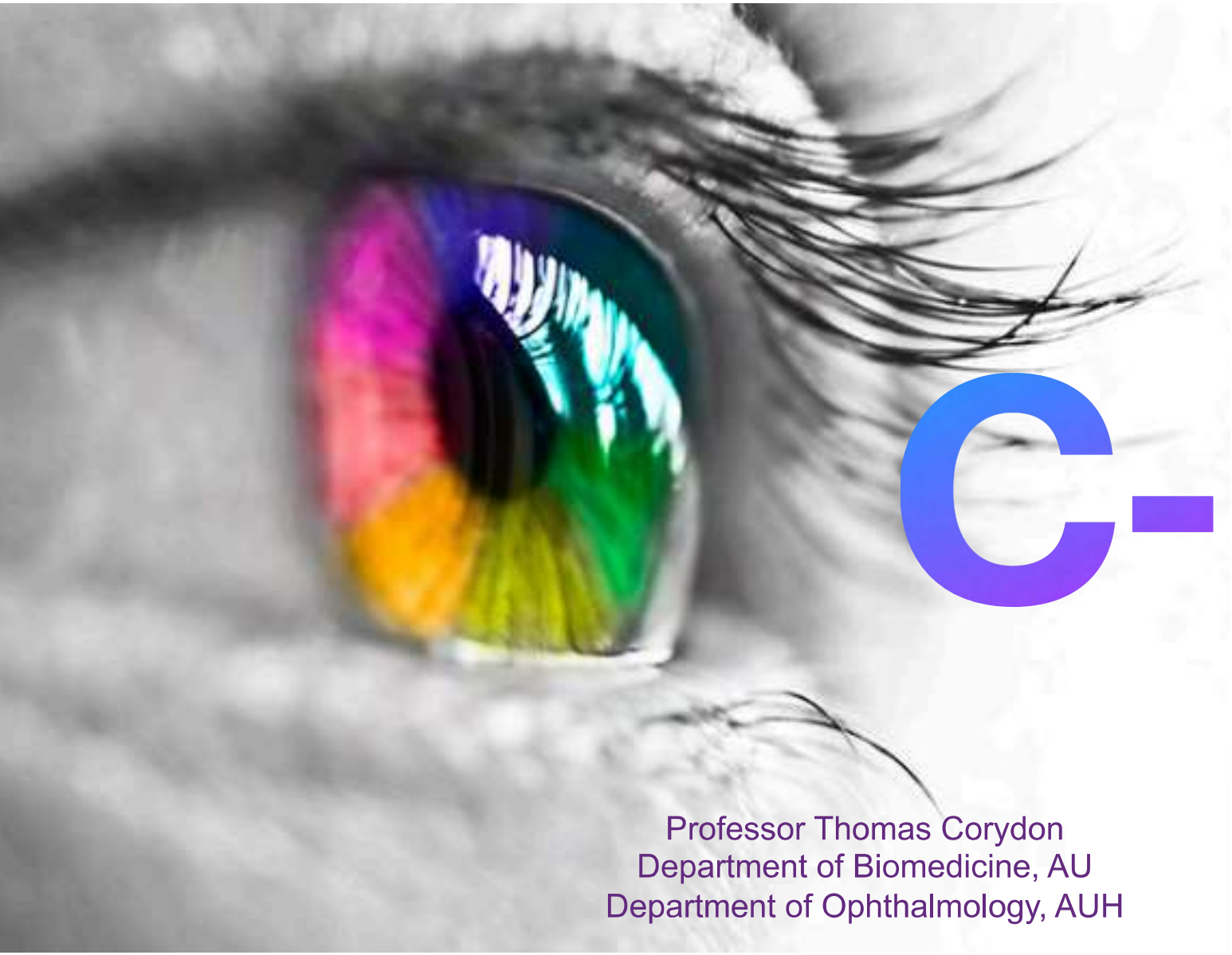


C-lab

Professor Thomas Corydon
Department of Biomedicine, AU
Department of Ophthalmology, AUH

