TOWARD PRECISION MEDICINE FOR SEPSIS

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GARCHES, FRANCE

DECLARATION OF INTEREST

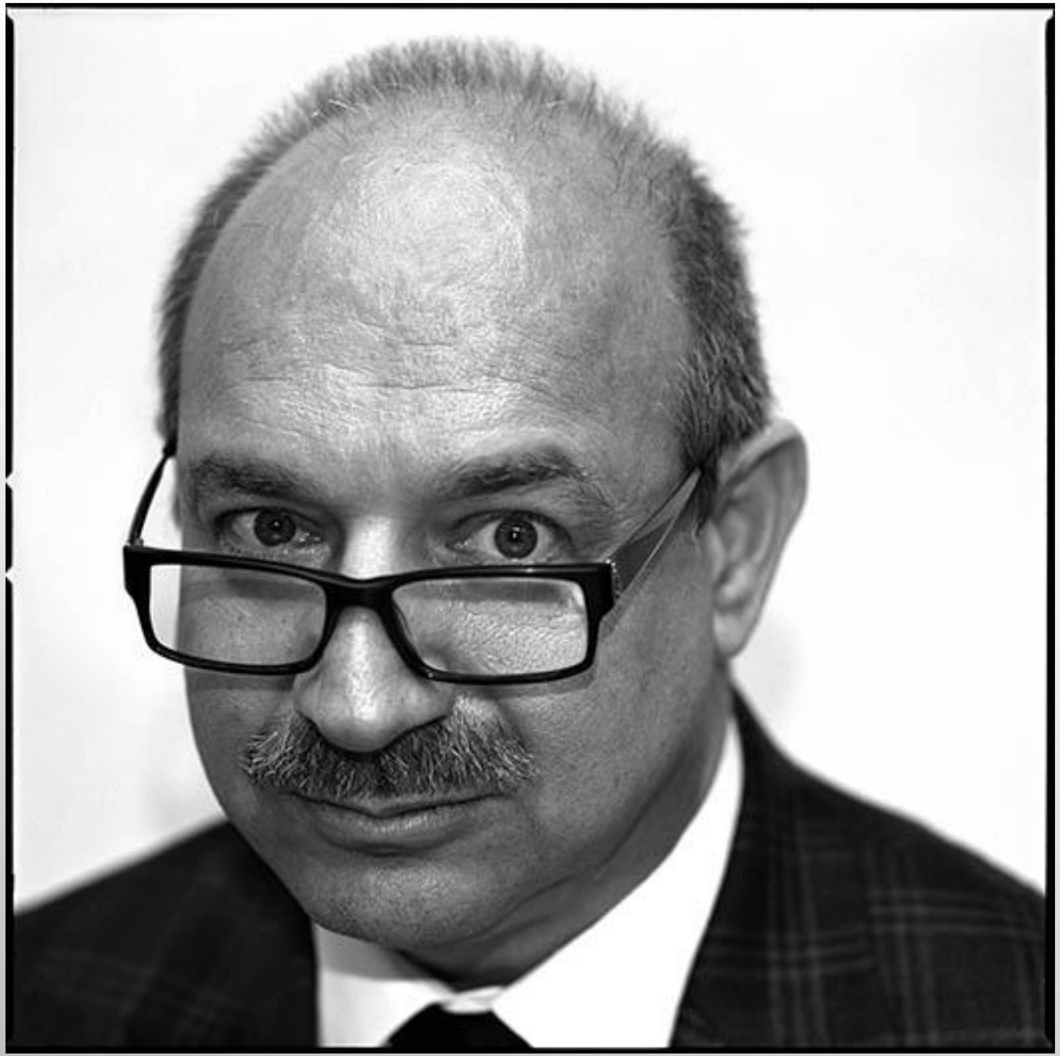
- FINANCIAL:
  - SINCE 1995 I HAVE RECEIVED MULTIPLE GRANTS FROM FRENCH MINISTRY OF HEALTH, FRENCH MINISTRY OF HIGHER EDUCATION, RESEARCH AND INNOVATION, VARIOUS EUROPEAN RESEARCH PROGRAMS, FROM CHARITY ENTITIES - FRENCH NATIONAL PROGRAMME D'INVESTISSEMENT D'AVENIR: ANR RHU 004
  - PAST 5 YEARS: RECEIVED HONORARIUM TO CONTRIBUTE TO ADVISORY BOARD ON CORTICOSTEROIDS FOR SEPSIS (PFIZER), BIOMARKERS FOR SEPSIS (BAXTER, BIOMERIEUX, VOLITION, SPHINGOTEC), VACCINES (JANSSEN), DRUGS (VIATRIS)
- ACADEMIC :
  - I CONTRIBUTE TO SSC 2008/2012/2016 UPDATES
  - I CO-CHAIR THE TASK FORCE OF CIRCI/CORTICOSTEROIDS IN THE ICU GUIDELINES, SINCE 2008
  - CORTICOSTEROIDS FOR ACUTE INFLAMMATION IS THE MAIN TOPIC OF RESEARCH OF MY GROUP SINCE 1991

Raphael Rodriguez
Curie Institute, Paris

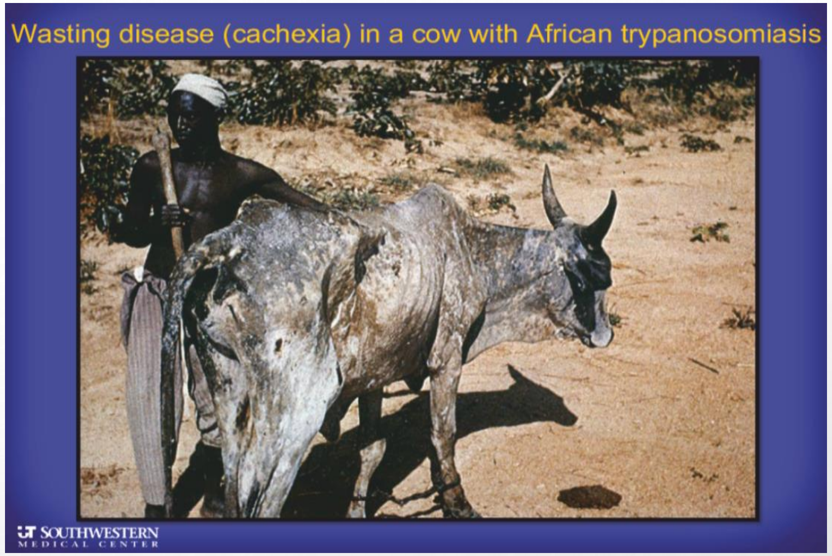
Elias Fattal
University Paris Saclay

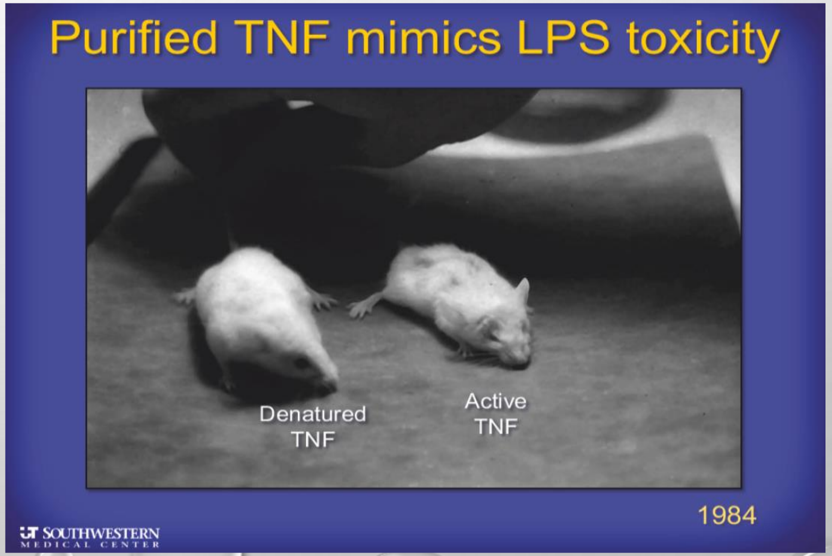
Eric Azabou
University Paris Saclay-Versailles

2011 Nobel Prize in Physiology or Medicine

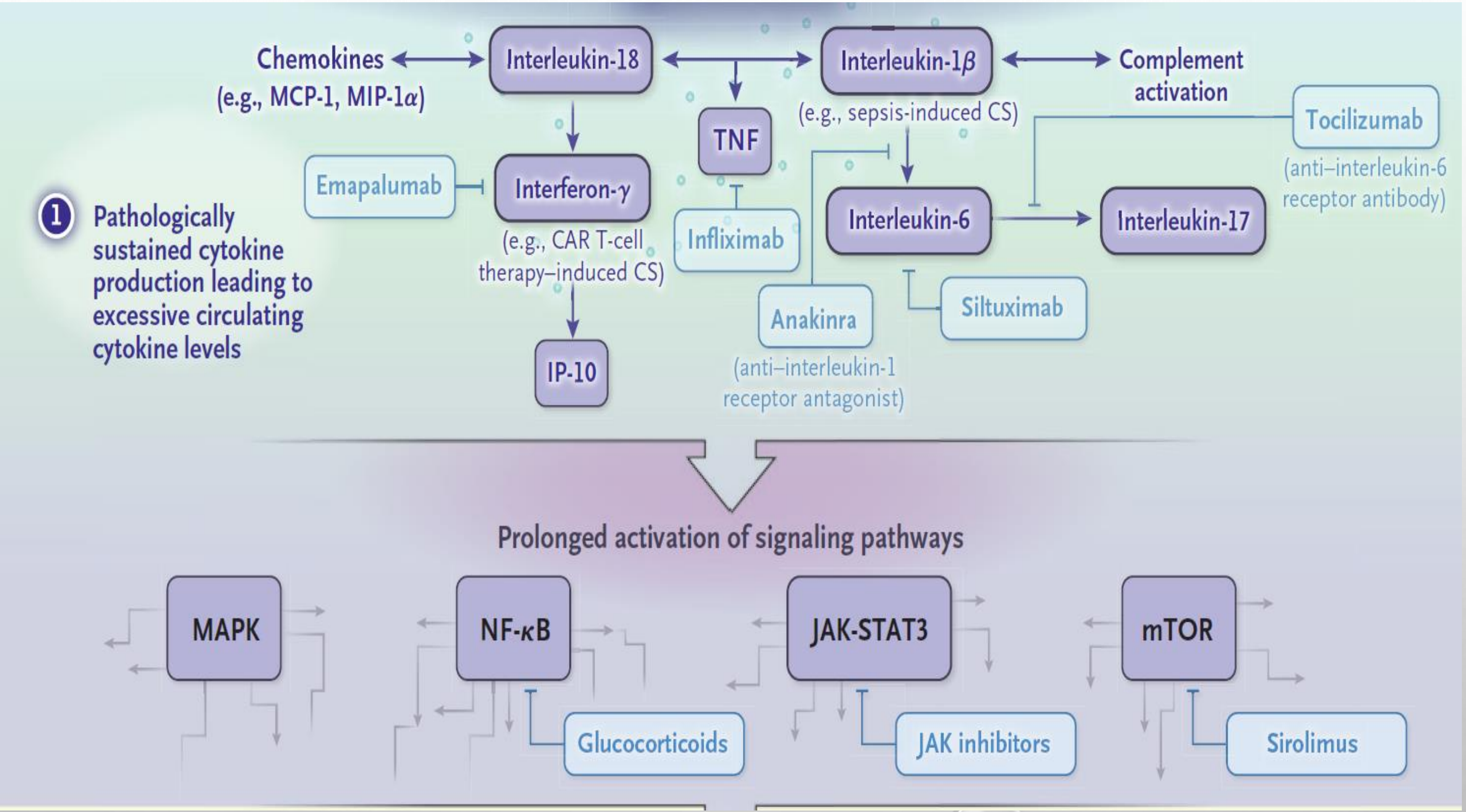


Bruce Beutler





Therapeutic intervention for CRS



Fajgenbaum, NEJM 2021

Treatment strategy	Therapeutic agent	Rationale	Stage	Clinical trial identifier	References
IL-6 or IL-6R inhibition	Siltuxumab, tocilizumab	IL-6 highly elevated during CRS, produced by activated myeloid cells, key cytokine in CRS development	Clinical, standard of care (tocilizumab)	–	(9) (10) (58) (19)
Corticosteroids	Dexamethasone, methylprednisolone	Immunosuppression to quiet overactive immune cells	Clinical, standard of care	–	(10) (18, 61) (33) (7)
GM-CSF depletion	Lenzilumab, GM-CSF gene knockout	GM-CSF involved in stimulation of myeloid cells, which are implicated in CRS and ICANS	Preclinical, clinical trial initiated	NCT04314843	(62) (63)
IL-1 inhibition	Anakinra	IL-1 elevated during ICANS, produced by activated myeloid cells, precedes IL-6 secretion	Preclinical, clinical trial initiated	NCT04148430, NCT04150913	(57) (55)
TNFα inhibition	Etanercept	TNFα elevated during CRS, produced by activated CART cells, key cytokine in CRS development	Clinical trials ongoing	NCT03050190	(67) (68) (69) (70)
JAK/STAT inhibition	Ruxolitinib, itacitinib	JAK/STAT pathway utilized by IL-6 and GM-CSF	Preclinical, clinical trial ongiong	NCT04071366	(72) (73) (74)
ITK inhibition	Ibrutinib	Retrospective analysis showed patients previously treated with ibrutinib had improved CART cell therapeutic outcomes, ITK inhibition dampens inflammatory cytokines but enhances Th1 functions	Clinical trials ongoing	NCT02640209, NCT03960840, NCT01865617, NCT04234061, NCT03331198, NCT03310619	(75) (77) (78) (79) (80)
Pharmacological T cell activation switch	Dasatinib	T-cell receptor kinases utilized in CART cell signaling, reversibly inhibited to dampen immune overactivation	Preclinical	–	(98) (99)
Endothelial cell protection	Defibrotide	Endothelial cell activation from systemic inflammation a key driver of ICANS	Clinical trial initiated	NCT03954106	(92)
Suicide genes and selection markers	Inducible caspase 9, truncated EGFR, CD20	CART cells selectively ablated if dangerously overactivated	Clinical trials ongoing	NCT02107963, NCT01822652, NCT03373071, NCT03618381, NCT03084380, NCT02937844, NCT03710421, NCT02159495, NCT02844062	(93, 94, 95, 96, 97)

Siegler, Front Immunol 2020

FHU SEPSIS
Personalized Interventions

Cell Reports Medicine

Article

Toward personalized immunotherapy in sepsis: The PROVIDE randomized clinical trial

