Gene Therapy in Ophthalmology

the TargetAMD Project

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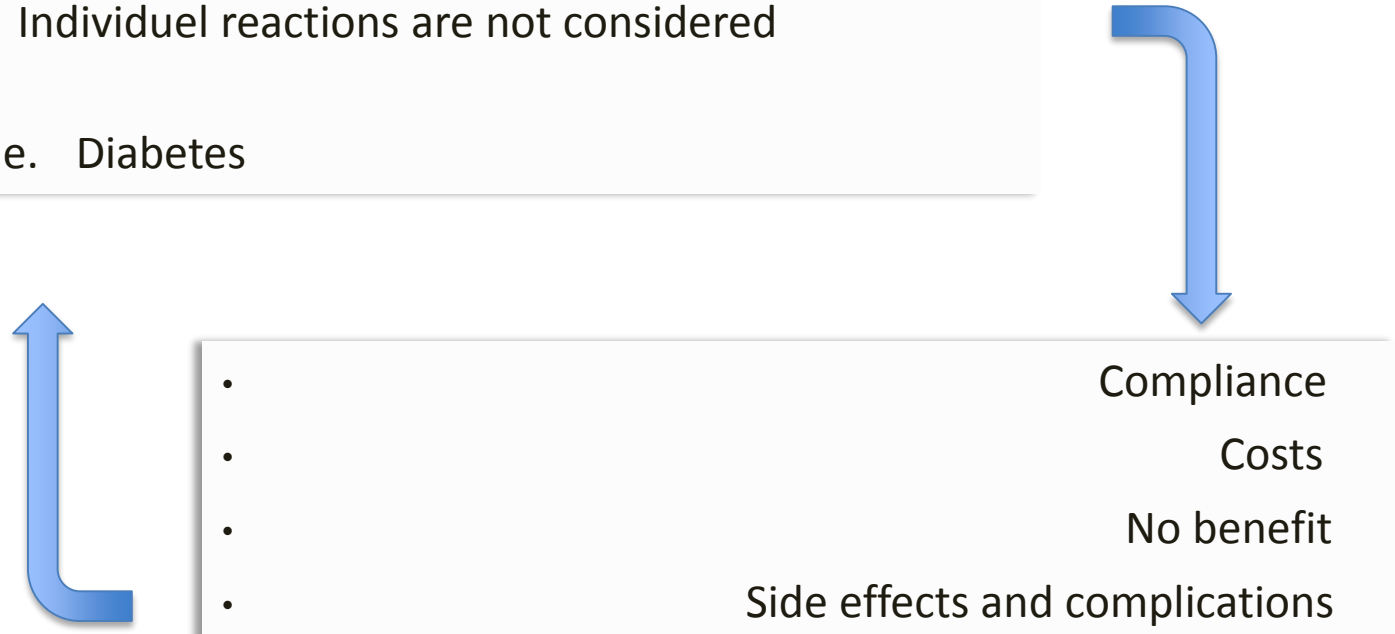
CLASSICAL THERAPY

- 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 **VIRAL**

- 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

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VIRAL

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NON-VIRAL

Advantages

- No limits in size
- Easy production
- Weak immune response
- Weak toxicity

Drawbacks

- Less efficient
- No guaranty of stable genetic expression

SUCCESSES AND FAILURES

Ashanti DeSilva
1st succsseful 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

Blaese RM, et al. T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. Science. 1995 Oct 20;270(5235):475-80. Maguire AM, et al. Age-dependent eff ects of RPE65 gene therapy for Leber's congenital amaurosis: a phase I dose-escalation trial. Lancet. 2009; 374: 1597–605; http://permanent.access.gpo.gov/lps1609/www.fda.gov/fdac/features/2000/500_ gene.html

