Programa Cooperación Farma-Biotech

Neurociencias

LSD1

Inhibitors of Lysine specific demethylase 1, a novel target in neurodegenerative disease

ORYZON

Barcelona, 15 de febrero 2011

CataloniaBIO
Associació catalana d'empreses de biotecnologia

MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española

farmaindustria

Content

1. The Company

2. The Product

a) Therapeutic focus
b) Innovative mechanisms of action
c) Differential features facing the market
d) Current status of development
e) IPR protection
f) Pitfalls & Risks to be considered

3. Availability for cooperation


ORYZON
DIAGNOSTICS AND DRUGS FOR A BETTER WORLD
www.oryzon.com

THE COMPANY

Oryzon a R&D focused company

Oryzon is a BIOMARKER and TARGET DISCOVERY company with IVD & drug discovery programs for:

- oncology and
- neurodegenerative diseases

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Oryzon at a Glance

Oryzon is a BIOMARKER and TARGET DISCOVERY company with IVD & drug discovery programs for:

- **Oncology and**
- **Neurodegenerative diseases**

- Venture backed from 2003
- 22 M € fresh cash-in achieved in 2008
- 3 M € income in 2009
- International team of 60 scientist
- 2200 m^2 labs HQ in Cornellà (Barcelona, Spain)
- 2008: With the acquisition of Crystax we gained fragment-screening and crystallography capabilities and achieve one of the most completes platforms in Europe: from genomic/biomarker discovery till clinical candidates

A Risk Balanced Business Model

recurrent incomes to minimize the funds needed for R&D activities

upside in the longterm scenario

1. **Oryzon diagnostic platform** should become a recurrent source of revenues for the company, we expected to launch our two first products in the Spanish market in 2011

2. **Fee for service**. We can offer to the industry a unique suite of capabilities for their open-innovation needs ranging from target discovery, target validation, crystallography, fragment screening and medicinal chemistry to develop rapidly progressing and focused programs providing new drug candidates.

3. **Early Licensing-out and Alliances on third party programs.** Oryzon is able to identify relevant targets in diseases with unmeet clinical needs and to develop fast and competitive drug discovery / antibody programs.

4. **Internal / Proprietary R&D.** The company goal is to develop their own molecules until Phase I or Phase II

5. **Alliance Policy.** We are ready as well to partner earlier our internal discovery program focused on identifying epigenetic molecules in oncology and neurodegeneration if this provides a faster development and shortens T2M.

Alliances with the industry: Fee for Service

- **Revenue recurrence** Oryzon has a track record of continuous collaborations with Pharma, Biotech, Agrofood and chemical industry.


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THE PRODUCT

The product we offer

Oryzon has an innovative FIRST IN CLASS DRUG DISCOVERY PROGRAM in preclinical satge ready to partner for:

- oncology and / or
- neurodegenerative diseases

The main indications in CNS are

- Alzheimer's disease
- Parkinson's disease
- Huntington's disease

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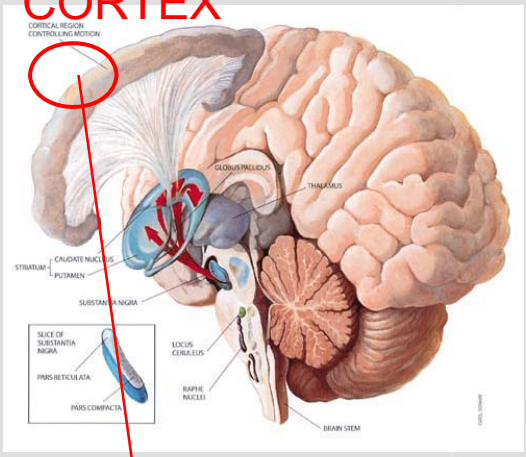
THE MECHANISM OF ACTION

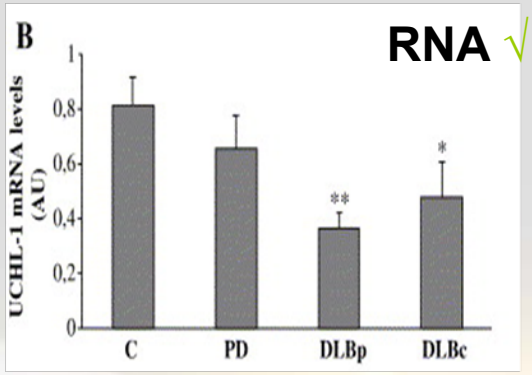
2005-2007 Identification of Biomarkers in CNS (DLB, PD &AD)

CORTEX

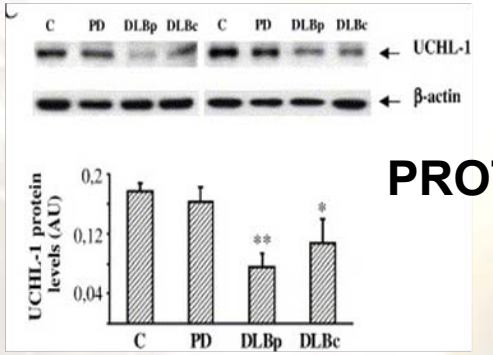


Several genes differentially expressed

Biomarker Validation



RNA √



PROTEIN √

GE Studies in postmortem affected brains: Oryzon

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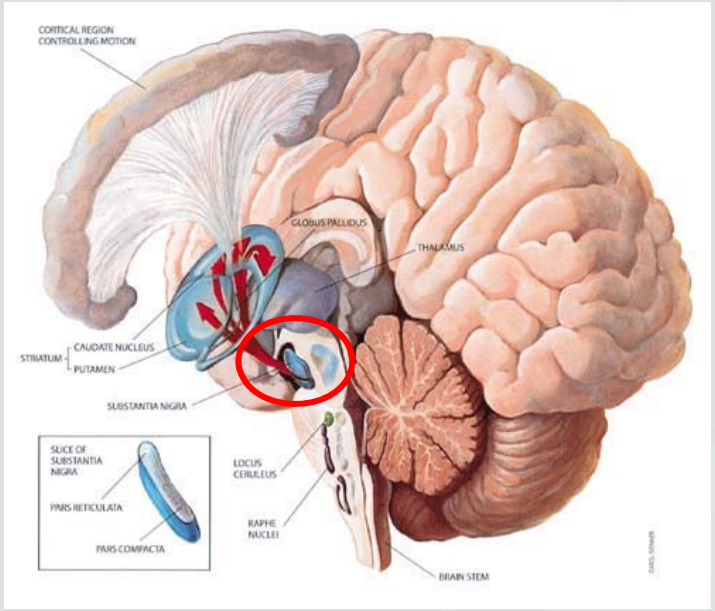
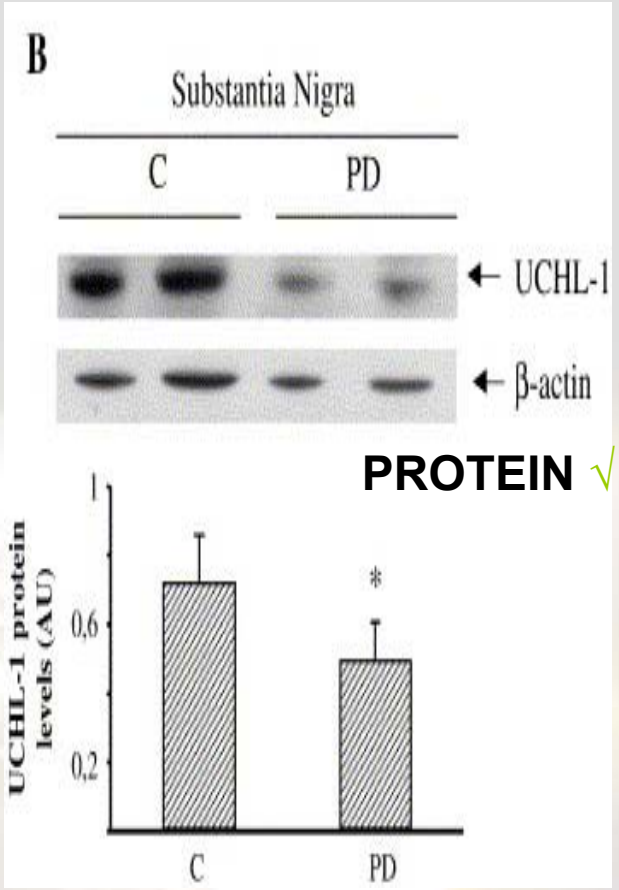
2005-2007 Identification of Biomarkers in CNS (DLB, PD &AD)



SUBSTANTIA NIGRA

GE Studies in postmortem affected brains: Oryzon



Evidence for a role of UCHL1 in neurodegenerative disease

- As a Biomarker:

  We (and others) have observed Reduced expression in several neurodegenerative disorders

  - Parkinson Disease
  - Dementia with Lewy Bodies
  - Alzheimer disease (Choi et al 2004)
  - Frontotemporal Dementia (Schweitzer et al 2006),

- Genetics/disease:

  Individuals carrying a mutant allele of UCHL1 (I93M) with 50% reduced enzymatic activity develop a familial version of DOPA responsive Parkinson's disease (PD), recent studies have shown classical Lewy pathology and cognitive defects in a deceased sibling of a family affected by the I93M UCHL1 mutation (Auburger et al., 2005).

