Suprachoroidally delivered non-viral DNA nanoparticles transfect chorioretinal cells in non-human primates and rabbits

Nancy Holekamp, MD[1]
Thomas Ciulla, MD, MBA[2]
Viral Kansara, PhD[2]

1. Pepose Vision Institute, St. Louis, MO. 2. Clearside Biomedical, Inc.

Disclosures

NH: Consulting fee: Allergan, Acucela, Lineage Cell Therapeutics, Clearside Biomedical, Gemini, Genentech, Gyroscope, Katalyst Surgical, Nacuity, Notal Vision, Novartis, Regeneron
Speakers Bureau: Allergan, Genentech, Novartis, Regeneron, Spark
Contracted Research: Genentech, Gemini, Gyroscope
Intellectual Property/Patent: Katalyst Surgical

TC: Clearside Biomedical (employee, personal financial interests)
VK: Clearside Biomedical (employee, personal financial interests)

Summary: Supachoroidal Injections of DNA Nanoparticles

May address an unmet needs in ocular gene delivery:

- Potentially safer and efficient in office delivery versus risk associated with surgical procedure
- Non-immunogenic, potential for repeat dosing
- Transfer large genes, allowing for gene therapy in common inherited retinal diseases (IRDs), ie. Stargardt disease and Usher syndrome
- Additional research evaluating SC injection in non-human primates and delivery of a therapeutic transgene is needed

Core Advantages of Treating Via the Suprachoroidal Space

TARGETED

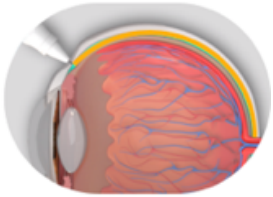
The back of the eye is the location of many irreversible and debilitating visual impairments[1]

for efficacy

COMPARTMENTALIZED

Drug is compartmentalized in the suprachoroidal space, which helps keep it away from non-diseased tissues[2]

for safety



BIOAVAILABLE PROLONGED PK

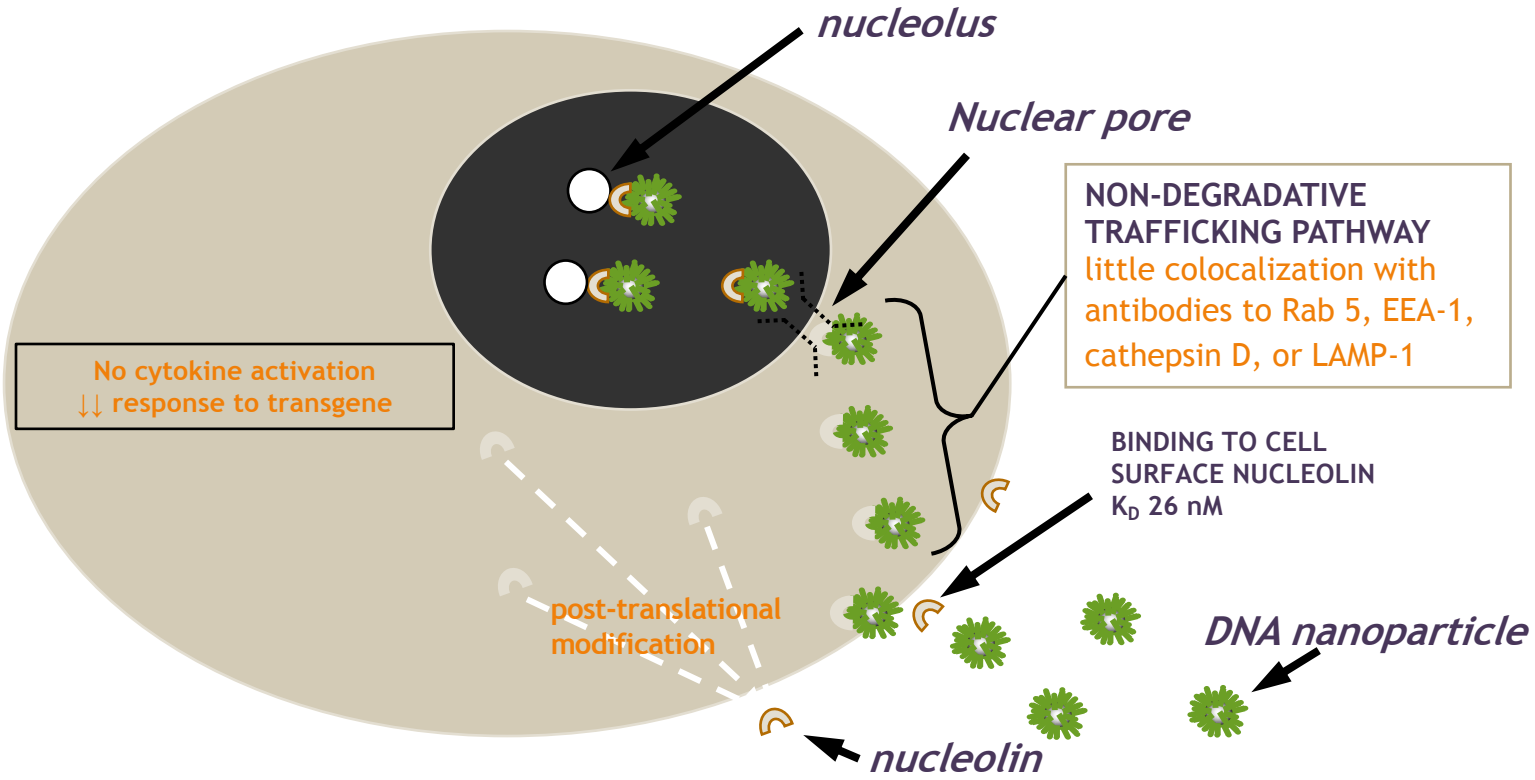
Fluid spreads circumferentially and posteriorly when injected within the suprachoroidal space, bathing the choroid and adjacent areas with drug[3]

