ProMIS™ Neurosciences

NOVEL APPROACH TO OPTIMIZATION OF ALPHA-SYNUCLEIN VACCINE COMPOSITION FOR MAXIMAL TARGETING OF TOXIC ALPHA-SYNUCLEIN SPECIES

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Johanne Kaplan, PhD*
Chief Development Officer

*Disclosure: Employee of ProMIS Neurosciences

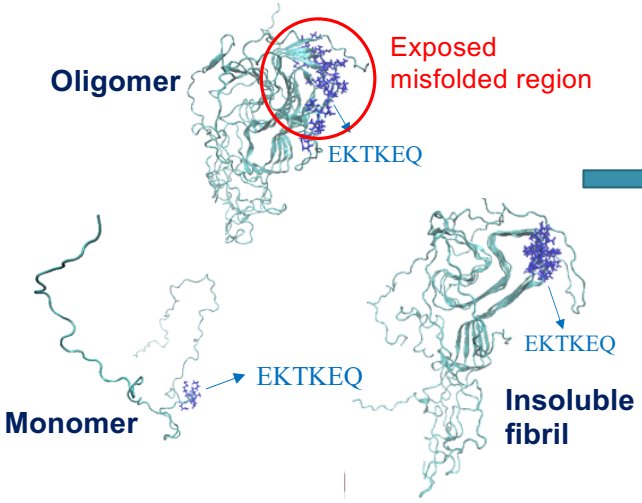
Designing an optimal alpha-synuclein vaccine

- Toxic alpha-synuclein (ASyn) aggregates drive the pathogenesis of Parkinson's disease and other synucleinopathies
  - ASyn toxicity resides primarily with the oligomeric form as opposed to monomers or insoluble fibrils (Lewy bodies/dendrites)[1,2]
  - Disease progression is driven by oligomers and small soluble fibrils of ASyn that possess seeding activity and propagate from cell to cell in a prion-like manner *in vitro*[3] and *in vivo*[4]
- Vaccination against pathogenic species of Asyn has the potential to protect against synucleinopathies
- ProMIS approach:
  - Use conformational ASyn B cell epitopes exposed only on pathogenic species of ASyn (oligomers, small fibrils)
    - Maximizes the dose of antibody reaching the pathogenic target -> No antibody wasted to cross-reactivity with the more abundant non-toxic forms of ASyn in blood and CNS
    - Preserves normal ASyn function
  - T helper epitopes provided by a carrier protein (KLH) not expressed in the brain
    - Circumvents the risk of T cell inflammatory response in the brain

[1]Fusco et al, 2017, Science; [2]Westphal & Chandra, 2013, J Biol Chem; [3]Choi et al, 2018, Cell Reports; [4]Peelaerts et al, 2015, Nature

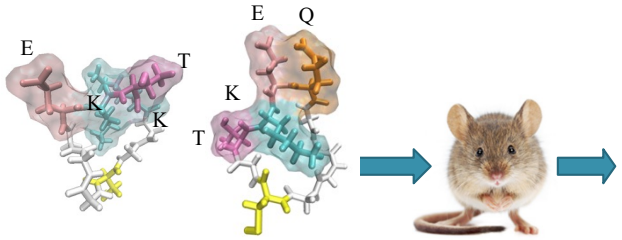
ProMIS platform applied to alpha-synuclein vaccine design

Computational Modeling



Identification of regions (conformational epitopes) likely to be exposed in toxic ASyn oligomers and small seeding fibrils but not in monomers or insoluble fibrils (Lewy bodies)