Evidence for a role of UCHL1 in neurodegenerative disease

- Animal models:
  - Transgenic gad/gad mice (KO allele of UCHL1) expression the human UCHL1 I93M variant show loss of dopaminergic neurons in the substantia nigra and reduced dopamine content in the striatum at 20 weeks of age (Setsuie et al., 2007).
  - in the APP/PS1 mouse model of AD. UCHL1 has also been found to be down-regulated. Gong et al., 2006, reported that UCHL1, is required for normal synaptic and cognitive function, and that transfection of UCHL1 protein fused to the transduction domain of HIV-transactivator protein (TAT) restores normal enzymatic activity and synaptic function both in the mouse model and in hippocampal slices treated with oligomeric Abeta.
  - in the APP/PS1 mouse model of AD intraperitoneal injections of the **UCHL1-TAT** fusion protein improves the retention of contextual learning in APP/PS1 mice over time, and that the beneficial effect of the UCHL1 fusion protein is associated with restoration of normal levels of the PKA-regulatory subunit IIα, PKA activity, and CREB phosphorylation.

Pharmacological Relevance of UCHL1

- It is a neurodegenerative Biomarker: Reduced expression can be observed in PD, DLB, AD and FTD
- There is genetic link and a biochemical rationale: UCHL1 is an attractive target.
- But, What is the ultimate cause that downregulates UCHL1 gene expression in neurons of the affected areas?
- And, Could it become a Target?: Classically, a pharmacological target needs to be inhibited not enhanced.
- A possible way to enhance it is to inhibit the inhibitors (if any) of such a target.

UCHL1 and NRSF/REST expression levels are inversely correlated

Transcriptional regulation of the UCHL1 gene in human post-mortem brain samples of PD patients.

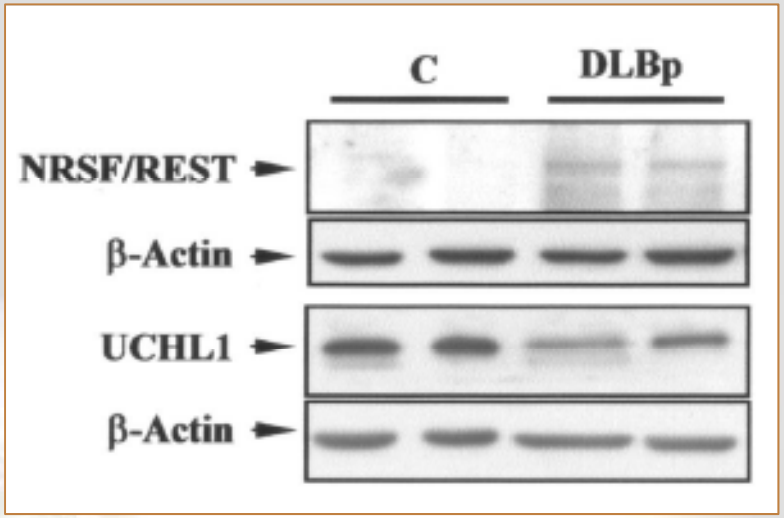
The UCHL1 gene contains a binding site for the silencer transcription factor (NRSF/REST) in the 5'UTR and 2 weak binding sites in the first intron.

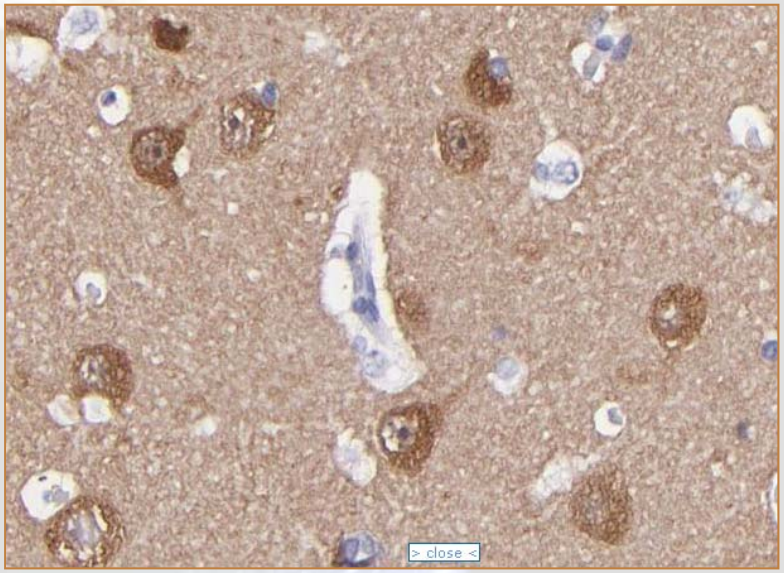
REST protein expression was inversely correlated to UCHL1 expression in the frontal cortex of DLBp patients.

The same tendency could be observed in GE data from AD brains (Blalock et al., 2004)

Inhibition of REST in undifferentiated ES cells leads to differentiation without the need for retinoic acid, and to expression of UCHL1.

Vice versa, REST impedes the expression of neuronal genes in non-neuronal cells.





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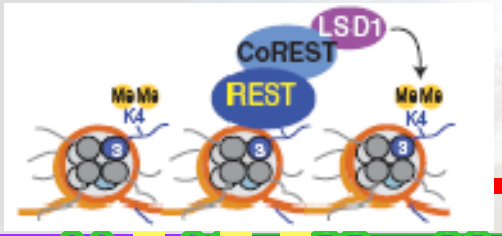
Deregulation of Proteasome is involved in several Neurodegenerative disorders

Parkinson
Dementia Lewy Bodies
Alzheimer

Huntington Disease
PolyQ diseases

UCHL1 ↔ **LSD1** ↔ **REST-HDAC**



• Low expression of UCHL-1 is probably caused by transcriptional inhibition driven by REST

