LP226A1 for the treatment of Alzheimer's disease

LIPOPHARMA
Lipid Science for life

Next generation medicines!

Vicenç Tur. CEO, Co-founder

Barcelona, 20 de octubre de 2015

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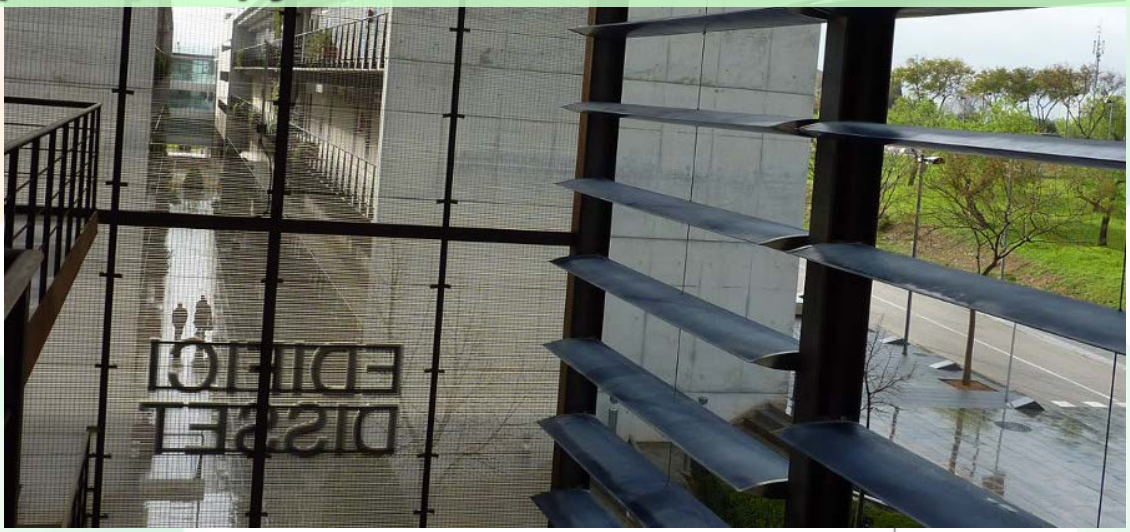
Next generation medicines!

Lipopharma, a pioneering clinical-stage biopharmaceutical company based in Palma de Mallorca (Spain) focusing on the discovery, design and development of next-generation medicines associated with a novel breakthrough therapeutic approach: the Membrane-Lipid Therapy (MLT)



LP226A1, novel bio-active lipid based on the MLT platform for the treatment of Alzheimer's disease (AD)

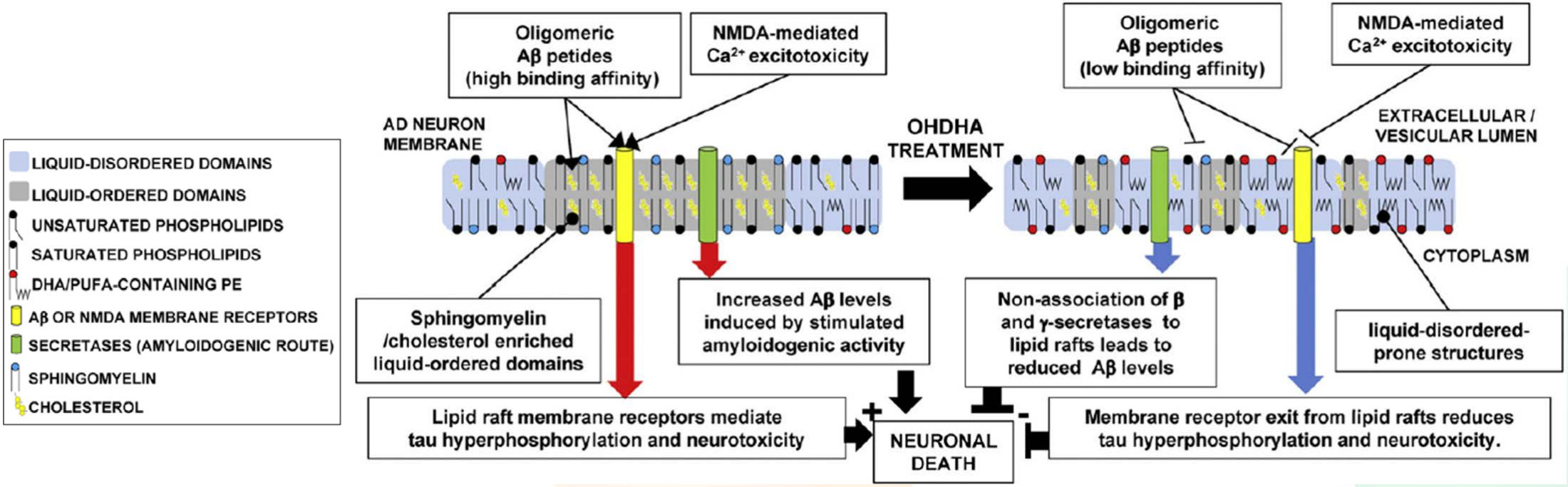
PIPELINE

PRODUCT	THERAPEUTIC AREA	DEVELOPMENT PHASE: RESEARCH	PRECLINICAL	PHASE I	PHASE II	PHASE III	REGIST.
Minerval	Cancer (GBM,...) [1]	■	■	■	■		
LP226A1	Alzheimer's Disease, CNS pathologies	■	■				
LP204A1	Inflammation, CV	■					
LP10218	Cancer [2]	■	■	■			
LPA181	Spinal Cord Injury, Pain	■					
LP205A1	CNS, Metabolic & Cardiovascular diseases	■					
LP30171	Metabolic disorders, Cancer	■					

(1) partnered with Praxis Pharmaceutical for SP, PT, CH, TU, IL & COL

(2) partnered with Ability Pharmaceuticals

MOA hypothesis: LP226A1 modulates lipid composition of Neuron membranes



LP226A1 (OHDHA) enriches brain membranes in PE, specially those carrying DHA and other PUFAs. These lipid changes influence the structure of the cell membrane, leading to the formation of liquid-disordered-prone structures and to the reversion of the PE and DHA loss during the late onset of AD. LP226A1 modulates the cellular signaling associated with AD by i) downregulating amyloidogenic processing and AB-dependent tau protein hyperphosphorilation and ii) decreasing neuron vulnerability to extracellular toxic agents. In addition, LP226A1 also induced proliferation of neuron stem cell in the mouse brain.

