XII Encuentro de Cooperación Farma-Biotech

Santiago de Compostela, 26 de septiembre de 2014

Novel strategy for symptomatic and disease-modifying treatment of Alzheimer's Disease

cima
CENTER FOR APPLIED MEDICAL RESEARCH
UNIVERSITY OF NAVARRA

GOBIERNO DE ESPAÑA MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

MEDICAMENTOS INNOVADORES
Plataforma Tecnológica Española

biospain 2014

farmaindustria

Outline

- **Institution: CIMA**
- **Project**
- **Partnering Opportunities**

CIMA

The **Center for Applied Medical Research** (CIMA) is private non-profit biomedical research institution of the University of Navarra, based in Pamplona, Spain.

CIMA carries out high quality scientific work with a strong **translational** focus.

CIMA. De-risking Drug Discovery Process

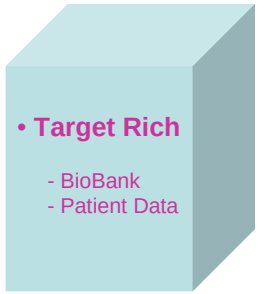
CUN

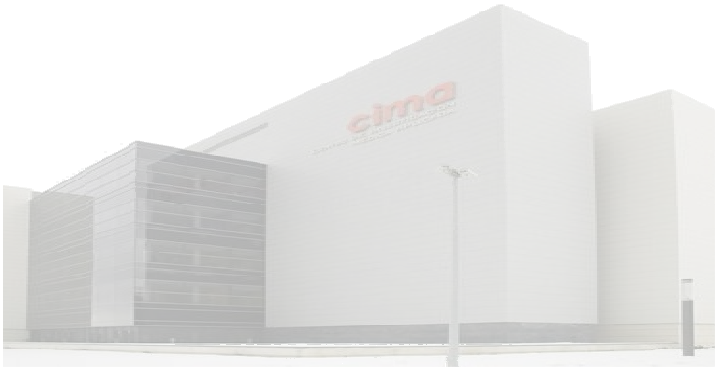
Medical Center

CIMA

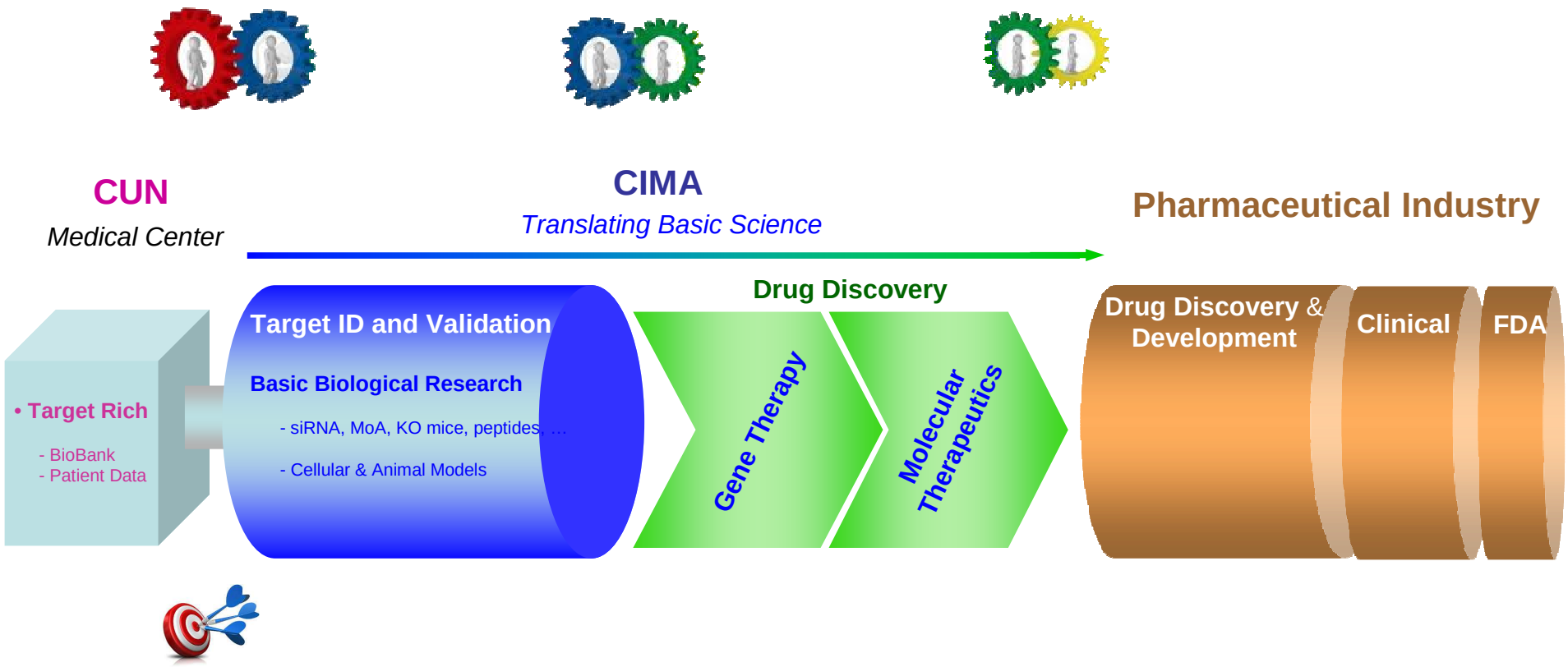
Translating Basic Science

Pharmaceutical Industry





CIMA. De-risking Drug Discovery Process



• Translational Medicine

Bidirectional data analysis to identify and/or prioritize clinically relevant molecular targets or pathways.

• Basic Science

Advanced basic research to decipher MoA underlying clinical evidence.

Implementation of *in-vitro* or/and *in-vivo* assays for unequivocal assessment: PoC

• Drug Discovery

Proprietary tool(s), biologics or/and small molecules, for *in-vivo* PoC: efficacy & safety

CIMA. De-risking Drug Discovery Process

From Bed to Bench and Back

CUN

Medical Center

CIMA

Translating Basic Science

Pharmaceutical Industry

Drug Discovery

- **Target Rich**
  - BioBank
  - Patient Data

Target ID and Validation

Basic Biological Research

- siRNA, MoA, KO mice, peptides …
- Cellular & Animal Models

Gene Therapy

Molecular Therapeutics

Goal: Partnering Licensing

Drug Discovery & Development

Clinical

FDA

- Expected Deliverables:

  i.- **Novel Target / MoA**

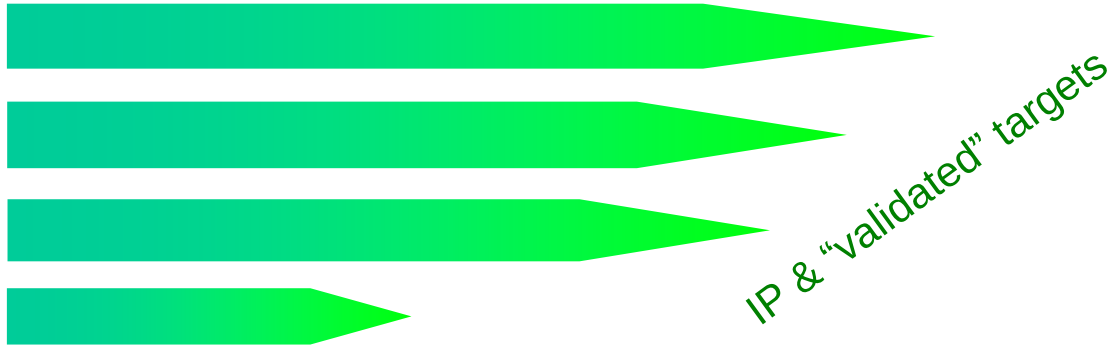
  ii.- In-vitro & **In-vivo PoC** with "drug-like" molecules or biologics: **Efficacy & Safety** → Advanced **Lead(s)**

  iii.- Lead(s) with **proprietary IP** *(Availability for further development)*

  iv.- **"Know-how"**

Projects Overview

Target(s)	Therapeutic effect
A & B	Alzheimer`s Disease
PMT & DNMT	Anti-neoplastic
Gene7	Wilson´s Disease
Gene70	Anti-coagulant



