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

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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
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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\(^1\)

**COMPARTMENTALIZED**
Drug is compartmentalized in the suprachoroidal space, which helps keep it away from non-diseased tissues\(^2\)

**BIOAVAILABLE PROLONGED PK**
Fluid spreads circumferentially and posteriorly when injected within the suprachoroidal space, bathing the choroid and adjacent areas with drug\(^3\)

**for efficacy**
**for safety**
**for durability**

Suprachoroidal Injection is an In Office, Repeatable Delivery Method
Uptake and Trafficking of DNA Nanoparticles

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

DNA nanoparticle

Nucleolus

Nuclear pore
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>
</tr>
</thead>
<tbody>
<tr>
<td>RDS (peripherin 2) RP</td>
<td>SR</td>
<td>photoreceptor</td>
<td>+ERG</td>
<td>+</td>
<td>4 mo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ vision behavior</td>
<td></td>
<td>1 year</td>
</tr>
<tr>
<td>Stargardts (ABCA4) macular degeneration</td>
<td>SR</td>
<td>RPE</td>
<td>+ERG</td>
<td>+</td>
<td>8 mo</td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPE65 RP</td>
<td>SR</td>
<td>RPE</td>
<td>+ERG</td>
<td>+</td>
<td>15 mo</td>
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<tr>
<td>Rhodopsin RP</td>
<td>SR</td>
<td>photoreceptor</td>
<td>+ERG</td>
<td>+</td>
<td>8 mo</td>
</tr>
<tr>
<td>Diabetic retinopathy (miRNA 200b)</td>
<td>IVT</td>
<td>vasculature</td>
<td>normal</td>
<td>+</td>
<td>3 mo</td>
</tr>
<tr>
<td>RPE marker gene</td>
<td>SR</td>
<td>RPE</td>
<td></td>
<td>+</td>
<td>2.5 yr</td>
</tr>
<tr>
<td>AAV versus DNA NP</td>
<td>SR</td>
<td>PR and RPE</td>
<td></td>
<td>+</td>
<td>4 mo</td>
</tr>
<tr>
<td>NHP</td>
<td>Baboon</td>
<td>SR, IVT</td>
<td>RPE</td>
<td>+ ERG</td>
<td></td>
</tr>
</tbody>
</table>
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)

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.

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
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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Test article</th>
<th>Route of Administration (OS only)</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>SC Injection</td>
<td>100 µL</td>
</tr>
<tr>
<td>2</td>
<td>Ellipsoid DNPs Luciferase</td>
<td>SC Injection</td>
<td>100 µL</td>
</tr>
<tr>
<td>3</td>
<td>Rod DNPs Luciferase</td>
<td>SC Injection</td>
<td>100 µL</td>
</tr>
<tr>
<td>4</td>
<td>Rod DNPs Luciferase</td>
<td>Sub-retinal injection</td>
<td>50 µL</td>
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</tbody>
</table>
SC injection produced activity comparable to that seen from subretinal injections of luciferase DNPs
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

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<tr>
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<tbody>
<tr>
<td>1</td>
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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
May address an unmet needs in ocular gene delivery:

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THANK YOU