Achieving an optimal profile for immunotherapy of alpha-synucleinopathies: Rational generation of monoclonal antibodies selective for pathogenic forms of alpha-synuclein – Abstract 1836

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Therapeutic imperative: selectively target only the toxic $\alpha$-synuclein aggregates

- Alpha-synuclein exists in different forms including normal, physiologically important conformations and toxic forms
- Maximal efficacy and safety is expected to require selectivity for the toxic forms of $\alpha$-syn, oligomers and/or small soluble fibrils, while avoiding physiologic forms of $\alpha$-syn

Disclosure: JK is an employee of ProMIS Neurosciences
PMN antibodies raised against predicted conformational epitopes display the desired binding profile by surface plasmon resonance (SPR)

**Representative PMN clone:**
- No binding to monomers
- No binding to physiologic tetramers
- Robust binding to soluble toxic oligomers
- Reactivity with sonicated, soluble PFFs

SPR sensorgrams for immobilized antibody and various concentrations of oligomers or tetramers injected over the surface

**Binding response units at 30 s post-dissociation**

- **PMN antibody**
- **Pan a-SYN 4D6**

![Graph showing binding responses](image)
Immunohistochemistry: PMN antibodies show greater selectivity for small aggregates over Lewy bodies (insoluble fibril deposits)
PMN antibodies react with native pathogenic α-syn in diseased brain from LBD and MSA patients

**DOT BLOT**

- Strong reactivity of PMN antibodies with LBD brain extract
- Background reactivity with normal brain

**SPR**

- Binding of immobilized antibodies to MSA brain extract
- PMN antibodies show binding response equivalent to or greater than the pan-α-syn antibody control (4D6)
- Murine IgG1 isotype control shows low background binding
PMN antibody neutralizes the seeding activity of human \(\alpha\)-syn pre-formed fibrils

100\(\mu\)M \(\alpha\)-syn protein monomers incubated with 10nM human \(\alpha\)-syn PFF seeds in 25\(\mu\)M Thioflavin T

Incubation at 37\(^\circ\)C. Shaking for 30s every hour (prior to each fluorescence reading)

For neutralization studies, PMN antibody was added at 0.1 nM
PMN antibodies protect dopaminergic neurons against α-synuclein oligomer toxicity *in vitro*

- Multiple antibodies provide neuroprotection in the same range as the brain-derived neurotrophic factor (BDNF) positive control.
- As expected, antibodies alone had no effect on viability (not shown).

\*p<0.05, \**p<0.01 vs α-syn oligomers alone
PMN antibodies inhibit \( \alpha \)-syn propagation: Reduced PFF uptake and formation of aggregates

**Human soluble \( \alpha \)-syn fibrils +/- PMN antibody**

![Diagram showing the inhibition of \( \alpha \)-syn propagation by PMN antibodies](image)

**ProMIS antibodies significantly decrease formation of \( \alpha \)-syn aggregates**

![Graph showing decreased formation of \( \alpha \)-syn aggregates](image)

\*p<0.05 vs fibrils alone (PFF)

**Staining for human \( \alpha \)-syn aggregates**

![Images of stained neurons](image)

Human \( \alpha \)-syn aggregates stained red
Neurons stained green
ProMIS antibodies inhibit $\alpha$-syn propagation: decreased recruitment of endogenous rat $\alpha$-syn into pathogenic phosphorylated aggregates

**Human PFF +/- PMN antibody**

Staining for rat phosphorylated $\alpha$-syn aggregates

ProMIS antibodies significantly decrease recruitment into $\alpha$-syn phosphorylated aggregates

* $p<0.05$ vs fibrils alone (PFF)

Endogenous rat phospho-aggregates stained yellow (denoted by arrows)

Human $\alpha$-syn aggregates stained red

Neurons stained green
Conclusions

• Identification of predicted disease-associated epitopes through computational modeling allowed for the generation of monoclonal antibodies with selectivity for pathogenic, aggregated species of α-synuclein
  ▪ Binding to toxic oligomers and soluble fibrils
  ▪ Binding to pathogenic α-syn in LBD and MSA brains
  ▪ No binding to monomers or physiologic tetramers
  ▪ No binding to insoluble inert aggregates of α-syn (Lewy bodies)

• Activity assays indicate that the antibodies can inhibit oligomer neurotoxicity as well as the seeding activity and propagation of aggregation by soluble pre-formed fibrils

• Selectivity of antibodies for α-syn pathogenic species, as opposed to pan- α-syn reactivity, is expected to provide better efficacy and safety by preserving normal α-syn function and minimizing “soaking up” of active antibody by more abundant non-toxic forms of the protein