Authors

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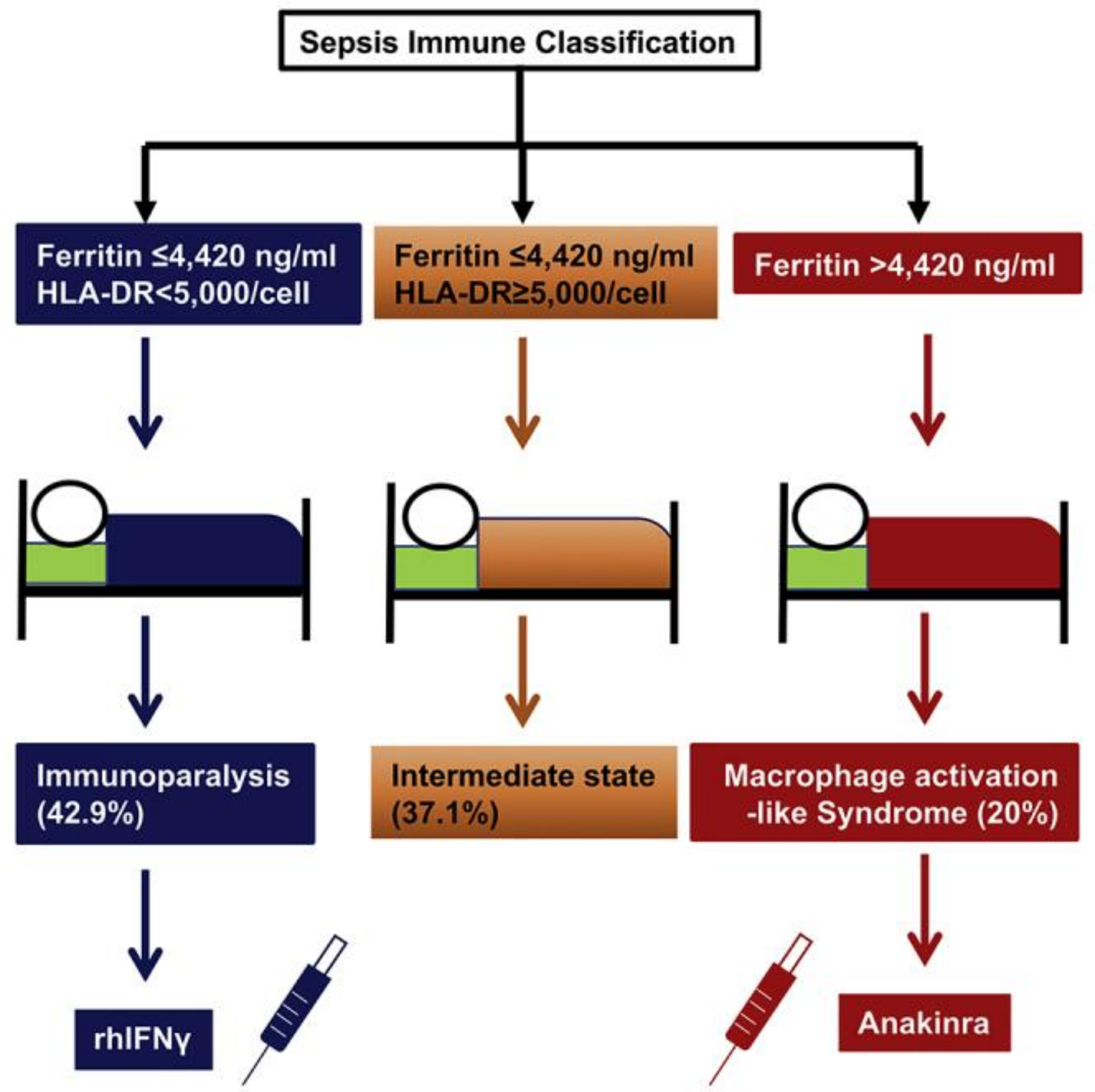
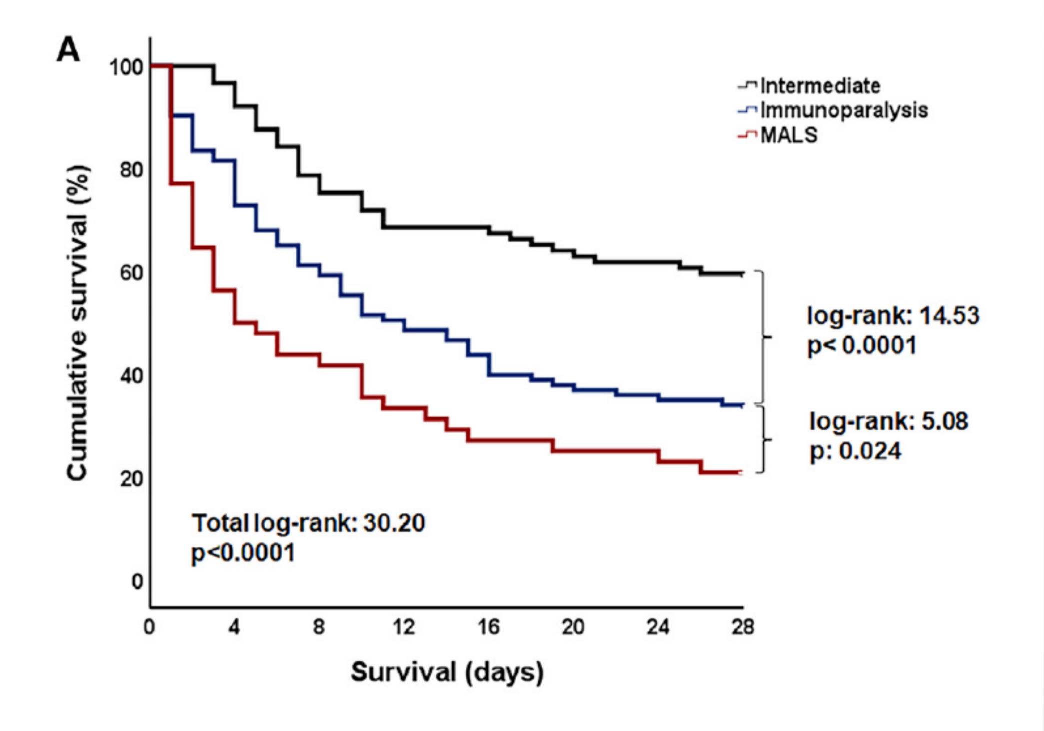
Evangelos J. Giamarellos-Bourboulis

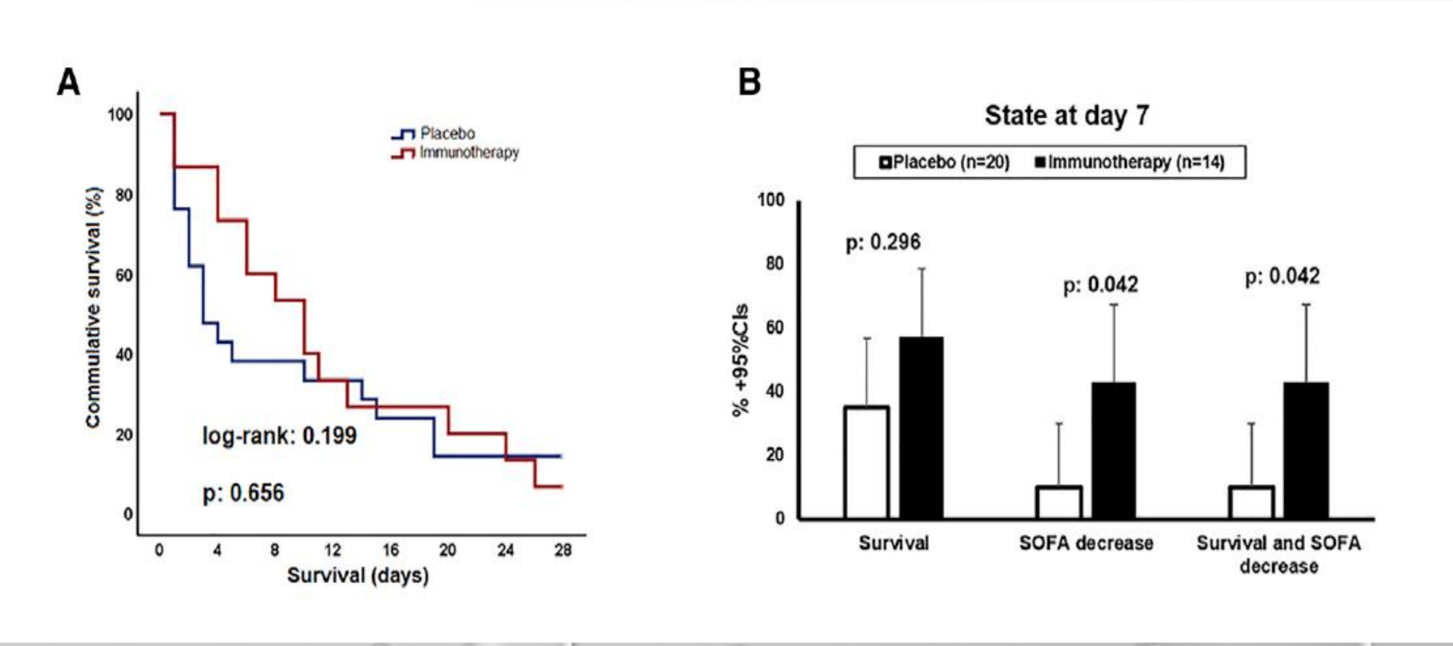


FHU SEPSIS
Personalized Interventions

Table 2. Classification of the immune states of sepsis used in the PROVIDE study

	Macrophage activation-like syndrome	Sepsis-induced immunoparalysis	Intermediate
Ferritin	>4,420 ng/mL	≤4,420 ng/mL	≤4,420 ng/mL
Percentage of CD45/CD14- monocytes that express HLA-DR	All values	<30%	≥30%





ADREN-OSS-2 POST HOC

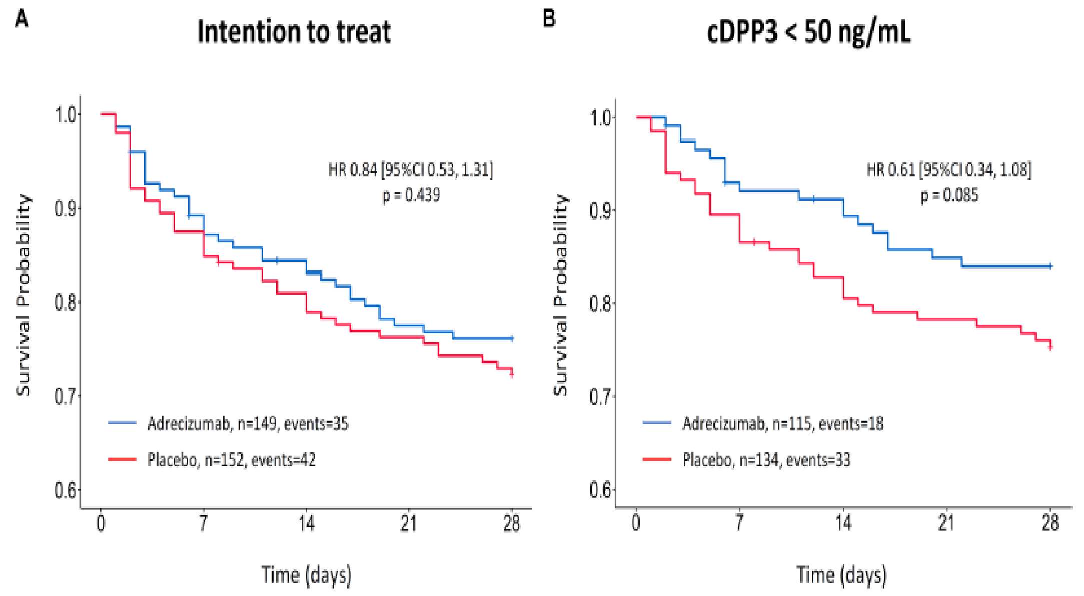
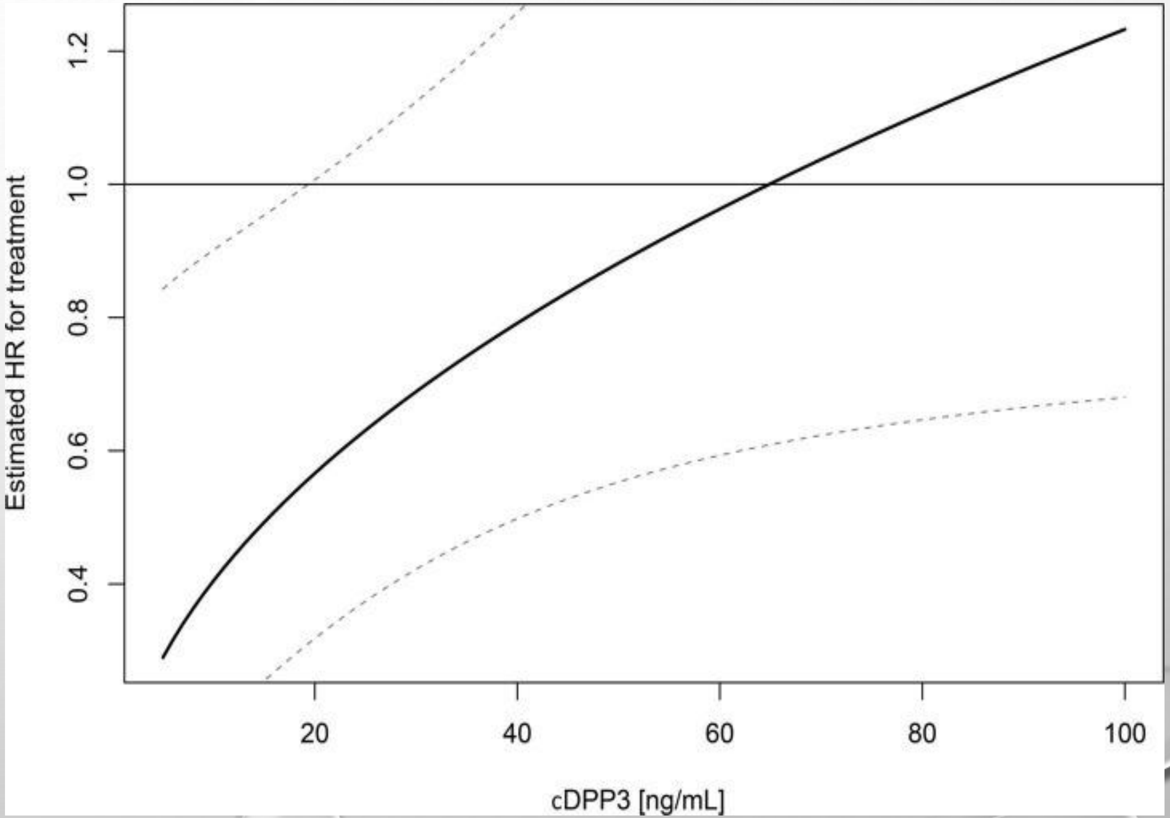


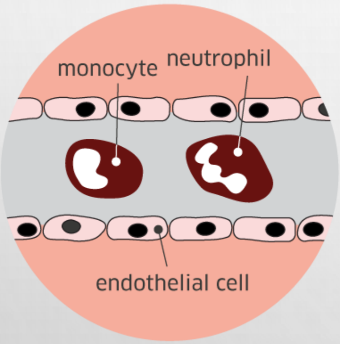
FIGURE 1
Kaplan–Meier of 28-day mortality after Adrecizumab/placebo infusion, based on circulating dipeptidyl peptidase 3 (cDPP3) stratification. Data is displayed for the original intention to treat (ITT) analysis (15) **(A)** and the cDPP3 <50 ng/mL subgroup **(B)**. Both Adrecizumab dosing groups were combined. Crosses represent censored patients.



