Antisense oligonucleotide mediated increase of OPA1 expression using TANGO technology for treatment of autosomal dominant optic atrophy

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Background

Autosomal dominant optic atrophy (ADOA) is one of the most commonly diagnosed optic neuropathies. This optic nerve disease is associated with structural and functional mitochondrial deficits that lead to degeneration of the retinal ganglion cells and progressive, irreversible loss of vision. A majority of ADOA patients carry mutations in *OPA1* and most mutations lead to haploinsufficiency (Lenaers G. et al. Orphanet J Rare Dis 2012). *OPA1* encodes a mitochondrial GTPase with a critical role in mitochondrial fusion, ATP synthesis and apoptosis. Currently, there is no approved disease-modifying treatment for ADOA patients. Here, we employ TANGO (Targeted Augmentation of Nuclear Gene Output), a novel therapeutic approach, that uses antisense oligonucleotides (ASOs), to increase the endogenous expression of *OPA1*.

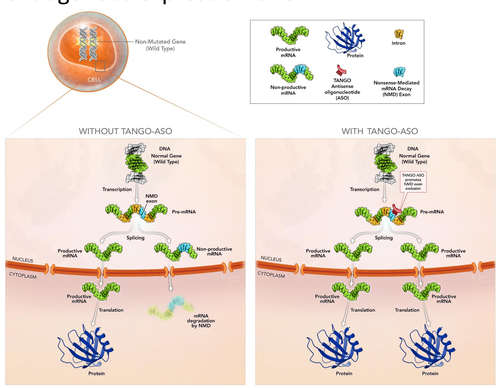


Figure 1: **Mechanism of TANGO:** TANGO reduces non-productive messenger RNAs (mRNA), which are normally targeted for degradation by nonsense-mediated mRNA decay (NMD) as shown in **Figure 1**. In turn, TANGO increases productive mRNA and protein. TANGO specifically increases expression of canonical target mRNA and full-length protein, only in tissues with endogenous gene expression. As these events are naturally-occurring, TANGO can upregulate the wild-type alleles in the context of autosomal dominant haploinsufficiency diseases such as ADOA.

Additionally, TANGO offers the following advantages for treating ocular diseases:

- ✓ Intravitreal injection of ASOs permits diffusion throughout the eye, including retinal neurons
- ✓ Long-term efficacy (>1 year in mouse retina) after single intravitreal injection (Kach et al, ARVO Poster Presentation May 2019)
- ✓ No specialized formulation or encapsulation required for ASO therapy
- ✓ Potential to target large genes not amenable to AAV-based gene therapy

OPA1 non-productive splicing event identification and validation

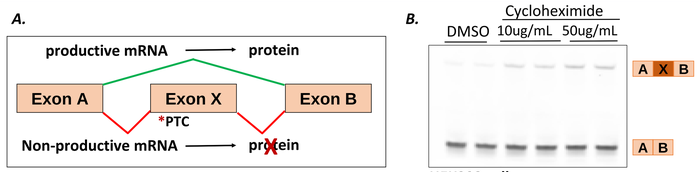


Figure 2. Novel NMD exon inclusion event (Exon X) identified in the *OPA1* gene which leads to the introduction of a premature termination codon (PTC) resulting in a non-productive mRNA transcript degraded by non-sense mediated decay (NMD) (Panel A). As NMD is a translation-dependent process, the protein synthesis inhibitor cycloheximide (CHX) was used to evaluate the true abundance of the event. Panel B shows an increase in *OPA1* transcripts containing the NMD exon in HEK293 cells with increasing CHX dose. Other ocular cell lines also validated for the presence of the NMD exon (ARPE-19, Y79).

OPA1 NMD event is conserved in the primate and rabbit eye

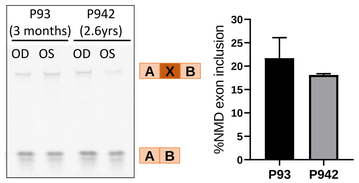


Figure 3: RT-PCR data from posterior segment of eye of *Chlorocebus sabaeus* (green monkey) with accompanying quantification of NMD exon abundance at 3 months and 2.6 years of age (N=1/age). Data represents average of OD and OS values for each animal. OD: oculus dextrus (right eye); OS: oculus sinister (left eye); P: post-natal day

NMD event also conserved in the rabbit retina (See Figure 7, Panels B and C).

