# A Quantitative Systems Pharmacology Platform of Brain and Serum Progranulin (PGRN) to Investigate Targets in Frontotemporal Dementia (FTD).

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## Introduction

- Frontotemporal dementia (FTD) is the second most common form of neurodegenerative dementia, characterized by extensive neuronal loss, TDP-43 pathology, and gliosis
- FTD can be caused by loss of function mutations in the *GRN* gene that results in a haploinsufficiency of the progranulin (PGRN) protein
- Restoration of normal PGRN levels is a therapeutic strategy of interest, and plasma PGRN is of interest as a possible biomarker
- It is unclear which pool of PGRN (e.g., intra- vs. extracellular) should be increased to achieve optimal therapeutic efficacy, and which functional pathway should be targeted

## Objectives

- Elucidate and document PGRN production, uptake, clearance, and transport in the brain and periphery
- Formulate hypotheses for unknown or uncertain aspects of PGRN life cycle
- Identify experiments to test hypotheses, resolve uncertainties, identify/prioritize potential targets
- Support development of therapies designed to restore the expression and distribution of PGRN

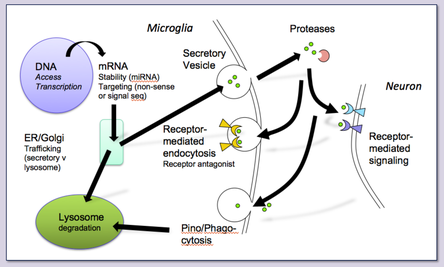


Figure 1. PGRN synthesis, secretion, uptake, and clearance. Microglia are the highest producers of PGRN on a per cell basis. Neurons are thought to be the relevant target for clinical manifestation of disease.

## Methods

- Explore the role of PGRN in FTD by collaborating on the development of a PGRN PhysioMap®, a qualitative, graphical model of PGRN's known and hypothesized functions
- Develop a PGRN PhysioPD™ research platform to quantitatively integrate public and proprietary data sets into a mechanistic representation of PGRN dynamics
- Reproduce key results in simulated experiments
- Facilitate identification of knowledge gaps, generation of hypotheses, and identification of assays to resolve uncertainties and test hypotheses

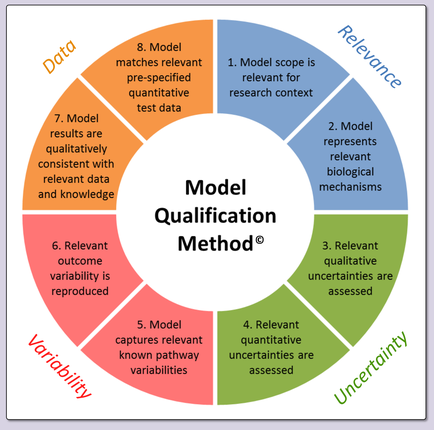


Figure 2. Rosa's Model Qualification Method (MQM)[1] ensures that models are qualified to support development decisions. The MQM is used for project management for the duration of the project.

## Results

- The PGRN PhysioMap was designed and curated by a multidisciplinary team from Rosa and FORUM to represent PGRN life cycle in brain, CNS, and periphery (Figure 3)
- Forum quantitative assay data were reproduced in the PGRN PhysioPD Research Platform
- The systematic process of integrating information in the PGRN Platform led to insights, development of hypotheses, and identification of experiments to resolve uncertainty

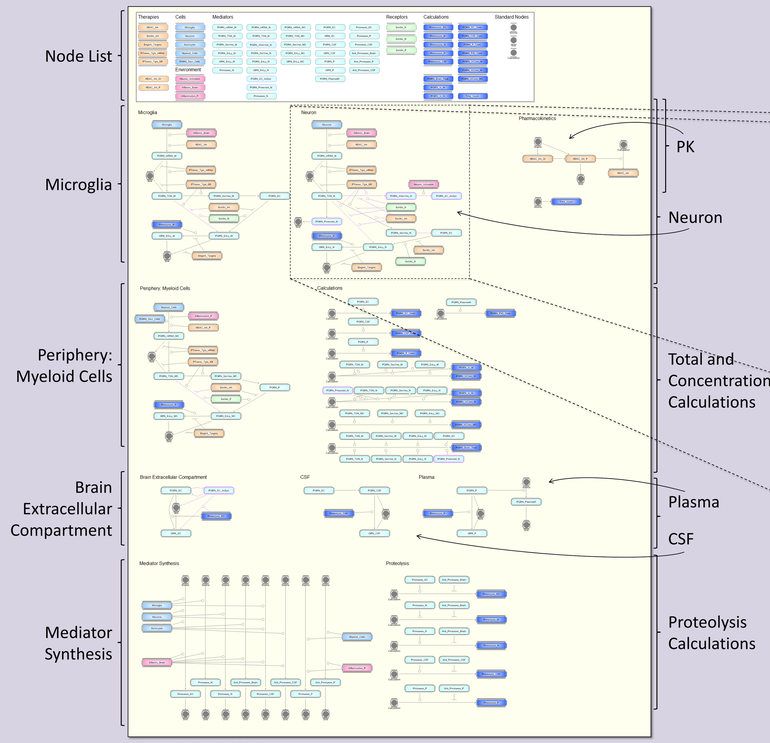


Figure 3. The PGRN PhysioMap.

