PR006 gene therapy increased progranulin levels and improved lysosomal related phenotypes in model systems

February 13, 2020
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Overview

- Gene therapy approach
- Scientific rationale for the FTD-GRN program

- PR006 is an AAV9-GRN vector
  - *In vitro* efficacy data in an iPSC-derived neuron model
  - *In vivo* efficacy and safety data in a Grn KO mouse model and in NHPs

- Planned Phase 1/2 clinical trial design
Precision genetic medicine approach to neurodegenerative diseases

- Human genetic studies have identified genes that cause or increase risk of neurodegenerative diseases
- Many of these genes are involved in lysosomal function
- Gene therapy can enable delivery of a functional gene to the CNS
AAV9 is well-suited to deliver functional genes or gene knockdown to the CNS

- AAV9-based therapy has shown transformative efficacy and safety and is now approved for SMA
- The effectiveness of AAV9-based therapies is due to its ability to distribute broadly across the CNS
- AAV9 manufacturing process is well-characterized and scalable
Intra-cisternal delivery route improves brain distribution for CNS indications

- Intra-cisternal (cisterna magna, at top of spinal cord) location for intrathecal delivery results in better brain distribution than lumbar-intrathecal.
Restoring progranulin expression in neurons is necessary and sufficient to restore functional deficits in Grn KO mice

- Conditional genetic deficiency of GRN in neurons led to behavioral deficits and astrogliosis, neither of which were exacerbated by GRN deficiency in microglia
- AAV2/1-Grn injection in Grn KO mice resulted in neuron-specific Grn expression, and led to suppression of lipofuscinosis and reduction in microgliosis in the hippocampus and thalamus
- Studies in conditional knockout mice demonstrate that endogenous brain progranulin is predominantly produced by neurons

Source: 1Arrant et al., 2017, 2Arrant et al., 2019, 3Arrant et al., 2018
Frontotemporal dementia with *GRN* mutation is caused by progranulin deficiency

- FTD pathology includes neurodegeneration and inflammation
- Progranulin is a secreted factor that is enriched in lysosomes
- Progranulin deficiency leads to lysosomal dysfunction

Number of GRN mutations causes varying degrees of progranulin deficiency resulting in spectrum of disease

Number of GRN mutations → Decreasing progranulin → Increasing disease severity

- 0 mutations → Healthy Individuals
- 1 mutation (Heterozygous) → Frontotemporal Dementia (Adult onset)
- 2 mutations (Homozygous) → Neuronal Ceroid Lipofuscinosis (Childhood onset)
## PR006 overview

<table>
<thead>
<tr>
<th>Table Title</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PR006</strong></td>
<td>● AAV9 viral vector delivering the <em>GRN</em> gene, which encodes progranulin</td>
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<tr>
<td><strong>Route of Administration</strong></td>
<td>● Single intra-cisterna magna (ICM) injection</td>
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</table>
| **Lead Indications**        | ● Frontotemporal dementia with *GRN* mutation (FTD-GRN)  
  ○ Rapidly progressive dementia impacting behavior and language  
  ○ Leads to death within 3-10 years |
| **Progress and Status**     | ● Mouse efficacy and NHP safety and biodistribution studies conducted  
  ● On track to initiate Phase 1/2 clinical trial in 2020 |
# PR006 preclinical studies

<table>
<thead>
<tr>
<th>Model</th>
<th>Study</th>
<th>Key Findings</th>
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</thead>
<tbody>
<tr>
<td>FTD-GRN iPSC-derived neurons</td>
<td><em>In vitro</em> PR006 efficacy study</td>
<td>• PR006 restored defective maturation in the lysosomal enzyme, Cathepsin D, and improved abnormal TDP-43 pathology in FTD-GRN neurons</td>
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<tr>
<td><em>Grn</em> KO mice</td>
<td>Aged ICV PR006 efficacy study</td>
<td>• PR006 demonstrated efficacy in aged <em>Grn</em> KO mice</td>
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<td>Dose-ranging ICV PR006 efficacy study</td>
<td>• PR006 demonstrated efficacy in a dose-dependent manner in adult <em>Grn</em> KO mice</td>
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<tr>
<td>Healthy NHPs</td>
<td>GLP NHP safety and biodistribution study of ICM PR006</td>
<td>• Administration of PR006 was well-tolerated in the NHPs and resulted in widespread biodistribution, with exposure levels comparable to efficacious doses in the mouse model</td>
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</table>

