ProMIS™ Neurosciences

Vaccination with Conformational Epitopes Derived from Computational Modeling Elicits Active Antibody Response Selective for Toxic Alpha-Synuclein Species

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*Disclosure: Employee of ProMIS Neurosciences

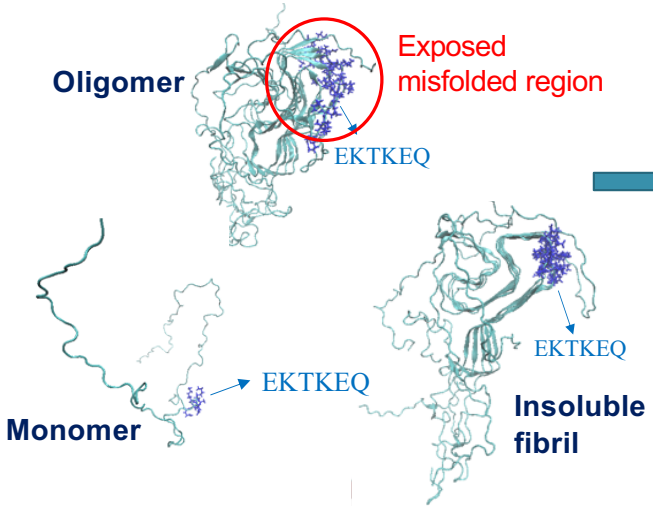
Designing an optimal alpha-synuclein vaccine

- Toxic alpha-synuclein (ASyn) aggregates drive the pathogenesis of Parkinson's disease and other synucleinopathies
  - **ASyn toxicity** resides primarily with **oligomers** 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 toxic species of ASyn (oligomers, small soluble 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
KLH: keyhole limpet hemocyanin

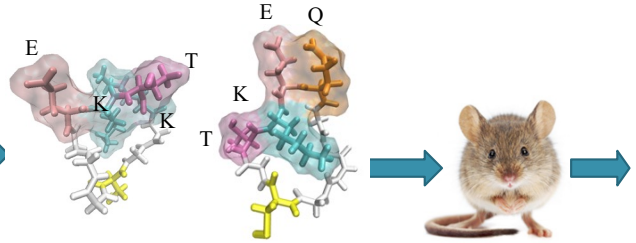
ProMIS platform applied to alpha-synuclein vaccine design

EpiSelect™ 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

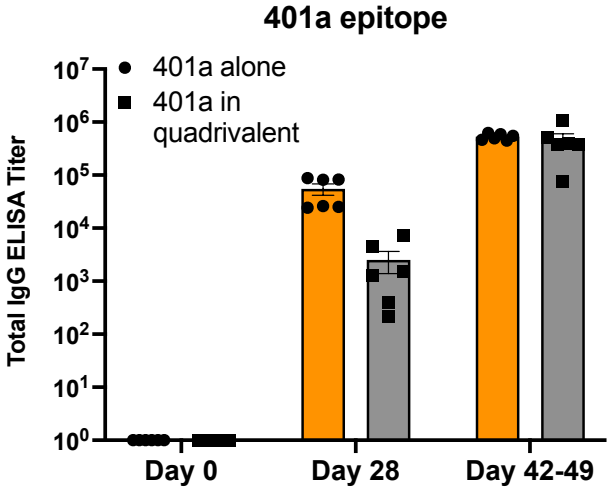


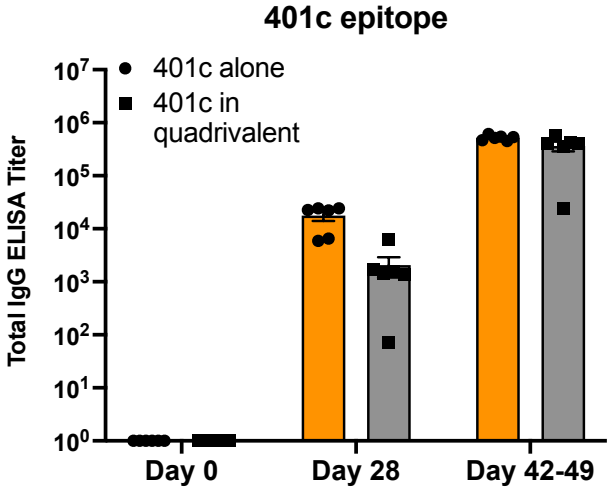
- Conformation of exposed, misfolded epitopes reproduced with cyclized peptides
- Coupled to KLH for T cell help
- QS-21 adjuvant

