Novel conjugation of oleic acid with an amphetamine derivative for prevention and treatment of steatohepatitis

Madrid, 15 de noviembre de 2016

GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española
farmaindustria

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2. The Product
   a) Target Indications
   b) Innovative mechanisms of action
   c) Differential features facing the market
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3. Partnering Opportunities

THE INSTITUTION

ibima Instituto de Investigación Biomédica de Málaga

The Institute of Biomedical Research in Malaga (IBIMA) is a space for multidisciplinary biomedical research that brings together:

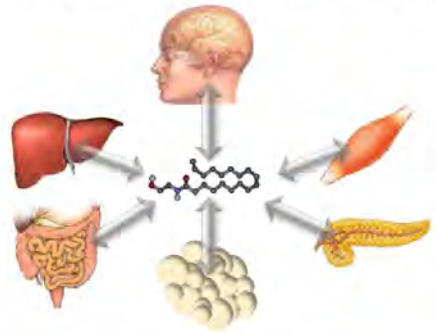
1. **The Regional and Virgen de la Victoria Malaga University Hospitals**

2. **The primary care units and**

3. **The biotechnology groups at the University of Malaga.**

THE RESEARCH GROUP "NEUROPSYCHOPHARMACOLOGY"

- Estudio de los mediadores lipídicos como moléculas de trasmisión y su papel principal en trastornos como la adicción, obesidad y metabolismo, así como su relación con la ansiedad, esquizofrenia, estrés, dolor y enfermedades inflamatorias



Doctores (8)

- Fernando Rodríguez de Fonseca (Dr. Medicina y Cirugía) Coordinador del grupo
- Francisco Alén Fariñas (Dr. Psicología) Investigador contratado CEIC - A.Conocimiento
- Miguel Angel Lucena Robles (Dr. Biología)) Investigador contratado CEIC- Internacional
- Francisco Javier Pavón Morón (Dr. Biología) Investigador contratado ISCIII-RTA
- Patricia Rivera González (Dra. Biología) Investigadora contratada PND
- Miguel Romero Cuevas (Dr. Biología y Lcdo. Bioquímica) Project Manager-Investigador contratado FP7-UE
- Antonia Serrano Criado (Dra. Biología) Investigadora contratada MICINN-PI
- Juan Suárez Pérez (Dr. Biología) Investigador principal ISCIII-SARA BORRELL

Licenciados (5)

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- María Jesús Luque Rojas (Lcda. Psicología) Investigadora predoctoral ISCIII-PREDOC
- Esther Martín García (Lcda. Periodismo) Técnico superior ISCIII-RTA
- Margarita Vida Botella (Lcdo. Biología) Técnico superior ISCIII-RTA

Diplomados (1)

- Monserrat Calado Romero (Diplomada Universitaria Enfermería) Investigadora PAIDI-CEIC

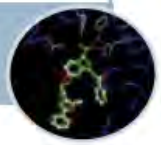
We are a research group on pharmacology and therapeutics, focused in lipid transmitters, and with a long experience in medicinal chemistry.

We have patented 14 molecules or models for major chronic diseases

12 years of experience colaborating with pharmaceutical industry and biotechs

THE RESEARCH GROUP "NEUROPSYCHOPHARMACOLOGY"

Modelos In silico
- Docking ligando-receptor
- Interacción de proteínas
- Dinámica Molecular
- Structure-Activity Relationships (SAR)



Modelos In vivo
- Ingesta y metabolismo
- Locomoción, ansiedad y depresión
- Condicionamientos, memoria, autoadministración
- *R. norvegicus*, *M. musculus*

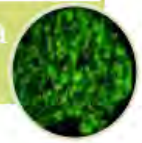
Líneas Celulares
- Insulinoma humano (INS-1E)
- Preadipocito humano
- Hepatocitos (HepG2)
- Islotes pancreáticos *R. norvegicus*
- Carcioma renal Hek293

Modelos Funcionales
- *M. musculus* KO
- PPAR-alfa
- IL-6
- CB1
- LPA-1

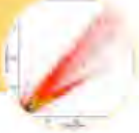
Biología Molecular
- PCR a tiempo real
- Espectrofotometría y luminómetría
- Electroforesis de ácidos nucléicos
- Análisis de imágenes
- ELISA

Bioquímica Proteómica
- Electroforesis proteínas
- Horno de hibridación
- Geles 2D
- Escáner 2D y Software análisis proteómico
- Citoquinas

Microscopía Histología
- Microscopía óptica y fluorescencia
- Analizador de imágenes
- Microtomo de precisión y Criostato
- Arrayer de tejidos
- Inmunohistoquímica



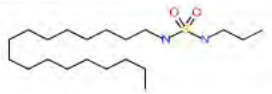
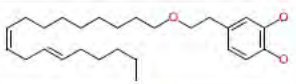
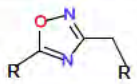
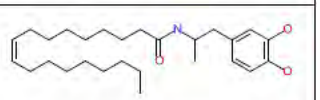
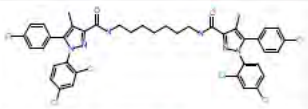
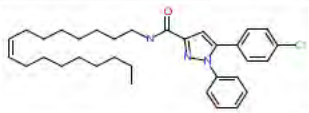
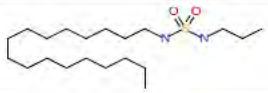
Citometría de flujo
- Citómetro Cyan 3 Láseres
- Robot DISIS
- Robot EPS



Pruebas clínicas
- Patología Dual PRISM
- Entrevista psiquiátrica DSM-IV
- Ansiedad y depresión Pfeiffer ACV-NIH, Barthel, Goldberg, Beck, GAD-7

THE RESEARCH GROUP "NEUROPSYCHOPHARMACOLOGY"

