INS1203: RNA End-Joining Enables a Dual AAV Approach for ABCA4 Gene Replacement in Stargardt Disease

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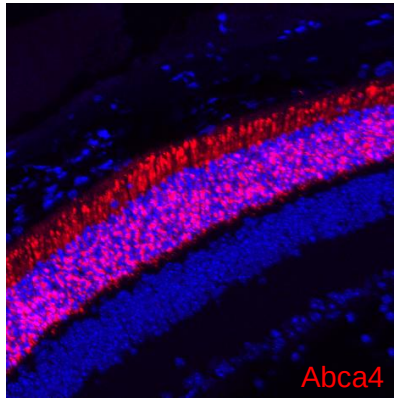
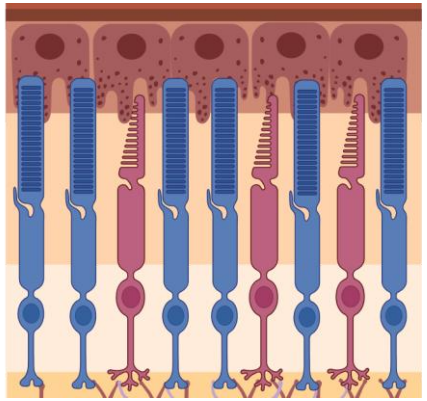
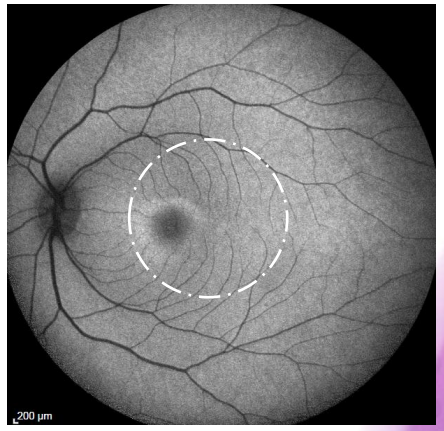
June 12th, 2025

Cell and Gene Therapy Products Symposium
June 10-12, 2025

Stargardt Disease: Mutations in Abca4 Lead to Macular Degeneration





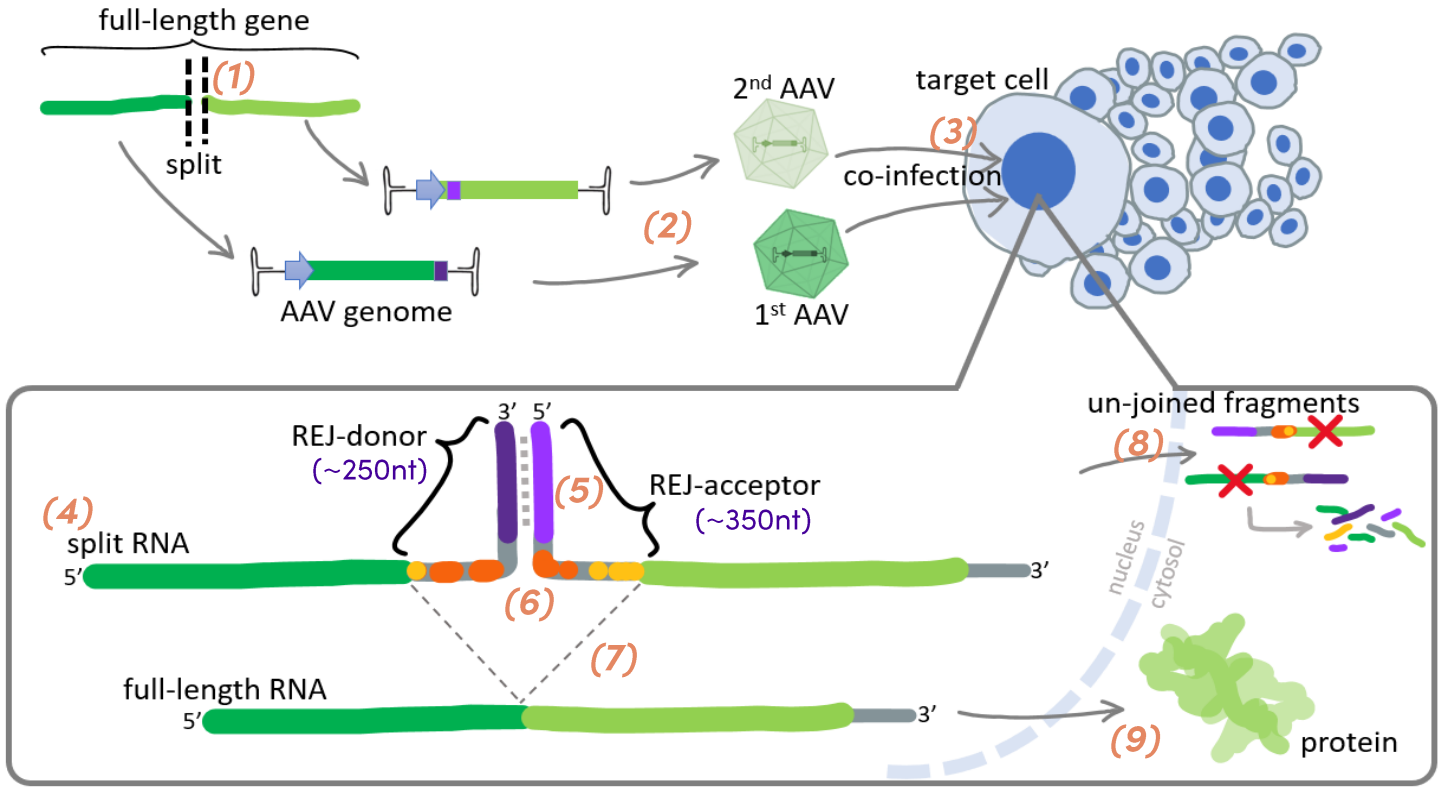


- Abca4 mutations result in accumulation of toxic bisretinoids (A2E) in the retina[1]
- Vision loss typically begins in childhood, with progressive loss of vision occurring within a few years of diagnosis[2-4]
- There are approximately 35,000 patients in U.S.[5]
- The global prevalence is 1 in 6,500 to 1 in 10,000[5]
- The *Abca4* gene (~6.8kb) exceeds the cargo capacity of a single adeno-associated virus