Van Lier Front Med 22

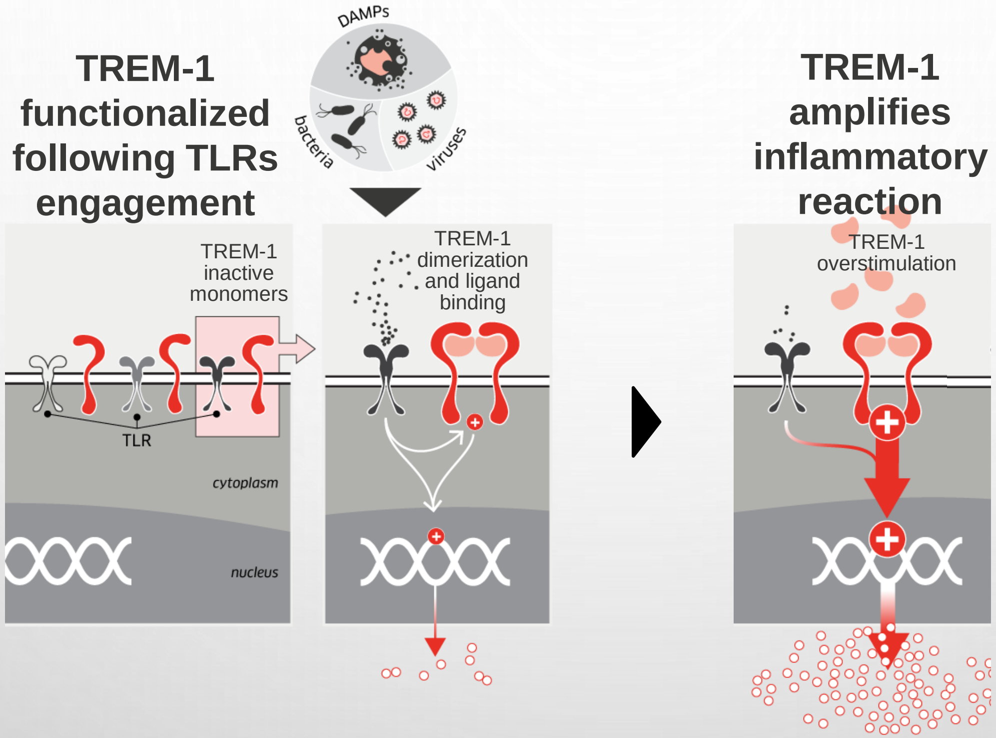
TREM-1: universal innate immune amplifier and inflammatory response regulator

Expressed by innate immune cells



TREM-1 functionalized following TLRs engagement

TREM-1 amplifies inflammatory reaction



INFLAMMATION

Production of pro-inflammatory cytokines, chemokines and free radicals

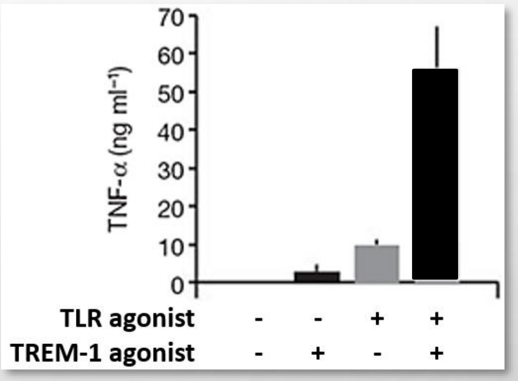
INFLAMMATION

IMMUNE DYSREGULATION

Excessive production of pro-inflammatory cytokines, chemokines and free radicals

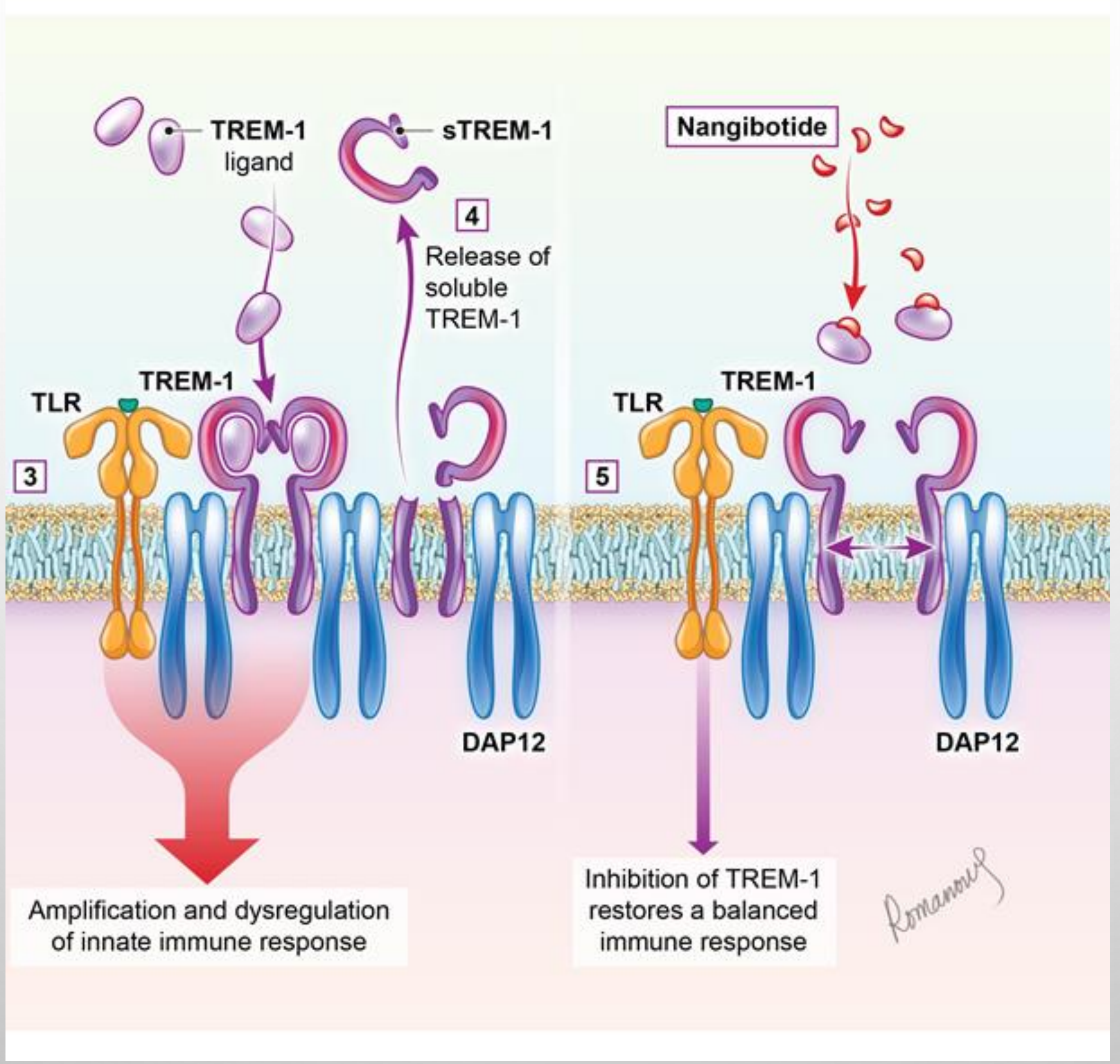
TNF-α release

human monocytes



BOUCHON, NATURE 2001

Nangibotide/LR12 acts as a decoy receptor to inhibit TREM-1



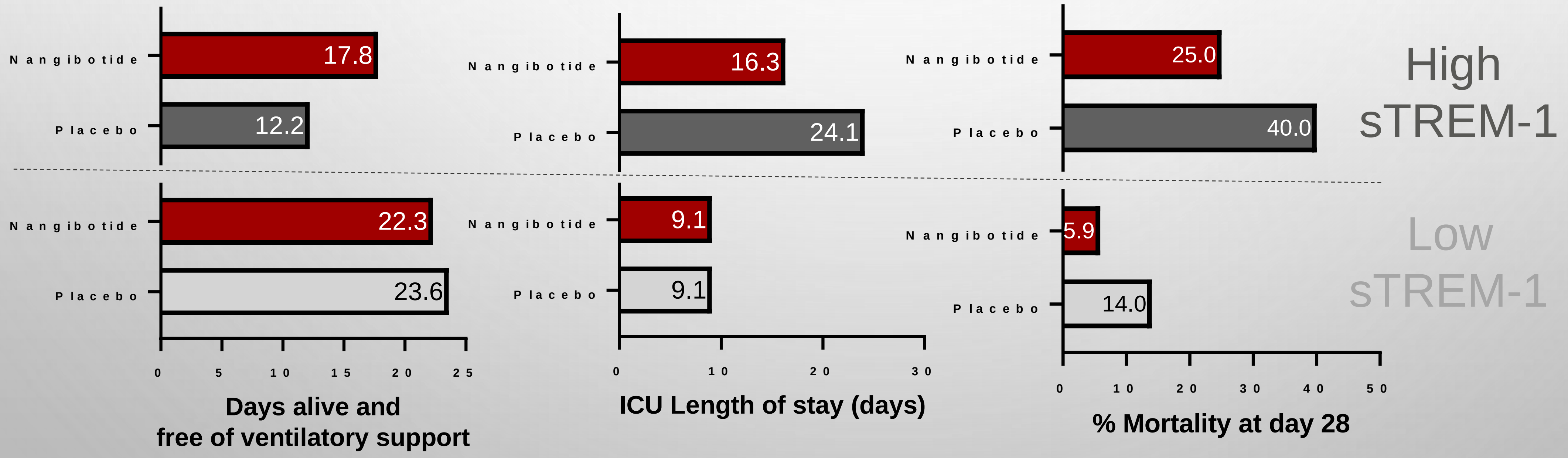
MOT-C-201 phase IIa septic shock trial

Results

sTREM-1 – A potential prognostic and predictive biomarker for nangibotide

Prognostic: sTREM-1 positive patients have higher acute morbidity, length of ICU stay & mortality

Predictive: Early phase signals support a preferential effect of nangibotide in high sTREM-1 patients



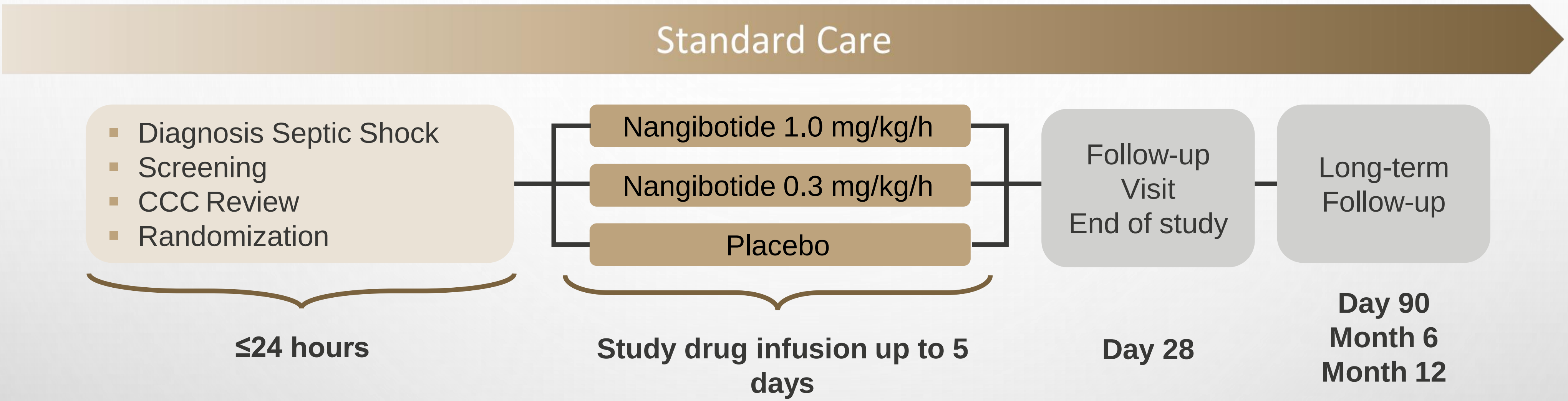
FRANÇOIS, ICM 2020

ASTONISH phase IIb septic shock trial

Study design

Prospective evaluation of the efficacy, safety, and optimal biomarker enrichment strategy for nangibotide, a TREM-1 inhibitor, in patients with septic shock (ASTONISH): a double-blind, randomised, controlled, phase 2b trial

*Bruno François, Simon Lambden, Tom Fivez, Sebastien Gibot, Marc Derive, Jean-Marie Grouin, Margarita Salcedo-Magguilli, Jérémie Lemarié, Nicolas De Schryver, Ville Jalkanen, Tarik Hicheur, Jean-Jacques Garaud, Valérie Cuvier, Ricard Ferrer, Morten Bestle, Ville Pettilä, Jean-Paul Mira, Camille Bouisse, Emmanuelle Mercier, Joris Vermassen, Vincent Huberlant, Isabelle Vinatier, Nadia Anguel, Mitchell Levy, Pierre-François Laterre on behalf of the ASTONISH investigators**



Biomarker driven precision medicine

- Analysis on overall and high sTREM-1 (≥400 pg/mL)

AND

- Optimal sTREM-1 cut-off level search

Robust endpoint selection

Primary endpoint in phase IIb

Organ function improvement:

