XI Encuentro de Cooperación Farma-Biotech

Antibodies & Peptides for the Diagnosis, Prevention and Treatment of Diseases Involving Alteration of the Inflammatory Response

Madrid, 2 de julio de 2014

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1. The Institution

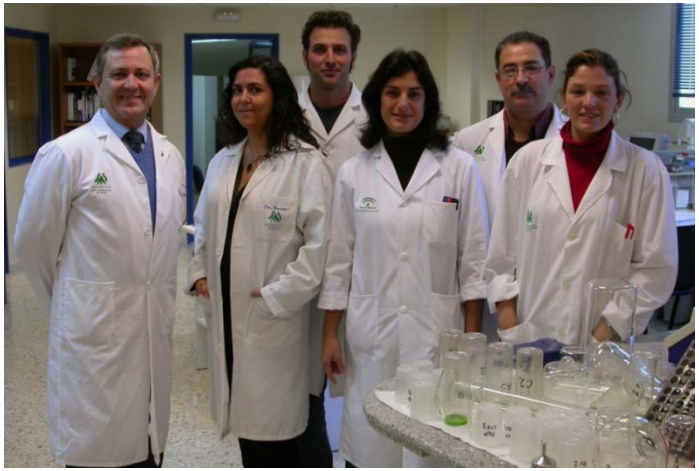
The University Hospital of Valme is one of the largest hospitals in Seville (coverage for more than 300,000 people), and is also affiliated to the Biomedical Research Institute of Seville (IBiS).

The Clinical and Experimental Pharmacology Research Unit is broad-based, and includes basic research of molecular mechanisms underlying normal and abnormal physiology to define novel therapeutic targets; translational research; and clinical research to examine the behavior of novel therapeutics in humans.

XI Encuentro de Cooperación Farma-Biotech

1. The Institution

Team and Collaborators



Neuropharmacology Research Group

Clinical and Experimental Pharmacology Research Unit. IBIS Valme University Hospital & University of Seville.

Prof. Javier Miñano. Group leader
Eva Tavares. Principal investigator
Rosario Maldonado. Postdoctoral Researcher

Group of Neuroscience/Neurology

Centre for Biomedical Research. The 12 de Octubre Hospital . CIBERNED. Madrid.

Eva María Carro. Principal investigator
Desiree Antequera. Laboratory technician
Consuelo Pascual. Laboratory technician
Teresa San Juan Díaz. Predoctoral Researcher
Fernando Bartolomé. Postdoctoral Researcher
Joana Figueiro. Postdoctoral Researcher
Félix Bermejo-Pareja. Neurologist
José Antonio Molina. Neurologist
Julián Benito León. Neurologist
Alberto Villarejo. Neurologist
Rocío Trincado. Statistics

2. The Product

- ✓ **AMINOPROCALCITONIN (NPCT) is a 57-aminoacid peptide derived from the prohormone procalcitonin (ProCT) encoded by the CALCA gene on chromosome 11.**
- ✓ **NPCT is a highly conserved peptide during evolution with a structural homology over 85% in all mammals.**
- ✓ **NPCT is a neuropeptide involved in energy homeostasis.**
- ✓ **NPCT is ubiquitously and uniformly expressed in multiple tissues throughout the body in response to sepsis.**

NPCT more than a harmful biomarker.

NPCT as a new therapeutic target

New Approaches

SIRS*, severe sepsis and septic shock

Acute lung injury (ALI) secondary to sepsis

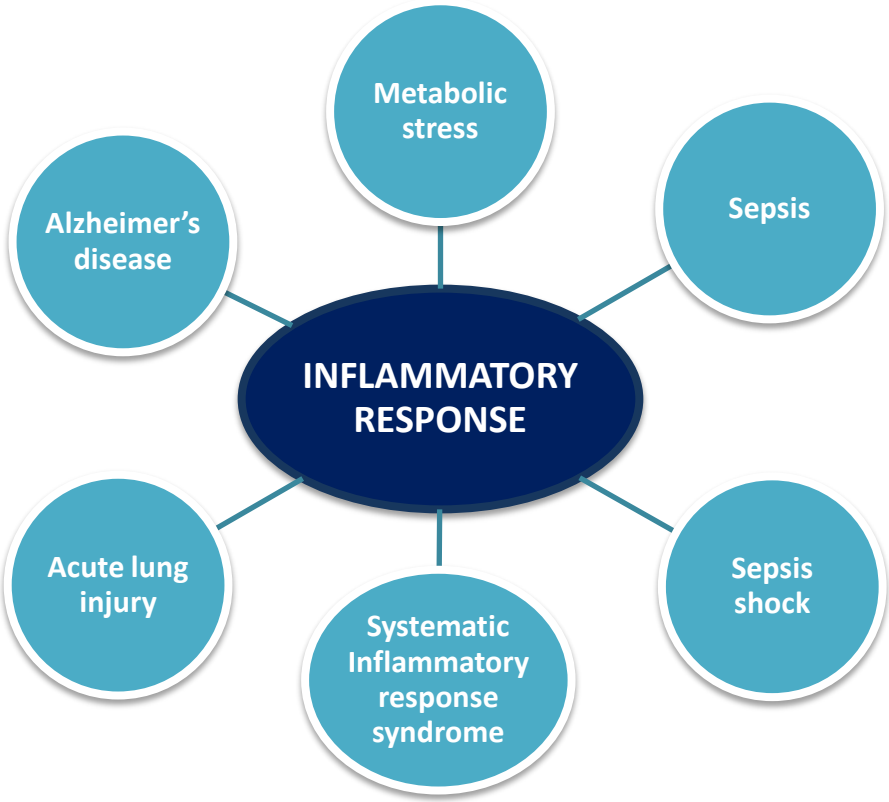
Neurodegenerative diseases Alzheimer Disease

*SIRS: Systemic inflammatory response syndrome

2. The Product

a) Target Indications

Diagnosis, treatment and prevention of diseases, which occur with alteration of the systemic inflammatory response.



There is **no ideal markers** for these indications, even though the **earliest possible diagnosis and treatment is decisive** in the patient's survival.

Even though the **improvement and acceleration of diagnostic** procedure has been researched worldwide for decades, there has been **no trailblazing development in this field**.

2. The Product

a) Target Indications

- **Sepsis** is a **major cause of mortality** throughout the world, killing about 1.400 people every day.
- **Over 18 million cases of severe sepsis worldwide each year.**
- Up to **135,000 European** and **215,000 American** deaths each year.
- Approximately **28.6% average overall mortality** for sepsis, severe sepsis and septic shock Death is common among sepsis patients, with around **28–50% of patients dying within the first month** of diagnosis.
- Sepsis affects **over 35% of ICU patients**, and manifests in approximately 2/3 of these patients as severe sepsis or septic shock.
- Up to **82% mortality** for patients with **septic shock**.

Delayed diagnosis leads to progression of disease and the need for more invasive and costly treatment

2. The Product

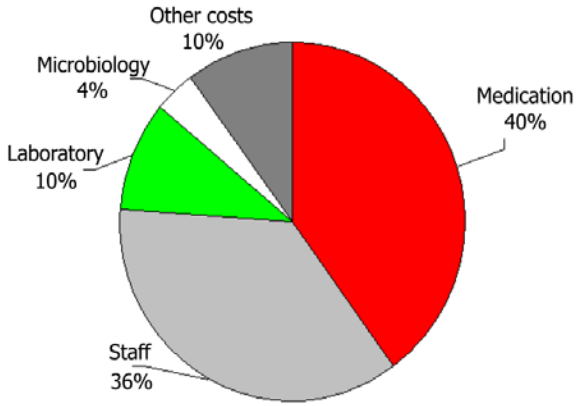
a) Target Indications

SEPSIS

Sepsis exacts a high toll

- Each year the cost of treating sepsis patients increases and is currently as high as **€7.6 billion in Europe** and **€17.4 billion in the US.**
- The treatment of sepsis places a significant burden on healthcare resources, accounting for **40% of total ICU**s expenditure.

- Costs per case: **22.100 US$**
- Length of stay in ICU: **18 days** (vs. 5 days in pts without sepsis)
- Costs per day: **1650 €/day (death)** / 1160 € (survived)

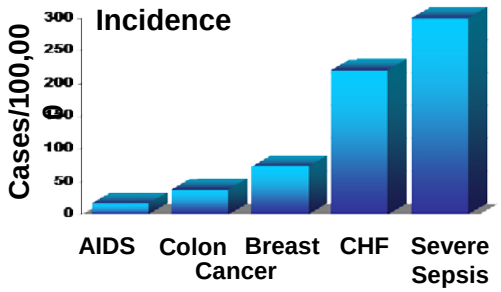


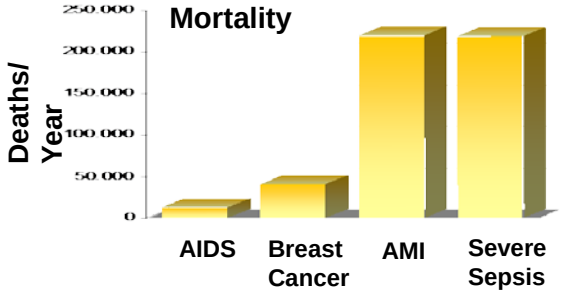
Global sepsis therapeutics is forecast to grow at a CAGR (Compound Annual Growth Rate) of 5.8%

2. The Product

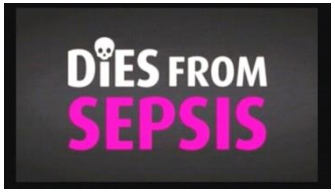
a) Target Indications

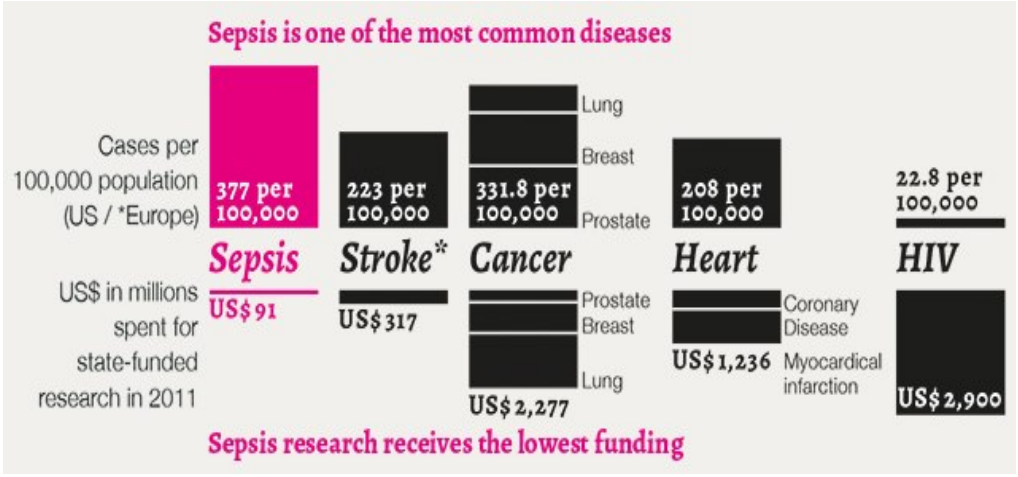
Comparison with Other Major Diseases





Every 3 min, someone …





No existing effective treatments

2. The Product

a) Target Indications

ACUTE LUNG INJURY (ALI) - ACUTE RESPIRATORY DISTRESS SYNDROME (ARDS)

- True incidence unknown ranging from **1.5 to 75 per 100,000** population per year.
- ALI/ ARDS affects more than 150,000 people in the US annually with a mortality rate projected at **30-40%** owing to **multiple organ failure**.
- About **16%** of all patients ventilated in the ICU for 24 h or more **develop ALI**.
- ALI is diagnosed clinically and radiologically, which are non-specific methods and are subject to interobserver variability.

