

A Novel Approach to Ocular Gene Therapy: Evaluation of Suprachoroidally Administered Non-Viral DNA Nanoparticles in Rabbits

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Disclosures

- **Consultant and Research Funding** – Adverum, RegenXBio, Spark
- IP related gene therapy

DNA nanoparticles offers the potential for safe, efficacious, and repeat dosing ocular gene therapy

Potential advantages: DNA Nanoparticles versus viral vector-mediated gene therapy

- Unlike AAV (payload capacity of 5 kb), can transfer large genes (up to ~20 kb)
- Safety
 - Non-immunogenic, without viral capsid proteins or pre-existing immunity
 - Potential for repeat and greater dosing
- Efficacy in numerous ocular animal models
 - Higher doses may be used to enhance transfection
- Manufacturing
 - Simpler than viral-based gene therapy

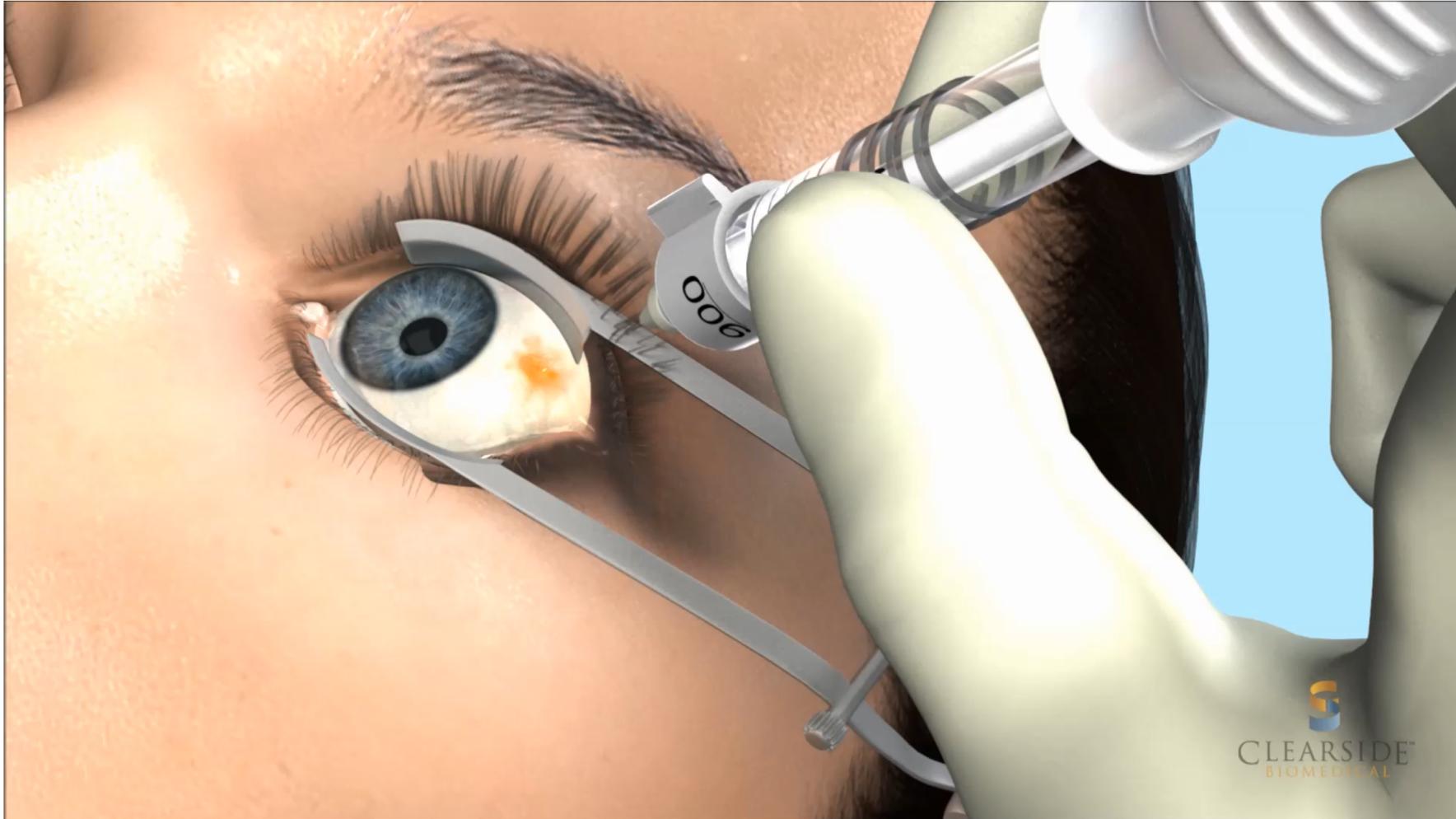
Potential disadvantages: DNA Nanoparticles versus viral vector-mediated gene therapy