Vaccination with conformational alpha-synuclein epitopes

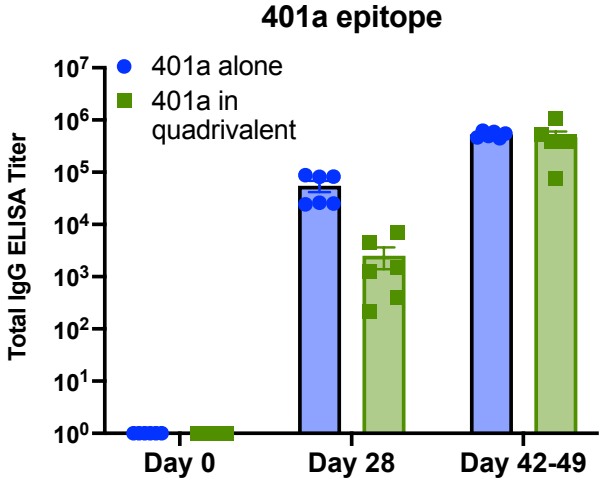


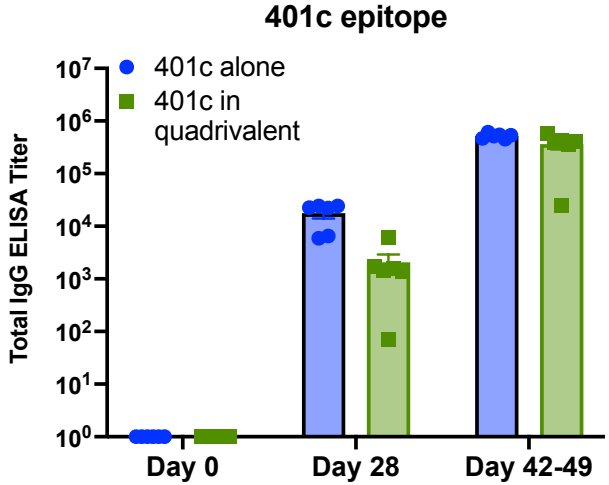
- Conformation of exposed, misfolded epitopes reproduced with cyclized peptides
- Coupled to KLH for T cell help
- QS-21 adjuvant

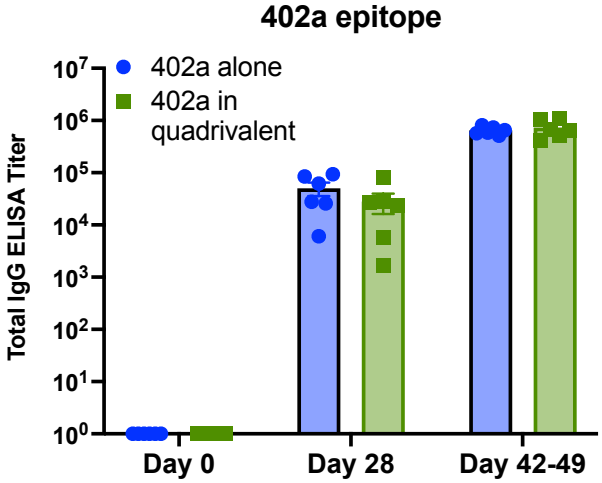
Read-outs

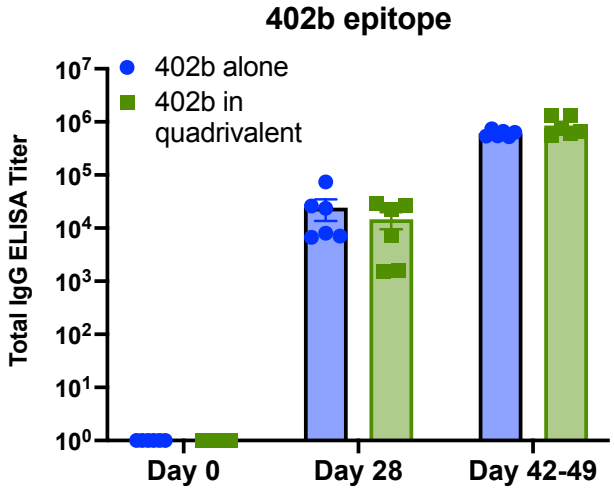
- ELISA IgG titers
- Selectivity profile
  - Pathogenic alpha-synuclein vs monomers (SPR)
  - Lewy bodies/neurites (IHC)
- Selection of optimal vaccine design
  - Reactivity of immune IgG with soluble toxic species from dementia with Lewy bodies (DLB) brains (SPR)

Pathogenic alpha-synuclein conformational peptide epitopes elicit a robust antibody response