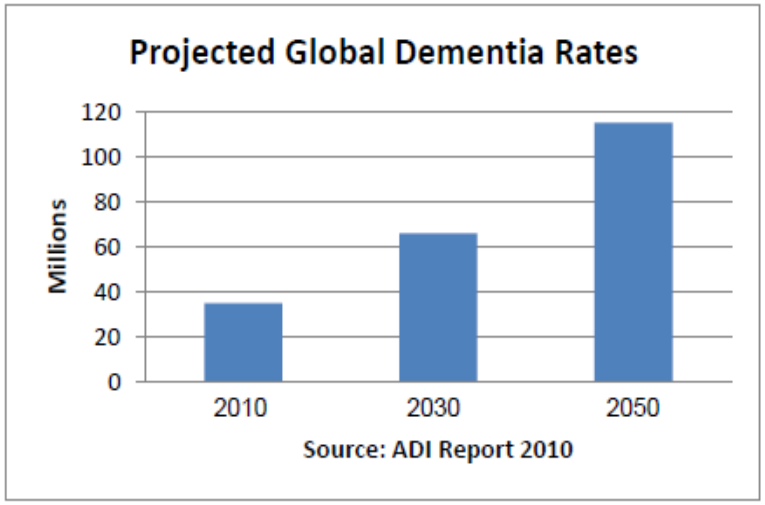
No existing effective treatments

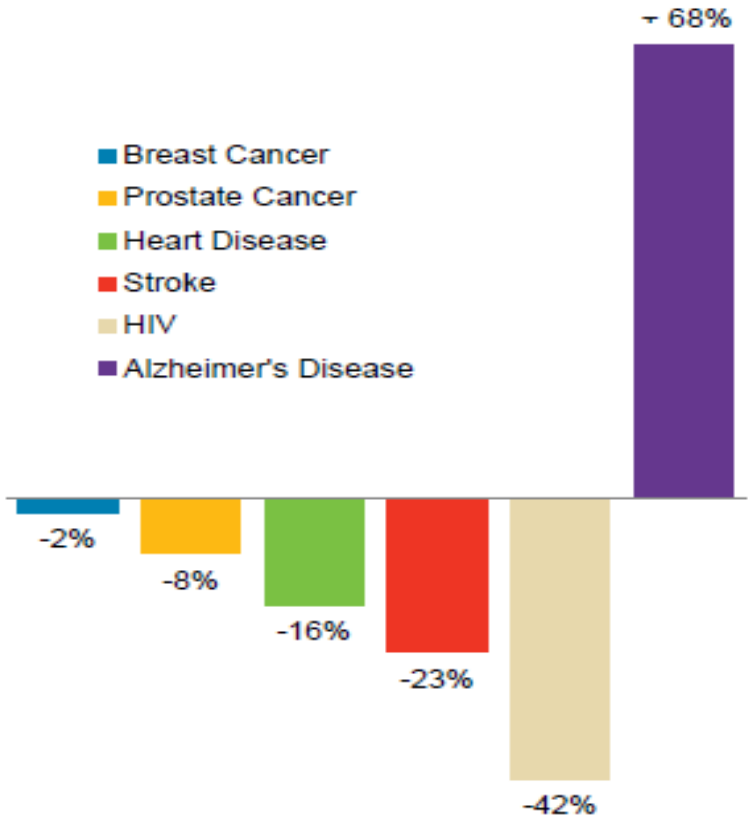
ALI/ ARDS is believed to be a public health crisis representing high costs to healthcare systems.

2. The Product

a) Target Indications

ALZHEIMER'S DISEASE (AD)





"Somewhere in the world, someone develops Alzheimer's every 7 seconds"

Percentage Changes in Selected Causes of Death (all ages) 2000-2010

2. The Product

a) Target Indications

ALZHEIMER'S DISEASE (AD): UNMET MEDICAL NEED

- **Current average diagnosis rate** in main pharma major markets is around **56.4%** > However, diagnostics and biomarkers segment is estimated to reach $2.9 billion in 2014 and is expected to increase at a 5-year CAGR of nearly 20%.

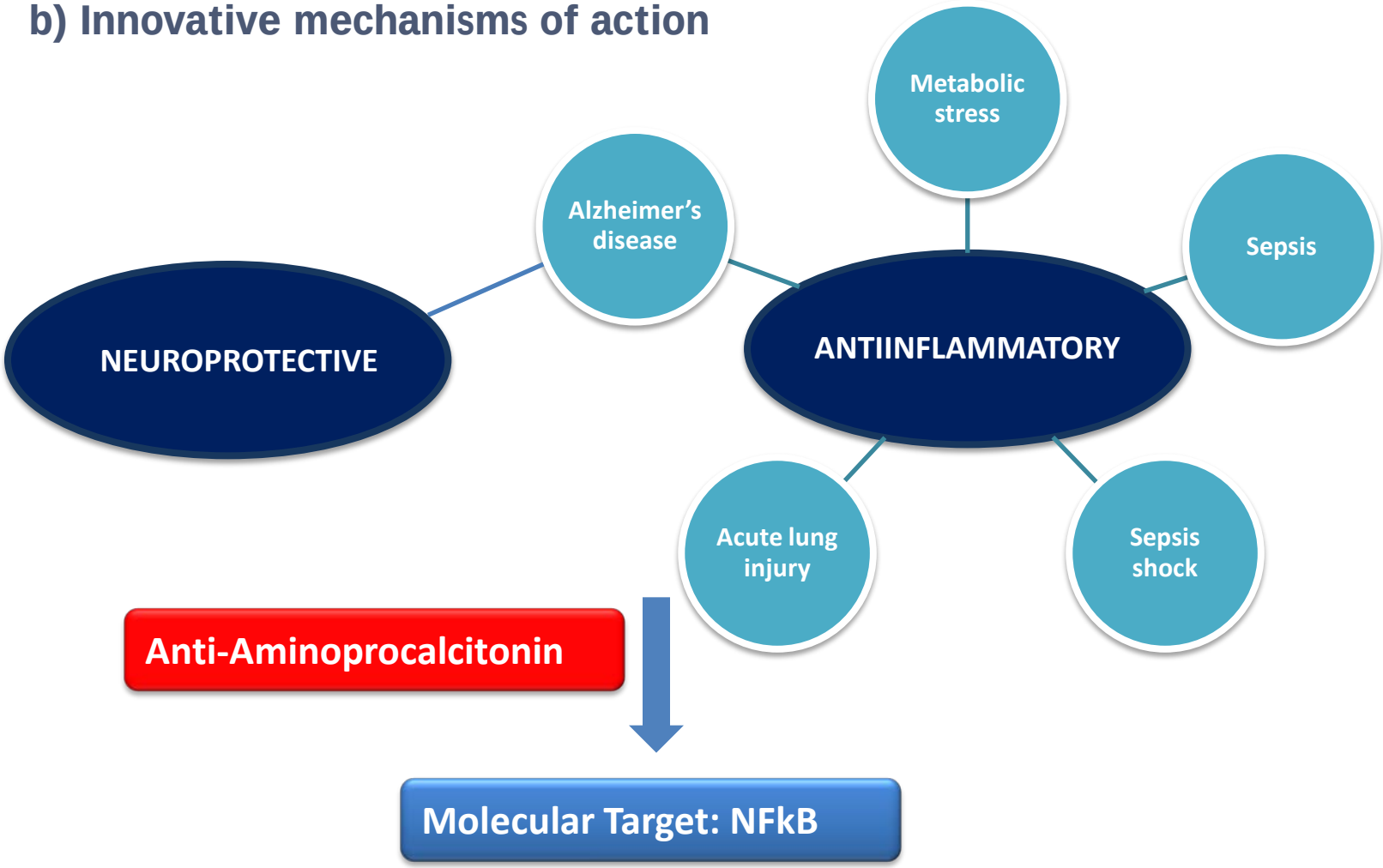
- **Current treatments offer only slight improvements for a limited period of time (12-14 months) >** Nevertheless, **AD market is currently estimated at $5 bn annually** and expected to rise to **$20 bn by 2020**, with sales expectations for the first disease modifying-treatment between $3 and $5 bn.

- **Lack of alternative therapies:** a new drug able to delay the progression of the disease is expected to favorably compete for this AD market.

