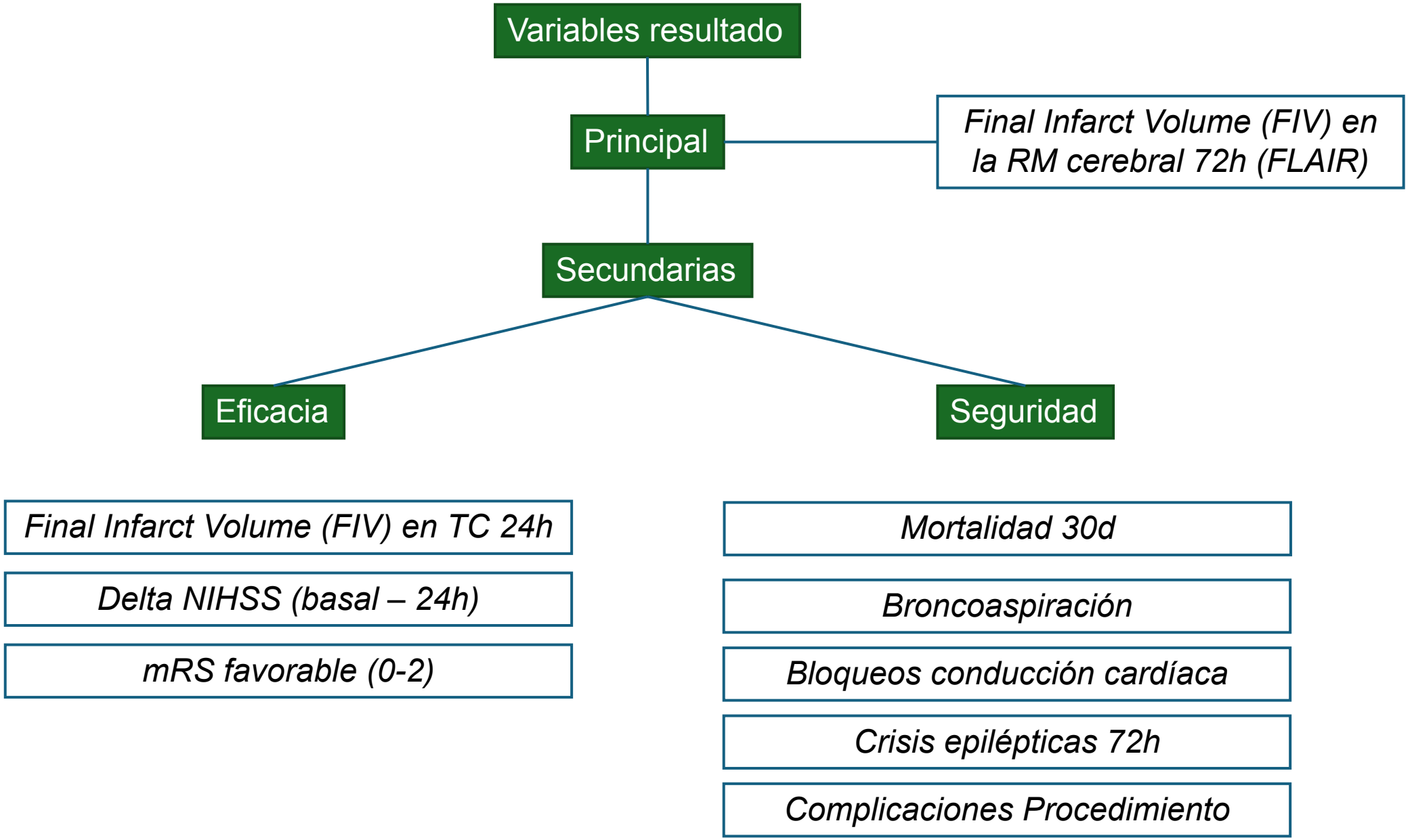
Criterios de Inclusión

- ≥18 años
- Ictus isquémico agudo <24h des de LTSW
- OGV de circulación anterior (TICA, M1, M2 o Tàndems)
- mRS previo 0-3
- TEV con AG (iniciada <30’ desde el inicio)
- ASPECTS >6

