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OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.
Toward the intra-ocular delivery of anti-amyloid antibody for dry AMD

Ruslan Grishanin, Ph.D.
Age-Related Macular Degeneration: Significant Unmet Medical Need

Demographic/Prevalence Data

- **1.8 million** Americans 40 years and older have advanced AMD.
- **3.8%** of Americans aged 50 to 59 years have either intermediate or advanced AMD. By age 70 to 79 years, this increases to **14.4%**. Overall, today **7.3 million** people with intermediate AMD are at substantial risk for vision loss.
- By 2020 there will be **2.95 million** individuals with advanced AMD.
- There has been much focus and subsequent success generating treatments targeted at wet age-related macular degeneration.
- Minimizing the treatment burden and enhancing treatment efficacy remain unmet needs in therapy for this chronic disease.

There are no treatments available to fight dry AMD and to prevent its development into neovascular form or into geographic atrophy.
Amyloid deposits have been found to accumulate in the drusen of AMD

Aβ localized in the Alzheimer’s disease brain

- Drusen are the hallmark of dry AMD pathology.
- Aβ deposits are found in drusen.
- Aβ deposits in drusen may be associated with the complement activation and inflammation.

Aβ is a component of drusen in eyes with AMD

Aβ localized in close vicinity of complement activation products in AMD drusen

Johnson et al. PNAS, 2002, 99:11830
POTENTIAL MECHANISM OF Aβ INVOLVEMENT IN AMD

Accumulation of proteolipid particles in BM

Opsonization of proteolipid particles with Aβ

Aβ–dependent activation of complement attack (eg through C1q or inactivation of negative complement regulators Factor I or CD46, CD55)

Potential involvement of Aβ in the inflammatory process and AMD pathology provides rationale for the clinical development of Aβ antibody for the dry AMD treatment
Pfizer’s RN6G antibody capable of recognizing both Aβ40 and Aβ42 cytotoxic peptides but not APP

- RN6G epitope in APP is hidden in the lipid bi-layer and in-accessible for the antibody.
- RN6G recognizes and binds to Aβ_{40-42} released from the membrane.
- RN6G Fc domain is engineered not to engage complement and Fc＿γ receptors of the immune cells.
High fat/High cholesterol APOE4/APOE4 knock-in mouse model of dry AMD

- Advanced age (> 60 weeks)
- APOE4 gene knock-in
- High fat and cholesterol (HF-C) diet for 8 weeks
- Anatomical change - RPE abnormality
- Functional change - ERG deficits

RPE changes in the AMD model mice

Subretinal Aβ deposition in the AMD model mice

Malek et al, 2005; Ding et al, 2008,
Aβ antibody suppresses RPE inflammation and RPE pathology in the animal model of dry AMD

RN6G suppresses complement activation \textit{in vivo} in the linear deposits in the mouse AMD model

RN6G suppresses basal deposit formation and RPE cell loss and cell-size expansion

Collaboration with Lincoln Johnson (UCSB), Catherine Bowes Rickman (Duke University), Ding et al PNAS 2011.
Amyloid β antibody delivery by the "gene therapy" approach

- Recent Phase 1 clinical trials have demonstrated excellent safety profile of RN6G.

- However, systemic or intravitreal delivery of antibody for the chronic and initially asymptomatic disease will impose significant burden on healthcare system and patients. Development of chronic delivery modalities for biologics is essential.

- AAV-mediated gene delivery to the eye has been successfully explored as a potential long-term therapeutic delivery system.
  - RPE65 for Leber Congenital Amaurosis (Applied Genetic Technologies Corp)
  - sFlt1 for wet AMD (Avalanche Biotechnologies, Inc)

- Sustained elimination of amyloid β from retina could be achieved by the antibody expression in the eye.

RN6G designed as a “polyprotein” expressed as full length IgG with the un-altered properties

- Furln
- "Self-cleavage"
- RAKRLLNFDDLKLAGDINESPGP
- Signal
- Heavy chain
- Light chain
- 2A sequence

Expression of RN6G from monocistronic cassette in vivo
- HC
- LC
- Reombinant RN6G from mouse serum

Analysis of interactions of RN6G expressed from monocistronic cassette as self-processing polyprotein
- Anti-hFc (ab)2
- hFcrn pH6
- Biotin-Abeta (1-40)
- Streptavidin
- RN6G
- ABeta (1-40)
- Anti-hFc


HIGHLIGHTS
a. RN6G is effectively produced from monocistronic polyprotein cassette.
b. As an antibody it is structurally identical to the original RN6G.
c. Binds amyloid-beta equally to the “original” RN6G.
d. Retains Fc-properties
RN6G delivered to mouse eye intravitreally or subretinally, is expressed in the distinct retinal compartments

**AAV2.2-RN6G**
Effectively transduces inner retinal cells if delivered IVT

**AAV2.5-RN6G**
Effectively transduces outer retina/RPE if delivered subretinally

Intravitreal vector delivery, RN6G localization in retina

Subretinal vector delivery, RN6G localization in retina

Red = Alexa568-goat-anti-human IgG (H+L)

GCL

INL

RPE

α-hulgG

DAPI
Delivery of AAV-IgG to RPE results in the sustained expression of antibody in the eye with low systemic exposure
Full length RN6G Expressed in the RPE in vivo retains amyloid-β-binding activity

Levels of active RN6G measured by Aβ1-40 ELISA approximates to a concentration detected by human IgG ELISA

AAV-mediated delivery of polyprotein cassette of full length RN6G may provide long-term passive immunization of the eye against pathogenic protein
Expression of full length amyloid β antibody in RPE does not affect function of outer retina

Effect of retina transduction with AAV2.5-RN6G or AAV2.5-GFP on scotopic ERG B-wave

Subretinal inj. group average (n=4)
Summary

• Amyloid $\beta$ is a potential therapeutic target for the treatment of dry AMD.
• Full-length therapeutic anti-amyloid $\beta$ IgG can be successfully delivered to the retina via rAAV vector.
• After subretinal delivery, full length pharmacologically active RN6G is stably expressed in RPE.
• Intraocular levels of the antibody are significantly higher than its serum levels. Intraretinal expression of RN6G does not impair retinal function measured by the electroretinogram.
• Since full length IgGs are stable molecules with low immunogenicity, the technology can provide a safe and effective way for the continuous delivery of therapeutic antibodies to treat chronic retinal degenerative diseases, if proven safe and effective in humans.
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