- Durability
 - May not represent one time therapy

Suprachoroidal (SC) injection offers the potential for safe, targeted, and efficient ocular gene therapy

- **Targeted treatment** of posterior tissues possible via SC injection
 - Spread of injectate flows circumferentially and posteriorly
- **Safety**
 - Avoids the risks of sub-retinal surgery
 - Does not require detachment of the photoreceptors from the RPEs, without associated risk of iatrogenic injection to already compromised disordered retina
 - SC injection procedure training is minimal
- **Access to care**
 - Does not require specialized gene therapy surgery treatment centers
 - In-office SC injection procedure is less expensive than surgical procedures
 - Procedure time is significantly less than standard sub-retinal procedure

Suprachoroidal Injection as a Novel Delivery Method



Evaluation of Suprachoroidally Injected DNPs in Rabbits

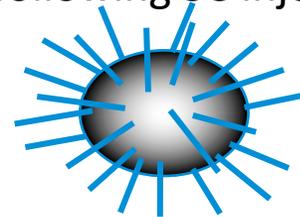
Key Questions

- Does SC injection potentially facilitate effective administration of non-viral DNP gene therapy?
- Does SC injection potentially fulfill an unmet need? Can sub-retinal surgery be avoided?

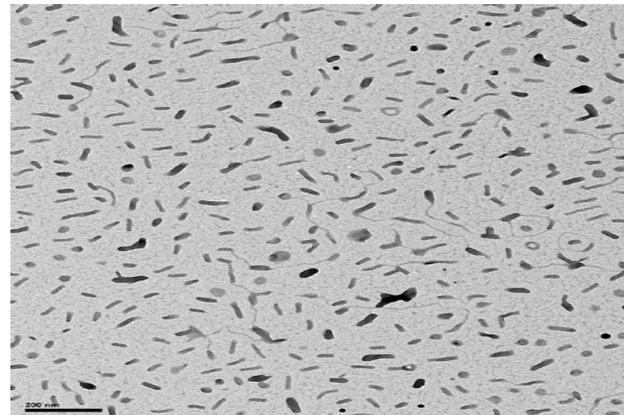
Study Objective

Evaluate the safety, tolerability, and retinal cell transfection following SC injection of DNPs in NZW rabbits

Ellipsoids

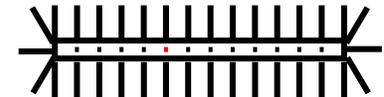


Colloidally stable DNPs Suspended in Saline

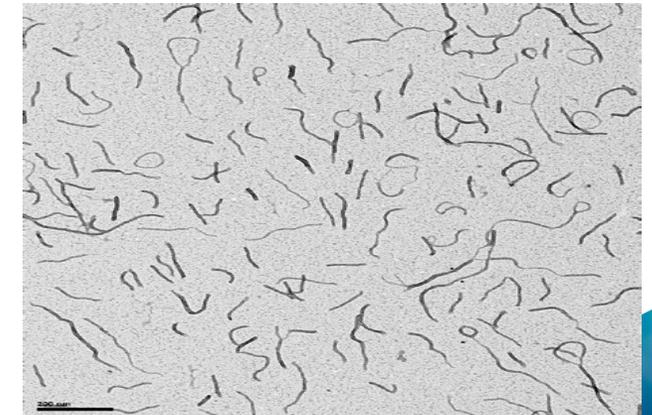


8-10 nm in diameter

Rods



Colloidally stable DNPs Suspended in Saline



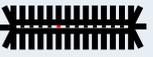
DNA
+
modified polylysine peptides
formulated with
polyethylene glycol (PEG)