No existing effective diagnostics & treatments

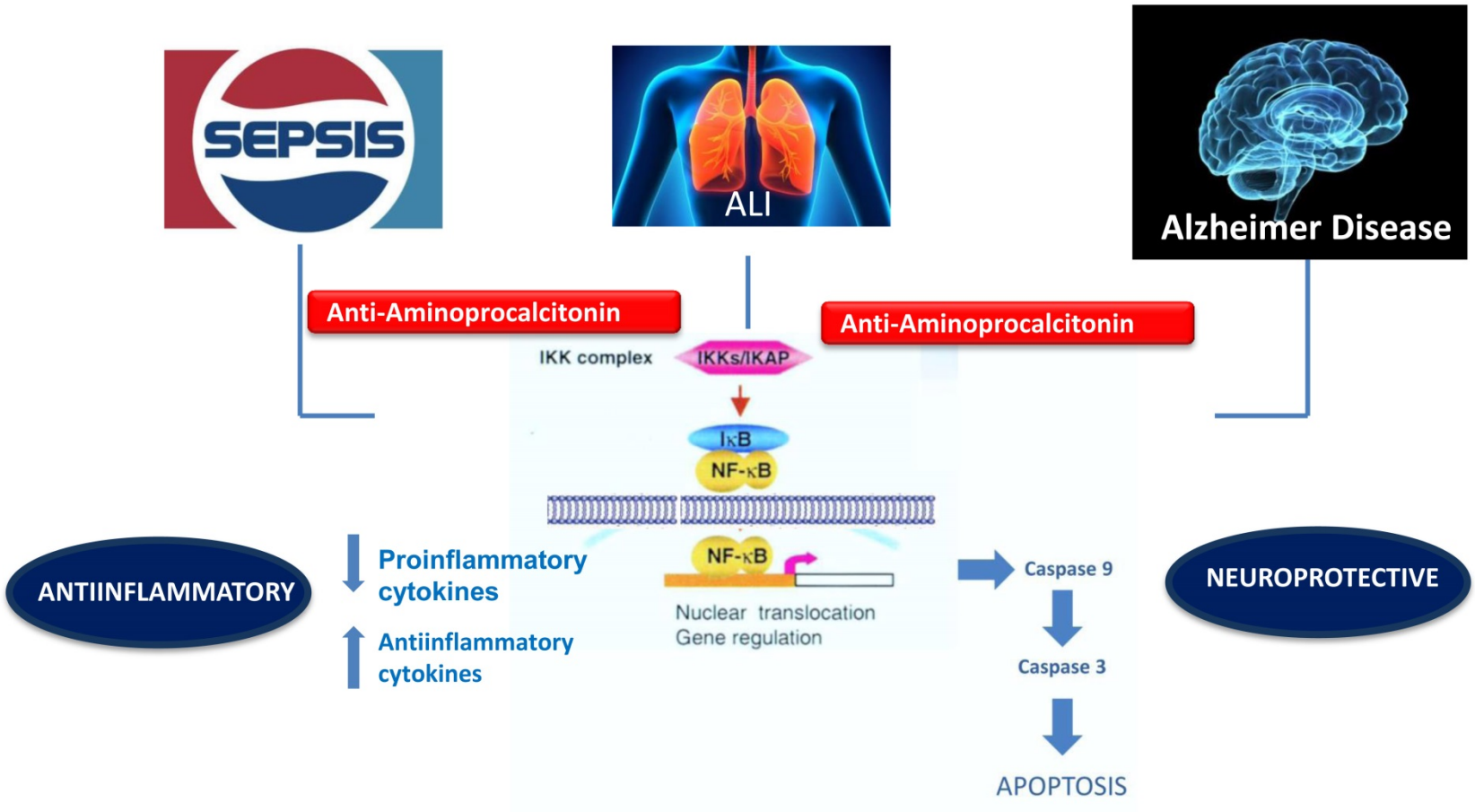
2. The Product

b) Innovative mechanisms of action



2. The Product

b) Innovative mechanisms of action



2. The Product

c) Differential features facing the market

DIAGNOSTICS

- Currently available diagnostic systems **lack specificity due to cross-reactions** with other molecules, such as PCT. Although diagnostic effectiveness of this marker is better than that of other infection markers, such as C-reactive protein, studies have not yet sufficiently proven that the use of the PCT marker can lower the mortality rate from sepsis.
- These systems may also require the use of two antibodies.
- N-PCT is the most abundant CALCA gene product in plasma of septic patients and has the greatest specificity for differentiating patients with systemic inflammatory response syndrome (SIRS) from those with sepsis, when compared with other biomarkers such as IL-2, IL-6, IL-8, CRP and TNF-α.
- Methodological approach provides a reliable and very specific way to detect those diseases involving an alteration of the inflammatory response or metabolic stress.

2. The Product

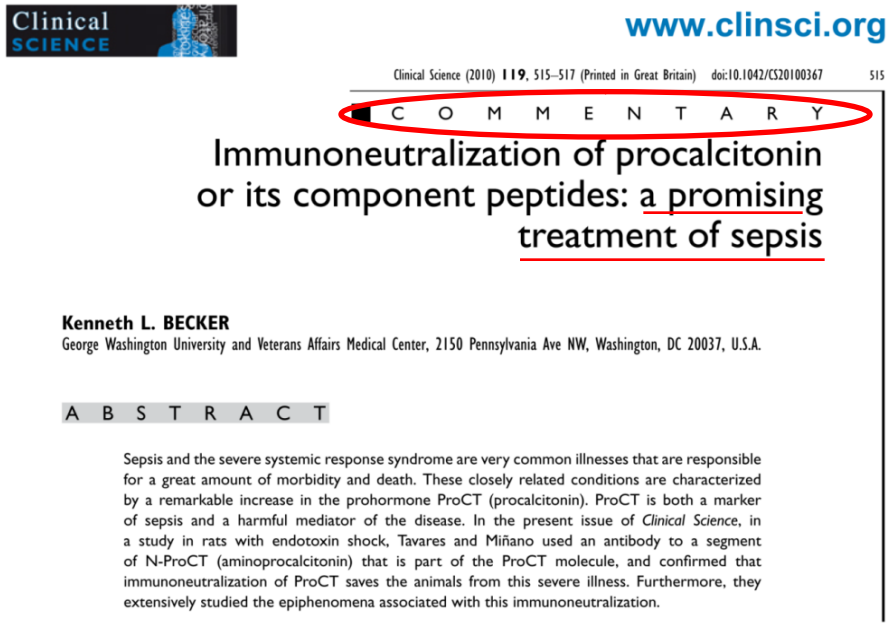
c) Differential features facing the market

THERAPY

- Synthetized antibodies influencing the physiological reaction caused by N-PCT open a **new therapeutic approach in sepsis** since serum concentrations of N-PCT are significantly higher than those of PCT in systemic inflammatory processes and severe/ sepsis septic shock, and apparently N-PCT is actively involved in these processes.
- These antibodies may be also useful for the treatment of other diseases with unmet medical needs since it has been shown that neutralization of the circulating N-PCT normalizes the levels of PCT and cytokines in animals with long-term survival.
- Safe and well tolerated due to their high specifity.
- Small monoclonal antibodies (7-13 aa.): Facilitates their scaled-up production.
- Existing **competition in the sepsis therapeutics market is weak**. Therefore the **unmet need remains high**.

2. The Product

d) Current status of development



Clinical SCIENCE

www.clinsci.org

Clinical Science (2010) 119, 515–517 (Printed in Great Britain) doi:10.1042/CS20100367 515

COMMENTARY

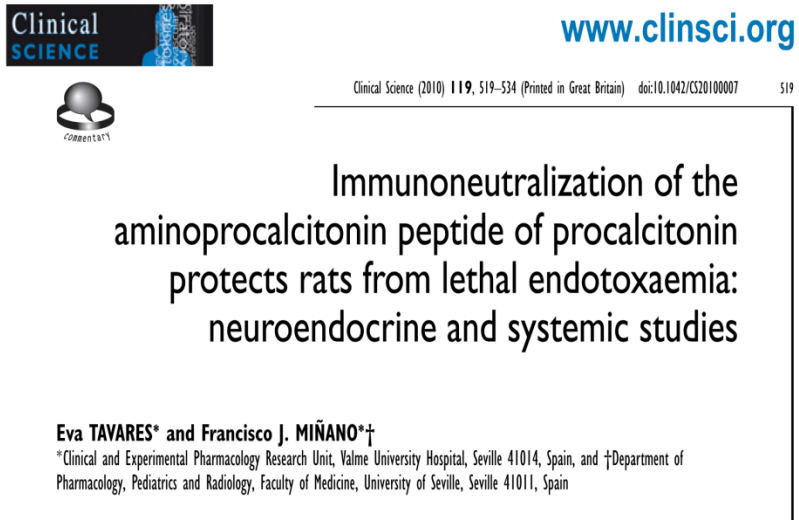
Immunoneutralization of procalcitonin or its component peptides: a promising treatment of sepsis

Kenneth L. BECKER
George Washington University and Veterans Affairs Medical Center, 2150 Pennsylvania Ave NW, Washington, DC 20037, U.S.A.

ABSTRACT

Sepsis and the severe systemic response syndrome are very common illnesses that are responsible for a great amount of morbidity and death. These closely related conditions are characterized by a remarkable increase in the prohormone ProCT (procalcitonin). ProCT is both a marker of sepsis and a harmful mediator of the disease. In the present issue of *Clinical Science*, in a study in rats with endotoxin shock, Tavares and Miñano used an antibody to a segment of N-ProCT (aminoprocalcitonin) that is part of the ProCT molecule, and confirmed that immunoneutralization of ProCT saves the animals from this severe illness. Furthermore, they extensively studied the epiphenomena associated with this immunoneutralization.

US13/527,069



Clinical SCIENCE

www.clinsci.org

Clinical Science (2010) 119, 519–534 (Printed in Great Britain) doi:10.1042/CS20100007 519

Immunoneutralization of the aminoprocalcitonin peptide of procalcitonin protects rats from lethal endotoxaemia: neuroendocrine and systemic studies

Eva TAVARES* and Francisco J. MIÑANO*†
*Clinical and Experimental Pharmacology Research Unit, Valme University Hospital, Seville 41014, Spain, and †Department of Pharmacology, Pediatrics and Radiology, Faculty of Medicine, University of Seville, Seville 41011, Spain

«…. Tavares and Miñano used a specific anti NPCT in rats with severe endotoxic shock... They confirmed that imunoneutralization of NPCT saves the animals from this severe illness.

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2. The Product

d) Current status of development

Lethal endotoxemia
LPS (15 mg/kg, ip)

SEPSIS
ALI

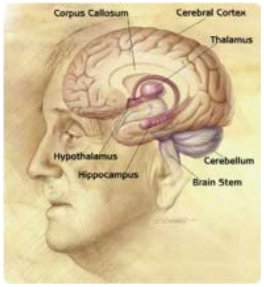
Anti-Aminoprocalcitonin


i.p.


