Macula Society Annual Meeting 2020

Suprachoroidal Delivery for Ocular Gene Therapy: Nonclinical Experiments Evaluating Non-Viral DNA Nanoparticles in Non-Human Primates

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Financial Disclosures

Consulting Fees

Regeneron, Genentech, Novartis, Allergan, Alimera, Optos, Clearside Medical

Research Grant

Regeneron, Apellis, Novartis, Clearside Medical

Salary

National Eye Institute (DRCR Network Vice-Chair)

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
  - Bioavailable with prolonged pK

- **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 comparable to other ocular injections

- **Access to care**
  - Does not require specialized gene therapy surgery treatment centers
  - In-office SC injection procedure which permits repeat dosing
  - Procedure time and expense are significantly less than sub-retinal procedure

Viral Vectors delivered via the suprachoroidal space

Ab-Externo AAV-Mediated Gene Delivery to the Suprachoroidal Space Using a 250 Micron Flexible Microcatheter

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Ophthalmology, University of Florida, Gainesville, Florida, United States of America

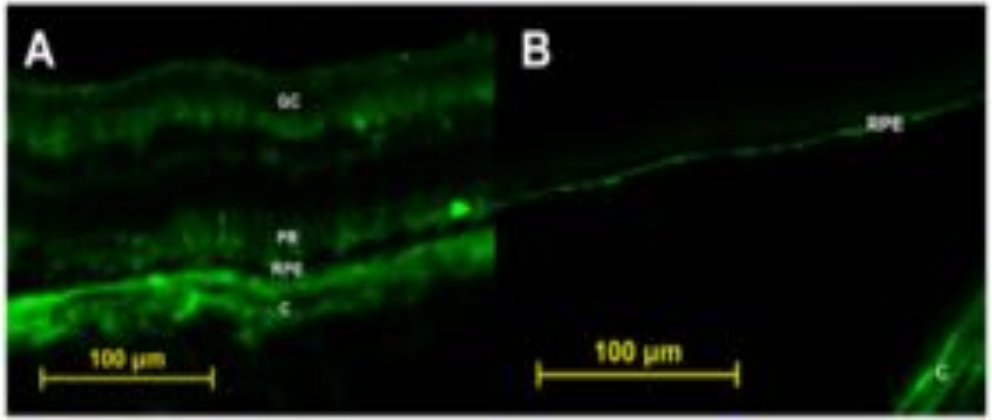


Figure 3. Fluorescent light micrographs of retinal cross sections. A. Treated eye of a Dutch Belted rabbit demonstrating immunofluorescence likely occuring at the level of choroid (C), retinal pigmented epithelium (RPE), photoreceptors (PR), and retinal ganglion cells (GC). **B.** Untreated eye of a Dutch Belted rabbit with autofluorescence seen at the level of choroid (C) and retinal pigmented epithelium (RPE).
doi:10.1371/journal.pone.0017140.g003

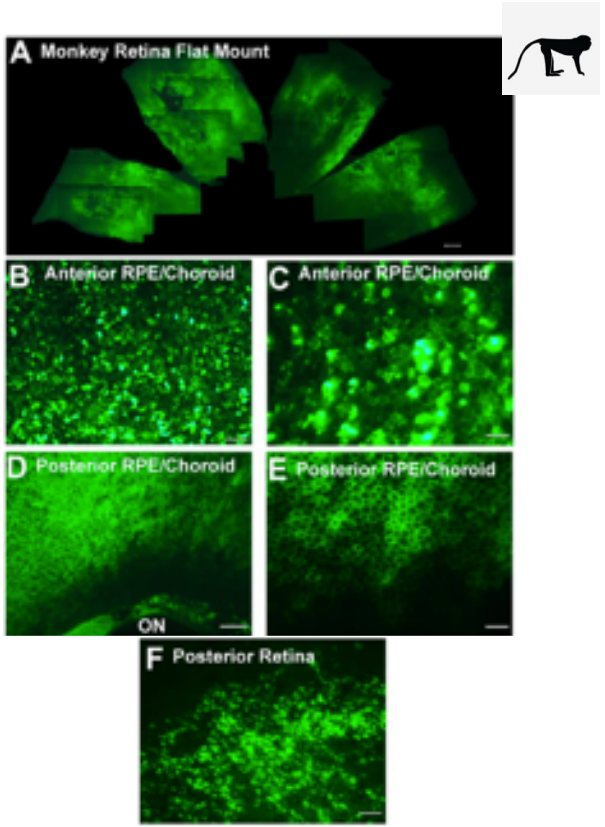
Methods:

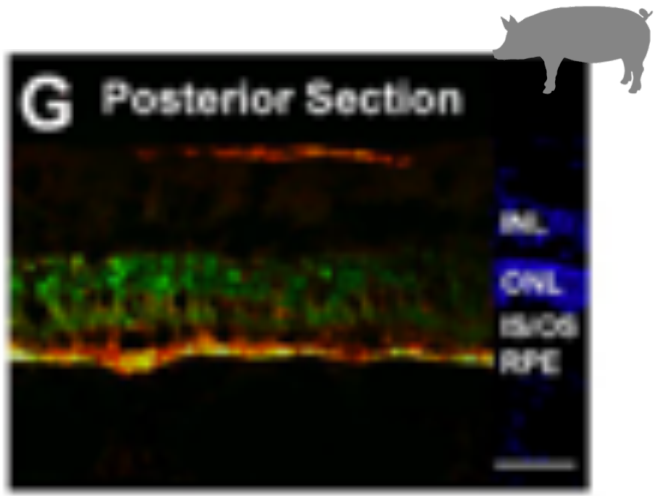
- 6 New Zealand White rabbits, 2 Dutch Belted rabbits
- sc-AAV5-smCBA-hGFP
- Illuminated iTrackTM 250A
- microcatheter
- 6 weeks after surgery, immunofluorescent antibody staining of GFP.

Results:

- Transfection in all treated eyes
- diffusely in both the choroid and the retina.
- No apparent adverse effects.

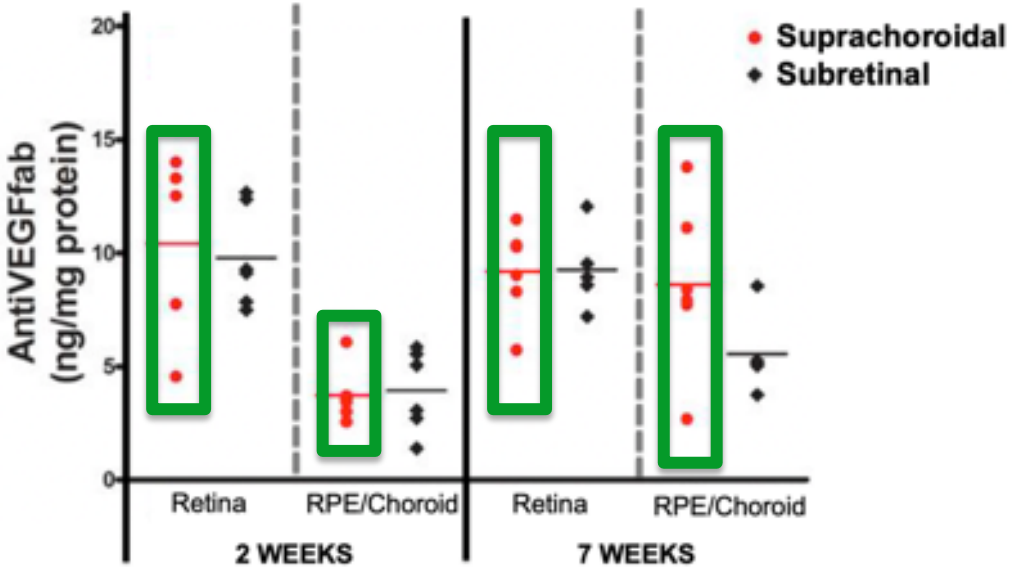
Widespread expression of GFP three weeks after SC injection of AAV8.GFP