SC Injection of DNPs in Rabbits

Design

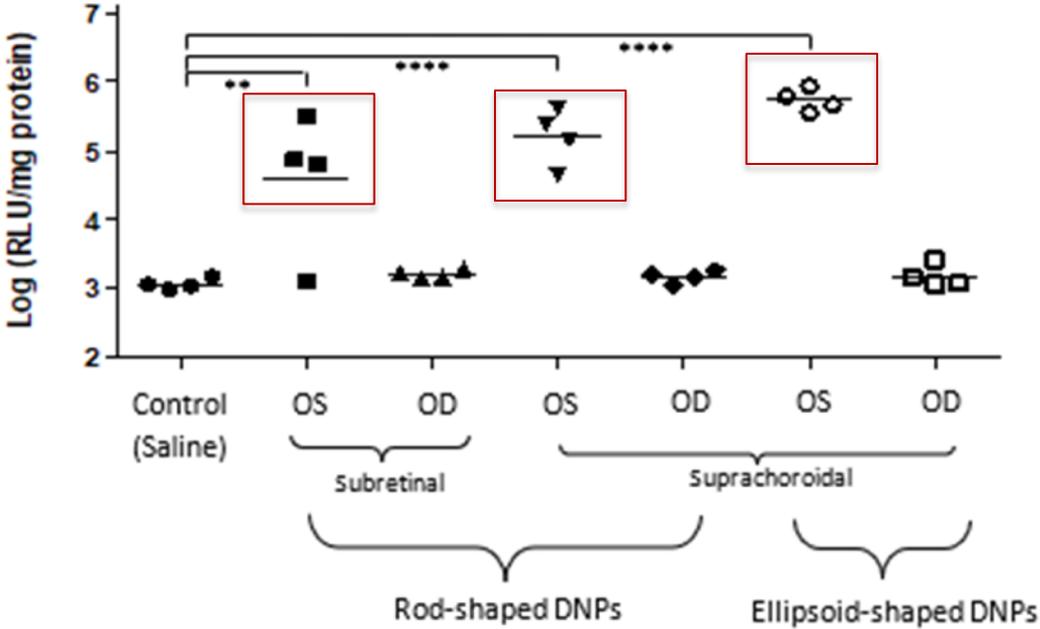
- Four animals per group injected into the right eye only
- Ophthalmic examinations Days 0, 1, and 7:
 - Assessed surface morphology, anterior segment inflammation, IOP and ERG
- One-week post-injection:
 - Eyes enucleated, choroid and retina separated, processed for evaluation of luciferase activity



Groups	Test article	Route of Administration (OS only)	Volume
1	Vehicle	SC Injection	100 μ L
2	 Ellipsoid DNPs Luciferase	SC Injection	100 μ L
3	 Rod DNPs Luciferase	SC Injection	100 μ L
4	 RodDNPs Luciferase	Sub-retinal injection	50 μ L

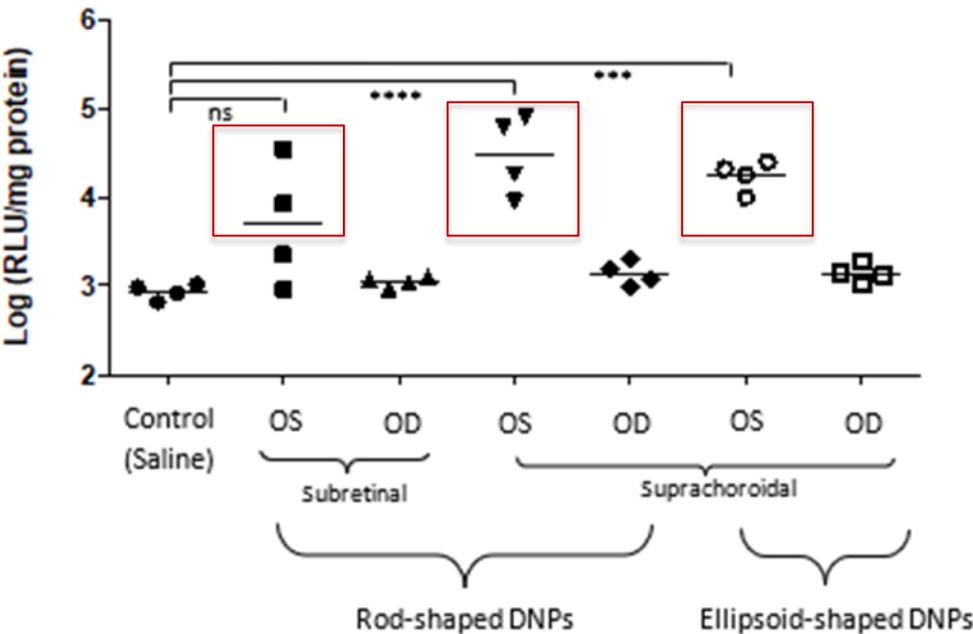
DNA Nanoparticles Transfect the Retina and Choroid

