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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ProMIS™ Neurosciences

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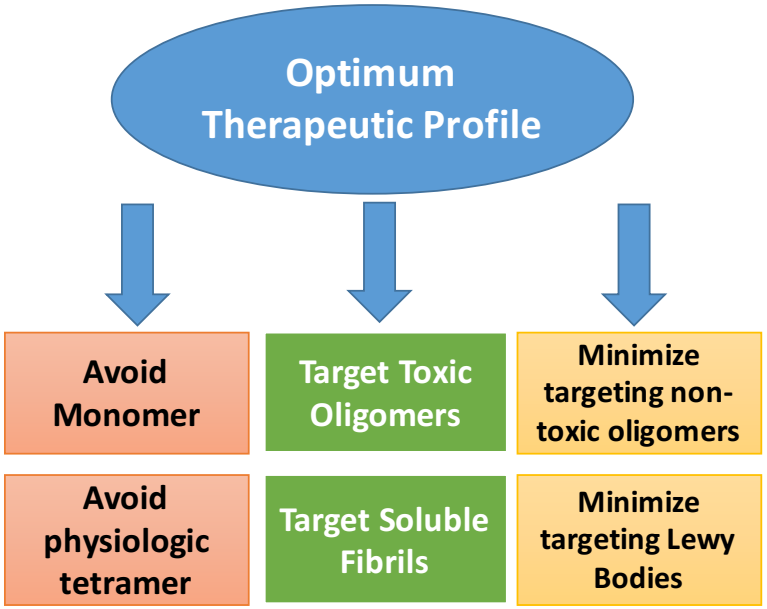
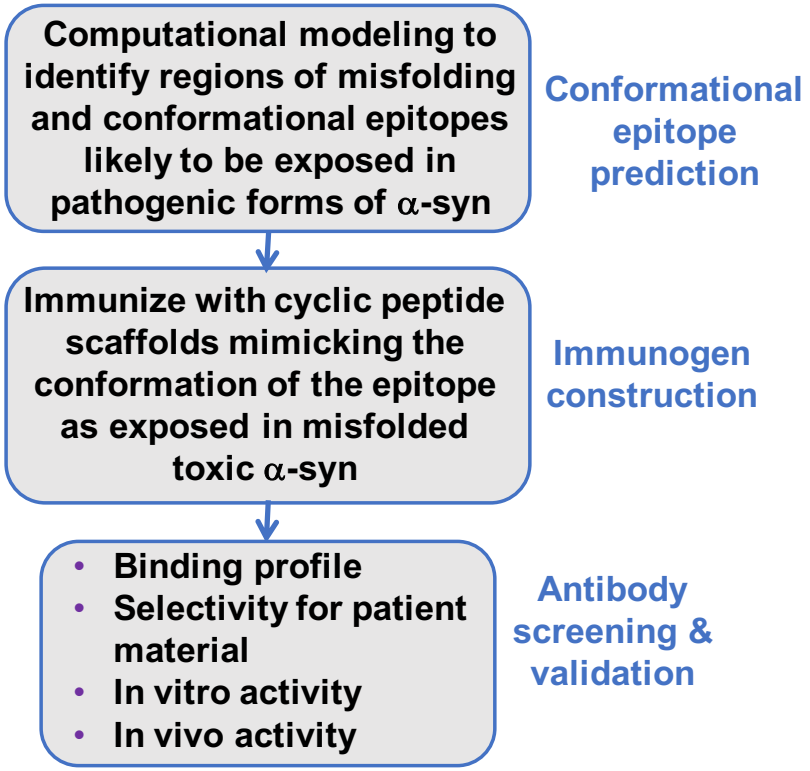
Therapeutic imperative: selectively target only the toxic α-synuclein aggregates