- i+d **APLICADA**: descubrimiento fármacos-patentes

2011	Uso de derivados de sulfamidas como neuroprotectores	P201130486	
2010	Éteres de Hidroxitirosol	PCT/ES2011/070880	
2010	Derivados de oxadiazol como fármacos moduladores del receptor para el péptido GLP-1	PCT/ES2011/070042	
2009	Derivados de amida de ácidos grasos con anfetaminas para el tratamiento de desordenes alimenticios	WO2011076966(A1)	
2009	Derivados de pirazol bivalentes como inhibidores de ingesta	WO2010128191(A1)	
2008	Derivados pirazólicos de amidas de ácidos grasos como activadores específicos de receptores PPAR-alfa, procedimiento de preparación y utilización	WO2009050318(A1)	
2006	Derivados acíclicos saturados e insaturados de cadena larga de sulfamidas como activadores específicos de receptores PPAR-alfa	WO2007085469(A1)	

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TARGET INDICATION: THE PROBLEM OF NASH

I. Non Alcoholic Steatohepatitis (NASH) is the most common chronic liver disease.

II. Multistep disease that progress from steatosis towards steatohepatitis, fibrosis, cirrhosis and liver cancer.

III. Its first step, Liver steatosis, has a high prevalence, ranging from Europe 28.04% (Europe), 19.24%, (East Asia) to 12.95%, in Middle East

