cima
CENTER FOR APPLIED MEDICAL RESEARCH
UNIVERSITY OF NAVARRA

Alzheimer's Disease treatment:
a novel approach

- **Alzheimer's disease (AD)** is particularly devastating since there is no cure, no way to prevent it and no proven way to slow its progression.
- Management of AD represents a huge unmet **need**; thus, discovery and development of more effective therapies are critical for worldwide public health and health-care systems.
- **Novel strategy** for symptomatic treatment of Alzheimer's disease.
  - Novel target: GPCR-X (not yet related to AD)
  - Discovery of GPCR-X antagonists
  - *Target validated using a novel animal model (KOGPCR-X/APPSwe" mice)*

Scope of the problem

- Currently, approximately 18 million people worldwide are afflicted with this disease and it is projected to reach over 30 million by 2025.
- 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.

Patient need addressed Substantially improve symptoms of Alzheimer's disease.

Therapeutic Hypothesis *(from experimental facts)*

- *h*GPCR-X is over-expressed in AD patients (Figure 1A)
- *m*GPCR-X is over-expressed in AD transgenic mice model Tg2576 (APPswe) (Figure 1B)

Thus, a GPCR-X antagonist might become therapeutic agent for the treatment of AD.

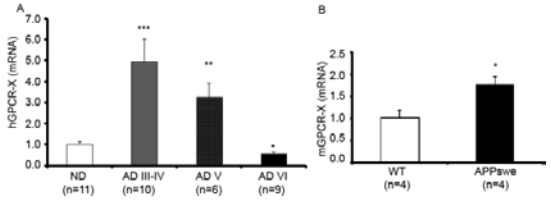

Figure 1. A) GPCR-X expression in hippocampal tissue of AD (Braak stages III to VI) and in 12 month old APPswe mice line B). Bars represent the receptor expression normalized to that of the corresponding 3B6 ribosomal internal control. Values are expressed as mean ± SEM. A) One way ANOVA followed by Tukey's test *p<0.05; **p<0.005; ***p<0.0001 vs ND (non-demented controls). B) Student T test *p<0.05 vs WT (wild type)

Target

- Although it is a decade since this Gi coupled GPCR-X was deorphanized, few ligands have been reported for the receptor; just weak agonists (EC_{50}>10μM).
- Only 6 antagonists have been reported so far, patent focused on a different therapeutic area (2004 as priority date), and no IC_{50} values were described.

Project Status

Validation process using a new animal model:

- mGPCR-X knock-out (KO) in Tg2576 (APPswe) mice was achieved: "KOGPCR-X/APPSwe" mice
- "KOGPCR-X/APPswe" mice showed a restoration of memory deficits in Morris Water Maze (MWM) test vs APPswe mice (Figure 2) – behavioural task
- AD pathological marks analysis. "KOGPCR-X/APPswe" mice showed a reduction in amyloid and Tau pathology (Figure 3)
- Synaptic plasticity analyses. "KOGPCR-X/APPswe" mice showed restoration in pGluR1 and PSD95 levels (Figure 4).

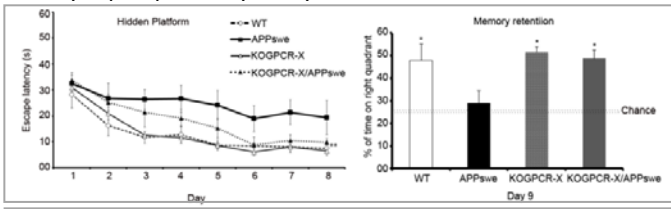

Figure 2. A). Behavioural analysis by MWM in the hidden platform. B) % of time spent searching for the target quadrant of the probe test on da. Results are expressed as mean ± SEM (n=10-12 per group). A)Two-way repeated measures ANOVA followed by Turkey's test. **p<0.005 vs APPswe. B) One-way ANOVA followed by Turkey's test. *p<0.05 vs APPswe

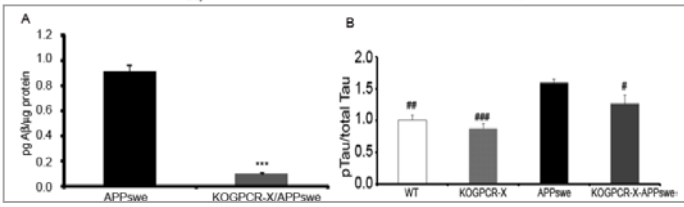

Figure 3. A)Aβ levels in hippocampus measured by ELISA. Bars represent the mean ± SEM (n=6). Student T test. ***p<0.0001 vs APPswe. B) Levels of pTau by western blotting. Bars represent the pTau/total Tauβ ratio (mean ±SEM, n=6) normalized to WT . One way ANOVA followed by Turkey's test. #p<0.05; ##p<0.005; ###p<0.0001 vs APPswe.

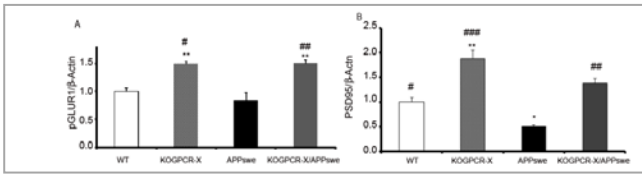

Figure 4. Levels of pGluR1 A) and PSD95 B) in hippocampal membrane preparations by western blotting. Bars represent the pGluR1 or PSD95 to β-Actin ratios (mean ±SEM, n=6) normalized to WT . One way ANOVA followed by Turkey's test. #p<0.05; ##p<0.005; ###p<0.0001 vs APPswe. *p<0.05; **p<0.005 vs WT.

Screening Assay *h*GPCR_X1 stable cell line HEK293 is available; then, Fluorometric Imaging Plate Reader (FLIPR) assay can be performed for the measurement of intracellular calcium mobilization. Thus, high-throughput screening campaign can be run to identify *h*GPCR_X1 antagonists; as confirmation, further hit validation may be performed using cAMP assay.

Contact: Business Development | Email: bdcima@unav.es; Tel: +34 948 194 700