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

Johanne M. Kaplan¹, Ebrima Gibbs², Beibei Zhao², Jing Wang², Clay Shyu², Steven S. Plotkin¹,², Neil R. Cashman¹,²

¹ProMIS Neurosciences, Toronto, Ontario, Canada, ²University of British Columbia, Vancouver, BC, Canada
Therapeutic imperative: selectively target only the toxic α-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 α-syn, oligomers and/or small soluble fibrils, while avoiding physiologic forms of α-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
Immunohistochemistry: PMN antibodies show greater selectivity for small aggregates over Lewy bodies (insoluble fibril deposits)
PMN antibodies react with native pathogenic $\alpha$-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-$\alpha$-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

Thioflavin T seeding assay

- 100μM \( \alpha \)-syn protein monomers incubated with 10nM human \( \alpha \)-syn PFF seeds in 25μM Thioflavin T
- Incubation at 37°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 \textit{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

Staining for human $\alpha$-syn aggregates

ProMIS antibodies significantly decrease formation of $\alpha$-syn aggregates

Human $\alpha$-syn aggregates stained red
Neurons stained green

*p<0.05 vs fibrils alone (PFF)
ProMIS antibodies inhibit α-syn propagation: decreased recruitment of endogenous rat α-syn into pathogenic phosphorylated aggregates

**Human PFF +/- PMN antibody**

**Staining for rat phosphorylated α-syn aggregates**

ProMIS antibodies significantly decrease recruitment into α-syn phosphorylated aggregates

**Survival (dopaminergic neuron % of control)**

- **Control**
- **PFF**
- **PFF + Ab1**
- **PFF + Ab2**

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

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

**Human α-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