1: Zaydon YA, Tsang SH. The ABCs of Stargardt disease: the latest advances in precision medicine. Cell Biosci. 2024 Jul 26;14(1):98. doi: 10.1186/s13578-024-01272-y. PMID: 39060921; PMCID: PMC11282698.
2: https://lighthouseguild.org/stargardt-disease/
3: https://payments.visionbuddy.com/blogs/the-vision-buddy-blog/stargardts-disease-what-it-is-and-how-low-vision-aids-can-help;
4: Cross-Sectional and Longitudinal Assessment of Retinal Sensitivity in Patients With Childhood-Onset Stargardt Disease, Trans. Vis. Sci. Tech.. 2018;7(6):10. doi:10.1167/tvst.7.6.10
5: Ben-Yosef T. Inherited Retinal Diseases. Int J Mol Sci. 2022 Nov 3;23(21):13467. doi: 10.3390/ijms232113467. PMID: 36362249; PMCID: PMC9654499.

Schematic generated in Biorender

RNA-End Joining (REJ) Enables a Potential Therapeutic Approach for Stargardt Disease

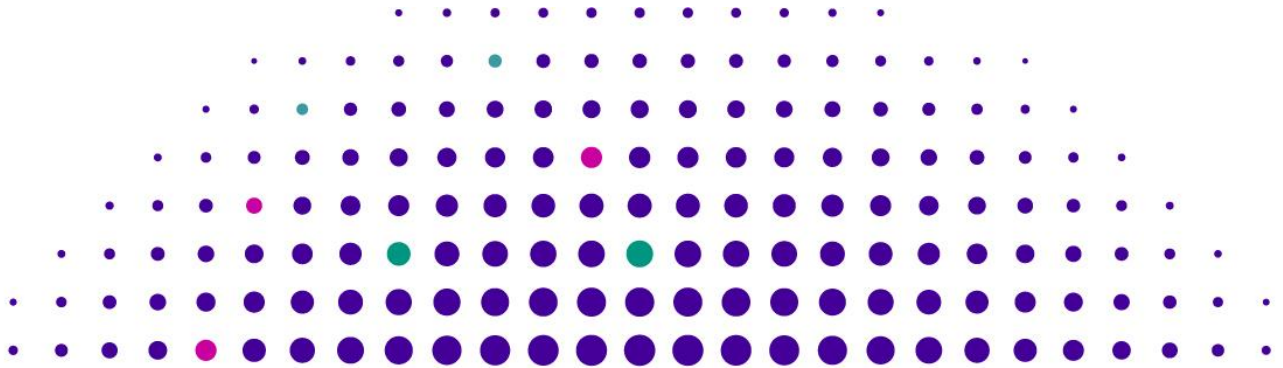


INS1203: DUAL AAV8 ENCODING ABCA4

Investigational INS1203 Drug Product:
1:1 formulation of REJ-Donor and REJ-Acceptor
Photoreceptor-specific GRK1 promoter

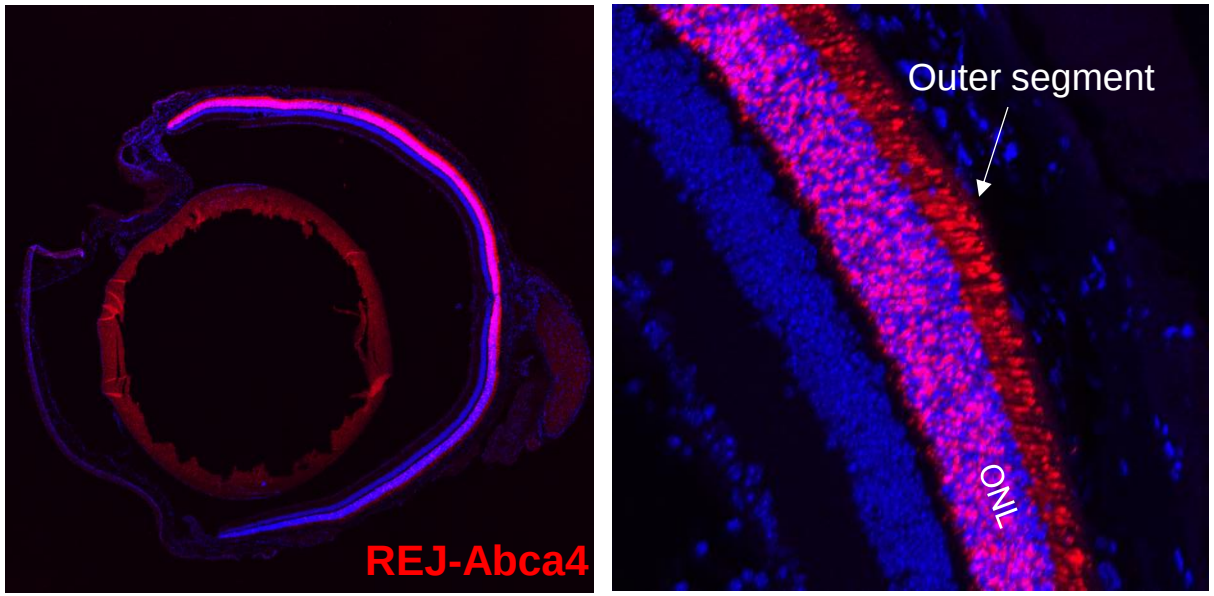
Characterization of Lead Candidate INS1203: REJ-Mediated Abca4 Gene Replacement Therapy

1. Proof of Concept Studies for INS1203 Candidate
   - Abca4 Expression in Stargardt Disease Mouse Model
   - Characterization of INS1203-Derived Gene Therapy Product
2. Dose-Ranging Efficacy Study in Mouse Model
3. Expression and Biodistribution in Nonhuman Primate