Source: Ding, K., Shen, J., Hafiz, Z., Hackett, S. F., Silva, R. L. E., Khan, M., ... Campochiaro, P. A. (2019). AAV8-vectored suprachoroidal gene transfer produces widespread ocular transgene expression. *Journal of Clinical Investigation*. doi: 10.1172/jci129085

SC injection of RGX-314 resulted in similar expression of anti-VEGF Fab



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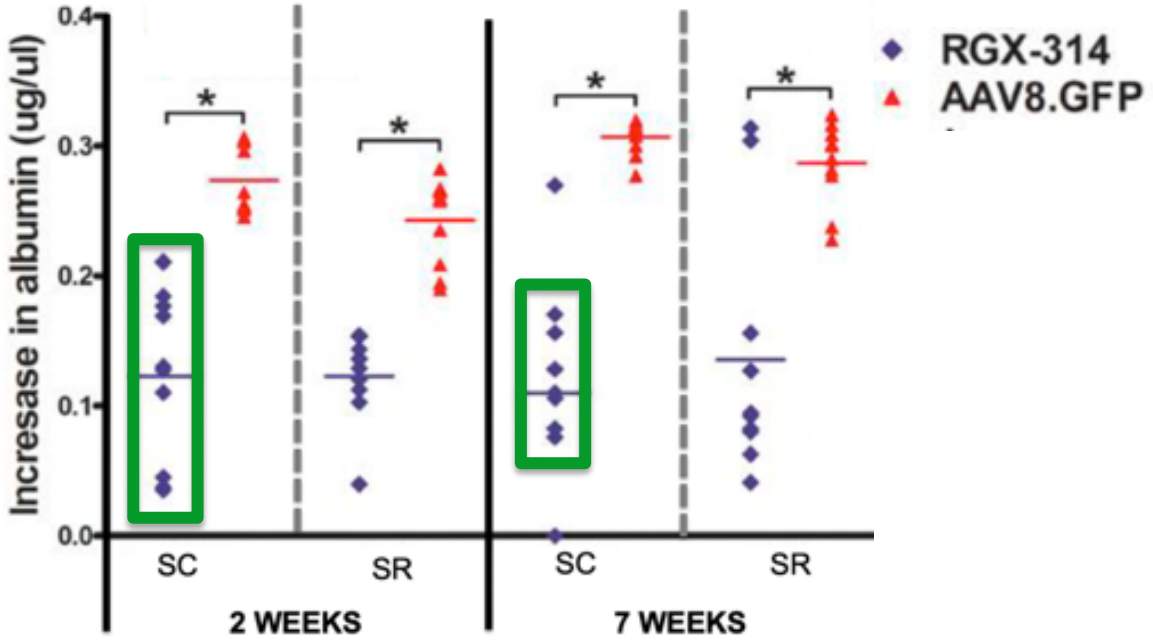
SC injection of RGX-314 resulted in similar suppression of VEGF-induced vascular leakage as subretinal delivery



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Phase II clinical trials for SC delivery to treat wet AMD and diabetic retinopathy expected to begin in 2020

REGENXBIO ANNOUNCES EXCLUSIVE WORLDWIDE OPTION AND LICENSE AGREEMENT WITH CLEARSIDE BIOMEDICAL FOR EVALUATION OF IN-OFFICE DELIVERY PLATFORM FOR RGX-314

January 9, 2020 at 7:00 AM EST

ROCKVILLE, Md., Jan. 9, 2020 /PRNewswire/ --

- RGX-314 programs for treatment of wet AMD and diabetic retinopathy continue to advance
  - Plans to initiate a pivotal program of subretinal delivery for wet AMD in 2H 2020, following the 12-month assessment of Cohort 5 patients in the Phase I/IIa trial
  - FDA removes partial clinical hold on Phase I/IIa trial of subretinal delivery for wet AMD
  - Phase II trials of suprachoroidal delivery for treatment of wet AMD and diabetic retinopathy expected to begin in 2020
- Enrollment continues in Cohort 2 of RGX-121 Phase I/II trial in MPS II, with additional interim data expected in 2020
- Interim update from RGX-501 Phase I/II trial in HoFH supports Cohort 2 safety with steroid prophylaxis; LDL-C measures expected in 1H 2020
- Utilization of new corporate, research and manufacturing headquarters expected to begin in late 2020, and cGMP manufacturing facility with capacity to produce NAV vectors at scales up to 2,000 liters expected to be fully operational in 2021
- Ended 2019 above guidance estimate with $400 million in cash, cash equivalents and marketable securities