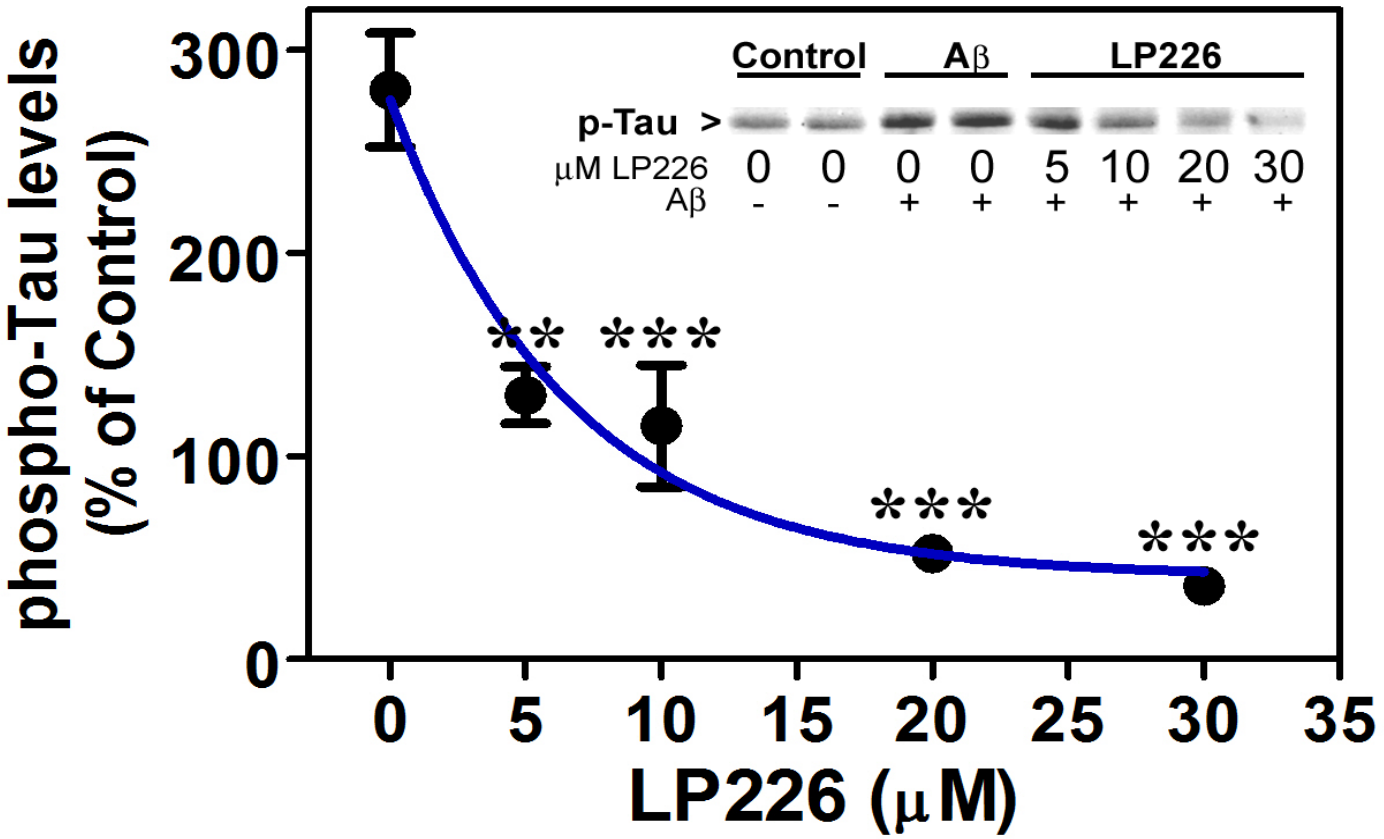
Torres M et al. BBA 2014

LP226A1 regulates the membrane lipid structure and binding of Aβ-42 to membranes

	Aβ42 oligomers		Aβ42 fibrils	
	SM-CHO membranes	SM-CHO + LP226	SM-CHO membranes	SM-CHO + LP226
Ka (M^{-1})	3.67×10^5	4.62×10^4	2.1×10^5	1.29×10^3
Kd (μM)	2,72	21,60	4,76	775.00

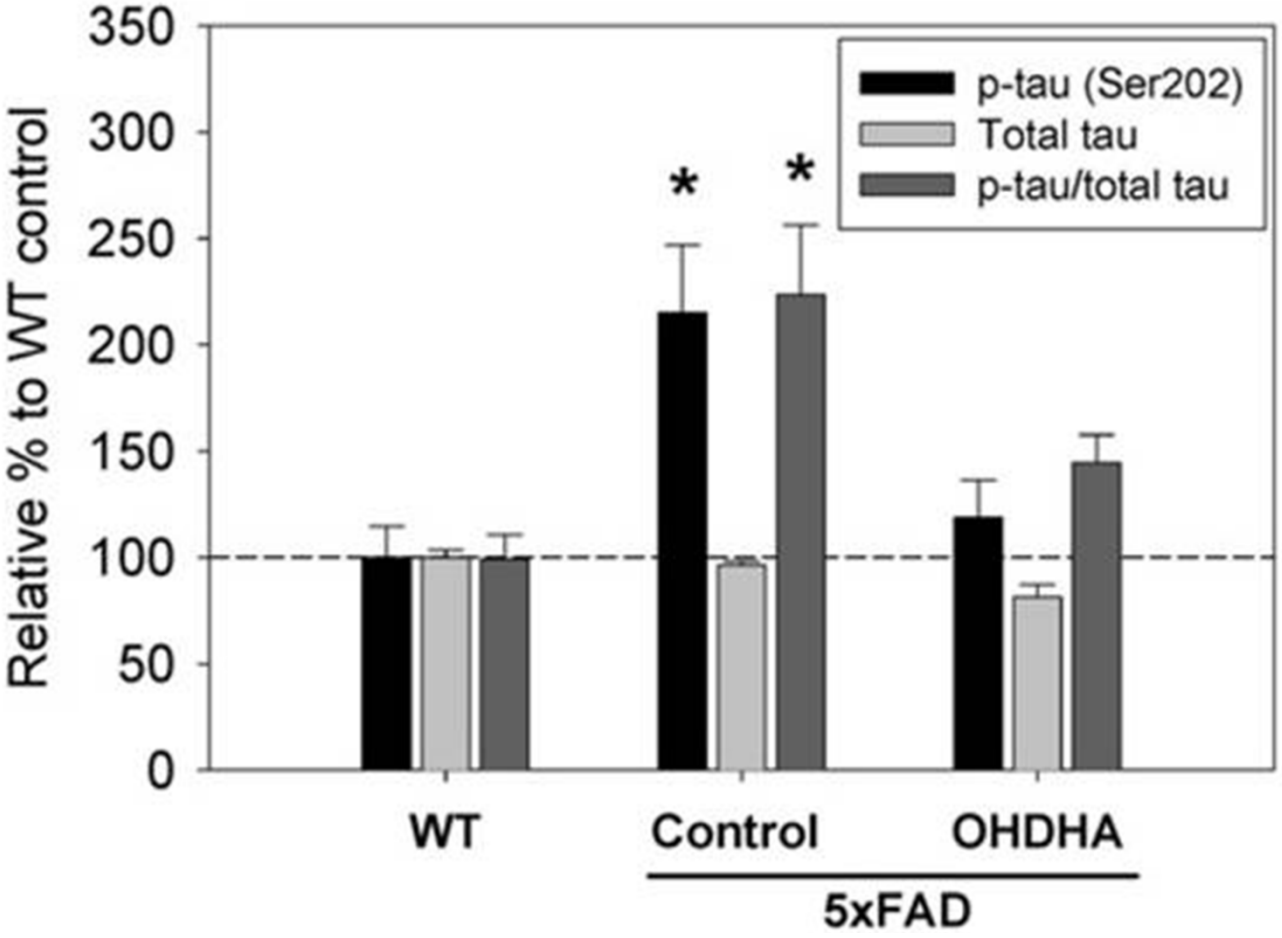
Binding affinity of Aβ-42 oligomers and fibrils to model membranes. Model membranes were formed with SM:CHO (1:1; mole:mole) in the presence or absence of LP226A1 (phospholipid:LP, 19:1, mole:mole). Binding affinity (Ka and Ks values) was determined by isothermal titration calorimetry (ITC)

LP226A1 regulates phospho-Tau expression *in vitro…*



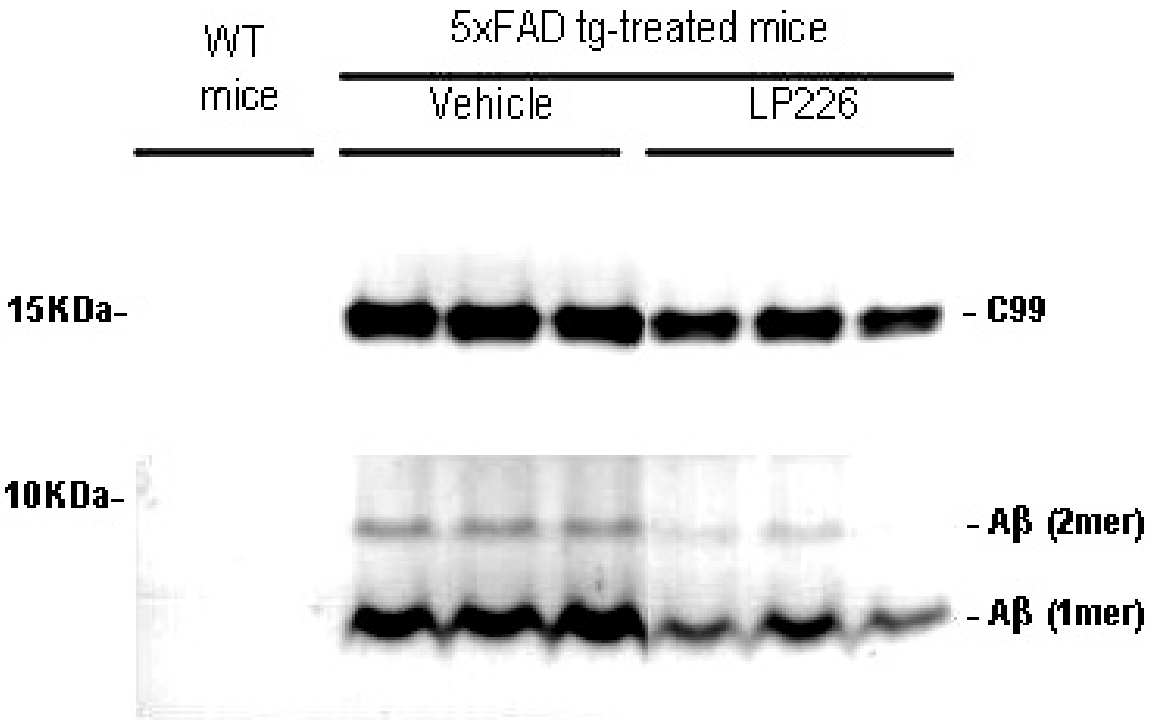
***In vitro* effect on phospho-Tau expression.** Differentiated SH-SY5Y cells were treated during 24h with Aβ 42 peptide (5 μM) in the presence or absence of LP226A1 (5-30 μM).