SOFA score

Relevant secondary endpoints

Key secondary endpoint

- Day 28 mortality

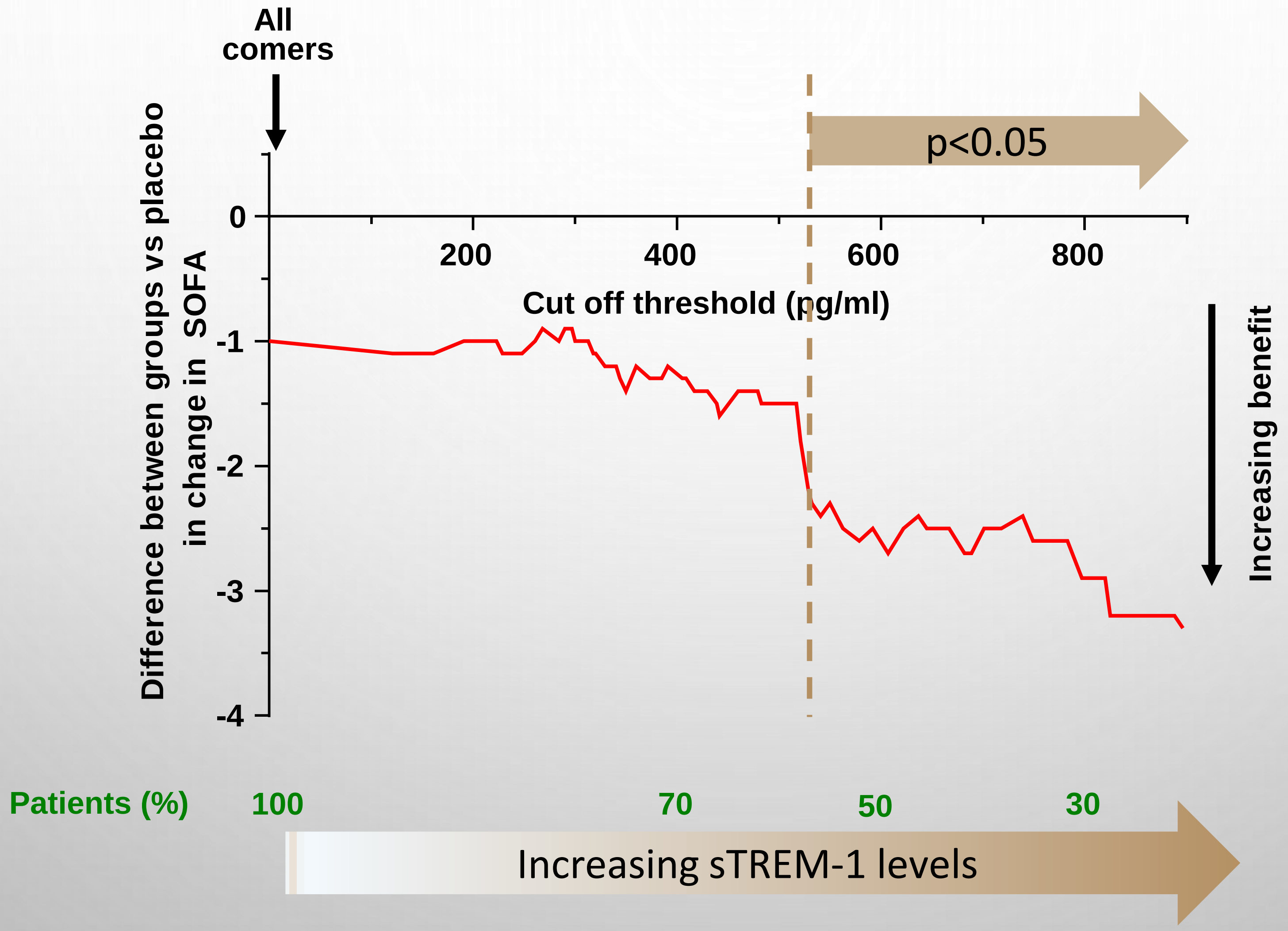
Other secondary endpoint

- Proportion death or on organ support
- Duration of organ support
- Superinfection and/or rehospitalization

FRANCOIS, LRM 2023

ASTONISH phase 2b septic shock trial

Change of SOFA depending on baseline sTREM-1 levels



Adjusted for baseline characteristics (SOFA, APACHEII, sTREM-1, IL6, Age, Gender, BMI, Infection site)

NEW DRUGGABLE PATHWAY OF REGULATION OF INFLAMMATION

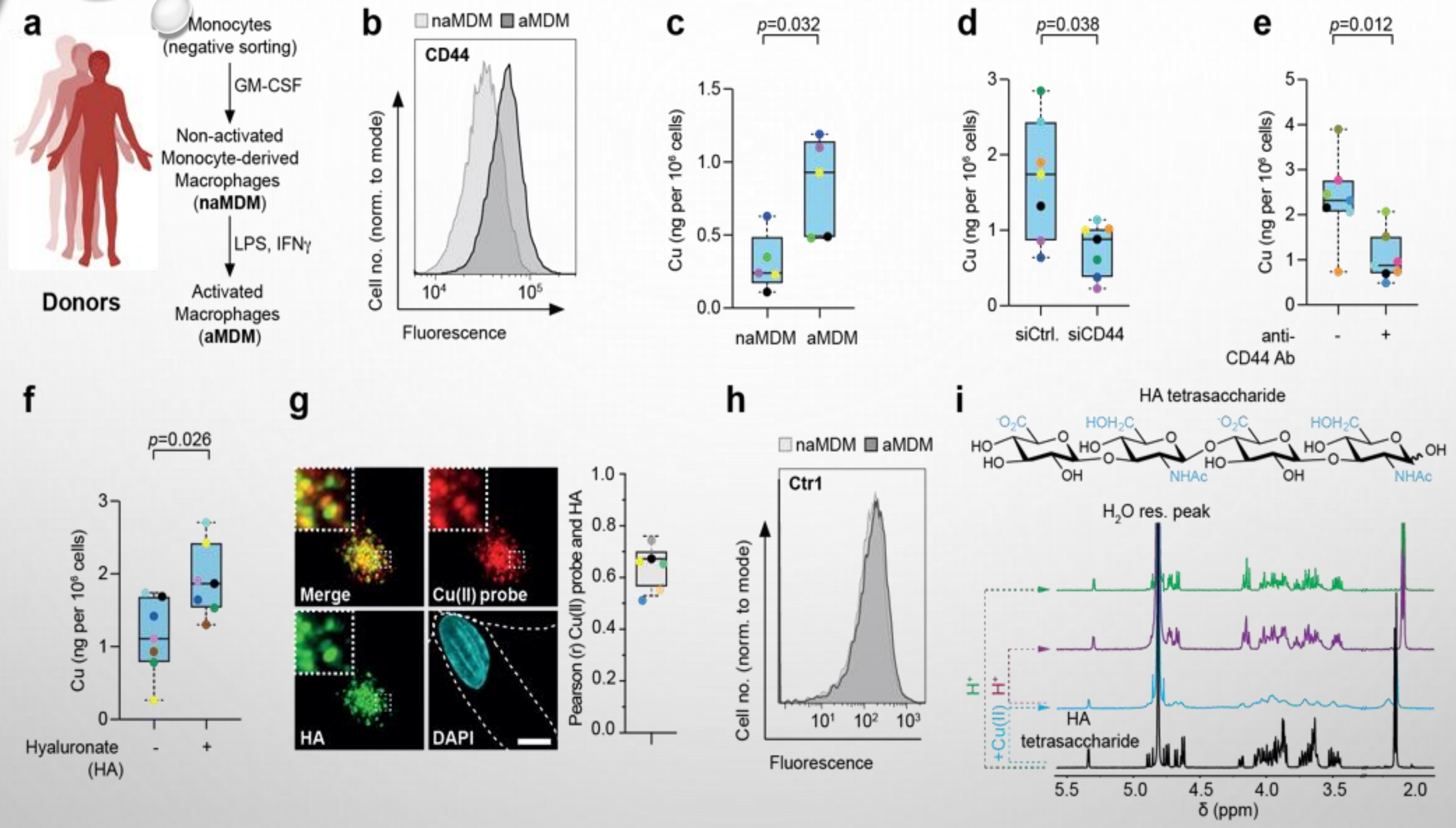


Figure 2. a, Experimental setup we used to generate inflammatory monocyte-derived macrophages (MDM).
b, CD44 increases in our primary cell model system. **c**, Copper increases in aMDM. **d**, CD44 siRNA reduces copper uptake.
e, CD44 blocking antibody reduces copper uptake. **f**, HA supplementation increases copper uptake. **g**, a fluorescent lysosomotropic copper(II) probe colocalizes with HA.
h, Copper transporter 1 and transferrin transporter 1 are not significantly increased in aMDM. **i**, NMR spectra showing that HA and copper(II) interact. **Nature 2023**.

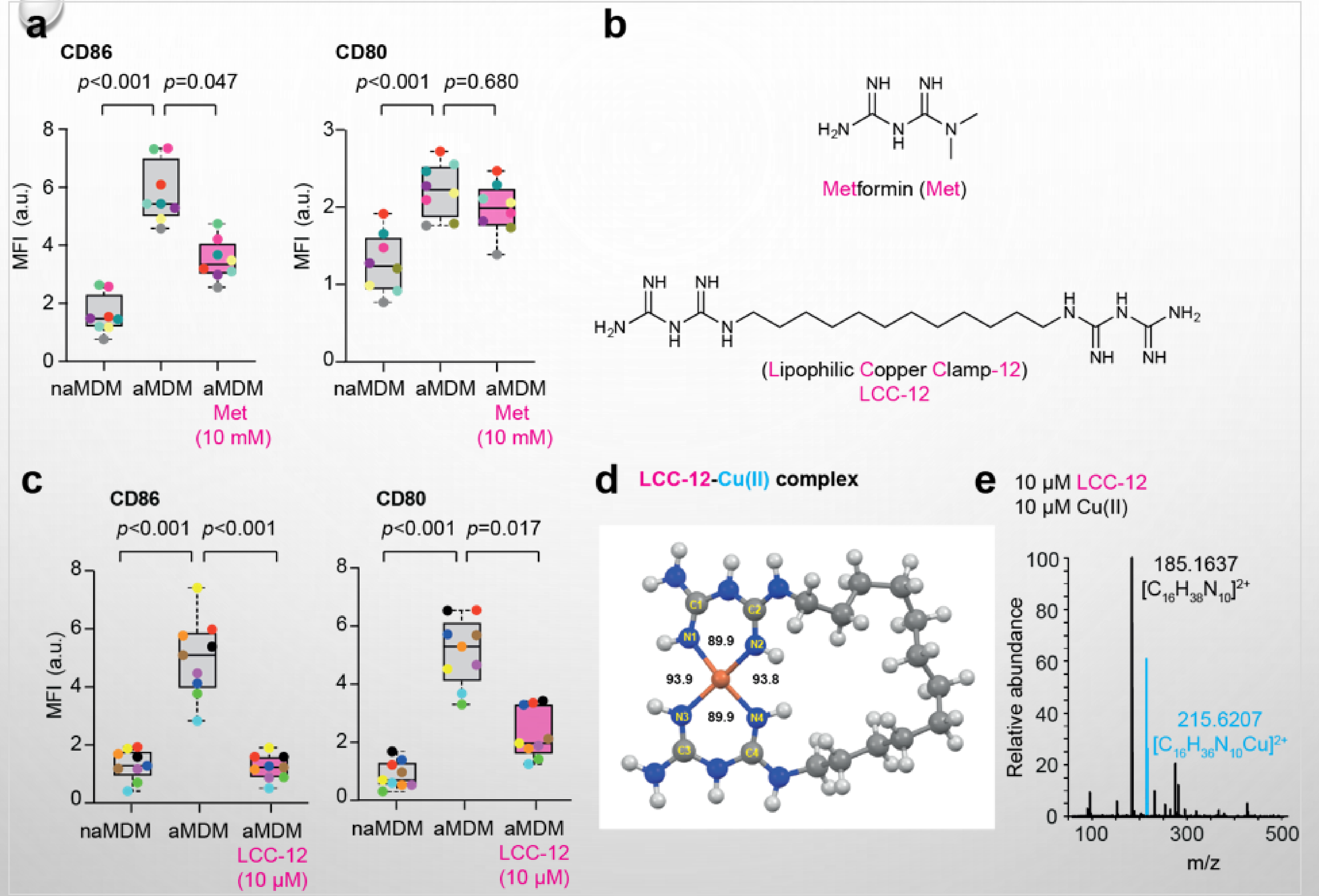


Figure 3. a, Metformin has a marginal effect on MDM activation. **b**, molecular structures of Met and LCC-12. **c**, LCC-12 antagonizes macrophage activation. **d**, Structural analysis of LCC-12-copper(II) complex by molecular modeling. **e**, High resolution mass spectrometry showing the LCC-12-copper(II) complex. **Nature 2023**.

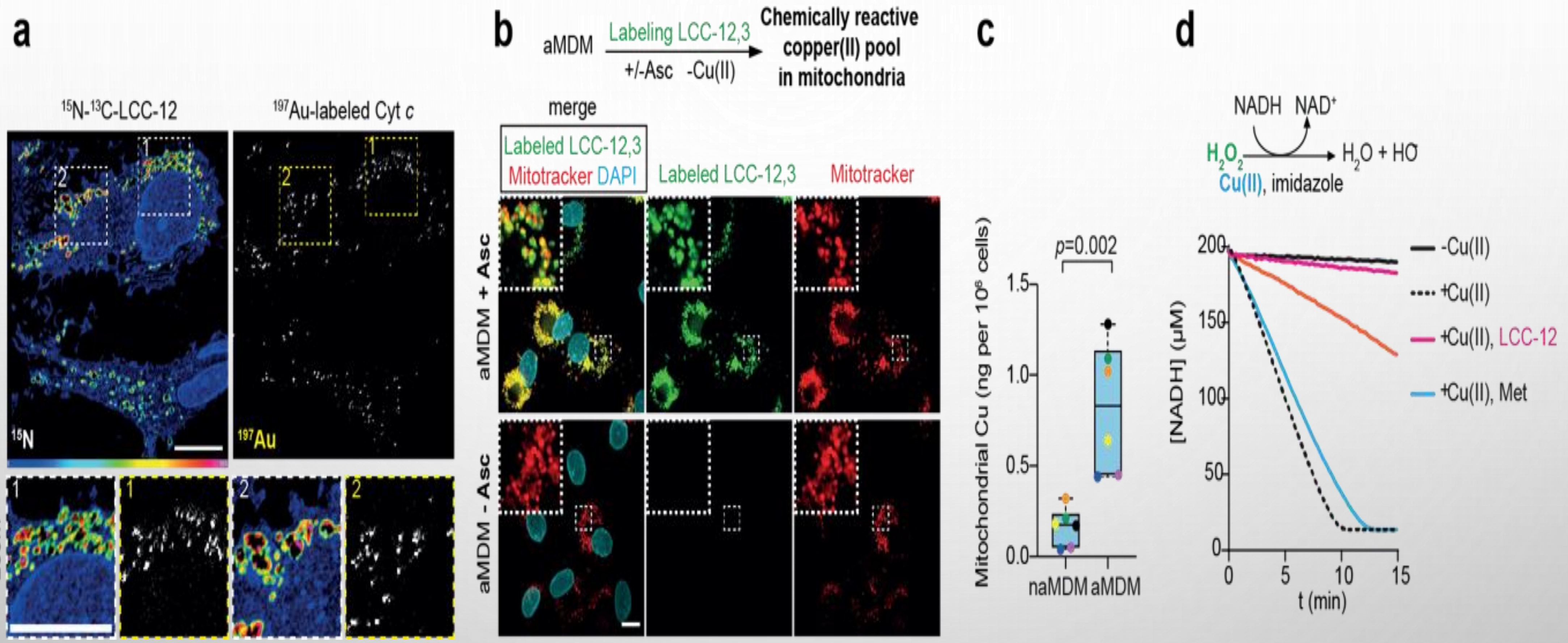


Figure 4. a, LCC accumulates in mitochondria marked by cytochrome *c*
b, Mitochondrial LCC-12 reveals the presence of a druggable mitochondrial copper(II) pool
c, Mitochondrial copper increases during macrophage activation
d, copper(II) catalyzes the conversion of NADH to NAD^+. LCC-12 blocks this reaction by chelating Cu(II). **Nature 2023**.

