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

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- 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.

- **DNA** + modified polylysine peptides formulated with polyethylene glycol (PEG)
- 8-10 nm in diameter
- Colloidal stability DNPs Suspended in Saline

![Ellipsoids](image1.png)

![Rods](image2.png)
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>RodDNPs Luciferase</td>
<td>Sub-retinal injection</td>
<td>50 µL</td>
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</tbody>
</table>
DNA Nanoparticles Transfect the **Retina** and **Choroid**

**Non Viral-Luciferase, Rabbit CHOROID**

**Non Viral-Luciferase, Rabbit RETINA**

[Graphs showing data for different conditions and treatments]
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
THANK YOU