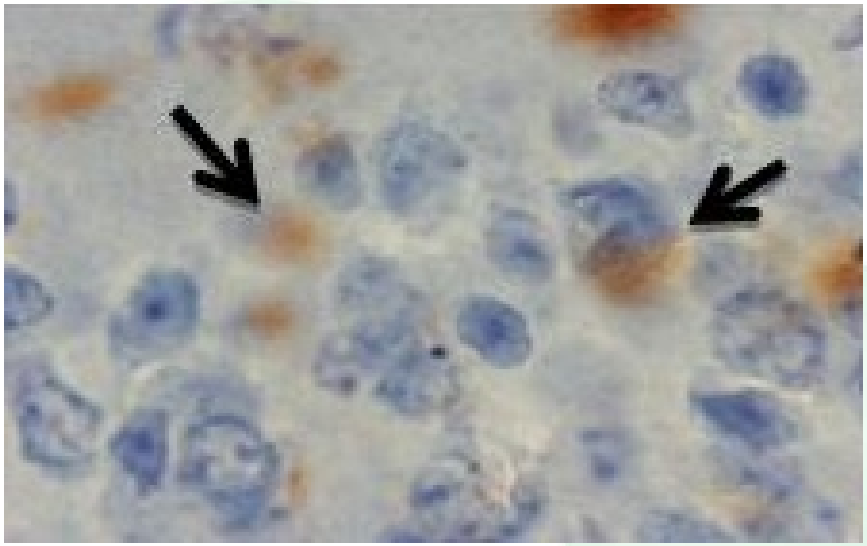
…and *in vivo.*



In vivo significant increase in **phospho-tau** levels in 5xFAD was majorly prevented in LP226A1-(OHDHA)-treated 5xFADmice, as compared with WT. Total-tau levels did not show any significant differences. Consequently, the phosho/total tau ratio was showed significantly increased in 5xFAD but not in LP226A1 (OHHDA)-treated 5xFAD mice (15 mg/Kg), as compared with WT.

LP226A1 inhibits the toxic accumulation of intraneuronal Aβ amyloid peptides

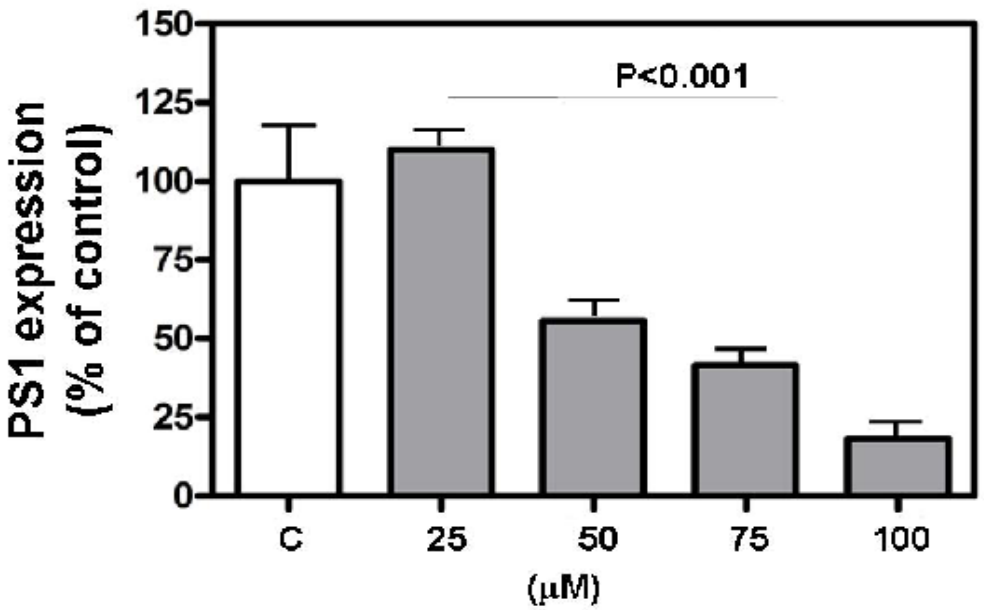


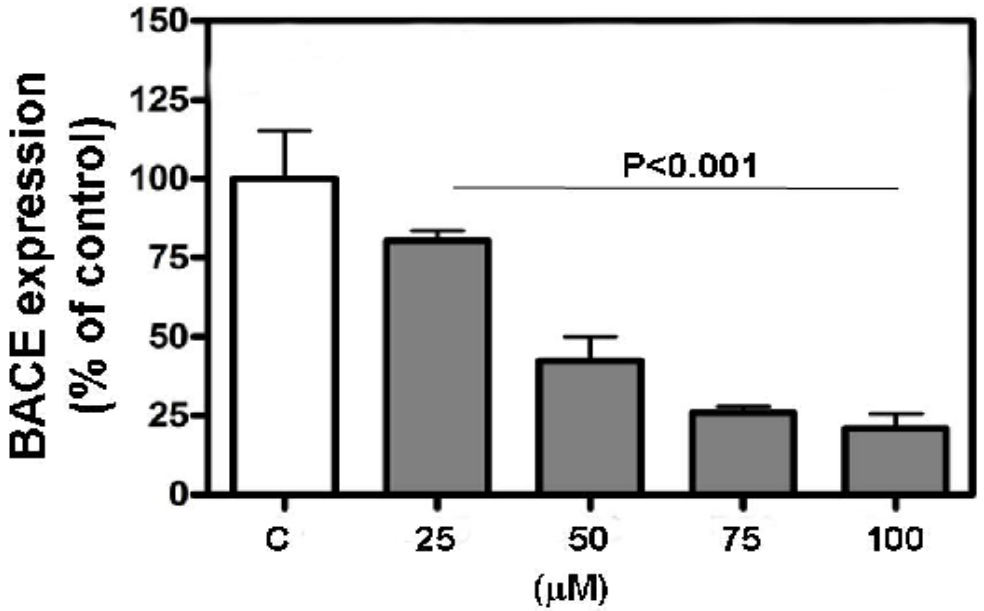


LP226A1 induced an important inhibition (≈ 50%) of intracellular C99 amyloid precursor protein and Aβ amyloid peptides

Left panel. A representative western blot showing the effect of LP226A1 in inhibiting C99 amyloid precursor protein and Aβ peptide accumulation in LP226A1-treated 5XFAD mice. Right panel. Aβ 42 accumulation (brown) in cortex neurons of untreated 5XFAD mice.

MOA [3.4]
LP226A1 regulates secretase activity





LP226A1 inhibits γ and β secretase expression and activity. Top. Dose-dependent inhibition of PS1 (**γ**) and BACE (**β**) secretase expression by LP226A1 in differentiated SH-SY5Y neuroblastoma cells. No effect in **α** secretase expression has been observed.

LP226A1 induces protective Autophagy markers

Markers	LP226A1 (24 h*)	DHA (24 h*)
Beclin-1	↑↑	↓↓
ATG5	↑↑	–
ATG12	↑↑	–
ATG7	↓	–
ATG3	↑↑	–
LC3BII	↑↑↑	↑

Level of induction of ER stress proteins (Beclin, ATG5, ATG12, ATG7, ATG3 and LC3BII) after treatment of differentiated SH-SY5Y cells with 10 μM of LP226A1 and DHA.