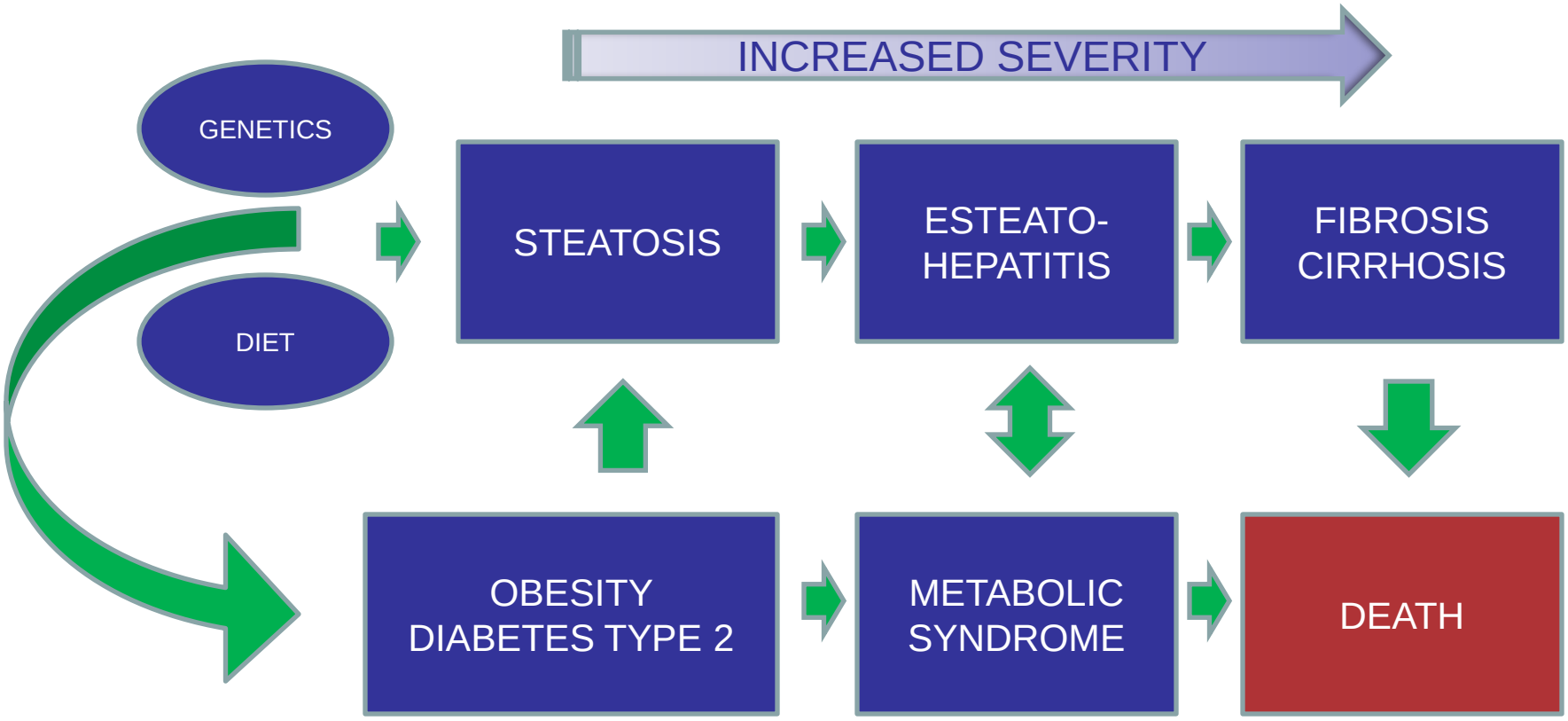
IV. Very high incidence in kids

V. There is a clear association in between obesity, diabetes type 2 and non-alcoholic steatohepatitis (NASH).

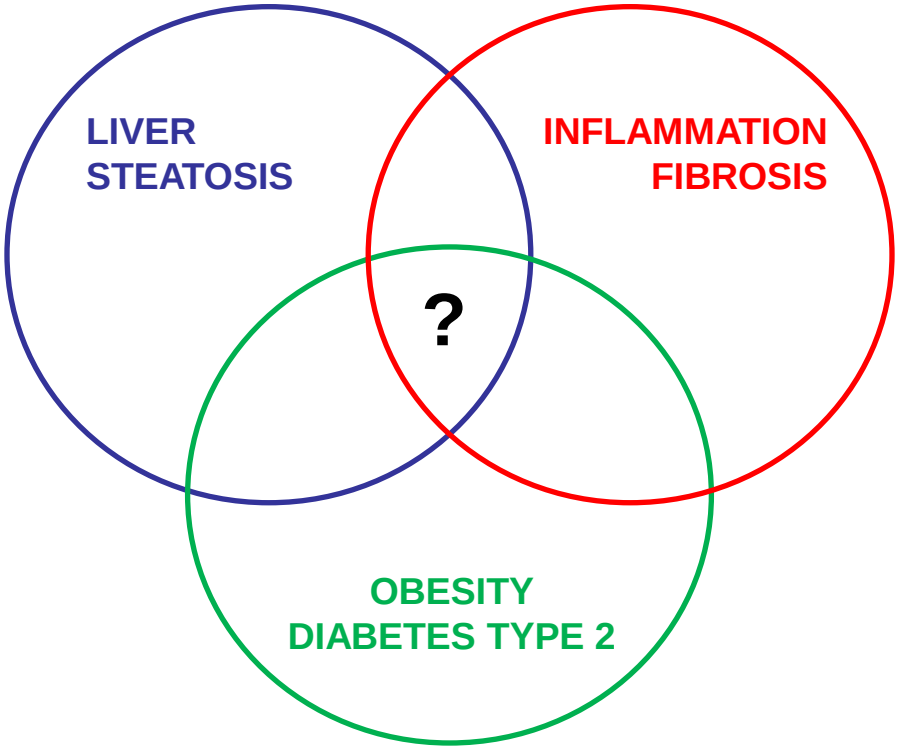
VI. Mortality is 10x normal population

VII. No effective therapy approved

TARGET INDICATION: THE PROBLEM OF NASH



TARGET INDICATION: THE PROBLEM OF NASH



THE IDEAL DRUG:

- **Reduction of liver steatosis and associated dyslipidemia**
- **Reduction of liver inflammation, fibrosis and prevention of cirrhosis**
- **Reduction of appetite and obesity**
- **Improvement of insulin resistance and better control of glucose homeostasis**

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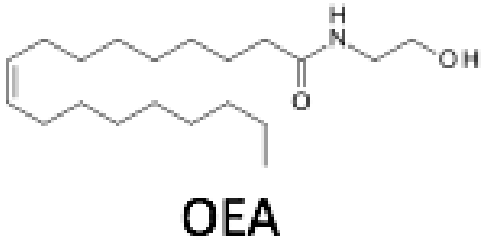
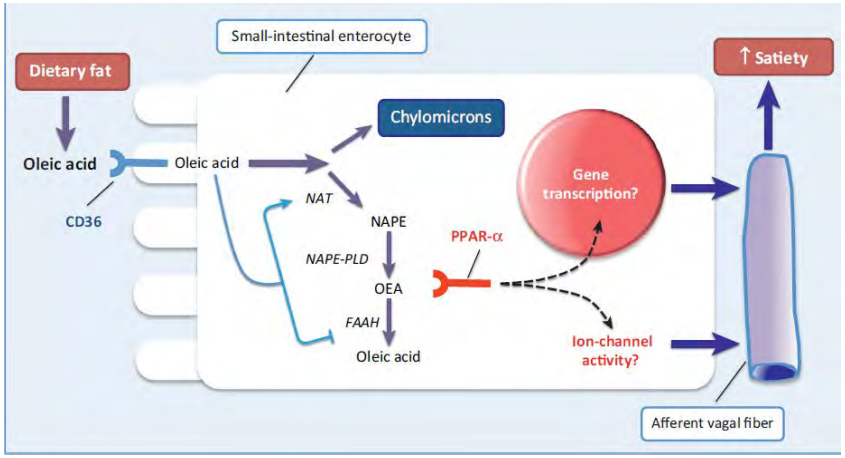
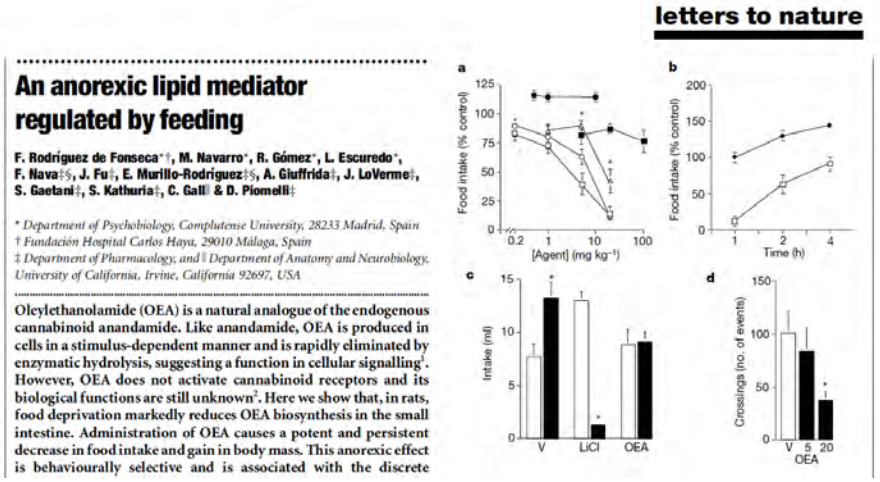
PHARMACOLOGY OF OLEOYLETHANOLAMIDE



