CHARACTERIZATION AND VALIDATION OF A NEW AMYLOID-β OLIGOMER AS A PHARMACOLOGICAL TARGET TO TREAT ALZHEIMER'S DISEASE

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1. Project summary

The brains of millions of people suffering from Alzheimer's disease (AD) are slowly and inescapably being depleted of neurons. However, the cause of neuronal death is still unknown. This is the main reason why **AD has no current cure**. Indeed, available treatments are only directed to slowing dementia symptoms and only help improve the quality of life for a short period of time. Therefore, **treatments directed to the causes of AD are an unmet medical need.**

Several studies have proposed that the interaction of a protein called amyloid-beta (Aβ) with the neuronal membrane is responsible for the observed neuronal death in AD.[1] However, the Aβ protein is a very difficult therapeutic target because it is very sticky, and consequently has a strong propensity to self-assemble leading to associations containing different numbers of Aβ molecules, which in turn can also adopt different shapes. Therefore, one of the main drawbacks in the development of effective therapies against AD is that the exact form of Aβ responsible for AD neurotoxicity is not known.[2] Without knowing the features that characterize this Aβ form, such as the number of Aβ molecules that make it and the shape they adopt, it is clearly difficult to design therapeutic strategies directed at the cause of AD.

In 2016, the group of Dr Carulla's Lab reported in vitro conditions to prepare a membrane-associated Aβ form, which involved the assembly of several Aβ molecules arranged in a barrel-shape structure.[3] Notably, this membrane-associated Aβ form has the capacity to form pores in cell membranes. On the basis of these properties, we named it β-barrel pore-forming Aβ oligomer (βPFO). Interpreted in the context of AD, this discovery suggests that βPFO can perforate the membrane of neurons, alter the equilibrium of ions between the inside and the outside of these cells, and consequently trigger their death. Therefore, we hypothesized that βPFO was the long-sought Aβ form responsible for the neurotoxicity in AD and consequently it was a potential new target to treat AD. A European patent application covers the procedure for the preparation of βPFO.

The research carried out during the project funded by La Marató combined the efforts of the research groups of Dr Natàlia Carulla at IRB Barcelona and the IECB in Bordeaux, of Dr Giovanni Maglia at the University of Groningen and Dr Serge

Muyldermans at the Vrije Universiteit Brussels in order **to characterize and validate βPFO as the Aβ form responsible for neurotoxicity in MA.** The strategy followed during the project is summarized in Figure 1. First, to characterize their mode of neurotoxicity and their structure, the latter meaning to establish the three-dimensional arrangement of all the atoms making the Aβ form under study. Second, to establish the presence of the newly characterized Aβ forms in vivo in relevant models of the disease to validate them as new AD targets. Third, to develop diagnostic and therapeutic strategies against the newly characterized and validated Aβ targets to contribute to the development of therapies against the cause of AD.