Projects Overview

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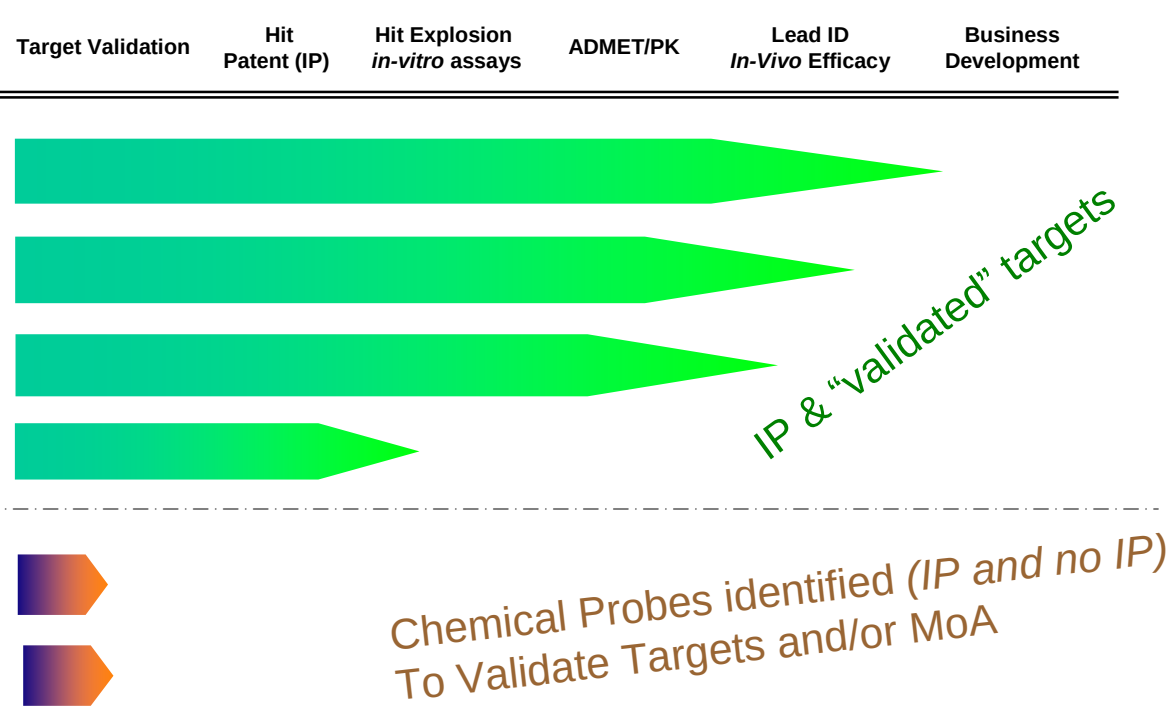
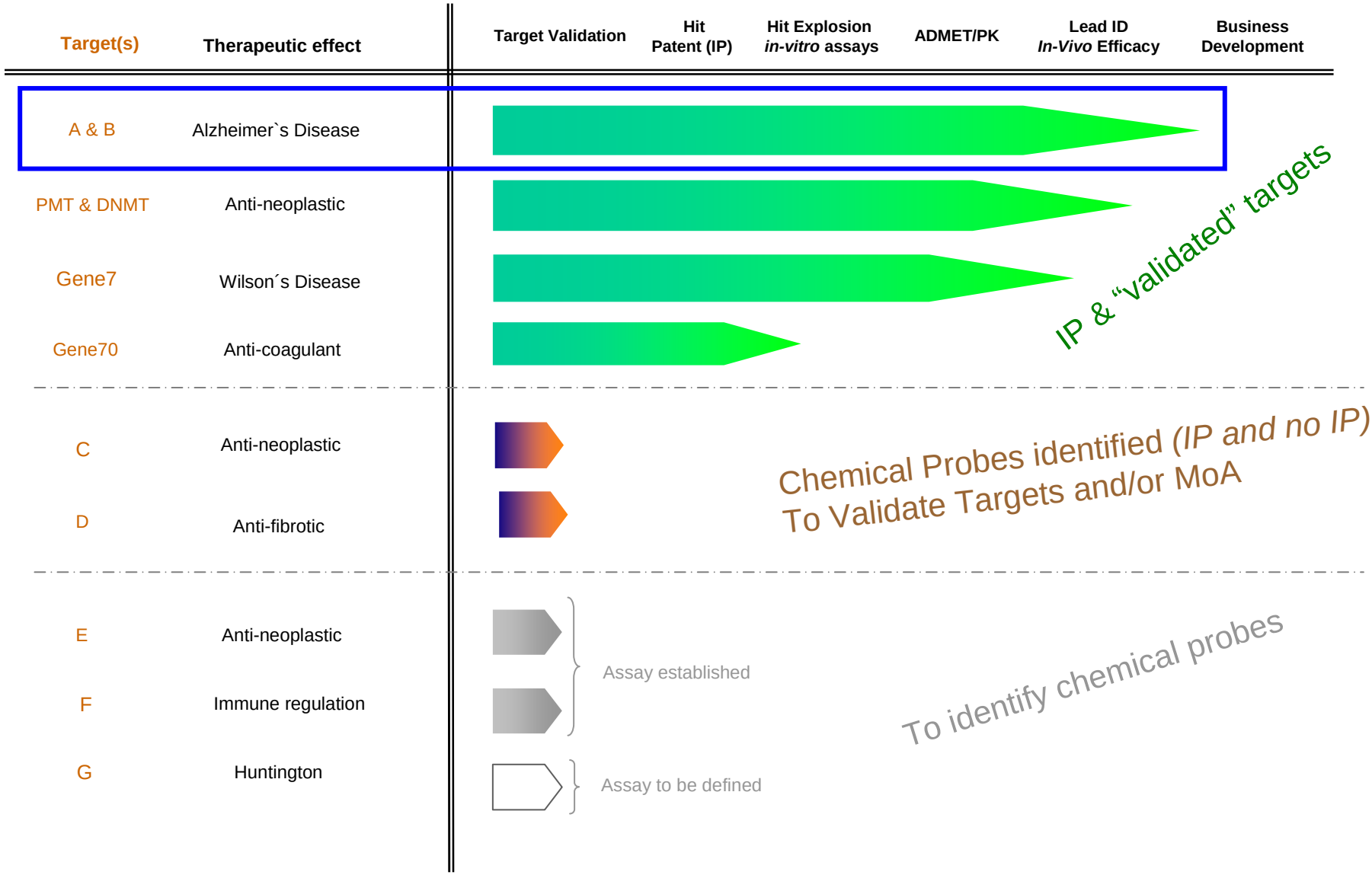
A & B	Alzheimer`s Disease
PMT & DNMT	Anti-neoplastic
Gene7	Wilson´s Disease
Gene70	Anti-coagulant
C	Anti-neoplastic
D	Anti-fibrotic



Projects Overview



Outline

- Institution: CIMA
- **Project**
- Partnering Opportunities

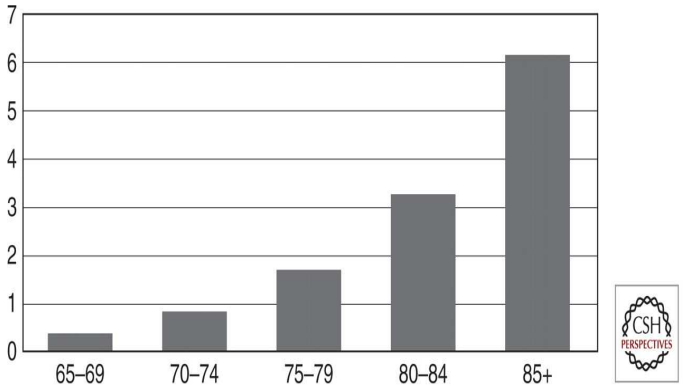
Alzheimer's Disease

Status

- Currently, approximately **18 million people worldwide.**
- At least **11 million Americans expected** to have the disease by the **middle of the century**, boosting the annual **costs of health care** to more than **US$1 trillion**

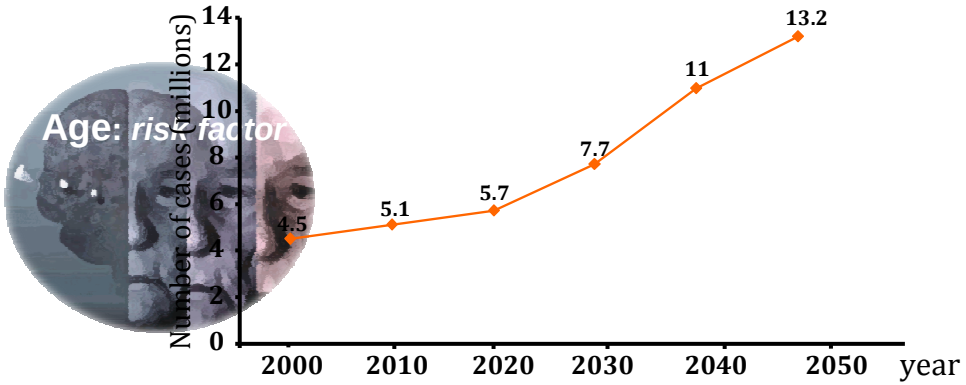
• Incidence

Annual incidence rate (per 100-person-years)



Mayeux R , Stern Y Cold Spring Harb Perspect Med 2012

• Prevalence

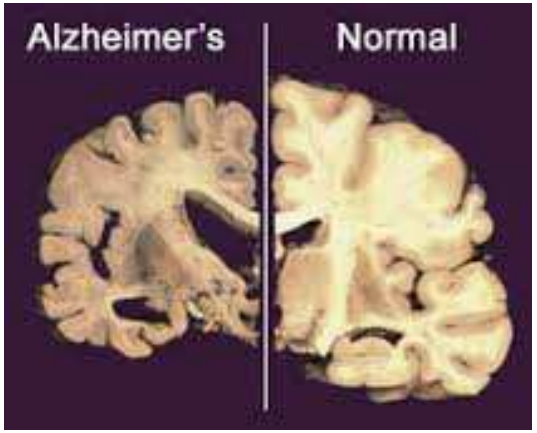


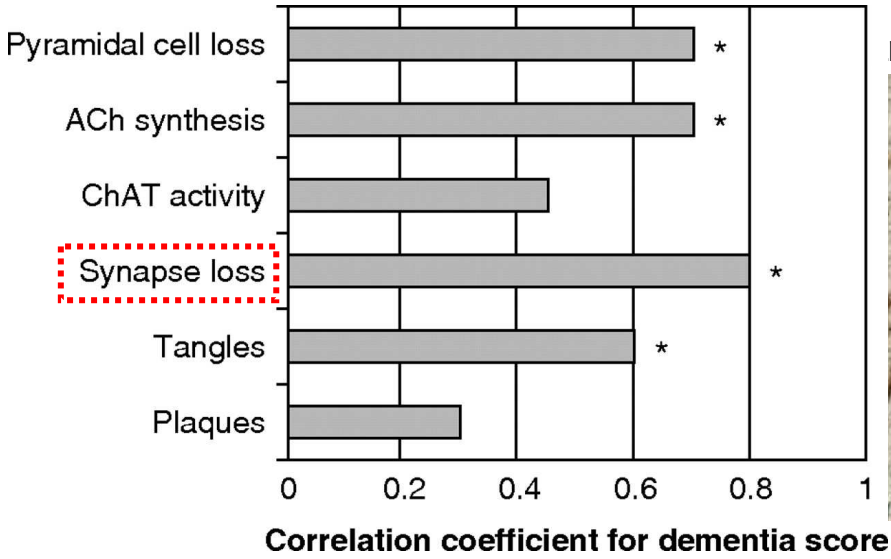
People Age 65 and Over in the U.S. with AD. Using the U.S. Census Bureau Estimates of Population Growth*