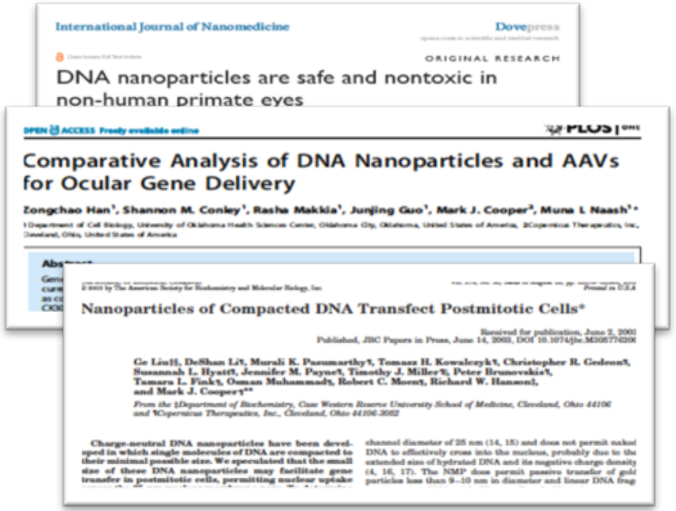
DNA Nanoparticles (DNPs)

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

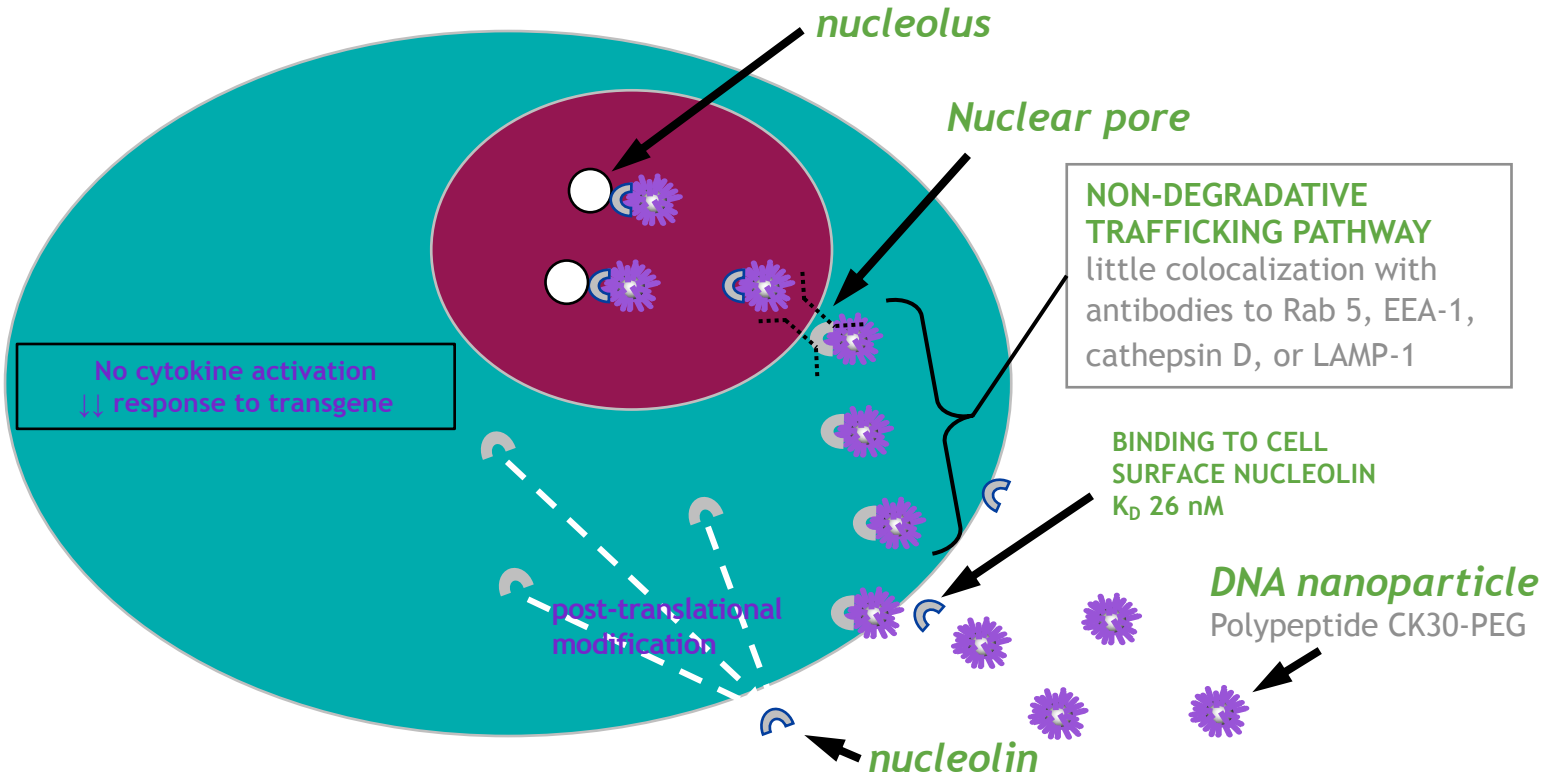
Potential advantages:

- 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

Well established literature on DNA nanoparticle gene therapy



Uptake and Trafficking of DNA Nanoparticles

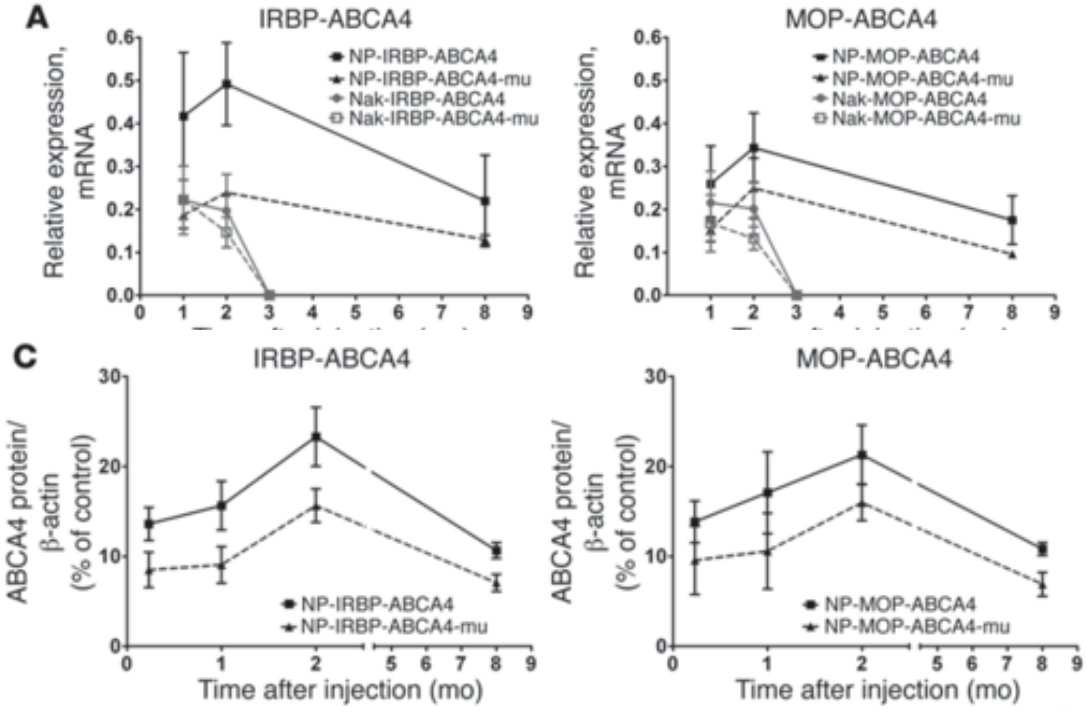


Non-viral DNP experience in ocular models

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
Baboon	Reporter gene	SR, IVT	RPE	+ ERG		

DNP-mediated ABCA4 gene delivery induces persistent gene expression in the retina in Abca4-/- mice



(A) ABCA4 mRNA levels were assessed by qRT-PCR and normalized to endogenous β-actin. Saline-injected and uninjected eyes were used as negative controls.