key CNS genes — **DNA**

key CNS genes — **RNA**

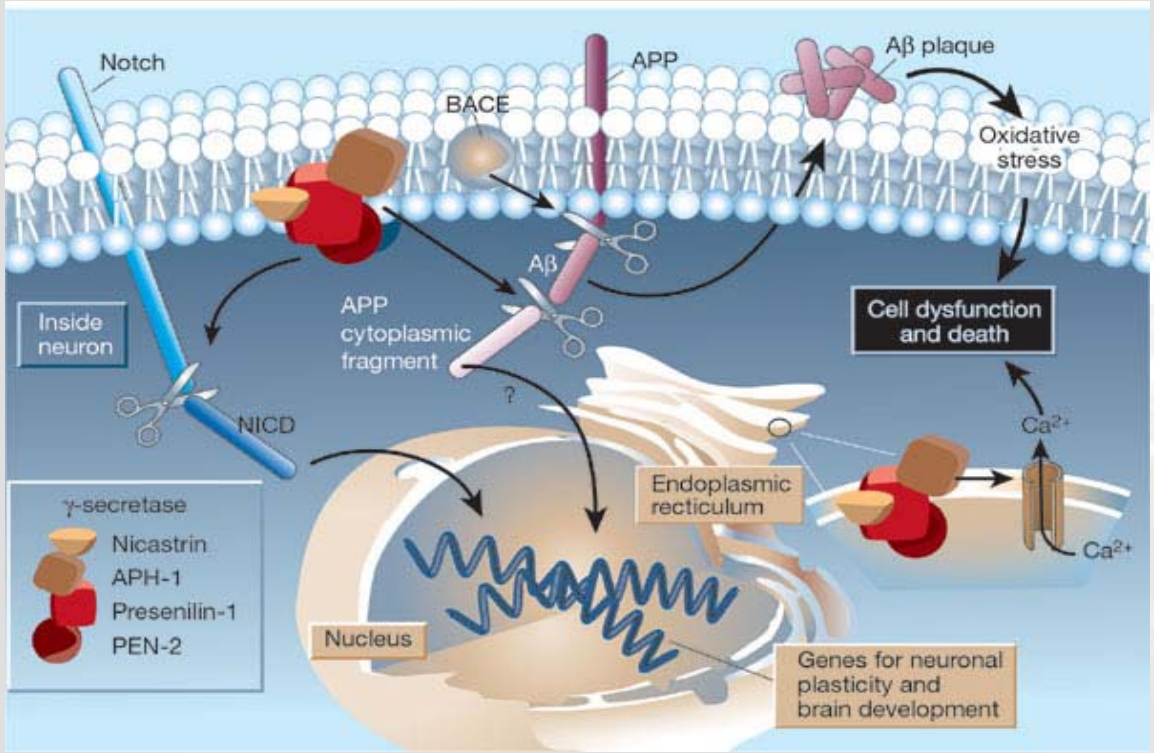
Key CNS PROTEIN

The REST complex comprises the chromatin modifying enzymes HDAC1, HDAC2, LSD1, CoREST and other components.

The transcriptional complex may be repressed directly or indirectly by altering the transcription, translation, sub-cellular localization or activity of one or several components of the complex.

Genetic link of LSD1 to AD in *C.elegans*

In *C.elegans*, loss of function mutants of sel-12, the worm homologue of PS1, a gene coding for a component of the g-secretase complex, can be suppressed by mutations of spr-5, the worm LSD1, through activation of hop-1 (PS2 homologue) transcription.



PS1 *sel-12* *egg laying defect*

PS2 *Hop-1*

Notch *Lin12*

Target genes

REST
COREST
LSD1

Spr-3/4
Spr-1
Spr-5

LSD1 A New Target for Oncology and Neurodegeneration

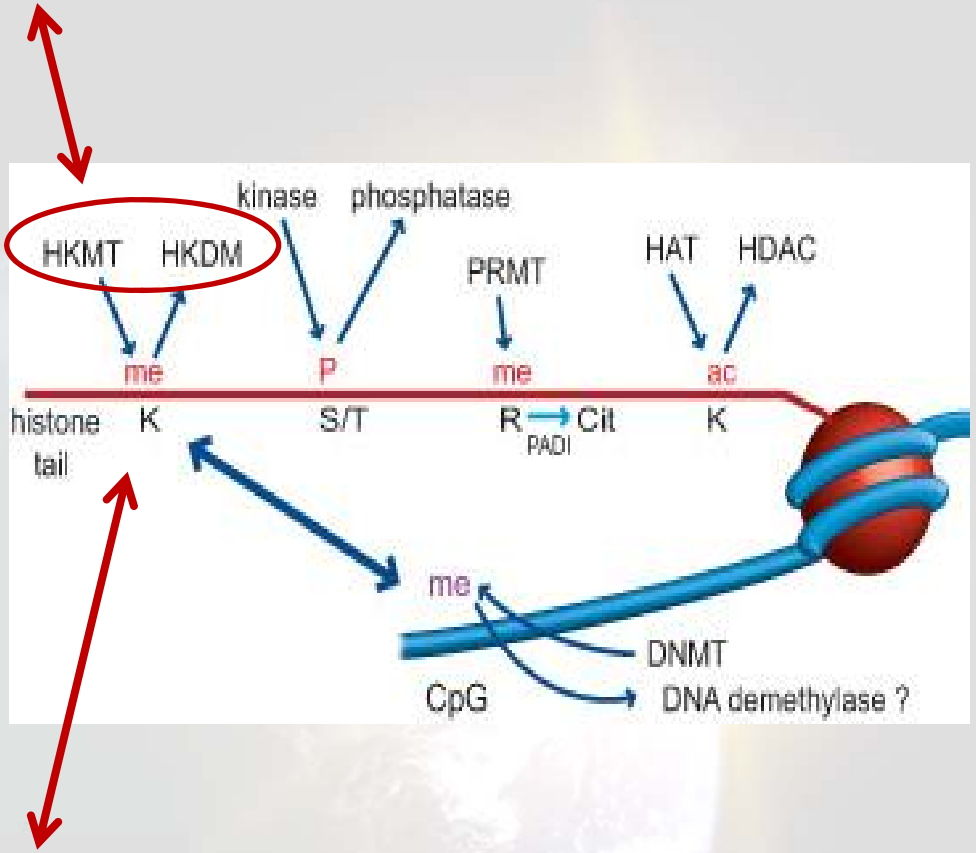
Histone lysine methyl transferases and histone lysine demethylases are involved in histone lysine modifications.

Lysine Specific Demethylase-1: *LSD1* (Shi *et al*. (2004) *Cell* 119:941) is involved in this crucial histone modification.

Increasing evidence suggests that LSD1 is a highly interesting biologically **relevant target in cancer,** which is complemented by additional evidences suggesting a key role in other fields

LSD1 interacts with key proteins in **neurodegenerative diseases**

LSD1 plays an important role in the **viral-host interaction biology**



Oryzon's epigenetic program is focused on inhibition of LSD1

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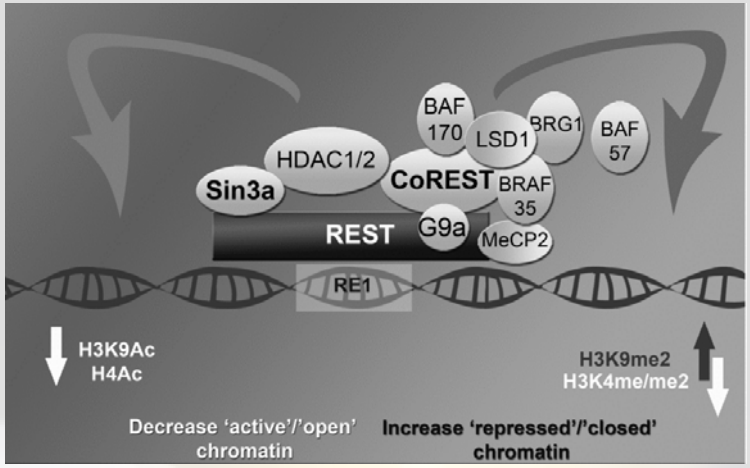
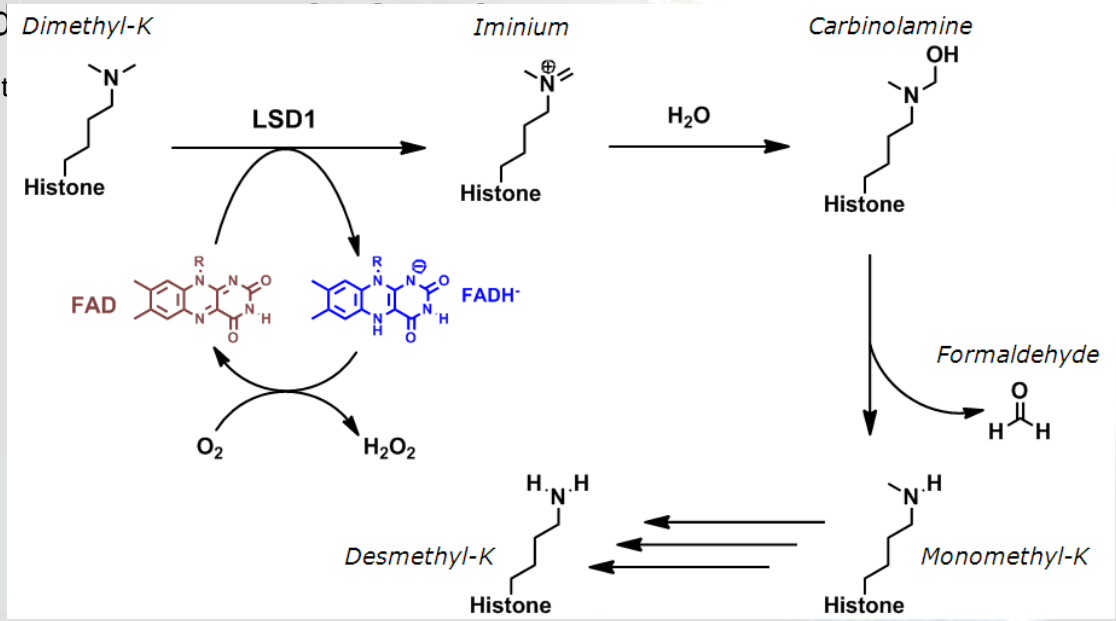
LSD1 Basics

