alzheimer's association
AAIC 20
ALZHEIMER'S ASSOCIATION INTERNATIONAL CONFERENCE®
July 27-31, 2020 > Virtual Event

Epitope prediction for oligomer-selective antibodies in tau and Aβ

Steven S. Plotkin[1,2], Aina Adekunle[1,2], Ebrima Gibbs[2,4], Andrei Roman[2,4], Xubiao Peng[1,3], Shawn Hsueh[1,2], Beibei Zhao[2,4], Sarah Louadi[2,4], Johanne Kaplan[2], Neil R. Cashman[2,4]
[1]Departments of Physics and Genome Sciences, University of British Columbia, [2]ProMIS Neurosciences, Toronto, Ontario, Canada, [3]Beijing Institute of Technology, [4] Department of Medicine, University of British Columbia

ABSTRACT

Oligomer-selective epitope predictions are presented for tau and Aβ, using a computational method that we have developed. Epitopes are conformationally distinct from those presented in functional healthy protein. Cyclic peptide epitope scaffolding is employed in animal immunizations. Preclinical antibodies have selectivity for pathogenic tau and Aβ species. SPR measurements confirm selective binding to synthetic oligomers and soluble pre-formed fibrils (PFFs), vs. native healthy protein. Antibodies showed little immunoreactivity to plaque or vascular Aβ deposits via immunohistochemistry. SEC fractionation of AD brain homogenate shows selective binding to toxic dimers, tetramers and dodecamers, in contrast to aducanumab and bapineuzumab. Tau antibodies recognized tau from AD brain extract, and inhibited seeding activity in a FRET assay. Aβ antibodies alleviated the cognitive deficits caused by oligomers in mouse NOR studies.

REFERENCES

1. See e.g. Rajamohamedsait, H et al. *Sci Rep* (2017), Dai, C-l et al. *Alz Res Therapy* (2017)
2. See e.g. Watts, JC and Prusiner SB, Cold Spring Harb Perspect Med (2018) Dujardin, S and Hyman, BT (2019). In Tau Biology, Ch 23. pp. 305–325.
3. Plotkin SS and Cashman NR *Neurobiol Dis* (2020).
4. Silverman, JM et al *ACS Chem Neurosci* (2018), Gibbs et al. Sci Rep (2019).
5. Peng, X et al. J Phys Chem B (2018).

CONTACT

Steven S. Plotkin
Associate Professor, Depts of Physics
and Genome Sciences & Technology
University of British Columbia
Email: steve@phas.ubc.ca
or steven.plotkin@promisneurosciences.com

BACKGROUND

Previous studies of Alzheimer's disease (AD) pathology point to cytotoxic tau as a cause of neuronal cell death, which is induced or exacerbated by soluble misfolded Aβ oligomers [1]. Soluble misfolded species of both tau and Aβ are both observed to propagate in prion-like fashion [2]. A method for generating antibodies to tau and Aβ that are conformationally-selective to propagative misfolded oligomeric forms, and which also have low affinity to isolated monomers or, particularly for Aβ, low affinity to fibrils, is thus a highly desired goal that holds significant promise for AD therapy.

Current antibody therapies to tau and Aβ are not selective for oligomers, but also bind monomer and plaque [3]. However, numerous lines of evidence point to oligomeric, prion-like conformational strains as the toxic, propagating species of these proteins [4].

Aβ and tau fibrils, as well as their oligomers, are polymorphic, thus they are difficult to experimentally characterize. We thus exploit computational modelling to predict solvent exposed- and conformationally specific epitopes on the surfaces of oligomers. These epitopes are then scaffolded for active immunization to generate oligomer-selective antibodies as passive immunotherapeutics.