ICV, intracerebroventricular; ICM, intra-cisterna magna
FTD-GRN patient-derived neuronal cell model

- iPSC-derived neural progenitor cells from humans with FTD-GRN mutations expressed and secreted less progranulin than neural progenitor cells derived from healthy control subjects

Statistics determined using an unpaired t-test. *** = p < 0.001. Data are mean ± SEM (n=3).
Cell lines from NHCDR, NINDS Materials ND50015, ND50060 and ND38555.

- NPCs for all lines were successfully differentiated into neuronal cultures
PR006 transduction increases secreted progranulin in FTD-GRN neuronal cultures

- PR006 transduction resulted in a robust, dose-dependent expression of secreted progranulin in FTD-GRN neurons

Data is presented as mean ± SEM (n=3-4).
**PR006 promoted Cathepsin D maturation in FTD-GRN neurons**

- Progranulin stimulates the maturation of cathepsin D (CTSD)

- PR006 treatment rescued the defective maturation of a key lysosomal protease, cathepsin D, in FTD-GRN neuronal cultures

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**Legend**

- Intramolecular Cleavage
- Intermolecular Cleavage
- Cleavage
- ProCTSD
- matCTSD
- CTSD propeptide
- Catalytic aspartates

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Source: Butler et al., JMB 2019; Statistics determined using a paired t-test; * = p < 0.05. Data is presented as mean ± SEM (n=3).

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**Graph**

- **Ratio matCTSD/proCTSD (%) Control**

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Control</th>
<th>FTD-GRN #1</th>
<th>FTD-GRN #2</th>
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</thead>
<tbody>
<tr>
<td>PR006</td>
<td></td>
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<tr>
<td>Excipient</td>
<td></td>
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</tbody>
</table>

- p=0.065
PR006 treatment decreases TDP-43 aggregates and normalizes soluble nuclear TDP-43 in FTD-GRN neurons

<table>
<thead>
<tr>
<th>TDP-43 overview</th>
<th>Insoluble TDP-43</th>
<th>Nuclear TDP-43</th>
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<tbody>
<tr>
<td><strong>Model of abnormal TDP-43 pathology:</strong> disease states have decreased nuclear TDP-43, leading to aggregation and downstream toxicity in neurons</td>
<td><strong>PR006 treatment decreased insoluble TDP-43, a hallmark of FTD-GRN pathology, in FTD-GRN neuronal cultures</strong></td>
<td><strong>PR006 treatment of FTD-GRN neuronal cultures increased nuclear TDP-43 expression levels to near WT Control levels</strong></td>
</tr>
</tbody>
</table>

Source: Figure adapted from Sugai et al., Front Neurosci 2018; Statistics determined using an unpaired t-test; ** = p < 0.01, *** = p < 0.001. Data is presented as mean ± SEM. (n=3 for soluble TDP-43; n=173-330 cells/group for nuclear TDP-43).
PR006 efficacy and biodistribution in aged mice

- Single dose aged Grn KO mouse study to evaluate PR006 effect on more severe progressive phenotypes
PR006 increased progranulin levels in aged progranulin knockout mouse model

14-16-month-old mice were dosed with ICV PR006, and after 2 months, mice were sacrificed and vector genomes and progranulin protein levels were quantified. Statistics determined using Kruskall-Wallis; * = p < 0.05, ** = p < 0.01. Data is presented as mean ± SEM (n=5/vehicle group and n=7/PR006 group). Vector genome levels below 50 (dotted line) were considered not positive. Vg = vector genomes; WT= wildtype.
PR006 reduced lysosomal-related neuropathology in aged progranulin knockout mouse model