for durability

Sources: PK = pharmacokinetic | 1. Rai UDJ, Young SA, Thrimawithana TR, et al. The suprachoroidal pathway: a new drug delivery route to the back of the eye. Drug Discov Today. 2015;20(4):491-495. 2. Chiang B, Jung JH, Prausnitz MR. The suprachoroidal space as a route of administration to the posterior segment of the eye. Adv Drug Deliv Rev. 2018;126:58-66. 3. Moisseiev E, Loewenstein A, Yiu G. The suprachoroidal space: from potential space to a space with potential. Clin Ophthalmol. 2016;10:173-178.

Suprachoroidal Injection is an In Office, Repeatable Delivery Method

Uptake and Trafficking of DNA Nanoparticles

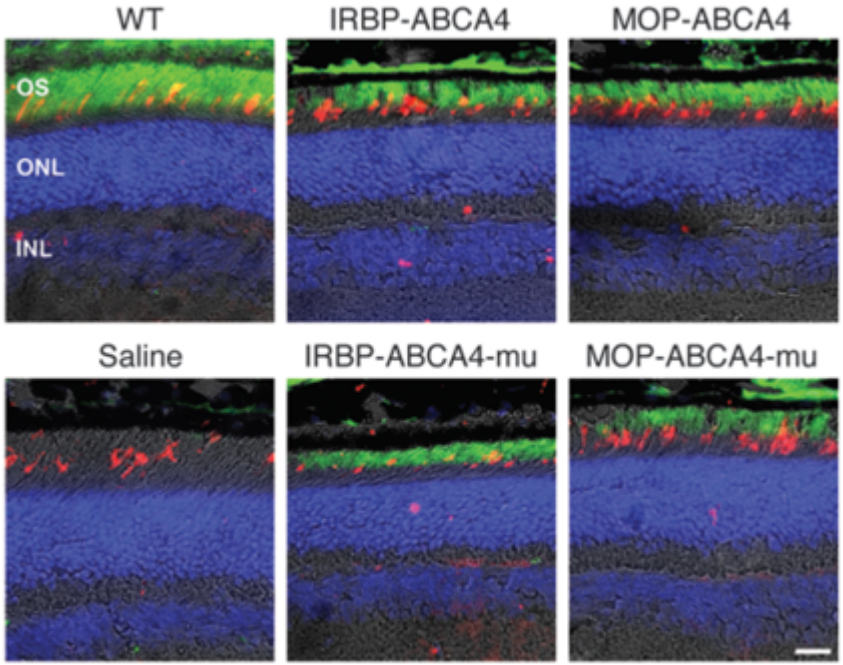


Non-viral DNP experience in ocular models

Safe and restores function in multiple mouse knock out models

	Model	Route	Target Cell Types	Function	Histology	Assay Time