LSD1 is a component of a number of different protein complexes which typically include REST, CoREST, HDAC 1/2 and are involved in regulation of gene expression



Biochemical Society Transactions (2009) 37, 1270-1275
Angela Bithell, Rory Johnson and Noel J. Buckley

LSD1 catalyzes demethylation H3K4me2 and H3K4me1 which is associated with **transcriptional repression** and with demethylation of H3K9me2 and H3K9me1 which is associated **activation** (Metzger et



Histone Lysine Methylation: Neuroblastoma

Target validation experiment of LSD1 inhibitors on tumor cell lines

Chromatin-modifying enzyme lysine-specific demethylase 1 (LSD1) is involved in maintaining the undifferentiated, malignant phenotype of neuroblastoma cells.

- **Inhibition of LSD1 reprograms the transcriptome of neuroblastoma cells and**
- **inhibits neuroblastoma xenograft growth.**

Published Online First on February 17, 2009 as 10.1158/0008-5472.CAN-08-1735

Research Article

Lysine-Specific Demethylase 1 Is Strongly Expressed in Poorly Differentiated Neuroblastoma: Implications for Therapy

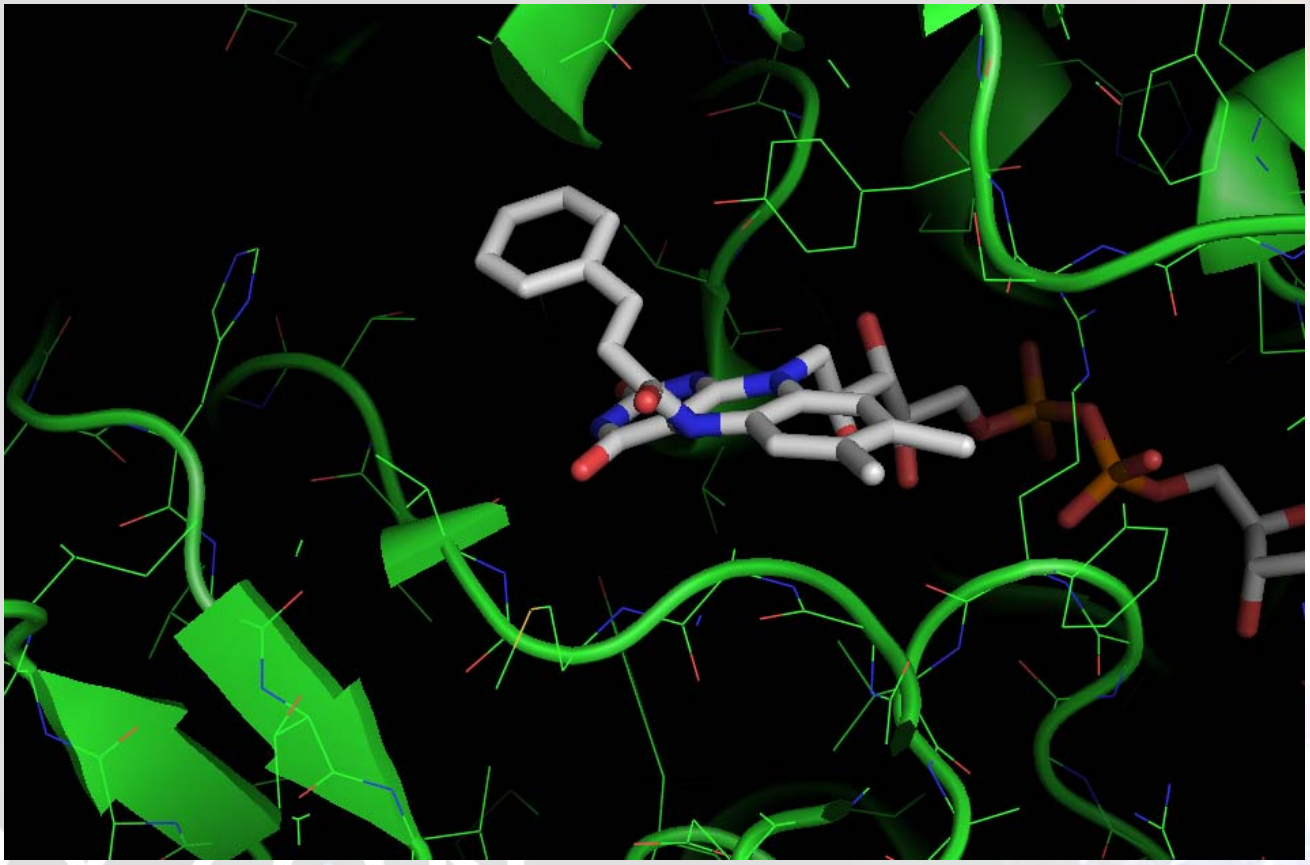
Johannes H. Schulte,[1] Soyoung Lim,[3] Alexander Schramm,[1] Nicolaus Friedrichs,[3] Jan Koster,[4] Rogier Versteeg,[4] Ingrid Ora,[4,5] Kristian Pajtler,[1] Ludger Klein-Hitpass,[2] Steffi Kuhfittig-Kulle,[1] Eric Metzger,[6] Roland Schüle,[6] Angelika Eggert,[1] Reinhard Buettner,[3] and Jutta Kirfel[3]

[1]Department of Paediatric Oncology and Hematology, University Children's Hospital Essen; [2]Institute of Cell Biology, University Hospital of Essen, Essen, Germany; [3]Institute of Pathology, University of Bonn, Bonn, Germany; [4]Department of Human Genetics, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands; [5]Department of Pediatric Oncology and Hematology, Lund University Hospital, Lund, Sweden; [6]Center for Clinical Research, Freiburg University Medical Center, Freiburg, Germany

LSD1 Inhibitors: + 600 cpds designed and tested

LSD1 Program. Starting Point.

LSD1 shares a high degree of homology with a family of flavin adenine dinucleotide (FAD)-dependent amine oxidases: certain inhibitors of monoamine a oxidases (MAOs) as TCPA are also capable of weakly inhibiting LSD1