RESULTS

HOW WE PREDICT OLIGOMER-SPECIFIC EPITOPES

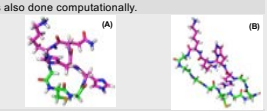
- **Hypothesis**: Regions of protofibrils that become solvent exposed when a protofibril is subject to partial denaturing stresses are solvent exposed on toxic oligomers.
- Since these conformational motifs are intermediate between fibrils and in a different conformation in the monomer, they are enriched *for oligomer-selective* epitopes.
- **Method**: Predict regions of low thermodynamic stability in the oligomer model, by challenging protofibril structures with a *"collective coordinate" global bias*, in a molecular dynamics simulation [5].
- This forces the protofibril to unfold, say, to be 1/3 unfolded. The protofibril energetics and entropics determine *where* it will unfold.
- Predicted epitopes are local regions that show increased solvent exposure, decreased interactions, and increased dynamics.
- Applying the method to Aβ yields 6 epitopes, two of which are ^{13}HHQK^{16} and ^{25}GSNKG^{29}.
- Applying the algorithm to tau allows epitopes to be identified.

RESULTS

Figure 1. Stressing **(A)** Aβ and **(B)** tau fibril structures, to computationally identify oligomer-selective epitopes.

- **Raising antibodies**: Scaffolded immunogens are constructed to present these epitopes in oligomer-like conformations. Cyclic peptides of the epitopes of different sizes to find the best construct, which is most distinct from the fibril or linear peptide ensembles. This predictive aspect is also done computationally.

Figure 2. Cyclic peptides of **(A)** CGHHQKG and **(B)** CGGGGHHQKGGGG.



- **Clustering analysis** is performed on computationally-generated ensembles of the isolated (linear) monomeric peptide, the fibril equilibrium ensemble, the stressed, disrupted protofibril ensemble, and the candidate cyclic peptide constructs.
- PDB structures in the human proteome are also analyzed for structural similarity, to avoid off-pathway targets.
- The analysis is done in high-dimension, but may be rendered in 3D:

RESULTS

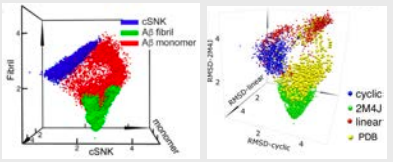
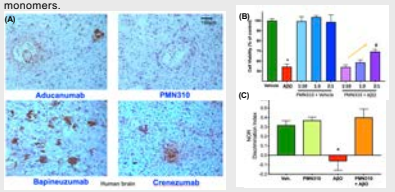


Figure 3. The cyclic **CGSNKGG** epitope is structurally distinct from fibrillar and monomeric Aβ. Too distinct is unphysical, yet physical conformations can lead to proteomic target distraction for proteins displaying the epitope. Scanning for the sequence across the human proteome, and analyzing the PDB for structural overlap, addresses this issue.

- SPR to screen clones against cyclic and linear epitopes, as well as *in vitro* Aβ oligomers and monomers.

Figure 4. (A) SPR results show that binding of antibodies (mAbs) to cyclic conformations of the Aβ epitope are preferred over linear conformations. **(B)** mAbs bind more strongly to oligomers than monomers **(C)** mAbs raised to the tau cyclic peptide epitope bind more strongly to tau oligomers than monomers.



RESULTS

Figure 5. (below left) **(A)** IHC staining of AD brain sections shows no binding by PMN310 to plaque, in contrast to other mAbs in clinical trials. Nuclei are stained with Haematoxylin. **(B)** PMN310 increases cell viability in an *in vitro* MTT assay, by neutralizing the toxic effects of Aβ oligomers. **(C)** In a novel object recognition (NOR) assay in mice, PMN310 completely prevents the loss of short-term memory formation caused by toxic oligomers.

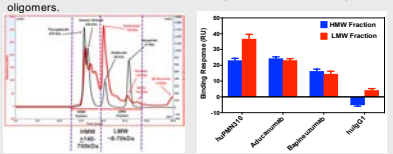


Figure 6. (A) Size exclusion chromatography (SEC) shows a reproducible pattern of brain extract fractionation. **(B)** PMN310 showed preferential binding by SPR to the LMW fraction of AD brain extract enriched for toxic dimers, tetramers, and dodecamers, in contrast to aducanumab and bapineuzumab

Figure 7. In an in-cell aggregation assay probed by FRET activity, most tau oligomer-selective mAbs inhibited intracellular aggregates induced by tau pre-formed fibrils (PFF).

CONCLUSIONS

Lead antibodies to tau and Aβ developed using rationally designed conformational epitopes are likely to achieve greater therapeutic potency by selectively targeting soluble toxic oligomers, and reducing the risk of target distraction and ARIA.