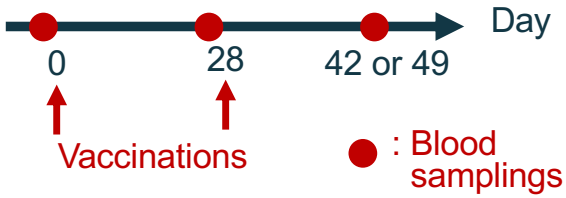






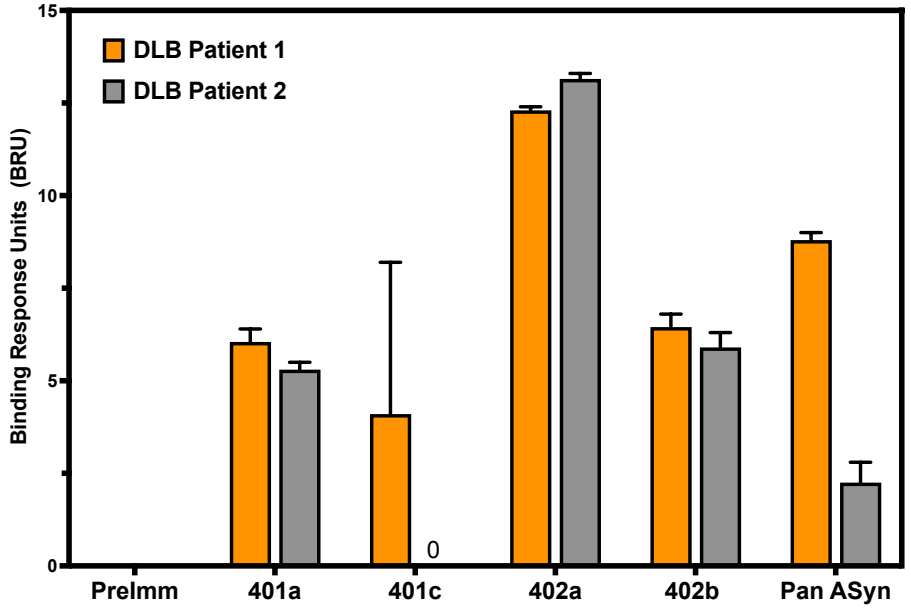
Total IgG ELISA titers

- All 4 alpha-synuclein conformational peptide epitopes elicited robust antibody titers when delivered alone or as part of a quadrivalent vaccine

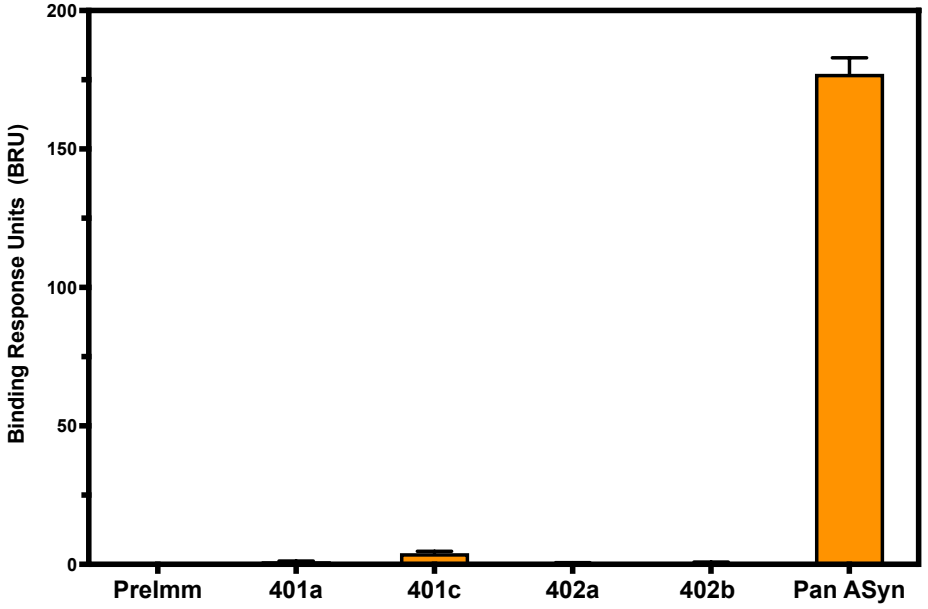


The antibodies induced by conformational alpha-synuclein epitopes are selective for soluble pathogenic species in DLB brain homogenates