Mouse	RDS (peripherin 2) RP	SR	photoreceptor	+ERG	+	4 mo
				+ vision behavior		1 year
	Stargardts (ABCA4) macular degeneration	SR	RPE	+ERG	+	8 mo
	RPE65 RP	SR	RPE	+ERG	+	15 mo
	Rhodopsin RP	SR	photoreceptor	+ERG	+	8 mo
	Diabetic retinopathy (miRNA 200b)	IVT	vasculature	normal	+	3 mo
	RPE marker gene	SR	RPE		+	2.5 yr
	AAV versus DNA NP	SR	PR and RPE		+	4 mo
NHP	Baboon	SR, IVT	RPE	+ ERG		

DNP-mediated ABCA4 gene delivery transfects retina photoreceptor cells in Abca4-/- mice

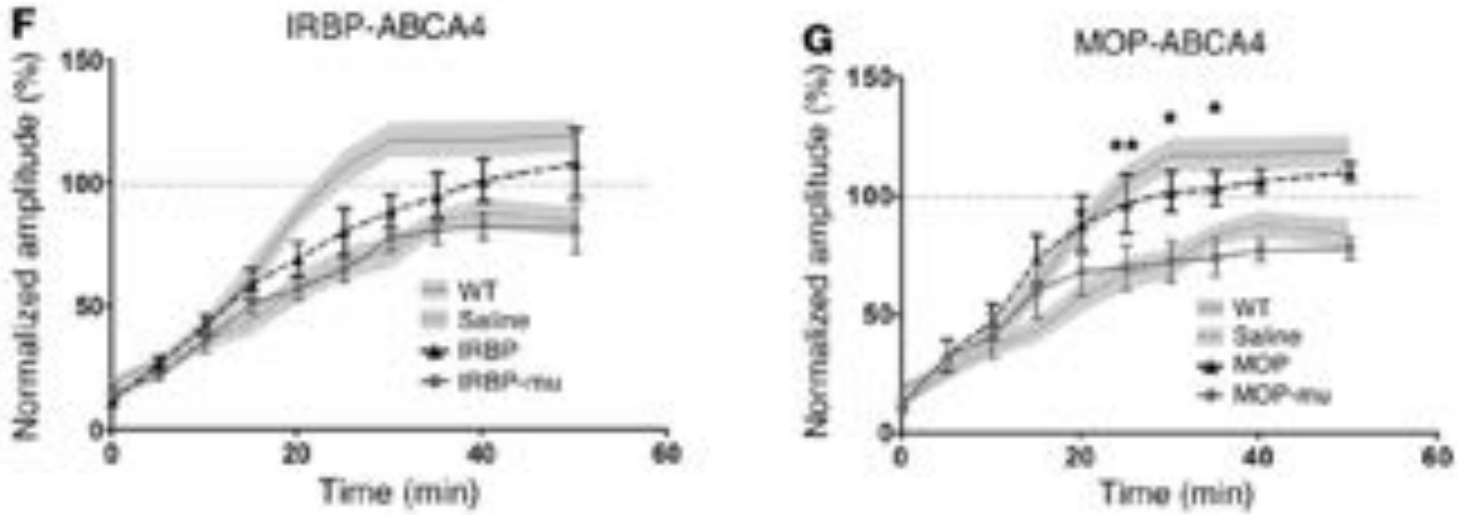


Retinal cryosections at 8 months PI co-labeled for ABCA4 (green), S-opsin (red) with DAPI (epifluorescent images/bright field)

Han Z, Conley SM, Makkia RS, Cooper MJ, Naash MI. DNA nanoparticle-mediated ABCA4 delivery rescues Stargardt dystrophy in mice. *J Clin Invest*. 2012;122(9):3221–3226. doi:10.1172/JCI64833