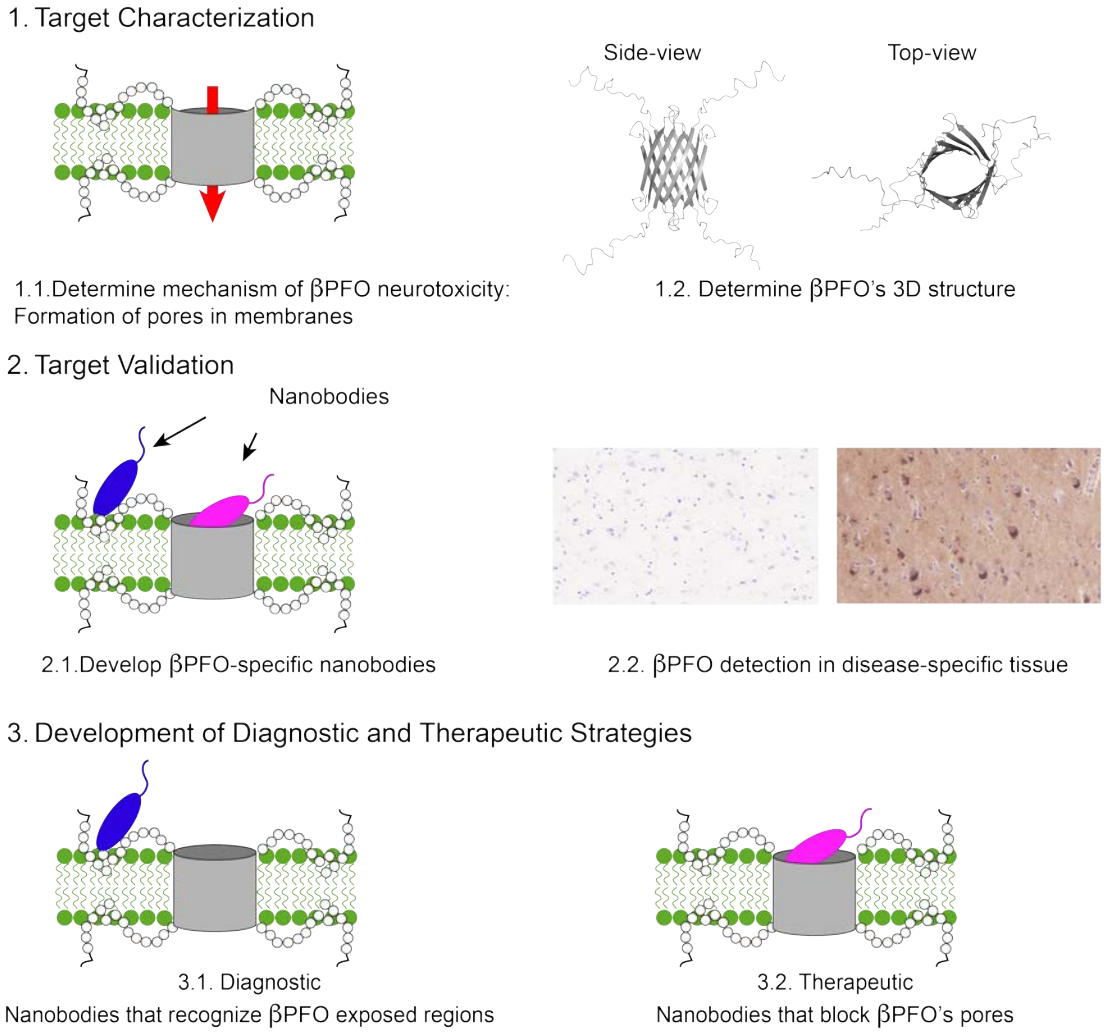

Figure 1. Schematics of the strategy used throughout the La Marató project to identify, validate, characterise and exploit βPFO as a new amyloid-beta target to treat AD.

2. Results obtained

Next, we summarize the progress achieved in the project:

- *Characterization of βPFO:* We have obtained the three-dimensional (3D) arrangement of all the atoms making βPFO. Obtaining this information will be crucial to design molecules that target βPFO formation and/or block the pores that βPFO forms.

- *Validation of βPFO in relevant AD models:* To detect βPFO in relevant AD models we have developed conformation-specific nanobodies. These are small antibody molecules that specifically recognize βPFO. After the in vitro characterization of the βPFO-nanobody interaction we are now using these nanobodies to establish the presence of βPFO in vivo in relevant AD. The specific detection of βPFO in AD brain tissue relative to control brain will allow us to validate βPFO as a new target for AD.

- *Development of diagnostic and therapeutic strategies against* βPFO: The nanobodies developed recognize different βPFO portions of the βPFO such as exposed regions and the pore cavity. Notably, some of them block the pores formed by βPFO. Nanobodies are being explored in diagnosis and as therapeutics in many fields of medicine, including oncology, inflammatory, infectious and neurological diseases.[4] Upon validation of βPFO, the βPFO-specific nanobodies generated throughout this project could contribute to the development of diagnostic (i.e. those that recognize membrane exposed regions) and therapeutic (i.e. those with pore-blocking activity) strategies against AD.

3. Relevance and potential future implications

In summary, our research has the potential to identify, characterize and validate new therapeutic targets for AD as well as to develop new AD drugs with new mechanisms of action directed at the causes of AD. The AD drug development pipeline of 2017 does not include any drug targeting Aβ pores[5] so **molecules targeting *βPFO* formation and/or blocking the pores it forms could aspire to be first in class.** Given this potential, we believe that the scientific and social interest of this project is enormous.

4. Literature generated

1. Serra-Batiste, M.; Bayoumi, M.; Gairí, M.; Ninot-Pedrosa, M.; Maglia, G., Carulla, N. *Aβ42 assembles into specific β-barrel pore-forming oligomers in membrane-mimicking environments* Proc. Natl. Acad. Sci. USA 113, 10866-10871 (2016). doi: 10.1073/pnas.1605104113. IF 9.7. Citations: 25. Q1.

2. Serra-Batiste, M.; Tolchard, J. Giusti, F.; Zoonens M.; Carulla N. *Stabilization of a membrane-associated amyloid-β oligomer for its validation in Alzheimer's disease.* Invited to contribute to the Research Topic "Structural and Molecular Biology of Alzheimer's Disease", Front. Mol. Biosci. 19, 5-38 (2018). doi: 10.3389/fmolb.2018.00038. IF: not available. Q1

3. Serra-Batiste, M.; Ninot-Pedrosa, M.; Puig. E.; Ciudad, S.; Gairí M.; Carulla, N. *Preparation of well-defined and stable β-barrel pore-forming Aβ42 oligomers.* Invited to contribute to the third edition of the volume on Amyloid Proteins in Method Mol. Biol. 1779, 13-22 (2018). doi: 10.1007/978-1-4939-7816-8_2. IF: not available. Q3

4. Ciudad, S#; Puig, E.#; Bardiaux, B; Botzanowski, T; Mayzel, M; Bayoumi, M; Chaignepain, S; Maglia, G; Orekhov, V; Cianferani, S; Carulla, N (#both authors have contributed equally to this work). *Structure of pore-forming amyloid-beta tetramers and octamers*. Manuscript in preparation.

Patent

TITLE: *Amyloid beta peptide oligomers and uses thereof*
INVENTORS: Serra-Batiste, M.; Ninot-Pedrosa, M.; Maglia, G., Carulla, N.
PATENT NO: EP16382123.4

References

1. Arispe, N., Diaz, J. C. & Simakova, O. Aβ ion channels. Prospects for treating Alzheimer's disease with Aβ channel blockers. *BBA-Biomembranes* **1768,** 1952–1965 (2007).

2. Benilova, I., Karran, E. & De Strooper, B. The toxic Aβ oligomer and Alzheimer's disease: an emperor in need of clothes. *Nat. Neurosci.* **15,** 349–357 (2012).

3. Serra-Batiste, M. *et al.* Aβ42 assembles into specific β-barrel pore-forming oligomers in membrane-mimicking environments. *Proc. Natl. Acad. Sci. U.S.A.* **113,** 10866–10871 (2016).

4. Steeland, S., Vandenbroucke, R. E. & Libert, C. Nanobodies as therapeutics: big opportunities for small antibodies. *Drug Discov. Today* **21,** 1076–1113 (2016).

5. Cummings, J., Lee, G., Mortsdorf, T., Ritter, A. & Zhong, K. Alzheimer's disease drug development pipeline: 2017. *Alzheimer's & Dement.* **3,** 367–384 (2017).