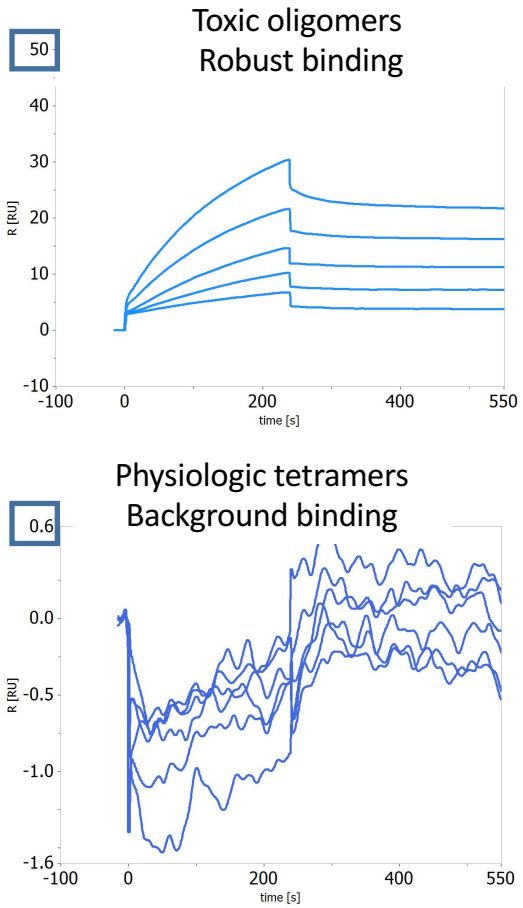
- Alpha-synuclein exists in different forms including normal, physiologically important conformations and toxic forms
- Maximal efficacy and safety is expected to require selectivity for the toxic forms of α-syn, oligomers and/or small soluble fibrils, while avoiding physiologic forms of α-syn

Disclosure: JK is an employee of ProMIS Neurosciences

ProMIS platform – Epitope design for generation of selective antibodies



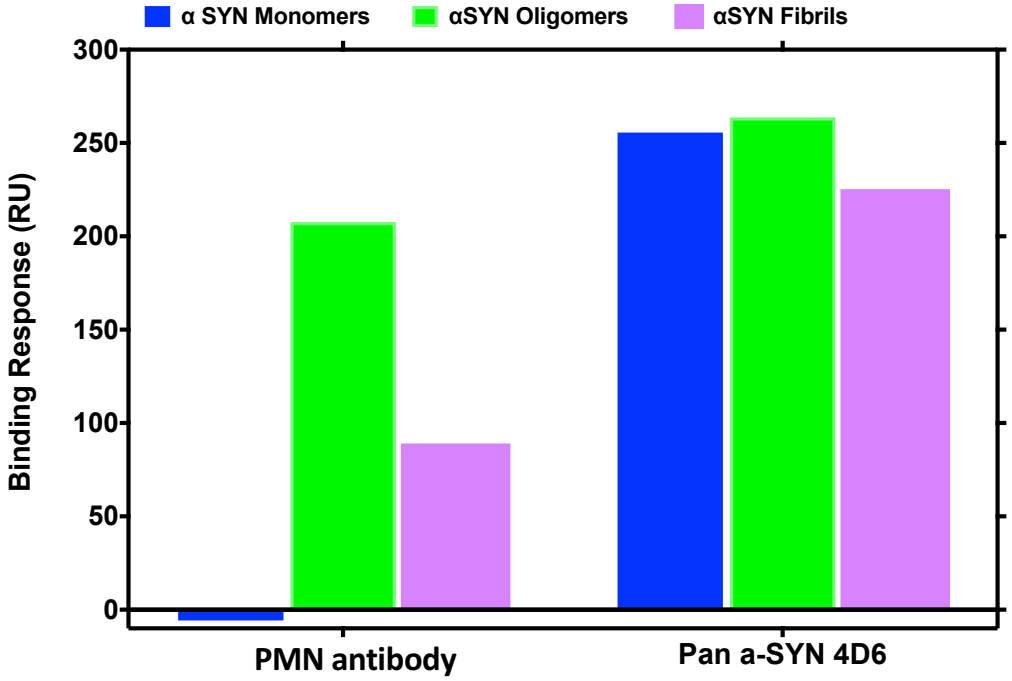
PMN antibodies raised against predicted conformational epitopes display the desired binding profile by surface plasmon resonance (SPR)



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

Representative PMN clone:

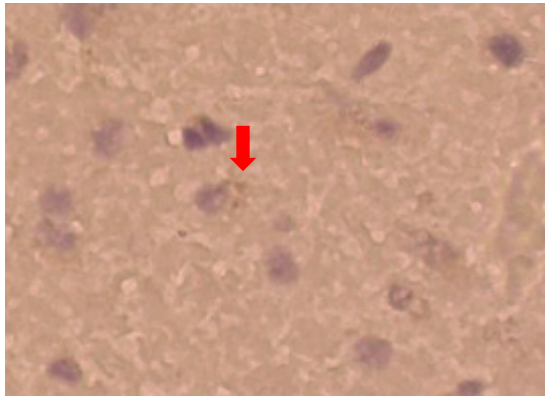
- **No binding to monomers**
- **No binding to physiologic tetramers**
- **Robust binding to soluble toxic oligomers**
- **Reactivity with sonicated, soluble PFFs**



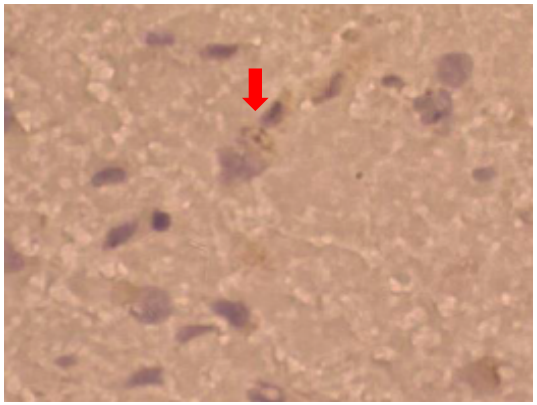
Binding response units at 30 s post-dissociation

Immunohistochemistry: PMN antibodies show greater selectivity for small aggregates over Lewy bodies (insoluble fibril deposits)

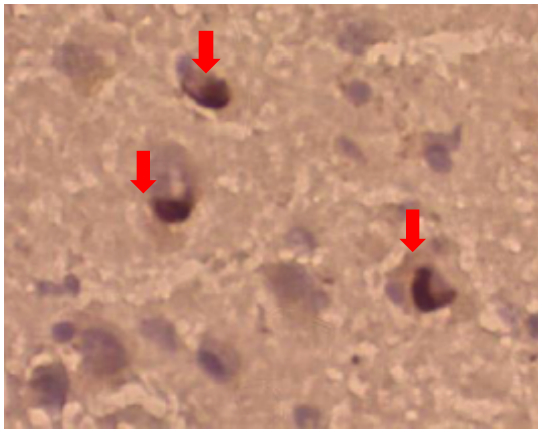
PMN Ab 1



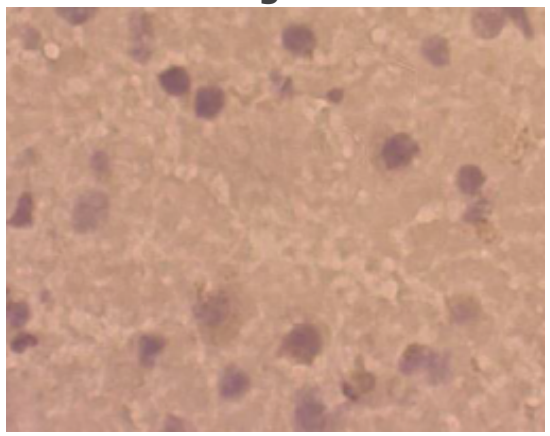
PMN Ab 2



Pan α-syn (4D6) - Lewy bodies

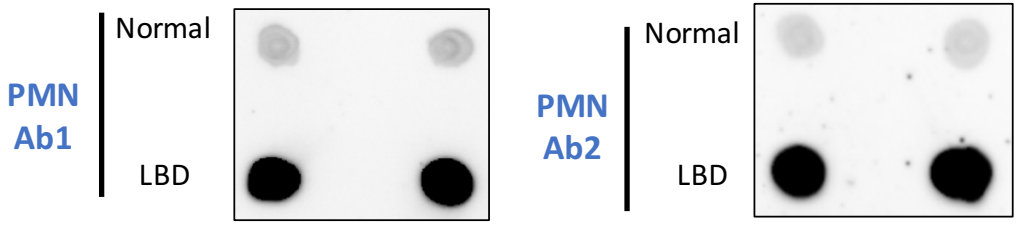


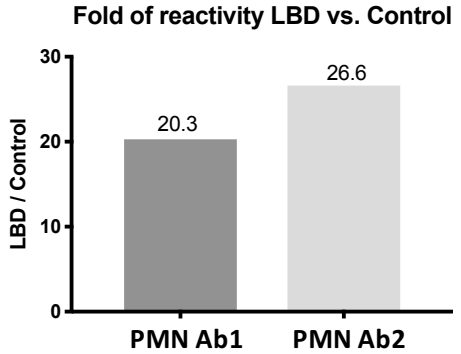
Mouse IgG1 control



PMN antibodies react with native pathogenic α-syn in diseased brain from LBD and MSA patients