Binding to soluble ASyn in DLB brains



No binding to ASyn monomers

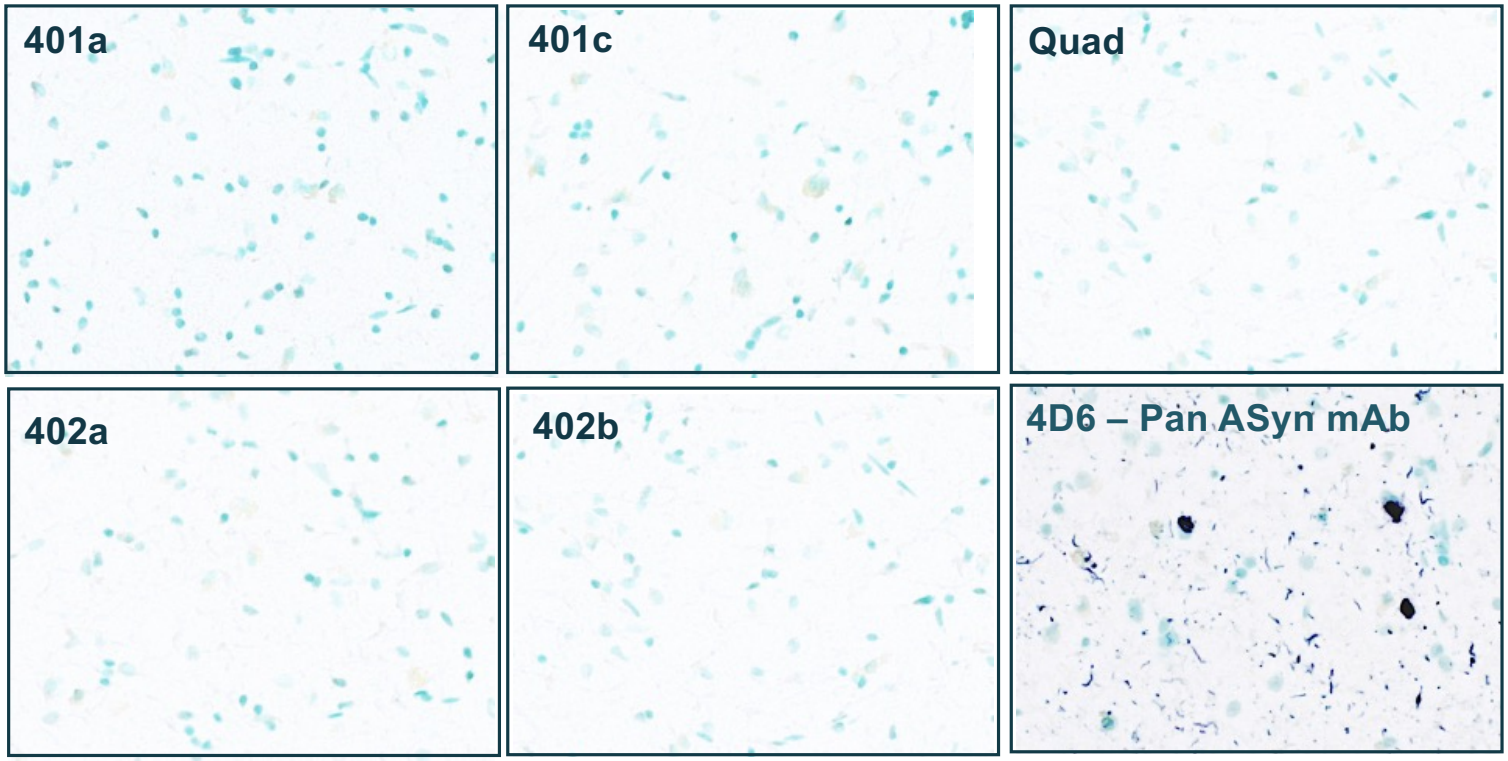


Antibodies in immune sera bind soluble pathogenic ASyn in DLB brain and not monomers by surface plasmon resonance (SPR)

- Day 42, purified immune IgG immobilized on sensor chip
- Preimm = Purified IgG from pre-immune serum
- Positive control: Pan Asyn antibody 4D6