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

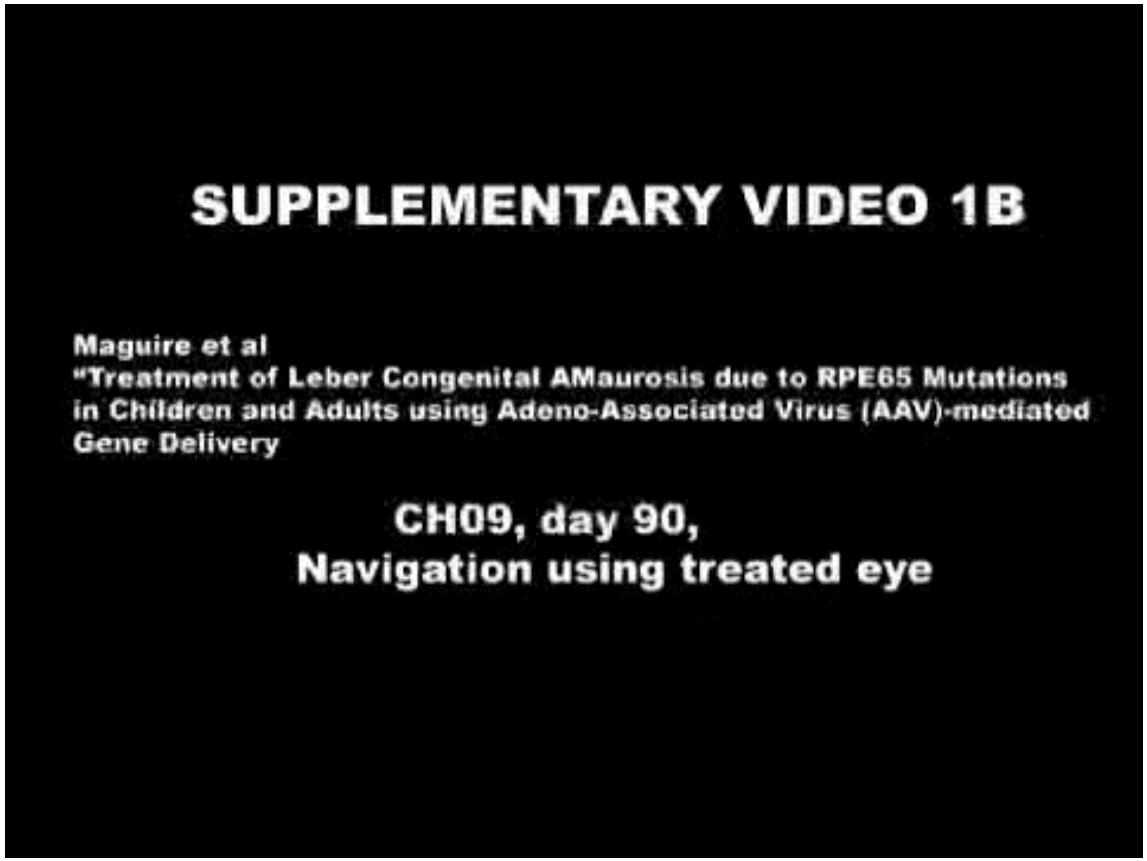
SUCCESS IN OPHTHALMOLOGY

Non-treated eye

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

SUCCESS IN OPHTHALMOLOGY

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

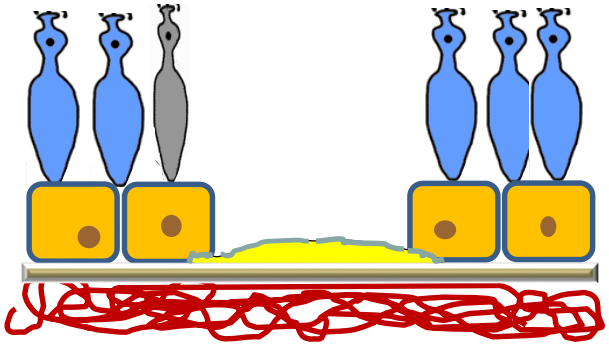
Age-related **M**acular **D**egeneration (AMD)

7 countries	13 partners
CH	University of Geneva
GER	Rheinisch-Westfälische Technische Hochschule Aachen
	Max-Delbrück-Centrum für Molekulare Medizin
	Paul-Ehrlich-Institut
	Universitätsklinikum Aachen
FR	Centre National de la Recherche Scientifique
	GenoSafe SAS
ESP	Universidad de Navarra
	3P Biopharmaceuticals, S.L.
IT	IGEA Clinical Biophysics
HUG	UD-Genomed Medical Genomic Technologies Ltd.
AUS	Krankenanstalt Rudolfstiftung
NL	AmBTU Stichting Amsterdam Biotherapeutics Unit