(C) Shown are 3 representative eyes at each time point/group (labels 1–3) injected with either WT or mutant NPs. (n = 4–6 eyes/group for A–C). Protein levels were normalized to β-actin and expressed as a percentage of levels found in uninjected WT mice. Results for WT and mu-NP treatment in A and C were analyzed by 2-way ANOVA with Bonferroni's post-hoc comparisons.

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

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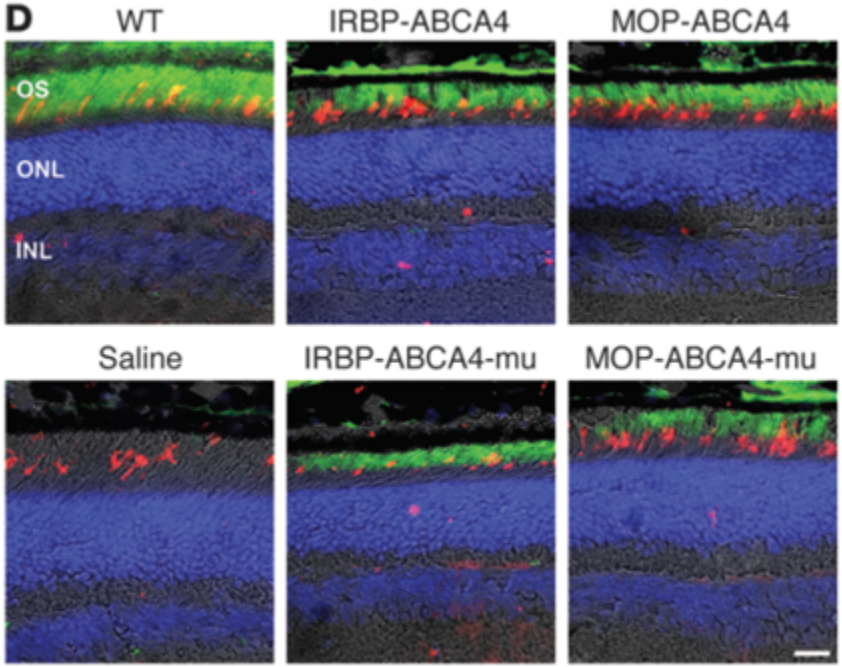
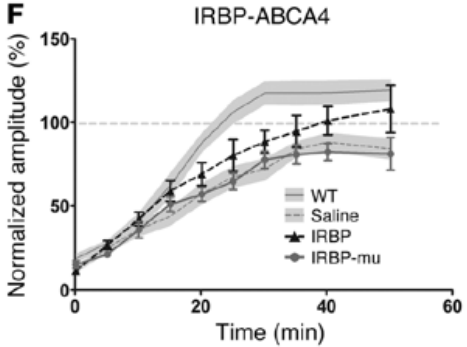
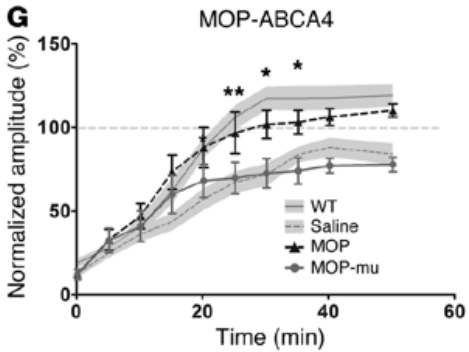


Retinal cryosections at 8 months PI were co-labeled for ABCA4 (green), S-opsin (red) with DAPI (epifluorescent images/bright field)

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 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