The natural ligand for PPARα receptor





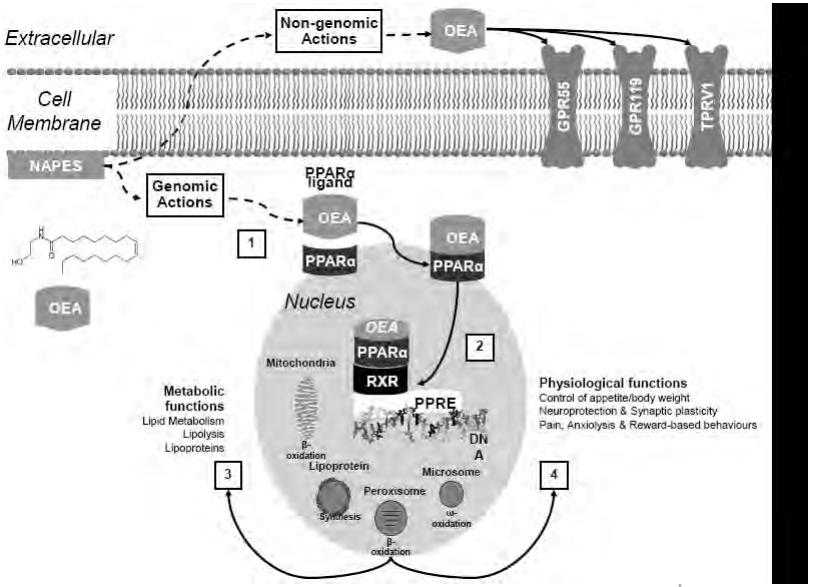
letters to nature

An anorexic lipid mediator regulated by feeding

F. Rodríguez de Fonseca*†, M. Navarro*, R. Gómez*, L. Escuredo*, F. Nava‡§, J. Fu‡, E. Murillo-Rodríguez‡§, A. Giuffrida‡, J. LoVerme‖, S. Gaetani‖, S. Kathuria‡, C. Gall‖ & D. Piomelli‡

* Department of Psychobiology, Complutense University, 28233 Madrid, Spain
† Fundación Hospital Carlos Haya, 29010 Málaga, Spain
‡ Department of Pharmacology, and ‖ Department of Anatomy and Neurobiology, University of California, Irvine, California 92697, USA

Oleylethanolamide (OEA) is a natural analogue of the endogenous cannabinoid anandamide. Like anandamide, OEA is produced in cells in a stimulus-dependent manner and is rapidly eliminated by enzymatic hydrolysis, suggesting a function in cellular signalling[1]. However, OEA does not activate cannabinoid receptors and its biological functions are still unknown[2]. Here we show that, in rats, food deprivation markedly reduces OEA biosynthesis in the small intestine. Administration of OEA causes a potent and persistent decrease in food intake and gain in body mass. This anorexic effect is behaviourally selective and is associated with the discrete



PHARMACOLOGY OF OLEOYLETHANOLAMIDE

I. Signal for fat sensing: inductor of satiety (Food and alcohol)

II. Activator of PPARα: activation of fat oxidation and inhibition of lipogenesis

III. Visceral analgesia and anti-inflammatory actions in brain and peripheral tissues

IV. Reduces fat depots in diet induced obesity, alcohol-induced liver steatosis and leptin deficiency

V. Antifibrotic

VI. Boosts antioxidant mechanisms in liver

PHARMACOLOGY OF OLEOYLETHANOLAMIDE

www.impactjournals.com/oncotarget/ Oncotarget, Vol. 6, No. 40

Oleoylethanolamide, an endogenous PPAR-α ligand, attenuates liver fibrosis targeting hepatic stellate cells

Ling Chen[1,4,*], Long Li[1,3,*], Junde Chen[2], Lei Li[1], Zihan Zheng[5], Jie Ren[1] and Yan Qiu[1]

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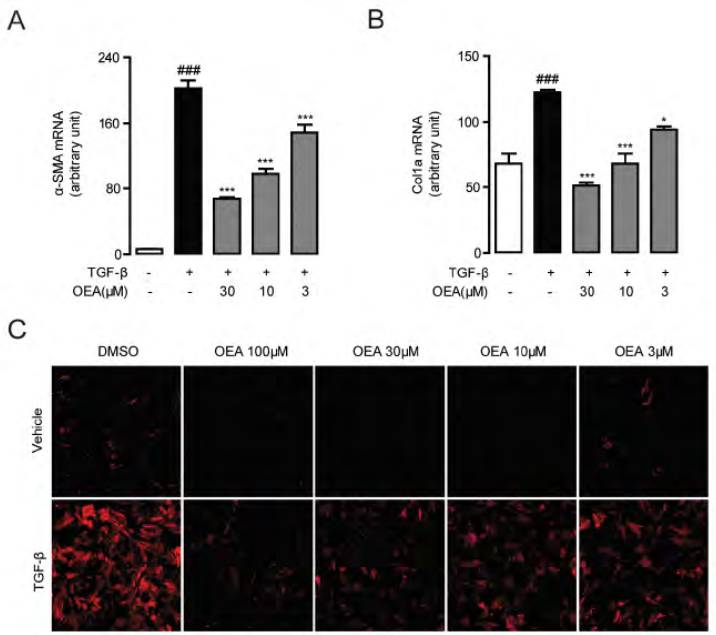
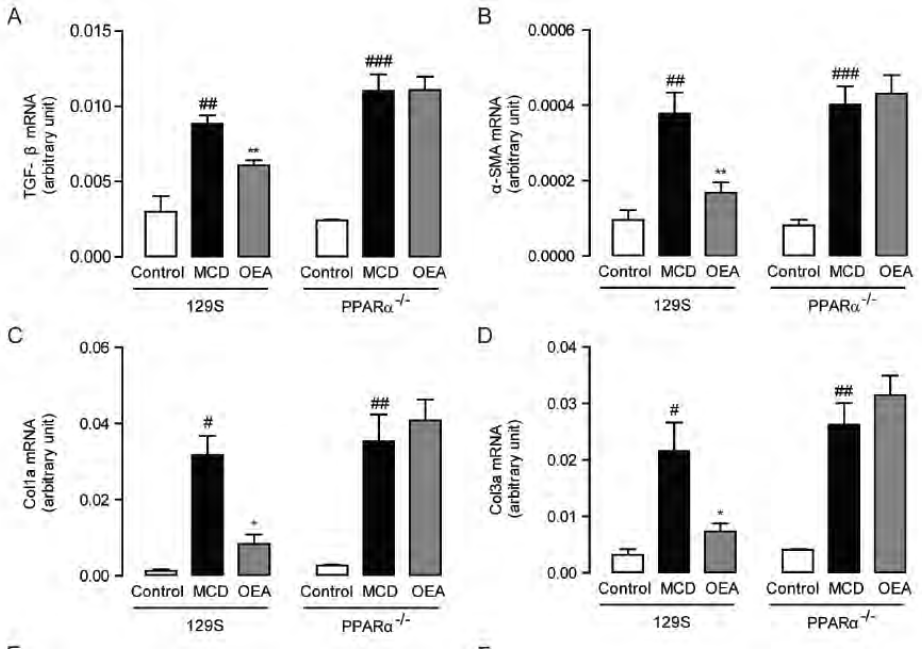
[2] Marine Biological Resource Comprehensive Utilization Engineering Research Center of the State Oceanic Administration, The Third Institute of Oceanography of the State Oceanic Administration, Xiamen, Fujian, China