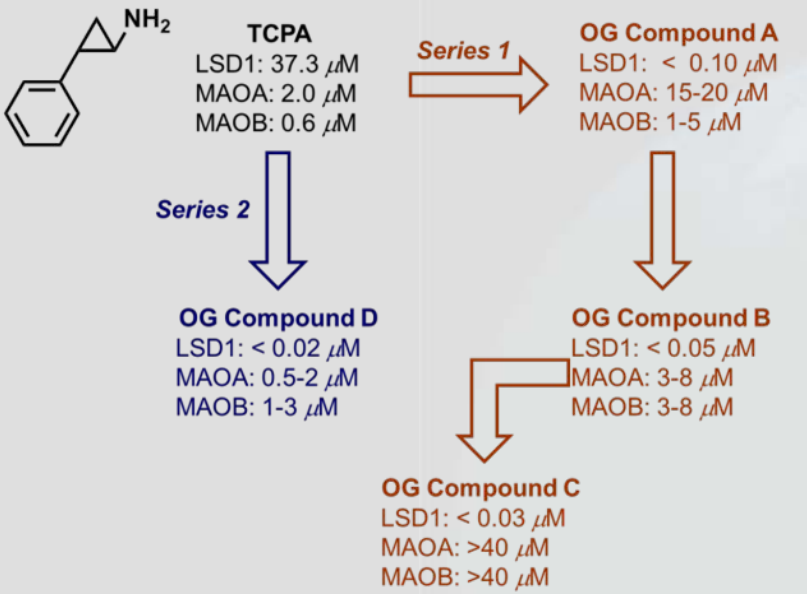


Biochem. Biophys. Res. Commun. 2008, *366*, 15–22

LSD1 Inhibitors: + 600 cpds designed and tested

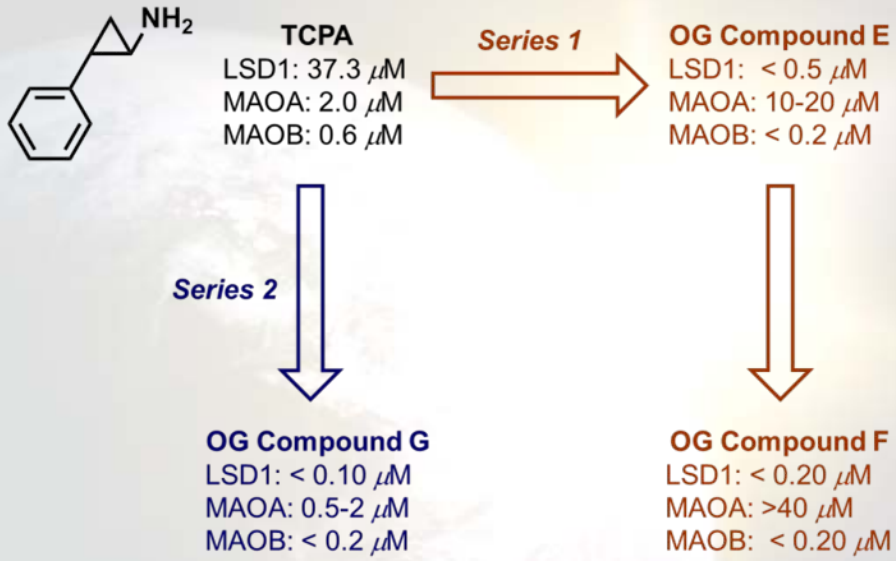
LSD1 shares a high degree of homolgy with a family of flavin adenine dinucleotide (FAD)-dependent amine oxidases: certain inhibitors of monoamine oxidases (MAOs) are also capable of inhibiting LSD1

SELECTIVE Program (ONCO & CNS)



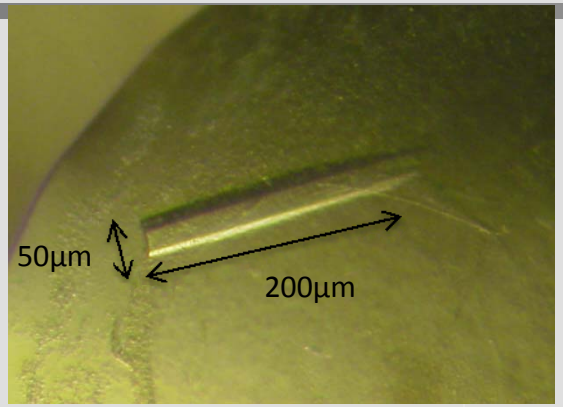
We have several leads with potency < 10 nM

DUAL Program (only CNS)



We have several leads with potency of around 50 nM and good selectivity against MAOA

LSD1 Co-crystals With Selective Inhibitor OG232



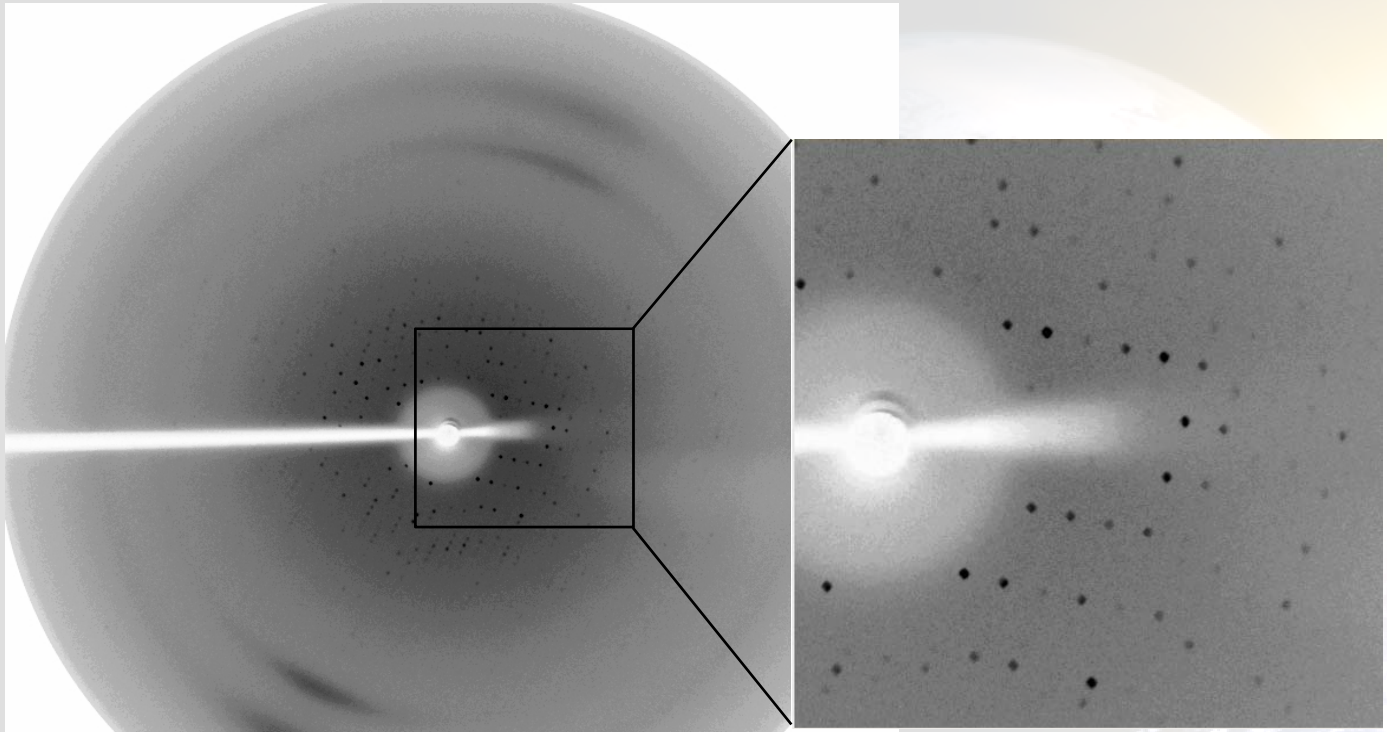
X-ray diffraction:

- Maximum resolution: 4,5-5Å
- Diffraction spots well defined
- Preliminary data:

  space group: P3 (or related)

  unit cell: a=185Å, b=185Å, c=108Å, α=ß=90, γ=120º

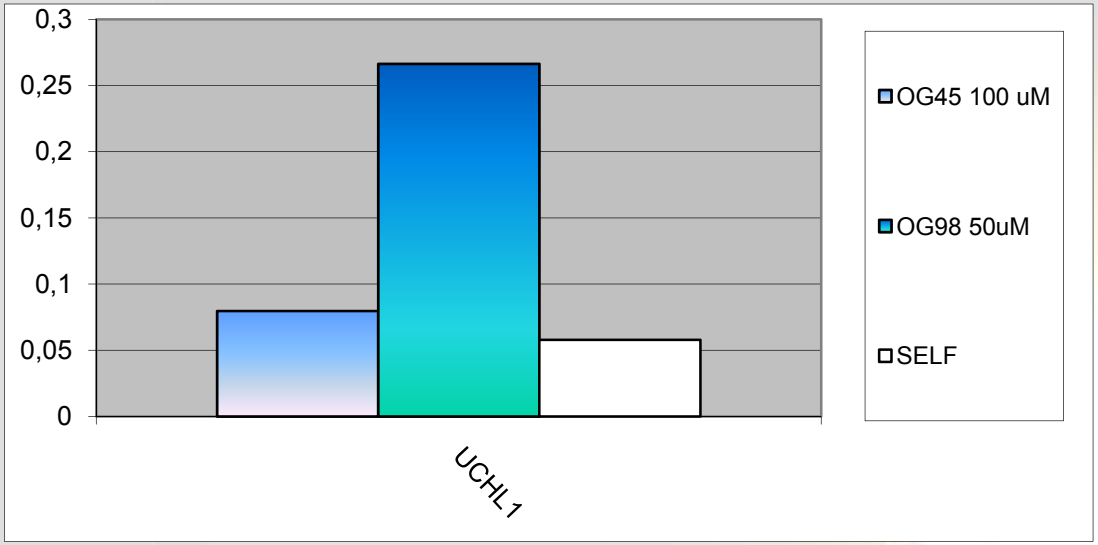
  (the same unit cell described by Stavropoulus et al., 2006, Chen et al., 2006 and Mimasu *et al*, 2008)