Abundance of event is likely to be higher *in vivo*, given that NMD is presumed active in the tissue

Specific ASOs reduce non-productive splicing and increase productive *OPA1* mRNA levels *in vitro*

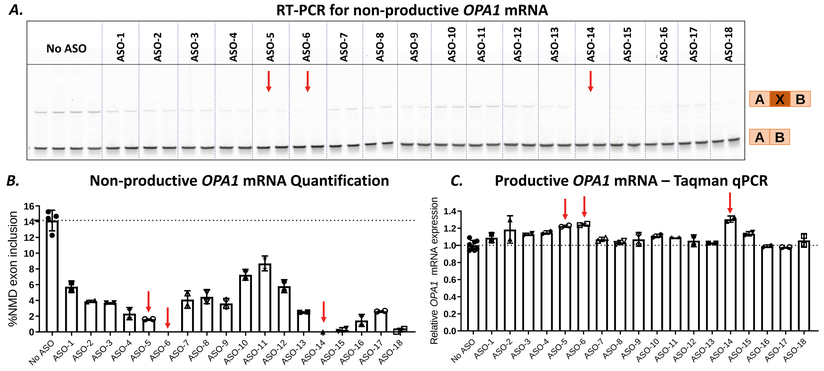


Figure 4. For *in vitro* screening, ASOs were transfected at 80nM dose into HEK293 cells using Lipofectamine RNAiMax as a transfection agent. For effect on NMD exon, cells were treated with CHX (50ug/mL, 3 hrs.) 21 hours after transfection. RNA was isolated and used for RT-PCR (Panel A with quantification in Panel B). For *OPA1* mRNA expression, non-cycloheximide treated cells were used for Taqman qPCR and mRNA expression of *OPA1* was normalized to *RPL32*. **Red arrows highlight ASOs that reduce non-productive splicing and increase *OPA1* mRNA expression by at least 20%. Among these, ASO-14 produces the most increase in *OPA1* mRNA (30%)**

ASO-14 decreases non-productive *OPA1* mRNA and increases OPA1 expression in a dose-dependent manner *in vitro*

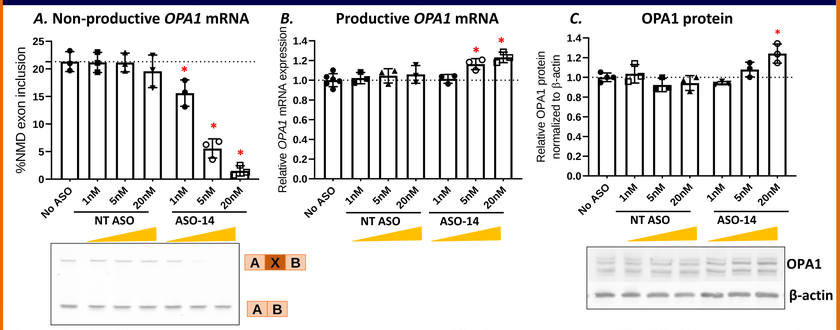


Figure 5. HEK293 cells were transfected with different doses of ASO-14 or non-targeting (NT) ASO. RNA was isolated 24 hours after transfection and analyzed for impact on non-productive *OPA1* mRNA (Panel A) and OPA1 mRNA expression (Panel B) similar to Figure 4. For protein analysis, cells were lysed with RIPA buffer 48 hours after transfection and western blots were probed with antibodies targeting OPA1 and β-actin (Panel C). Multiple bands correspond to different isoforms of OPA1. Data represent average of three independent experiments (* $P<0.05$ by one-way ANOVA compared to "No ASO" group). NT ASO targets unrelated gene.

ASO-14 increases OPA1 expression in an *OPA1* haploinsufficient (*OPA1*$^{+/-}$) cell line

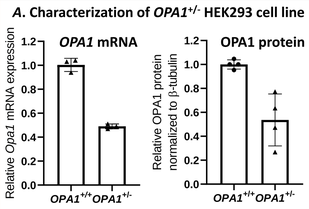
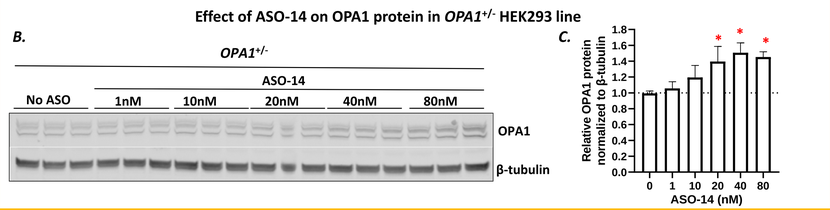
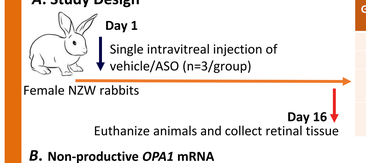