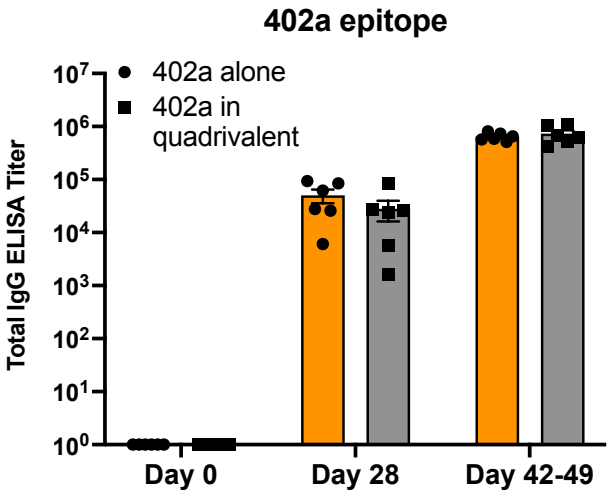
Read-outs

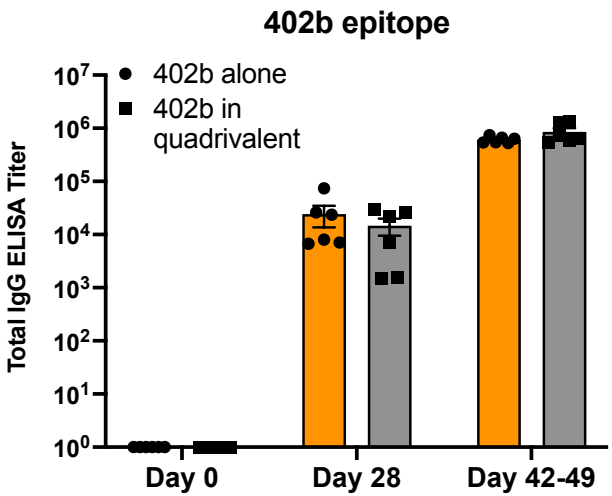
- ELISA IgG titers
- Selectivity profile
  - Pathogenic alpha-synuclein vs monomers (SPR)
  - Lewy bodies/neurites (IHC)
- Selection of optimal vaccine design
  - Reactivity of immune IgG with soluble toxic species from dementia with Lewy bodies (DLB) brains (SPR)

