Suprachoroidal delivery for ocular gene therapy: nonclinical experiments evaluating non-viral DNA nanoparticles.

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# **Financial Disclosures**

None relevant to this presentation

# DNA nanoparticles offer the potential for safe, efficacious, and repeat dosing of ocular gene therapy

#### **Potential advantages:**

- 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 model, higher doses may be used to enhance transfection
- Manufacturing, Simpler than viral-based gene therapy

#### **Potential disadvantages:**

• Durability: May not represent one time therapy

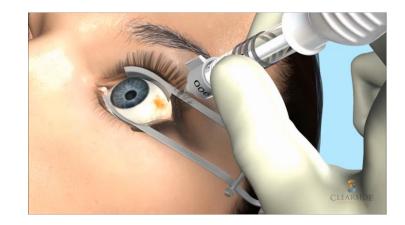
#### PLOSION Comparative Analysis of DNA Nanoparticles and AAVs for Ocular Gene Delivery Zongchao Han<sup>1</sup>, Shannon M. Conley<sup>1</sup>, Rasha Makk International Journal of Nanomedicin Dovepres ant of Call Biology, University of Oklahr ORIGINAL RESEARCH Abstract DNA nanoparticles are safe and nontoxic in Gene therapy is a critical tool for the non-human primate eves The decays, or Benjamen. Concerns: © 2011 by The American Society for Rochemistry and Melecular Society. In Vol. 276, No. 20, Issue of August 20, pp. 22270-22140, 201 Nanoparticles of Compacted DNA Transfect Postmitotic Cells<sup>4</sup> Ryan A Kelley Ransived for publication, June 2, 2003 Published, JBC Papers in Press, June 14, 2003, DOI 10.1074/be M205774200 Shannon M Conle Rasha Makkia<sup>1</sup> Ge Liuth, DeShan Lit, Murali K. Pasumarthyt, Tomasz H. Kowalezykt, Christ Susannah L. Hyatti, Jennifer M. Paynet, Timothy J. Miller & Peter Brunovek Tumara L. Finkt, Oman Muhammadt, Robert C. Moent, Richard W. Hanson, and Mark J. Coopert\*\* amie N Watson Zongchao Han' Mark I Cooper From the ±Department of Biochemistry, Case Western Reserve University School of Medicine, Cleveland, and Wesperatrus Therapeutics, Inc., Cleveland, Ohas 44106-3082 Muna I Naash<sup>2</sup> Desartment of Cell Bo nces Center, Okla in which single molecules of DNA are compared minimal possible size. We speculated that the small USA; \*Copernicus Therap Cleveland, OH, USA; \*Dep DNA to effectively cross into the nucleus, probably due to th extended aims of budgeted DNA and its a size of these DNA nanopart cles may facilitate gene did for ocular gene therapy due to their safety and ability t long-term gene expression [1,2,3,4]. AAV-based clinic icles can tran is for RPE65-associated Leber congenital amaurosis [4,5,6,7 ant side effects and some positive : offers [3,8] AAV is Lentiviral Vector Gene Transfer of Endostatin/Angiostatin on affer for Macular Degeneration (GEM) Study is although they LAVs are welly Peter A. Campochiaro,<sup>1,\*</sup> Andreas K. Lauer,<sup>2</sup> Elliott H. Sohn,<sup>3</sup> Tahreem A. Mir,<sup>1</sup> Avs are supported to a series of the series Stuart Naylor,<sup>4</sup> Matthew C. Anderton,<sup>5</sup> Michelle Kelleher,<sup>5</sup> Richard Harrop,<sup>5</sup> Scott Ellis,5 and Kyriacos A. Mitrophanous5 nanopartides (NPs) compacted with 10 k (CK30PEG) drive e AAV8-antiVEGFfab Ocular Gene Transfer for Neovascular Age-Related Macular [15,16]. These mal Degeneration PLOS ONE | www.pk uan Liu, 1-3-6 Seth D. Fortmann, 1-5 Jikui Shen, 1 Erik Wielechowski, 2 Anna Tretiakova, 2-7 Stephen Yoc Karen Kozarsky, 3,8 Jiangxia Wang,4 James M. Wilson,2 and Peter A. Campochiaro Cell-specific gene therapy driven by an optimized hypoxia-regulated vector reduces choroidal neovascularization Manas R. Biswal<sup>1,2</sup> · Howard M. Prentice <sup>3,4</sup> · George W. Smith<sup>1</sup> · Ping Zhu<sup>5</sup> · Yao Tong<sup>2</sup> · C. Kathleen Dore Development of an inducible anti-VEGF rAAV gene therapy strategy for the treatment of wet AMD 3

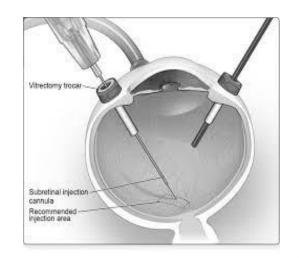
DNA nanoparticle Gene Therapy:

Well established literature

# 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 subretinal procedure





## Suprachoroidal Injection of DNPs in Non-Human Primates and Rabbits

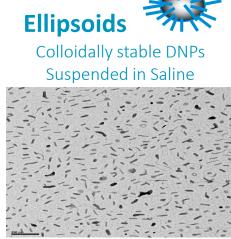
#### **Study Objective**

• Evaluate the safety, tolerability, and retinal cell transfection following SC injection of DNPs