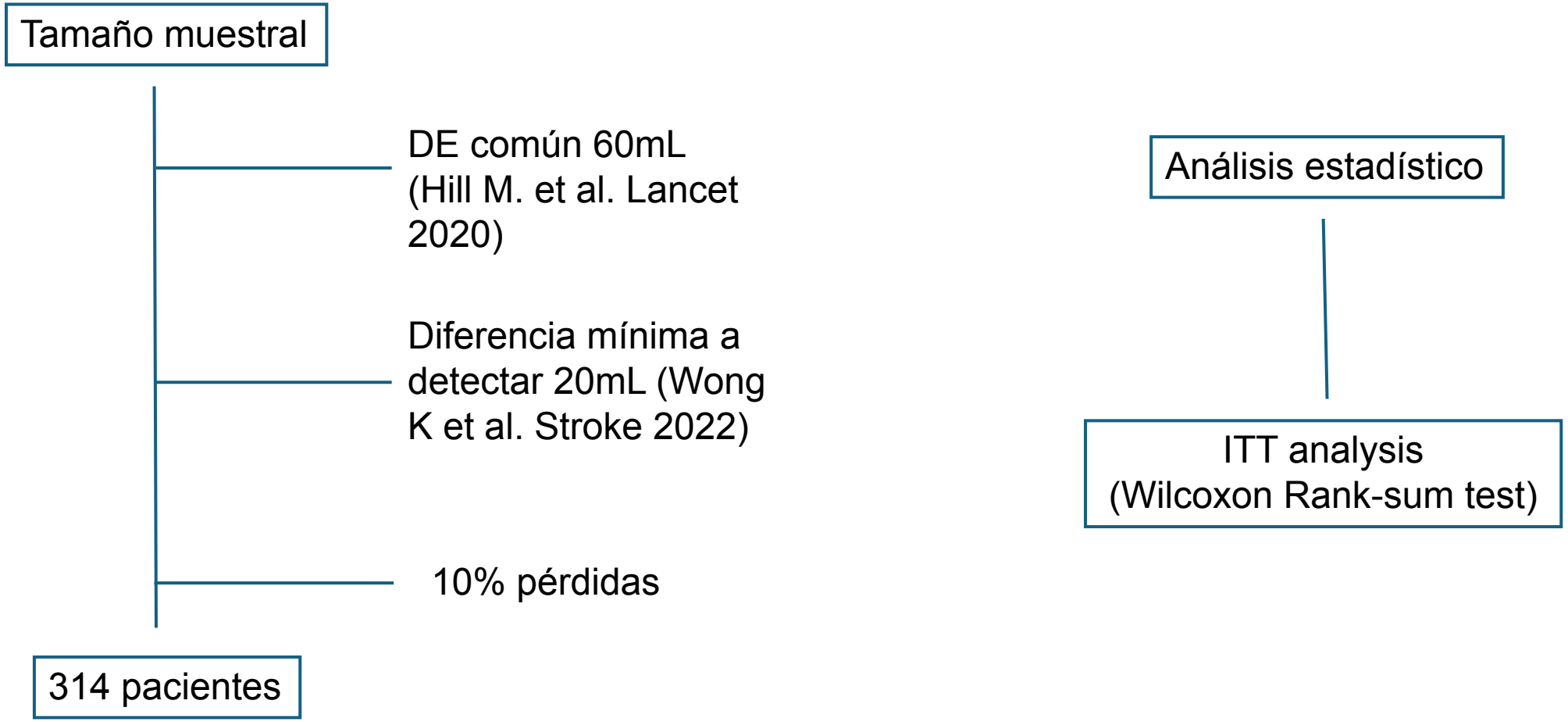
Criterios de Exclusión

- Inestabilidad hemodinámica y/o insuficiencia respiratoria
- Oclusión de ACA o segmentos distales de ACM (M3/M4)
- Oclusión de circulación posterior