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



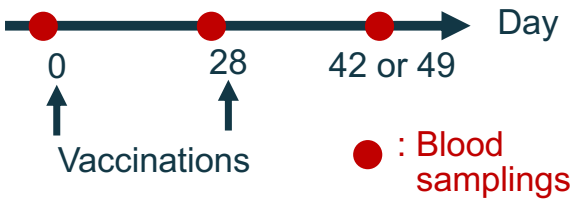




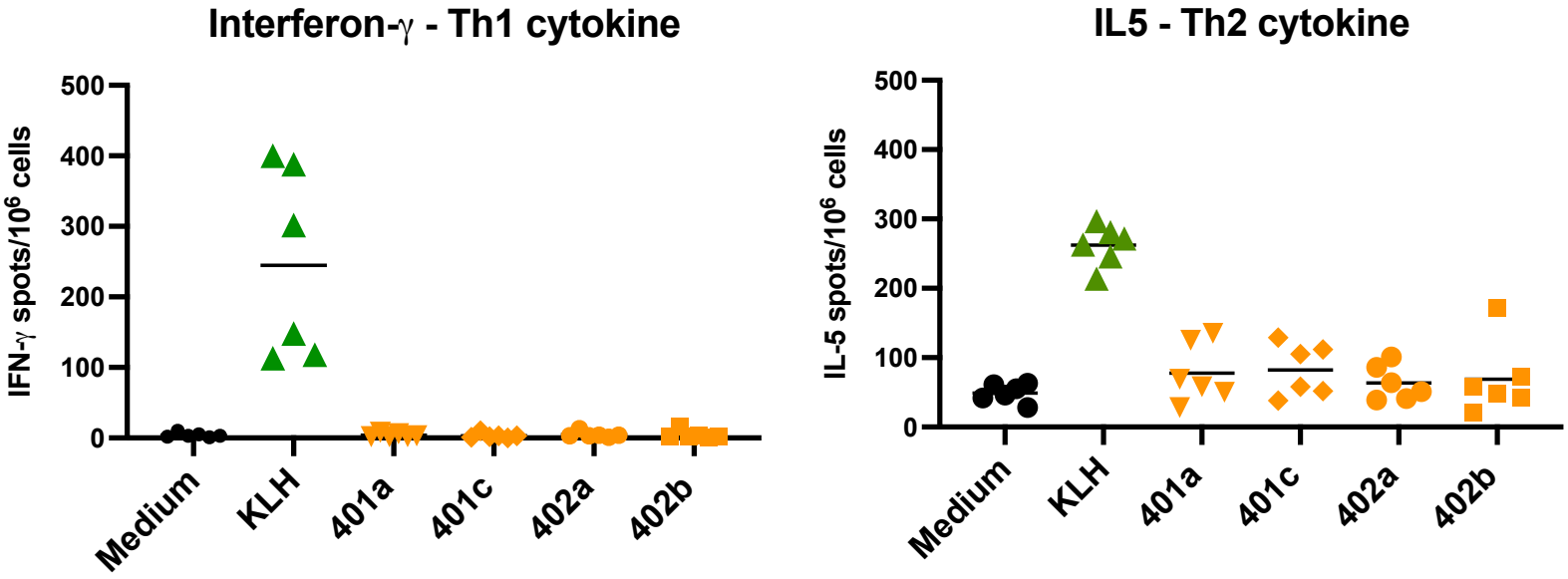


Total IgG ELISA titers

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



Only the KLH carrier protein, not conformational alpha-synuclein epitopes, elicits Th cell cytokines in ELISPOT assay – No detrimental inflammatory T cell response to alpha-synuclein

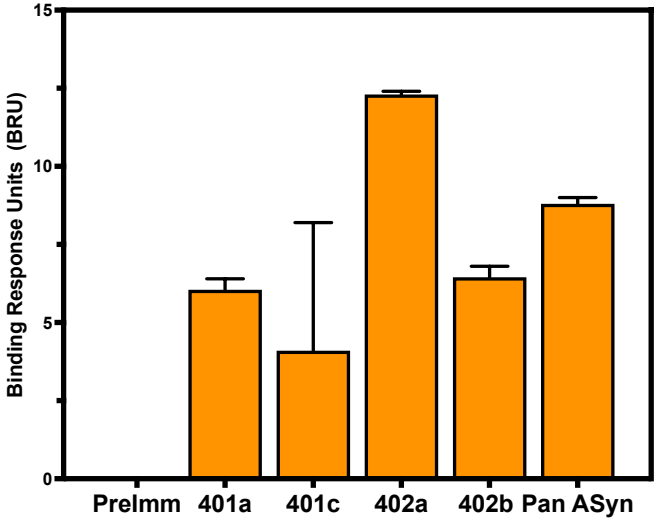


- The production of T helper cytokines in response to KLH stimulation confirms that KLH provides effective Th cell epitopes to support anti-pathogenic ASyn peptide antibody responses
- The background production of T helper cytokine in response to stimulation with conformational ASyn epitopes confirms that the peptides do not contain any Th cell epitope, only a B cell epitope