Landskursus for Øjensygeplejesker 2024 Vingsted

25–27 Januar

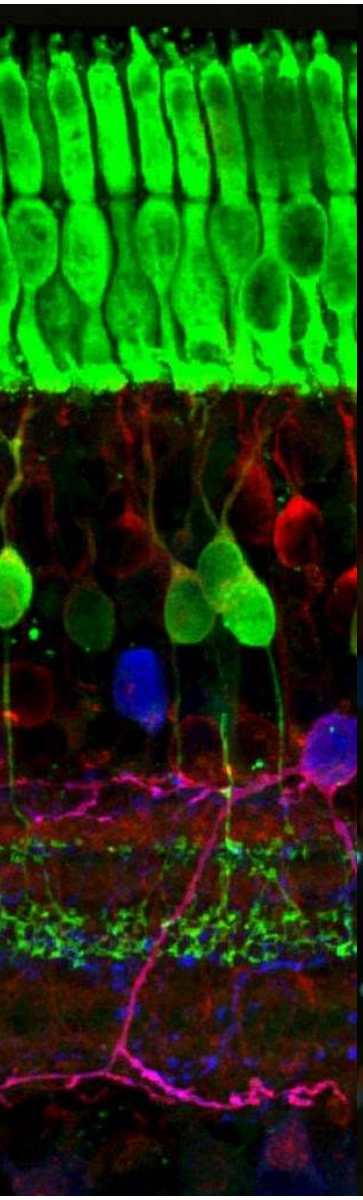
Experimental Gene Therapy For AMD

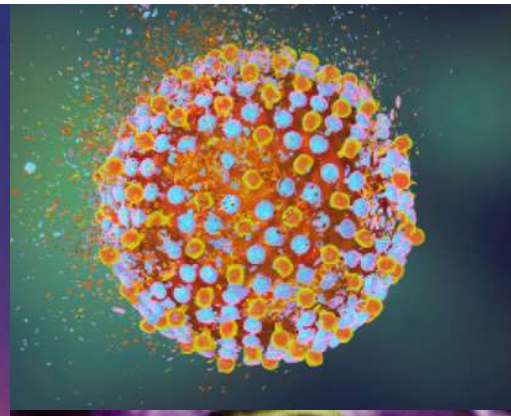
Professor THOMAS CORYDON

Department of Biomedicine
Aarhus University
DENMARK

Department of Ophthalmology
Aarhus University Hospital
Denmark

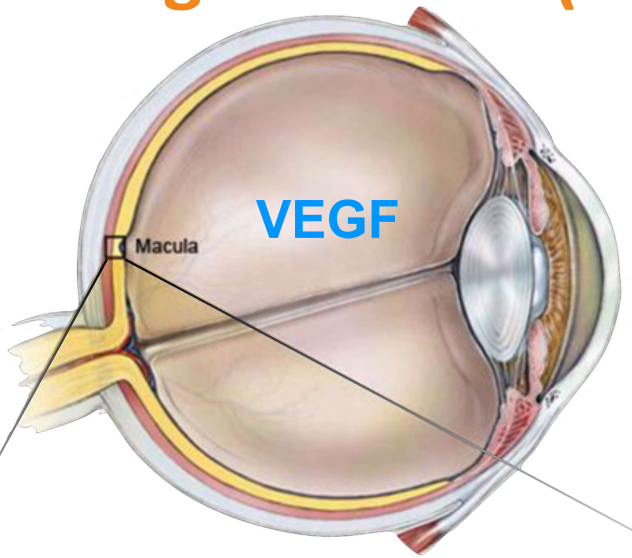
AARHUS UNIVERSITY

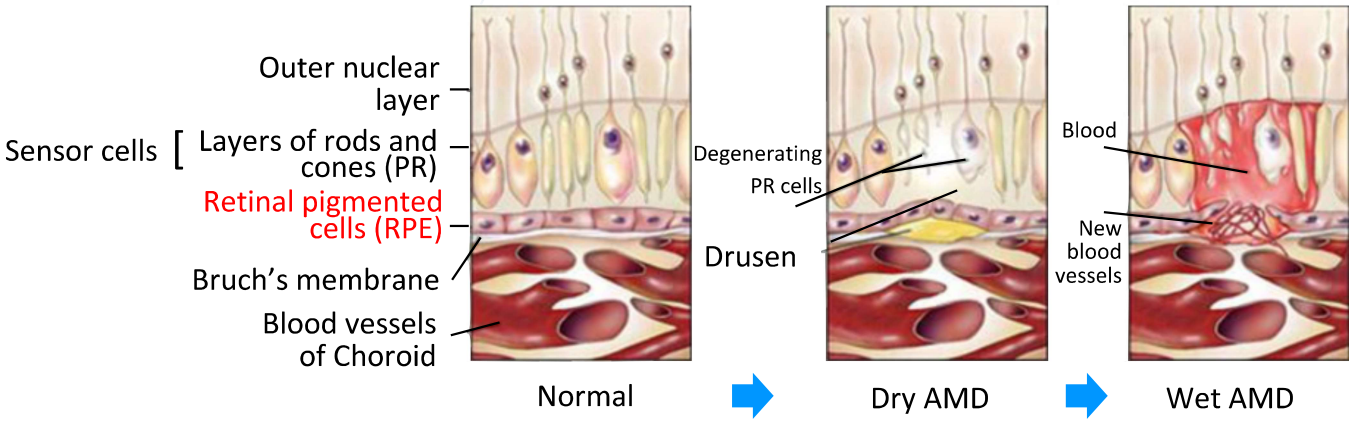




Age-related Macular Degeneration (AMD)

– a **multifactorial** slowly progressing disease, affecting approx. 50% of the elderly population





CNV

Bright Focus Foundation

AIM:

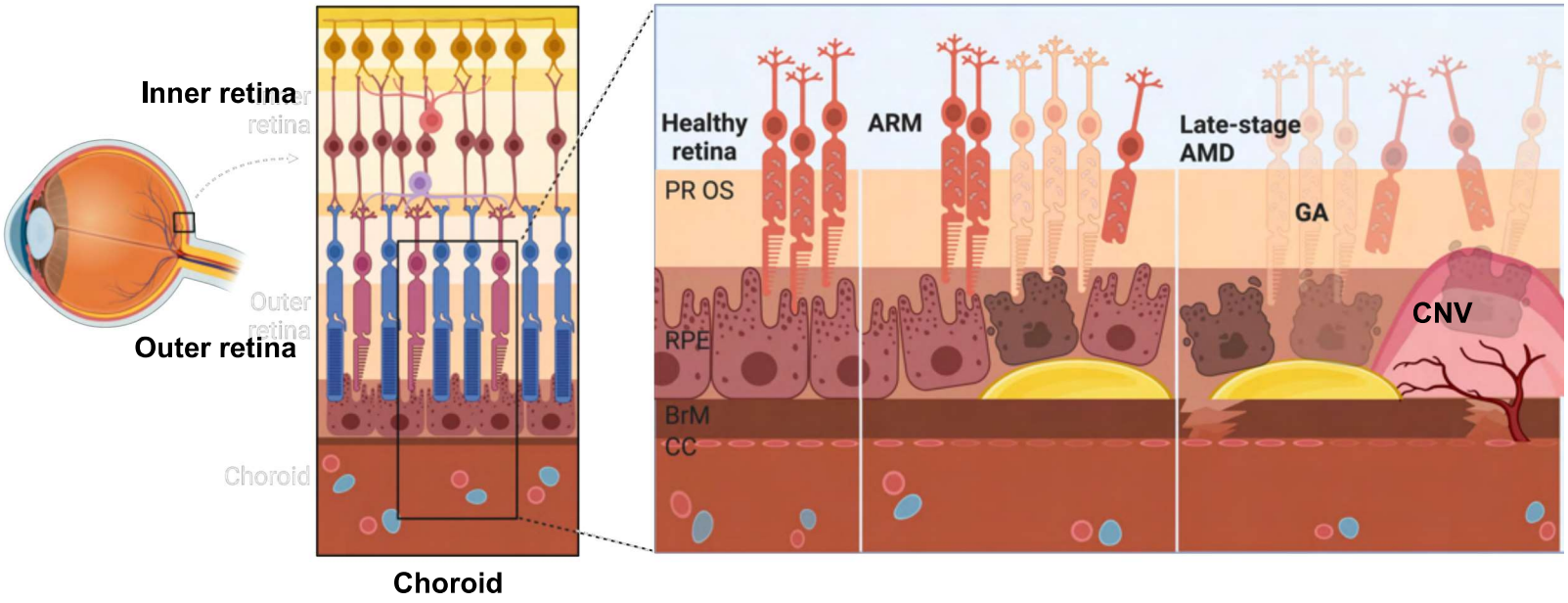
Reduce number of injections

Improve efficacy

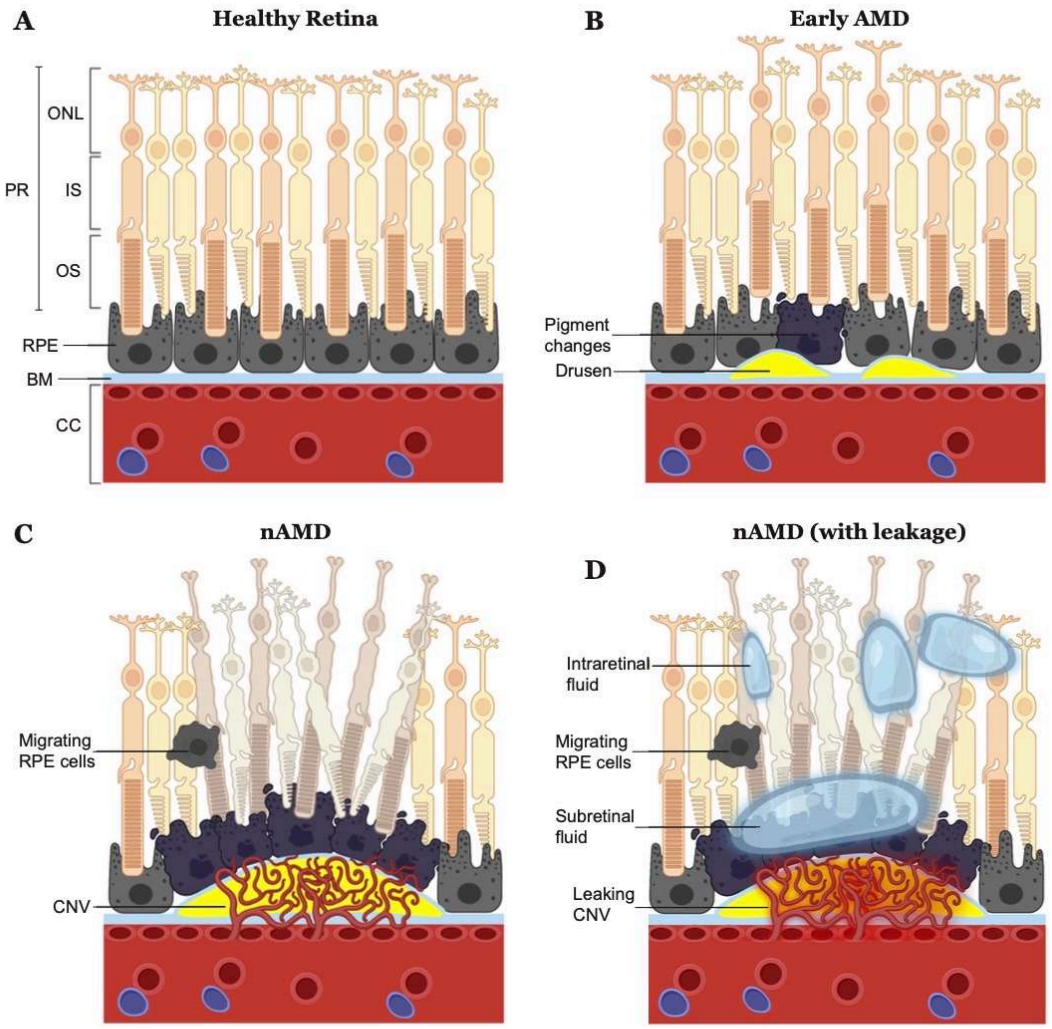