We can now OPTIMIZE our leads using crystallographic data

Inhibition of LSD1 Upregulates UCHL1 Expression

Influence of LSD1 inhibition on the expression of the original biomarker UCHL1
A small but significant increase is detected in cells treated with potent LSD1i.
(note: initial levels of UCHL1 are high in SH-SY5Y)



SH-SY5Y UCHL1

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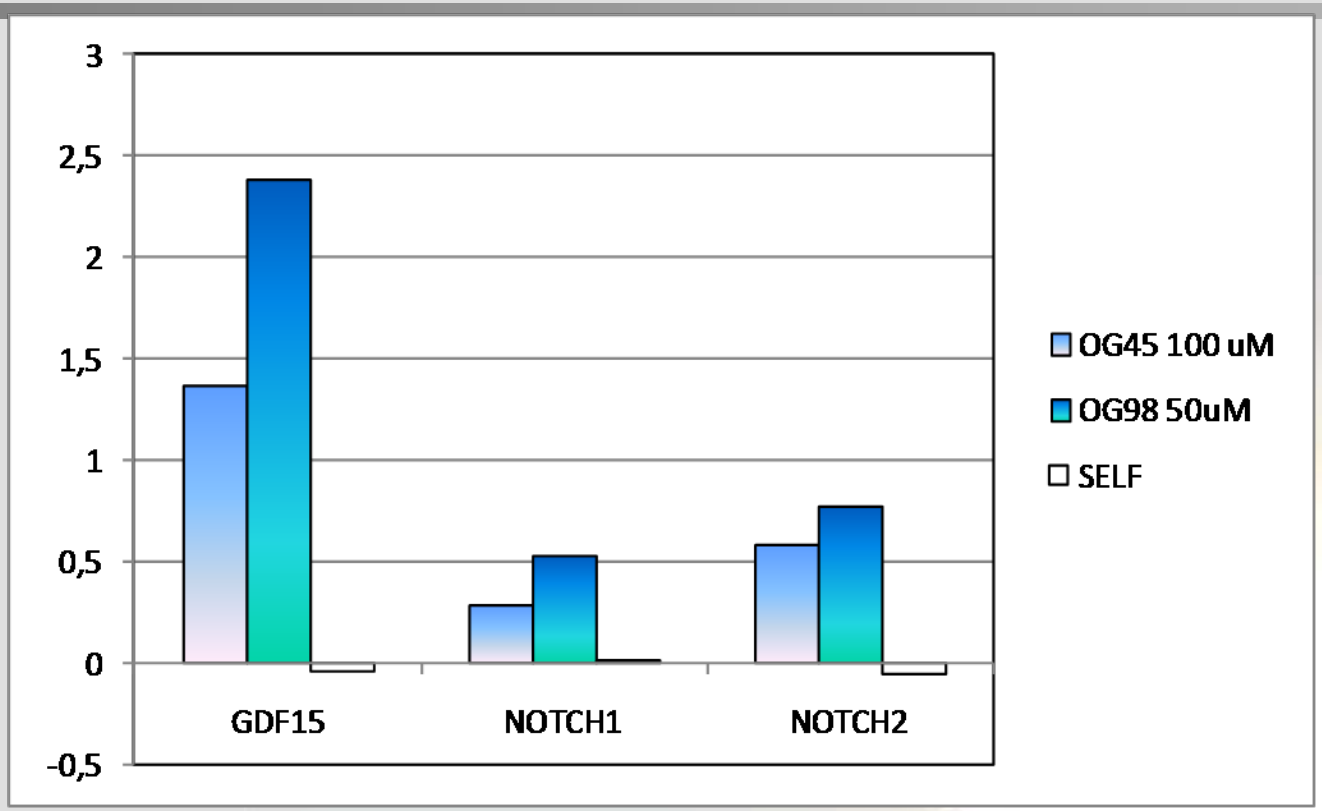
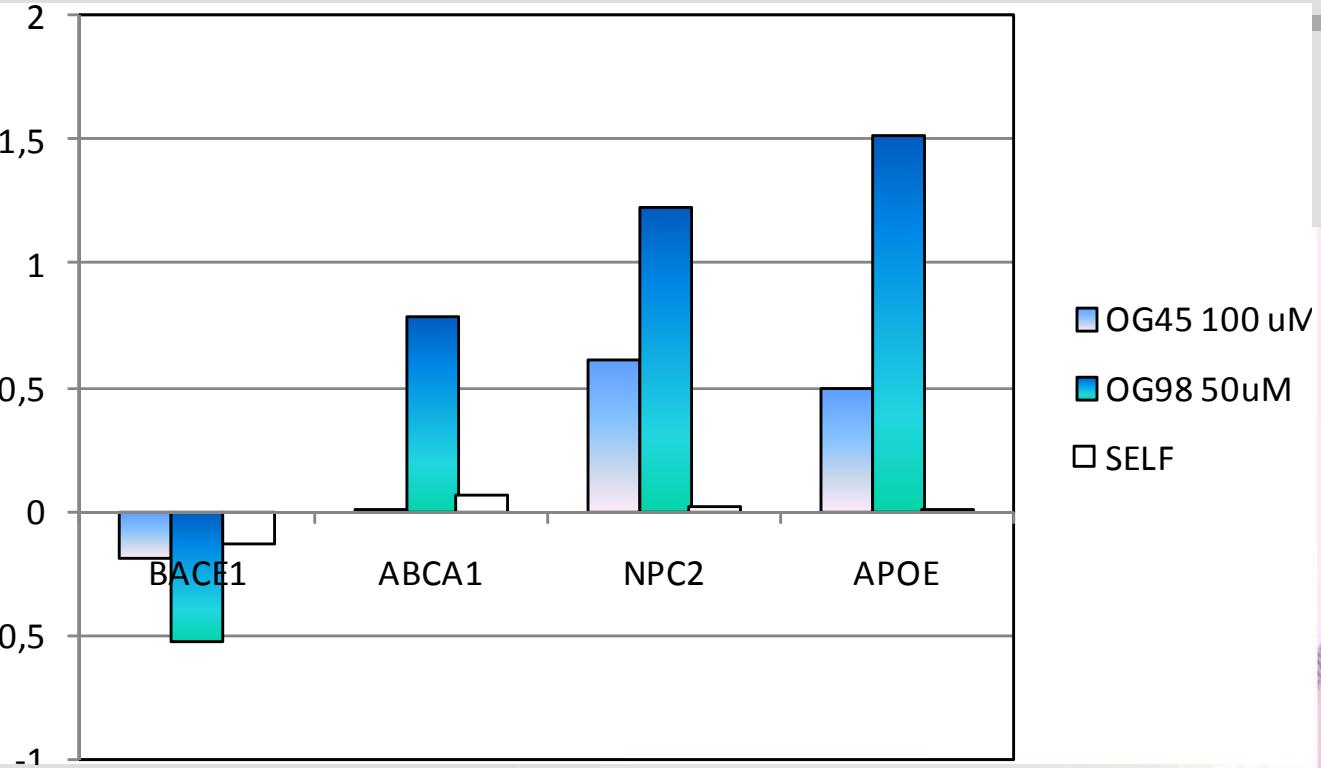
Pharmacological inhibition of LSD1 leads to upregulation of GDF15 and Notch expression