Minipumps

Lethal sepsis by Cecal Ligation and Puncture

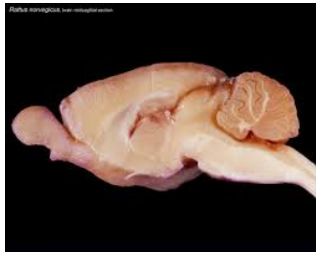
Postmortem brain tissue

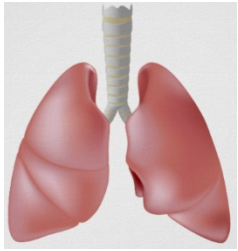


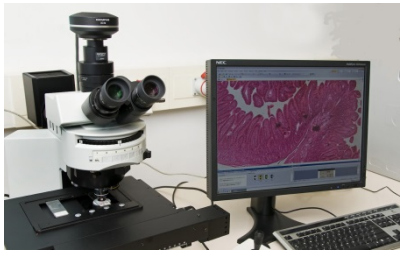
ALZHEIMER'S DISEASE

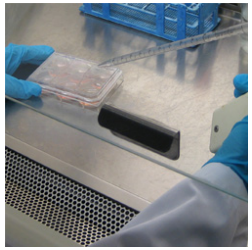
***APP/PS1* transgenic mice**

SURVIVAL







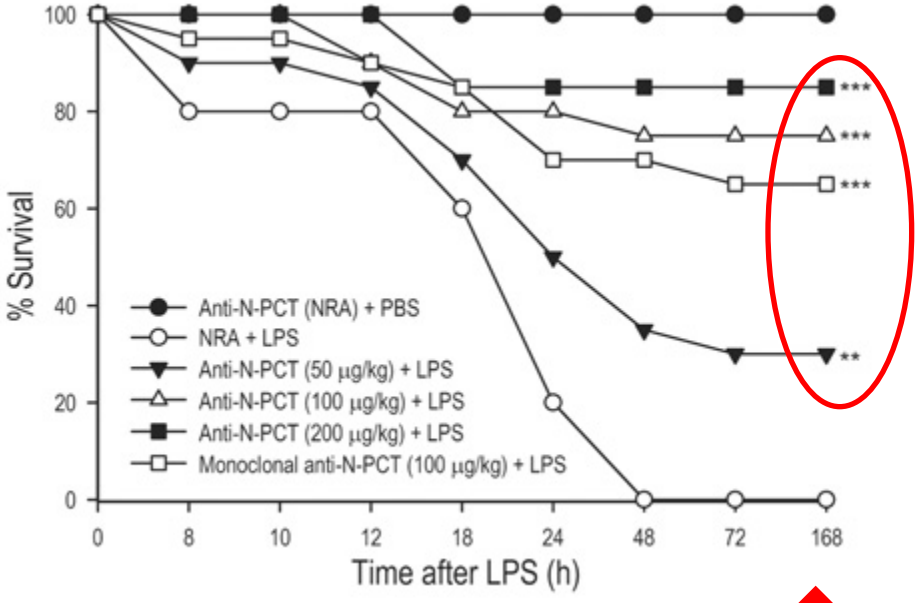


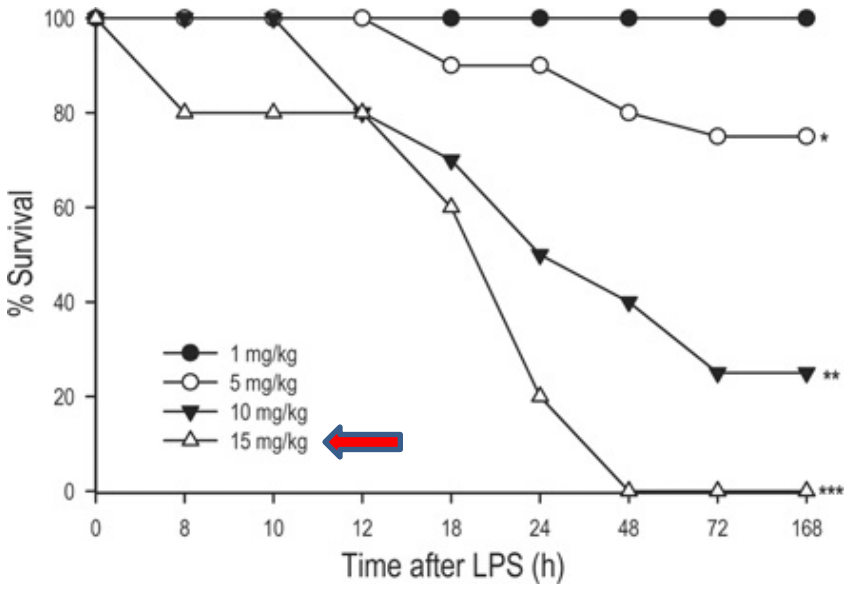
ELISA | Real Time-PCR, Western-Blot | Inmunohistochemistry | Celular culture

2. The Product

d) Current status of development

Effects of anti-NPCT on LPS-induced mortality



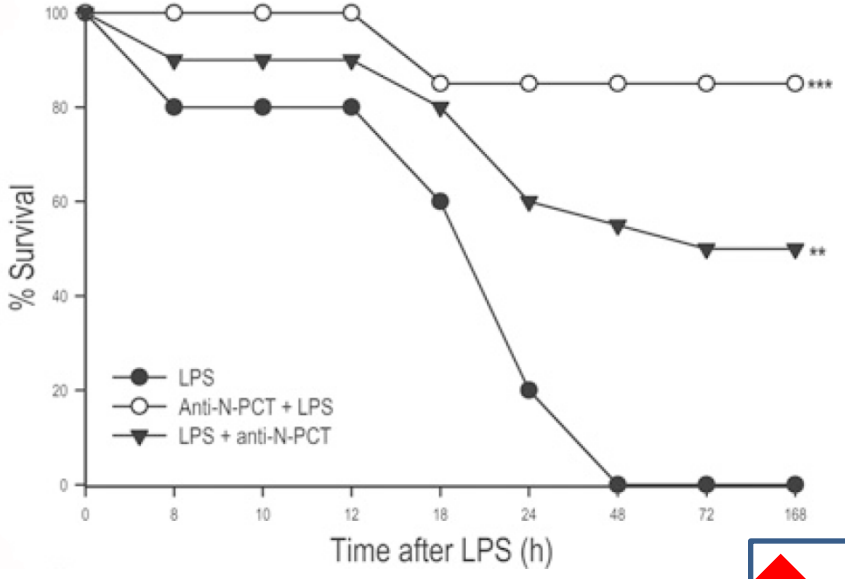


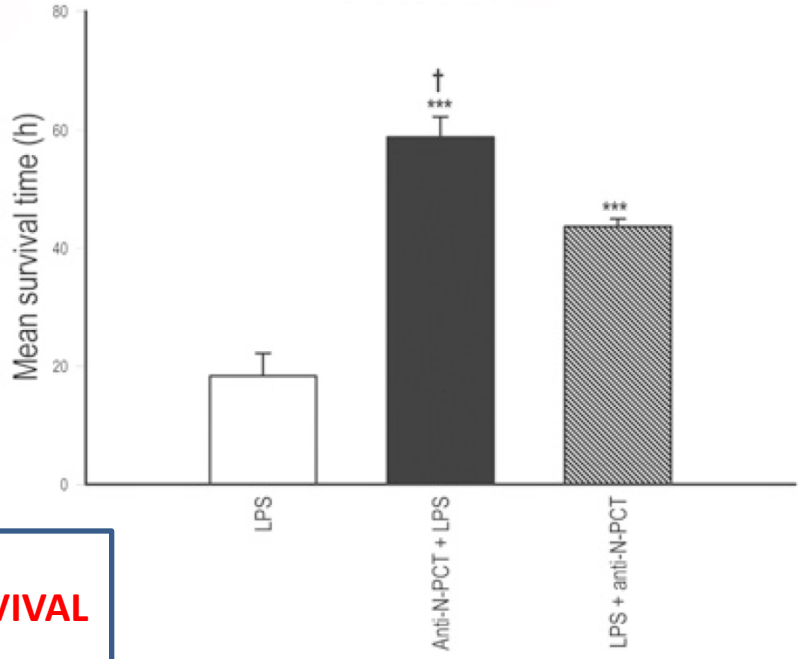
SURVIVAL

2. The Product

d) Current status of development

Effect of **prophylactic** and **therapeutic** anti N-PCT administration on survival after a lethal dose of LPS