DNA Nanoparticles (DNPs) for ocular therapies

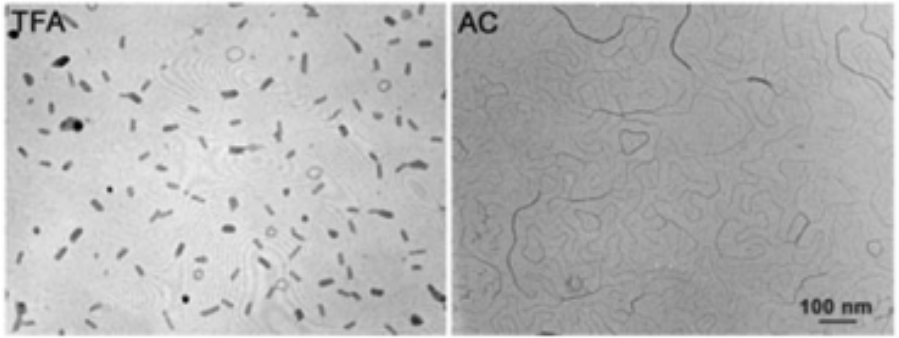


Figure 1. Differential shape of the trifluoroacetate (TFA) and acetate (AC) nanoparticles as visualized by electron microscopy. During compaction of the EGFP expression plasmid, the presence of TFA as the lysine counterion produces ellipsoidal nanoparticles with a minor diameter <18 nm whereas acetate produces rods with a minor diameter <8 nm. Scale bar, 100 nM.
doi:10.1371/journal.pone.0000038.g001

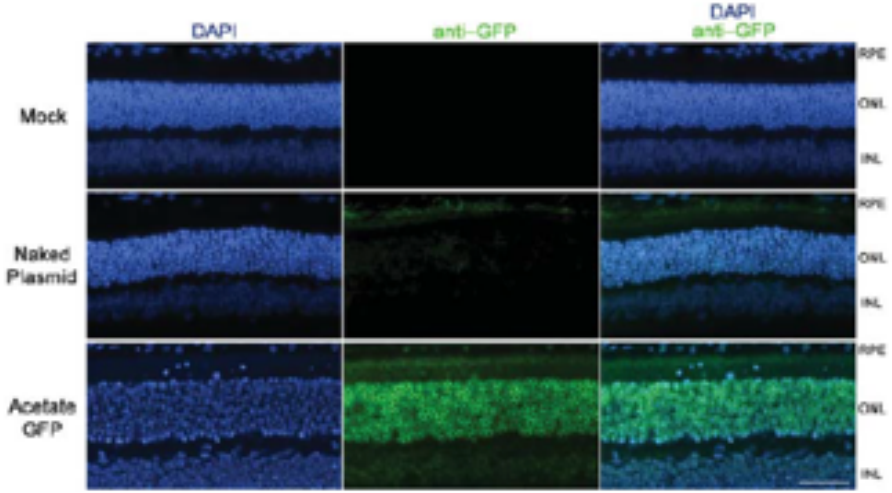


Figure 6. EGFP immunoreactivity following subretinal injection. To eliminate any artifacts due to retinal autofluorescence, immunohistochemistry was performed with a Cy-3-anti-GFP antibody on paraffin-embedded ocular sections from eyes taken at 2-days post-injection of 0.6 µg of compacted DNA. An absence of or minimal EGFP immunoreactivity was found in the mock-injected and naked plasmid-injected eyes, respectively. Examination of sections from eyes injected with the AC-GFP nanoparticle revealed high-level EGFP immunoreactivity in nearly all of the cells in the ONL. Lower levels of EGFP were also detected within the upper half of the INL. Scale bar, 10 µM.
doi:10.1371/journal.pone.0000038.g006

Farjo R, Skaggs J, Quiambao AB, Cooper MJ, Naash MI. Efficient non-viral ocular gene transfer with compacted DNA nanoparticles. *PLoS One*. 2006;1(1):e38.
Published 2006 Dec 20. doi:10.1371/journal.pone.0000038