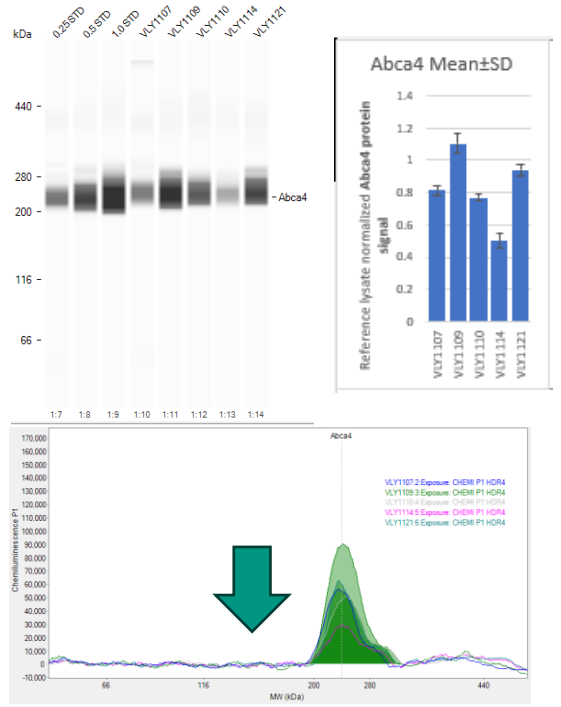


Subretinal Injection of INS1203 Results in Photoreceptor-Specific Expression of Abca4 Protein

PHOTORECEPTOR-SPECIFIC EXPRESSION



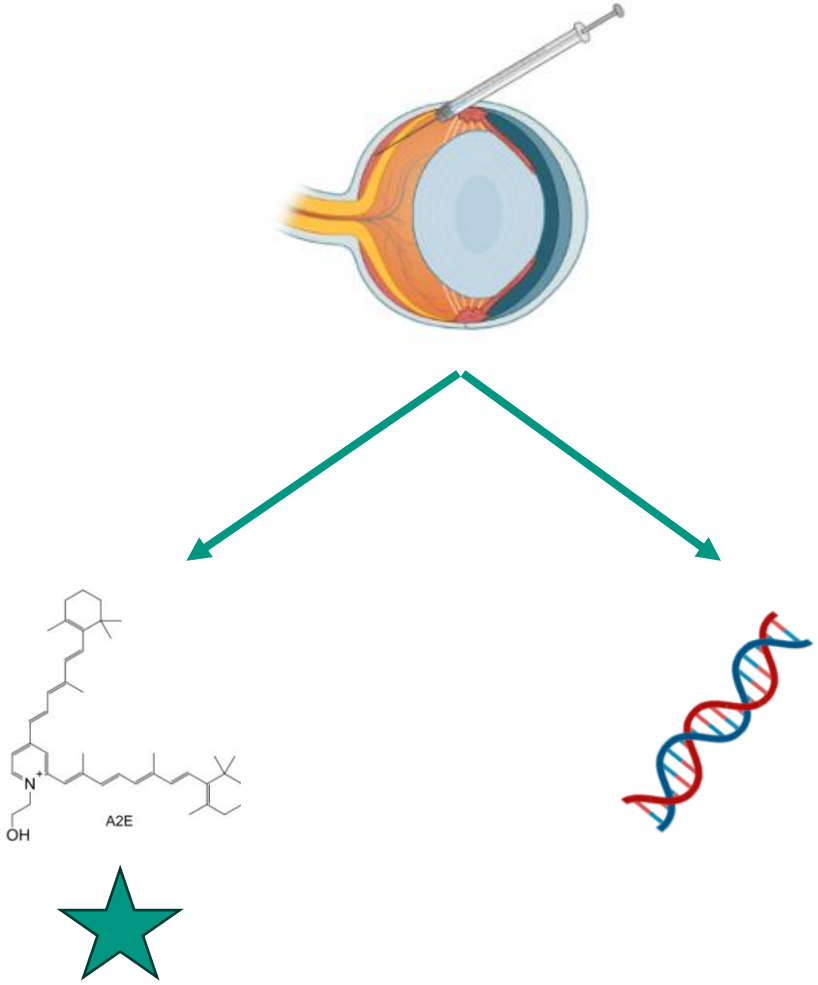
PROTEIN FRAGMENT SUPPRESSION



Subretinal injection of INS1203 (High Dose), Abca4 knockout mouse
Left: Immunofluorescent detection of Abca4 protein (Representatve individual)
Right: Jess capillary western blot detection of Abca4 protein (N=5 mice)

Dose-Finding Study in Stargardt Disease Mouse Model

Study Design and Objectives



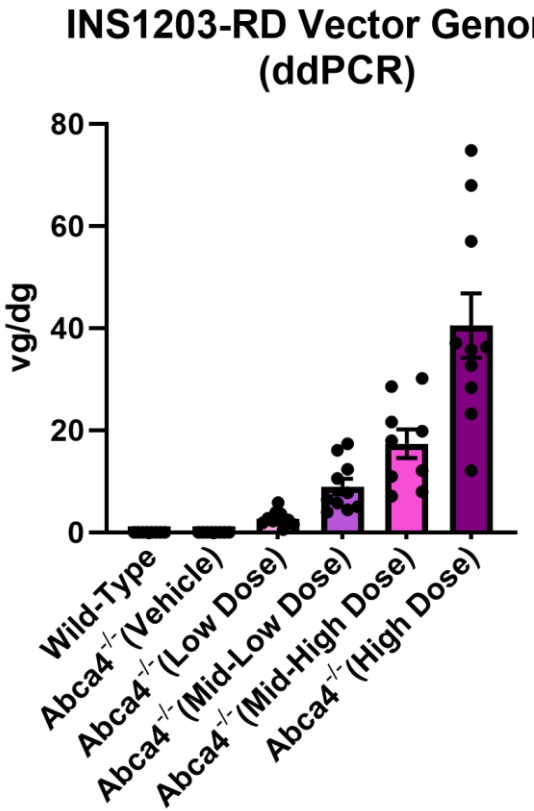
Subretinal administration of INS1203 into Abca4$^{-/-}$ Mouse at P28