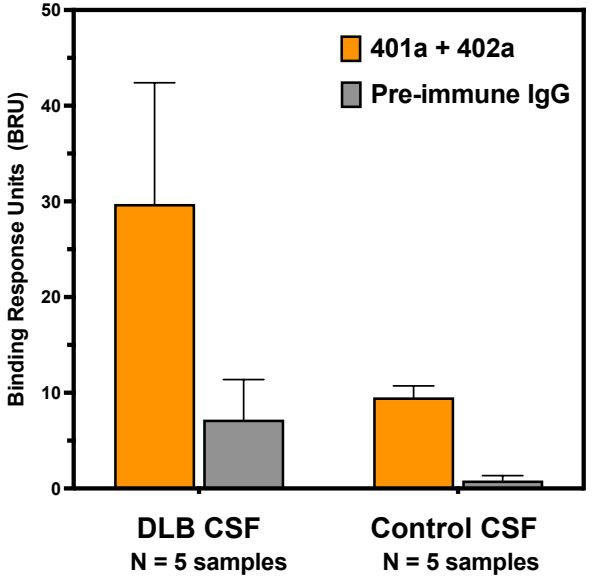
Spleens at day 49, quadrivalent vaccine shown here

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

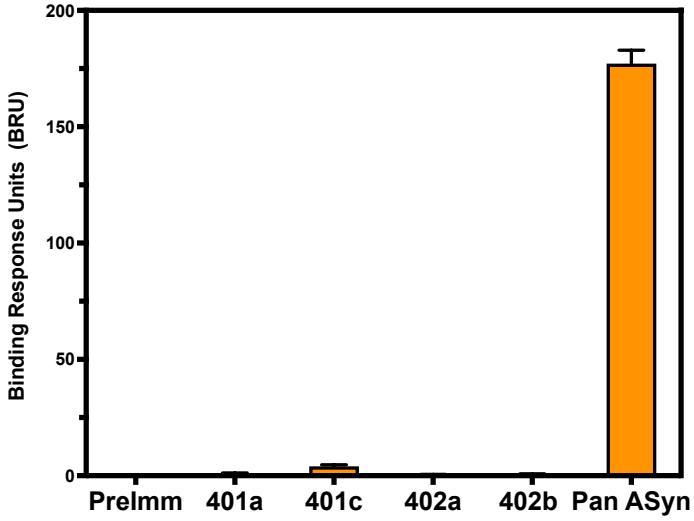
Binding to soluble ASyn in DLB brain homogenate