Development of Clinical Prediction Rule

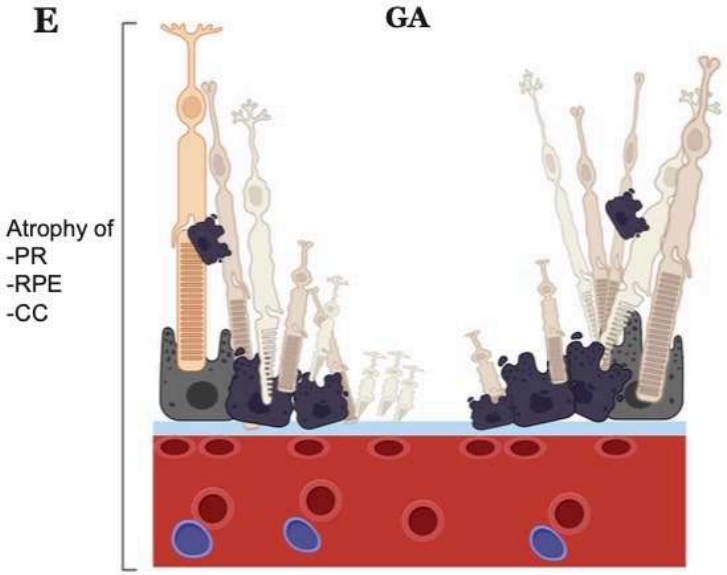
Who will benefit from GT?