*** Cells were incubated concomitantly with Aβ42 peptide.**

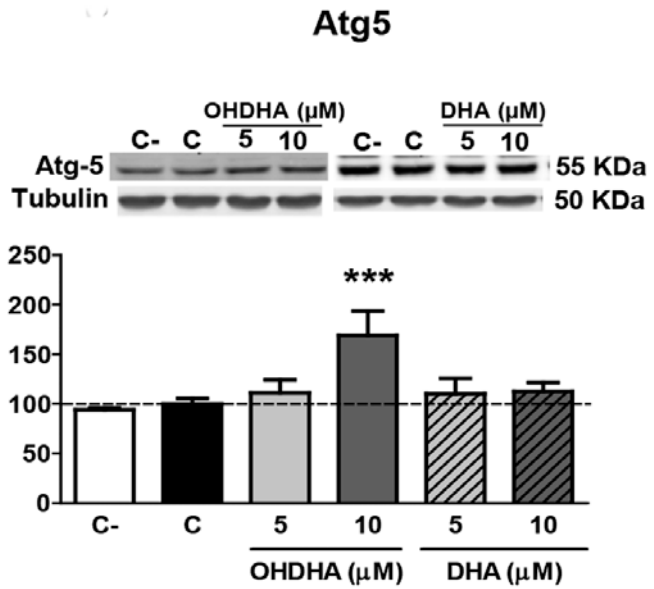
Meaning of symbols:

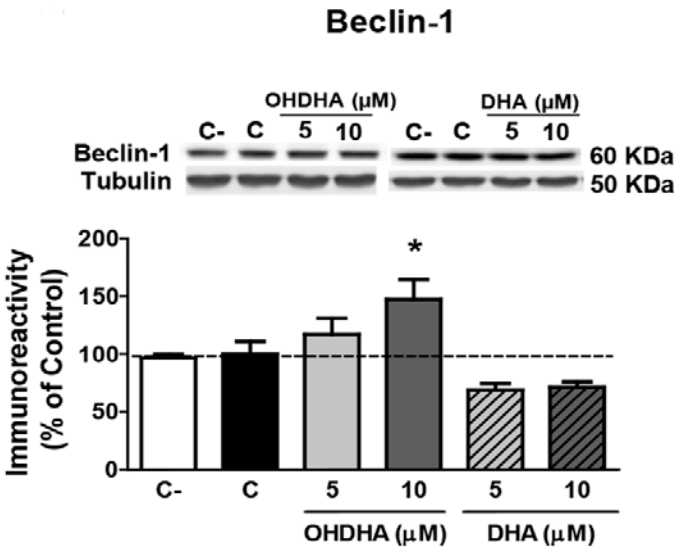
– no modulation; ↑ marked up-regulation; ↑↑ very marked up-regulation; ↑↑↑ very very marked up-regulation; marked down-regulation; ↓↓ very marked down-regulation.

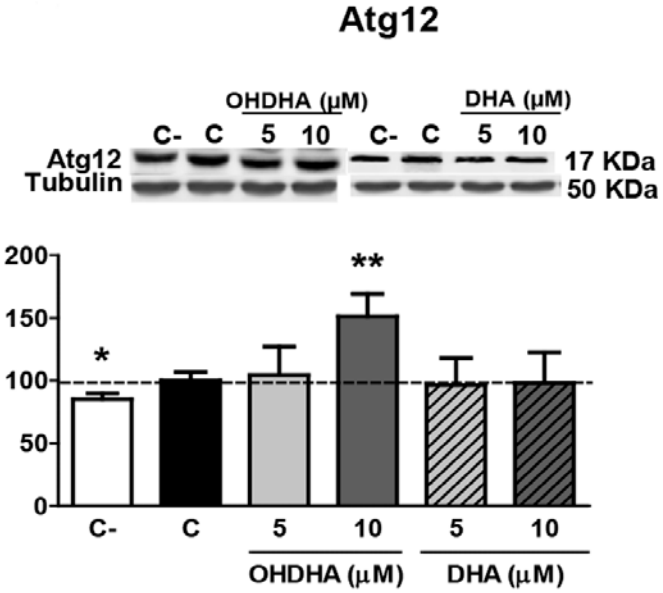
LP226A1 differentially induces Autophagy/cell reparation markers

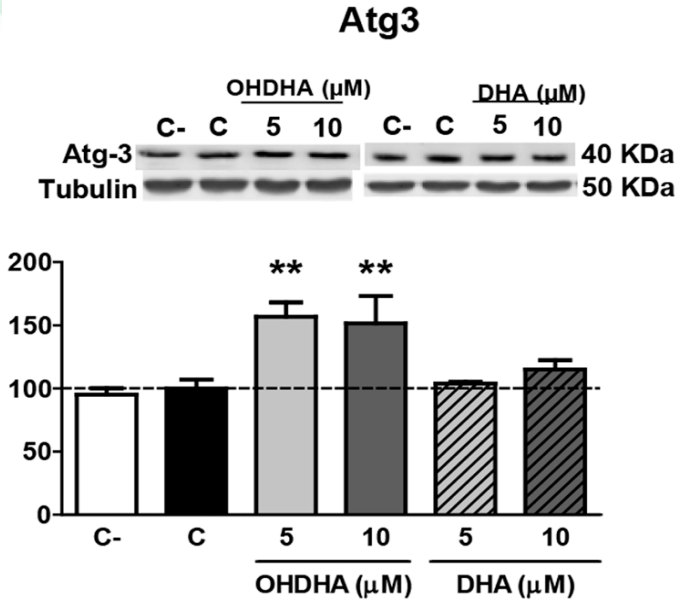
Key Autophagy / cell reparation markers are induced by OHDHA (but not by DHA). These experiments were carried out in Neuron-like phenotype cells (SH-SY5Y) in the presence of Aβ 42.