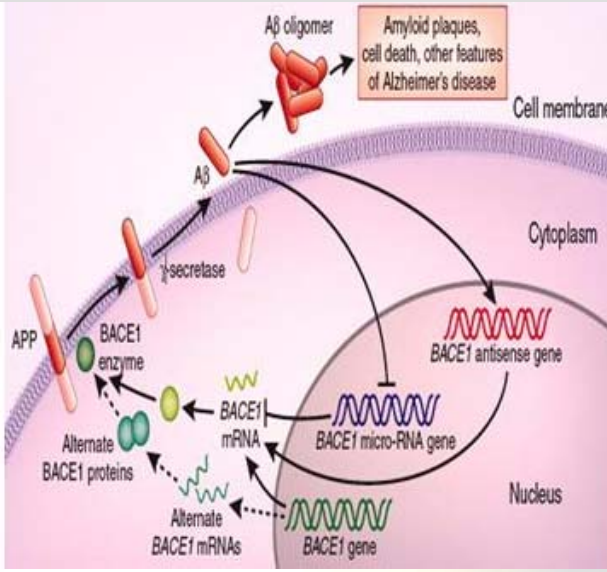


GDF15, a member of the TGF-beta superfamily, is a trophic factor for nigral dopamine neurons, both in vitro and in vivo. Specifically, GDF-15 promotes survival and differentiation of embryonic rat dopaminer-gic neurons, but not of other neuron populations, with the exception of serotonergic raphe neurons

Notch is a p53 target with a role in human tumor suppression through negative regulation of Rho effectors. Treatment of SH-SY5Y cells with ATRA also leads to Notch induction, and to differentiation of SH-SY5Y cells to cells with apparent neuronal morphology with long, extensively branched neurites.

Pharmacological inhibition of LSD1 leads to down-regulation of BACE and up-regulation of genes involved in cholesterol transport





BACE is a key enzyme in the formation of amyloid plaques

ABCA1 is a cholesterol and phospholipid transporter. Loss of ABCA1 impairs apoE lipidation and promotes amyloid deposition in AD mouse models. Selective overexpression of ABCA1 increases apoE lipidation in the central nervous system (CNS) and eliminates the formation of amyloid plaques in vivo.

APOE Apolipoprotein E-mimetics inhibit neurodegeneration and restore cognitive functions in a transgenic Drosophila model of Alzheimer's disease.

NPC2 is a cholesterol-binding lysosomal proteins required for export of lipoprotein-derived cholesterol from lysosomes

Genome-wide MoA studies of *in vitro* pharmacological inhibition of LSD1

As a conclusion, pharmacological inhibition of LSD1 leads to:

- Activation of neurotrophic factors and genes involved in neurogenesis
- Activation of genes involved in cholesterol / lipoprotein transport
- Reduction of BACE activity

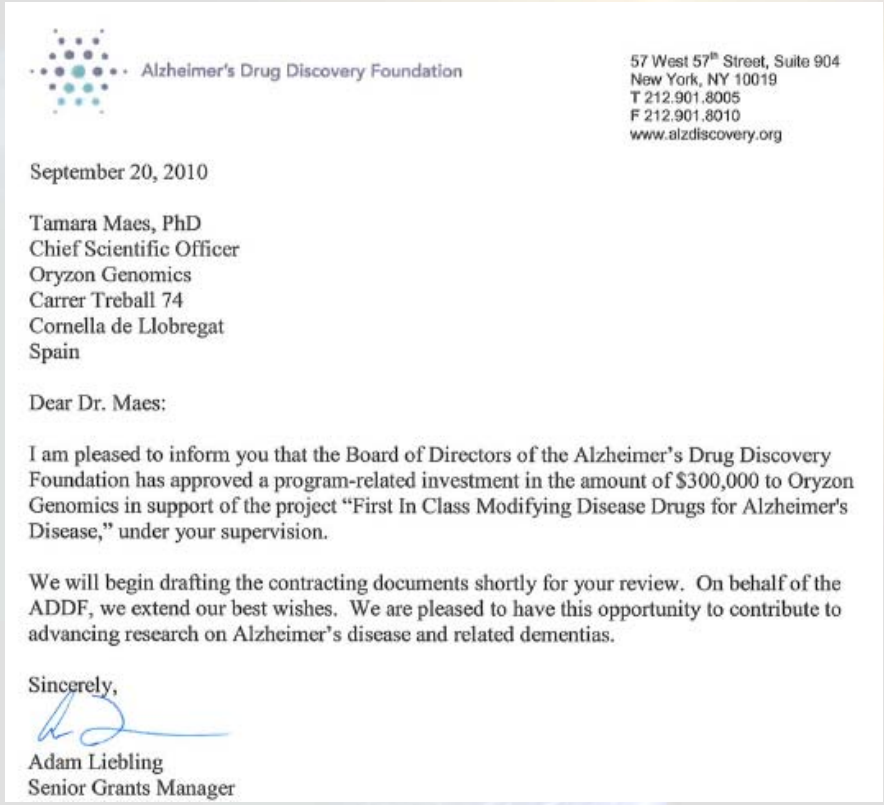
The GE changes observed after small molecule inhibition indicate a potential for the use of LSD1i or MAO-B/LSD1i for the treatment of neurodegenerative disease.

LSD1approach is recognized as an innovative CNS Program

ORYZON is the First Spanish company to have received an award from The Alzheimer's Drug Discovery Foundation.

The Alzheimer's Drug Discovery Foundation, a public charity to support the advancement of drugs to prevent, treat, and cure Alzheimer's disease, has a strategy of venture philanthropy based on the idea that our research grant recipients are engaged in projects that are potentially viable in the marketplace with a possible return on investment.
As of the end of 2008, ADDF has invested over $8 million in 35 biotechnology companies which have received follow-on commitments of over $1 billion

- Other 2010 Awardees
  - Columbia University, New York, NY Mitochondria-associated membranes in Alzheimer disease: a new target for drug discovery. Duration: 2010
  - Signum Biosciences, Inc. Phosphoprotein phosphatase 2A (PP2A): A novel therapeutic target for Alzheimer's disease. Duration: 2010
  - VIB, Zwijnaarde Drug Discovery for Progranulin-Mediated Frontotemporal Lobar Degeneration. Duration: 2010
  - Columbia University, New York, NY Tau Clearance by Autophagy. Duration: 2010
  - University of Rochester, Rochester, NY Early toxicology and ADME studies with our lead RAGE inhibitors. Duration: 2010



Alzheimer's Drug Discovery Foundation

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September 20, 2010

Tamara Maes, PhD
Chief Scientific Officer
Oryzon Genomics
Carrer Treball 74
Cornella de Llobregat
Spain

Dear Dr. Maes:

I am pleased to inform you that the Board of Directors of the Alzheimer's Drug Discovery Foundation has approved a program-related investment in the amount of $300,000 to Oryzon Genomics in support of the project "First In Class Modifying Disease Drugs for Alzheimer's Disease," under your supervision.

We will begin drafting the contracting documents shortly for your review. On behalf of the ADDF, we extend our best wishes. We are pleased to have this opportunity to contribute to advancing research on Alzheimer's disease and related dementias.

Sincerely,

Adam Liebling
Senior Grants Manager

More Advanced Program: Dual MAO-B/LSD1 Inhibitors

Advanced Lead Compound Properties

Dual Lead and followers

Ki (LSD1) = 15 nM
Ki (MAO-B) = 130 nM
MAO-A Ki > 5 uM

- Good pharmaceutical properties
  - Solid with satisfactory melting point
  - Possibility of salt formation
- Good *in vitro* ADMET data
  - No significant CYP inhibition: IC_{50} > 10 uM
  - Stable in human and mouse liver microsomes ($T_{1/2}$ > 1 h)
- No hERG issues
- No mutagenic potential: Clean in the Ames test
- Orally bioavailable with good half-life in mice
- Large safety window following per oral administration to mice
- Highly potent for LSD1 (low nanomolar)
- MAO-B potency equivalent to rasagiline/selegiline with the added benefit of LSD1 inhibition
- Improved selectivity over MAO-A
- Crosses the BBB (Haloperidol test PoC)
- Good Brain / Plasma ratio in mice

LSD1 Inhibitors: + 600 cpds designed and tested

DUAL Program (NEURO, Huntington, Parkinson and Alzheimer diseases)

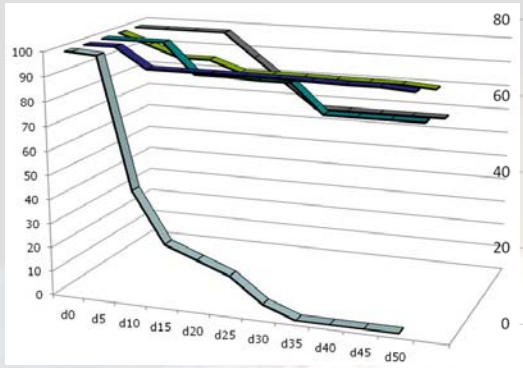
In Vivo PoC

In drosophila:
The compounds are able to rescue eye phenotypes and lethality in HD flies more effectively than SAHA.