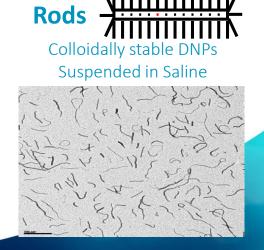
#### Design

- Ophthalmic examinations at Day 0, 1, and 7
  - Surface morphology, ocular inflammation, direct and indirect ophthalmoscopy, IOP, ERG
- Eyes were enucleated at Day 7 and 21
  - Choroid and retina separated and processed for evaluation of luciferase activity

| Species | Group<br>(n=4) | Test article                | Route of<br>Administration | Volume |
|---------|----------------|-----------------------------|----------------------------|--------|
|         | 1              | Vehicle                     | SC Injection               | 100 μL |
| 2 John  | 2              | 🗮 Ellipsoid DNPs Luciferase | SC Injection               | 100 μL |
| JR B    | 3              | Hat Rod DNPs Luciferase     | SC Injection               | 100 μL |
|         | 1              | Vehicle                     | SC Injection               | 100 μL |
| X       | 2              | 🗱 Ellipsoid DNPs Luciferase | SC Injection               | 100 μL |
|         | 3              | Hat Rod DNPs Luciferase     | SC Injection               | 100 μL |
|         | 4              | Rod DNPs Luciferase         | Sub-retinal injection      | 50 μL  |



8-10 nm in diameter

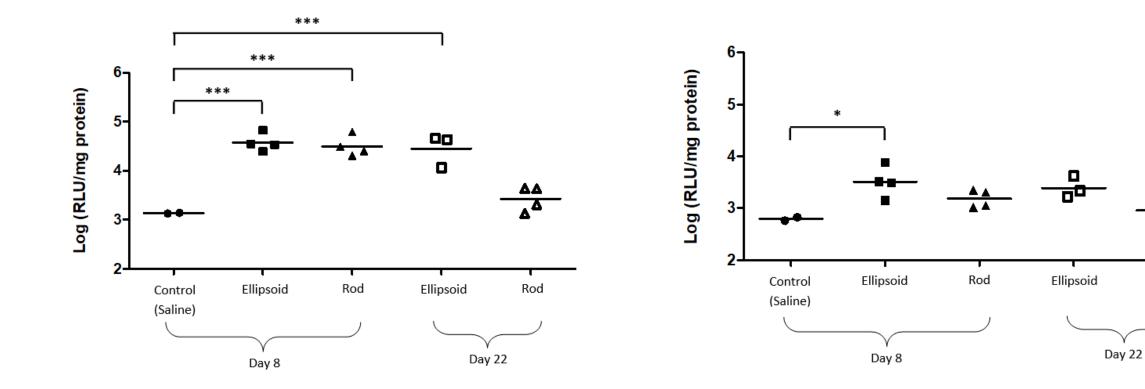


# NHP: DNA Nanoparticles Transfect RPE + Choroid and Retina



Rod

RETINA



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

**RPE-CHOROID** 

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

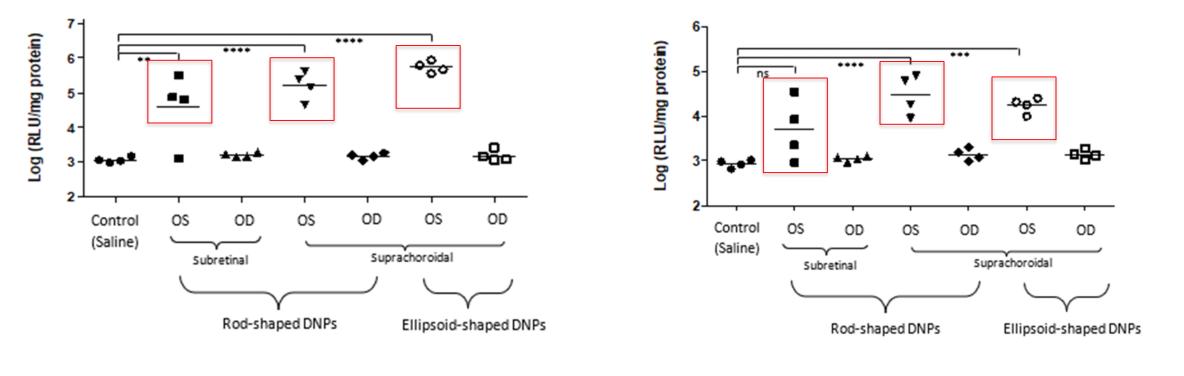
# Rabbit: DNA Nanoparticles Transfect the RPE + Choroid and Retina

Non Viral-Luciferase, Rabbit

RETINA



Non Viral-Luciferase, Rabbit CHOROID



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

## **Study Summary**

- Luciferase activity observed in the retina and RPE+choroid
- In rabbits, SC injection comparable to subretinal injections of luciferase DNPs produced activity
- SC injections of DNPs were **generally well-tolerated** across groups in both species
- Safety
  - SC injection of DNPs may address an unmet need in ocular gene delivery
  - Non-immunogenic, potential for repeat dosing
- Efficacy
  - Higher doses may be used to enhance transfection
  - 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