Torres M. et al, Apoptosis 2015

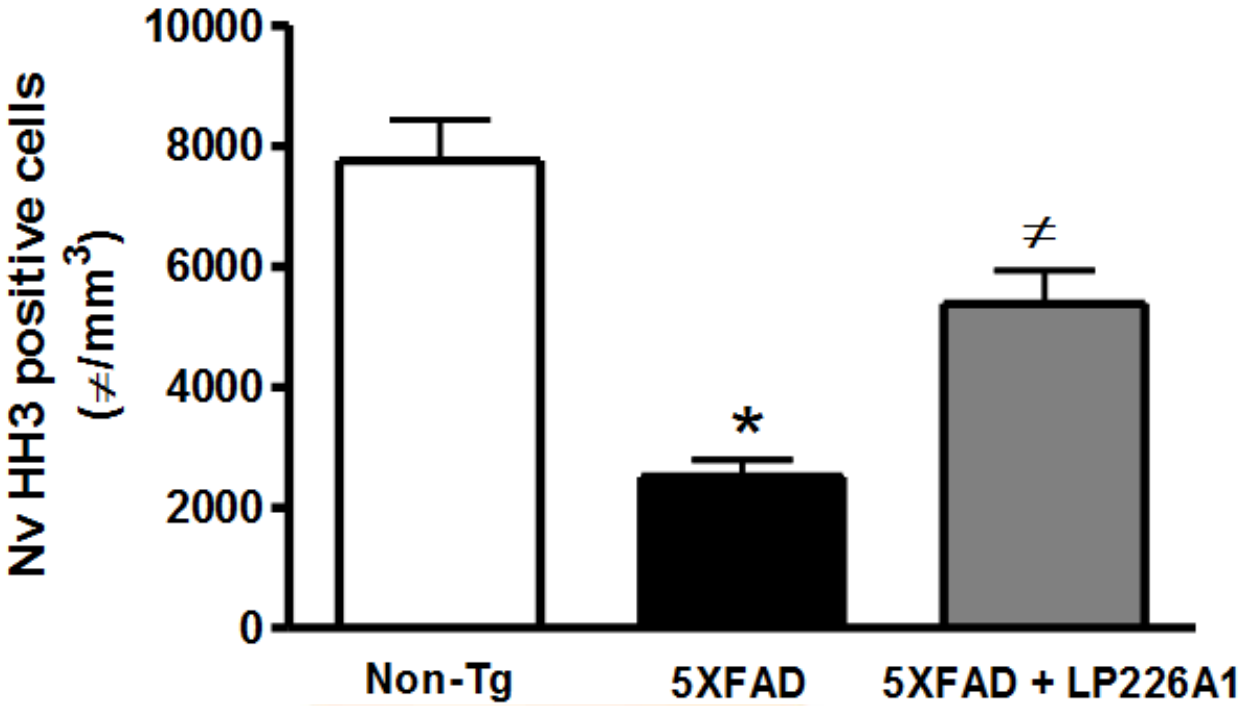








LP226A1 restores neurogenesis activity

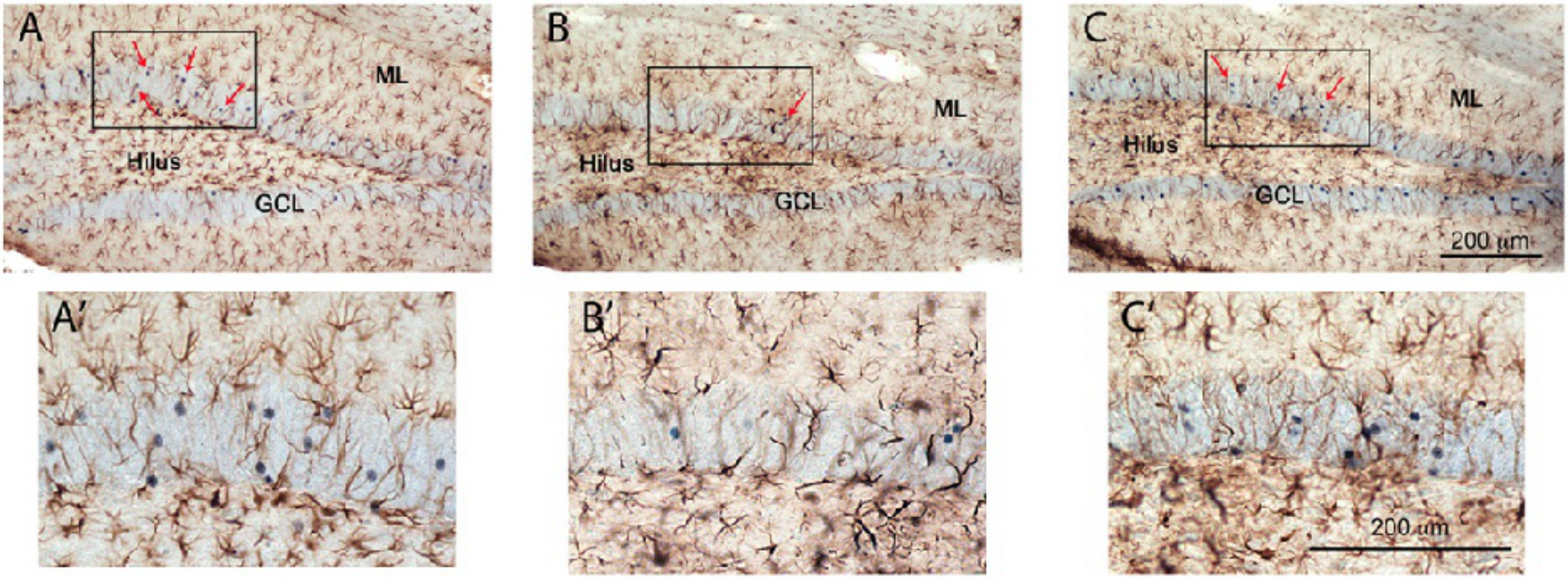


LP226A1 induces HH3 phosphorylation (neurogénesis) in the hippocampus of 5XFAD mice. Bar graph illustrating HH3-positive neurons (#/mm3) in the DG of the different experimental groups. Bars represent mean ± SEM, Non-Tg (n=4) 5XFAD (n=7), 5XFAD+LP226A1 (n=7). Treatments with LP226A1 induced marked and significant increases (270%, p=0.016) in the number of HH3-positive neurons in the hippocampus of 5XFAD mice

Fiol-deRoque et al. Biogerontology, 2013

LP226A1 restores neurogenesis activity

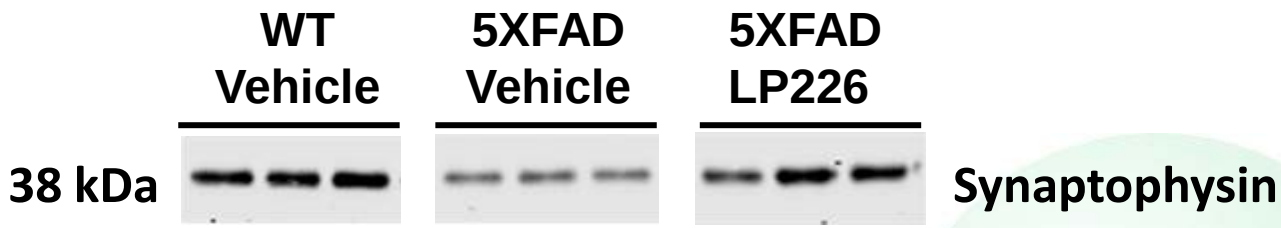
Hippocampal Dentate Gyrus cell proliferation in AD mice

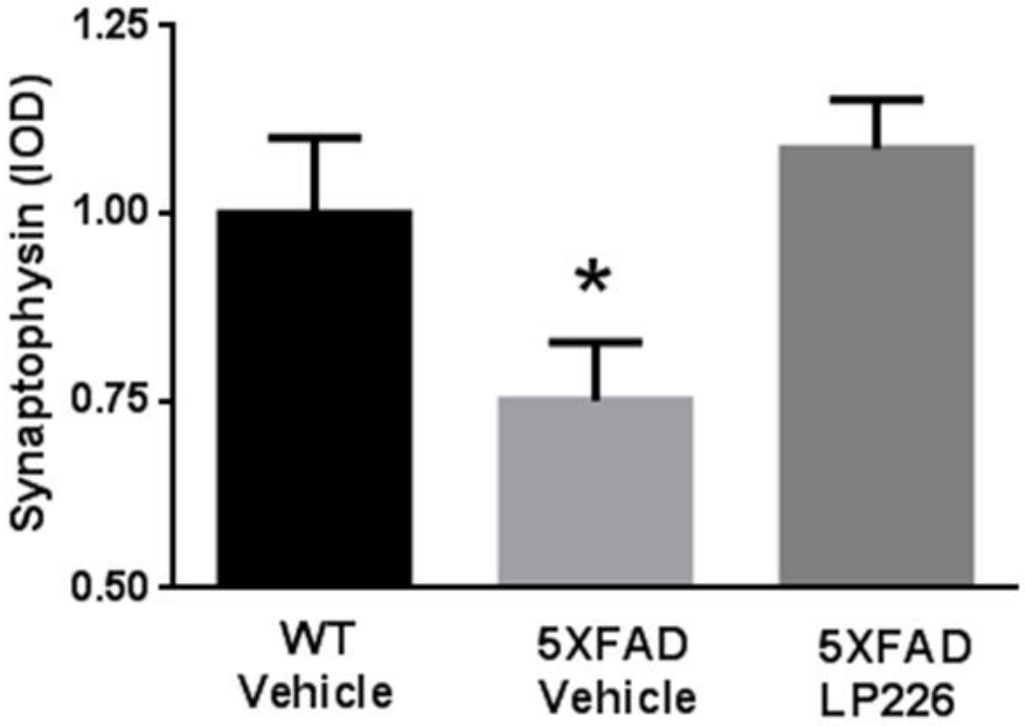


Imunohistochemical analysis of the dentate gyrus (DG) from control (A), 5XFAD (familial Alzheimer's disease mice) untreated (B), and 5XFAD micet reated with LP226A1 (C, 15 mg/Kg for 4 months- 5 days/week). It is noticeable that after LP226A1 treatment, the rate of neurogenesis is recovered , with no co-localization with GFAP positive glial cells (brown)