[3] Xiamen Diabetes Institute, The First Affiliated Hospital of Xiamen University, Xiamen, Fujian, China

[4] Clinical Research Institute, The First Affiliated Hospital, University of South China, Hengyang, Hunan, China

[5] College of Arts and Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA





OEA is antifibrotic in a methionine-choline Deficiency model.
Activity of liver stellate cells boosted by TGF-β is attenuated by OEA through PPARα

OLEOYLETHANOLAMIDE-MODELLED DRUGS

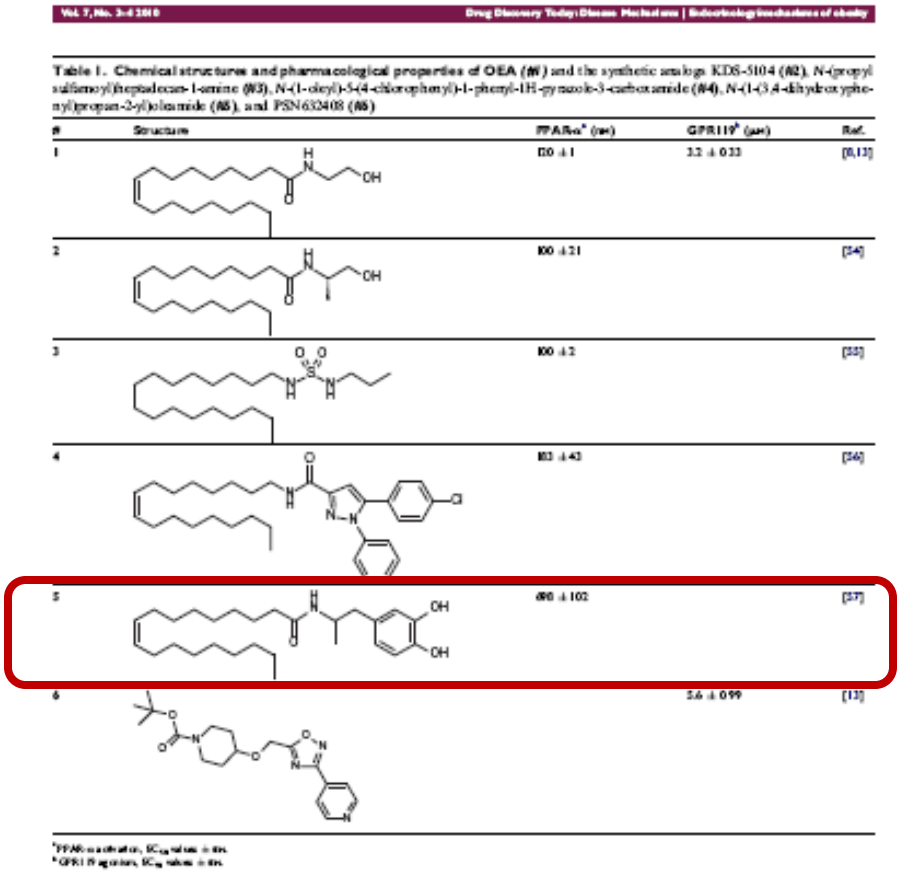


Table 1. Chemical structures and pharmacological properties of OEA (**#1**) and the synthetic analogs KDS-5104 (**#2**), *N*-(propyl sulfamoyl)heptadecan-1-amine (**#3**), *N*-(1-oleyl)-5-(4-chlorophenyl)-1-phenyl-1H-pyrazole-3-carboxamide (**#4**), *N*-(1-(3,4-dihydroxyphenyl)propan-2-yl)oleamide (**#5**), and PSN632408 (**#6**)

#	Structure	PPARα[a] (rel)	GPR119[b] (μM)	Ref.
1		100 ± 1	3.2 ± 0.33	[8,13]
2		100 ± 21		[34]
3		100 ± 2		[35]
4		103 ± 4.3		[36]
5		600 ± 102		[37]
6			5.6 ± 0.99	[13]

[a] PPARα activation, EC_{50} values in nM.
[b] GPR119 agonism, EC_{50} values in nM.

MEDICINAL CHEMISTRY PROGRAM DEVELOPED WITH THE GROUPS OF:

PILAR GOYA (IQM)

JESUS JOGLAR (CSIC)

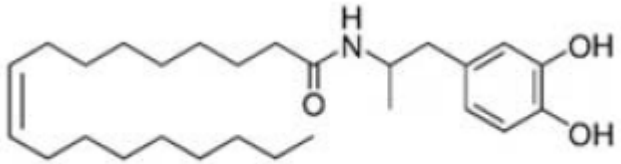
RAFAEL DE LA TORRE (IMIM)

PHARMACOLOGY: OLEOYLETHANOLAMIDE-MODELLED DRUG

Synthesis of Fatty Acid Amides of Catechol Metabolites that Exhibit Antiobesity Properties

Bruno Almeida,[a, b, f] Jesús Joglar,*[c] María Jesús Luque Rojas,[d] Juan Manuel Decara,[d] Francisco Javier Bermúdez-Silva,[d] Manuel Macías-González,[e, f] Montserrat Fitó,[f, g] Miguel Romero-Cuevas,[d] Magí Farré,[a, h] María Isabel Covas,[f, g] Fernando Rodríguez de Fonseca,[d, f] and Rafael de la Torre*[a, b, f]

In memory of Prof. Dr. José Manuel Concelión (Universidad de Oviedo)



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RESEARCH ARTICLE

SUBJECT COLLECTION: MODEL SYSTEMS IN DRUG DISCOVERY

Treatment with a novel oleic-acid–dihydroxyamphetamine conjugation ameliorates non-alcoholic fatty liver disease in obese Zucker rats