Late-stage AMD



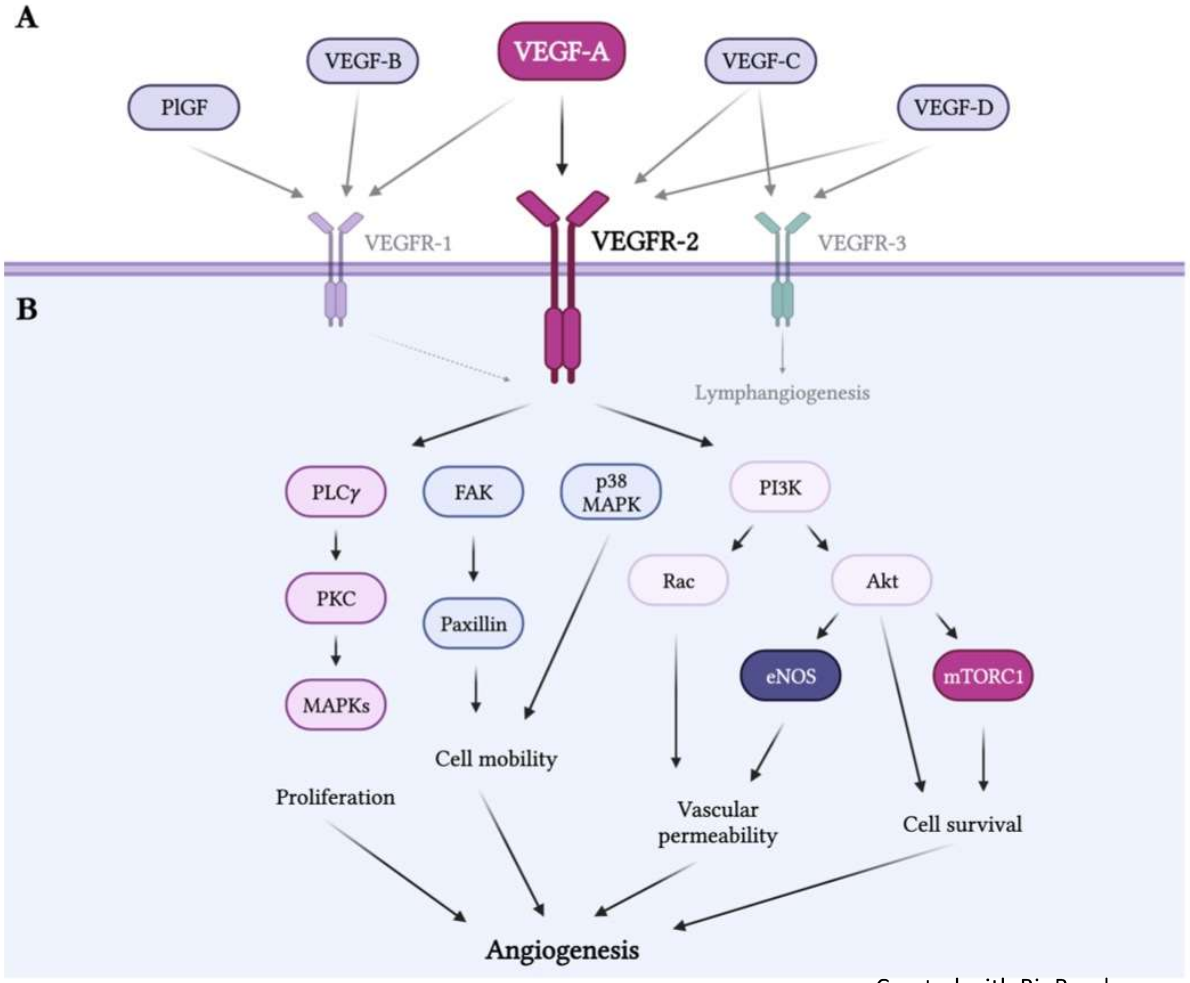
AMD PATHOGENESIS





MD Silja Hansen, PhD dissertation 2023

VEGF signaling cascade



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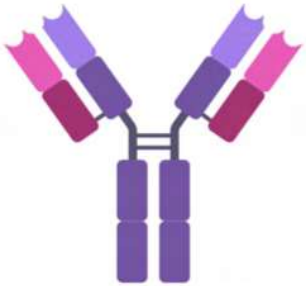
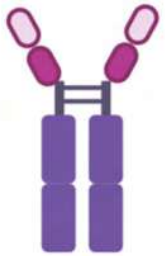
VEGF antagonists used in clinical practice

	Bevacizumab (Avastin)	Ranibizumab (Lucentis)	Aflibercept (Eylea)	Brolucizumab (Beovu)
				