PR006 treatment decreased lipofuscin accumulation in multiple brain regions

PR006 treatment reduced ubiquitin accumulation in multiple brain regions

16-month-old mice were dosed with ICV PR006, and after 2 months, mice were sacrificed and inflammatory markers were quantified. Statistics determined using an unpaired t-test; * = p < 0.05, ** = p < 0.01. Data is presented as mean ± SEM (n=4/group)
PR006 reduced neuroinflammation in aged progranulin knockout mouse cerebral cortex

**Inflammatory Marker Gene Expression**

PR006 treatment reduced cerebrocortical gene expression of the proinflammatory cytokine TNFα (*Tnf*) and the microglial marker CD68 (*Cd68*).

**Inflammatory Marker Protein Expression**

PR006 treatment reduced cerebrocortical protein expression of the proinflammatory cytokine TNFα.

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16-month-old mice were dosed with ICV PR006, and after 2 months, mice were sacrificed and inflammatory markers were quantified. Gene expression was measured by qRT-PCR. Statistics determined using an unpaired t-test; * = p < 0.05; ** = p<0.01. Data is presented as mean ± SEM (n=4/group).
PR006 dose-ranging efficacy and biodistribution in adult progranulin knockout mice

- Multi-dose adult $Grn$ KO mouse mouse study to determine efficacious PR006 dose
- Due to the progressive nature of $Grn$ deficiency, 7-month-old mice exhibit a less severe phenotype
**PR006 increased progranulin in cerebral cortex of adult progranulin knockout mouse model**

**Biodistribution**

Vector genomes were observed to be present in the cerebral cortex 3 months after ICV PR006 administration

**Progranulin mRNA**

PR006 dose-dependently increased expression of the *GRN* transgene in the cerebral cortex

**Progranulin protein**

PR006 increased progranulin protein levels in the cerebral cortex

Means are presented +/- SEM (n=8-10 per group). Statistics determined by ANOVA followed by Dunnett’s multiple tests correction comparing each group to vehicle-treated *Grn* KO mice; *** = p<0.001 . Vector genome levels below 50 (dotted line) were considered not positive. Vg = vector genomes; LLOQ = lower limit of quantitation.
**PR006 reduced lysosomal-related neuropathology in adult progranulin knockout mouse model**

Mild to moderate lipofuscinosis was observed in the hippocampus and thalamus of *Grn* knockout mice. PR006 dose-dependently reduced accumulation of lipofuscin, an indicator of lysosomal dysfunction, throughout the brain.

### Lipofuscinosis

#### Hippocampus

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Gm KO</th>
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**Lipofuscin Score**
- 0 (Normal; no finding)
- 1 (Minimal)
- 2 (Mild)
- 3 (Moderate)

Mild to moderate lipofuscinosis was observed in the hippocampus and thalamus of *Grn* knockout mice.

### Ubiquitin accumulation

#### Hippocampus

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<thead>
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<th></th>
<th>WT</th>
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**Ubiquitin Object Size (μm²)**

Means are presented +/- SEM (n=6-10 per group). Statistics determined by ANOVA followed by Dunnett’s multiple tests correction comparing each group to vehicle-treated *Grn* KO mice; ** = p<0.01; *** = p<0.001. Vg = vector genomes.
PR006 reduced neuroinflammation in adult progranulin knockout mouse model

- *Gm* knockout mice exhibited increased neuroinflammation in the cerebral cortex as measured by gene expression of the proinflammatory cytokine TNFα (*Tnf*) and microglial marker CD68 (*Cd68*)
- PR006 treatment reduced gene expression of proinflammatory markers *Tnf* and *Cd68* in the cerebral cortex