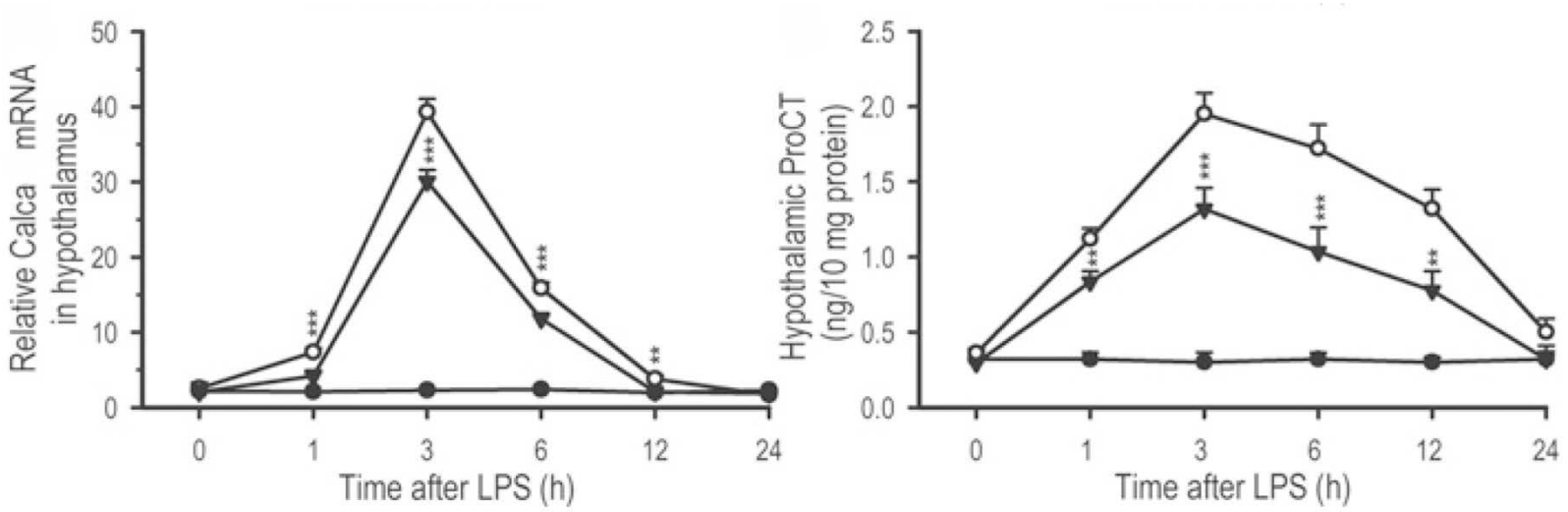


SURVIVAL

2. The Product

d) Current status of development

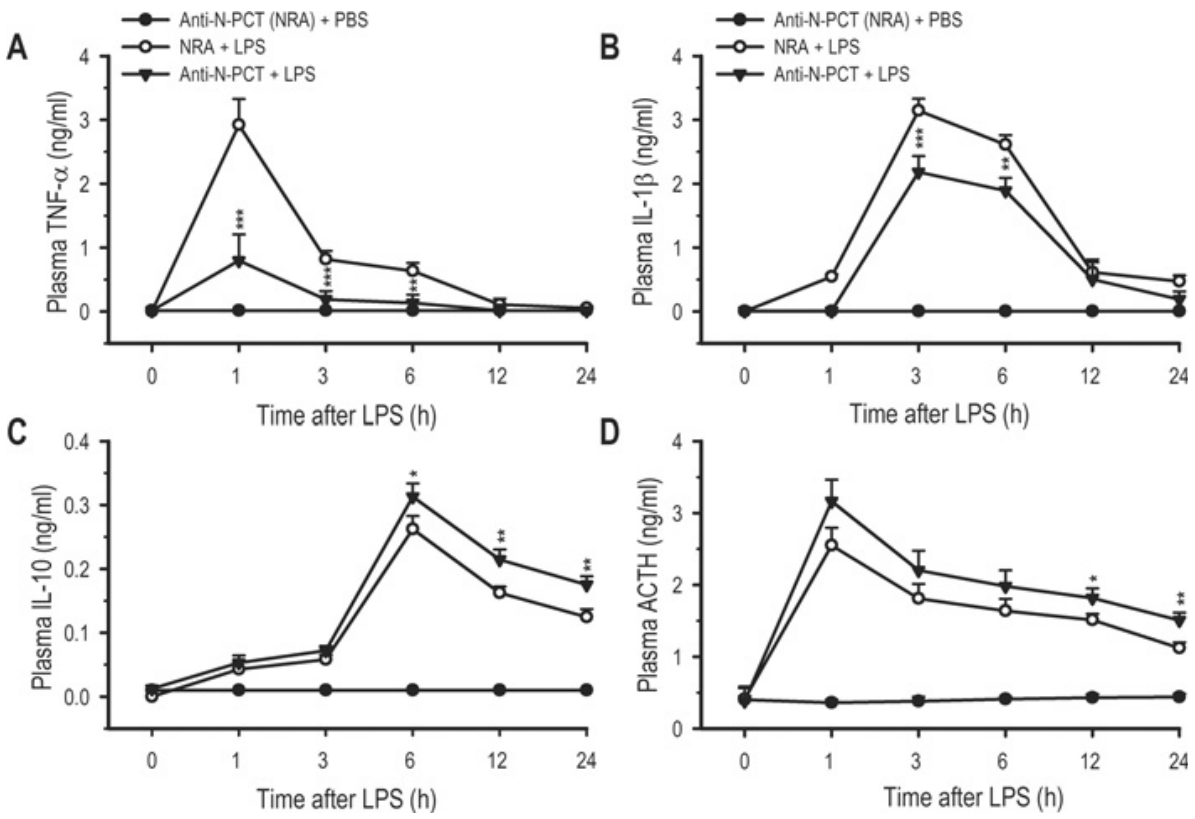
Anti-NPCT pre-treatment reduces LPS-induced hypothalamic *CALCA* gene expression and ProCT production in hypothalamus

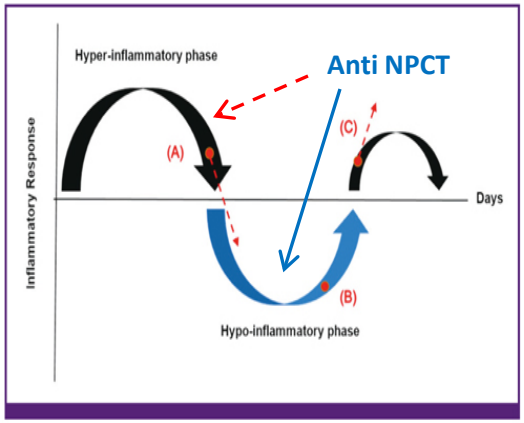


2. The Product

d) Current status of development

Effects of anti-NPCT on LPS-induced pro- and anti-inflammatory factors





EARLY PRO-INFLAMMATORY RESPONSE

Anti-Aminoprocalcitonin

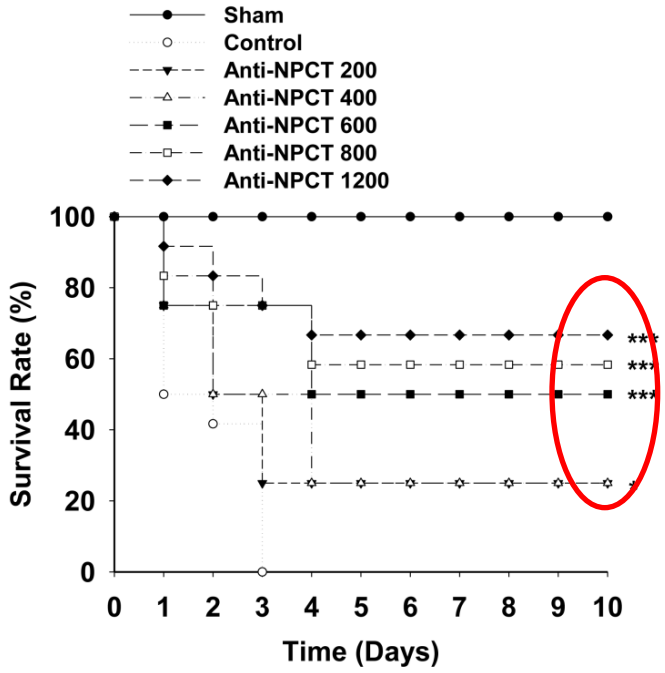
ANTI-INFLAMMATORY LATE RESPONSE

2. The Product

d) Current status of development

PCT/ES2013/070831

Anti-NPCT attenuates CLP-induced mortality



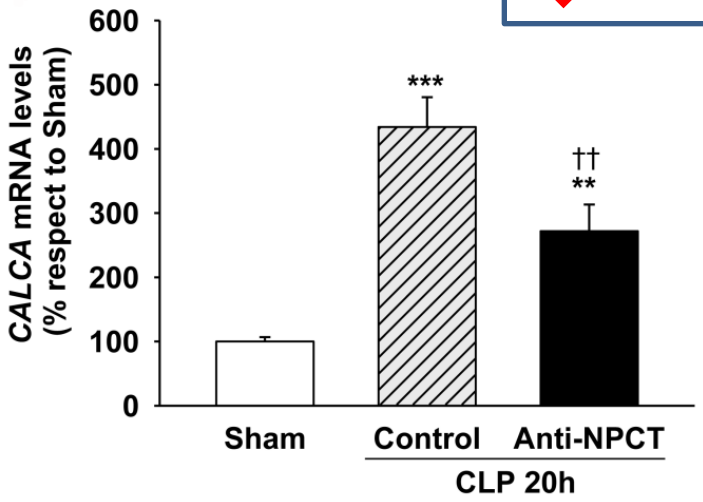
SURVIVAL

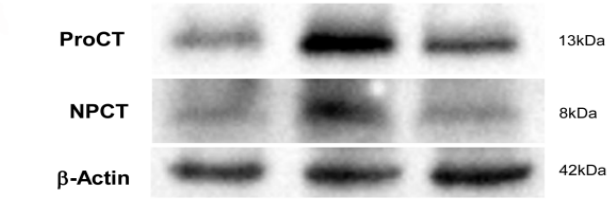
Immunoneutralization of Endogenous Aminoprocalcitonin Attenuates Sepsis-Induced Acute Lung Injury and Mortality in Rats.
Eva Tavares, Rosario Maldonado, and Francisco J. Miñano. *Am J Pathology. In press. 2014*