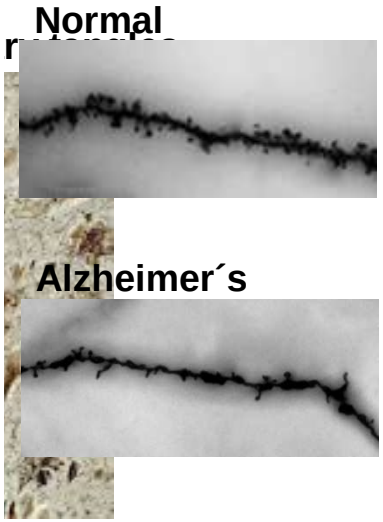
Alzheimer's Disease

Status

- The current treatment options are only moderately effective. There is an **unmet need** for therapies that halt of substantially slow disease progression.
- **Recent clinical trials** of various disease-modifying therapies for AD **failed** to demonstrate benefit. Thus, it is emerging the idea that *other pathways not directly linked to Aβ pathology should be explored.*







Novel Strategy for the Treatment of AD

Aim

Effective therapeutic agents for the *symptomatic and disease-modifying* treatment of AD

Novel Strategy for the Treatment of AD

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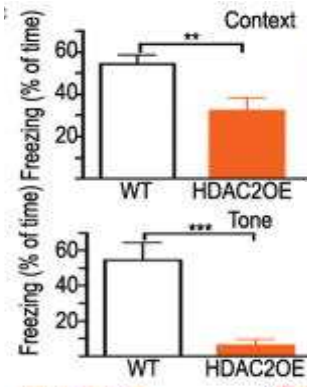
Effective therapeutic agents for the *symptomatic and disease-modifying* treatment of AD

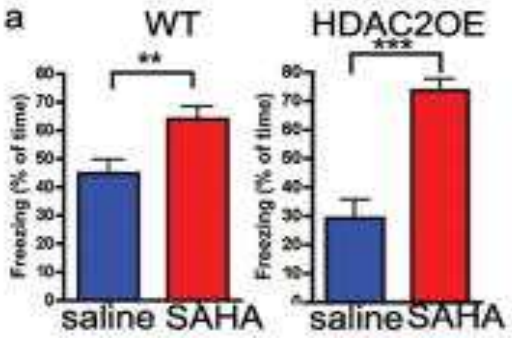
Approach

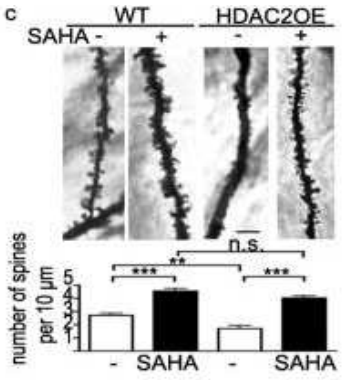
1. Targets proposal & identification: *Systems Therapeutics* ✓
2. Targets validation ✓
3. Hit ID (proprietary chemical series, IP). ✓
4. Hit Explosion and Lead(s) ID, acceptable PK, *in-vivo* PoC ✓
5. Lead Optimization ***On-going***

Targets Proposal

- **HDACs** *(Histone DeACetylases)*



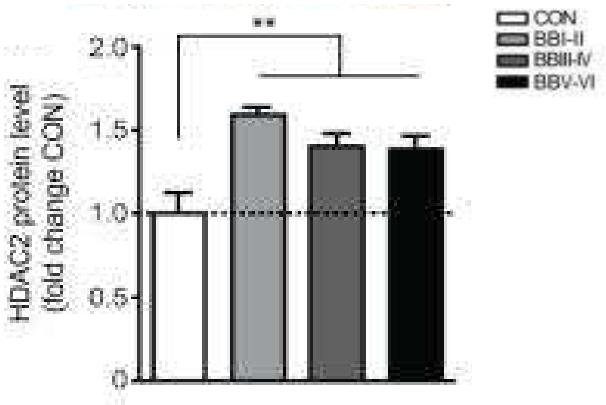




HDAC2 overexpression impairs memory formation whereas HDAC2 knockout mice exhibit enhanced memory formation

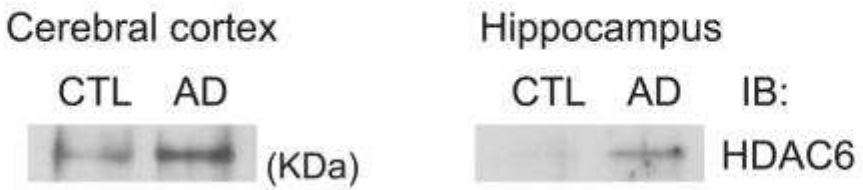
Guan et al. ***Nature*** *(2009)*

- **HDAC2 increases in AD patients**



Gräff et al., ***Nature*** *(2012)*

- **HDAC6 increases in AD patients**

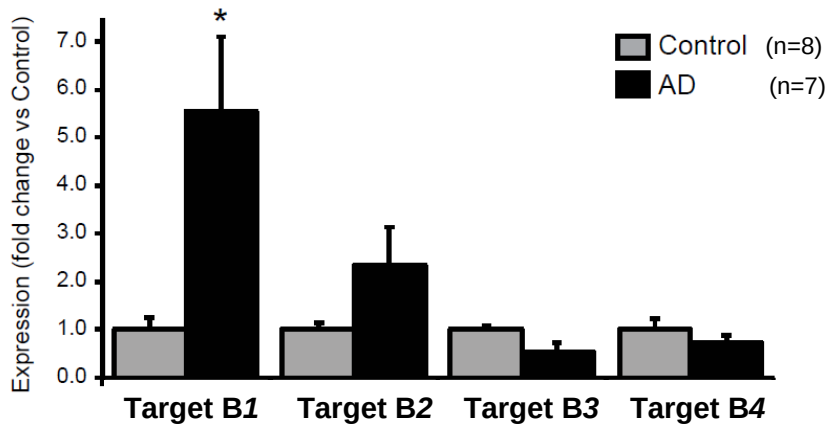


Ding et al., J. Neurochem (2008)

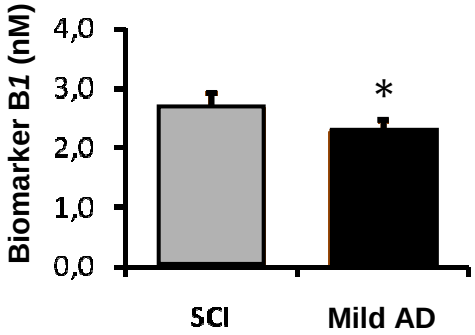
A. Garcia-Osta et al. Neuropsychopharmacology (2009)

Target Identification

- **"Target B"**
  - **"Target B" mRNA expression in hippocampus**



  - **CSF levels of "Target B*1*" biomarker**[1]



[1] Determined by LC-MS/MS

***** p≤0.05, n=83 patients

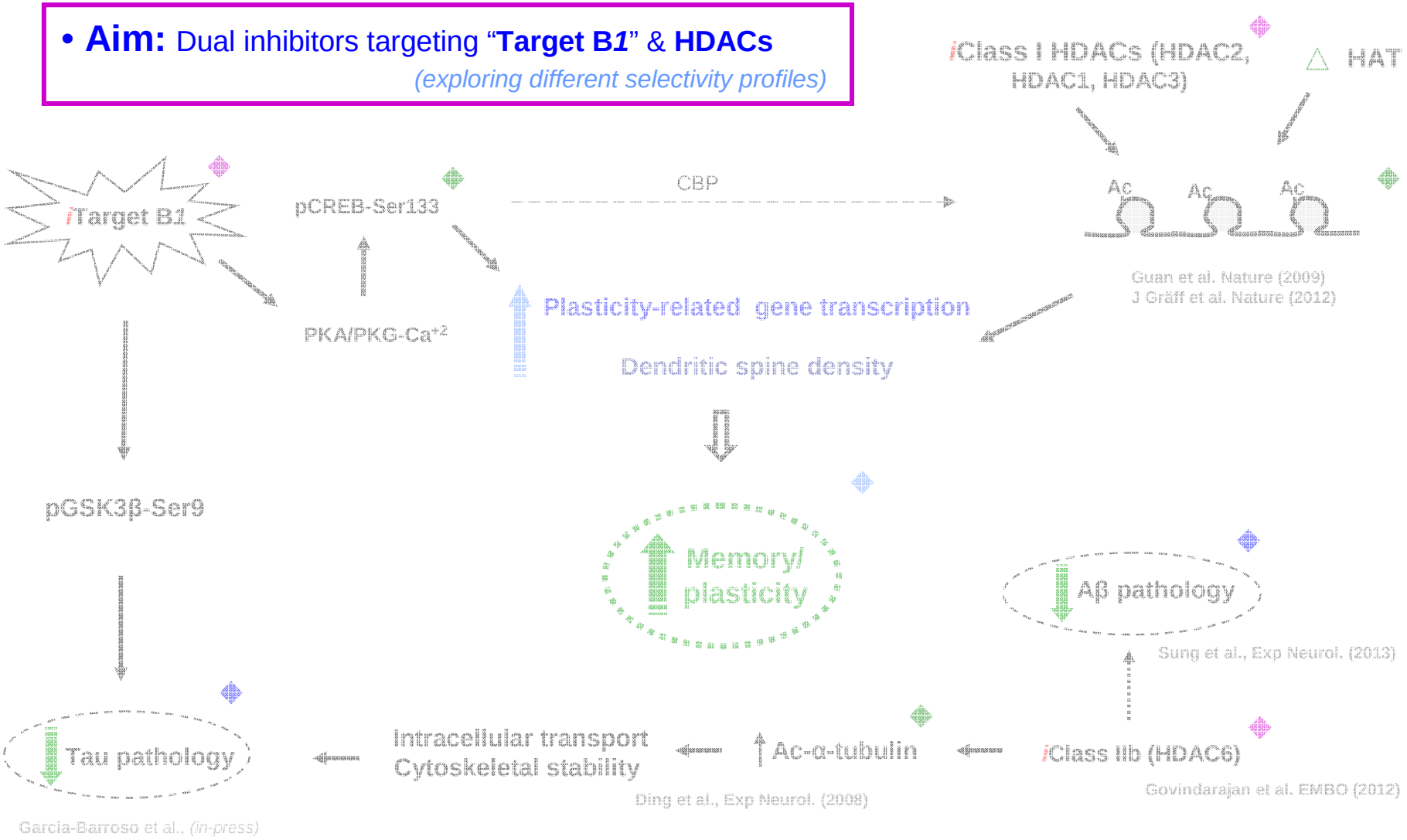
CSF levels of "Target B*1*" biomarker are significantly associated to cognitive status (MMSE score) and CSF levels of $A\beta_{1-42}$ in patients with AD, which may confirm its implications in AD