PATHOGENY OF AMD

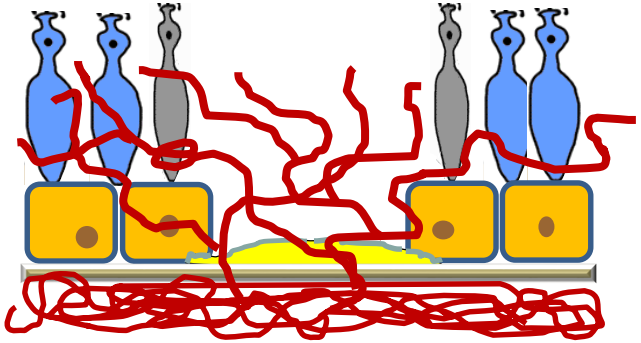
1 Disease – 2 Forms

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

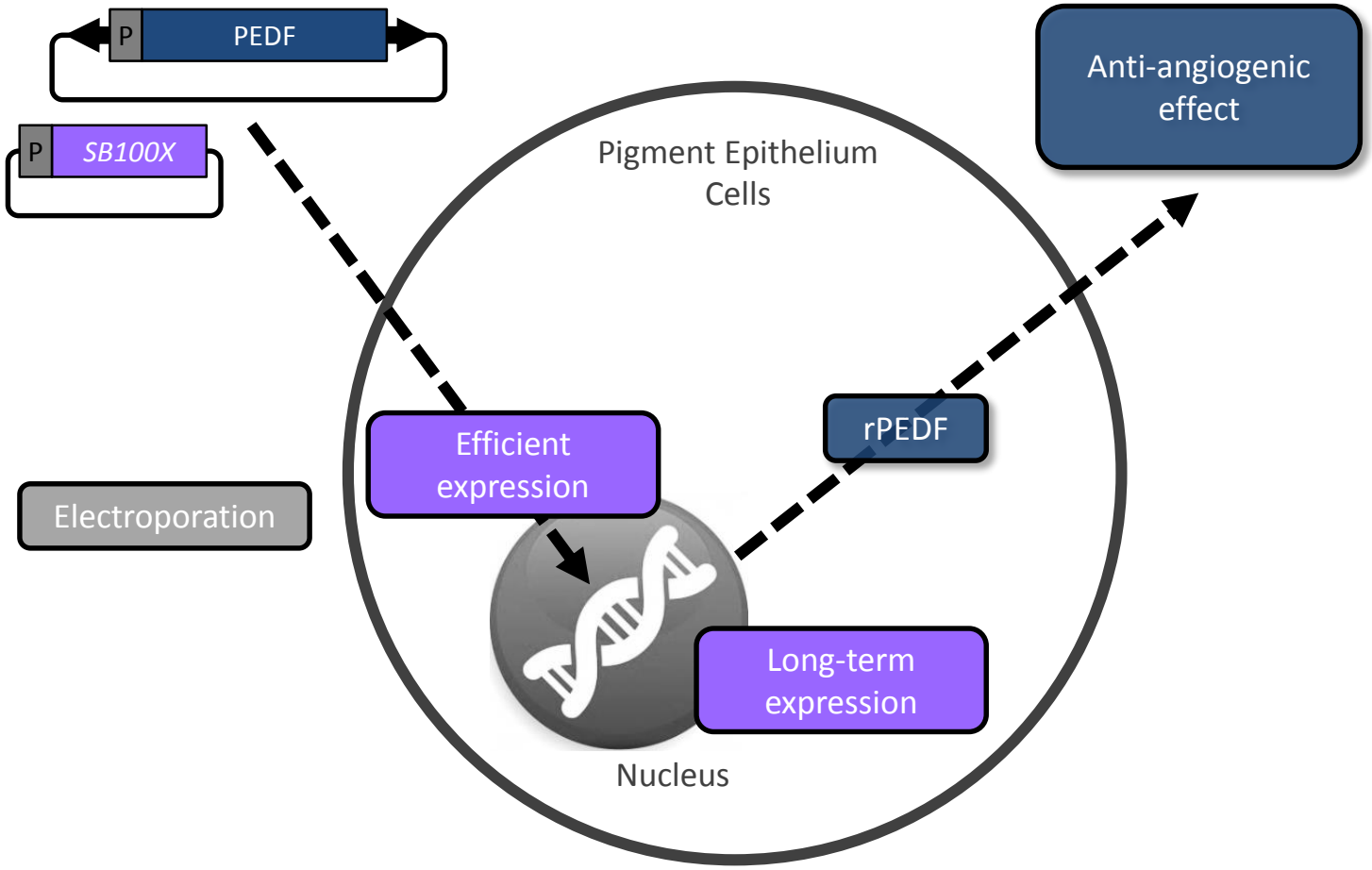
