Application of a Qualitative Model to Elucidate the Role of the Alpha-Synuclein System in Parkinson's Disease.

CM Friedrich[1], W Zago[2], S Gardai[2], G Tonn[2], MJ Reed[1]
[1]Rosa&Co, CA, USA; [2]Elan Pharmaceuticals, Inc, CA, USA

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Introduction

- Alpha-synuclein protein is a major component of Lewy bodies, the hallmark features of Parkinson's disease (PD)
- Alpha-synuclein's is thought to be involved in vesicle formation, though its functions in healthy brains or in patients with PD are not well understood
- Improper vesicle formation has been hypothesized to contribute to the pathophysiology of PD

Objectives

- Provide insight into alpha-synuclein function in vesicle trafficking
- Discover, discuss, and document the current state of knowledge within Elan
- Formulate hypotheses for unknown or uncertain aspects of alpha-synuclein function
- Identify future experiments to test hypotheses, resolve uncertainties and identify and prioritize potential targets

Methods

- Explore the role of alpha-synuclein in vesicle formation and PD, by collaborating on the development of an Alpha-Synuclein PhysioMap®, a qualitative, graphical model of alpha-synuclein's known and hypothesized functions
- Engage in a participatory process of developing the Alpha-Synuclein PhysioMap to curate, discuss, and integrate existing public and Elan-proprietary data and knowledge
- Facilitate identification of knowledge gaps, generation of hypothesis, and identification of assays to resolve uncertainties and test hypotheses

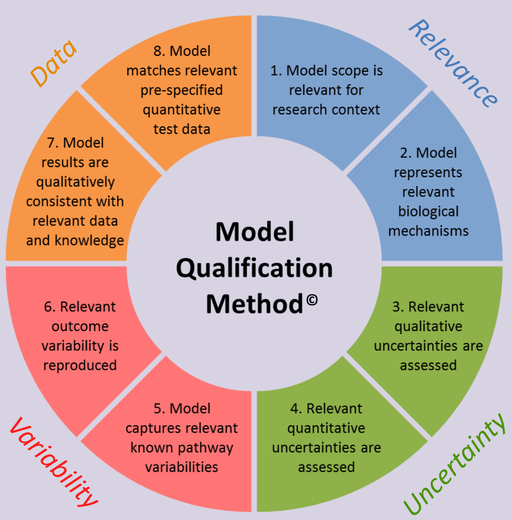

Figure 1. Rosa's Model Qualification Method (MQM)[1] ensures that models are qualified to support development decisions. The MQM is used for project management for the duration of the project.

Results

- The Alpha-Synuclein PhysioMap was designed and curated by a multidisciplinary team from Rosa and Elan to represent alpha-synuclein life cycle and function, including:
  - Alpha-synuclein synthesis and distribution within a neuron
  - SNARE complex formation
  - Phagocytosis
  - Cytokine and neurotrophic factor release
  - Mitochondrial function
- Each process was represented as one of a series of inter-connected modules, or sub-systems (Figure 2)
- The graphical interface facilitated identification and discussion among the multi-disciplinary team of current knowledge, available data, data gaps, and hypotheses

