Novel Aβ Oligomer-Specific Epitopes: A Hypothesis-Driven Approach to Alzheimer's Immunotherapeutics

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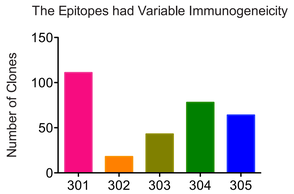
Background: Alzheimer's disease (AD) Aβ immunotherapeutics in humans have been hampered by negative side-effects including ARIA-E and cerebral microhemorrhage[1], mediated by antibody binding to non-toxic fibrils and/or monomers[2]. Recent advances have demonstrated the fundamental role of amyloid-β oligomers (AβO) in disease onset[3], synaptotoxicity[4] and progression[5,6]. Together with promising results from clinical trials targeting aggregated Aβ[7], these data suggest that by targeting AβOs, a blockade of cognitive decline in humans is possible. We used computational epitope discovery methods to identify immunologic targets on toxic and propagating AβO. The target profile of the AβO-specific antibodies is murine monoclonal antibodies that react with synthetic oligomers over monomers with an affinity of ~100/1, or react with native oligomers in brain and CSF with low affinity binding to monomers, and do not react with plaques.

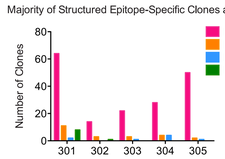
1 Panza, F. et al. Curr Opin Psychi (2014).
2 Winblad, B. et al. Lancet Neurol (2016).
3 Lesne, S. E. et al. Brain (2013).
4 Ferreira, S. T. et al. Front in Cell Neurosci (2015).
5 Jaunmuktane, Z. et al. Nature (2015).
6 Ye, L. et al. Brain Pathol (2015).
7 Patel, K. R. Manag Care (2015).

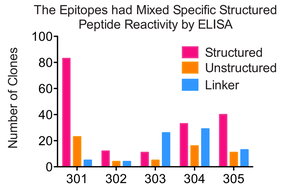
Methods: Proprietary algorithms have computationally predicted five AβO-specific epitopes (see Poster #12344, session P4-14). The epitopes were synthesized, structured through cyclization, and conjugated to immunogens, validated for sequence and structure, and used to produce monoclonal antibodies. ELISA and Surface Plasmon Resonance (SPR) was used to screen clones against structured and unstructured epitopes and synthetic AβOs and monomers.

Results:
Together the five epitopes produced more than 300 clones.



ELISA prescreen of hybridoma supernatants found specificity for the structured epitopes vs. unstructured epitopes was varied, and appeared to be maximal with the 301 epitope. A proportion of the clones were reactive to the KLH-epitope linker peptide. These were excluded from further investigation. The majority of the clones were of the IgG isotype.

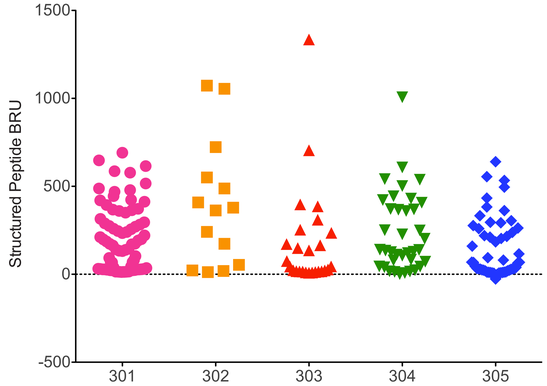


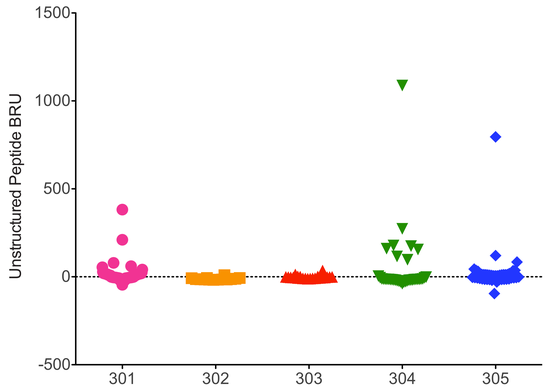


Surface Plasmon Resonance detailed screening of Hybridoma Supernatants

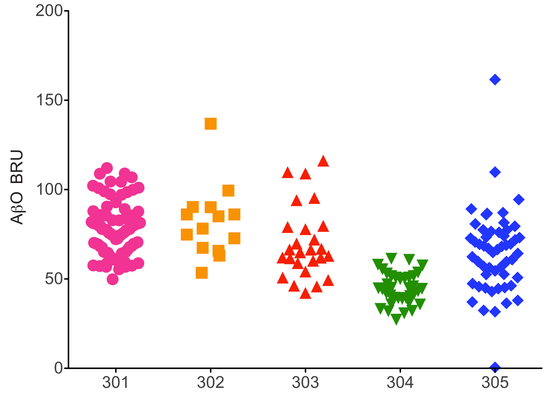
Only clones specific to structrured epitope by ELISA are shown.