Reduce

- Vascular permeability

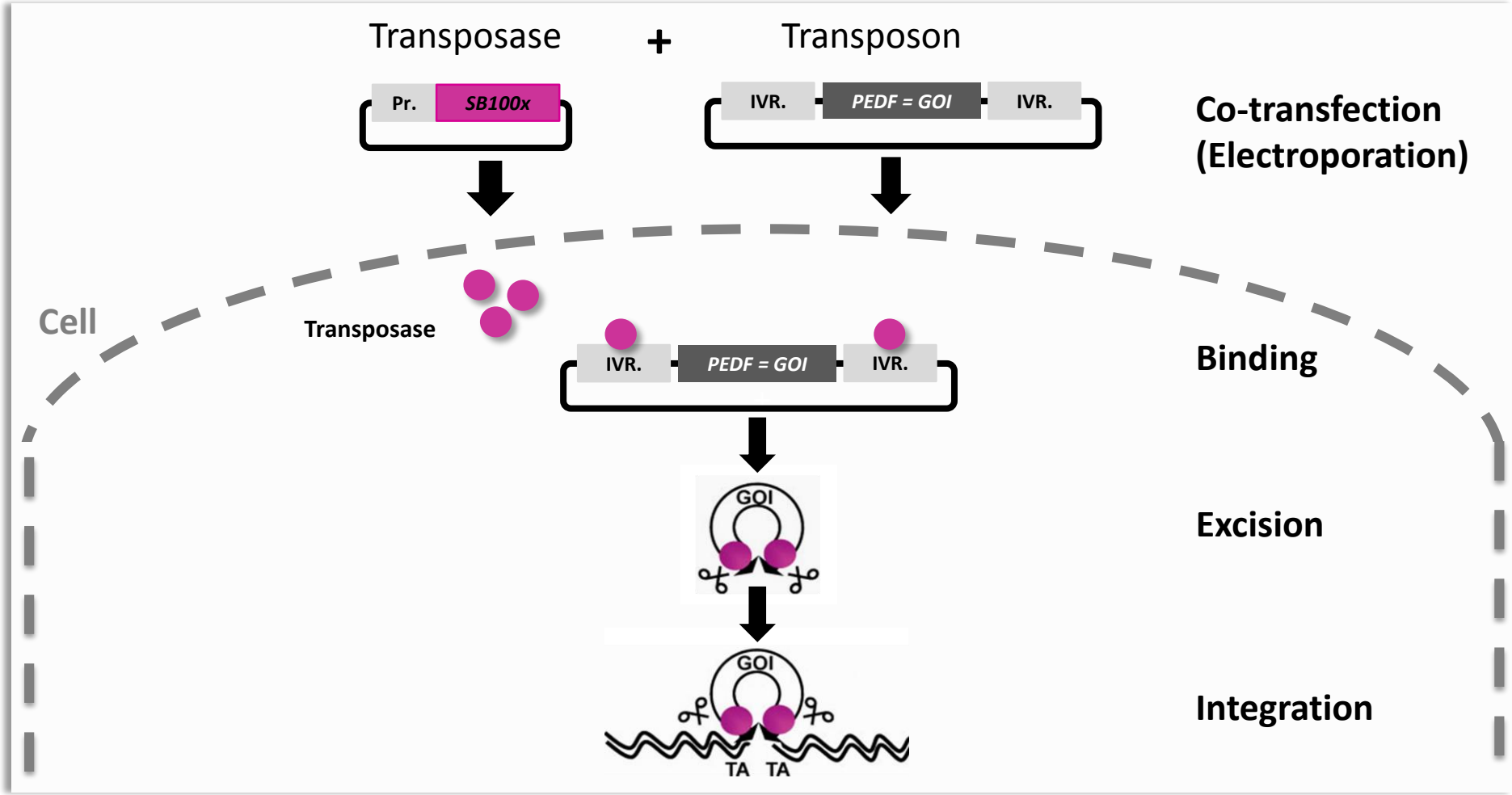
- Risks for infection
- Big effort for the patients
- Lifelong treatment