Binding to ASyn in DLB CSF



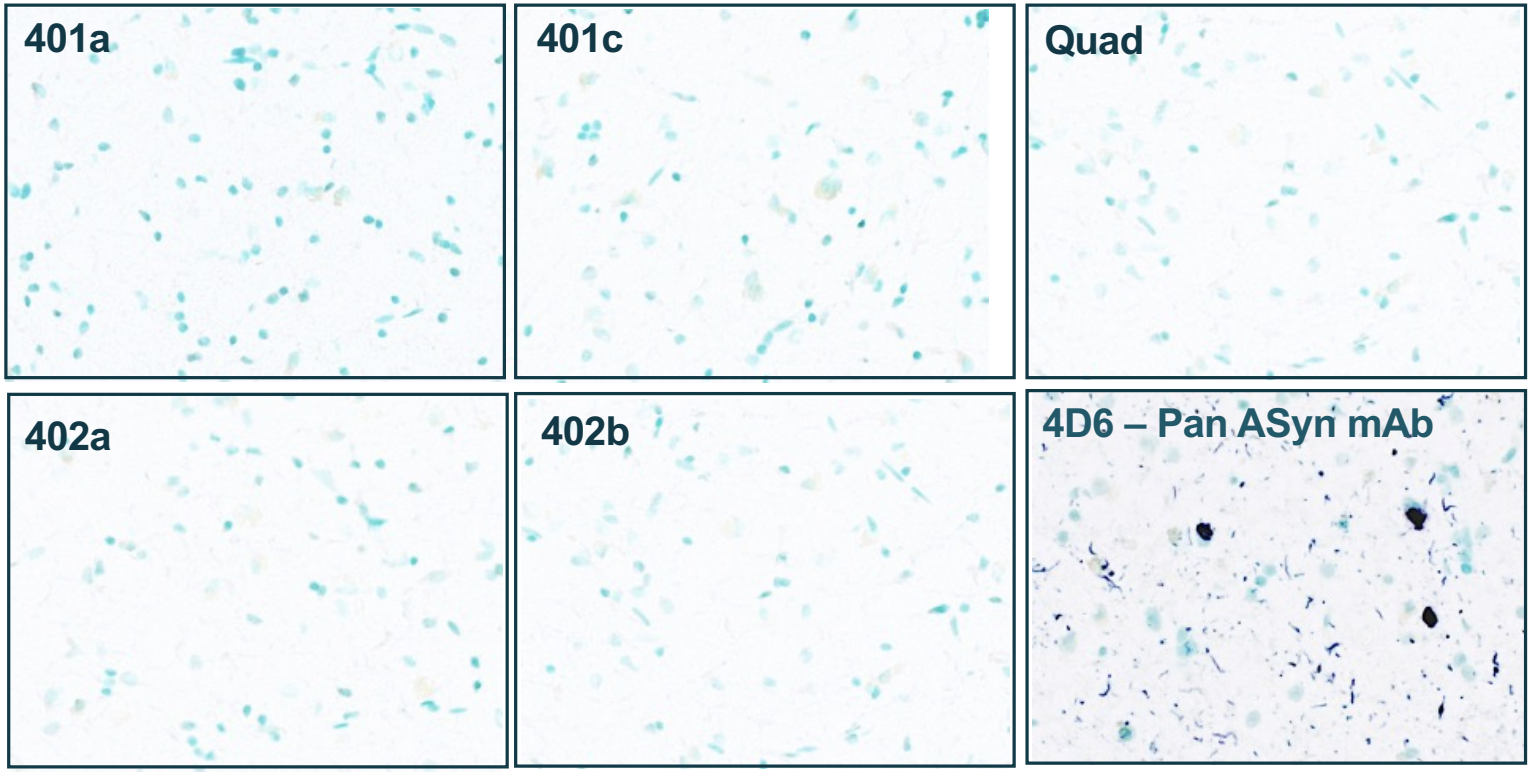
No binding to ASyn monomers



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

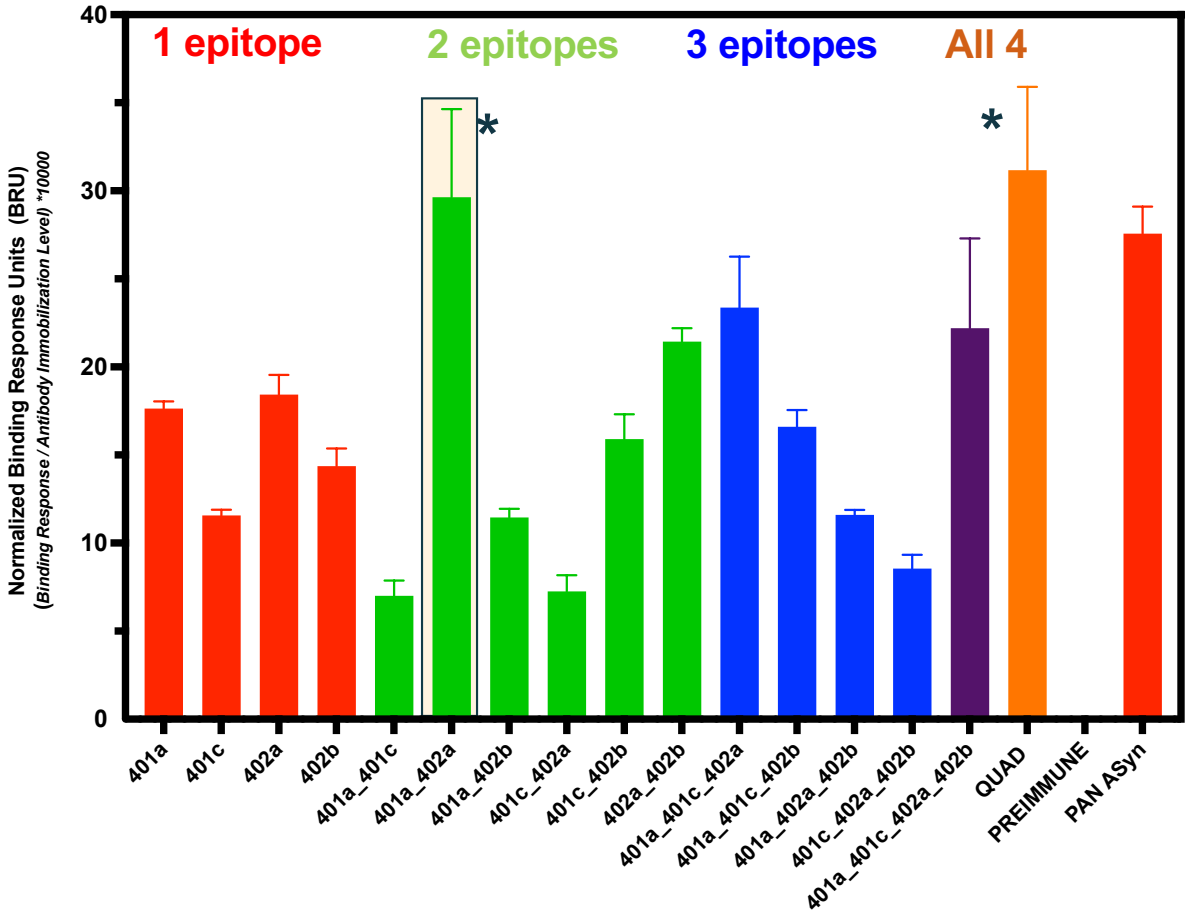
- Day 42, purified immune IgG immobilized on sensor chip
- Preimm = Purified IgG from pre-immune serum
- Pre-immune serum background subtracted
- Monomers or DLB soluble brain homogenate/CSF injected over the surface. DLB CSF from Dr. Stephen Pasternak, UWO
- Positive control: Pan Asyn antibody 4D6