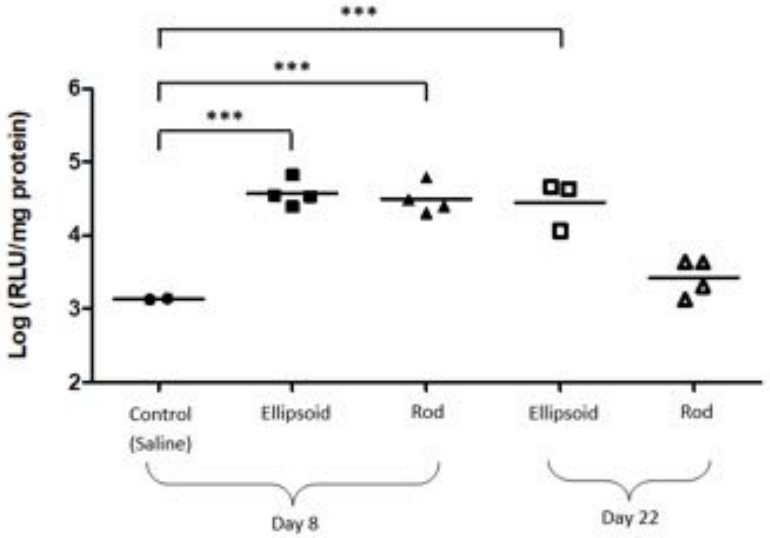
SC injection of Luciferase DNPs in non-human primates

- **Design**
  - Animals received a single bilateral **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

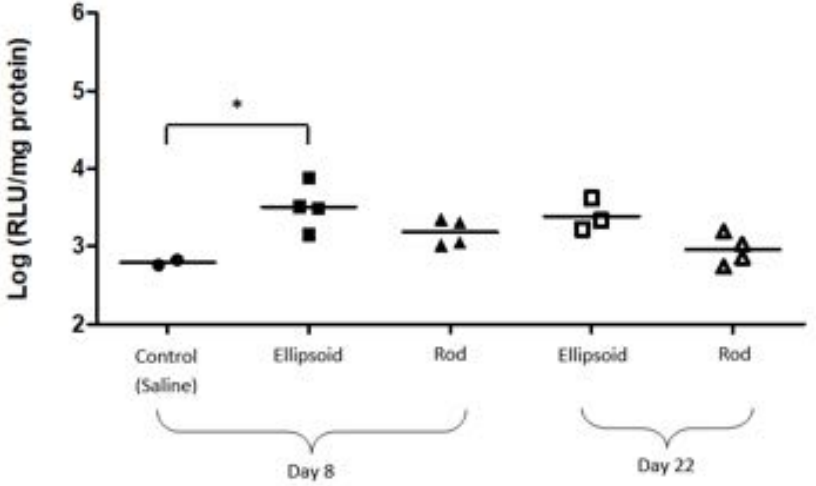
SC injection of Luciferase DNP transfect RPE + Choroid and Retina

RPE-CHOROID



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

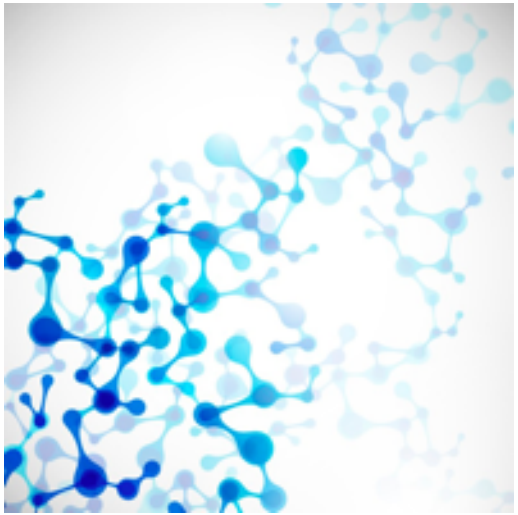
RETINA



1-way ANOVA, p=0.0088.
Bonferroni's multiple comparison test: * p<0.05, ** p<0.01

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 in a prior rabbit study
- SC injections on DNPs were **generally well-tolerated** across groups; no significant abnormalities observed on ophthalmic exams or ERGs
- Next step is to evaluate SC injection of **therapeutic transgene**
- **SC injections of DNPs offer the potential for safer and more efficient transgene delivery**




THANK YOU