DNP-mediated ABCA4 delivery promotes functional improvement in Abca4-/- mice

Scotopic ERGs: a-wave amplitudes



Scotopic ERGs were recorded from dark-adapted WT and Abca4–/– mice before and every 5 minutes after a 5-minute (400 lux) photobleach. Mean a-wave amplitudes ± SEM are shown for IRBP-ABCA4/IRBP-ABCA4-mu (F), MOP-ABCA4/MOP-ABCA4-mu (G), WT (solid line, shaded in gray), and saline (dashed line, shaded in gray). *$P < 0.05$; **$P < 0.01$ by repeated-measures 2-way ANOVA with Bonferroni's post-hoc tests. n = 4–10/group.

Han Z, Conley SM, Makkia RS, Cooper MJ, Naash MI. DNA nanoparticle-mediated ABCA4 delivery rescues Stargardt dystrophy in mice. *J Clin Invest*. 2012;122(9):3221–3226. doi:10.1172/JCI64833

DNPs offer the potential for safe, efficacious, and repeat dosing ocular gene therapy

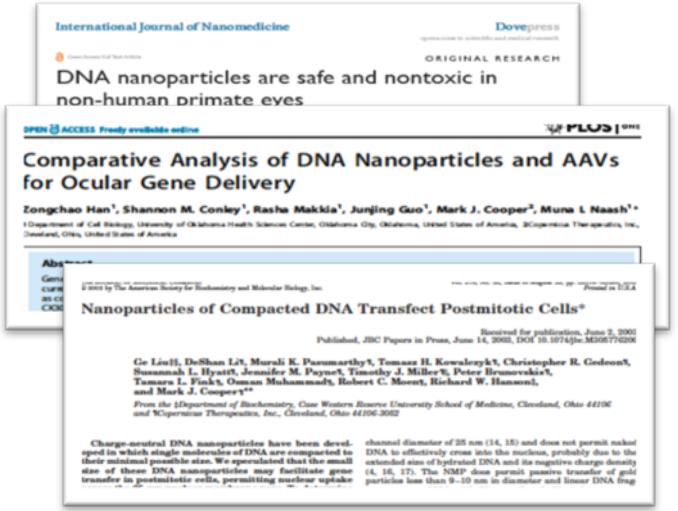
Potential advantages:

- Efficacy: demonstrated in numerous ocular animal models
  - Transfer large genes (up to ~20 kb)
- Safety: Non-immunogenic, without viral capsid proteins or pre-existing immunity.
  - Potential for repeat dosing
  - Higher doses possible to enhance transfection

Potential synergies with suprachoroidal injection:

- In office, repeat dosing as needed
- Targeted circumferential compartmentalized spread to large surface areas
  - Potentially ideal distribution for inherited retinal disease treatment or biofactory approach

Well established literature on DNA nanoparticle gene therapy



Preclinical studies demonstrate SC injections of DNA nanoparticles may offer the potential for a safe and efficient delivery method

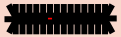
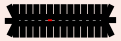
Purpose

The purpose of this research was to evaluate ocular tolerability and chorioretinal cell transfectability of suprachoroidally injected non-viral DNA nanoparticles (DNPs) in non-human primates (NHPs) and rabbits.