Juan M. Decara[1,*], Francisco Javier Pavón[1,*], Juan Suárez[1,2], Miguel Romero-Cuevas[1,2], Elena Baixeras[1], Mariam Vázquez[1], Patricia Rivera[1], Ana L. Gavito[1], Bruno Almeida[3,4], Jesús Joglar[5], Rafael de la Torre[2,3,4], Fernando Rodríguez de Fonseca[1,2,‡] and Antonia Serrano[1,2,‡]

1. **Analogue of oleoylethanolamide that reduces feeding**
2. **Small molecule that fits Lipinski's rules.**
3. **PPARα agonist**
4. **CB1 receptor antagonist**
5. **Potent antioxidant**
6. **No HERG interaction**
7. **Good profile on CYP tests: non toxic**
8. **Easy synthesis**

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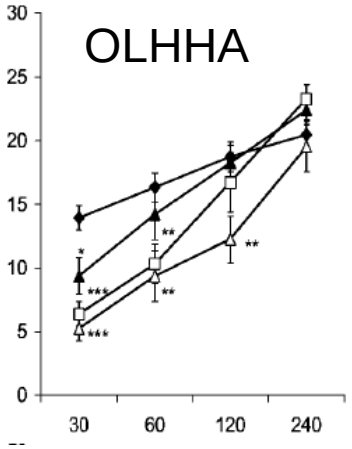
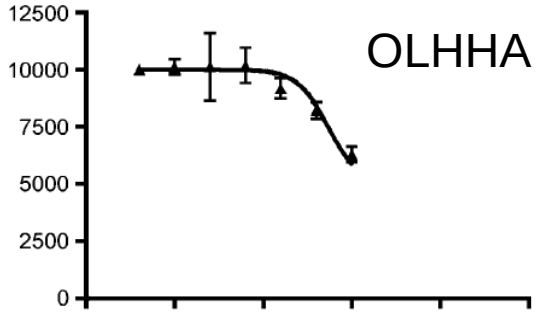
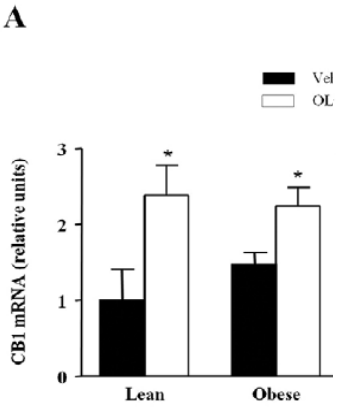
INNOVATIVE MECHANISM OF ACTION: triple action

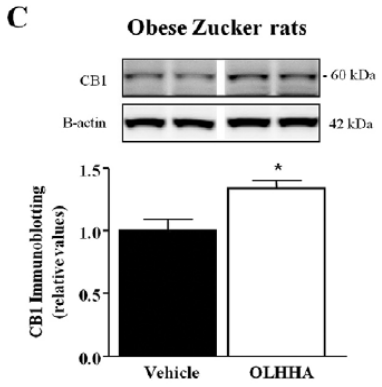


Reduction of feeding



Affinity for CB1





Upregulates Cb1 receptors

Table 1. Pharmacological properties of fatty acid amides.

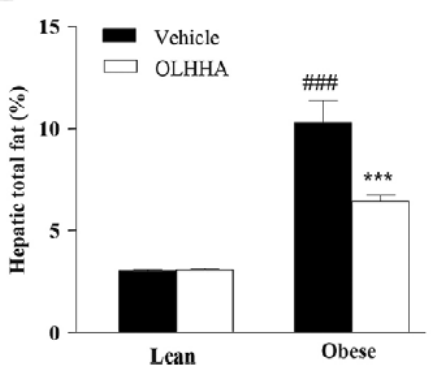
Compound	CB_1 K_i [M]	CB_1 pK_i	PPAR-α EC_{50} [nM][a]
SR141716	3.64×10^{-10}	9.44	–
anandamide	1.7×10^{-7}[b]	6.77	>10000
WIN55212-2	1.11×10^{-8}[b]	7.95	–
5	3.65×10^{-7}	6.44	698±102

Affinity for PPARα similar to that of fibrates.

