Antisense oligo targeting of GRN regulatory RNA upregulates progranulin: a potential therapeutic approach for granulin-related frontotemporal dementia

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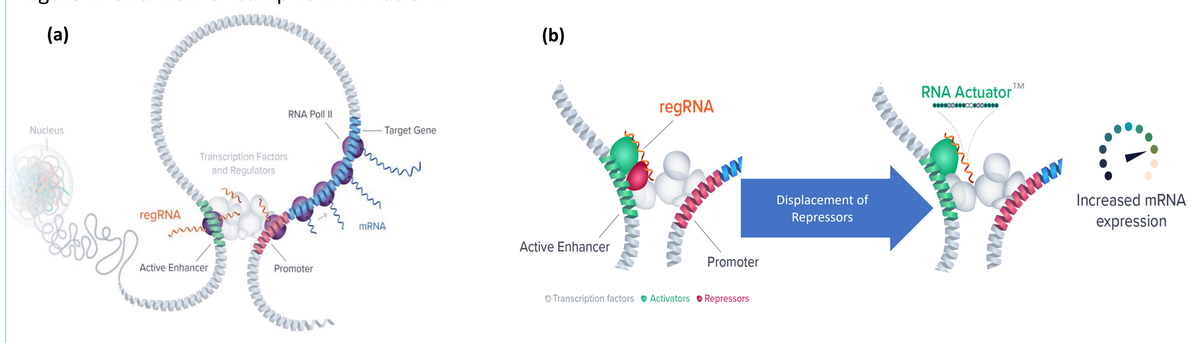
Abstract

Regulatory RNAs (regRNAs) are a class of noncoding RNAs that modulate gene transcription. Our proprietary RAP Platform™ enables the identification of regRNAs associated with a target gene and enables us to upregulate the gene by specifically targeting these regRNAs with antisense oligonucleotides (ASOs). Here we present data regarding the development of ASO drug candidates that target regRNAs and upregulate granulin (*GRN*) expression as a strategy for addressing the haploinsufficiency that is the cause of granulin-related frontotemporal dementia (*GRN*-FTD). We utilized next generation sequencing technologies to identify regRNAs associated with transcriptional control of the human *GRN* gene. We performed an *in vitro* screening of a library of ASOs targeting a regRNA of *GRN* and identified several ASOs that increased *GRN* transcription. We further optimized the ASOs using various techniques including basewalking, varying the length of the ASOs , as well as incorporating high affinity modifications and varied backbone chemistry. Lead ASOs demonstrated robust upregulation of *GRN* both *in vitro*, in *GRN*-FTD patient neurons and induced microglia-like (iMGL) cells, and *in vivo*, in a *GRN*-FTD mouse model when delivered via intracerebroventricular injection. This works exemplifies the ability of our platform to identify regulatory RNAs, and design and optimize ASOs to generate therapeutic candidates.

CAMP4's RAP Platform™ for gene upregulation

Figure 1: Overview of Camp4's RAP Platform

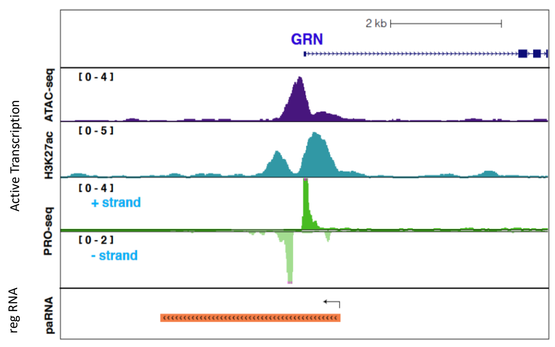


- In the nucleus, genes and their regulatory elements are organized into conserved 3D DNA structures known as insulated neighborhoods to control gene expression.
- Bidirectional transcription at enhancers and promoters produce non-coding RNAs termed regulatory RNA (regRNAs) that act as rheostats in transcriptional control of genes in the insulated neighborhood.[1, 2]
- Our RAP Platform enables the identification of the relevant regRNAs for a given gene and the upregulation of its expression using antisense oligonucleotides (ASOs).