Figure 6. OPA1 haploinsufficient (*OPA1* $^{+/-}$) HEK293 cells were generated using CRISPR-Cas9 gene editing. Similar to ADOA patient cells, *OPA1* $^{+/-}$ HEK293 cells show approximately 50% mRNA and protein levels of that observed in *OPA1* $^{+/+}$ cells (Panel A). *OPA1* $^{+/-}$ HEK293 cells were transfected with different doses of ASO-14, total protein was isolated 72 hours after transfection. Western blots were probed with antibodies targeting OPA1 and β-tubulin (Panel B, quantification in Panel C). Data represent average of two independent experiments (* $P<0.05$ by one-way ANOVA compared to "No ASO" group). **ASO-14 increases OPA1 protein levels in *OPA1* $^{+/-}$ HEK293 cells by 50%, which translates to 75% of wild-type levels.**

Effect of ASO-14 on OPA1 protein in *OPA1*$^{+/-}$ HEK293 line



Rabbit surrogate ASO decreases non-productive splicing and increases OPA1 expression in wild-type rabbit retinae following intravitreal injection



Group	N	Treatment	Dose (ug/eye)	Final conc. expected in vitreous* (uM)	Dose volume (uL/eye)	Dose and regimen	Matrices collected (OU)
1	3	Vehicle (PBS)	NA	N/A	30	IVT OU on Day 1	Retinae split along the nasal-temporal axes, temporal half used for RNA, nasal for protein
2	3	NT ASO	110	12			
3	3	Test ASO – Low Dose	39	4			
4	3	Test ASO – High Dose	116	12			

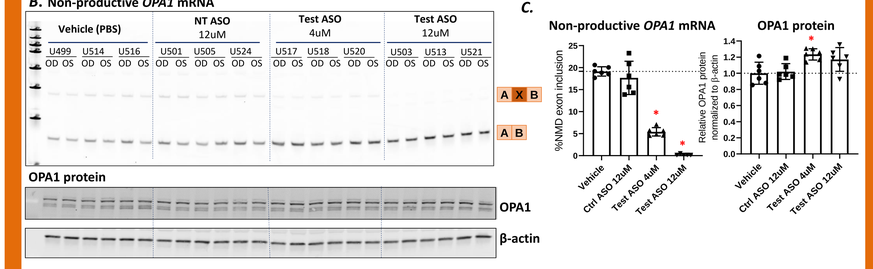


Figure 7. Rabbits were used as a surrogate for initial *in vivo* proof of concept studies to test if our ASO can increase OPA1 expression in the retina following intravitreal injection. Female New Zealand White (NZW) adult rabbits were injected with either vehicle, non-targeting (NT) or test ASO, and animals were euthanized after 15 days to obtain retinal tissue. Panel A outlines the study design while Panels B and C depict impact on NMD exon and OPA1 expression (* $P<0.05$ by one-way ANOVA compared to Vehicle group). **Data show that following intravitreal injection in the rabbit eye, our test ASO reduces non-productive *OPA1* mRNA and increases OPA1 expression in retinal tissue.**

OD: oculus dextrus (right eye); OS: oculus sinister (left eye); OU: oculus uterque (both eyes)

*Final concentration in the vitreous calculated assuming vitreal volume in the rabbit as 1.5mL

Conclusions

We have validated TANGO as a novel therapeutic approach to address ADOA caused by *OPA1* haploinsufficiency

- ✓We identified ASOs that reduce non-productive *OPA1* splicing, increase productive *OPA1* mRNA, and increase OPA1 protein levels
- ✓ASO-14 increased OPA1 protein up to ~75% of wild-type levels in *OPA1*$^{+/-}$ HEK293 cells
- ✓The rabbit surrogate ASO increased OPA1 protein *in vivo* in wild-type rabbit retinae and was well tolerated for up to 15 days after intravitreal injection
- ✓The approach allows us to leverage the wild-type allele and can be used to potentially treat ADOA in a mutation-independent manner

Ongoing work

Our ongoing work focuses on the following areas:

1. Development of *in vitro* assays to assess mitochondria function upon ASO treatment
   - High resolution imaging to characterize mitochondrial morphology
   - Mitochondrial bioenergetics assays to measure function
2. Development of additional *OPA1*$^{+/-}$ *in vitro* systems
   - ADOA patient fibroblast lines
   - Retinal ganglion cells differentiated from *OPA1*$^{+/-}$ iPSCs

Disclosures

AV, SA, AC, KHL, JK, JH, IA and GL are employees and hold equity in Stoke Therapeutics; ZL and RH received financial support from Stoke Therapeutics