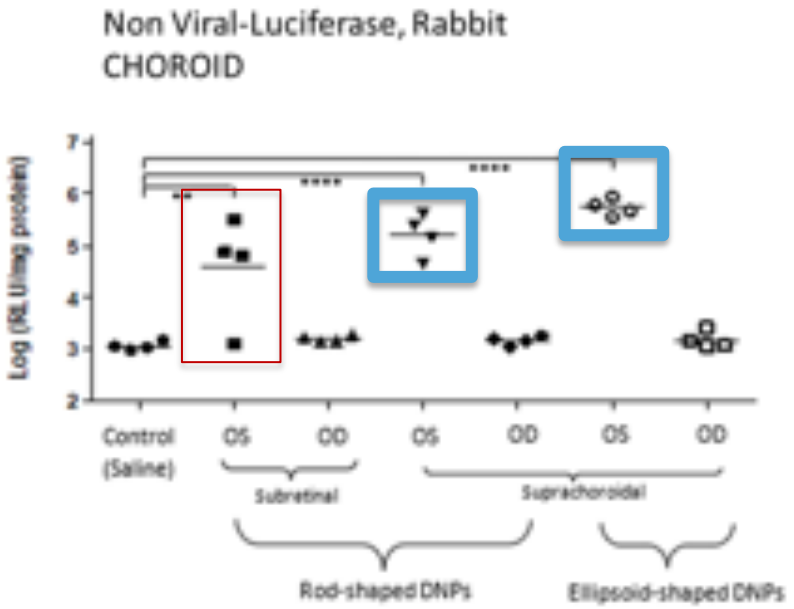
SC Injection of DNPs in Rabbits

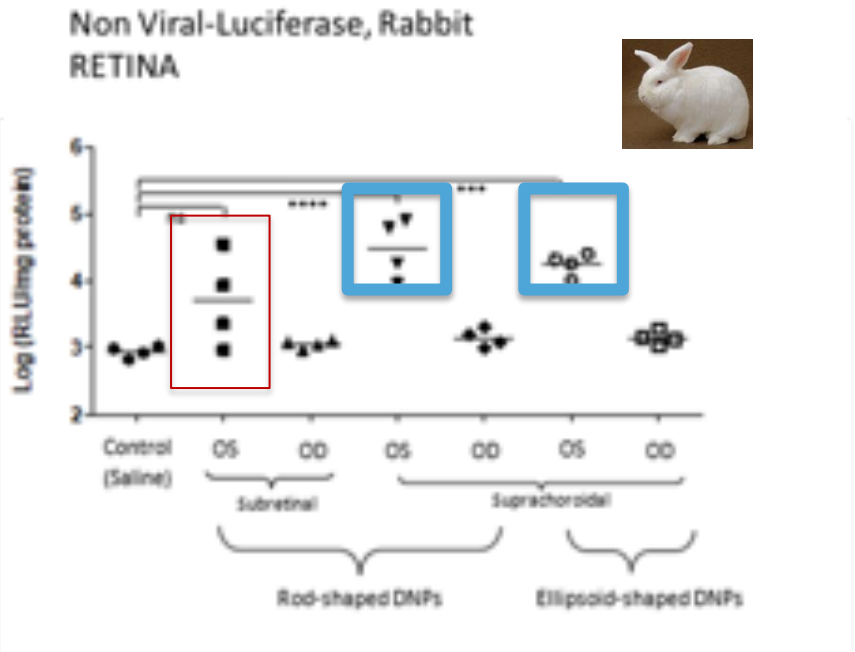
Design

- 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 µL
2 	Ellipsoid DNPs Luciferase	SC Injection	100 µL
3 	Rod DNPs Luciferase	SC Injection	100 µL
4 	Rod DNPs Luciferase	Sub-retinal injection	50 µL

SC injection produced activity comparable to that seen from subretinal injections of luciferase DNPs





OS: Dosed OD: Undosed	Bonferroni's test: ** p<0.01, ***p<0.001, **** p<0.0001 ns, non-significant

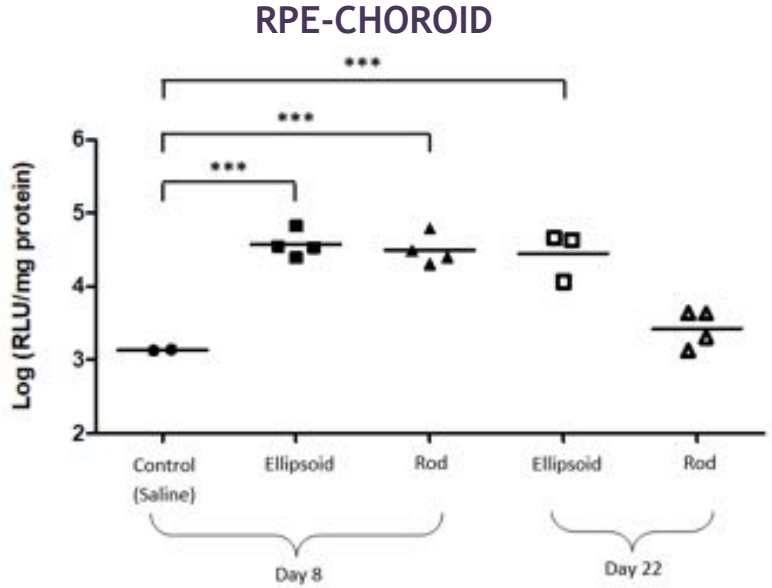
SC Injection of DNPs in Non-Human Primates (NHPs)