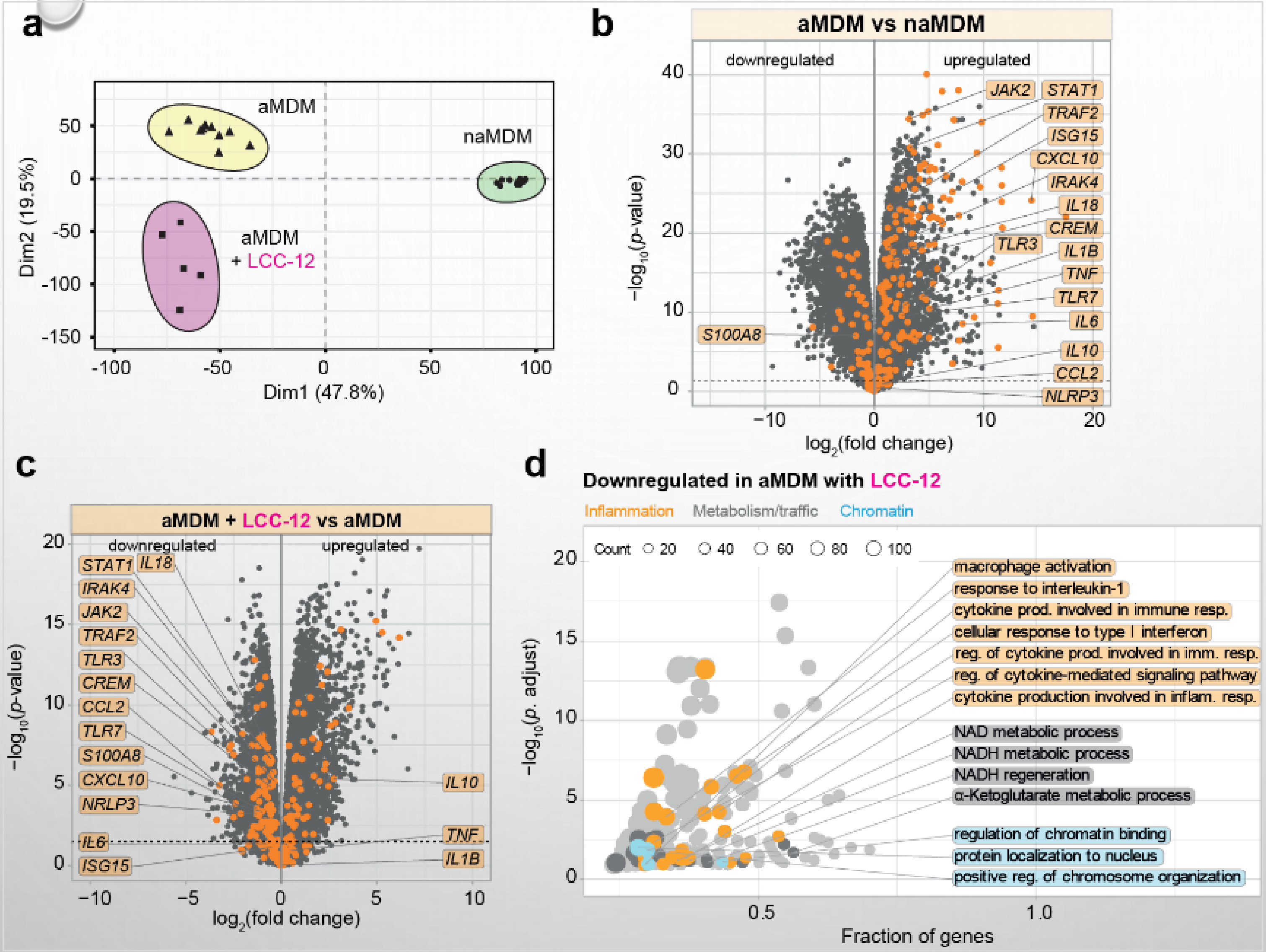


Figure 7. a, LCC-12 induces a different cell state. **b**, upregulation of the inflammatory gene signature during macrophage activation. **c**, downregulation of the inflammatory signature in aMDM treated with LCC-12. **d**, GO term analysis of genes in aMDM whose expression is downregulated upon treatment with LCC-12. **Nature 2023.**

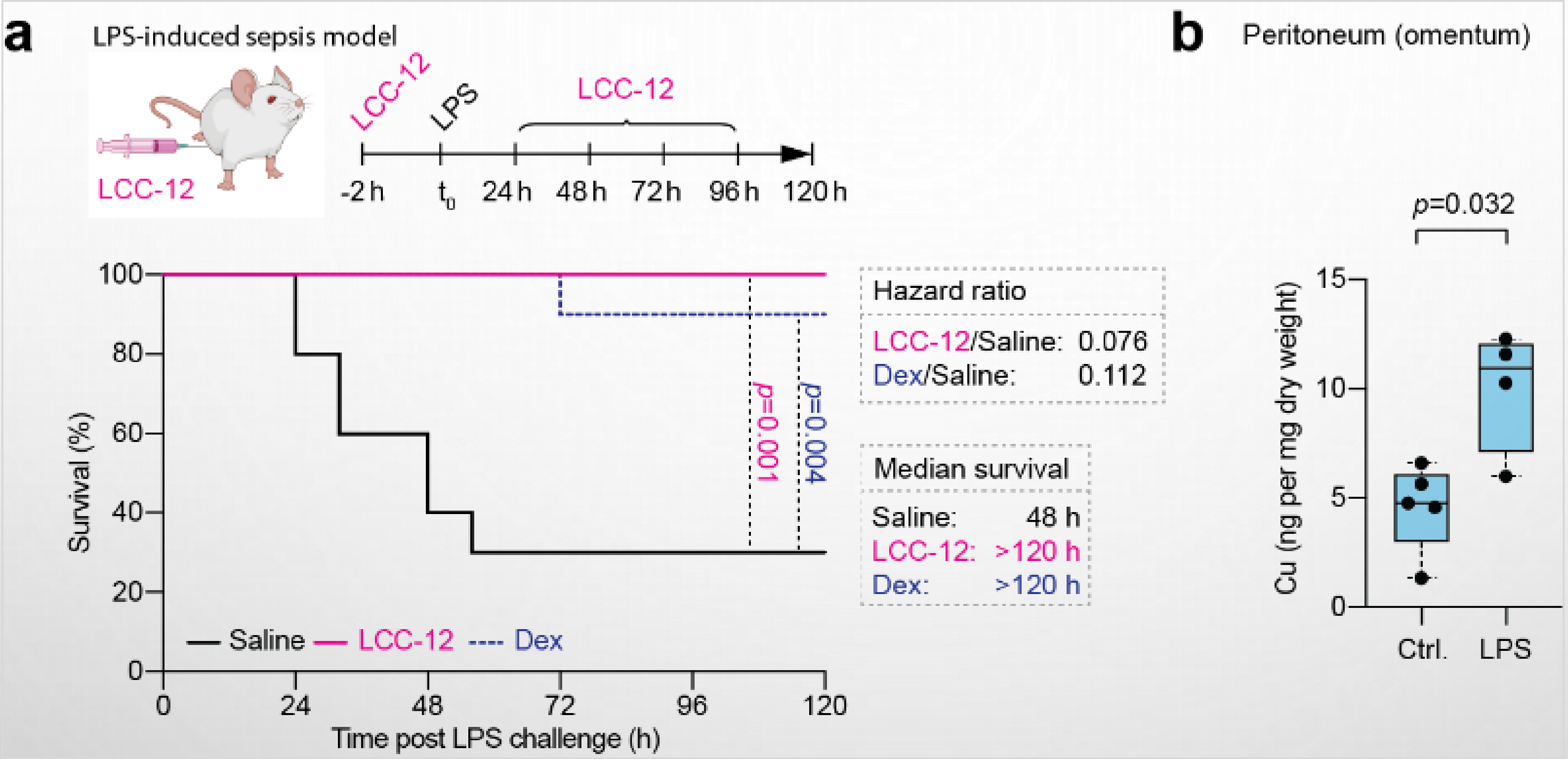
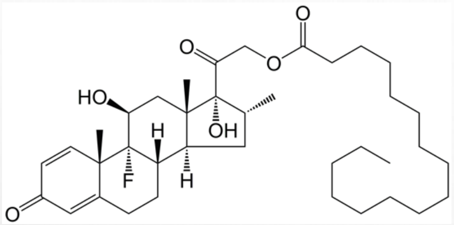


Figure 11. a, Kaplan-Meier survival curves of mice challenged with LPS (20 mg/kg/single dose, IP, *n*=10) and treated with LCC-12 (0.3 mg/kg, 2 h prior challenge, then 24 h, 48 h, 72 h and 96h post challenge, IP, *n*=10) or dexamethasone (10 mg/kg/single dose 1 h prior challenge, *per os* (PO), *n*=10). Mantel-Cox log-rank test. Hazard ratio calculated using Mantel-Haenszel.
, Copper increases in the peritoneum in LPS-challenged mice.

NANOMOLECULES
TOWARD PRECISION MEDICINE FOR CORTICOSTEROIDS

Encapsulated Corticosteroids

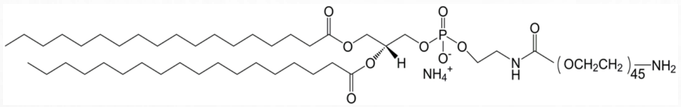
NANOPARTICULES OF DEXAMETHASONE :



Dexamethasone palmitate (DXP)