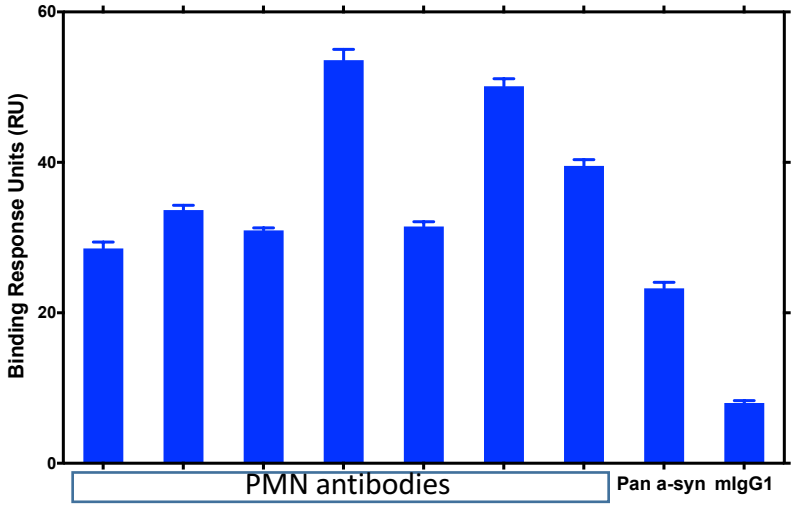
DOT BLOT





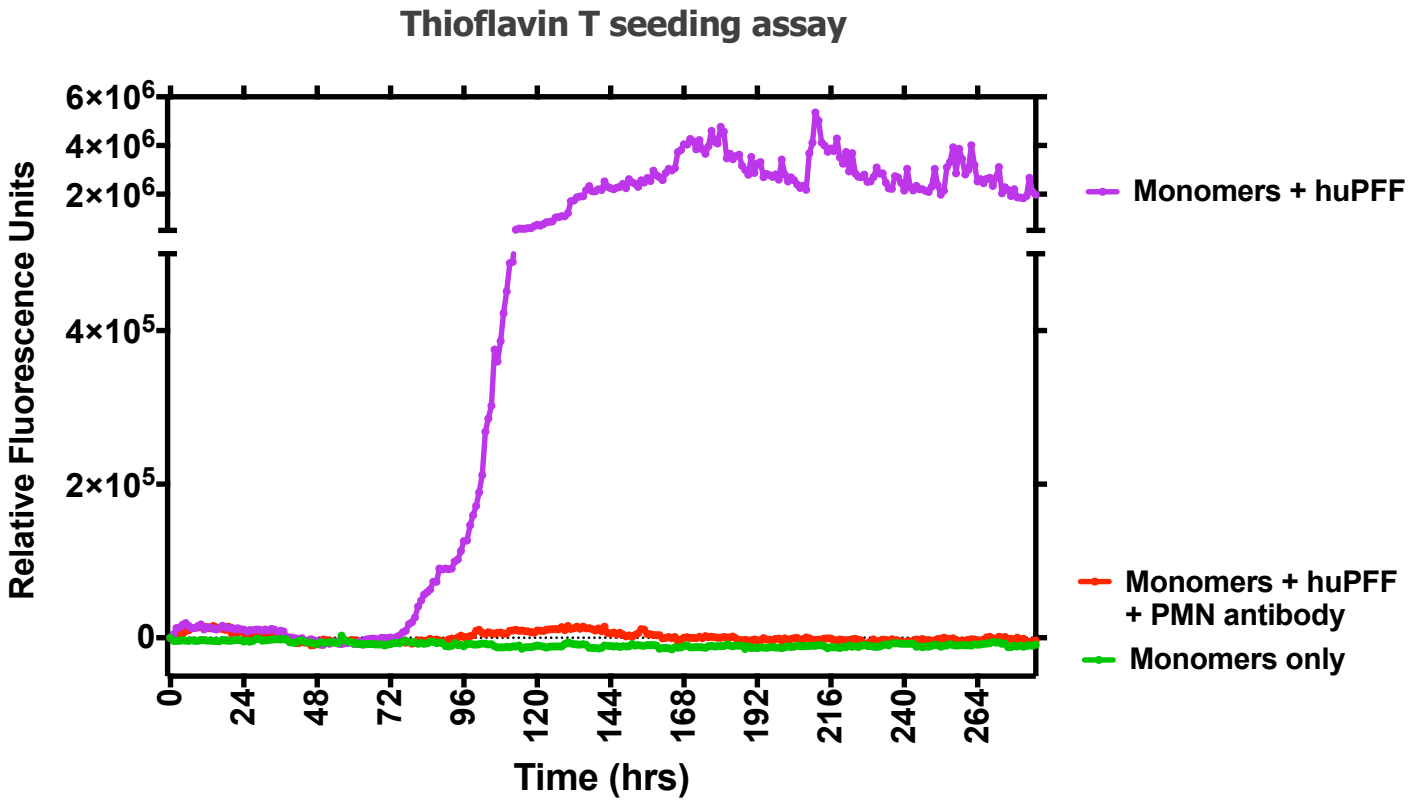
- Strong reactivity of PMN antibodies with LBD brain extract
- Background reactivity with normal brain