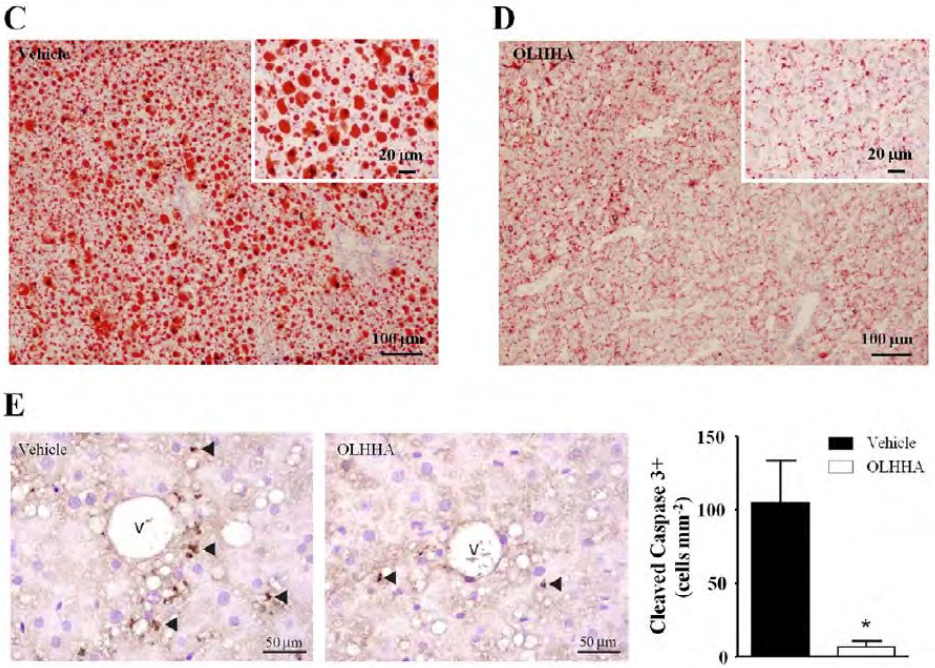
Antioxidant properties

INNOVATIVE MECHANISM OF ACTION

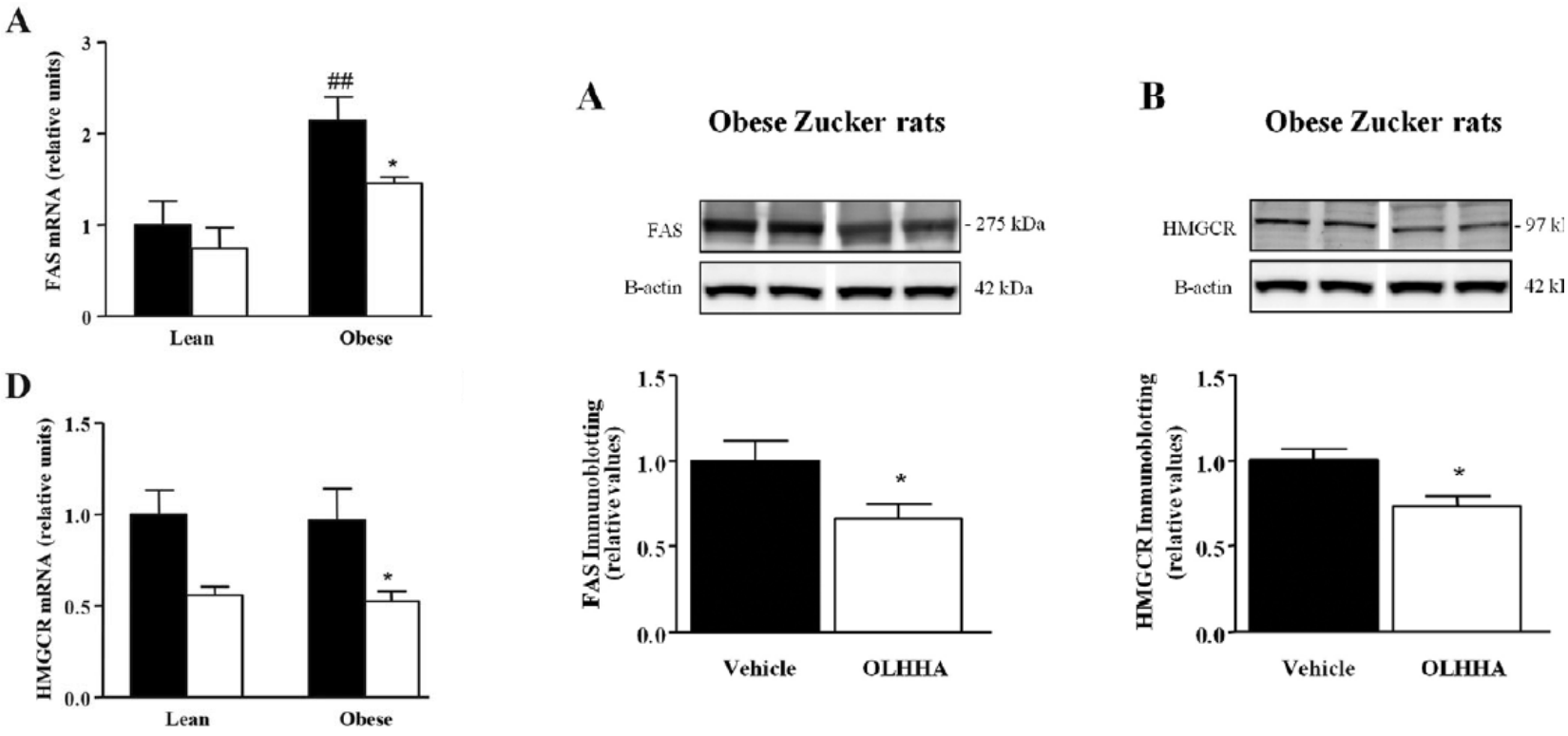
Plasma biochemical parameters	Obese Zucker	
	Vehicle	OLHHA
Uric acid (mg dl^{-1})	0.71±0.03	0.80±0.05
Urea (mg dl^{-1})	33.50±0.53	26.88±0.84***
Creatinine (mg dl^{-1})	0.89±0.01$^{\#\#}$	0.89±0.02
AST (U l^{-1})	175.63±10.93$^{\#\#\#}$	151.63±8.64
ALT (U l^{-1})	74.63±4.37$^{\#\#}$	61.88±2.71*
GGt (U l^{-1})	15.00±1.14$^{\#\#}$	17.75±0.60
Leptin (ng ml^{-1})	237.64±20.81$^{\#\#\#}$	188.39±10.05*
IL-6 (pg ml^{-1})	178.00±13.36	170.12±10.10
TNF-α (pg ml^{-1})	1.43±0.75	0.32±0.23
Glucose (mg dl^{-1})	173.00±5.42	192.63±16.22
Insulin (ng ml^{-1})	9.51±1.66$^{\#\#\#}$	6.22±0.79
Cholesterol (mg dl^{-1})	295.38±11.32$^{\#\#\#}$	269.75±21.28
HDL-C (mg dl^{-1})	35.00±2.24$^{\#}$	31.63±1.67
Triglycerides (mg dl^{-1})	573.88±70.87$^{\#\#\#}$	364.00±67.23*



1. Reduces fat depot in the liver
2. Reduces triglicerides, leptin and transaminases
3. Reduces apoptosis in the liver



INNOVATIVE MECHANISM OF ACTION



REDUCES LIPOGENIC ENZYMES IN LIVER

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DIFFERENTIAL FEATURES FACING THE MARKET

I. No effective therapy approved

II. Many phase 2 and 3 trials are underway. Drugs hoped to be effective are obeticholic acid, elafibranor, glucagon-like peptide-1 analogues and CCR2/5 inhibitors.