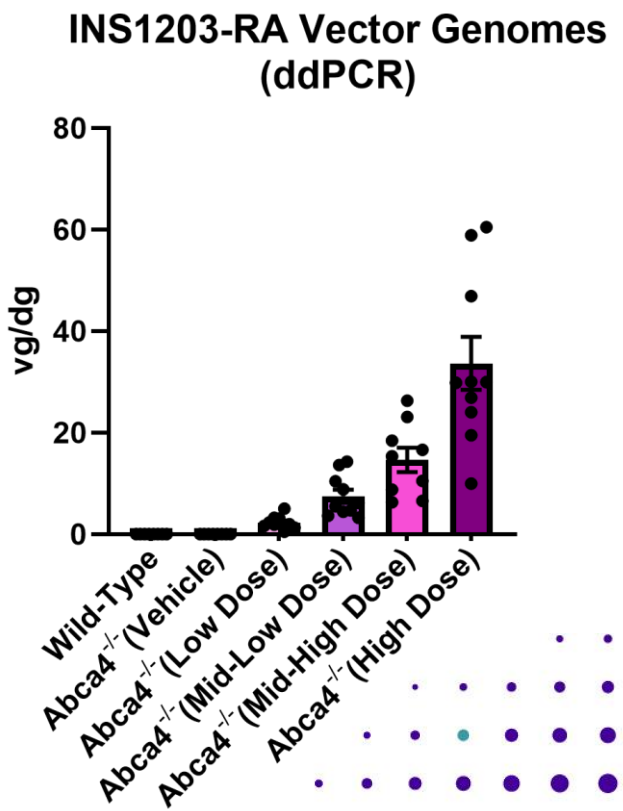
- 6 groups (n=10-12 eyes/group):
  - Wild-type, Naive
  - Abca4$^{-/-}$, Vehicle-Treated
  - Abca4$^{-/-}$, INS1203-Treated (High)
  - Abca4$^{-/-}$, INS1203-Treated (Mid-High)
  - Abca4$^{-/-}$, INS1203-Treated (Mid-Low)
  - Abca4$^{-/-}$, INS1203-Treated (Low)

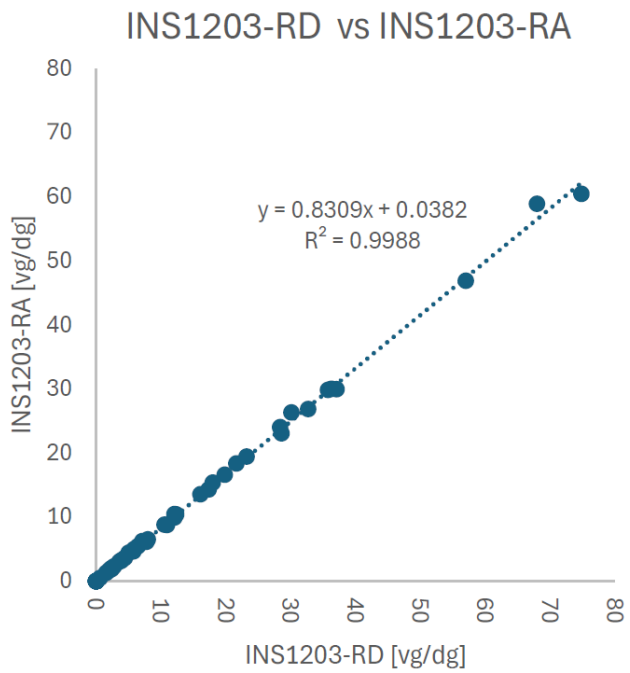
- 10 week in-life time course
- Combined analytical workflow to evaluate multiple readouts (LCMS/MS, Nucleic acids)
- Dose Range covers a ~33-fold reduction in dose (relative to high dose)

Dose-Dependent Increase in Transduction of INS1203-RD and INS1203-RA

ddPCR: Quantification of viral vector genomes

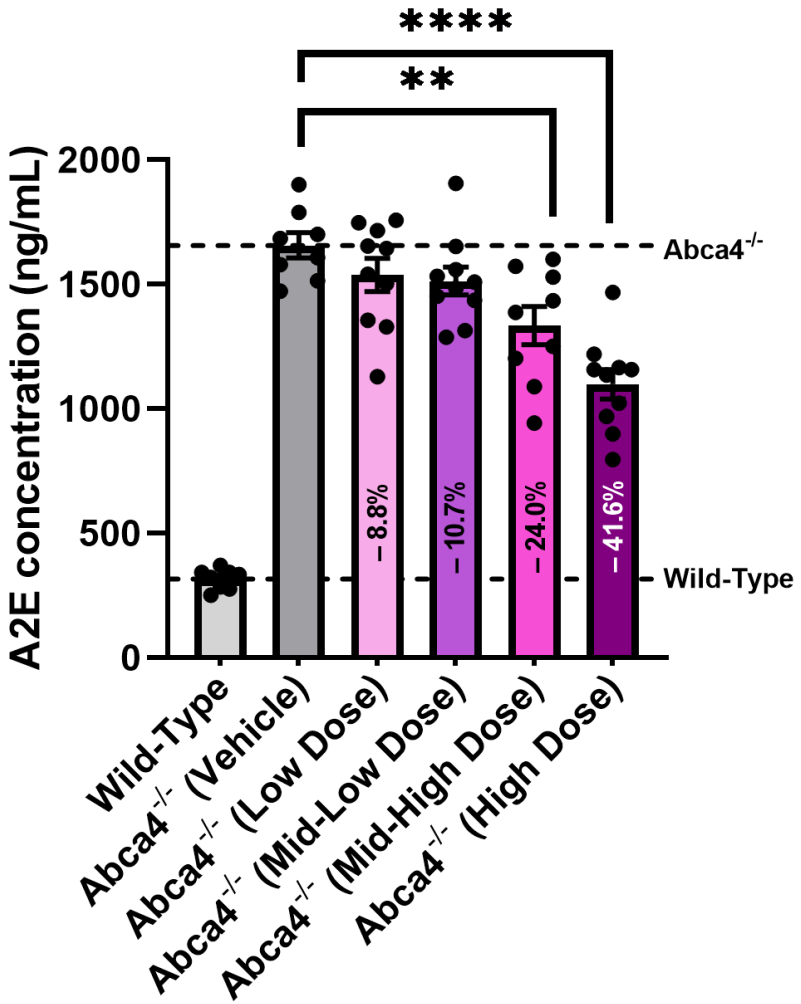






Dose-Dependent Reduction in Stargardt Disease Biomarker A2E

A2E: Stargardt Disease Biomarker



Subretinal delivery of INS1203 results in:

- High Dose: 41.6% reduction****
- Mid-High Dose: 24.0% reduction**
- Mid-Low Dose: 10.7% reduction
- Low Dose: 8.8% reduction

in A2E, relative to vehicle-injected Abca4$^{-/-}$

Interim Summary from Mouse Expression and Efficacy Studies

1. Subretinal injection of INS1203 results in **photoreceptor-specific, supraphysiological** expression of Abca4
2. INS1203 treatment results in **significant and dose-dependent reduction** of Stargardt Disease biomarker A2E
3. Combined analytical workflow allows for multidimensional evaluation of INS1203-treated eyecups
4. INS1203 formulation results in **equivalent levels** of INS1203-RD and INS1203-RA
5. Dose-ranging study was successful in **identification of efficacious dose** levels

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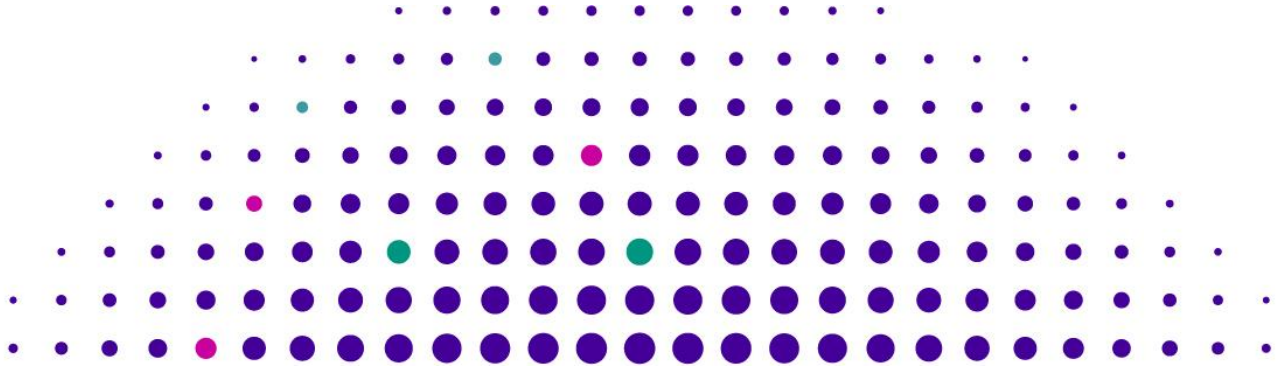
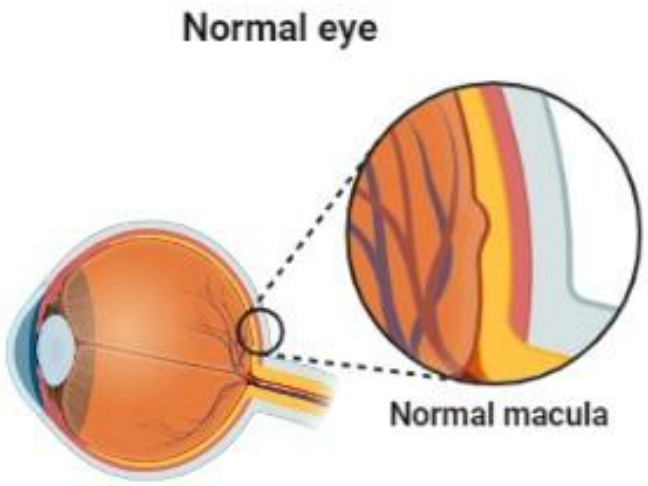
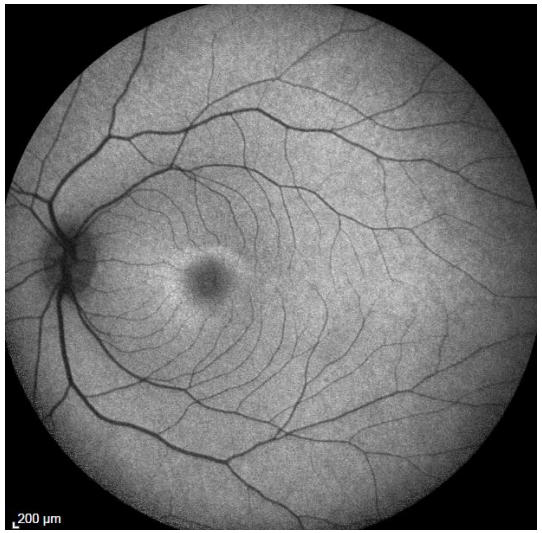
2. Dose-Ranging Efficacy Study in Mouse
3. **Expression and Biodistribution in Nonhuman Primate**



Proof of Concept Expression Study in Nonhuman Primate

INS1203 Delivery via Subretinal Administration



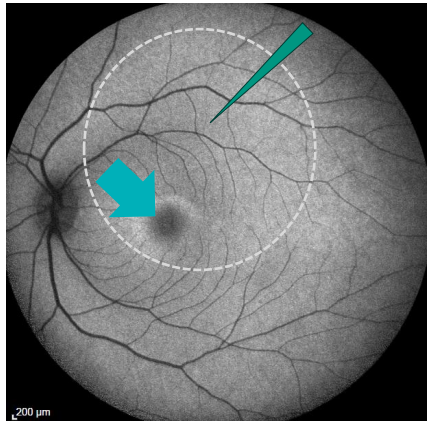


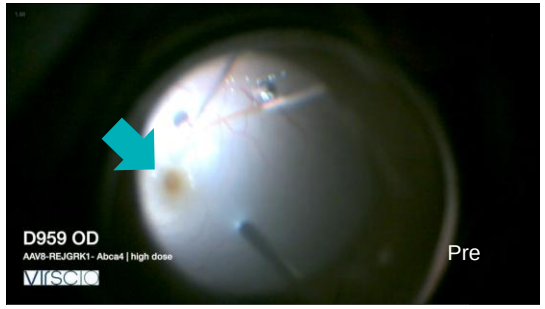
Subretinal administration of INS1203 into African Green Monkey (Chlorocebus sabaeus)

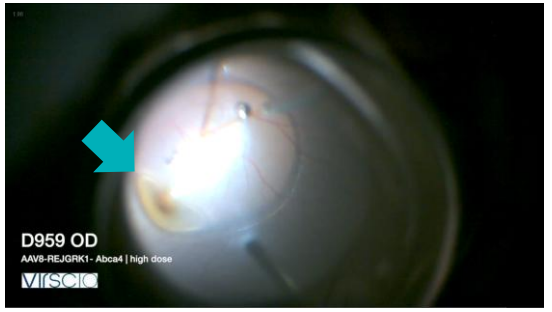
- 2 groups (1 eye/group):
  - Naïve, Low-Dose Treated
  - Naïve, High-Dose Treated
- 8 week in-life timecourse
- Histology: Abca4 mRNA, retinal coverage
- Preliminary Biodistribution: ddPCR