Means are presented +/- SEM (n=6-10 per group). Statistics determined by ANOVA followed by Dunnett’s multiple tests correction comparing each group to vehicle-treated *Gm* KO mice; *** = p<0.001 . Vg = vector genomes.
PR006 reduced neuroinflammation in adult progranulin knockout mouse model

- Grn knockout mice exhibited increased microgliosis in multiple brain regions as measured by IHC (protein expression) of the microglial marker Iba1
- All three doses of ICV PR006 reduced microgliosis throughout the brain of Grn knockout mice

Means are presented +/- SEM (n=8-10 per group). Statistics determined by ANOVA followed by Dunnett’s multiple tests correction comparing each group to vehicle-treated Grn KO mice; * = p<0.05; *** = p<0.001. Vg = vector genomes.
PR006 NHP safety and biodistribution study design

Non-diseased cynomolgus monkeys 2-4 years of age, including males and females

| PR006 Dose | Excipient | Low | High |

ICM Administration (Day 1) | Day 30 Cohort | N=3/group | Day 183 Cohort | N=3/group

**In-Life Safety**
- Clinical labs
- Daily observations
- Neurological assessments
- ECGs

**Post-Mortem Analysis**
- Biodistribution (qPCR)
- GRN expression
- Histopathology (H&E)
NHP biodistribution and progranulin expression following ICM administration of PR006

- ICM administration of PR006 resulted in widespread biodistribution throughout the CNS and periphery
- Exposure levels were comparable to the efficacious levels seen in the mouse model
- PR006 treatment dose-dependently increased progranulin expression in the CSF

Data presented from select representative regions from animals sacrificed 182 days post ICM administration of excipient. Transgene levels were analyzed by qPCR; each bar represents the mean ± SEM. Progranulin levels in the CSF are presented as fold change to the excipient-treated control animals; data is presented as the mean ± SEM. p-value: *p<0.05, by one-way dose dependence response analysis using William's trend test
Conclusions from GLP NHP study

- No adverse PR006-related findings were observed in any of the in-life assessments

- No adverse PR006-related findings (clinical and histopathological endpoints)
  - Third-party pathologist classified "extremely minor" degree of nerve fiber degeneration in spinal cord and glial cellularity in DRGs

- ICM administration of PR006 resulted in broad distribution of the transgene throughout the CNS and periphery

- Positive transduction was positively correlated with increased GRN mRNA in the CNS and periphery, as well as increased progranulin protein in the CSF
PR006 FTD-GRN PROCLAIM Phase 1/2 trial

Planned, open label

FTD-GRN Patients

- 30-80 years old
- Single pathogenic GRN mutation
- Symptomatic disease stage
- Stable background medications

PR006 (Open label, N=15)

- Safety and tolerability
- Key biomarkers: progranulin, NfL (CSF and blood)
- Additional biomarkers: volumetric MRI, markers of neuroinflammation and astroglial pathology
- Efficacy: CDR plus NACC FTLD; measures of behavior, cognition, language, function, QoL

ICM: intra-cisterna magna; NfL: neurofilament light chain; CDR plus NACC FTLD: Clinical Dementia Rating plus National Alzheimer’s Coordinating Center Frontal Temporal Lobar Dementia; QoL: quality of life
Conclusions

- PR006 reversed lysosomal abnormalities and reduced TDP-43 pathology in FTD-GRN mutation carrier iPSC-derived neurons
- PR006 demonstrated efficacy in adult and aged Grn KO mice
- PR006 administration via ICM injection was well-tolerated in NHPs
- A Phase 1/2 clinical trial is planned for 2020
Thank You!

Laura D. Heckman  Li Chin Wong
Patty Sheehan    Zhaohui Yang
Alissa Brandes   Stuart Nelson
Suzanne Burstein Jorge Haller

Jennifer Daily    Tim Fenn
Jason Politi      Sid Kamalakaran
Yong Dai         Swetha Garimalla
Franz Hefti      Zarah Aziz