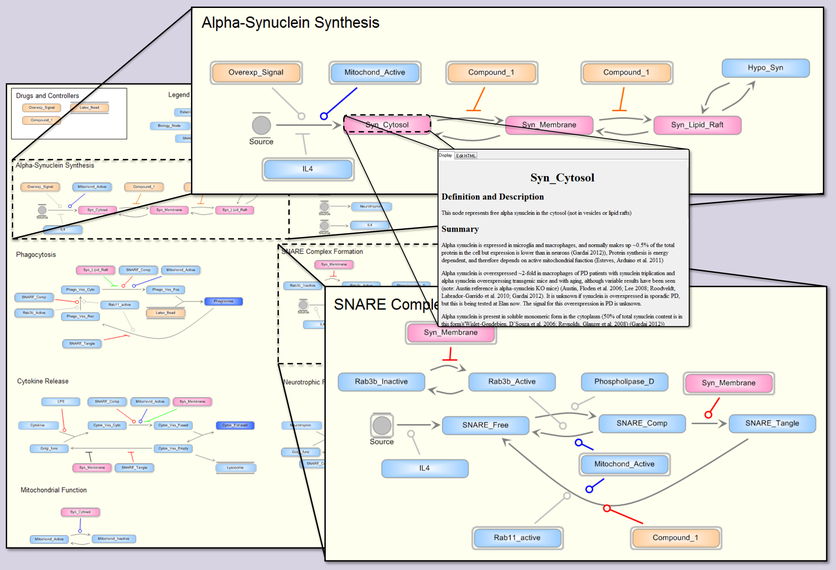

Figure 2. An Alpha-Synuclein PhysioMap.

Results

- The systematic process of capturing knowledge in the PhysioMap, the embedded notes, and the associated MQM[1] document led to identification of knowledge gaps and development of new hypotheses
- Several experiments were identified that could resolve knowledge gaps and test hypotheses identified by the team in the process of developing the PhysioMap:
  - Perform phagocytosis experiment challenging macrophages with small beads prior to large beads to exhaust recycling vesicles
  - Label alpha-synuclein to identify the location where alpha-synuclein is acting, i.e., if interfering with fusion or with recycling
  - Reduce ATP supply in wild type macrophages to determine contribution of mitochondrial dysfunction to alpha-synuclein-like effects
  - Add ATP to alpha-synuclein-overexpressed macrophages to determine whether the alpha-synuclein effects can be reduced, and to determine the proportion of alpha-synuclein effects that are due to mitochondrial dysfunction
  - Assess impact of alpha-synuclein on macrophage function using transfer model with alpha-synuclein null cells fed conditioned medium containing alpha-synuclein
    - Note that incorporation into cell might not recapitulate internal synthesis
  - Rescue experiments to increase free SNARE within macrophages to see if alpha-synuclein effects are overcome
    - Note that IL-4 stimulation does this and it does rescue

Conclusions

- Development of the Alpha-Synuclein PhysioMap facilitated:
  - Documentation and expansion of Elan's institutional knowledge of alpha-synuclein
  - Identification of key biological uncertainties and hypotheses
  - Recommendations for focused laboratory experiments to further elucidate alpha-synuclein function in vesicle formation and PD
- The Alpha-Synuclein PhysioMap could be expanded into a quantitative Alpha-Synuclein PhysioPD™ model, by adding mathematical relationships and parameters to the existing structure
- An Alpha-Synuclein PhysioPD Platform would enable further *in silico* hypothesis generation and testing, including using simulation-based what-if explorations

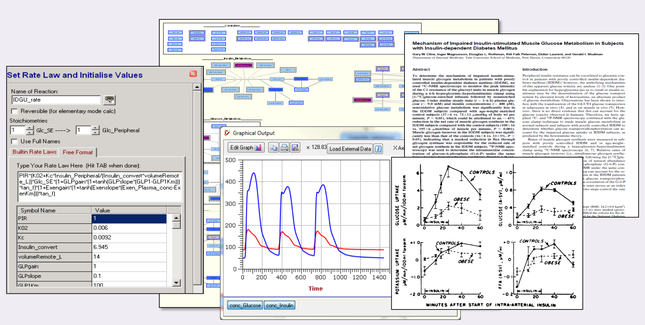

Figure 3. Example components of a quantitative PhysioPD Platform.

References

1 Friedrich, C. M. A model qualification method for mechanistic physiological QSP models to support model-informed drug development. *CPT Pharmacometrics Syst Pharmacol* **5**, 43-53, doi:10.1002/psp4.12056 (2016).

For more information about this work, please contact:
Mike Reed
Rosa & Co LLC
(650) 393-3738
mreed@rosaandco.com