SPR



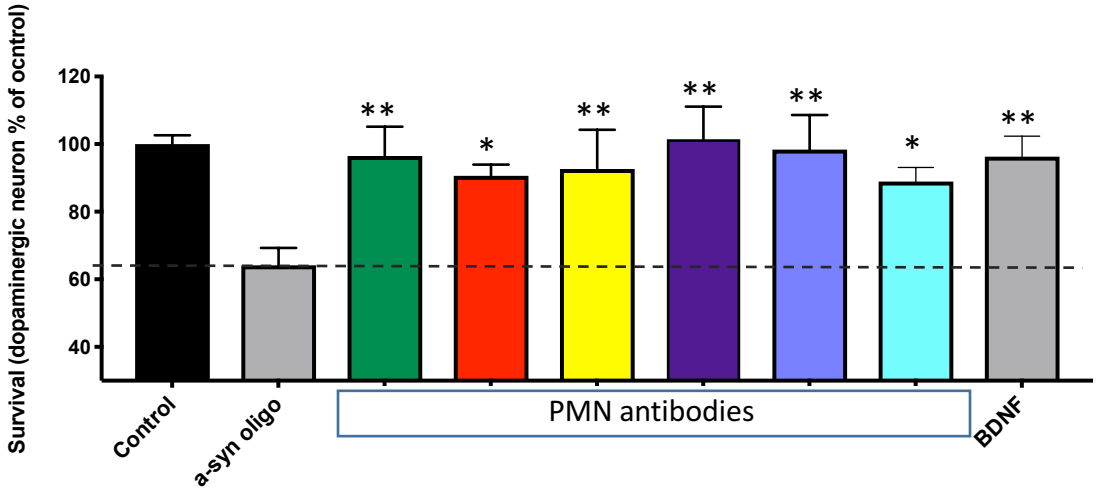
- Binding of immobilized antibodies to MSA brain extract
- PMN antibodies show binding response equivalent to or greater than the pan-α-syn antibody control (4D6)
- Murine IgG1 isotype control shows low background binding

PMN antibody neutralizes the seeding activity of human α-syn pre-formed fibrils



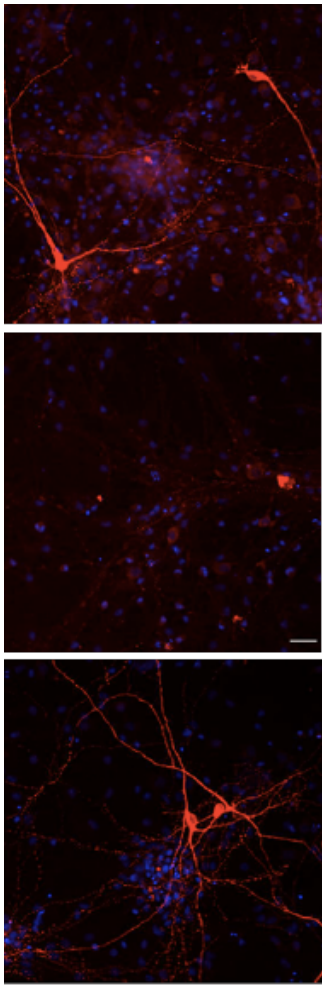
- 100µM α-syn protein monomers incubated with 10nM human α-syn PFF seeds in 25µM Thioflavin T
- Incubation at 37^{0}C. Shaking for 30s every hour (prior to each fluorescence reading)
- For neutralization studies, PMN antibody was added at 0.1 nM