Aisenbrey S, Walter P, Thumann G, Bartz-Schmidt KU. Macular translocation with 360 degrees retinotomy for exudative age-related macular degeneration. *Arch Ophthalmol*. 2002;120:451-459.; Aisenbrey S, Bartz-Schmidt KU, Walter P, Thumann G. Long-term follow-up of macular translocation with 360 retinotomy for exudative age-related macular degeneration. *Arch Ophthalmol*. 2007;125:1367-1372.

ADDITIVE GENE THERAPY



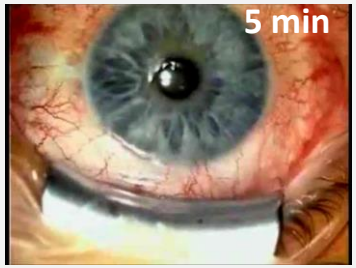
SLEEPING BEAUTY TRANSPOSON SYSTEM

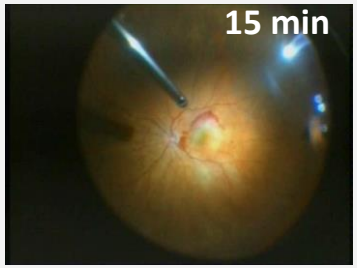


SURGICAL PROCEDURE

TargetAMD approach

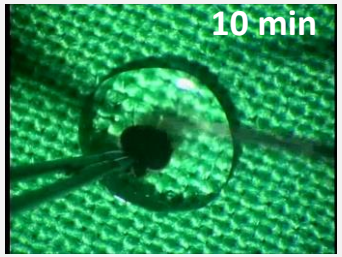
Iridectomy/Retinal Biopsy



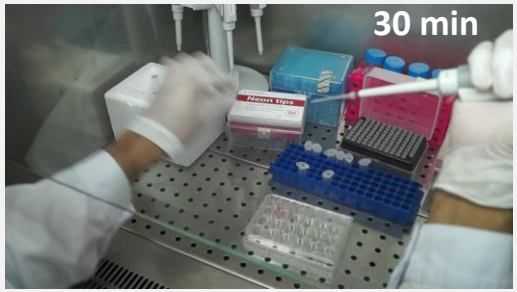


Subretinal Transplantation

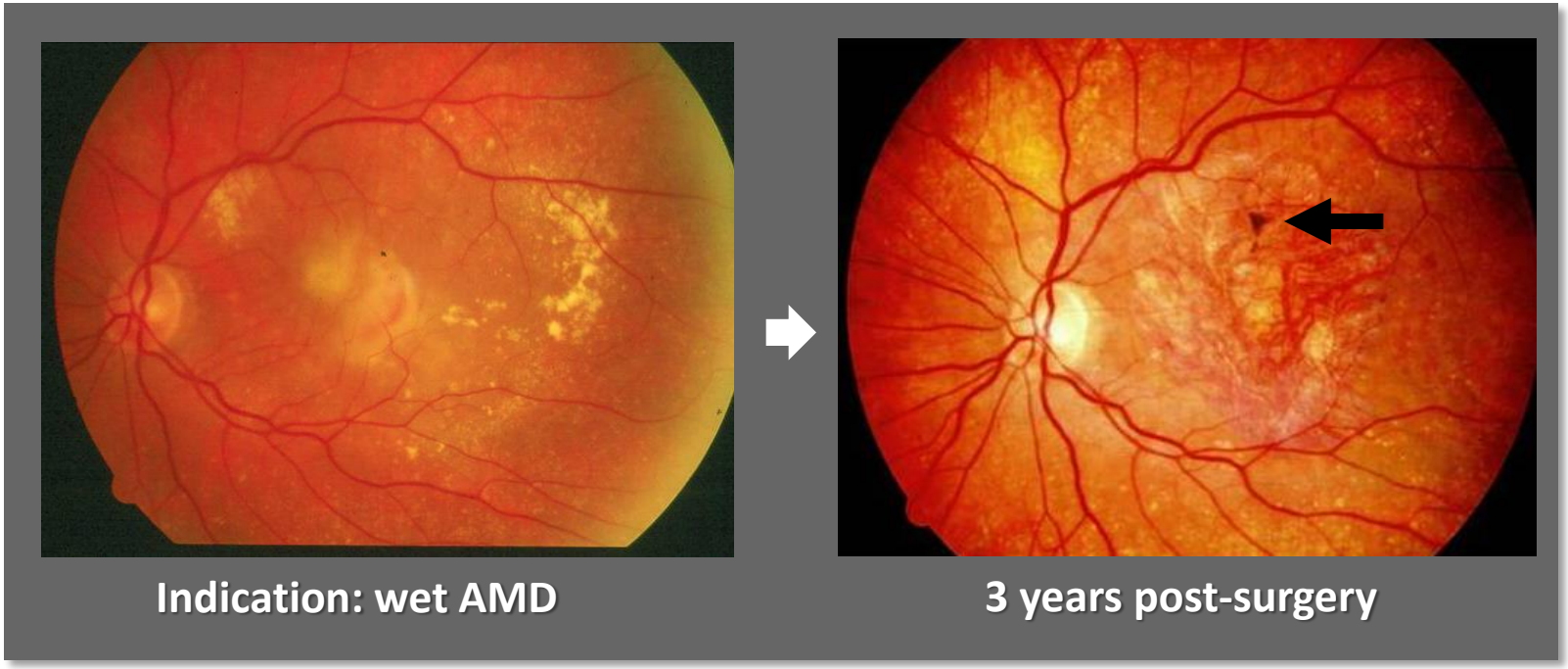
Cell Isolation



Electroporation



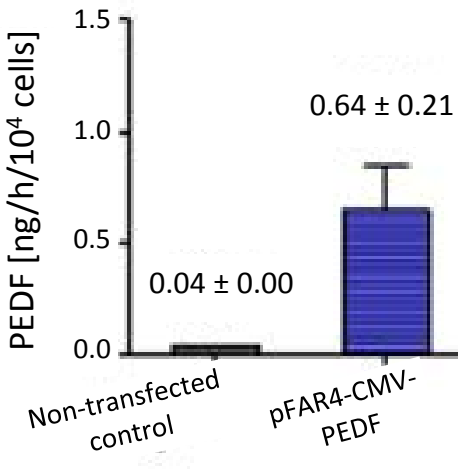
AUTOLOGOUS IPE CELL TRANSPLANTATION



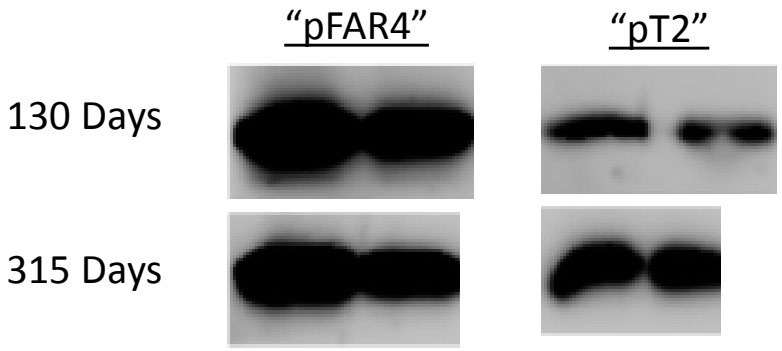
Thumann G, Aisenbrey S, Schraermeyer U, Lafaut B, Esser P, Walter P, Bartz-Schmidt KU. Transplantation of autologous iris pigment epithelium after removal of choroidal neovascular membranes. *Arch Ophthalmol*. 2000;118:1350-1355. Aisenbrey S, Lafaut BA, Szurman P, Hilgers RD, Esser P, Walter P, Bartz-Schmidt KU, Thumann G. Iris pigment epithelial translocation in the treatment of exudative macular degeneration - A 3-year follow-up. *Arch Ophthalmol*. 2006;124:183-188.