Fiol-deRoque et al. Biogerontology, 2013

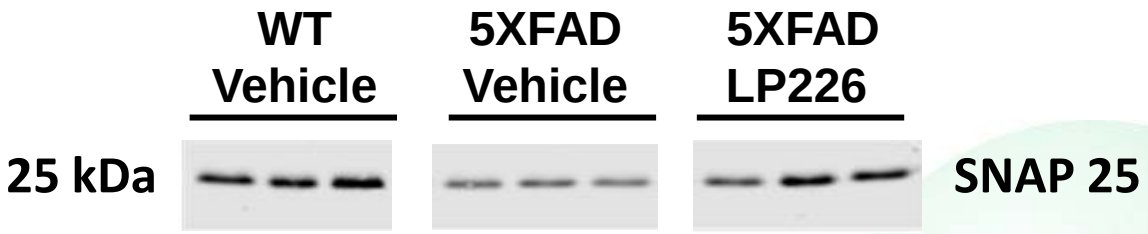
LP226A1 restores synapsis activity

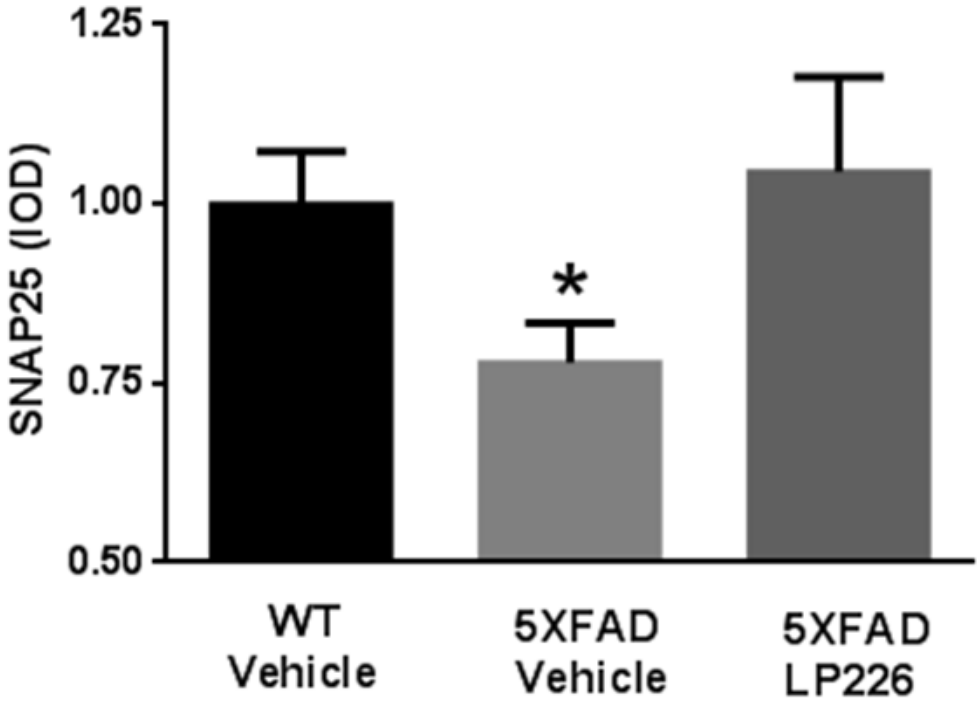




5XFAD mice,
Four month treatment,
20 mg/Kg. N=6

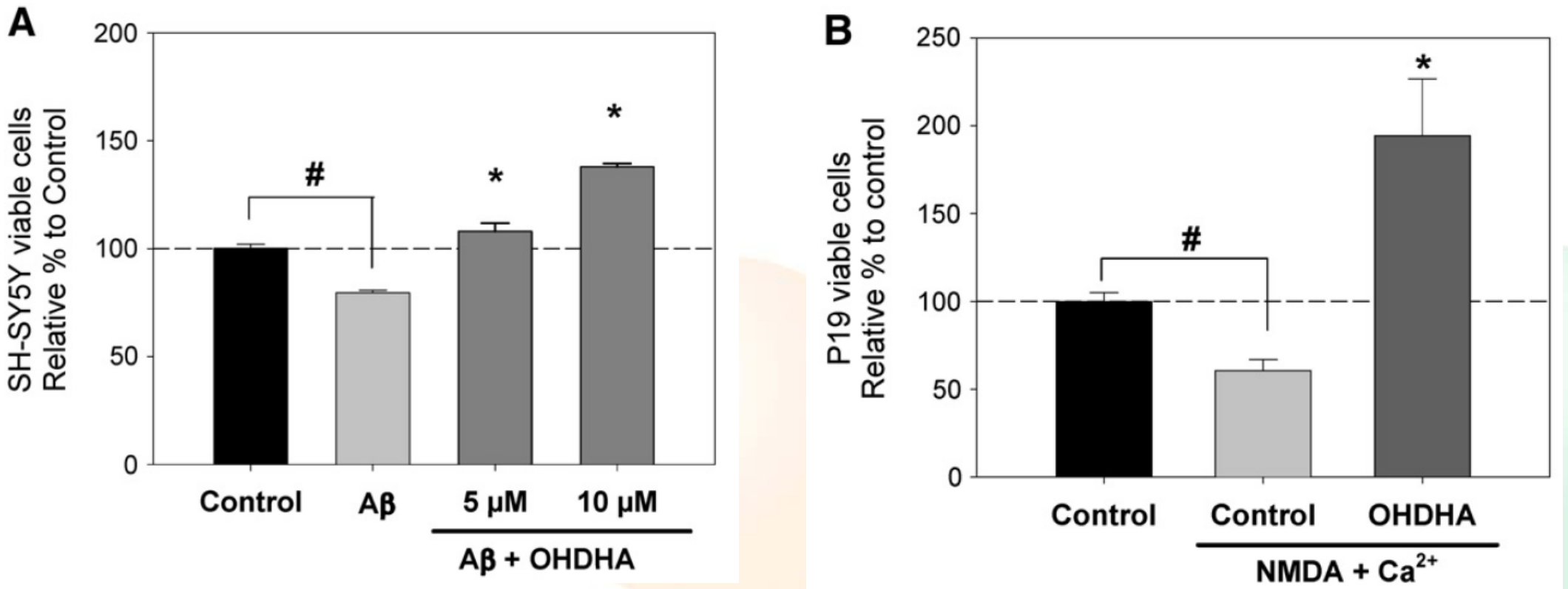
LP226A1 restores synapsis activity





5XFAD mice,
Four month treatment,
20 mg/Kg. N=6

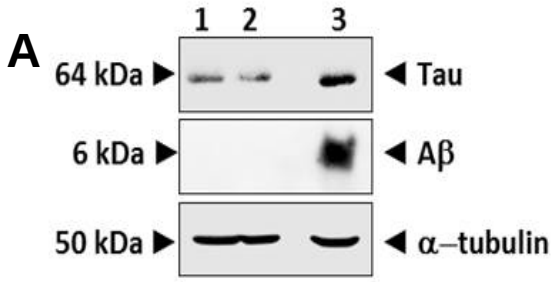
Neuroprotection screening in cell models



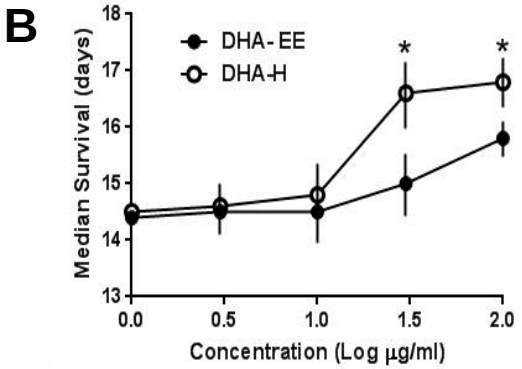
***In vitro* neuroprotective effects of LP226A1 (OHDHA).** Left panel. Protective effects of LP226A1 against soluble Aβ-2-induced neuron (SHSY5Y) death. Right panel. P-19 cell cultures were incubated in the presence of NMDA plus LP226A1. The bars correspond to mean±SEM values of live neurons after 24-h incubation.

Torres M et al. BBA 2014

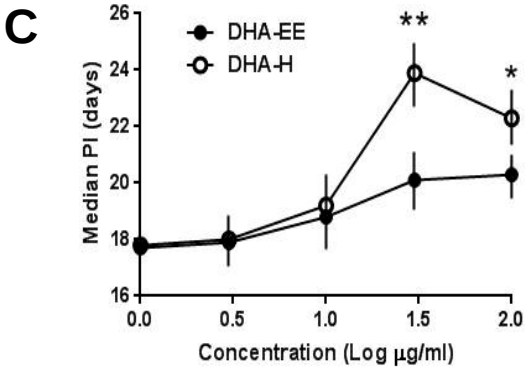
LP226A1 (DHA-H) restores aging-dependent behavioral motor functions in human transgenic (APP and Tau) *Drosophila melanogaster*.