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 a-syn oligomers alone



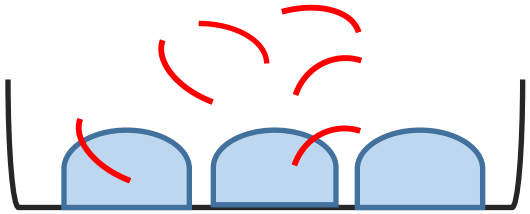
CONTROL
Normal neurons in bright red

α-SYN OLIGOMERS
Neurons killed by toxic oligomers

PMN ANTIBODY + α-SYN OLIGOMERS
Neuronal death blocked by PMN antibody

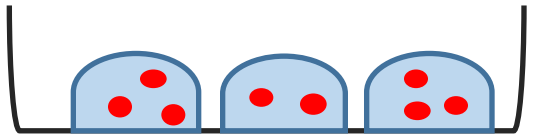
PMN antibodies inhibit α-syn propagation: Reduced PFF uptake and formation of aggregates

Human soluble α-syn fibrils +/- PMN antibody

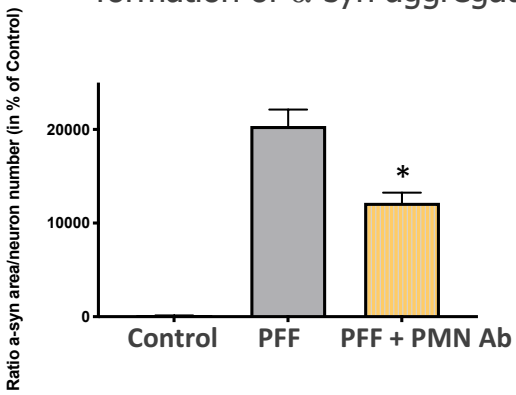


14 day incubation

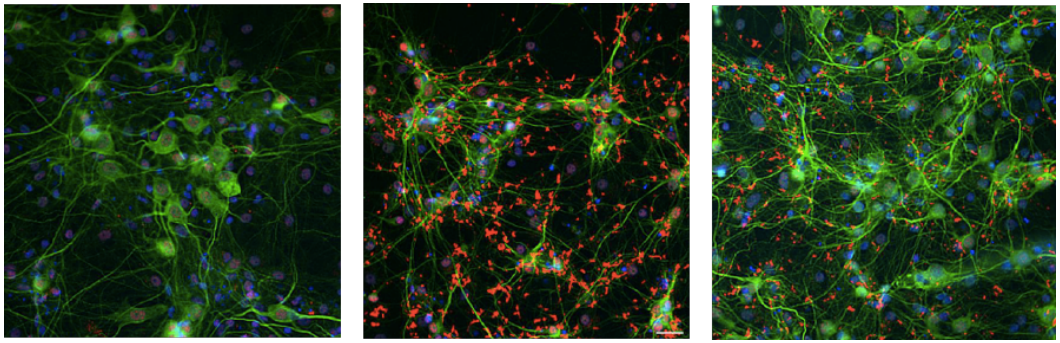
Staining for **human α-syn aggregates**