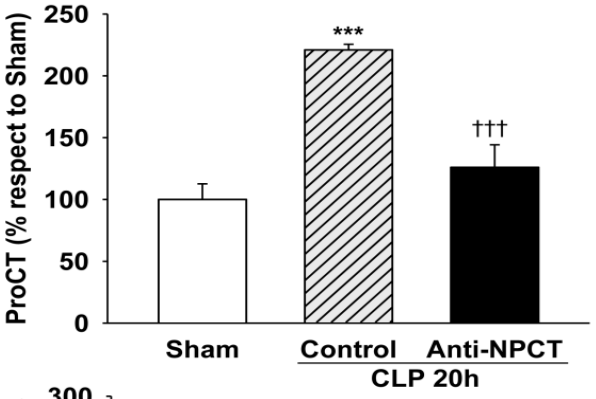
2. The Product

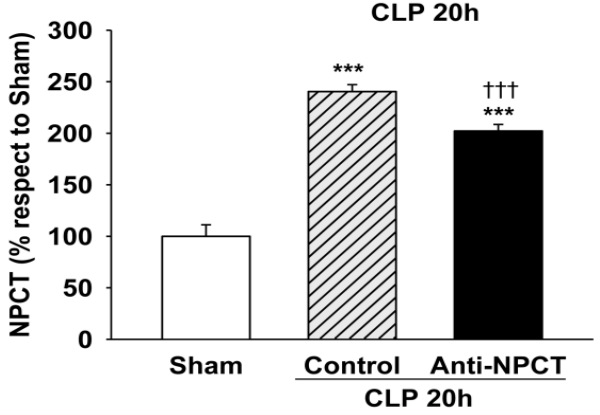
d) Current status of development

CALCA gen expression
ProCT
NPCT







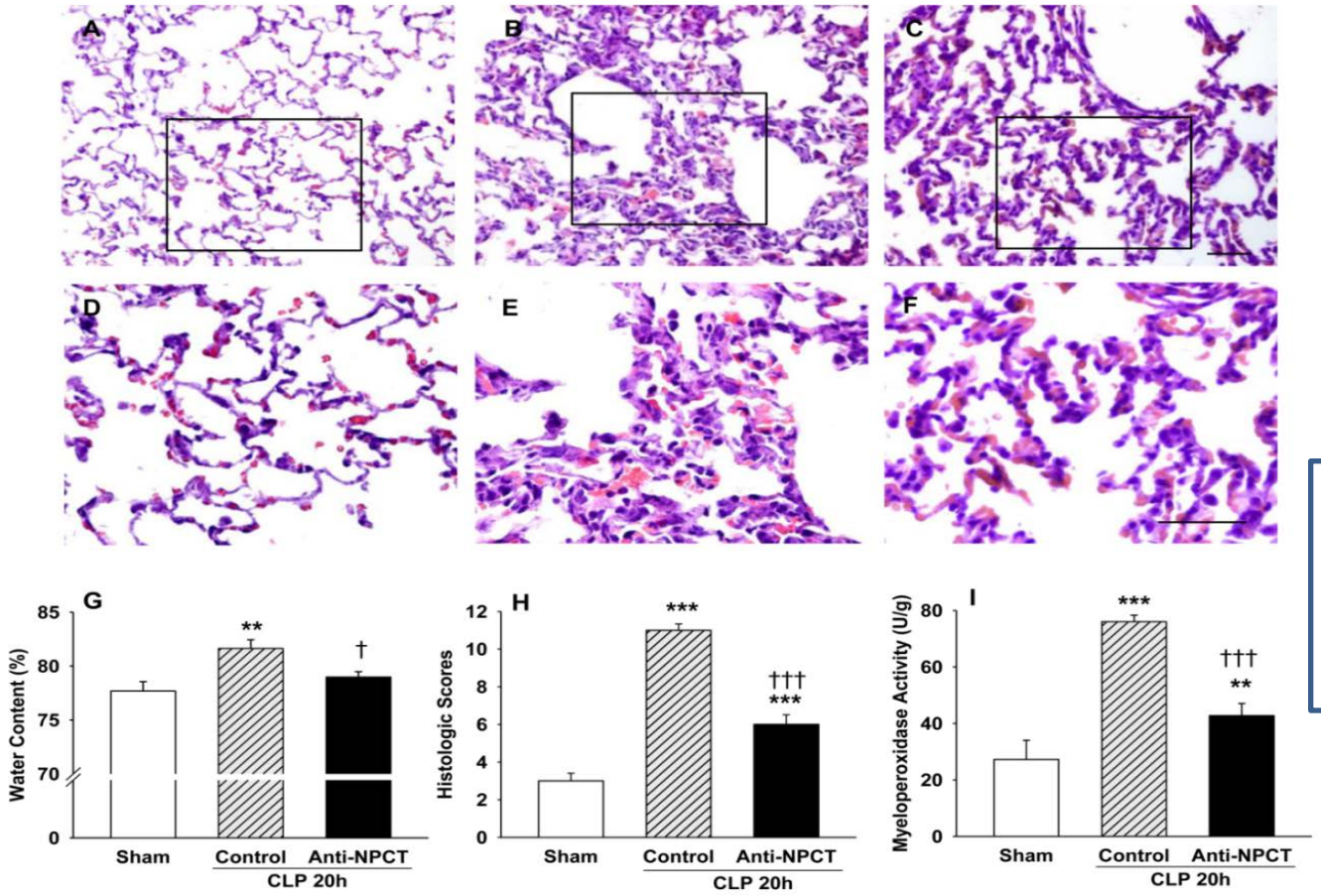


Immunoneutralization of NPCT reduces CLP-induced expression of CALCA, ProCT and NPCT in the lung tissues of the rats

2. The Product

d) Current status of development

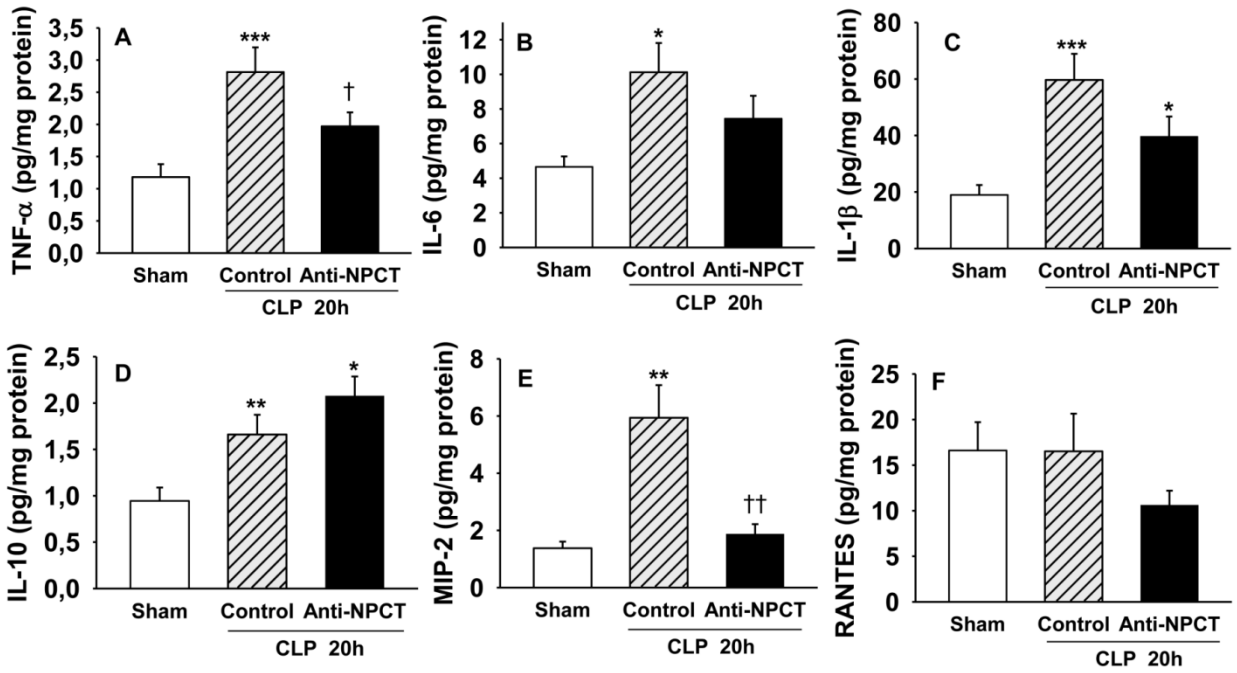
Inhibition of CLP-induced lung inflammation by anti-NPCT treatment

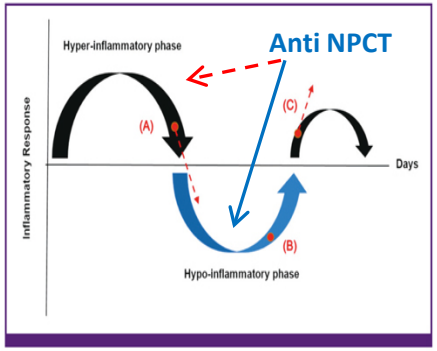


- Edema
- Lung injury
- Neutrophil infiltration
- Bacterial load

2. The Product

d) Current status of development





EARLY PRO-INFLAMMATORY RESPONSE

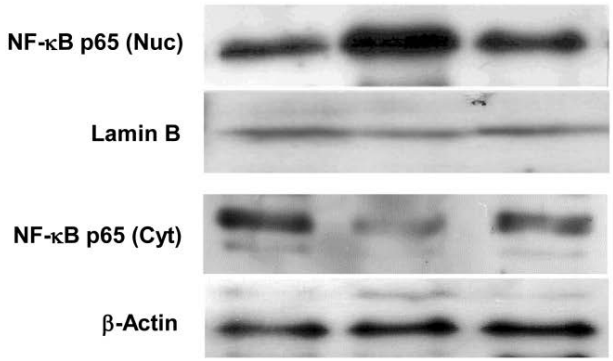
Anti-Aminoprocalcitonin

ANTI-INFLAMMATORY LATE RESPONSE

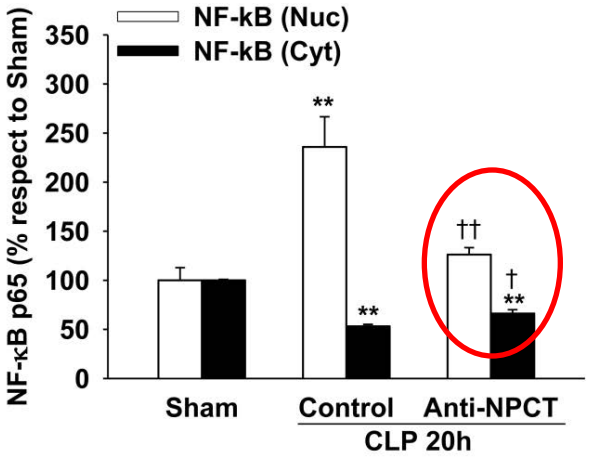
Anti-NPCT reduces the pulmonary production of inflammatory cytokines in septic rats

2. The Product

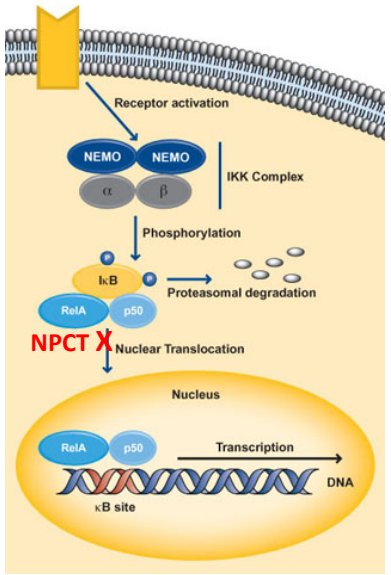
d) Current status of development

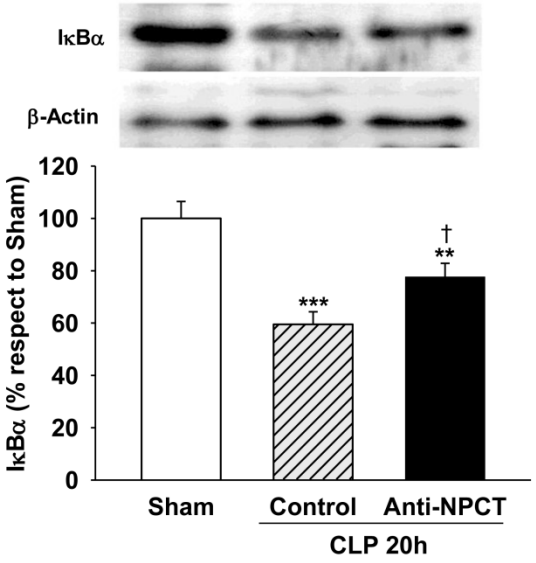


Immunoneutralization of NPCT **inhibits IκBα degradation and nuclear NFκ-B p65 translocation** in lung tissues of septic rats