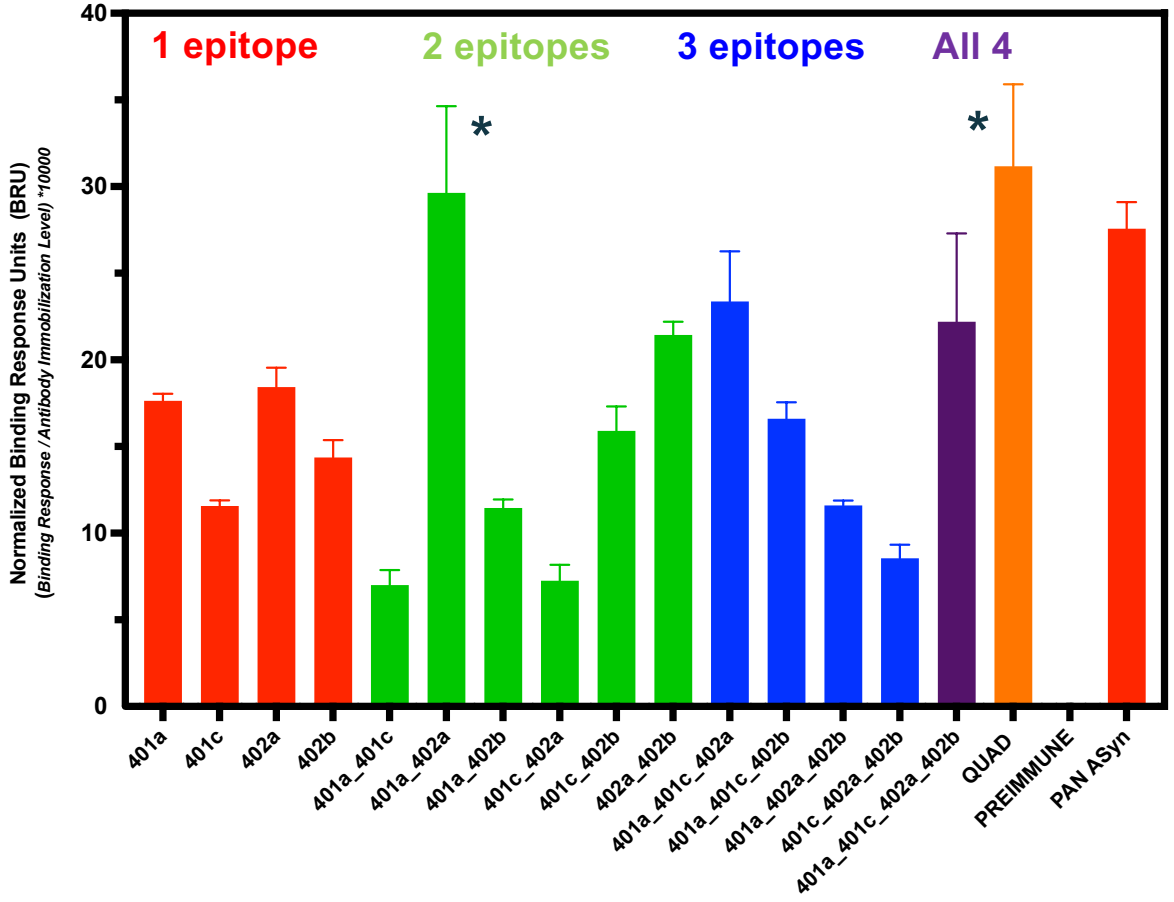
- Monomers or DLB soluble brain homogenate injected over the surface
- Pre-immune serum background subtracted

The antibodies induced by conformational alpha-synuclein epitopes do not bind Lewy bodies/neurites in DLB brain -> Selective for soluble toxic species



- Quad = Quadrivalent vaccine
- Day 42 (monovalent vaccine) or 49 (quadrivalent vaccine) antisera
- Positive control: 4D6, pan ASyn mAb
- 40X magnification
- No signal on normal, control brain

Maximal binding to DLB brain homogenate is achieved with immune IgG elicited by vaccination with two select conformational epitopes or a combination of all four