hRPE CELL TRANSFECTION USING pFAR4 PLASMIDS

ELISA



Western Blot



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

→ **P**lasmid **F**ree of **A**ntibiotic **R**esistance-4-CMV-PEDF is superior

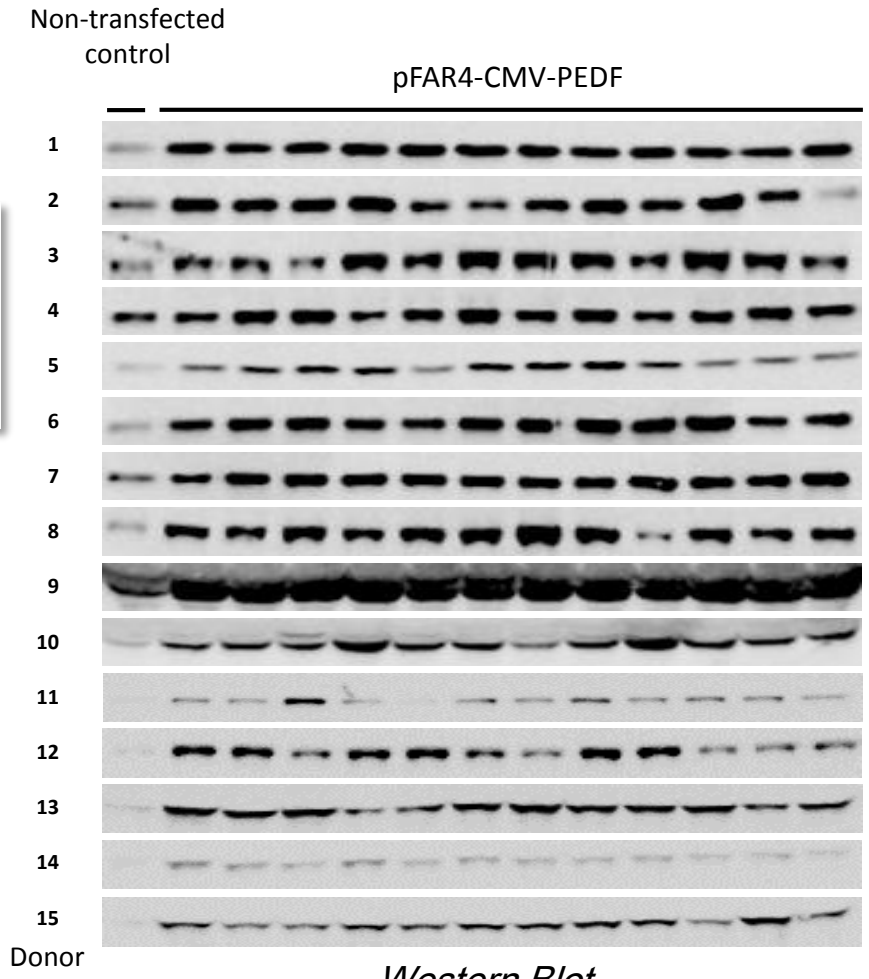
REPRODUCIBLE WITH SMALL CELL NUMBERS

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

→ Efficient transfection

→ Low intra-individual variances

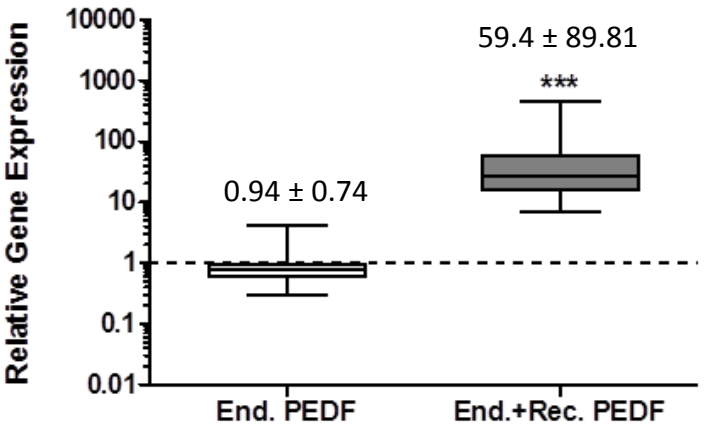
→ High reproducibility



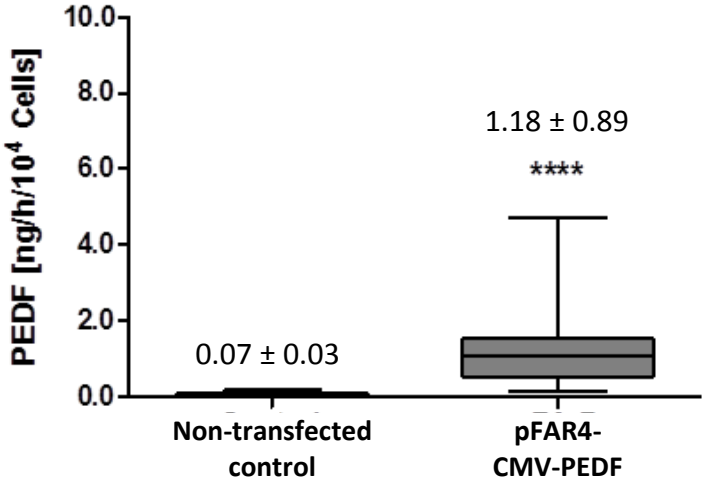
Western Blot

INCREASED PEDF EXPRESSION AND SECRETION

qRT-PCR



ELISA



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

→ 63.2 times increased expression

→ 16.9 times increased secretion

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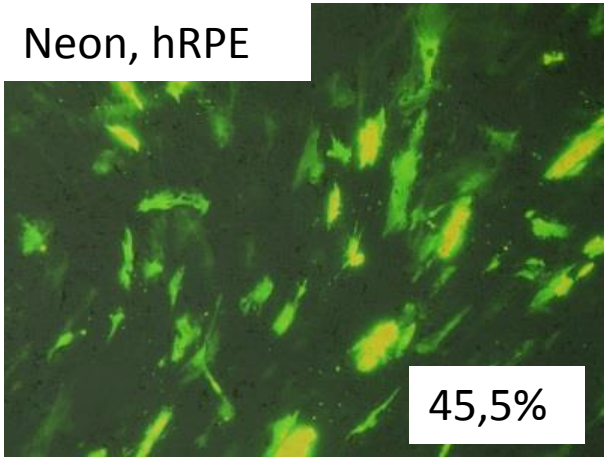
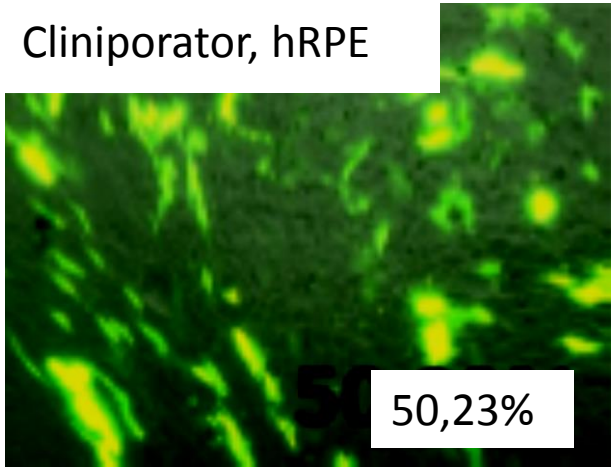
NEW ELECTROPORATOR – CLINIOPRATOR, IGEA