The antibodies induced by conformational alpha-synuclein epitopes do not show reactivity with 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 specific conformational epitopes or a combination of all four



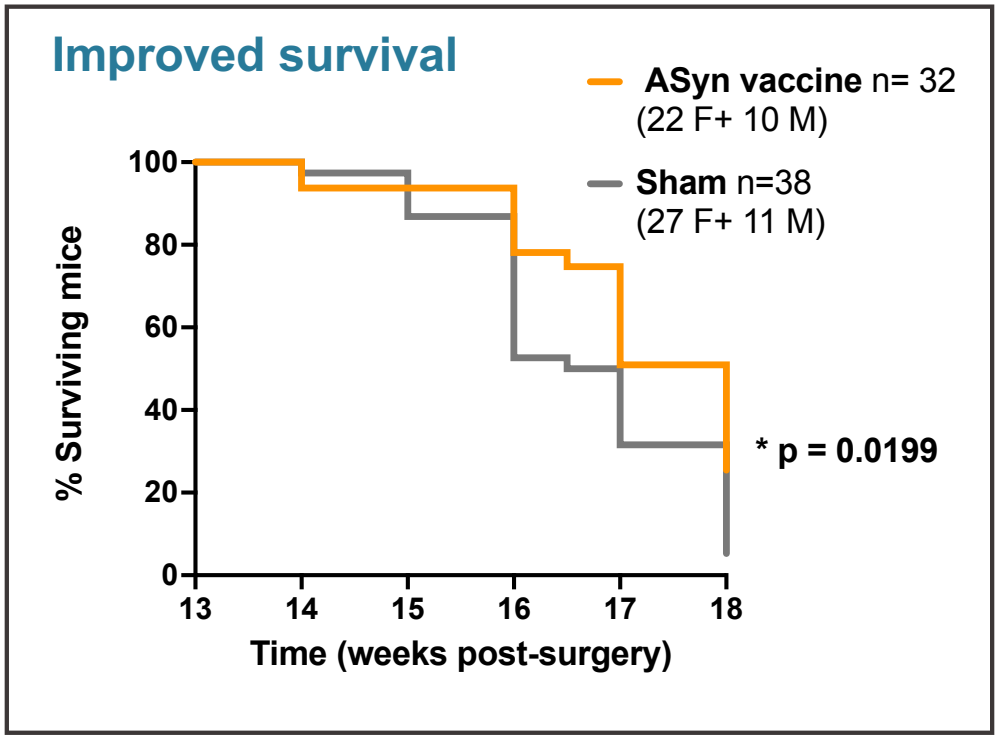
Evaluation of 15 possible vaccine configurations