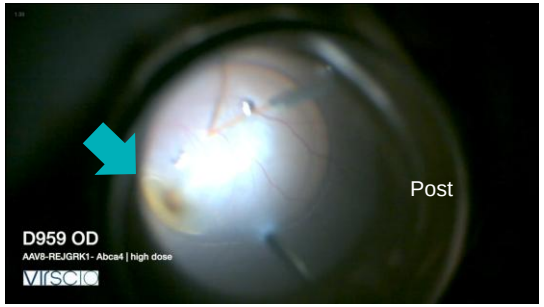
Evaluation of Retinal Structure Following Subretinal Delivery of INS1203

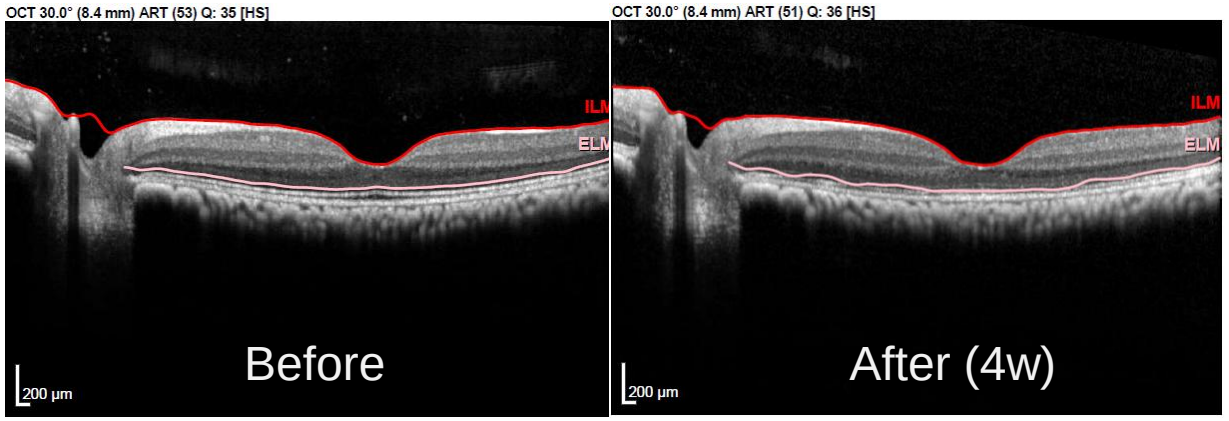
Post-operative imaging and histopathology