- **SCI**, subjective cognitive impairment
- **Mild AD**, patients with mild Alzheimer's dementia.

Ugarte et al., *(under revision)*

Hypothesis Proposal: *Systems Therapeutics*

- **Aim:** Dual inhibitors targeting "**Target B*1***" & **HDACs**
  (exploring different selectivity profiles)



Hypothesis Validation

- **PoC**

  ✓ ***i*HDAC & *i*"Target B*1*"**

  SAHA & "Compound B"

  In vitro (neuronal primary culture)

  WT neurons: AcH3, pCREB

  Tg2576 neurons: AcH3, pCREB, AcTub, C99 and pTau

  In vivo (AD mouse model: Tg2576 mice)

  Memory function: FC and WM

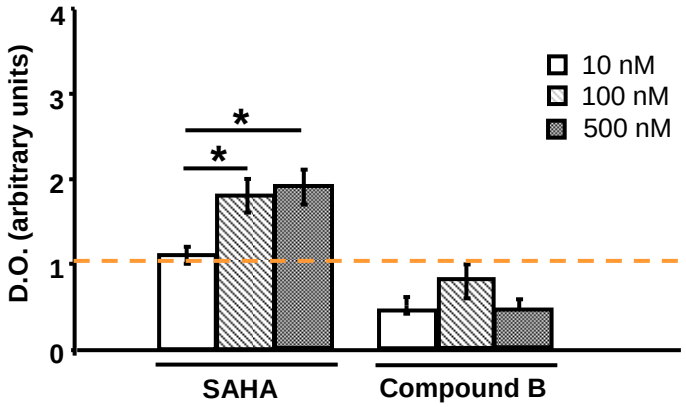
  Dendritic spine density (Golgi Cox)

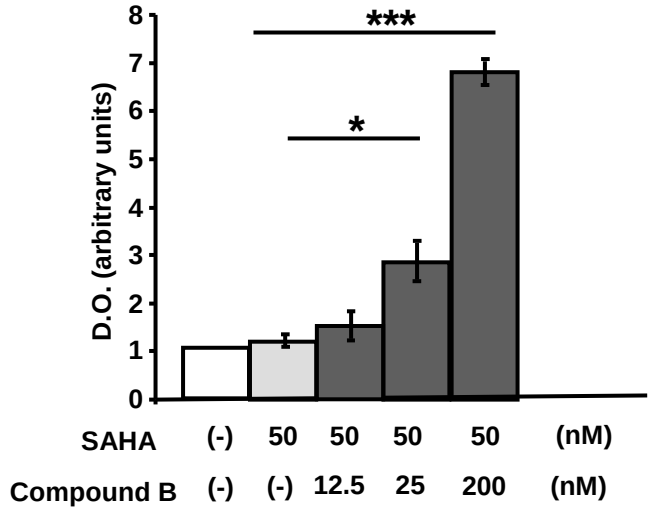
  Biochemical determinations: memory and AD-related marks

Hypothesis Validation

- **PoC:** ***In vitro*** assays in WT-primary neuronal culture

  - **AcH3-K9**





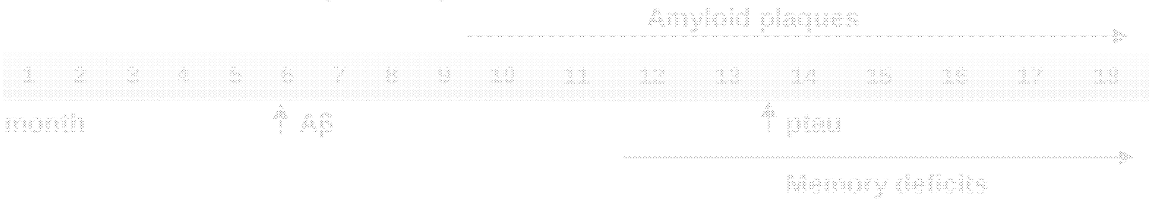
Hypothesis Validation

- **PoC:** ***In vivo*** studies using Tg2576 AD mouse model

Hippocampus

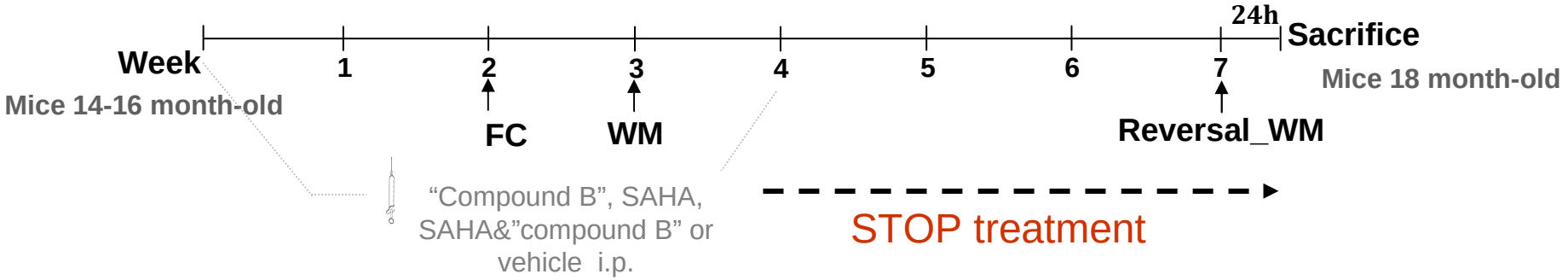
Tg2576
(hAPP^{Swe})

- Memory impairment
- Synaptic pathology
- Amyloid burden
- Tau pathology
- Neuroinflamation



Animals: (14-16-month)

i.- WT vehicle (n=7)
ii.- Tg2576 vehicle (n=8)
iii.- Tg2576 "Compound B" 1 mg/Kg (n=7)
iv.- Tg2576 SAHA 12,5 mg/Kg (n=7)
v.- Tg2576 "Compound B" & SAHA (n=8)



Hypothesis Validation

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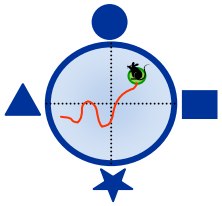
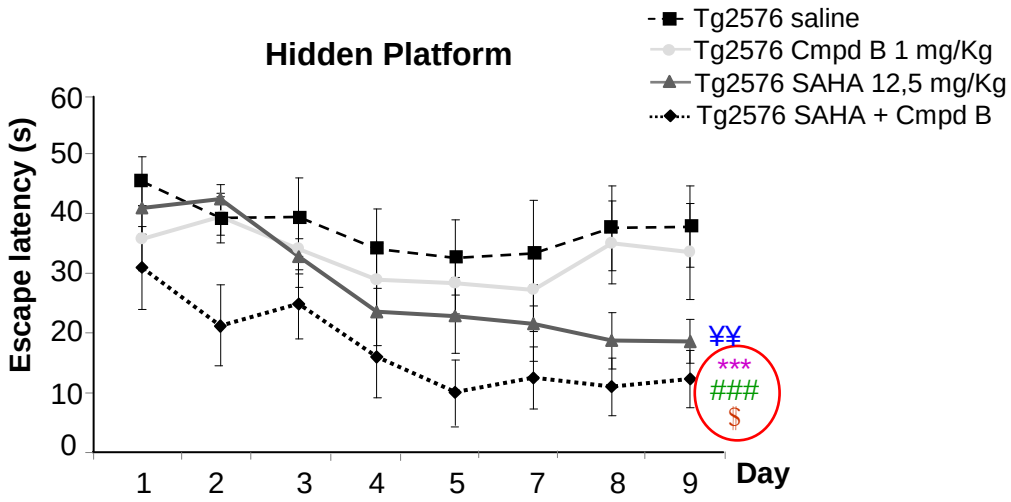