Format	Full monoclonal antibody	Antibody fragment	VEGRF1/2 recombinant fusion protein	Single-chain antibody fragment
Molecular mass	149 kD	48 kD	115 kD	26 kD
Target(s)	All VEGF-A isofom	All VEGF-A isofom	All VEGF-A, VEGF-B, and P1GF isoforms	All VEGF-A isofom

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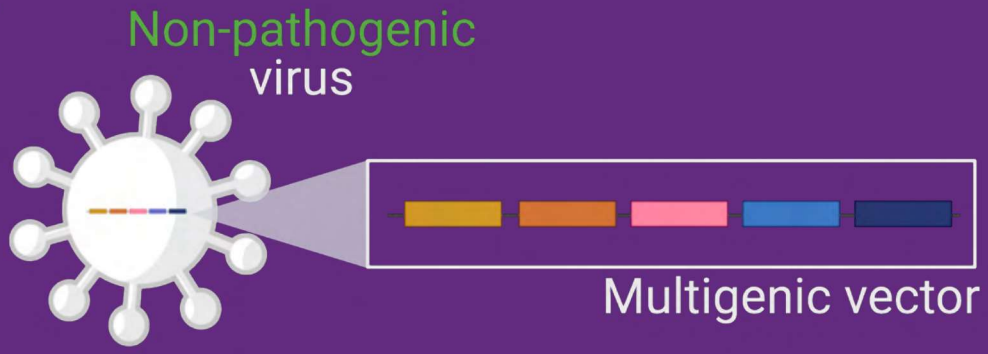
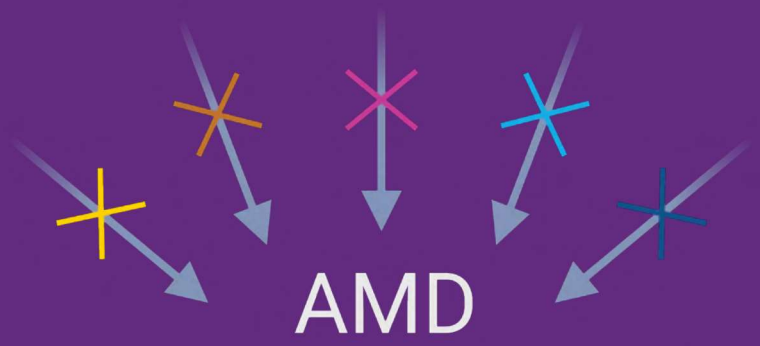
Our IDEA