Non Viral-Luciferase, Rabbit
CHOROID



OS: Dosed OD: Undosed	Bonferroni's multiple comparison test: ** p<0.01, *** p<0.001, **** p<0.0001 ns, non-significant
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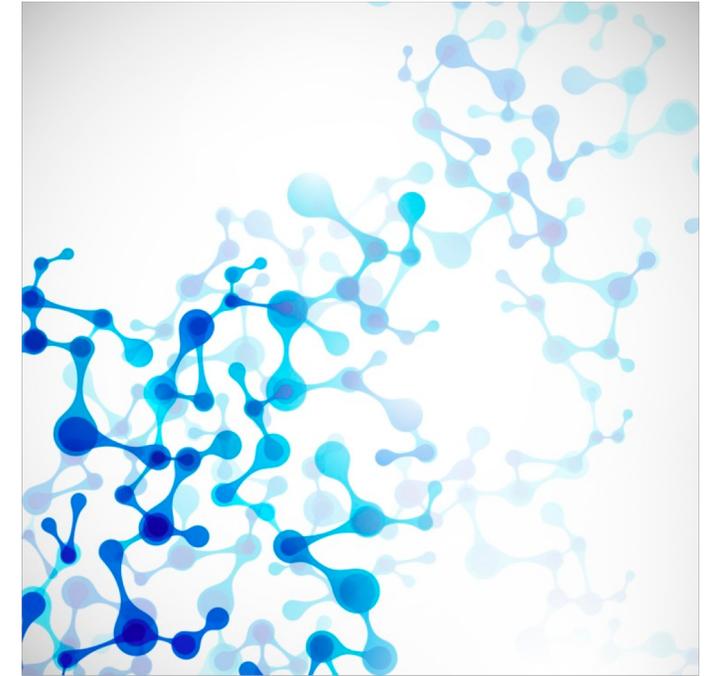
Non Viral-Luciferase, Rabbit
RETINA



OS: Dosed OD: Undosed	Bonferroni's multiple comparison test: *** p<0.001, **** p<0.0001 ns, non-significant
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Study Summary

- Luciferase activity observed in the **retina and choroid** of ALL eyes that received SC injection of DNPs
- SC injection of luciferase DNPs produced activity **comparable to that seen from subretinal injections** of luciferase DNPs
- SC injections on DNPs were **generally well-tolerated** across groups; no significant abnormalities observed on ophthalmic exams or ERGs



The Future of SC Injections of DNA Nanoparticles

- **Additional experiments needed**
 - Evaluate SC injection in non-human primates
 - Evaluate delivery of a therapeutic transgene
- **Why is this important?**
 - **Safety**
 - SC injection of DNPs may address an unmet need in ocular gene delivery
 - Non-immunogenic, potential for repeat doing
 - **Efficacy**
 - Higher doses may be used to enhance transfection
 - Sub-retinal procedure is 5-10 times more efficient in delivery than intravitreal injections, but has shortcomings that may be overcome with SC injections of DNPs
 - DNPs can transfer large genes which may allow for gene therapy in the most common inherited retinal diseases (IRDs) such as Stargardt disease and Usher syndrome
- **SC injections of DNPs offer the potential for a safer and efficient delivery method**

A glowing blue DNA double helix structure is shown against a dark blue background. The helix is composed of two strands connected by rungs, creating a spiral pattern. The text "THANK YOU" is centered over the middle of the image.

THANK YOU