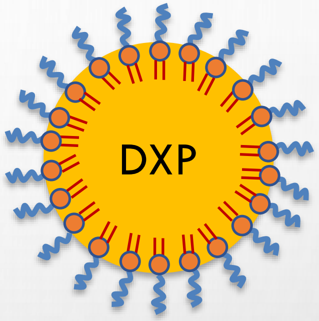
+

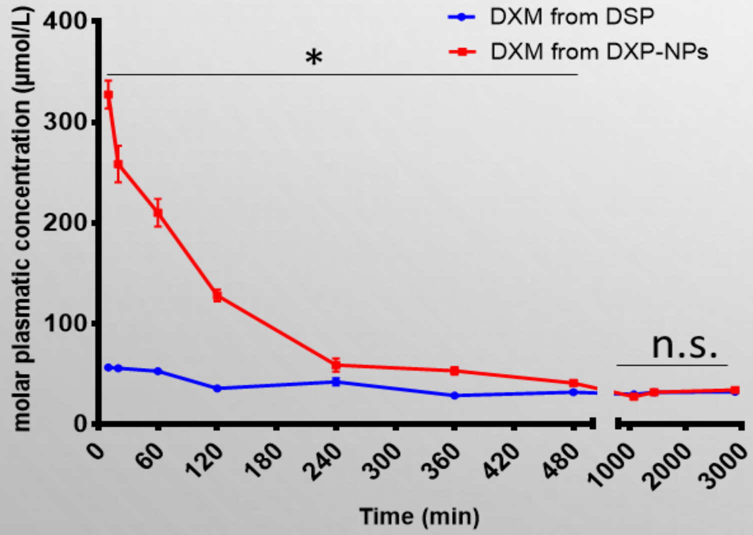
Prodrug based approach



DSPE-PEG $_{2000}$

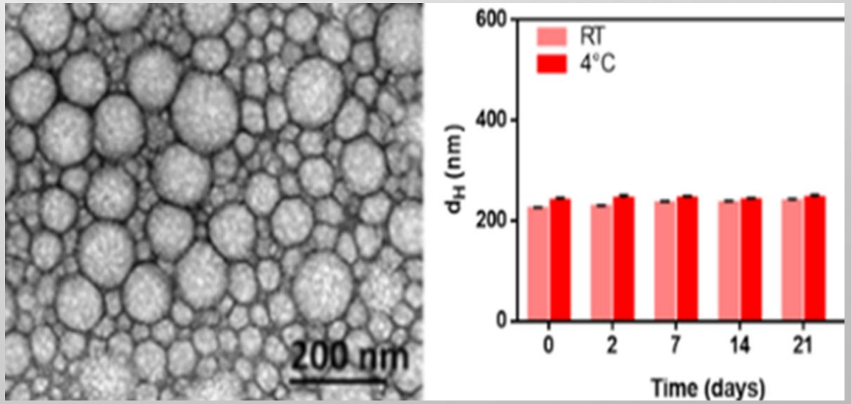
→ DXP-NPs



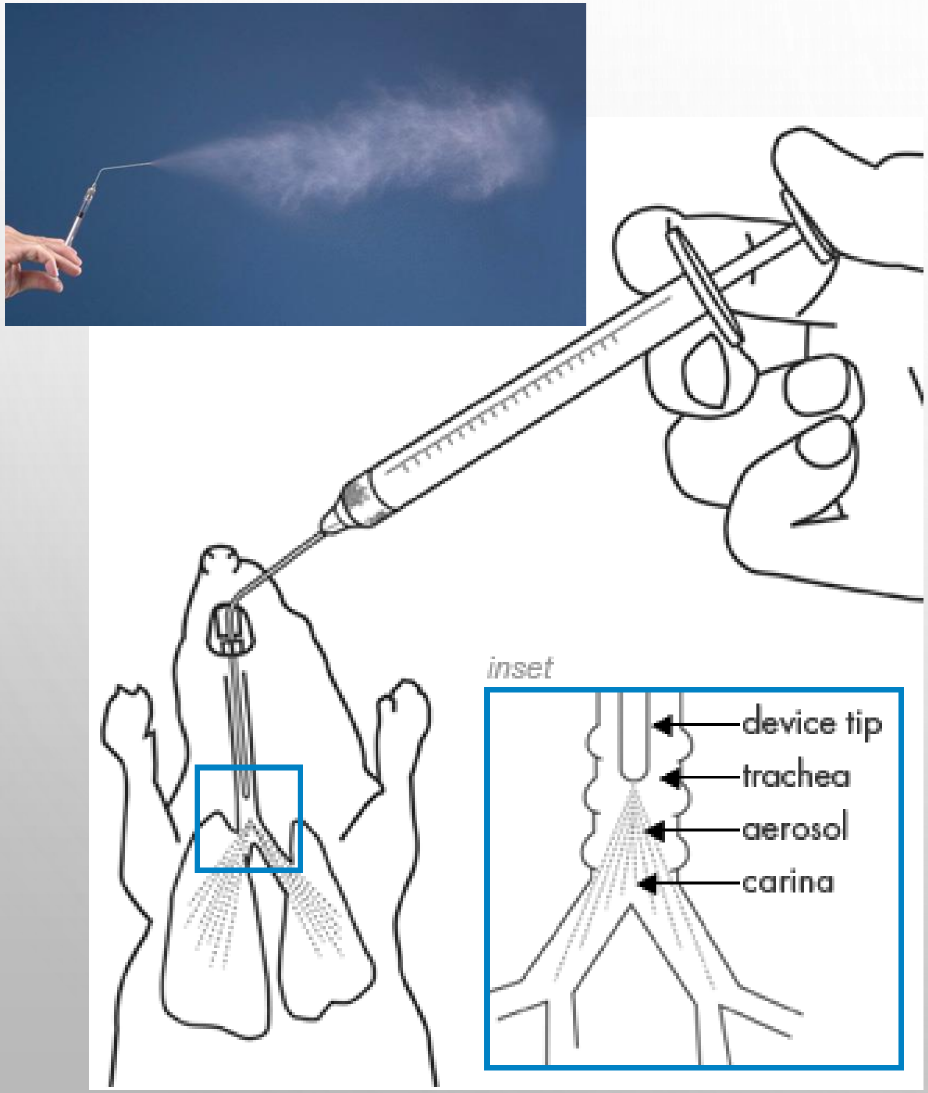


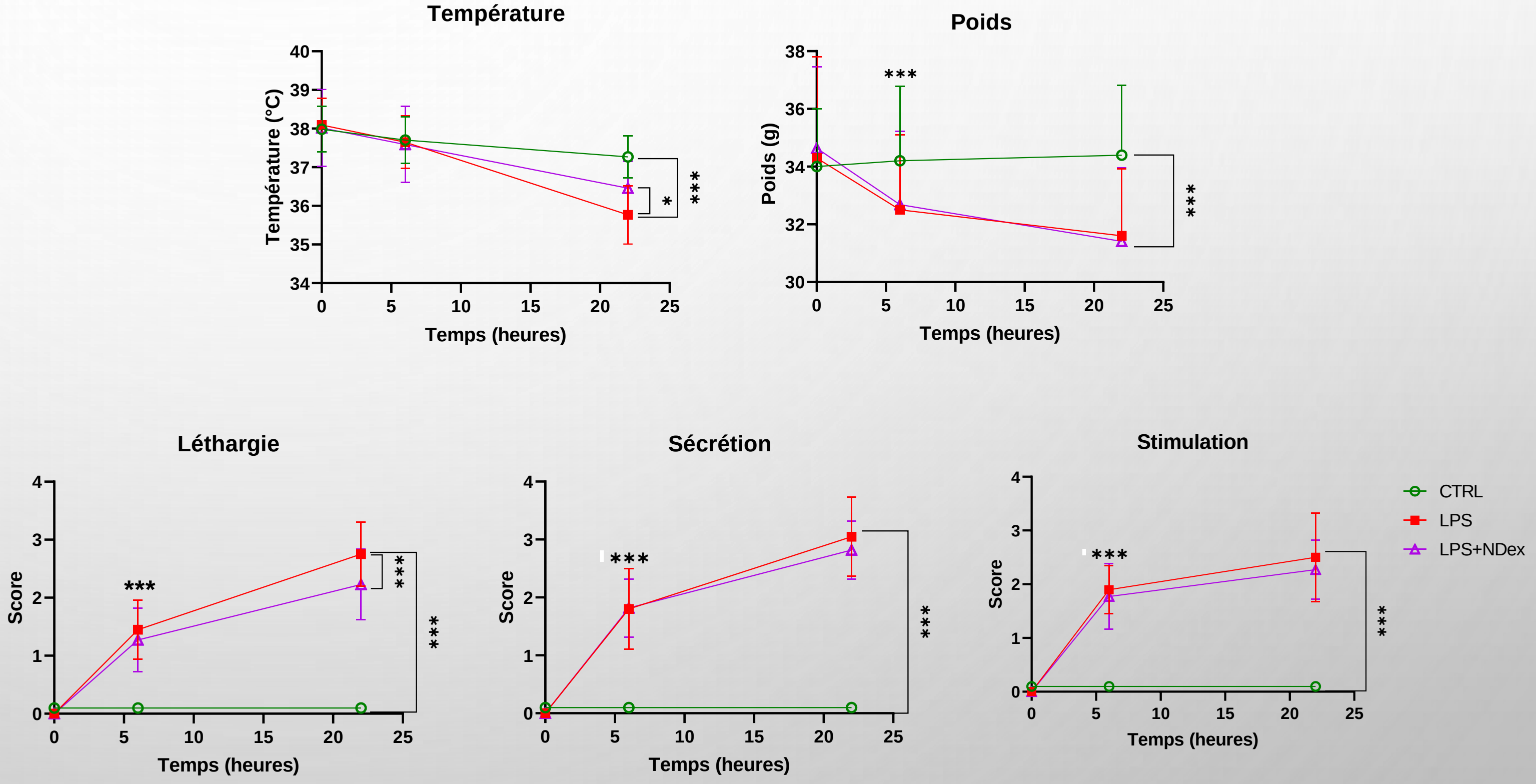
IV injection of DXP-NPs
Versus
Dexamethasone
Sodium Phosphate
(DSP) solution

12mg/kg (eq. DXM)



NANOPARTICULES DEXAMETHASONE : ENDO-TRACHEAL ADMINISTRATION





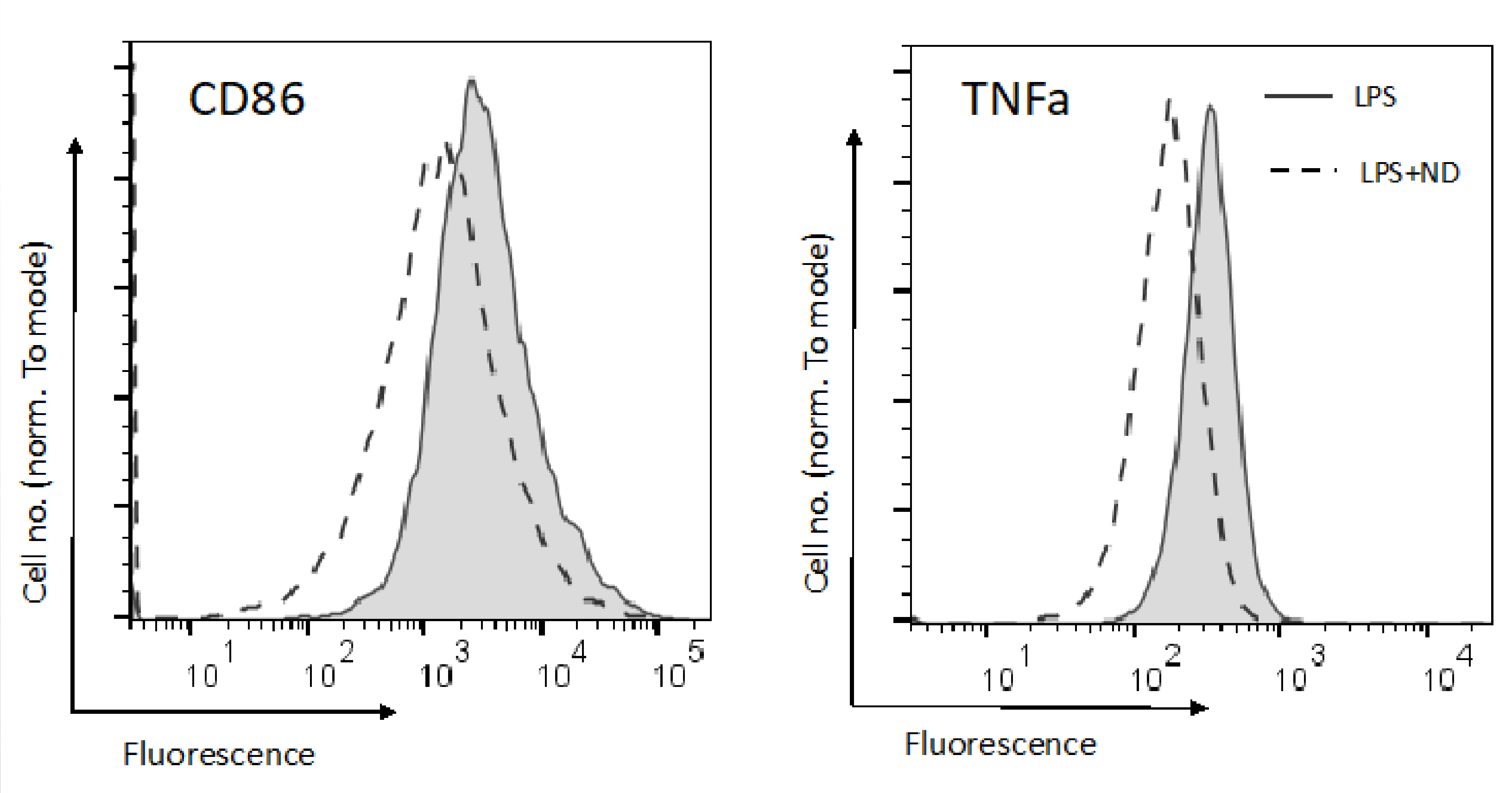
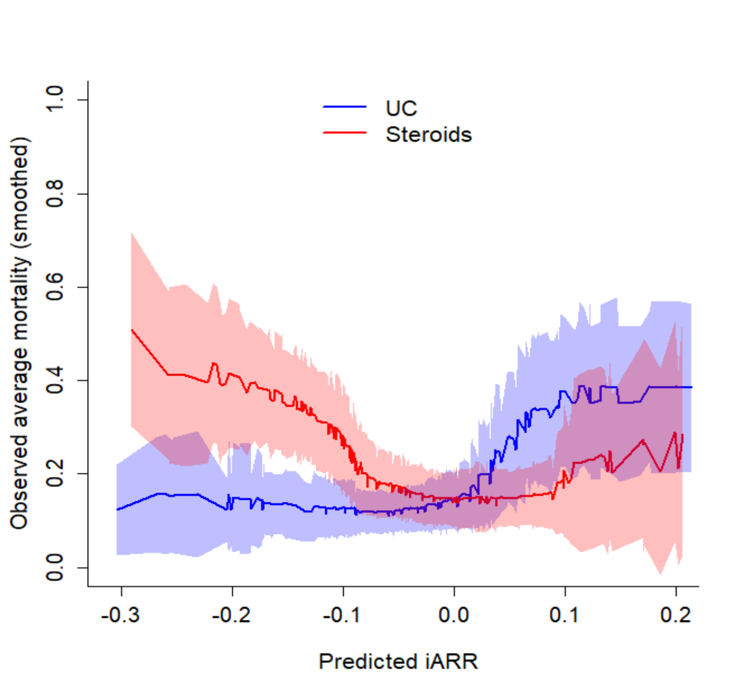


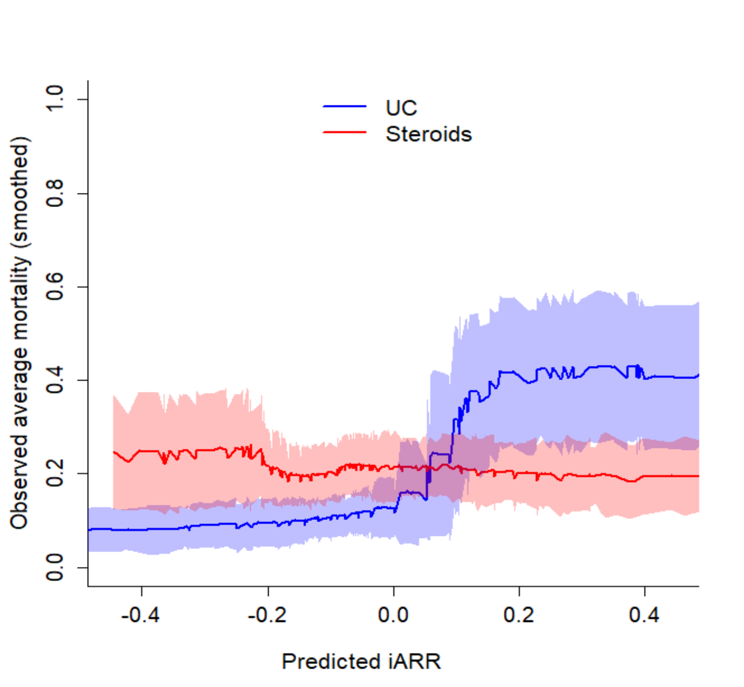
Figure 2 Flow cytometry of CD86 (cell surface marker) and TNF-alpha (intracellular marker) in lung macrophages. Data are representative of 8 animals in LPS group (22 mg/kg, IP) and 9 LPS animals treated by DXP nanoparticles (ND) 6 hours post model creation.

CLINICAL AND BIOMARKER DATA IN ADRENAL PREDICT TREATMENT RESPONSE

Clinical variables alone
(mortality risk, HGB, HR, CVP)



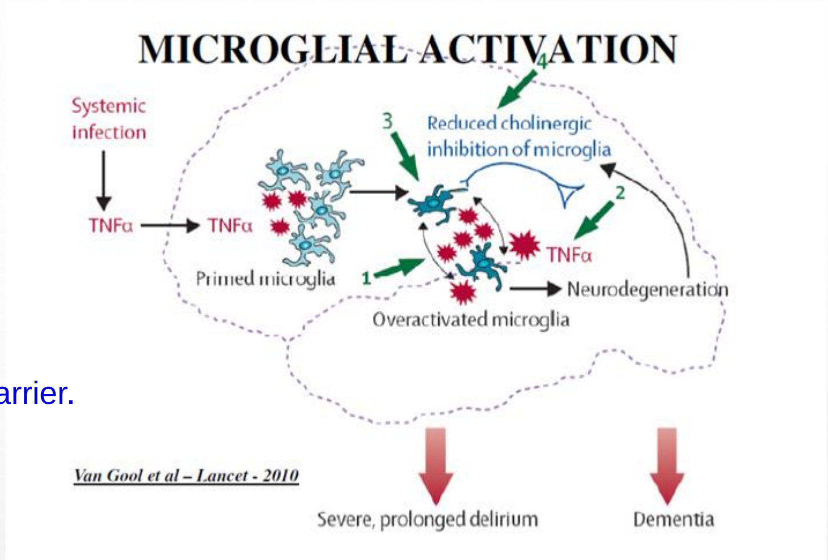
Endocrine markers alone
(Total and free cortisol)



Venkatesh, Angus, Annane

VAGAL NERVE STIMULATION

VAGAL NEUROMODULATION IN SEPSIS



Contexte:

SEPSIS INDUCED BRAIN DYSFUNCTION

- Acute, subacute or delayed impairment of brain function in sepsis: (coma, confusion, agitation, delirium, dementia like….)
- 32-60% of septic patients
- Associated with increased mortality, impaired societal quality of life
- Physiopathology: multifactorial, effects of excessive systemic
- inflammation, and neuro-inflammation, rupture of the blood-brain barrier.