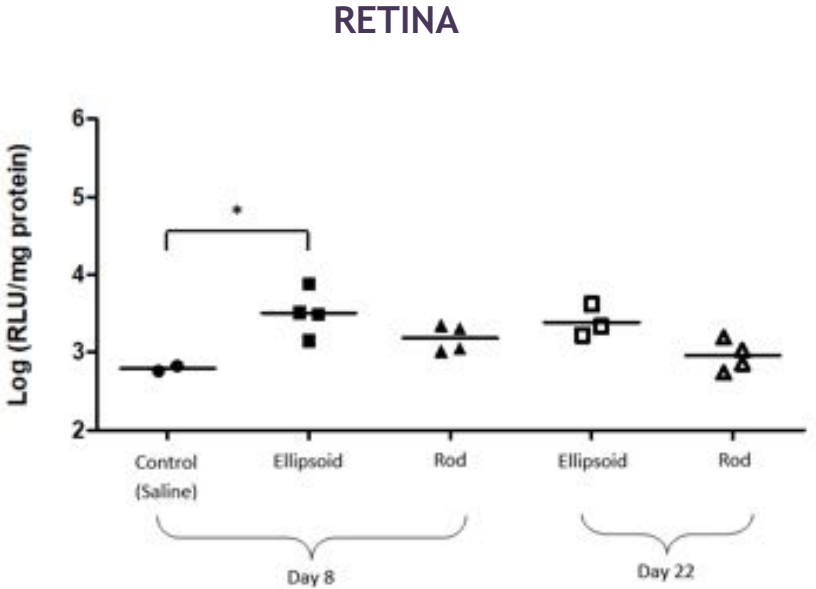
Design

- Animals received a single bi-lateral **suprachoroidal injection** (0.1 mL/ eye)
- Ophthalmic examinations Days 0, 1, and 7:
  - Assessed surface morphology, ocular inflammation – slit lamp, direct and in-direct ophthalmoscopy, IOP
- One-week and 3-weeks post-injection:
  - Eyes enucleated, choroid and retina separated, processed for evaluation of luciferase activity

Groups	n	Test article
1	2	Vehicle
2	4	Ellipsoid DNPs Luciferase
3	4	Rod DNPs Luciferase

DNA Nanoparticles Transfect RPE + Choroid and Retina

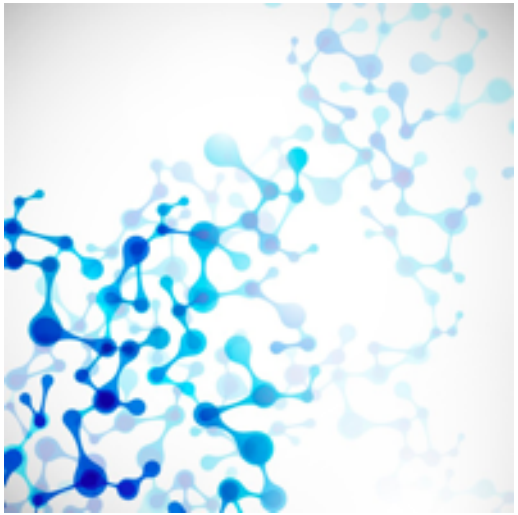




1-way ANOVA, $p<0.0001$.
Bonferroni's test: *$p<0.05$, ** $p<0.01$, ***$p<0.001$,

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



Summary: Supachoroidal Injections of DNA Nanoparticles

May address an unmet needs in ocular gene delivery:

- Offer potentially safer and efficient in office delivery versus risk associated with surgical procedure
- Non-immunogenic, potential for repeat dosing
- Transfer large genes, allowing for gene therapy in common inherited retinal diseases (IRDs), ie. Stargardt disease and Usher syndrome
- Additional research evaluating SC injection in non-human primates and delivery of a therapeutic transgene is needed


THANK YOU