III. Elafibranor is the most advanced, followed by Obeticholic (Both Phase III)

IV. GLP-1 peptide agonists well positioned with many drugs in the market for diabetes (and obesity)

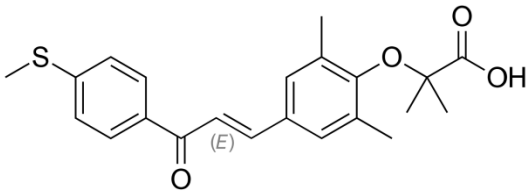
V. 7 clinical trials on course on GLP-1 analogues and NASH (the only type of approved drugs with patents active on the market of obesity)

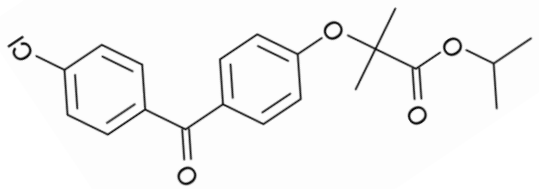
VI. All of them with potential carcinogenetic risks

DIFFERENTIAL FEATURES FACING THE MARKET

Elafibranor

Fibrate: a PPARα and δ receptor agonist
Reduces inflammation, steatosis and
Does not worsen fibrosis





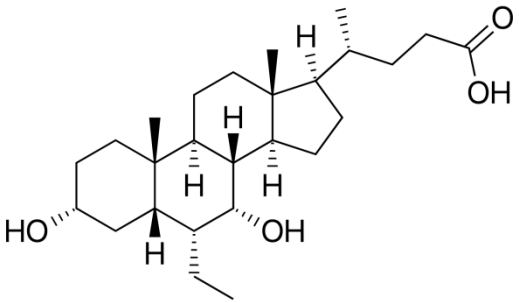
fenofibrate

Elafibranor
120 mg resolved NASH in larger proportions of patients than placebo based on the protocol definition (20% vs 11%; odds ratio ¼ 3.16; 95% confidence interval: 1.228.13; P ¼ .018)

DIFFERENTIAL FEATURES FACING THE MARKET

Intercept

Obeticholic Acid



A semisynthetic Farnesoid X Receptor agonist

Approved for Primary Cholangitis

Phase III clinical trial launched in 2016

GASTROENTEROLOGY 2013;145:574–582

CLINICAL—LIVER

Efficacy and Safety of the Farnesoid X Receptor Agonist Obeticholic Acid in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease

SUNDER MUDALIAR,[1] ROBERT R. HENRY,[1] ARUN J. SANYAL,[2] LINDA MORROW,[3] HANNS-ULRICH MARSCHALL,[4] MARK KIPNES,[5] LUCIANO ADORINI,[6] CATHI I. SCIACCA,[7] PAUL CLOPTON,[1] ERIN CASTELLOE,[7] PAUL DILLON,[8] MARK PRUZANSKI,[6] and DAVID SHAPIRO[7]

Obeticholic Acid

25 mg Phase II clinical trial. 25 or 50 mg OCA for 6 weeks was well tolerated, increased insulin sensitivity, and reduced markers of liver inflammation and fibrosis in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease. ClinicalTrials.gov, Number: NCT00501592.

DIFFERENTIAL FEATURES FACING THE MARKET

I. OLHHA is a multitarget drug, interacting with both PPARα and CB1 receptor and with reactive oxygen species production.

II. Synergic effects on obesity, dyslipidemia, fat depot and cell damage through multiple independent mechanisms

III. Unique profile as food intake inhibitor (Essential for prevention)

IV. No central effects observed

V. Good toxicity profile in vitro and in vivo

VI. Easy and scalable synthesis

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CURRENT STATUS OF DEVELOPMENT

I. Preclinical package being developed. Financial support for 2017-18 obtained through a ISCIII-DTS grant to IBIMA and IMIM teams

II. Two main preclinical analysis yet to be performed:

   I. Induction of fibrosis with thioacetamide

   II. Induction of fibrosis by high fat/high fructose diet

III. Backup molecule developed, protected and started to be tested (hydroxytyrosol linoleylether)

IV. DTS project included basic pharmacokinetics and toxicity in humans (Phase I)

V. Aim is to be ready for Phase II in 2018

VI. Additional studies on alcohol-induced liver disease on course

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IPR PROTECTION

1. Indication patent on steatosis for OLHHA:

National patent (priority date) – P201431739, 24 november, 2014.

International Patent (PCT) - PCT/ES2015/070848, 24 november, 2015.

International publication WO 2016/083646 A1, 2 june, 2016.

2. Hydroxtyrosol éter patent:

Granted in Spain: ES2384852 (B1) 2013-06-04

International application (WO2012080555 (A1)) and national phases in Europe EP2666761 (A4), and EEUU: US2013267588 (A1)

The Project development plans to to protect any chemical modification, formulation issues or use indication that might appear along the research course.

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PITFALLS AND RISKS TO BE CONSIDERED

I. Efficacy against liver fibrosis yet to be tested. However parent natural compound, Oleoylethanolamide, found to be effective on liver fibrosis

II. Pharmacokinetics studies in humans yet to be performed

   I. Metabolism might be different in humans

   II. Oral formulations yet to be developed. Previous IP developed for nanoemulsions for OEA

III. Competitors in phase III. Efficacy (partial) only demonstrated for elafibranor, but with limitations.

IV. The issue of carcinogenesis need to be addressed for long-term treatments (This constraint affects all Phase III studies).

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PARTNERING OPPORTUNITIES

I. Major metabolic disease without treatment: maximum interest for the main chronic liver disease.

II. Drugs being developed do not cover all the needs for solution. Good potential positioning in the market.

III. Market of alcohol-liver disease unexplored.

IV. We search a biotech/pharmaceutical company for co-development / licensing

V. Opportunities for additional IP development. Pipeline with three scaffolds active on the disease