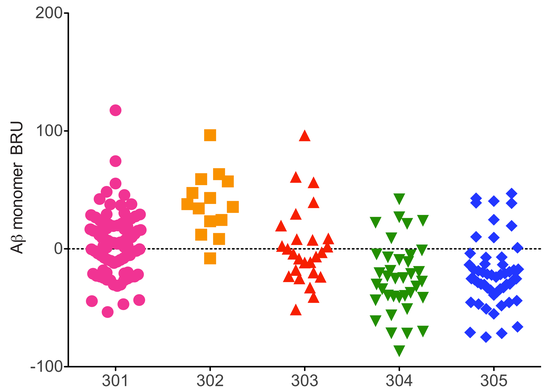
The clones had varied reactivity to cognate structured epitopes and minimal reactivity to unstructured peptide.



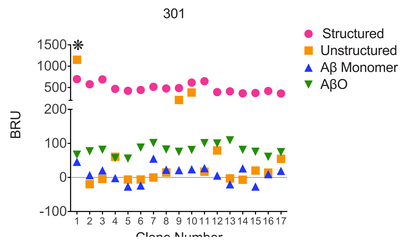


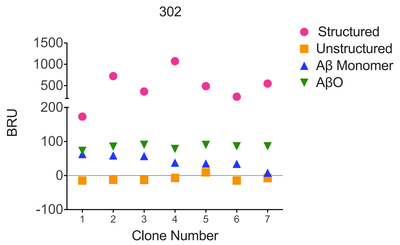
Reactivity to Aβ oligomers was evident, while monomer reactivity was almost completely absent in 3 of 5 epitopes.

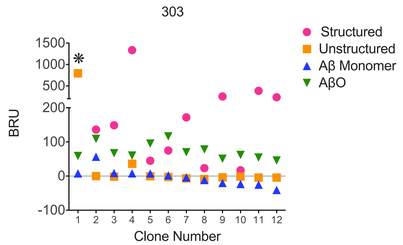




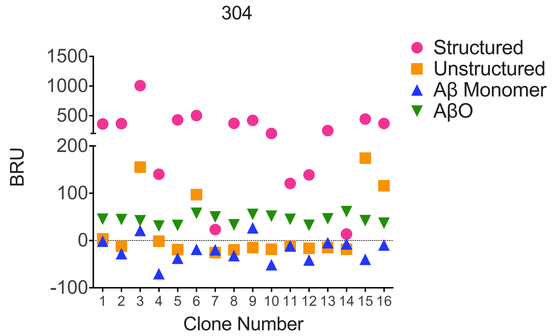
For each epitope the comparitve biding profiles of the selected clones are shown below. In some cases, clones reactive to the unstructured epitope were chosen as a control (indicated by ✻).

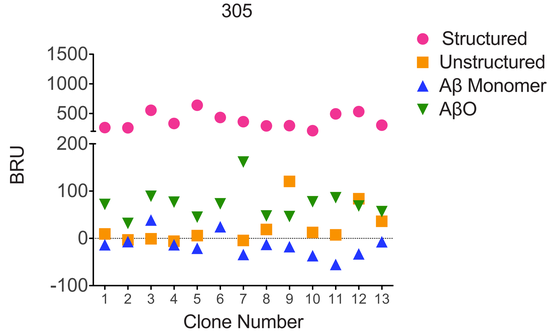






Comparitve biding profiles of the selected clones.





Next steps: Further validation will include screening of purified antibodies (SPR, immunoprecipitation, immunoblotting) against brain extract and CSF from AD patients and age matched human controls. Antibodies with cross reactivity to monomers and/or fibrils and plaques, determined by immunohistochemistry in brain tissue, will be rejected. Oligomer specific antibodies with no off-target binding will be evaluated in vitro and in vivo for the ability to neutralize neurotoxicity and propagation.

Conclusions: The computational epitope discovery approach utilized here may produce multiple AβO-specific antibodies. If successful, the approach may be applicable to other proteins and protein misfolding diseases.

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