A. F1-generation flies (lane 3) showed increased levels of human tau protein and Ab peptide as compared with their progenitors (lane 1: Gal4-expressing flies; lane 2: flies expressing Ab and tau under UAS promoter control).

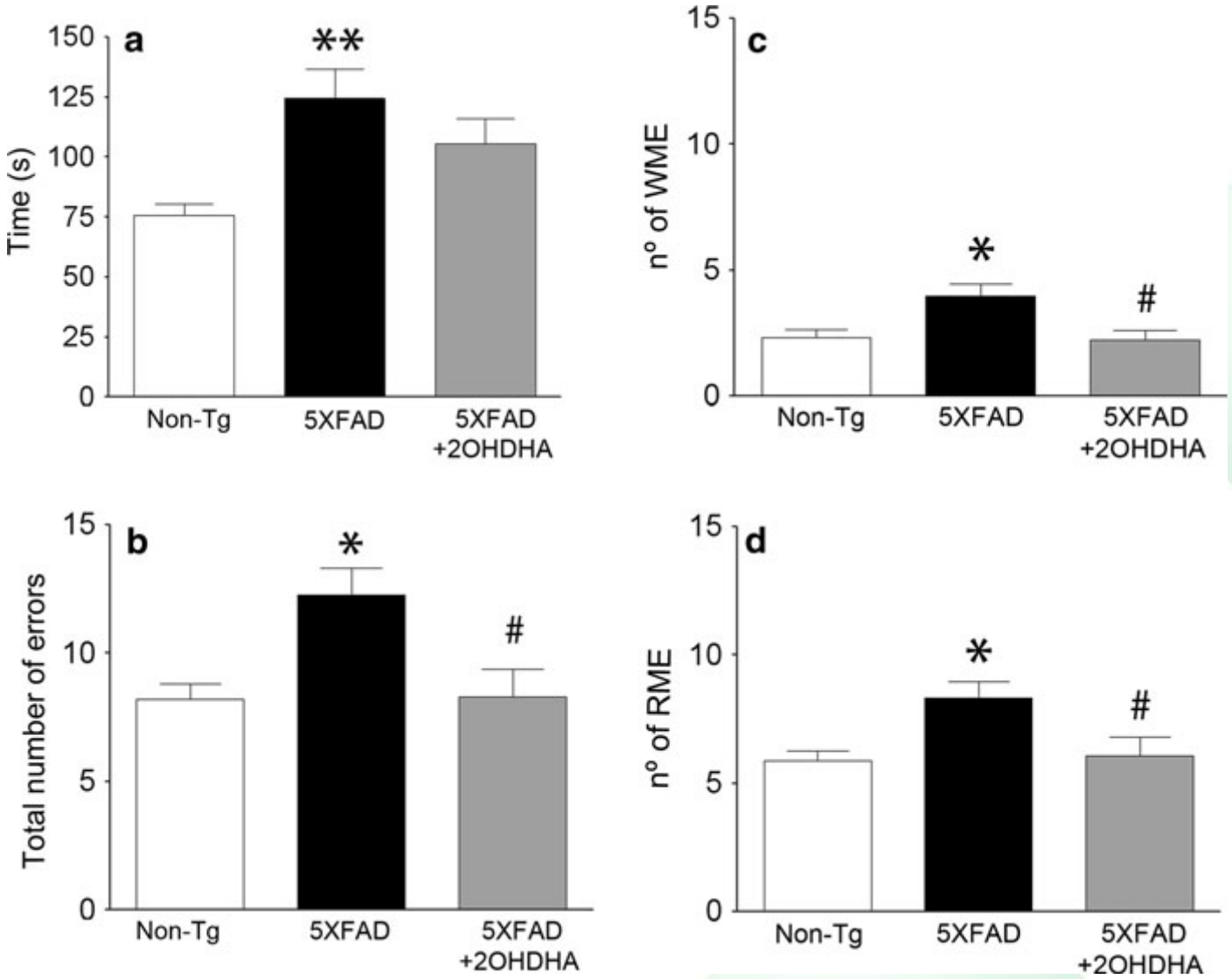


B. Median survival plots of F1 flies fed with DHA-EE (filled dots) and fed with LP226A1 (DHA-H). Food supplementation with LP226A1 demonstrated to be more effective than food supplementation with DHA-EE in terms of increasing the lifespan of these flies



C. Median locomotor performance index plots of F1 flies fed with DHA-EE (filled dots) and fed with LP226A1 (DHA-H). Food supplementation with LP226A1 demonstrated to be more effective than food supplementation with DHA-EE in terms of increasing the locomotor performance.

LP226A1 (2OHDHA) in a mouse model of AD (5XFAD) 4-month treatment



Learning ability on the Radial Arm Maze (RAM).

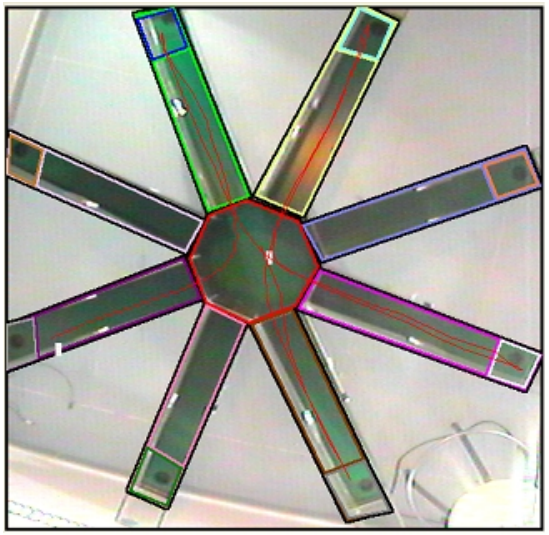
Bar graphs showing the RAM performance at the fourth week of trial (5 trials/week) at 7 months of age after **4 months of treatment** (15mg/kg/day, 5 days/week): Non-Tg (n = 10), 5XFAD (n = 11) and 5XFAD+2OHDHA (n = 12).

a) Time performance, b) total number of errors, c) number of working memory errors (WME, re-entrance in visited arms) and d) number of reference memory errors (RME, entrance in un-baited arms).

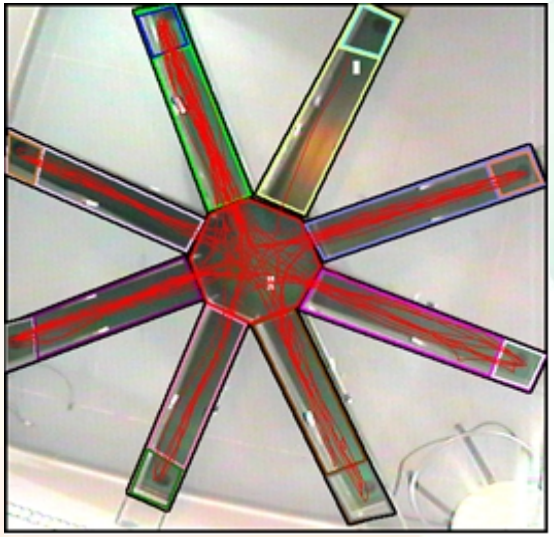
Bars represent mean ±SEM. One-way ANOVA followed by Bonferroni post hoc test. *p ≤ 0.05; **p ≤0.01; difference from Non-Tg group; #p ≤ 0.05 difference from 5XFAD group

Fiol-deRoque et al. Biogerontology, 2013

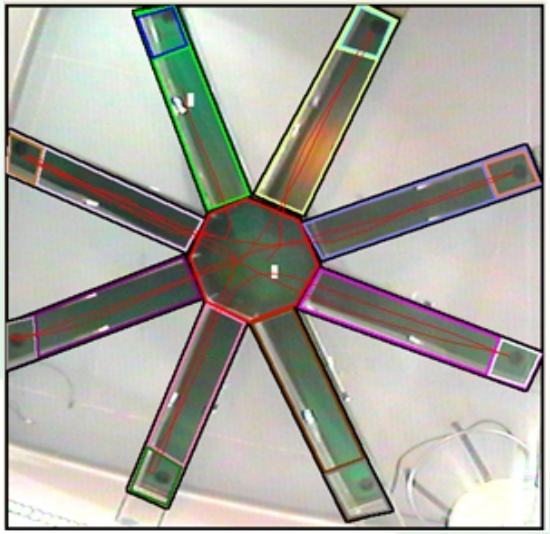
Efficacy of LP226A1 in a 5XFAD Alzheimer's disease mouse model