Targeted upregulation of progranulin for treatment of frontotemporal dementia

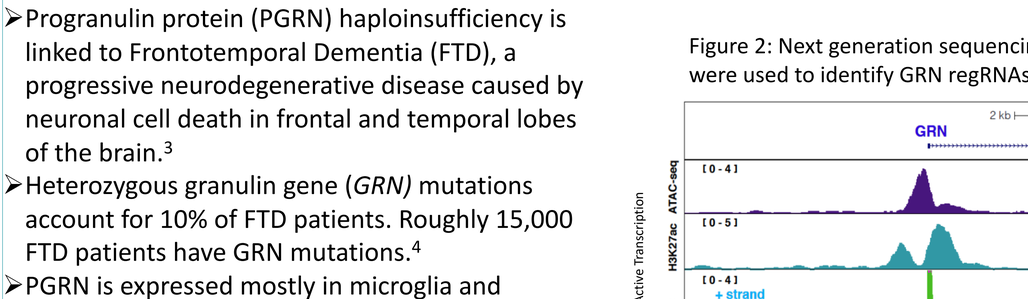
- Progranulin protein (PGRN) haploinsufficiency is linked to Frontotemporal Dementia (FTD), a progressive neurodegenerative disease caused by neuronal cell death in frontal and temporal lobes of the brain.[3]
- Heterozygous granulin gene (*GRN)* mutations account for 10% of FTD patients. Roughly 15,000 FTD patients have GRN mutations.[4]
- PGRN is expressed mostly in microglia and neurons, making delivery to the central nervous system imperative for treatment.
- Upregulation of endogenous wild type PGRN in subjects with heterozygous *GRN* mutations is a potential therapeutic strategy for FTD.
- Using CAMP4's RAP Platform, we identified a regRNA for ASO targeted upregulation of PGRN.

Figure 2: Next generation sequencing techniques were used to identify GRN regRNAs



Design, primary screening, and confirmation of ASOs hits

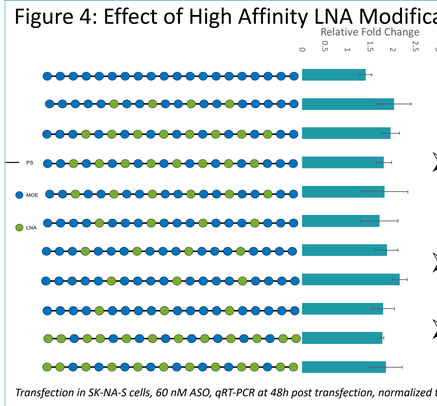
Figure 3: Design and synthesis pipeline for primary screen ASOs

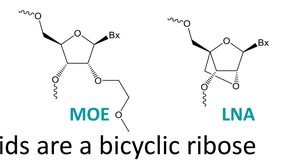


- ASOs targeting GRN regRNA were designed and synthesized using a high-throughput MerMade™ synthesizer (LGC Biosearch Technologies, Inc.).
- The ASOs contained 2'-O-methoxyethyl modifications at all positions and phosphorothioate internucleotide linkages and were screened for *in vitro* activity in a HepG2 cell line via transfection
- Identified hits were confirmed in follow-up dose response experiments in SK-N-AS cells.

Optimizing ASO chemistry for increased upregulation of GRN mRNA

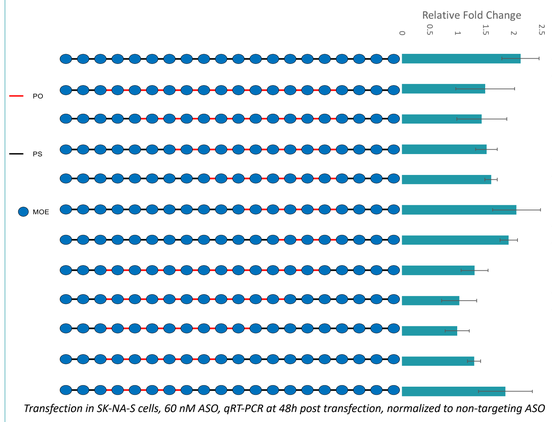
Figure 4: Effect of High Affinity LNA Modifications on ASO Activity

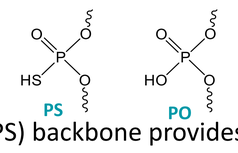




- LNA or Locked Nucleic Acids are a bicyclic ribose modification which increases the binding affinity of ASOs towards complementary sequences.[5]
- Addition of 2 or 3 LNAs residues within the sequence improved efficacy compared to parent ASO sequence.
- Greater number of LNA residues did not improve activity.

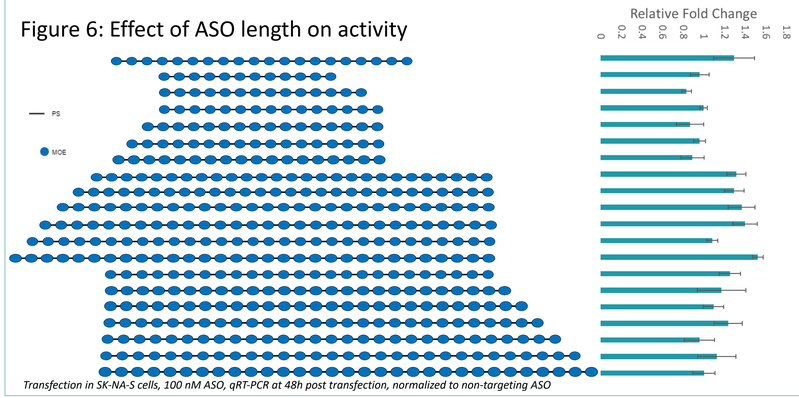
Figure 5: Effect of Replacing PS Bonds with PO Linkages on ASO Activity





- Phosphorothioate (PS) backbone provides resistance against nucleases and is necessary for *in vivo* distribution and uptake of ASOs.[6]
- Toxicity of some ASO sequences is ascribed to a fully PS backbone.
- Limited substitution of PS with phosphodiester (PO) linkages is known to mitigate toxicity.
- A parent ASO targeting GRN regRNA was modified to have various configurations of PS and PO internucleotide linkages and screened in SK-N-AS cell line.
- Several ASOs were identified that maintained activity compared to the parent ASO.

