GENE THERAPY IN OPHTHALMOLOGY

THE TARGETAMD PROJECT

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CLASSICAL THERAPY

- Half-life of the drugs necessitates repeated doses
- We can treat but often not cure
- Individuel reactions are not considered
i.e. Diabetes

Compliance
Costs
No benefit
Side effects and complications
Worsening of the disease and/or its consequences
GENE THERAPY - PRINCIPLES

Goals
- Genetic defects
- Augment faint activities
- New genes
- Supplementary functions

Substitution
RPE65 gene in Leber’s Congenital Amaurosis (LCA)

Silencing
Rhodopsin gene in Retinitis Pigmentosa

Addition
PEDF gene in Age-related Macular Degeneration

Correction
Factor VIII gene in hemophilia
GENE THERAPY – METHODS

Ex vivo vs. In vivo

Ex vivo

Example: ADA gene in SCID

In vivo

Example: RPE65 gene in LCA
GENE THERAPY – METHODS

**Viral vs. Non-viral**

**Advantages**
- Efficient DNA packaging
- Highly efficient

**Drawbacks**
- Limited size
- Expensive and complex production
- Immune responses
- Frequent distribution of the transgene
- Preferred integration into active gene loci
- Cancerogenicity
- Cell death
**GENE THERAPY – METHODS**

### VIRAL vs. NON-VIRAL

#### Advantages

**VIRAL**
- Efficient DNA packaging
- Highly efficient

**NON-VIRAL**
- No limits in size
- Easy production
- Weak immune response
- Weak toxicity

#### Drawbacks

**VIRAL**
- Limited size
- Expensive and complex production
- Immune responses
- Frequent distribution of the transgene
- Preferred integration into active gene loci
- Cancerogenicity
- Cell death

**NON-VIRAL**
- Less efficient
- No guaranty of stable genetic expression
SUCCEEDEDS AND FAILURES

Ashanti DeSilva
1st successful treatment
1990
SCID = severe combined immuno deficit

Jesse Gelsinger †
Fatal issue
1999
Ornithine transcarbamylase deficiency

Corey Haas
Successful treatment
2009
LCA = Leber’s Congenital Amaurosis

SUCCESS IN OPHTHALMOLOGY

Leber’s Congenital Amaurosis

Ausotomal recessive pathology
2 carriers → 25% risk to fall ill

Clinical study (phase I)
♦ 15 patients
♦ 3 centers
♦ 11-30 year old patients
♦ 3 years follow-up
♦ rAAV2-hRPE65

Results
♦ Safety - systemic and ocular
♦ Absence - systemic distribution
♦ Improvement - in all patients (but variable)

Feasible and efficient
SUCCESS IN OPHTHALMOLOGY

Non-treated eye

Maguire et al. Treatment of Leber Congenital Amaurosis due to RPE65 Mutations in Children and adults using Adeno-Associated Virus (AAV)-mediated Gene Delivery
SUCCESS IN OPHTHALMOLOGY

Treated eye

SUPPLEMENTARY VIDEO 1B

Maguire et al
“Treatment of Leber Congenital AMaurosis due to RPE65 Mutations in Children and Adults using Adeno-Associated Virus (AAV)-mediated Gene Delivery

CH09, day 90,
Navigation using treated eye
WHY THE EYE?

Accessibility

Local application
- intravitreal → DR, glaucoma
- intracameral → Inflammation reduction after corneal transplantation
- Sub-conjunctival → Neovascular retinal macular diseases
- Sub-retinal → Retinal degeneration

Size

Immune privilege
**TARGET AMD**

*Transposon-Based, Targeted* Ex Vivo Gene Therapy to Treat Age-related Macular Degeneration (AMD)

<table>
<thead>
<tr>
<th>7 countries</th>
<th>13 partners</th>
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<tr>
<td>CH</td>
<td>University of Geneva</td>
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<tr>
<td>GER</td>
<td>Rheinisch-Westfälische Technische Hochschule Aachen</td>
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<tr>
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<td>UD-Genomed Medical Genomic Technologies Ltd.</td>
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<tr>
<td>AUS</td>
<td>Krankenanstalt Rudolfstiftung</td>
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<tr>
<td>NL</td>
<td>AmBTU Stichting Amsterdam Biotherapeutics Unit</td>
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PATHOGENY OF AMD