ProMIS antibodies significantly decrease formation of α-syn aggregates



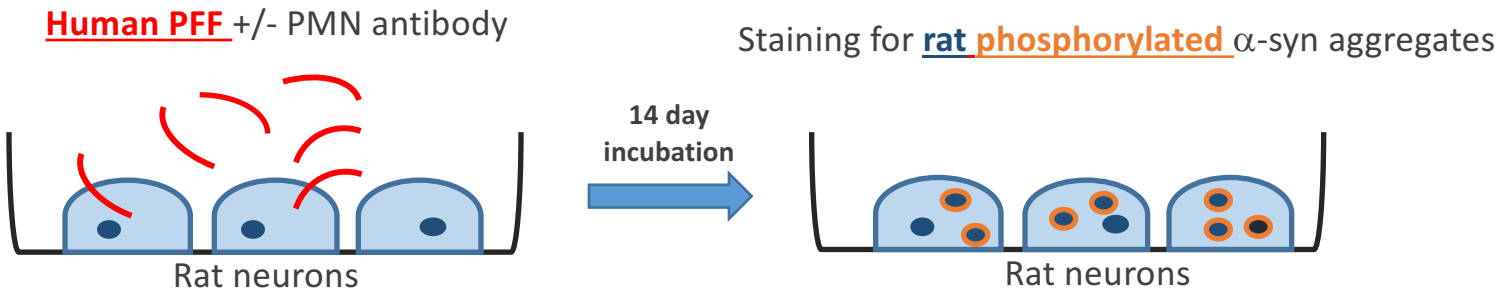
*p<0.05 vs fibrils alone (PFF)

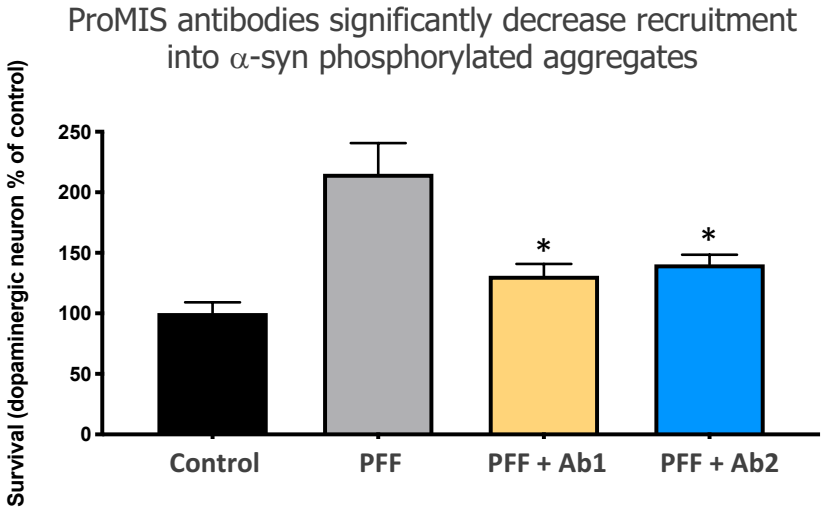


CONTROL **α-SYN SOLUBLE FIBRILS** **α-SYN SOLUBLE FIBRILS + PMN ANTIBODY**

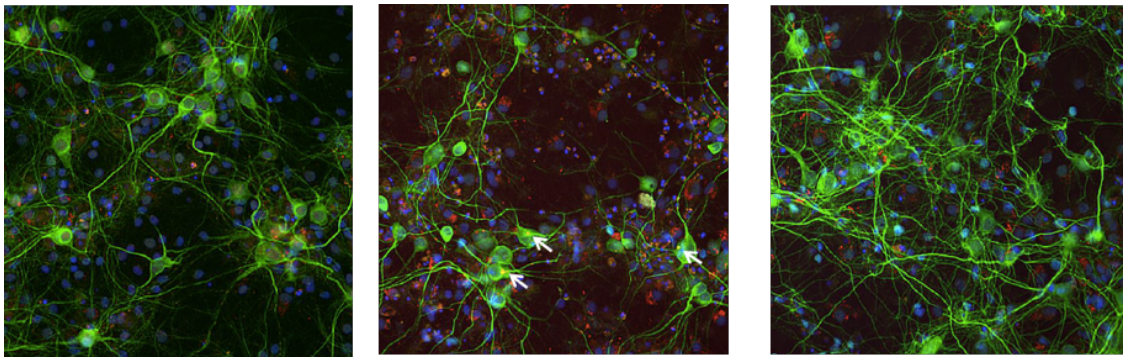
Human α-syn aggregates stained red
Neurons stained green

ProMIS antibodies inhibit α-syn propagation: decreased recruitment of endogenous rat α-syn into pathogenic phosphorylated aggregates





*p<0.05 vs fibrils alone (PFF)



CONTROL | α-SYN SOLUBLE FIBRILS | α-SYN SOLUBLE FIBRILS + PMN ANTIBODY

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