Neon, hRPE



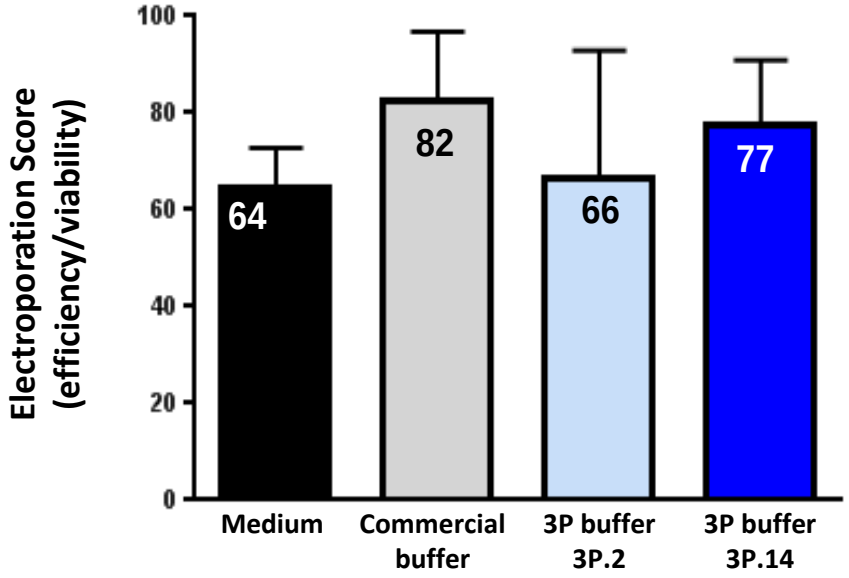
Cliniporator, hRPE



- Modified **Cliniporator** and **Microcuvette** for small cell numbers

→ Efficient transfection using the Cliniporator with the microcuvette

NEW ELECTROPORATION BUFFER, 3P



- **3P buffer**
- human RPE cells

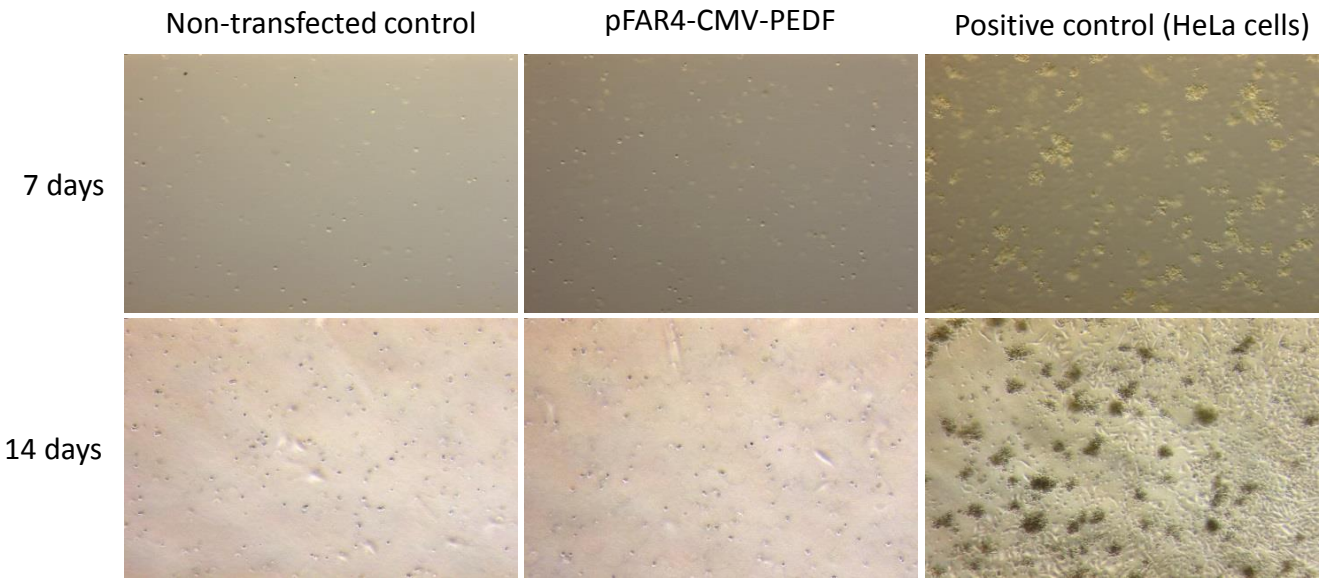
→ Efficient transfection

→ High viability of the cells

→ Defined composition of the buffer

SAFETY STUDY ON TUMORIGENICITY

Soft-Agar Colony Formation Assay

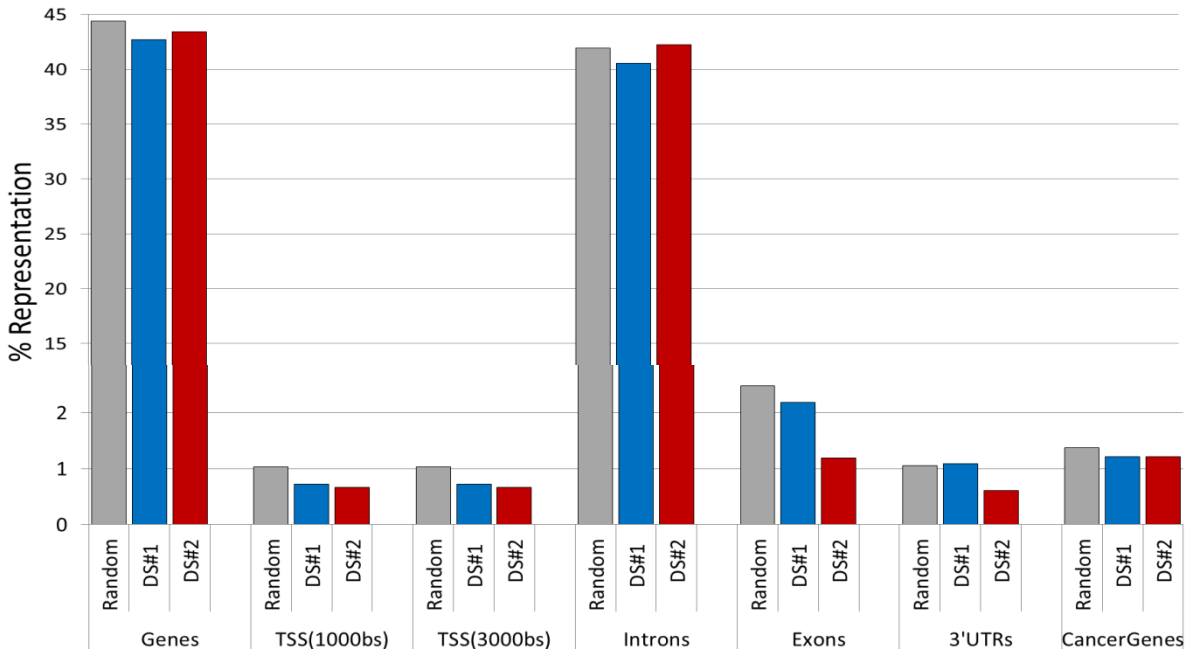


- Human RPE cells

→ No tumorigenicity