Healthy Control (B6) Mice



Alzheimer's Disease (5XFAD) Mice



5XFAD Mice treated with LP226A1

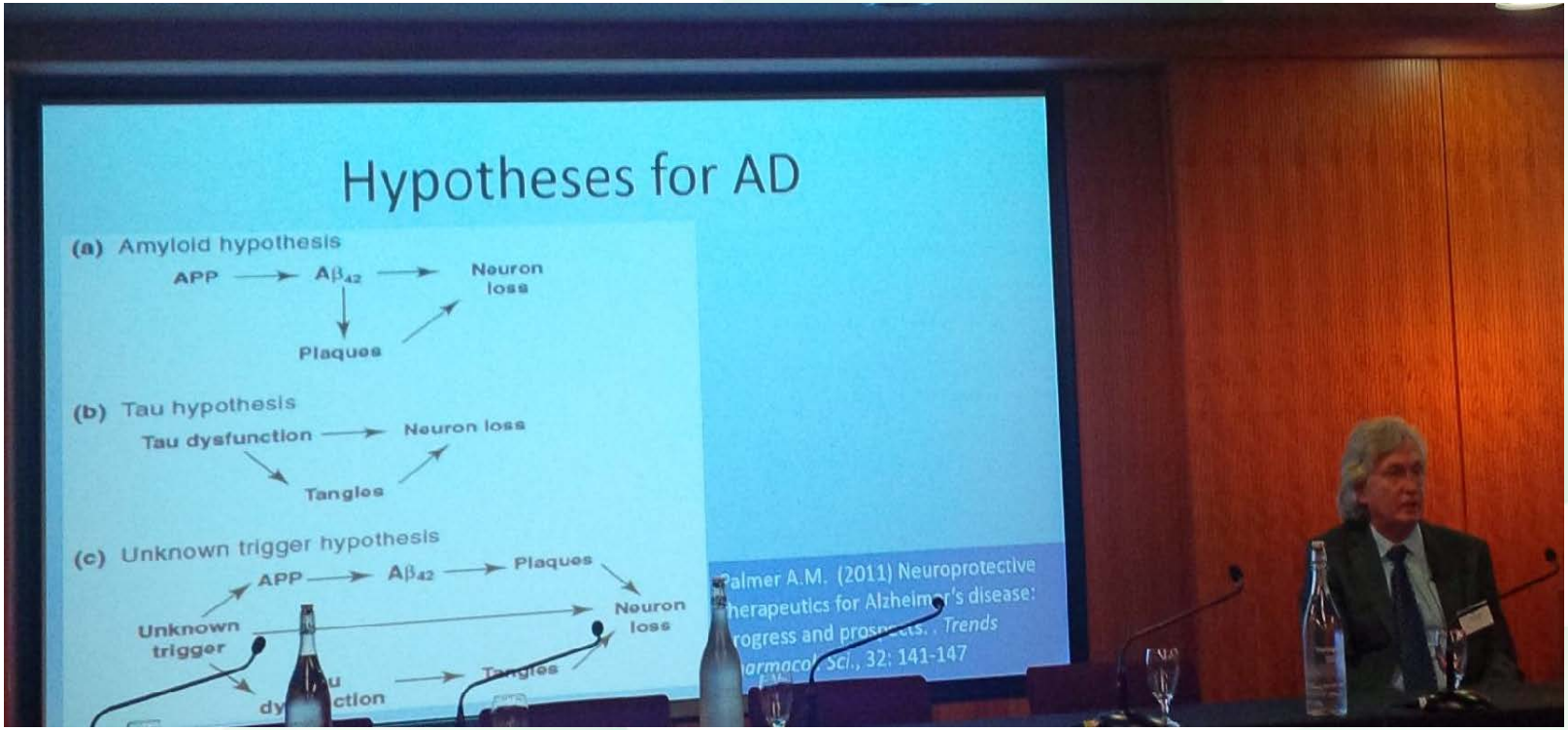
Upper view of the Radial Arm Maze (RAM) showing a representative computer-generated itinerary (red line) of one average healthy mouse (left), of an Alzheimer's disease (5XFAD) mouse (center) and of an Alzheimer's disease (5XFAD) mouse treated during 4,5 months with LP226A1 (right)

LP226A1 for the treatment of AD: What's different?

✓**Oral formulation, safe** at therapeutic doses (safety profile similar to DHA!)

✓ **Disease modifying** capacity demonstrated in 2 different animal models

✓Novel **mechanism of action**: lipid modulation of neuron membranes → could be the "**unknown triger**" that, moduled upstream is able to regulate both **Tau** and **Amyloid** pathways



Prof. Alan Palmer. London, Oct. 2014

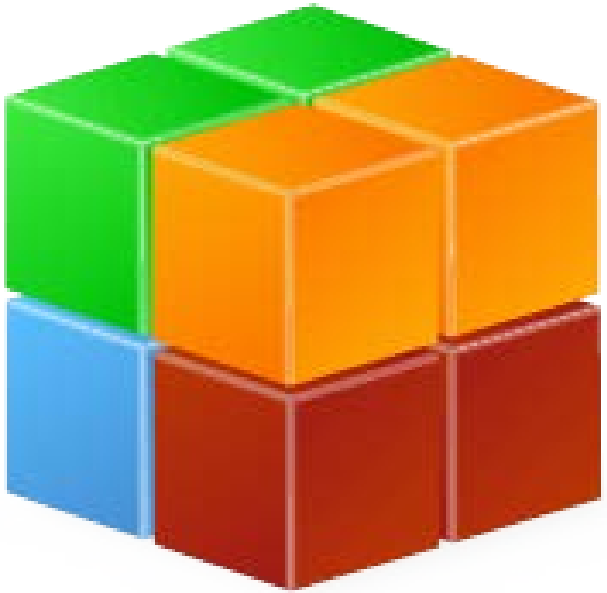
LP226A1 for the treatment of AD: current status

- Product in **preclinical** stage.
- **RETOS** project awarded to advance regultory preclinical & CMC development (1,3M€, 2015-2018)
- **Nutraceutical formulation** (enriched purified DHA + DHA derivatives) also in development by partner companies Pharmaconcept and Praxis Pharmaceutical

LP226A1: IP position

Global Patent Family with priority date of **March 2010** covering the therapeutic and nutraceutical applications of PUFA derivatives in examination proceedings in all major markets.

Patent for LP226A1 in AD already granted in Russia and Mexico.



LP226A1. Challenges ahead:

- preclinical stage asset

- Novel MOA (not yet fully understood)

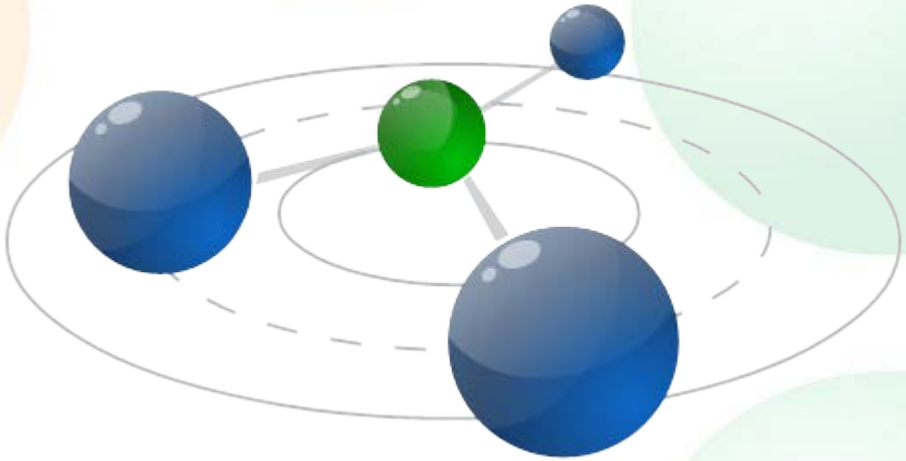
- Clinical development in AD very challenging

- Clinical & Financial risk

- too nice to be true (again)!!

- ...

Considering collaborations with strong and experienced partners to advance clinical development of LP226A1 in AD.



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