Two-way repeated measures ANOVA followed by Scheffè's test

Tg2576 SAHA vs Tg2576 saline **¥¥** (p<0,01)

Tg2576 SAHA+Cmpd B vs Tg2576 saline *** (p<0,001)
Tg2576 SAHA+Cmpd B vs Tg2576 Cmpd B 1 mg/kg ### (p<0,001)
Tg2576 SAHA+Cmpd B vs Tg2576 SAHA $ (p<0,05)

Animals: (14-16-month)

i.- WT vehicle (n=7)
ii.- Tg2576 vehicle (n=8)
iii.- Tg2576 "Compound B" 1 mg/Kg (n=7)
iv.- Tg2576 SAHA 12,5 mg/Kg (n=7)
v.- Tg2576 "Compound B" & SAHA (n=8)

Biological Chemistry: Hit ID

- **Aim:**

  i.- First-in-class dual inhibitors: molecules targeting "**Target B**" & **HDACs**

  ii.- Novel chemical series with proprietary IP

- **Approach:**

  - Knowledge-based
  - Structure-based

  } ***de-novo* design**

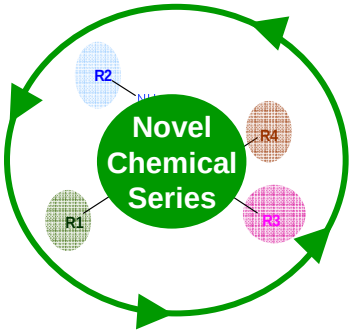
- **Achievement:**

  ✓ • Hit ID: Synthesis and biochemical evaluation

  ✓ • IP – *patents filed in 2013 & 2014* *(4 different chemical series)*

Medicinal Chemistry: Lead ID

- **Aim:** From Hit Explosion to Lead ID



- **Approach:**

a) Synthesis of new compounds to establish Structure Activity/Property Relationships (SAR/SPR)

b) Workflow,

SAR

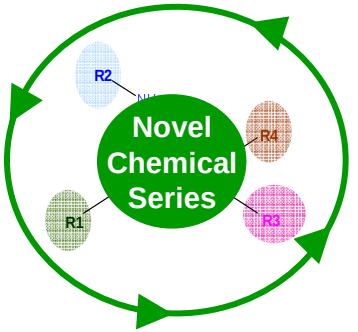
i.- Design
ii.- Synthesis
iii.- *In-vitro* binding assays (vs “Target Bs” & HDACs) *(initial decision point)*
iv.- *In-vitro* functional assay (primary neuronal culture, WT & Tg2576):
pCREB, AcH3, AcTub, pTau and C99 *(decision point)*

SPR

v.- Toxicity (THLE-2, neuron/glia & PBMC) *(decision point)*
vi.- ADME profiling
vii.- Pharmacokinetics: crossing BBB & functional response (pCREB, AcH3) *(decision point)*
viii.- *In-vivo* efficacy model(s)

Medicinal Chemistry: Lead ID

- **Aim:** From Hit Explosion to Lead ID



- **Results:**

  - Currently, >180 new compounds have been synthesized
  - CM-414 was selected as the first lead compound for *in-vivo* studies
  - Three additional compounds with different selectivity profiles have been also selected for *in-vivo* studies – corresponding studies are currently *on-going*

Lead ID. *CM-414 profiling*

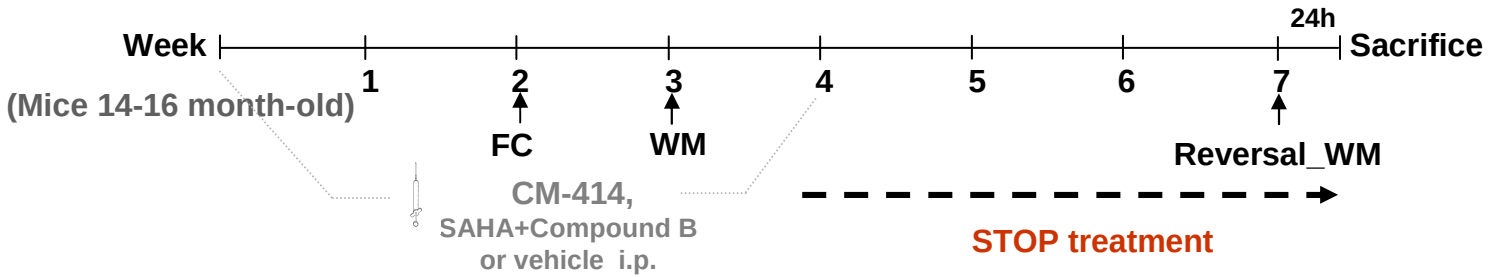
In-Vitro	**Efficacy**	Binding affinities (HDAC2, HDAC6 & Target B): AcH3 (functional assay, WT neurons) AcTub (functional assay, WT neurons) pCREB (functional assay, WT neurons) APP processing (functional response, Tg2576 neurons) pTau (functional response, Tg2576 neurons)	IC_{50} (nM): 492, 110 & 61 EC_{max} (nM): 10 *(190%)* EC_{max} (nM): 100 *(110%)* EC_{max} (nM): 100 *(360%)* 50% reduction @ 50nM 50% reduction @ 100nM	→ Therapeutic window
	ADME	P450s: 1A2, 2C19, 2C9, 2D6, 3A4 (<50% @ 10mM) Plasma Protein Binding (% unbound) Brain protein binding (% unbound) Solubility (at pH=7.4) PAMPA (Pe 10^{-6} in nm/s) Liver Microsomal Stability ($t_{1/2}$ estimation) *in minutes*	OK, *except 3A4 (75.2%)* 1.8 (H), N.D. (M) 8.4% (M) 29.8 µg/mL *(intermediate)* 0.52 (Low) 40.1(H), 3.3(M)	~ 3 log units
	CV safety	hERG binding Patch Clamp	IC_{50}: >100 µM IC_{50}: *on-going*	
	Toxicity	THLE-2 @ 72 hours Neurons @ 72 hours PBMC @ 72 hours	LC_{50}: 7.2µM *(>100 µM @ 24 h)* LC_{50}: 17.7 µM *(>100 µM @ 24 h)* LC_{50}: 72.6 µM *(>100 µM @ 24 h)*	→ Therapeutic window
In-Vivo	**PK**	Pharmacokinetics (e.g. Vz & $t_{1/2}$) @ 40 mg/Kg *in mice* (i.p.) Brain tissue/Plasma Ratio @ T_{max} from 40 mg/Kg *in mice* (i.p.)	0.5 (L/Kg) and 2.8 (h) 1.4 % *(248 nM)*	→ Short half-life Functional response achieved, Increment in *AcH3 and pCREB in hippocampus @ 2 hours*

Lead ID. *In-Vivo* Proof-of-Concept (PoC)

Animals utilized in this study: **14-16 month-old**

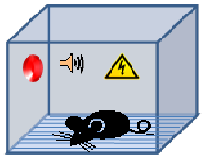
- WT vehicle (n=12)
- Tg2576 vehicle (n=11)
- Tg2576 **SAHA** (12.5 mg/Kg) **+Compound B** (1 mg/Kg) (n=12)
- Tg2576 **CM-414** (40 mg/Kg) (n=11)

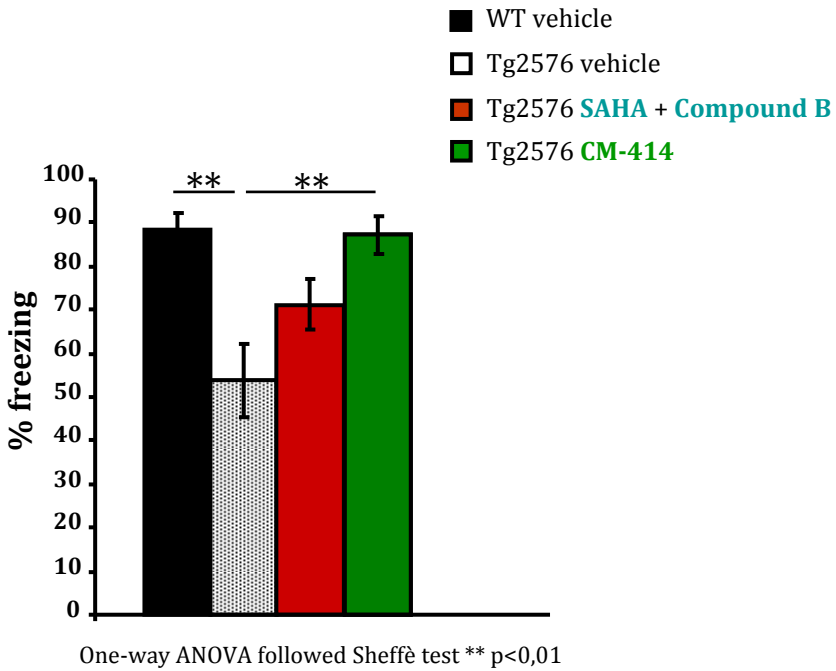
***In-vivo* efficacy (aged-Tg2576 mice)**



Lead ID. *In-Vivo* Proof-of-Concept (PoC)

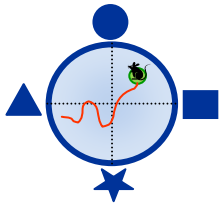
- **Behavior:** Fear Conditioning test after 2 weeks of treatment