Molecular Target: NFkB





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2. The Product

d) Current status of development

P201430015

RELATIONSHIP BETWEEN AMINOPROCALCITONIN AND BETA-AMYLOID DEPOSITION IN ALZHEIMER´S DISEASE

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Increasing evidence has suggested that inflammation in the brain is closely associated with the pathogenesis of several degenerative neurologic disorders, including Alzheimer's diseases (AD). The role of neuroinflammation in the development and progression of AD is, however, not clear. Aminoprocalcitonin (N-PCT), a neuroendocrine peptide derived from the amino-terminal half of rat prohormone procalcitonin, is able to block inflammatory effects induced by inflammatory agents, such as a LPS, diminishing plasma levels of pro-inflammatory cytokines. N-PCT exerts a neuroinmune role as a catabolic mediator in neuroendocrine and metabolic mechanisms that regulate the intake and energy homeostasis in systemic inflammation, playing an important role in the control of the hypothalamus-pituitary-adrenal axis. In the central nervous system (CNS), under normal physiological conditions, the N-PCT is expressed, in hypothalamic regions involved in the control of energy homeostasis. To date, its presence in other brain areas has not been described. Thus, we hypothesize that N-PCT expression could fluctuate according to the neuroinflammatory status in AD, probably mediated by amyloid beta (Aβ) peptides. In this study we evaluated NPCT expression in several experimental models of AD, including double transgenic APP/PS1 mice, neuronal cultures, and brain samples from AD patients. We found that NPCT is expressed in several cell populations including neurons, astrocytes and microglial cells, and that its expression is up-regulated by amyloid β (Aβ). These findings suggest that NPCT could be involved in the Aβ-induced pathological cascade.

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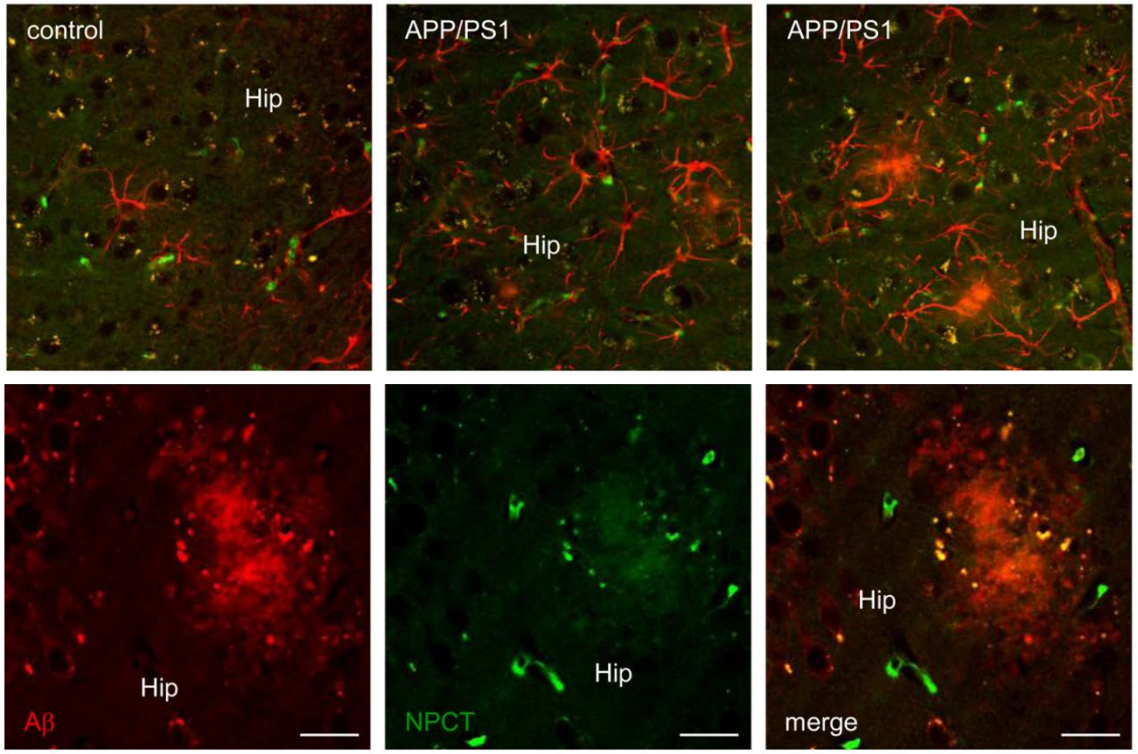
NPCT expression in APP/Ps1 mouse brain



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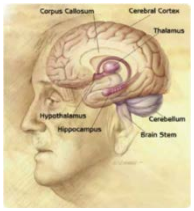
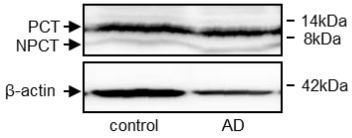
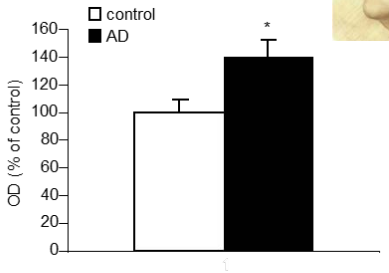
Alzheimer Disease patients

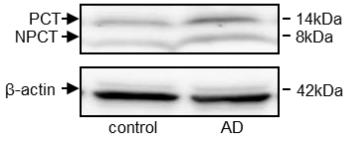


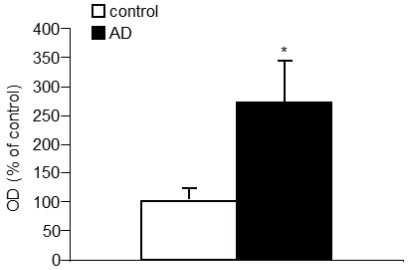
Hippocampus

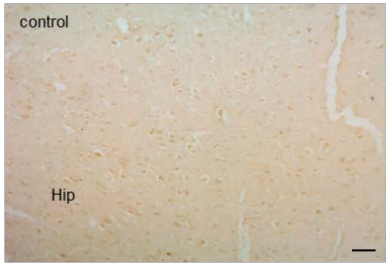


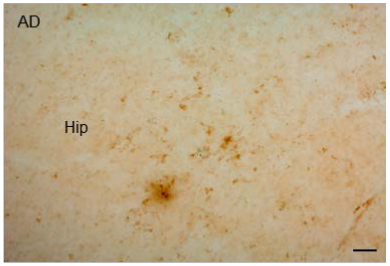


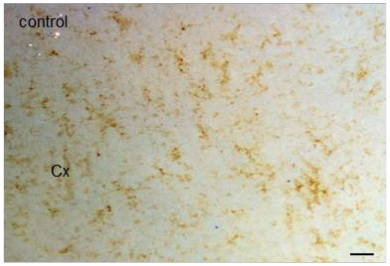
Cerebral Cortex

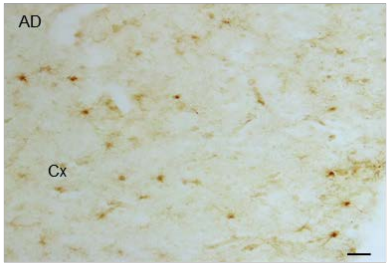


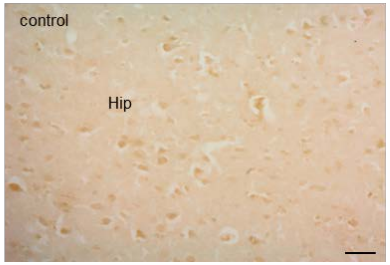


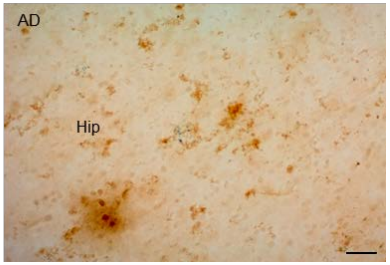


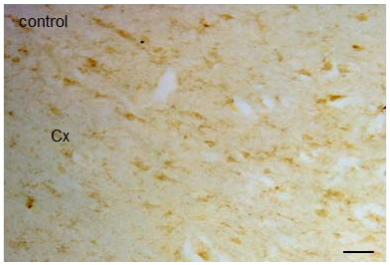


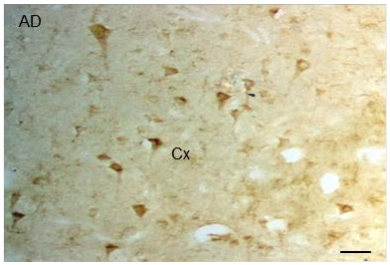












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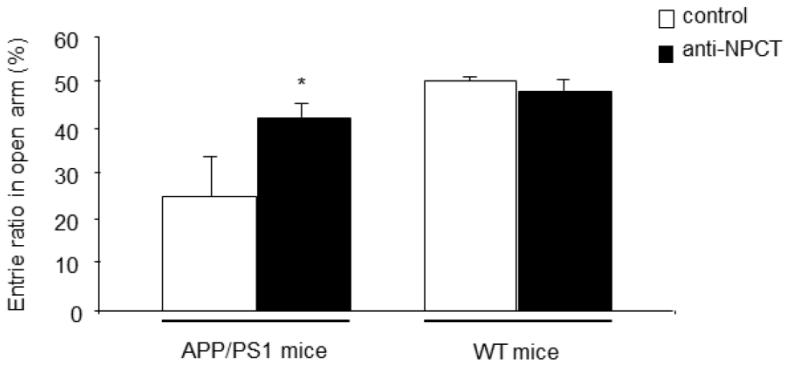
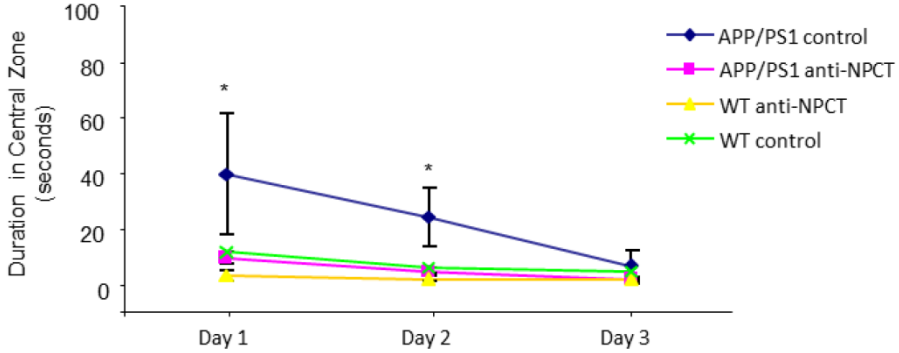
d) Current status of development