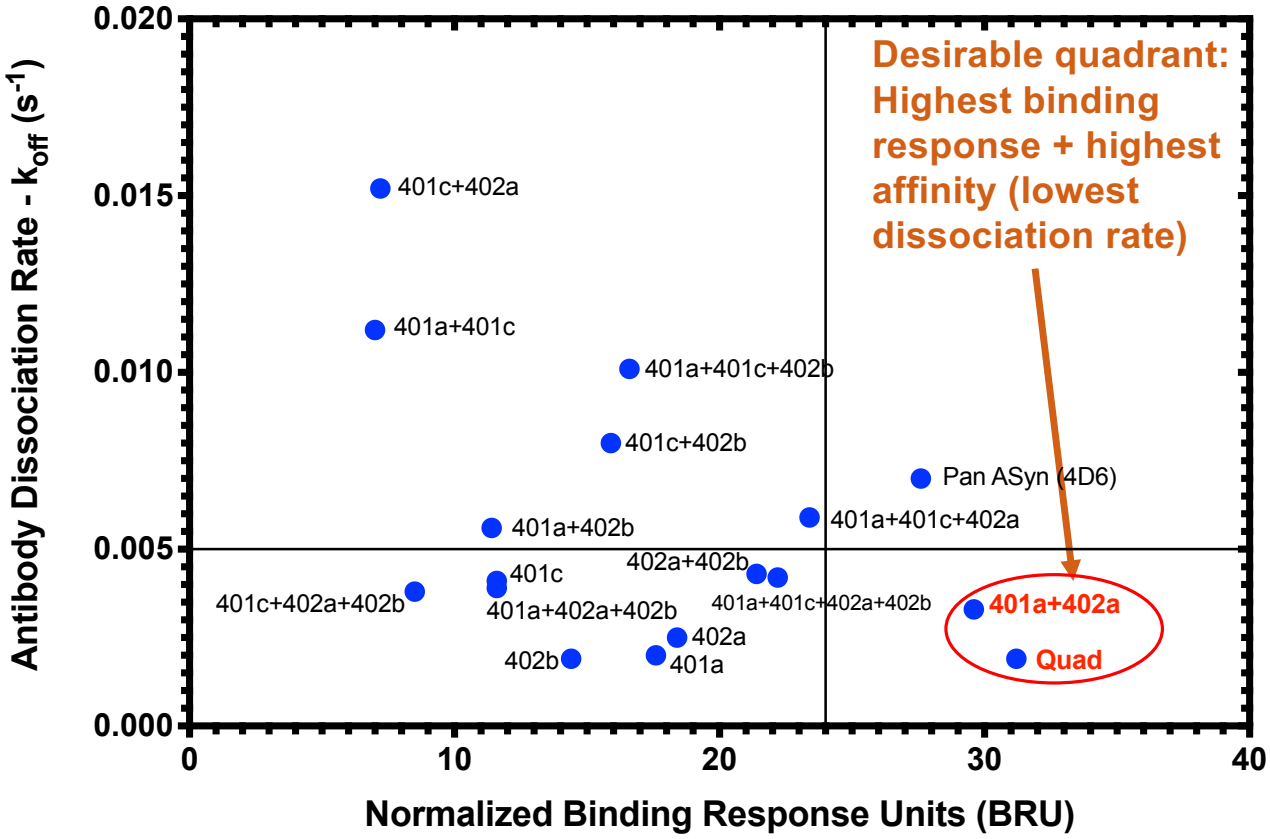
Evaluation of 15 possible vaccine configurations

- IgG from immune serum of monovalent vaccines vs mixtures of 2, 3 or 4 sera were tested by SPR for binding to soluble pathogenic species in DLB brain homogenate
- Maximal reactivity was achieved with immune IgG against two of the epitopes or a combination of all four.

*Highest reactivity

- Day 42, purified immune IgG immobilized on sensor chip
- Pooled soluble homogenates from 3 DLB brains injected over the surface
- Binding response units normalized to the actual amount of immobilized IgG for each combination

Maximal reactivity and highest affinity for DLB brain homogenate is achieved with immune IgG elicited by vaccination with two select conformational epitopes or a combination of all four



Summary

- A robust antibody response was elicited by vaccination with 4 different conformational peptide epitopes of pathogenic ASyn conjugated to KLH and formulated with QS-21, an adjuvant approved for human use
- The serum antibodies elicited were selective for soluble pathogenic species of ASyn in DLB brain, with no detectable binding to monomers or Lewy bodies/neurites
- Evaluation of all 15 possible combinations of immune IgG to the 4 conformational epitopes indicated that maximal reactivity and highest affinity for pathogenic ASyn in DLB brain was achieved with immune IgG against a select two conformational epitopes or a combination of all four.

❖ Vaccination with conformational B cell epitopes elicited antibodies with the desired selectivity for pathogenic ASyn.

❖ The advantage of this approach, as opposed to inducing pan-ASyn reactivity, is the potential to preserve normal ASyn function and minimize the diversion of active antibody by the more abundant non-toxic forms of ASyn in blood and CNS.

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