SAFETY STUDY ON INTEGRATION PROFILE

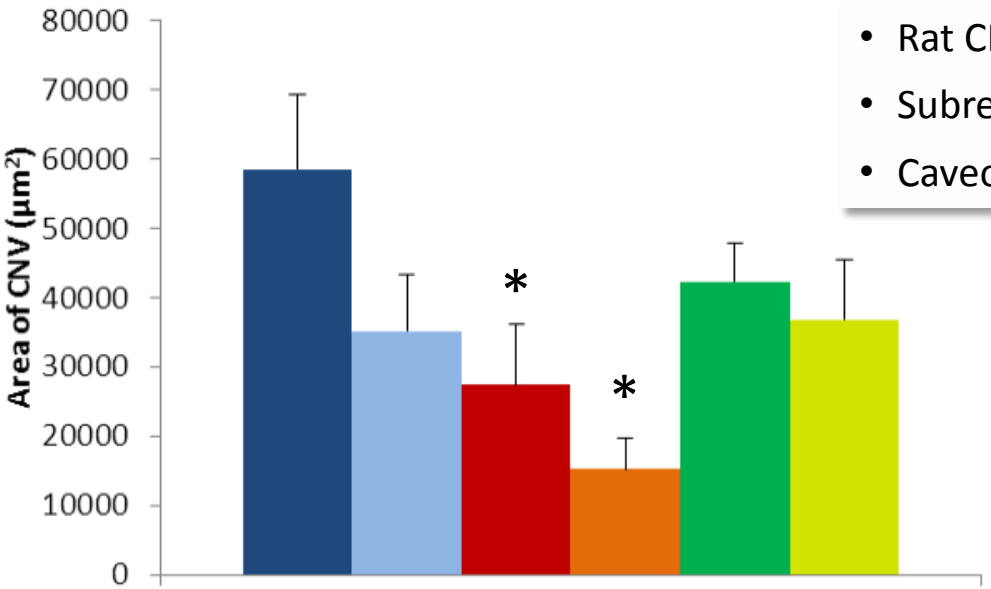
SB transposon integration profile in transfected hRPE cells.



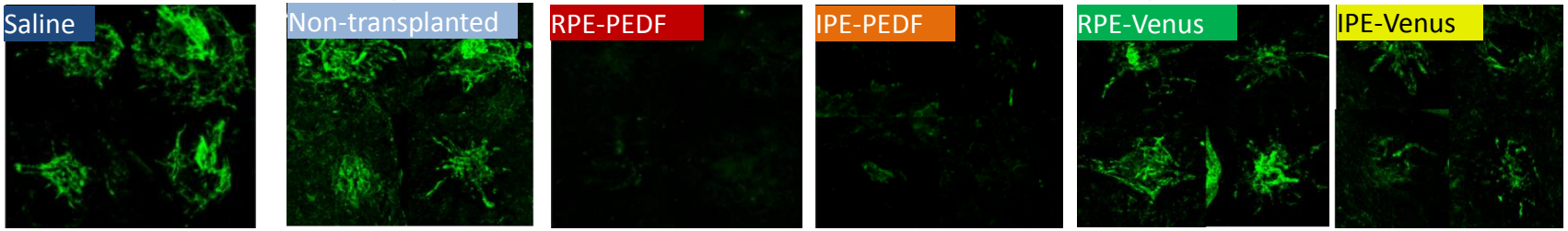
→ Random integration profile

→ ***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 Clinicial Trials

PARTNERS AND COLLABORATORS

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- Max-Delbrück-Centrum für molekulare Medizin (MDC), Germany
- Centre national de la recherche scientifique (CNRS), France

Industry

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