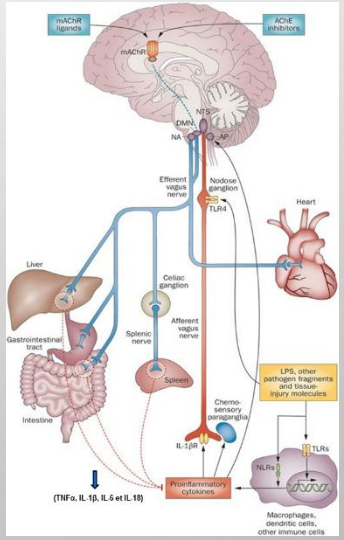
Why ?

The cholinergic anti-inflammatory reflex provides neuroprotective effects:

Controls inflammation, protects brain, heart, lung and blood functions

Vagal efferent fibers: release acetylcholine (Ach) which interacts with nicotinic receptors (α-7) in tissue macrophages and other immune cells

to inhibit cytokine synthesis/release pro-inflammatory *(TNFα, IL-1β, IL-6, and IL- 18)*



(Maier et al 1998; Thayer, 2009, Borovikova et al., 2000, Rosas-Ballina et al., 2008).

VAGAL NEUROMODULATION IN SEPSIS

How in Animals ?
Vagus nerve stimulation therapy models

Meneses et al. *Journal of Inflammation* (2016) 13:33
DOI 10.1186/s12950-016-0140-5

Journal of Inflammation

RESEARCH Open Access

Electric stimulation of the vagus nerve reduced mouse neuroinflammation induced by lipopolysaccharide

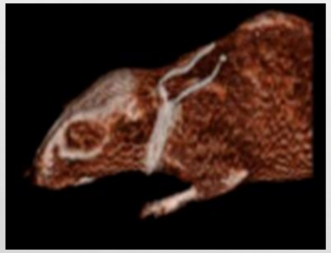


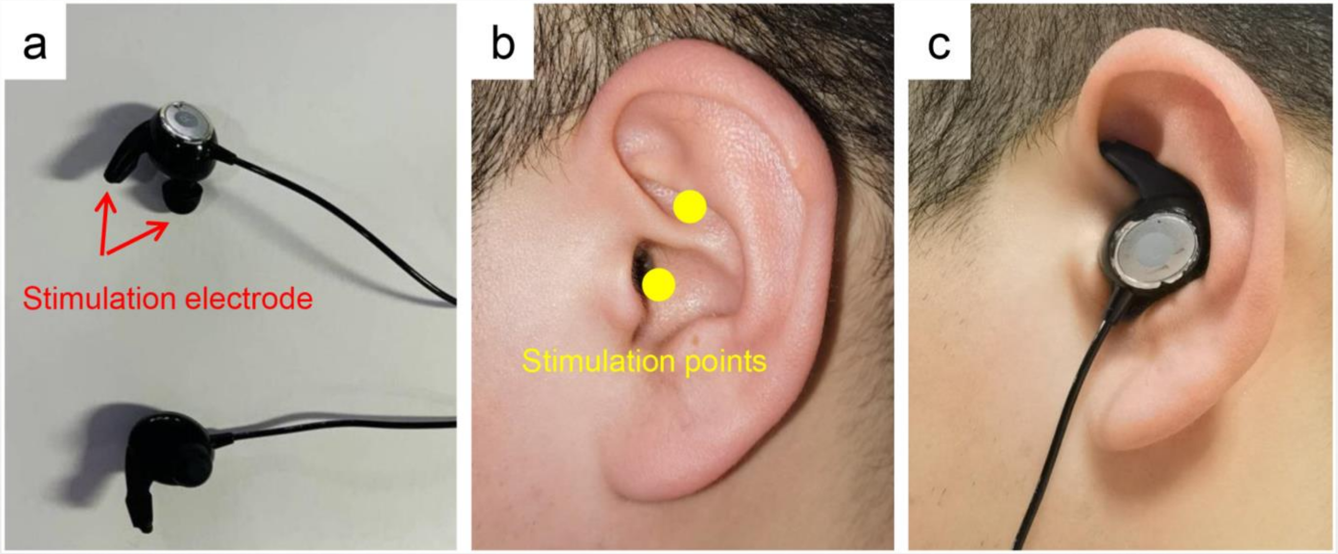
Table 2 Cytokine (pg/mg of protein) levels in brain extracts of sham and treated mice

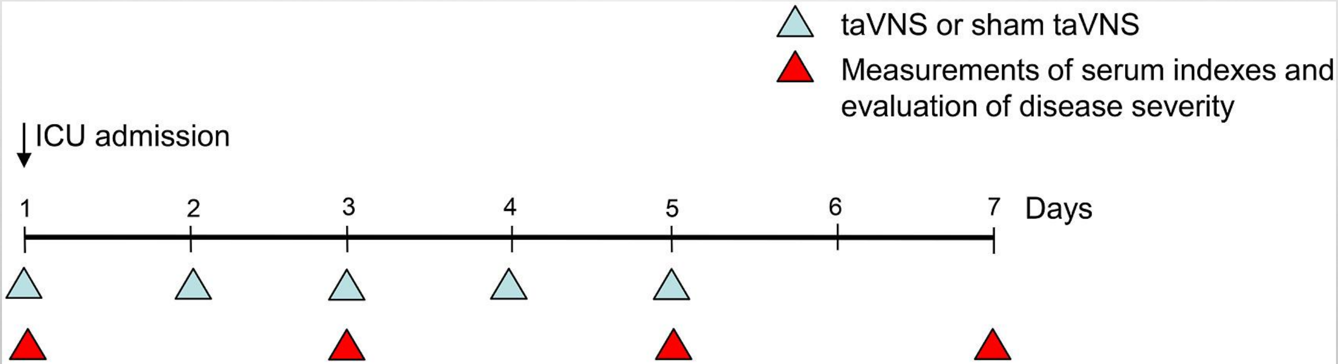
	TNFα	IL-1β	IL-6
Sham	0.94 ± 0.25[a]	10.54 ± 3.7[a]	0.78 ± 0.20[a]
ISS	0.34 ± 0.3[a]	4.1 ± 0.7[a]	0.62 ± 0.03[a]
VNS	0.06 ± 0.06[a]	7.4 ± 3.8[a]	0.28 ± 0.06[a]
††LPS	3.7 ± 0.38[b]	184 ± 1.87[b]	22.3 ± 0.72[b]
†LPS + VNS	1.47 ± 0.04[a]	20.4 ± 1.55[a]	1.19 ± 0.08[a]

Mean ± SEM of brain cytokine levels in groups of five C57BL/6 male mice each († one and †† two mice dead after LPS injection). VNS and LPS + VNS reported levels of central cytokines 24 h after VNS treatment in mice injected or not with LPS 3 days before (Fig. 2b). Different literals indicate significant differences in each cytokine level between the different treatments using the Kruskal-Wallis test (non- parametric ANOVA), TNFα ($P = 0.025$), IL-1β ($P = 0.004$), IL-6 ($P = 0.005$). Data are from one out of four experiments

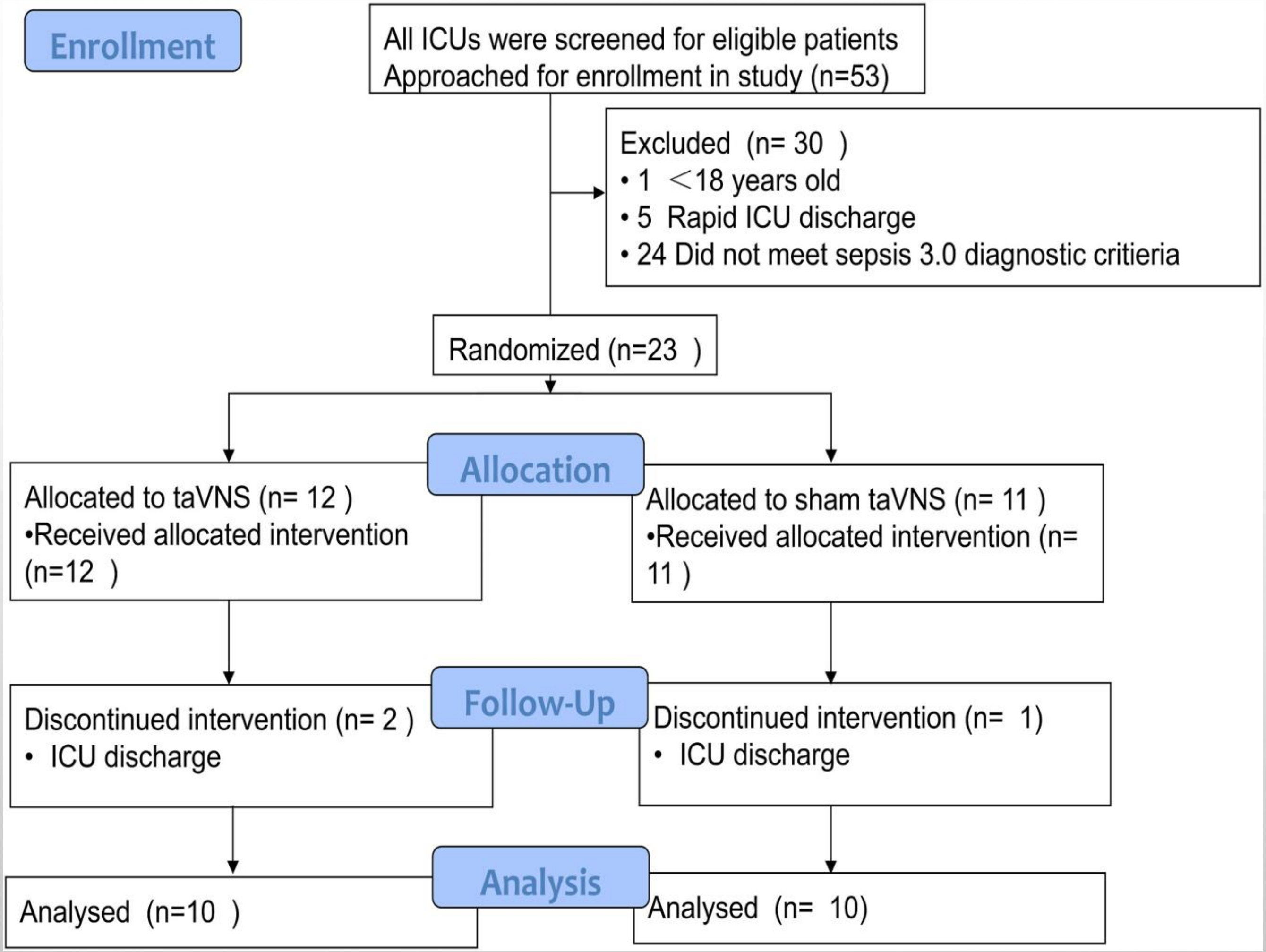
LPS :5 mg/kg (i.p.) from Escherichia coli serotype
ISS: 0.9 % NaCl (isotonic saline solution)