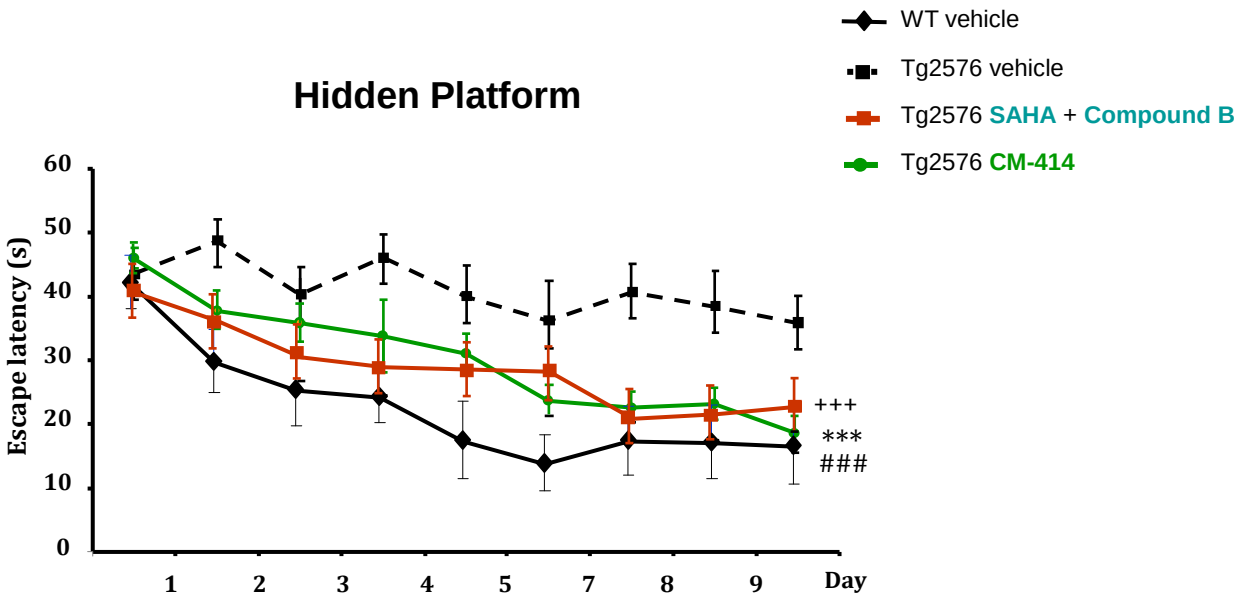




Lead ID. *In-Vivo* Proof-of-Concept (PoC)

- **Behavior:** Water-Maze (WM) test after 4 weeks of treatment

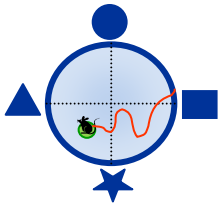


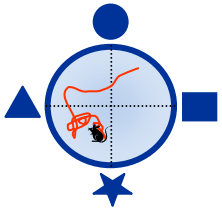


Two-way repeated measures ANOVA followed Sheffe test
WT vehicle vs Tg2576 vehicle ### (p<0,001)
Tg2576 **SAHA+Compound B** *vs* Tg2576 vehicle +++ (p<0,001)
Tg2576 **CM-414** vs Tg2576 vehicle *** (p<0,001)

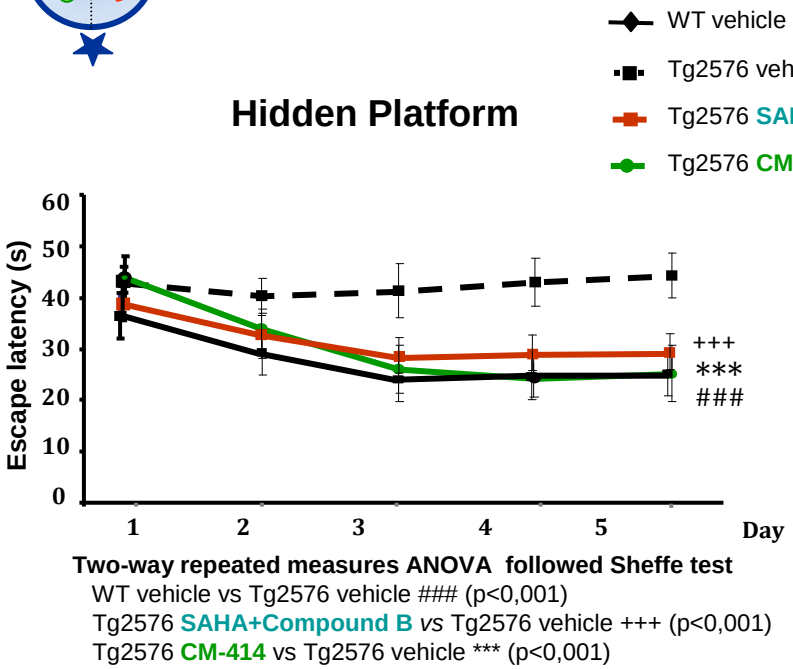
Lead ID. *In-Vivo* Proof-of-Concept (PoC)

- **Behavior:** Water-Maze (WM) test after a washout period of 4 weeks of treatment





Hidden Platform



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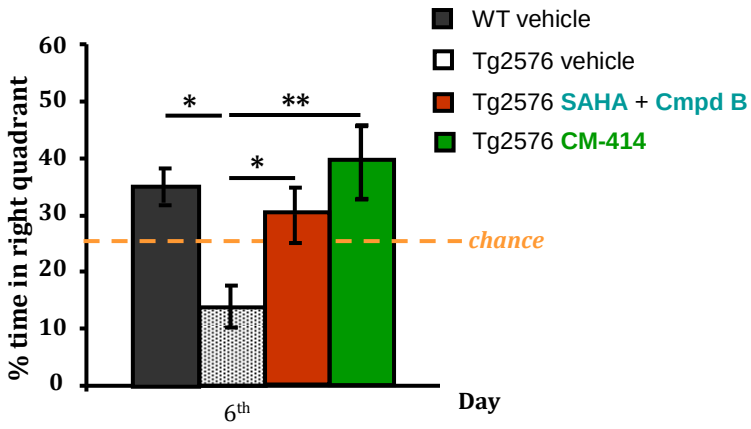
Tg2576 **SAHA+Compound B** *vs* Tg2576 vehicle +++ (p<0,001)
Tg2576 **CM-414** vs Tg2576 vehicle *** (p<0,001)

Memory retention (probe)

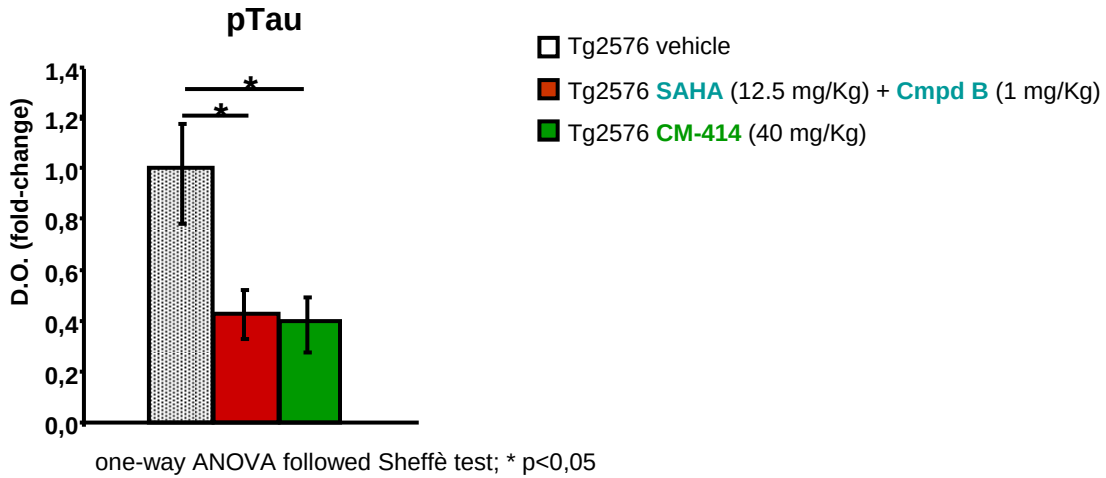


One-way ANOVA followed Sheffè test * p<0,05 ** p<0,01

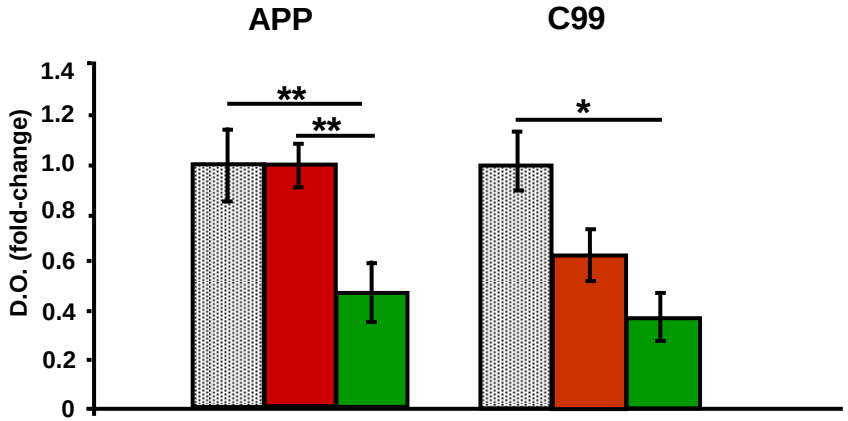
The memory recovery induced by CM-414 was maintained after a washout period of 4 weeks in aged Tg2576