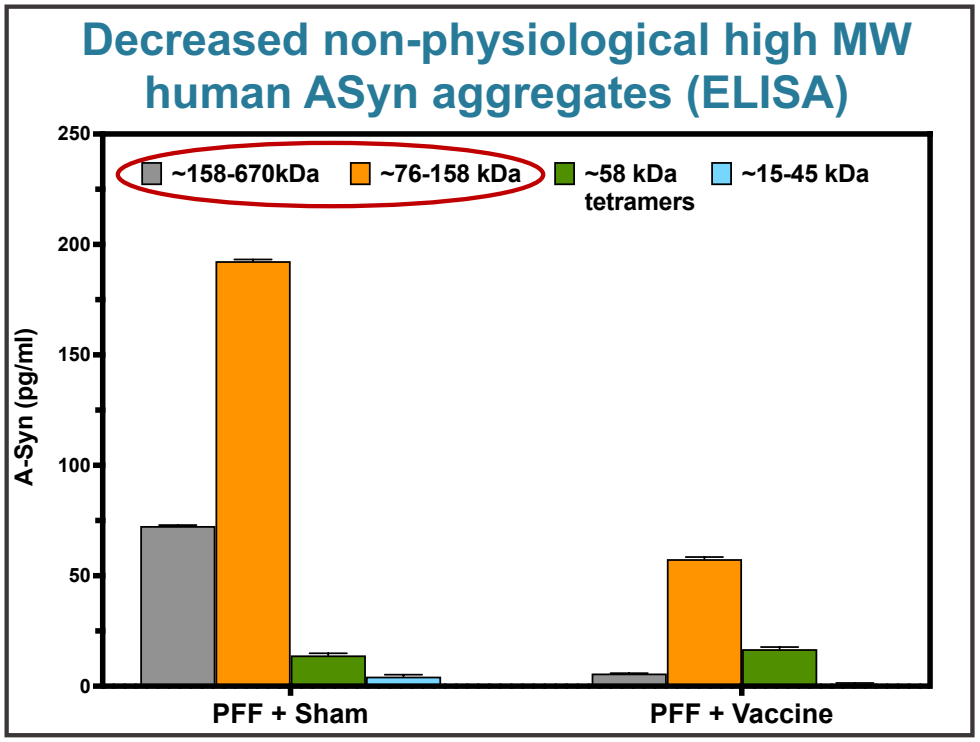
- IgG from immune serum of monovalent vaccines vs mixtures of 2, 3 or 4 sera were tested by SPR for binding to soluble pathogenic species in DLB brain homogenate
- Maximal reactivity was achieved with immune IgG against two of the epitopes or a combination of all four.
- Two-epitope vaccine chosen as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

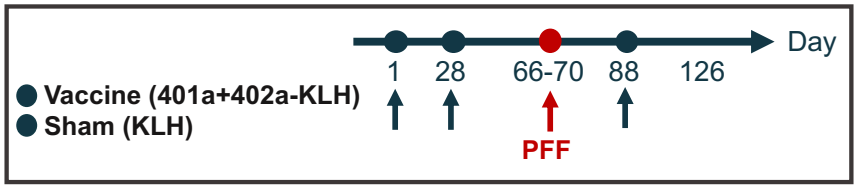
*Highest reactivity

- 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

Vaccination improves survival and reduces ASyn brain aggregates in M83 hemizygous mouse model of PFF-induced disease







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 & CSF, 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 for pathogenic ASyn in DLB brain was achieved with immune IgG against two specific conformational epitopes or a combination of all four.
- Bivalent vaccine improved survival and reduced levels of aggregated ASyn in M83 mouse model of disease

❖ 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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