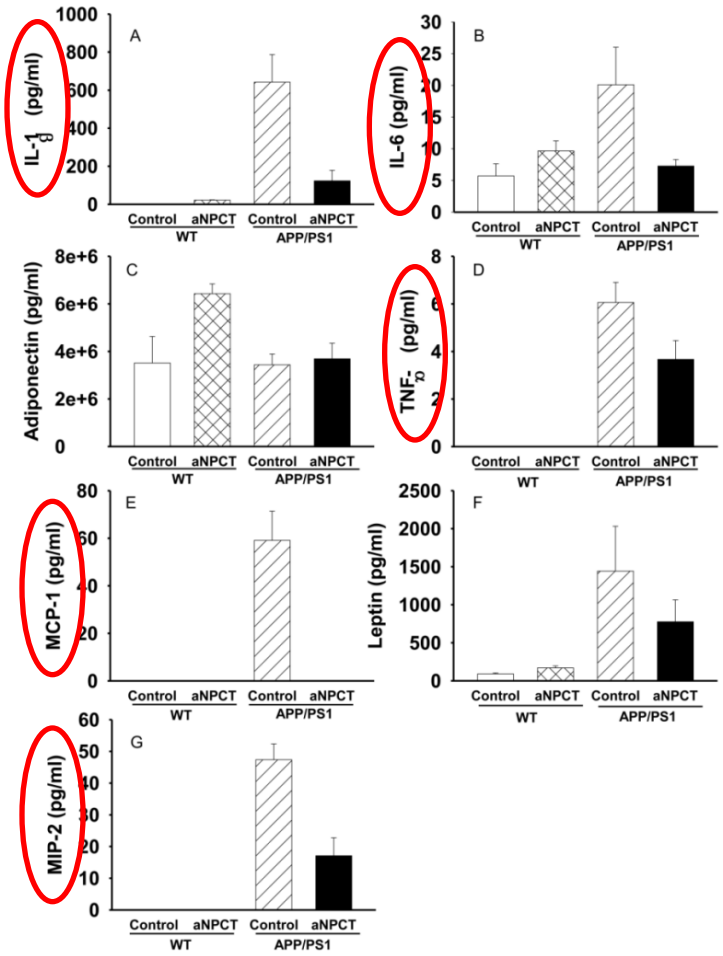




Immunoneutralization of NPCT. Behavioral effects in APP/PS1 mice

2. The Product

d) Current status of development



EARLY PRO-INFLAMMATORY RESPONSE

Anti-Aminoprocalcitonin

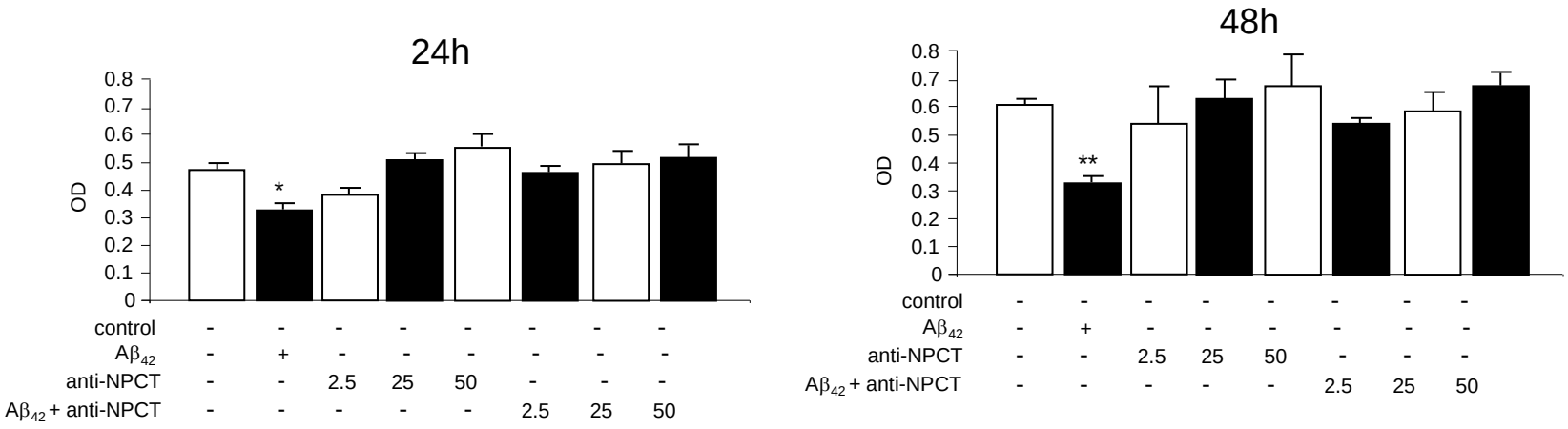
ANTI-INFLAMMATORY LATE RESPONSE

"Immunoneutralization of NPCT reduces the plasma production of inflammatory cytokines in AD"

2. The Product

d) Current status of development

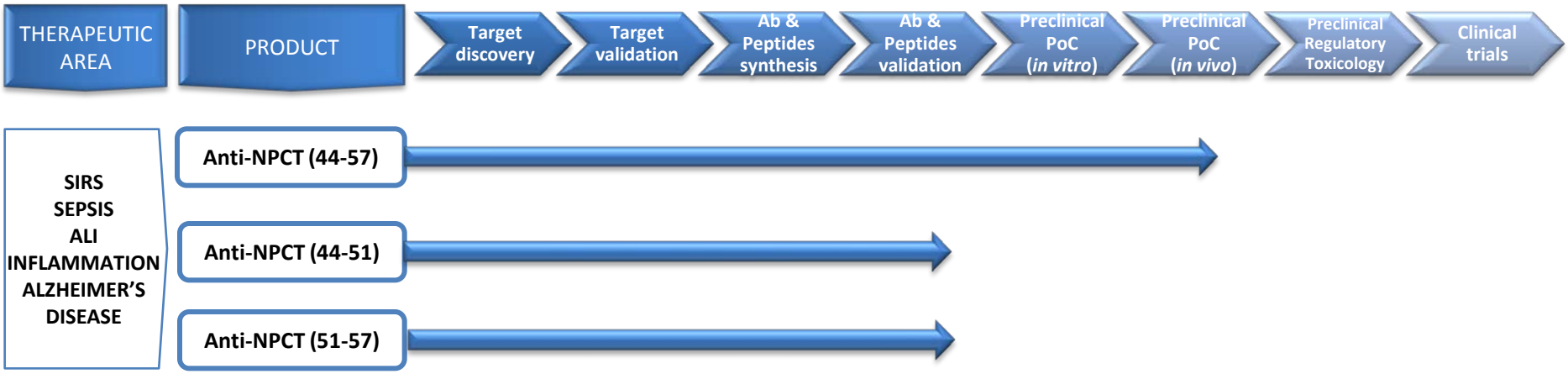
NPCT regulate Aβ-induced cytotoxicity in vitro



Viability evaluation in SK-SY-5Y neuroblastome cells treated with or without $A\beta_{1-42}$ (10 µM), and anti-NPCT (2.5, 25, 50 µg/ml)

2. The Product

d) Current status of development



- **Mechanism of action, toxicity and *in vitro* and *in vivo* efficacy studies have been successfully completed with very promising results.**
- **<u>Following step</u> would be to design and produce an humanized Ab for subsequent clinical trials.**

2. The Product

e) IPR protection

US13/527,069

Filed in Jun 2012
(Prov. Appl. Filed on Jun 2011)

- **Isolated nucleotides, peptides and antibodies obtained from said peptides.**
- Their **use for the early diagnosis & treatment of diseases that develop alterations of the inflammatory or metabolic stress**.
- **2nd medical uses:** sepsis, septic shock, cardiogenic shock, post-surgical complications, peritonitis, transplants, autoimmune diseases, obesity, diabetes, bacterial meningitis, neoplasias or neurodegenerative diseases that develop with inflammation.

PCT/ES2013/070831

Filed in Nov 2013
(priority date: Nov 12)

- Use of said peptides or antibodies in the treatment of **lung injury**, including **sepsis-derived lung injury or septic shock** (2nd medical use).
- A **fusion protein** comprising said peptides and a second peptide ables to carry a peptide into the cell.

P201430015

Filed in Jan 2014

- Use of said peptides or antibodies in the **prevention or treatment of a neurodegenerative disease**
- A **fusion protein** comprising said peptides and a second peptide ables to carry a peptide into the cell for said 2nd medical use.
- Use of said peptides, Abs, fusion proteins, as well as pharmaceutical compositions comprising them, for the treatment **of Alzheimer's disease**, among others neurodegenerative diseases.

2. The Product

f) Pitfalls & Risks to be considered

- Preliminary evidence that these antibodies prepared against the disclosed sequences have high affinity and specificity on (the) antigen (s) described, so that bind specifically to the free N-PCT and not to the whole molecule of PCT (ELISA), must be confirmed (pending results of studies *in vitro* and *in vivo* activity).

- N-PCT function and mechanism of action remain to be fully elucidated.

- Design and production of an humanized antibody for preclinical regulatory toxicology and clinical trials are needed.

- Regarding the diagnostic use, the design and development of the kit, as well as the scaling-up of the antibody production, should be performed.

3. Partnering opportunities

- Looking for a partner interested in a **license and/or a collaboration agreement (First Option Agreement)** to further develop and exploit this innovative technology.

- Open to establishing partnerships for **co-development** of the technology before reaching the market and highly interested in applying to different funding calls, mainly to **Horizon 2020.**

NEXTS STEPS OF DEVELOPMENT

- Further perform the *in vitro* and *in vivo* PoC studies with Anti-NPCT (44-51) & Anti-NPCT (51-57).
- Develop an humanized antibody for preclinical regulatory toxicology and clinical trials.
- Further develop a kit for quantifying the levels of N-PCT, as well as scale-up the current antibody production.

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CONTACT INFORMATION

Thank You!

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