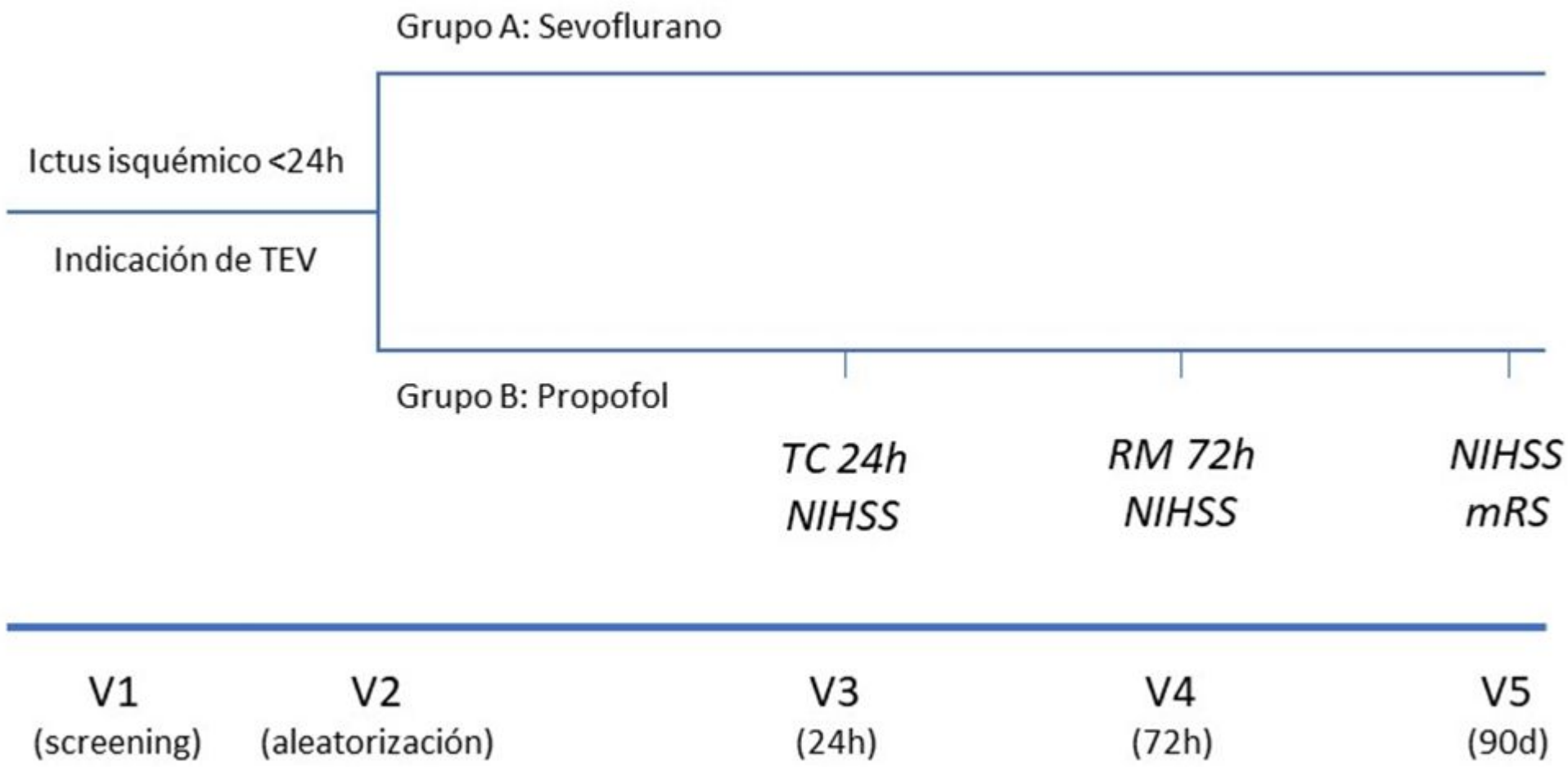
03. Endpoints

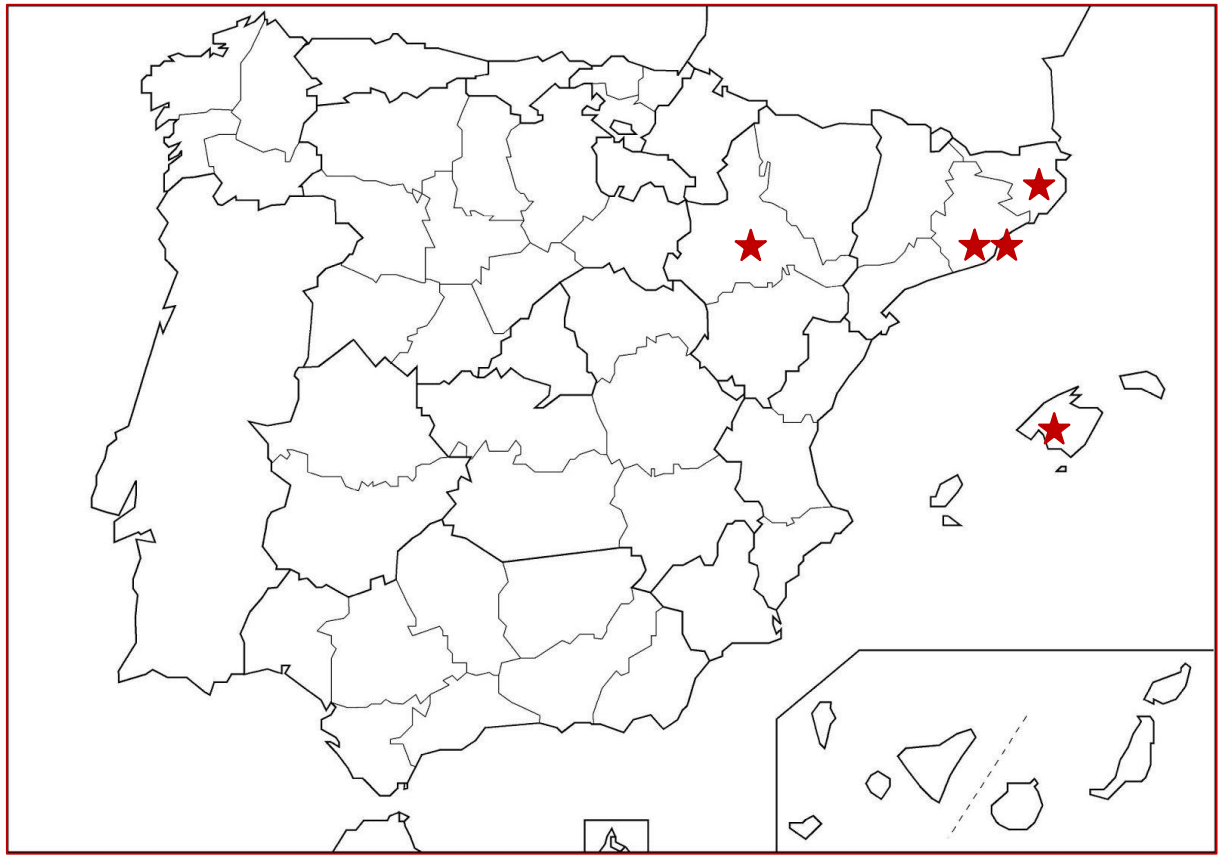

Variables resultado
Principal
Final Infarct Volume (FIV) en la RM cerebral 72h (FLAIR)
Secundarias
Eficacia
Seguridad
Final Infarct Volume (FIV) en TC 24h
Delta NIHSS (basal – 24h)
mRS favorable (0-2)
Mortalidad 30d
Broncoaspiración
Bloqueos conducción cardíaca
Crisis epilépticas 72h
Complicaciones Procedimiento

03. Cálculo tamaño muestral y anàlisis estadístico


Tamaño muestral
DE común 60mL (Hill M. et al. Lancet 2020)
Diferencia mínima a detectar 20mL (Wong K et al. Stroke 2022)
10% pérdidas
314 pacientes
Análisis estadístico
ITT analysis (Wilcoxon Rank-sum test)

04. Intervención y flujo del estudio


Grupo A: Sevoflurano
Ictus isquémico <24h
Indicación de TEV
Grupo B: Propofol
TC 24h
NIHSS
RM 72h
NIHSS
NIHSS
mRS
V1
(screening)
V2
(aleatorización)
V3
(24h)
V4
(72h)
V5
(90d)



Centros participantes:

- Hospital de la Santa Creu i Sant Pau
- Hospital del Mar
- Hospital Josep Trueta
- Hospital Son Espases
- Hospital Miguel Servet

Stroke Pharmacogenomics and Genetics

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de Recerca
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Fundació
Privada Hospital
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