Suprachoroidally delivered non-viral DNA nanoparticles produce hMyo7A Protein in RPE-choroid in rabbits

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Purpose
- Suprachoroidal (SC) delivery offers the potential to target chorioretina while avoiding surgical risks associated with a subretinal injection and may offer a novel alternative for office-based gene therapy for the treatment of inherited retinal diseases.
- Non-viral based gene delivery offers the potential for repeated (if needed) gene therapy and affords delivery of large size plasmid (>10kb) that cannot be accommodated by a single AAV vector.
- Functional deficiency of Myosin (Myo) 7A protein is implicated in the pathogenesis of Ushers syndrome, an inherited retinal disease and a form of retinitis pigmentosa.
- The purpose of this research was to evaluate ocular tolerability and chorioretinal cell transfectability of SC and intravitreal (IVT) injected non-viral DNA nanoparticles (DNPs) containing plasmid encoded for hMyo7A.

Methods
- Dutch-Belted pigmented rabbits (N=4 eyes per group) received a single SC (0.1 mL) or IVT injection (0.05 mL) of DNPs (either 4 or 6 mg/mL of DNA).
- The DNPs consisted of a single copy of plasmid DNA encoding hMyo7A with a polyubiquitin C transcriptional cassette.
- DNP-1 consist of pDNA encoding natural hMyo7A, DNP-2 consist of pDNA encoding codon optimized hMyo7A.
- Ocular tolerability was assessed via slit lamp, indirect ophthalmoscopy, intraocular pressure (IOP), optical coherence tomography (OCT), electroretinography (ERG) and fundus photography (FP) for up to 3 months.
- Protein (hMyo7A) and RNA levels were measured in ocular tissues via ELISA and qRT-PCR, respectively, at 3 months.
- Expression pattern of eGFP was assessed longitudinally via in-vivo imaging method.

Results

1. SC injection resulted in reversible opening of suprachoroidal space

Representative optical coherent tomography images of rabbit eyes

Baseline
Immediately after SC injection
Day 30

2. Suprachoroidally injected DNPs exhibited better ocular tolerability with milder and less frequent ocular findings compared to IVT injected DNPs

Cumulative ocular exam scores
(0-2: minimal; >2-4: mild; >4-6: moderate; >6: marked; >8: severe)

Fundus Photographs (representative images)

3. SC and IVT injected DNPs produced hMyo7A protein in the RPE-choroid

In-Vivo Transfection (hMyo7A Protein and mRNA Levels)
- SC and IVT injected DNPs produced hMyo7A protein in the RPE-choroid
- Protein and mRNA levels were near or below level of detection in retina

In-Vivo Imaging
- OCT imaging confirmed reversible opening of SC space immediately after the SC injection.
- IVT injected DNPs exhibited higher incidence and degree of intraocular inflammation, lens opacity, and retinal detachment/ degeneration.
- Acute increase in intraocular pressure was observed after either SC or IVT injection of DNPs which returned to the baseline level at the next assessment timepoint.
- OCT imaging confirmed reversible opening of SC space immediately after the SC injection.

Key Findings
- Suprachoroidally injected non-viral DNPs were generally well-tolerated in rabbits.
- IVT injected DNPs exhibited higher incidence and degree of intracocular inflammation, lens opacity, and retinal detachment/ degeneration.
- Acute increase in intraocular pressure was observed after either SC or IVT injection of DNPs which returned to the baseline level at the next assessment timepoint.
- OCT imaging confirmed reversible opening of SC space immediately after the SC injection.
- The hMyo7A protein was detected in the RPE-choroid (68-105 ng/gm) at 3 months after SC or IVT injection with no statistically significant difference between the routes of administration.
- The hMyo7A protein levels were detected in sporadic retina samples.
- The RT-PCR data indicate that DNPs produce mRNA levels in the RPE-choroid that is in the range of 45%-160% of the endogenous rabbit Myo7A.

Conclusions
- SC DNPs containing transgene encoded for hMyo7A produced efficient and durable levels of hMyo7A in RPE-choroid.
- Photoreceptor (PR) specific promoters will be assessed to transfection in PR in future studies.
- The immune response to human Myo7A in rabbits may have impacted the levels observed in this study.
- Further studies in clinically relevant higher species (monkeys) are warranted.
- SC non-viral DNP-based gene delivery has potential as an office-based repeatable therapy for large-gene disorders.

References