VNS with constant voltage (5 Hz, 0.75 mA, 2 ms) during 30s

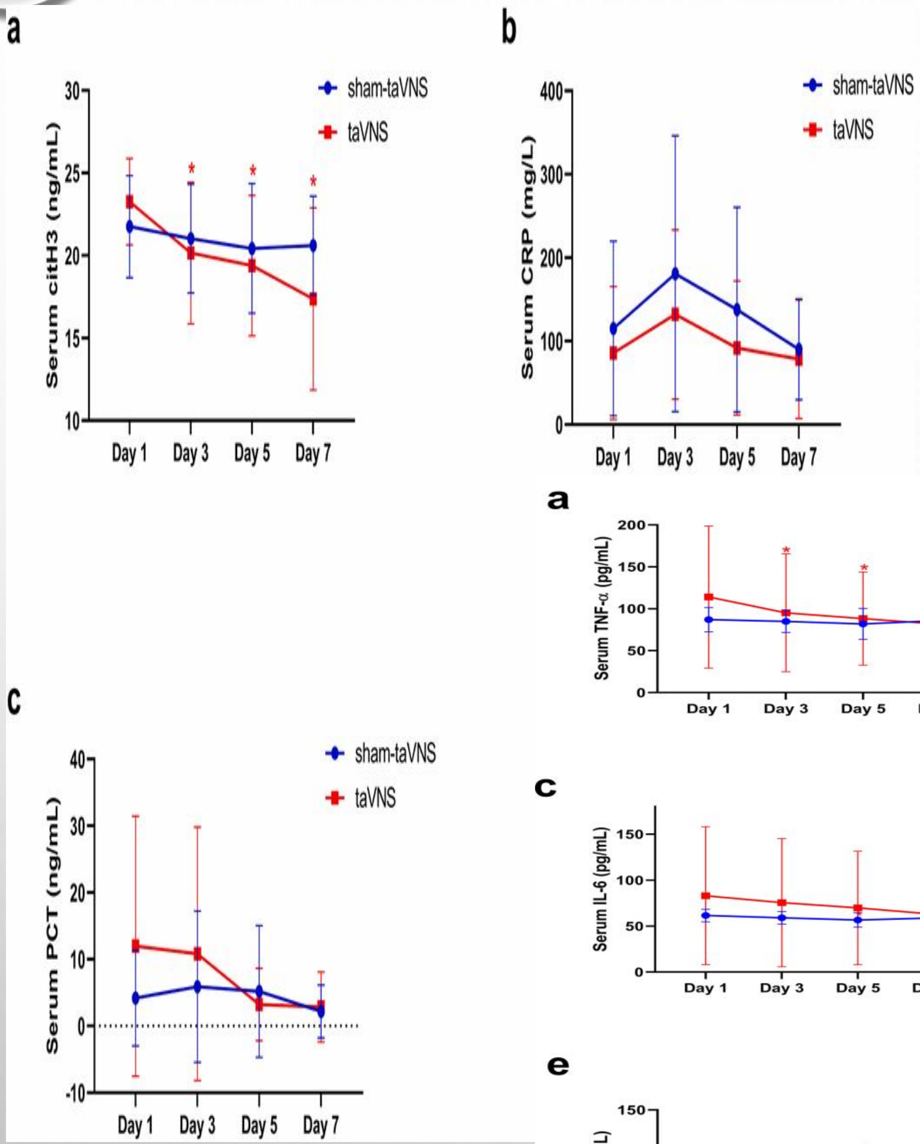


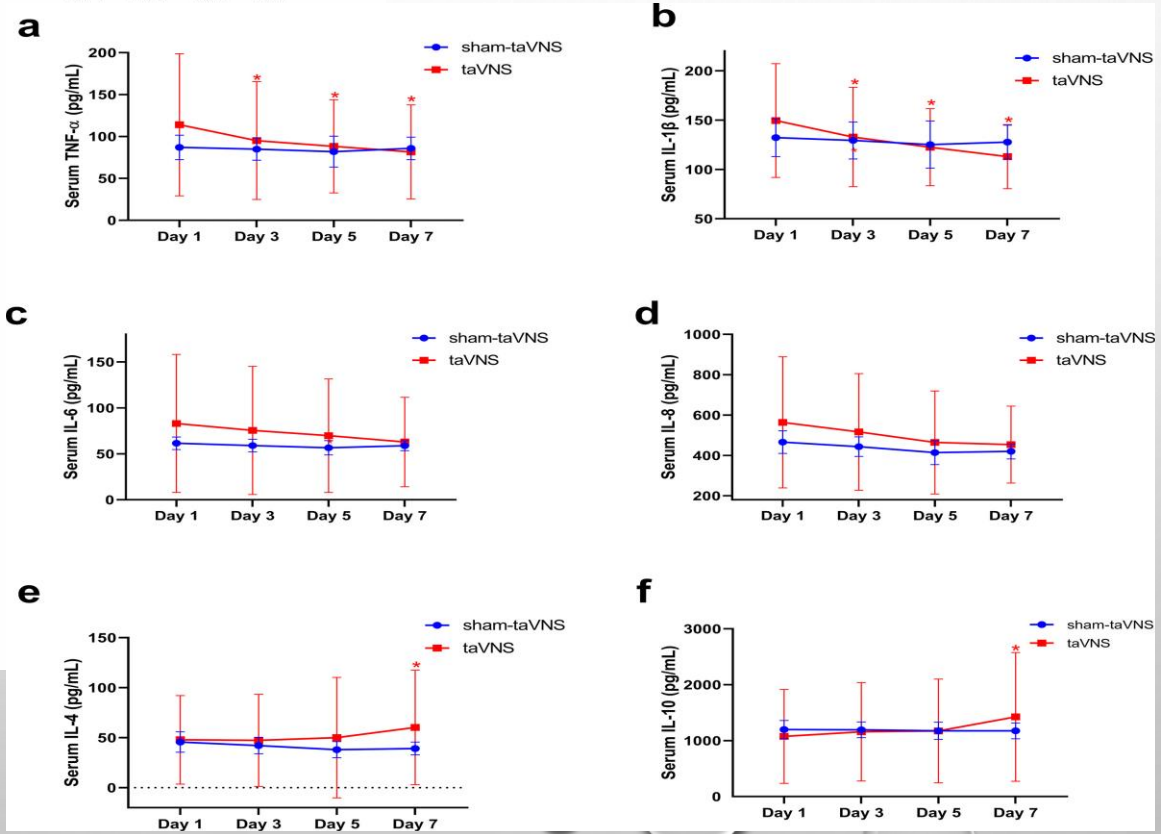


Wu, *Brain Stimulation:* 2023



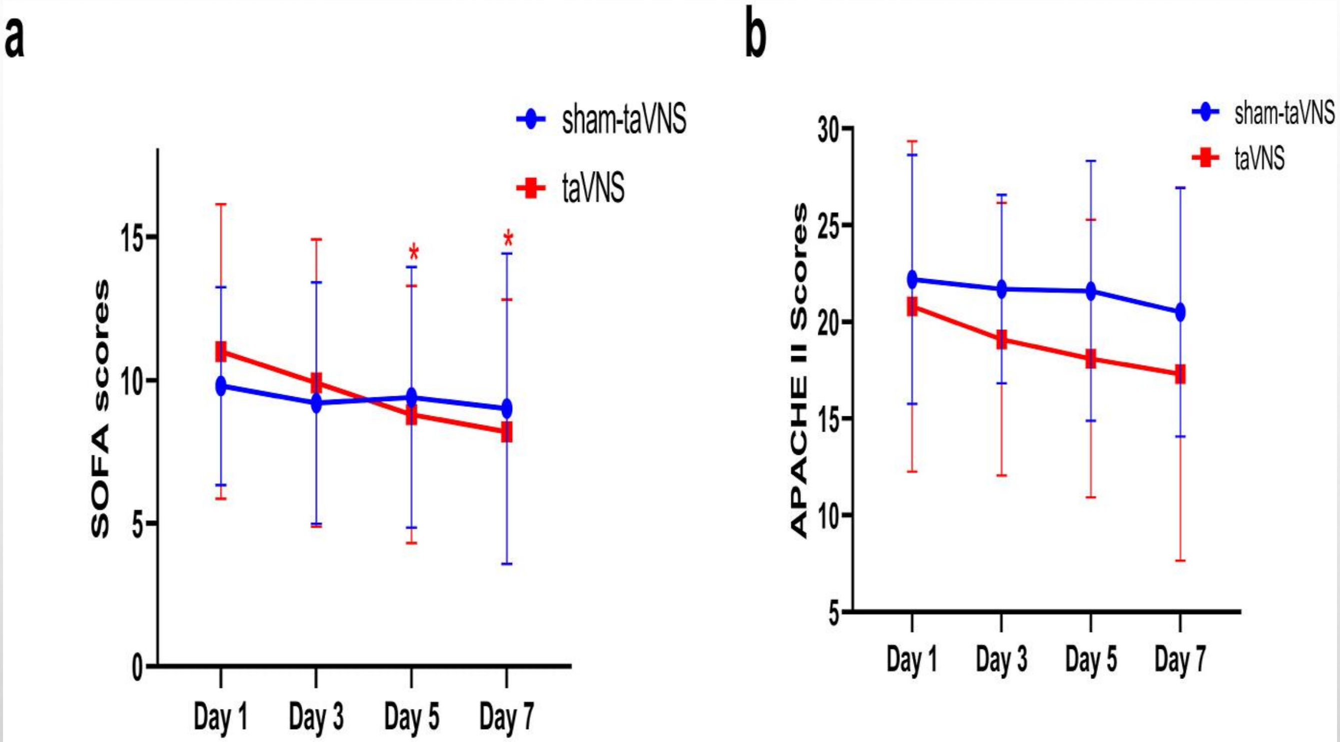
Wu, Brain Stimulation: 2023





Brain Stimulation: Basic, Translational, and Clinical Research in Neuromodulation 2023 16507-514DOI: (10.1016/j.brs.2023.02.008)

Wu, Brain Stimulation: 2023

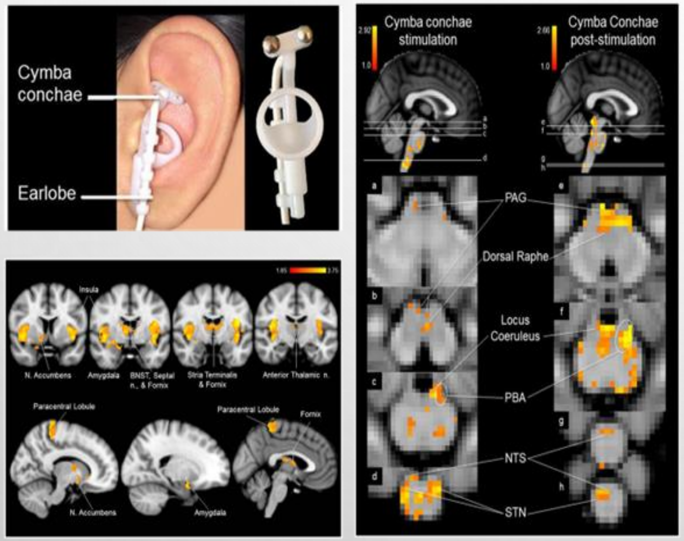


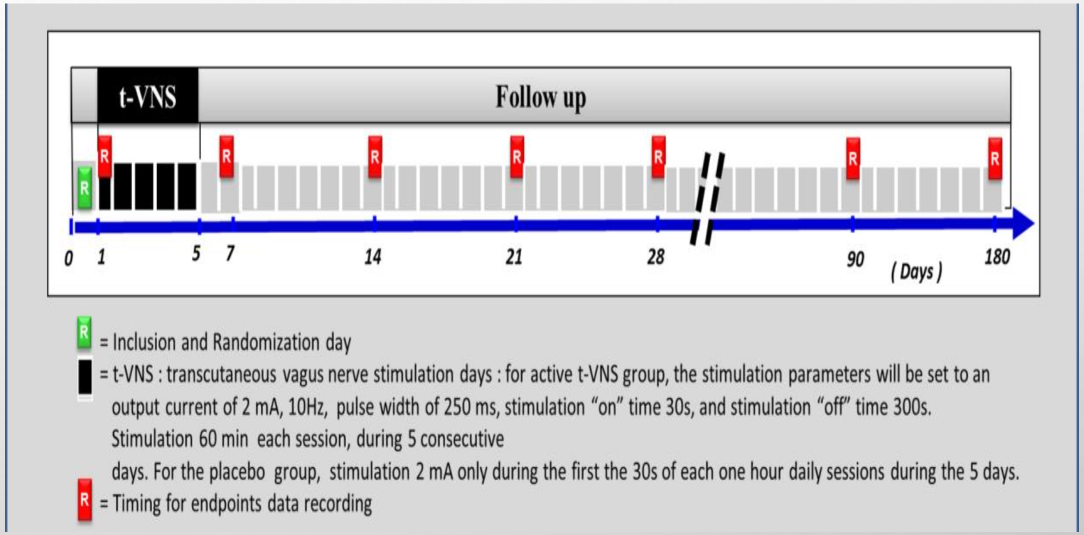
Wu, Brain Stimulation: 2023

VAGAL NEUROMODULATION IN SEPSIS

How in Human ?
Non invasive vagus nerf stimulation therapy

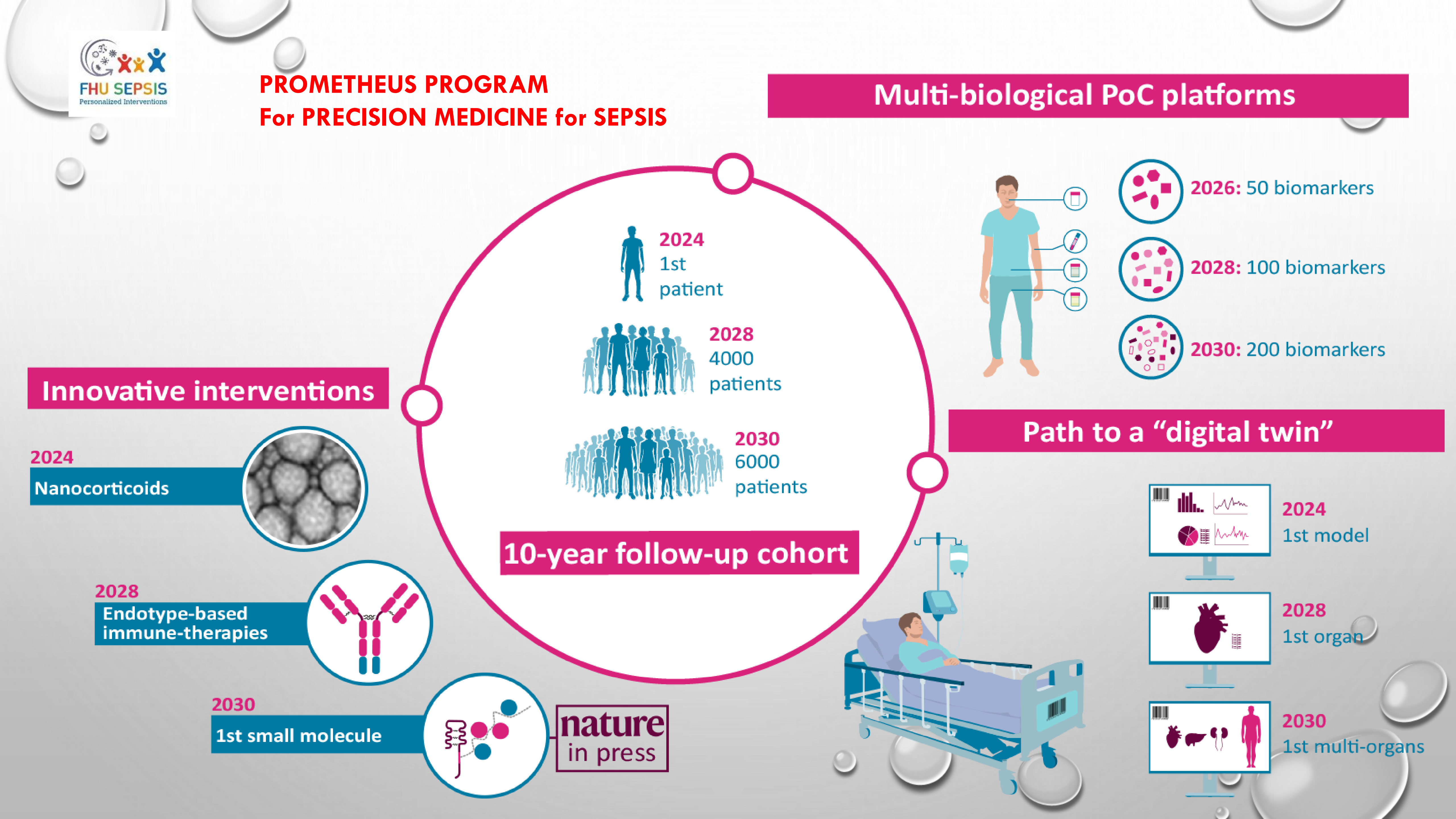
SNV-Sepsis trials : Effectiveness of Non-invasive Vagus Nerve Stimulation as an Adjuvant Treatment in Patients With Sepsis in Intensive Care.





- DR ERIC AZABOU
- PR DJILLALI ANNANE
- PR NICOLAS HEMING
- CLINICALTRIALS.GOV IDENTIFIER: NCT04774705
- NOW RECUITING

Wu, Brain Stimulation: 2023


FHU SEPSIS
Personalized Interventions
PROMETHEUS PROGRAM
For PRECISION MEDICINE for SEPSIS
Multi-biological PoC platforms
2026: 50 biomarkers
2028: 100 biomarkers
2030: 200 biomarkers
2024
1st
patient
2028
4000
patients
2030
6000
patients
10-year follow-up cohort
Innovative interventions
2024
Nanocorticoids
2028
Endotype-based
immune-therapies
2030
1st small molecule
nature
in press
Path to a "digital twin"
2024
1st model
2028
1st organ
2030
1st multi-organs