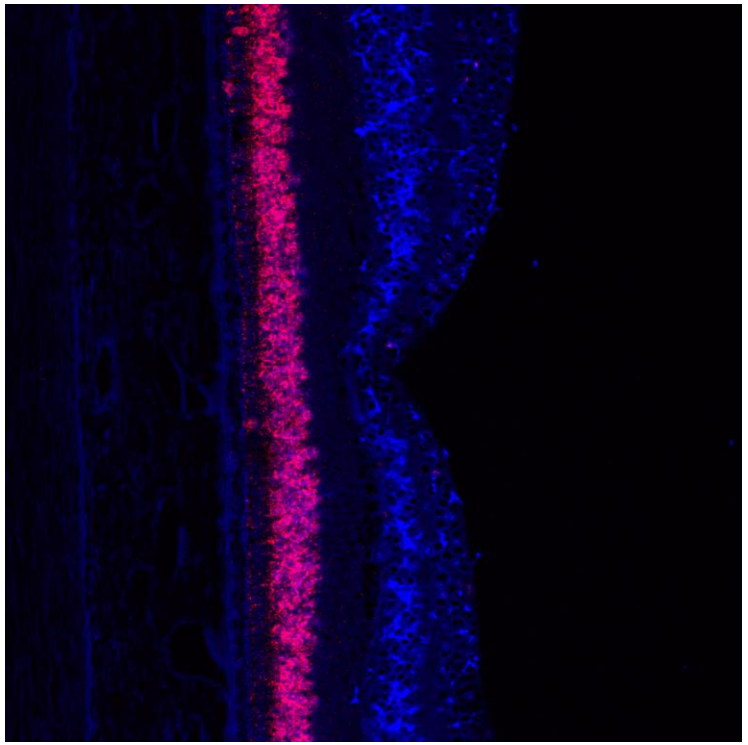




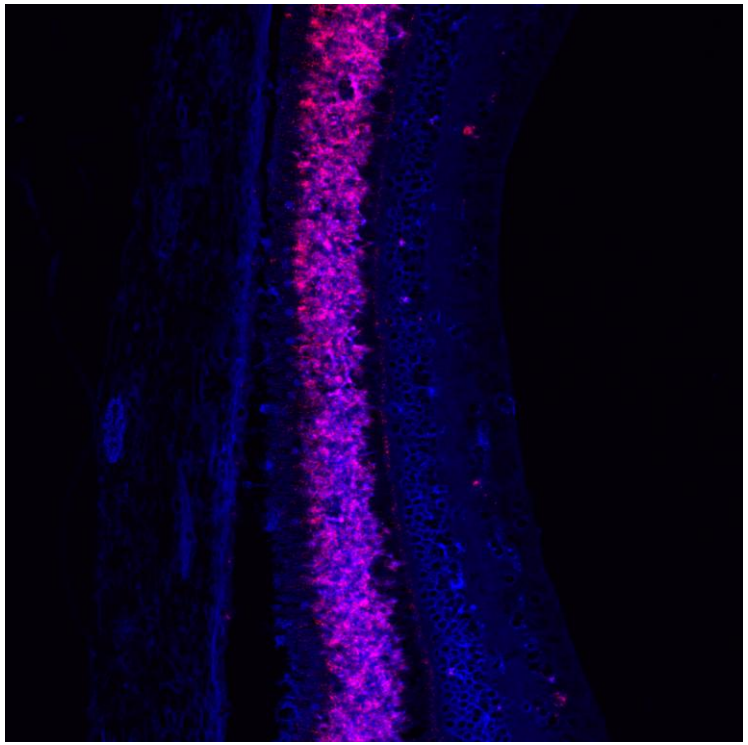


Cell Type-Specific Expression of Human Abca4 in Nonhuman Primate Photoreceptors

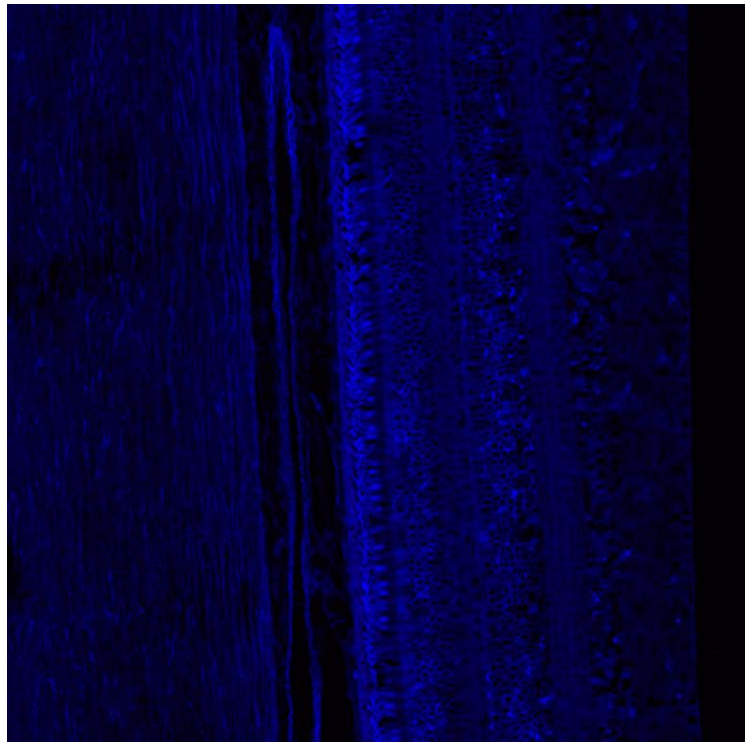
Fluorescent in situ hybridization (full-length human Abca4)



D959 OS (High Dose)



D959 OD (Low Dose)



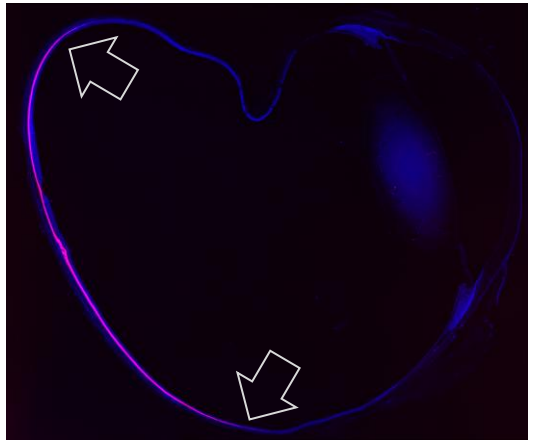
A761 OD (Naïve, untreated)

BaseScope Abca4 (joined mRNA)

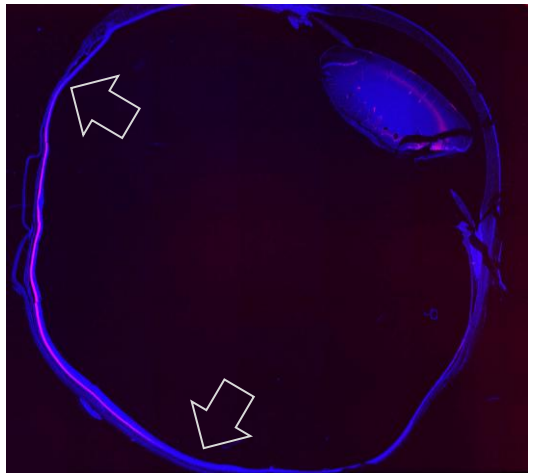
Dose-dependent Transduction and Expression of the Nonhuman Primate Retina

INS1203 Delivery via Subretinal Administration

Subretinal – INS1203 (High Dose)



Subretinal – INS1203 (Low Dose)



Retinal Coverage*	
OS retina (high dose; %)	43.40
OD retina (low dose; %)	37.39
OS retina (high dose; mm)	16.42
OD retina (low dose; mm)	13.28

Tissue Sample	INS1203-RD gDNA (vg/dg)	INS1203-RA gDNA (vg/dg)
OS retina (high dose)	43.06	39.28
OD retina (low dose)	24.25	22.30
OS optic nerve (high dose)	0.39	0.22
OD optic nerve (low dose)	0.01	0.01
OS extraocular muscle (high dose)	1.61	0.98
OD extraocular muscle (low dose)	0.01	0.01
Left ventricular wall	0.02	0.01
Right liver lobe	0	0
Frontal cortex	0	0
Left lateral geniculate nucleus	0	0
Occipital cortex	0	0
Left ovary	0	0
Spleen	0.08	0.08
Left mandibular lymph nodes	0.01	0.01
Cervical spinal cord	0	0
Left cervical (C3) dorsal root ganglion	0	0

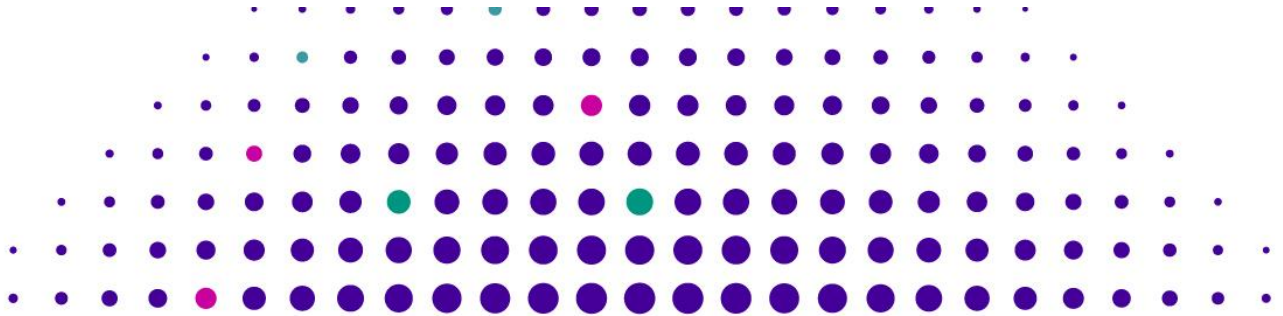
Key takeaways:

- Subfoveal delivery of INS1203 results in extensive coverage of the nonhuman primate retina*
- INS1203 formulation results in equivalent transduction of N- and C-terminal fragments
- Dose-dependent transduction of INS1203, as quantified by vector genome counts
- Limited drug product biodistribution following subretinal delivery of INS1203

*For reference, in human:
macula = 5.5mm
fovea = 1.5mm
foveal pit = 150μm

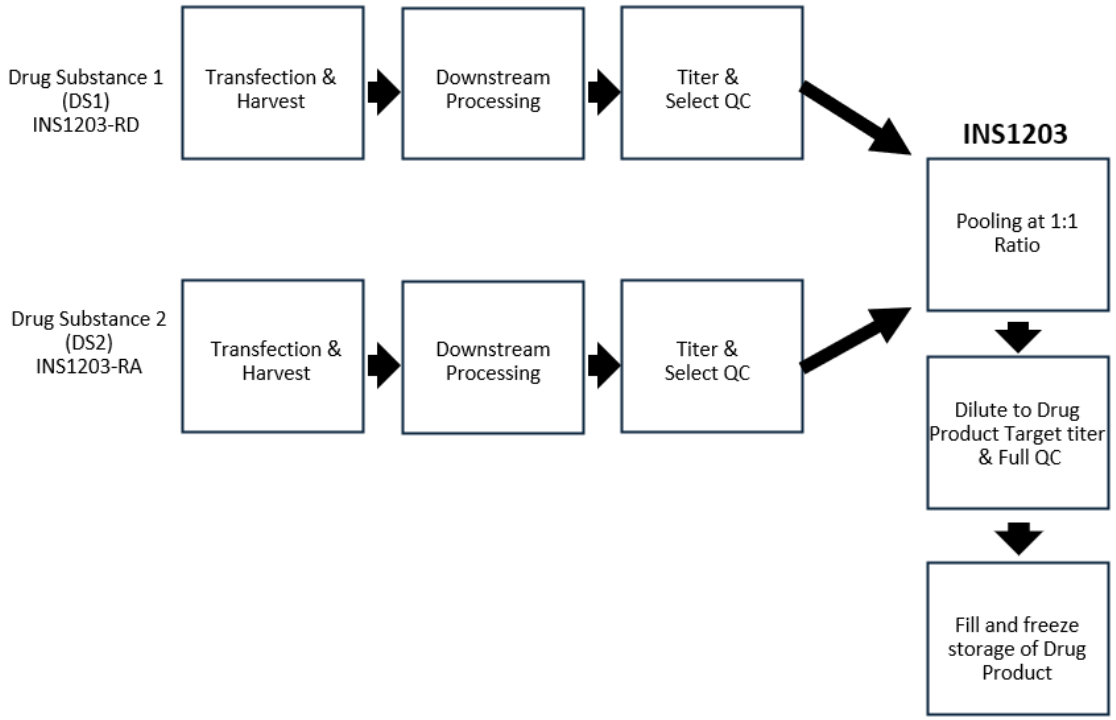
Conclusions from Nonhuman Primate Proof of Concept Study

1. Subretinal injection of INS1203 results in **photoreceptor-specific expression of full-length Abca4** in the nonhuman primate
2. INS1203 treatment is **generally well-tolerated** with minimal biodistribution in extraocular and systemic tissues
3. INS1203 formulation results in **equivalent levels** of INS1203-RD and INS1203-RA in the nonhuman primate
4. Collectively these nonclinical studies demonstrate **successful delivery of large genes to the nonhuman primate retina** and **support further development of INS1203** as an investigational gene replacement therapy for Stargardt Disease

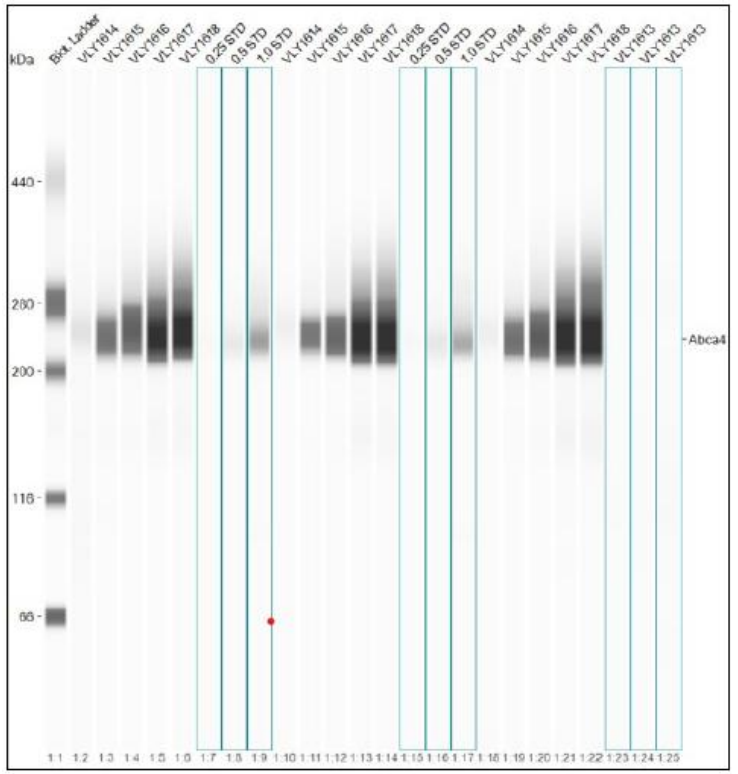


Manufacturing Considerations for a Dual AAV Drug Product

POOLING OF INS1203-RD AND INS1203–RA DRUG SUBSTANCE



ENGINEERED CELL MODEL: YIELD-BASED POTENCY ASSAY



Expected size of Abca4 signal: 230 kDa.

INS-1203 Global Asset Team

Stargardt R&D Team

Count us in.

Disclaimer: Sam Pfaff is employed by the Salk Institute. All other authors are employed by and are shareholders of Insmed Incorporated, San Diego, CA and Bridgewater, NJ. This project is funded by Insmed Incorporated.