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

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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

Marc C. Peden, Jeff Min, Craig Meyers, Zachary Lukowski, Qihong Li, Sanford L. Boye, Monica Levine, William W. Hauswirth, Ramakrishna Ratnakaram, William Dawson, Wesley C. Smith, Mark B. Sherwood

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

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

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

Phase II clinical trials for SC delivery to treat wet AMD and diabetic retinopathy expected to begin in 2020.
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
Uptake and Trafficking of DNA Nanoparticles

- **Nucleolus**
- **Nuclear pore**
- **DNA nanoparticle**
- **Polypeptide CK30-PEG**
- **BINDING TO CELL SURFACE NUCLEOLIN**
  - $K_D$ 26 nM
- **NON-DEGRADATIVE TRAFFICKING PATHWAY**
  - little colocalization with antibodies to Rab 5, EEA-1, cathepsin D, or LAMP-1

No cytokine activation ↓↓ response to transgene

Post-translational modification
Non-viral DNP experience in ocular models
Safe and restores function in multiple mouse knock out models

<table>
<thead>
<tr>
<th>Model</th>
<th>Route</th>
<th>Target Cell Types</th>
<th>Function</th>
<th>Histology</th>
<th>Assay Time</th>
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<tbody>
<tr>
<td>RDS (peripherin 2) RP</td>
<td>SR</td>
<td>photoreceptor</td>
<td>+ERG</td>
<td>+</td>
<td>4 mo</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>+ vision behavior</td>
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<td>1 year</td>
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<td>Stargardts (ABCA4) macular degeneration</td>
<td>SR</td>
<td>RPE</td>
<td>+ERG</td>
<td>+</td>
<td>8 mo</td>
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<tr>
<td>RPE65 RP</td>
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<td>+ERG</td>
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<td>vasculature</td>
<td>normal</td>
<td>+</td>
<td>3 mo</td>
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<tr>
<td>RPE marker gene</td>
<td>SR</td>
<td>RPE</td>
<td></td>
<td>+</td>
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<tr>
<td>AAV versus DNA NP</td>
<td>SR</td>
<td>PR and RPE</td>
<td></td>
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<tr>
<td>Baboon</td>
<td>SR, IVT</td>
<td>RPE</td>
<td>+ ERG</td>
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</table>
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.

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

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

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

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Test article</th>
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<tr>
<td>1</td>
<td>2</td>
<td>Vehicle</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>Ellipsoid DNPs Luciferase 🐶</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>Rod DNPs Luciferase 🐶</td>
</tr>
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SC injection of Luciferase DNP transfect RPE + Choroid and Retina

RPE-CHOROID

RETINA

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

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