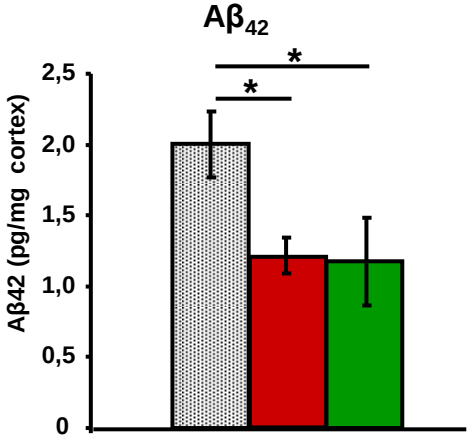
Lead ID. *In-Vivo* Proof-of-Concept (PoC)

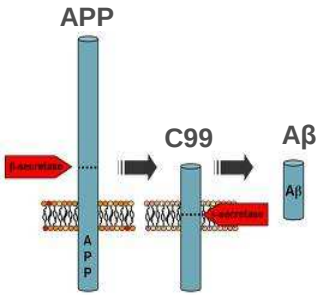
- **Tau and Amyloid pathology** *(parieto-temporal cortex)*



one-way ANOVA followed Sheffè test; * p<0,05





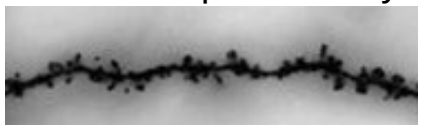


one-way ANOVA followed Sheffè test; * p<0,05 ** p<0,01

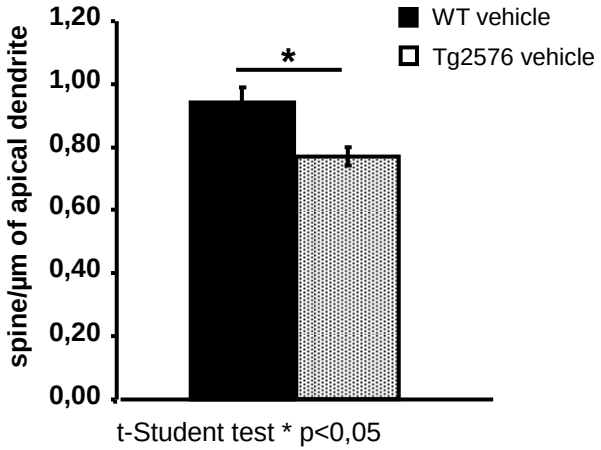
Lead ID. *In-Vivo* Proof-of-Concept (PoC)

- **Reversal of defects in spine density**

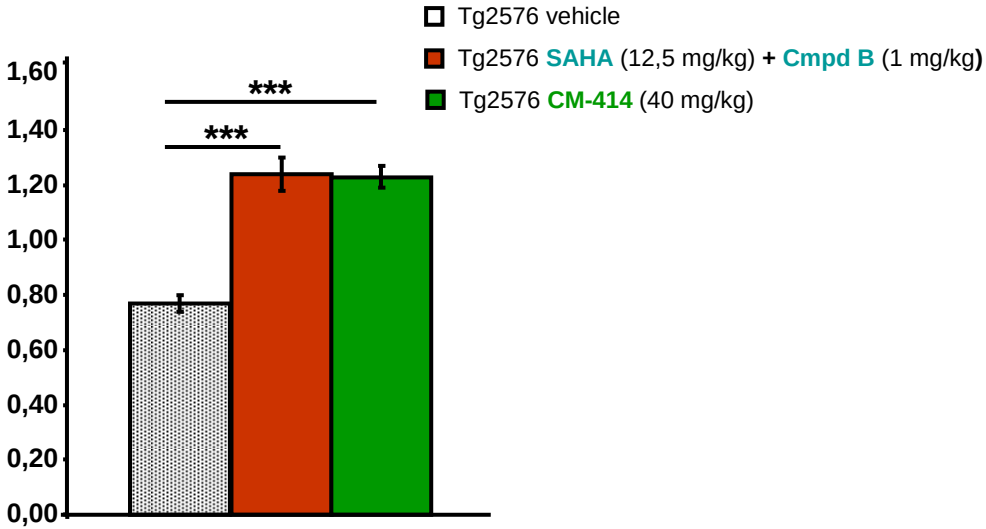
Dendritic spine density



CA1 pyramidal neurons *(hippocampus)*



t-Student test * p<0,05

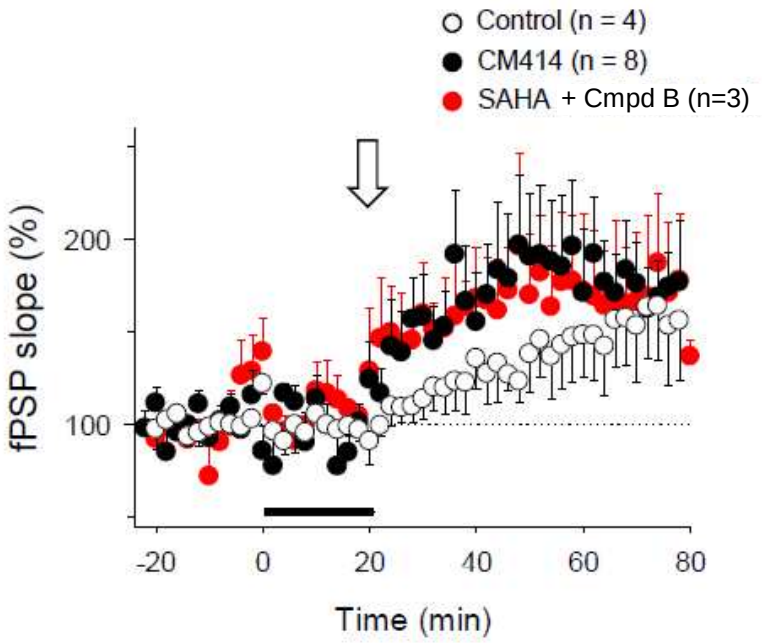


One-way ANOVA followed Sheffè test *** p<0,001

Lead ID. *In-Vitro* Proof-of-Concept (PoC)

- **Functional *in-vitro* screening by Long-Term Potentiation (LTP)** – *on-going*

Consequences on synaptic plasticity in APP/PS1 mouse model – *preliminary results*



Lead ID. *In-Vivo* Proof-of-Concept (PoC)

- **Gene-expression profiling after a 4-weeks washout period**

✓ Gene expression profiling in hippocampus of aged-Tg2576 mice after the washout period using Affymetrix microarray-based gene-expression technology (*Mouse Gene 2.0 ST Array)*

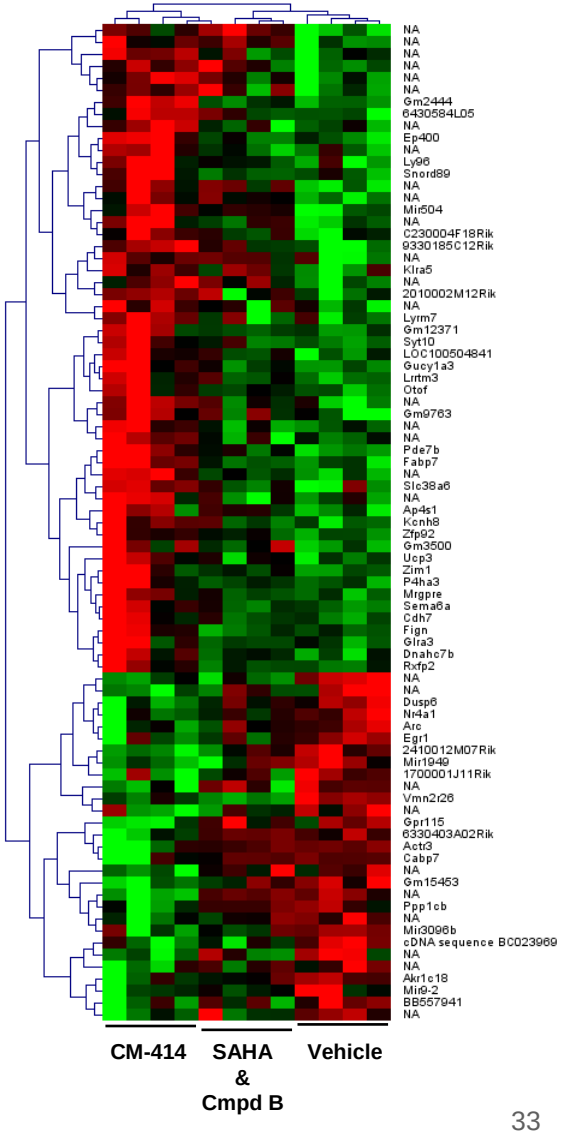
-Saline

-Compound B

-SAHA

-SAHA+Compound B

-CM-414



Lead ID. *In-Vivo* Proof-of-Concept (PoC)

- **Gene-expression profiling after a 4-weeks washout period**

Physiological System Development and Function

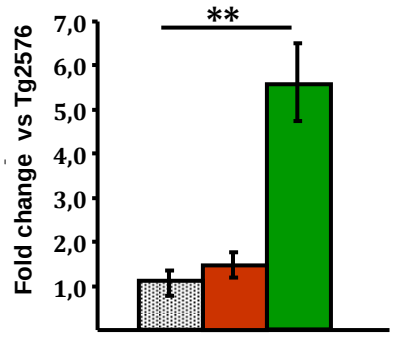
Name	p-value	# Molecules
Organismal Development	4.92E-05 - 4.54E-02	22
→ Nervous System Development and Function	4.89E-04 - 4.27E-02	33
Organ Morphology	5.26E-04 - 4.09E-02	22
Reproductive System Development and Function	5.26E-04 - 3.73E-02	17
→ Behavior	5.39E-04 - 3.45E-02	28