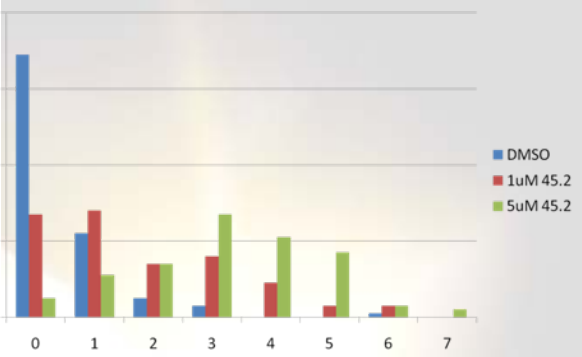
In mice:
The compounds are orally available and cross the BBB. The LSD1/MAO-B dual compounds have **demonstrated an efficacy in the mouse haloperidol-induced catalepsy model for PD**.

In the Huntington transgenic mice model, R6/2, treatments with one of our dual lead compounds increases time of survival and improves partially some tested HD cognitive and motor parameters

Tg -Drosophila survival



Tg -Drosophila eye phenotype recovery



Interested in discussing positive mouse model results in more detail with potential partners under confidentiality

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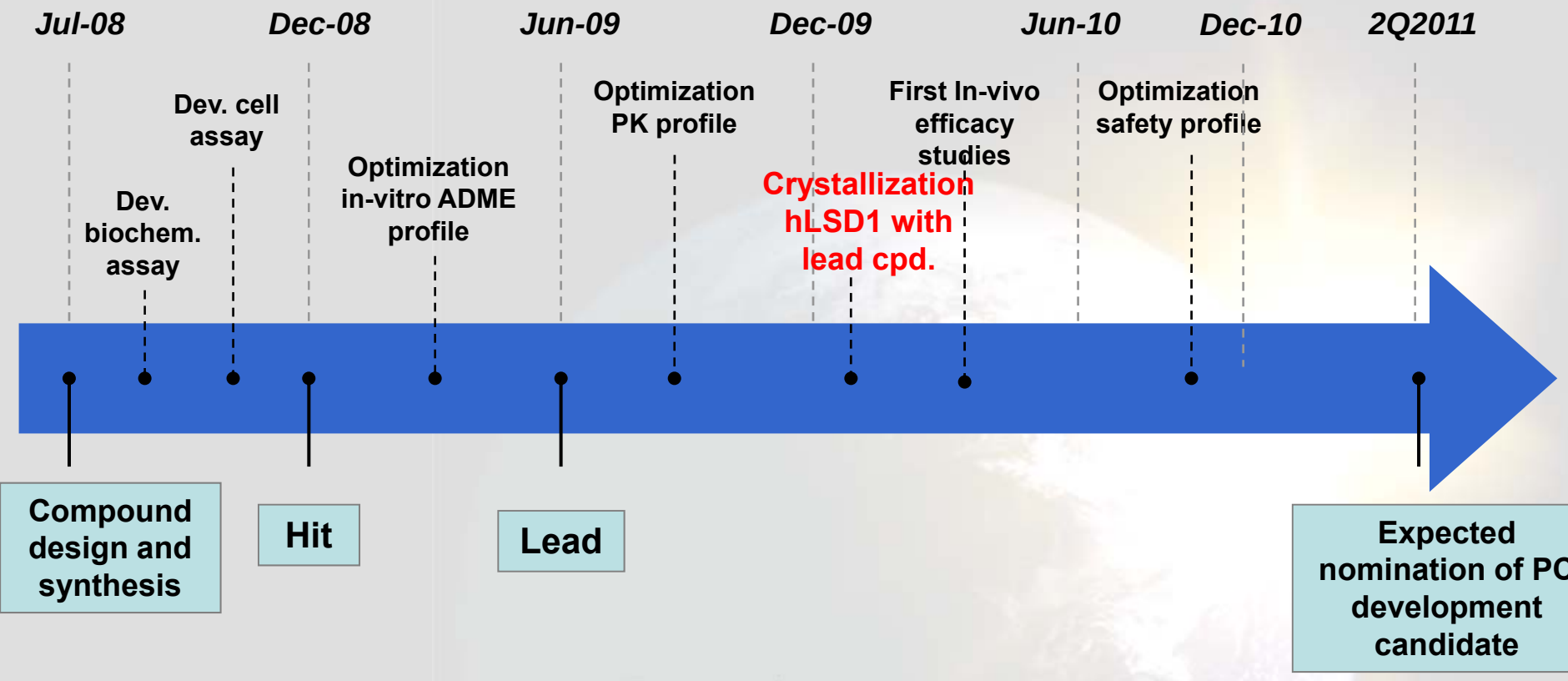
CURRENT STATUS OF DEVELOPMENT

LSD1-MAO inhibitors are in advanced LO to enter in PCDC

Discovery of LSD1 inhibitors



Expected IND in 1H 2012

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IPR protection and Competitive Landscape

LSD1: Competitive Landscape

Company or Group	Compound Class – Patent Status
Progen Pharmaceuticals (Marton, Casero, & Woster et al. WARF & Johns Hopkins)	(Bis)guanidine, (Bis)guanides, (Bis)ureas & (Bis)thioureas Patent Application
Frontier Pharma (Ueda et al., Nagahama Institute Bio-Science Technology)	Substituted phenylcyclopropylamines Patent Application
Yokoyama & Umehara et al. (RIKEN)	Substituted phenylcyclopropylamines Patent Application
Binda et al. (University of Pavia)	Substituted phenylcyclopropylamines No patent application detected

Progene had a program based on selective oligoamines as LSD1 inhibitors for cancer and is reported to be discontinued.

LSD1: Competitive Landscape

Group	Compound	LSD1 IC_{50}	LSD1 K_i	MAO-A K_i	MAO-B K_i	GI_{50} cancer cell lines
Frontier	1	2.5	5.7	230	740	6-45
	2	1.9	3.1	250	1700	17-70
Progen	1c, 2d	< 1	ND	ND	ND	ND
Binda	14e	ND	1.3	1.2	None	None
Yokoyama Umehara	S2101	0.99	0.61	17	110	ND
	S2107	4.1	1.6	150	27	ND

*Reported values are all micromolar

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Orzyon LSD1 Patent Applications

ORYZON LSD1 Patent Applications:

15 patent families covering LSD1:

- 8 Patent application filed related to new series of compounds and method of treatment.
- 7 Patent application filed related to methods of treatment and new uses of LSD1 inhibitors (antiviral and other diseases).

2 patent applications in preparation

Search reports from 2 published applications were positive.

3 more patent applications will be published this year.

LSD1: Competitive Landscape

- No public reports of Big Pharma developing LSD1 inhibitors

- Several small biotechs and academic groups are investigating LSD1 inhibitors

- To the best of our knowledge, Oryzon has:

  - The most potent small molecule LSD1 inhibitors
  - The most selective LSD1 inhibitors (LSD1 vs. MAO-A and MAO-B)
  - The only potent dual inhibitors of LSD1 and MAO-B

- Oryzon's expertise in the field coupled with our strong patent portfolio positions us as a clear leader in this area

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AVAILABILITY FOR COOPERATION

- Oryzon is looking for a experienced partner that help us to move the program forward in a faster and more efficient way

- There are several architecture deals done in preclinical programs in the last two years that could be of interest for us

  - GSK option deal with Epyzyme
  - Elli-Lilly option deal with Alizé Pharma
  - Elixir Pharmaceuticals Inc. with Bristol-Myers Squibb
  - GSK option deal with Supergen
  - GSK option deal with Vernalis
  - Lilly discovery deal with Neurosearch
  - Novartis option deal with Forma Therapeutics

Muchas Gracias por su atención…!

En el networking a las 13.45 estarán a su disposición:

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