1 Disease – 2 Forms

**DRY**

Causes unknown
Suspicion: oxidative stress & inflammation

**WET**

Imbalance
angiogenic *VEGF* & anti-angiogenic *PEDF*
CURRENT TREATMENT OF AMD

Monthly injections of Anti-VEGF
- Lifelong -

Inhibits
- Proliferation
- Survival
- Migration of endothelial cells

Reduce
- Vascular permeability
- Risks for infection
- Big effort for the patients
- Lifelong treatment
ADDITIVE GENE THERAPY

Pigment Epithelium Cells

Efficient expression

Long-term expression

rPEDF

Anti-angiogenic effect

Electroporation

Nucleus

PEDF

SB100X

P
SLEEPING BEAUTY TRANSPOSON SYSTEM

Co-transfection (Electroporation)

Binding

Excision

Integration

Transposase

Transposon

Cell
SURGICAL PROCEDURE

TargetAMD approach

Iridectomy/Retinal Biopsy
- 5 min

Subretinal Transplantation
- 15 min

Cell Isolation
- 10 min

Electroporation
- 30 min
AUTOLOGOUS IPE CELL TRANSPLANTATION

hRPE CELL TRANSFECTION USING pFAR4 PLASMIDS

ELISA

<table>
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<th>Non-transfected control</th>
<th>pFAR4-CMV-PEDF</th>
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<td>PEDF [ng/h/10^4 cells]</td>
<td>0.04 ± 0.00</td>
<td>0.64 ± 0.21</td>
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Western Blot

“pFAR4”

130 Days

“pT2”

315 Days

- PEDF-transfected human RPE cells
- pFAR4- vs. pT2-CMV-PEDF-HIS

→ Plasmid Free of Antibiotic Resistance-4-CMV-PEDF is superior
REPRODUCIBLE WITH SMALL CELL NUMBERS

- 5’000 primary human RPE cells
- 15 different donors
- 21 days after transfection

→ Efficient transfection
→ Low intra-individual variances
→ High reproducibility

Western Blot
INCREASED PEDF EXPRESSION AND SECRETION

**qRT-PCR**

- **5’000 hRPE cells**
- **qRT-PCR and ELISA**

**ELISA**

- 63.2 times increased expression
- 16.9 times increased secretion
Modified Cliniporator and Microcuvette for small cell numbers

→ Efficient transfection using the Cliniporator with the microcuvette
NEW ELECTROPORATION BUFFER, 3P

- Efficient transfection
- High viability of the cells
- Defined composition of the buffer

• 3P buffer
• Human RPE cells
SAFETY STUDY ON TUMORIGENICITY

Soft-Agar Colony Formation Assay

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<th>pFAR4-CMV-PEDF</th>
<th>Positive control (HeLa cells)</th>
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- Human RPE cells
  → No tumorigenicity
SAFETY STUDY ON INTEGRATION PROFILE

*SB transposon integration profile in transfected hRPE cells.*

→ Random integration profile

→ *Especially important with respect to a clear lack of preferred integration into cancer genes.*
DECREASED CHOROIDAL NEOVASCULARIZATION

- Rat CNV laser model
- Subretinal transplantation
- Caveolin stain

→ Significant reduction in CNV lesion size
PERSONALIZATION AND SAFETY

- Safe harbours
- Insulators
- mRNA transposase
- Suicide gene
- Tet-On system

→ Increasing controllability
→ Personalizing the treatment
NEXT STEPS

• Completion of preclinical analyses
• Validation of GMP grade production of the cell product
• GMP grade plasmid production
• 2 Phase Ib/IIa Clinical Trials
PARTNERS AND COLLABORATORS

**University of Geneva and HUG**
- Univ.-Prof. Dr. Gabriele Thumann
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- Antje Schiefer

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- IGEA medical GmbH, Italy
- 3P Biopharmaceuticals, Spain
- UD-GenoMed Medical Genomic Technologies Ltd, Hungary