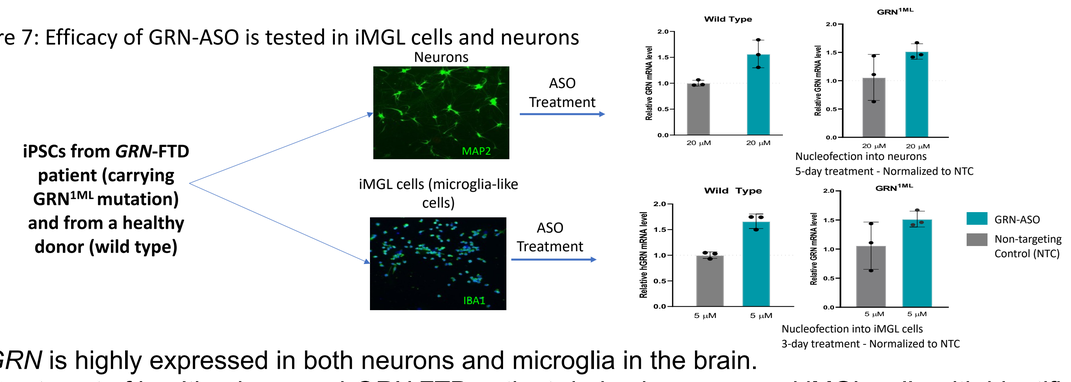
Figure 6: Effect of ASO length on activity



- The length of the parent strand was decreased as well as extended on both the 3' and 5' end and screened for activity.
- Changed ASO length did not improve activity compared to parent ASO.

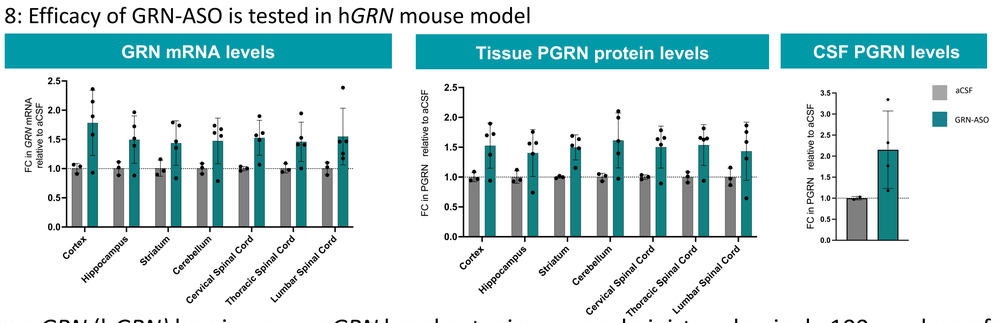
Upregulation of GRN mRNA in target cells and *in vivo*

Figure 7: Efficacy of GRN-ASO is tested in iMGL cells and neurons



- *GRN* is highly expressed in both neurons and microglia in the brain.
- Treatment of healthy donor and *GRN*-FTD patient-derived neurons and iMGL cells with identified ASO targeting *GRN* regRNA induced significant upregulation of *GRN* mRNA levels.

Figure 8: Efficacy of GRN-ASO is tested in h*GRN* mouse model



- Human *GRN* (h*GRN)* hemizygous, m*GRN* knockout mice were administered a single 100 mg dose of ASO via intracerebroventricular (ICV) injection.
- Animals were sacrificed 3 weeks post ICV dosing.
- Consistent ASO-mediated induction of *GRN* mRNA and PRGN protein was observed across brain regions as well as in secreted CSF.

Summary

- A regRNA associated with human *GRN* associated regRNA was identified.
- Several ASOs targeting this regRNA that successfully induced *GRN* upregulation were discovered.
- ASOs optimized to incorporate LNA residues resulted in improved upregulation of *GRN* expression.
- The optimized ASOs showed upregulation of GRN mRNA in multiple CNS cell lines, including in *GRN*-FTD patient derived neurons and microglia.
- ASOs targeting *GRN* regRNA delivered via ICV injection also showed PGRN upregulation in a h*GRN* hemizygous rodent model and induced *GRN* upregulation across various regions of the brain.

References

1. Henninger et al. (2021) *Cell* 184(1): 207-25.
2. Sartorelli and Lauberth (2020) *Nature Structural & Molecular Biology* 27: 521-8.
3. Kao et al. (2017) *Nature Reviews Neuroscience* 18: 325–333.
4. Rademakers et al. [2012] *Nature Reviews Neurology* 8:423–434.
5. Hagedorn et al. (2018) *Drug Discovery Today* 23: 101-14.
6. Crooke et al. (2020) *Nucleic Acids Research* 48: 5235-53.