### Finding #1: PGRN protein in the brain is mostly stored in neurons

- Modeling confirmed that microglial supply most of the PGRN in the brain, but total neuronal secretion rate was also significant (Figure 4)
- Neurons take up more PGRN than microglia
- Because PGRN in neurons has a long intracellular half-life, it is expected that most of the PGRN measured in brain tissue is stored in neurons (Figure 5)
- This has important implications for interpreting PGRN protein measurements in brain

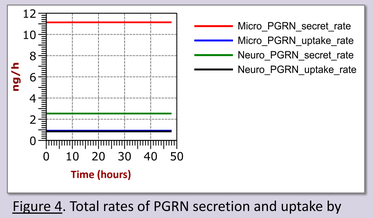


Figure 4. Total rates of PGRN secretion and uptake by neurons (Neuro_PGRN_secret_rate, Neuro_PGRN_uptake_rate) and microglia cells (Micro_PGRN_secret_rate, Micro_PGRN_uptake_rate) in the brain.

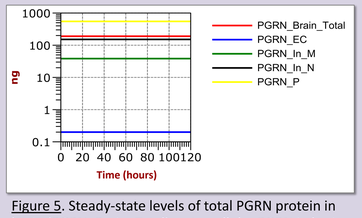


Figure 5. Steady-state levels of total PGRN protein in brain (PGRN_Brain_Total), extracellular PGRN in brain (PGRN_EC), intracellular PGRN in microglia cells (PGRN_In_M), intracellular PGRN in neurons (PGRN_In_N), and PGRN in plasma (PGRN_P).

## Results

### Finding #2: PGRN synthesized vs. taken up has very different intracellular half-life.

- Modeling of two proprietary datasets revealed an apparent inconsistency in the intracellular half-life of PGRN in neurons
  - Cyclohexamide chase experiments suggested long intracellular half-life
  - Blocking sortilin-mediated PGRN uptake increased extracellular PGRN concentration compared to control, but had no effect on intracellular PGRN levels in neurons
- The team formulated 5 hypotheses to explain the apparent contradiction (Figure 6)
- One hypothesis that is consistent with the given data is that there is a protected pool of PGRN inside the neuron that is subject to slow turn-over dynamics
- The team discussed specific experiments that could be used to test the hypotheses

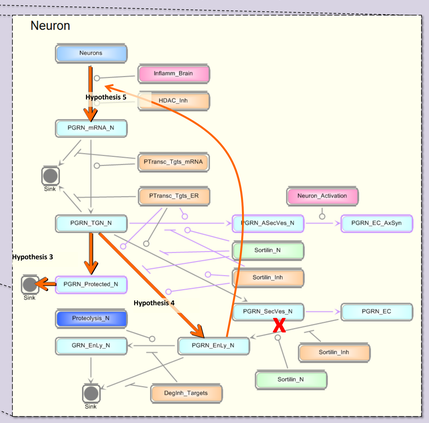


Figure 6. The PGRN PhysioMap facilitated hypothesis development and initial testing to uncover possible mechanistic explanations for constant intracellular PGRN levels in neurons when uptake is blocked.

Hypotheses:

1. EnLy pool is much smaller than 33% and does not contribute much to total IC PGRN
   Con: Shown to be inconsistent with CHX data
2. Much longer IC half-life
   Con: Shown to be inconsistent with sortilin data
3. Separate protected (IC) PGRN pool, not connected to sortilin uptake
   Pro: Tested, consistent with data
4. PGRN is shuttled from TGN to EnLy, and this flow rate increases when sortilin is inhibited
   Requires further testing
5. PGRN transcription is upregulated when sortilin is inhibited/EnLy pool drains
   Requires further testing

### Finding #3: Dynamic analysis suggests significant revision of plasma PGRN half-life.

- The only available data suggested a 40 hour plasma PGRN half-life
- FORUM preclinical data showed that when PGRN production in CNS is increased, plasma concentration increases significantly (> 20%) within 4 hours
- Dynamic modeling illustrated that this level of increase at 4 hours is inconsistent with a long plasma half-life (Figure 7)
- This suggests that caution should be used in interpreting serum PGRN level as a biomarker for brain PGRN level, consistent with recent evidence[2]

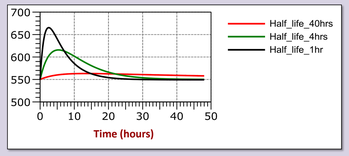


Figure 7. Changes in plasma PGRN level in response to a temporary increase in CNS PGRN production are influenced by plasma PGRN half-life.

## Conclusions

- The process of constructing the PGRN PhysioPD Research Platform produced novel insights and recommendations
- Several experiments were identified that could resolve knowledge gaps and test hypotheses identified by the team in the process of developing the Platform
- The PGRN PhysioPD Research Platform can be used to simulate the effects of modulating different targets on increasing PGRN in intracellular and extracellular compartments in support of developing effective treatments for FTD
- PhysioPD research supports focused pharmaceutical R&D and reduces development risk

## References

1. Friedrich CM. A model qualification method for mechanistic physiological QSP models to support model-informed drug development. *CPT Pharmacometrics Syst Pharmacol*. 2016;5(2):43-53.
2. Wilke C, Gillardon F, Deuschle C, et al. Serum levels of progranulin do not reflect cerebrospinal fluid levels in neurodegenerative disease. *Curr Alzheimer Res*. 2016;13(6):654–662.

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