INGENUITY SYSTEMS

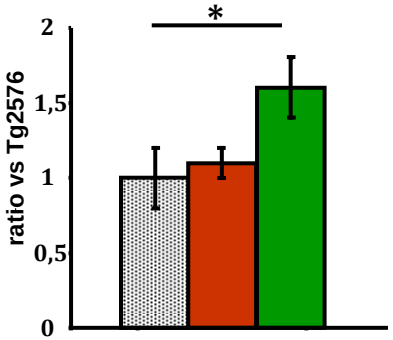
EphB2

Tg2576 vehicle
Tg2576 **SAHA** + **Cmpd B**
Tg2576 **CM-414**

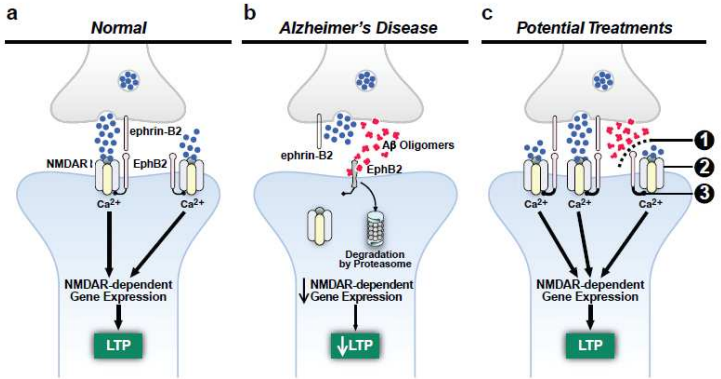
mRNA expression



Protein expression



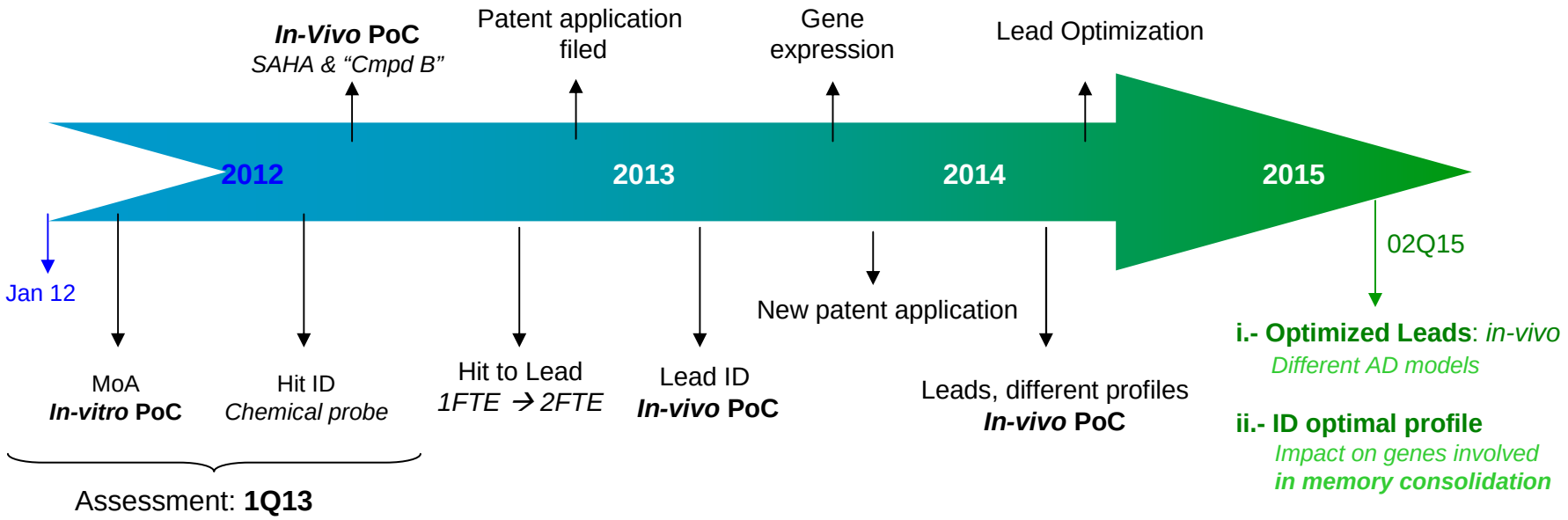
one-way ANOVA followed Sheffè test; * p<0,05 ** p<0,01



Nature **469**, 47–52 (2011)

Timeline & Next Steps: *Lead Optimization*

- **Timeline**



- **Lead Optimization** process is *on-going*, mainly focused on:

  i.- Improving crossing BBB; thus, reducing dose til 10mg/Kg (aim: FIH PoP)

  ii.- Pharmacokinetics (oral admin; mainly focused on solubility and permeability)

- **ID optimal compound's target profile**. Balance among HDACs and "Target B", *impact on gene-expression*

Outline

- Institution: CIMA
- Project
- **Partnering Opportunities**

Partnering Opportunities

- **Value Proposition**

  - Novel Mode of Action, a system therapeutics strategy: “Target B” & HDACs

    a) Targets and their corresponding functional marks dysregulated in AD patients.

    b) This dual inhibition leads to synergistic effect in epigenetic mechanistic pathway.

  - First-in-class dual inhibitors targetting “Target B” and HDACs

  - Proprietary chemical series; *patents filed in 2013 & 2014*

  - Identified lead compound for *in-vivo* Proof of Concept:

    a) Adequate safety window and PK to perform chronic *in-vivo* PoC, *no toxicity issue*

    b) Remarkable efficacy from three perspectives:

    i.- Behavioural models: FC, WM and reversal WM after washout period (4 weeks)
    ii.- Disease modifying markers: Amyloid & Tau pathology
    Reversal in deficits in spine density *(and LTP)*
    iii.- Over-expression of memory related genes: e.g. EphB2

Partnering Opportunities

- **Partnering**

Two scenarios are initially envisioned:

1.- Product license (IP)

2- Stepwise research investment & first option (right of first refusal)

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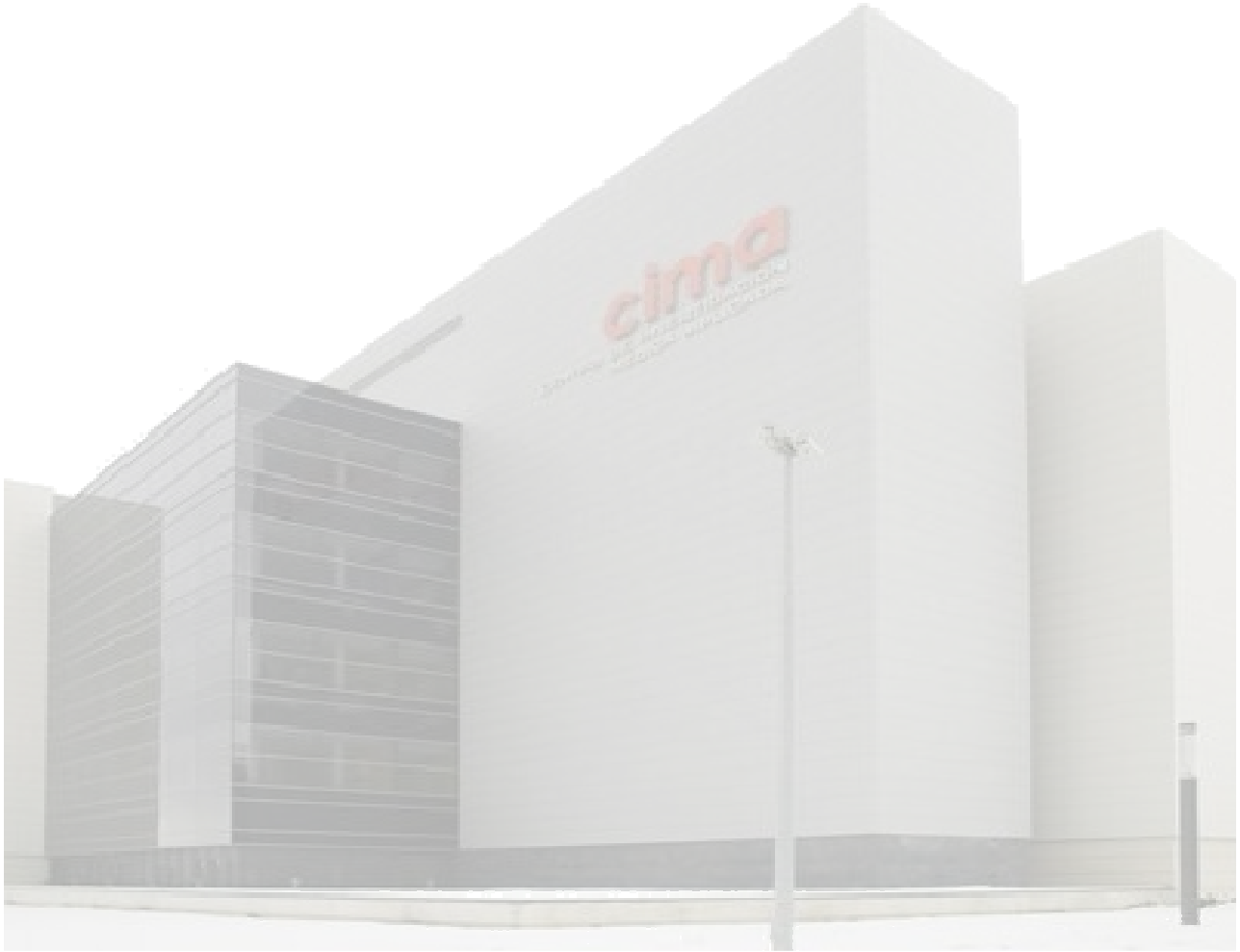
Thank you !



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