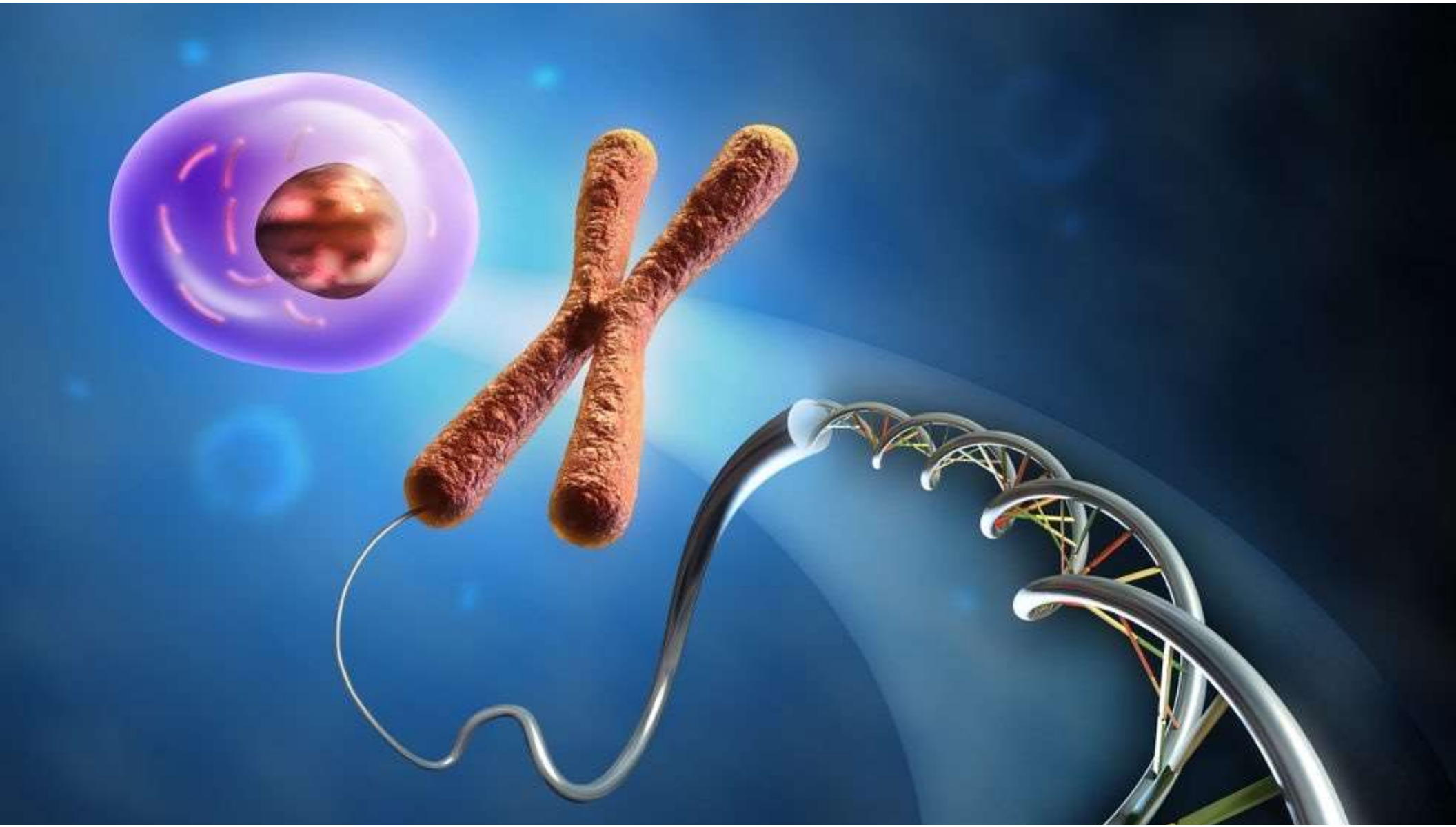
... targets multiple pathways involved in AMD

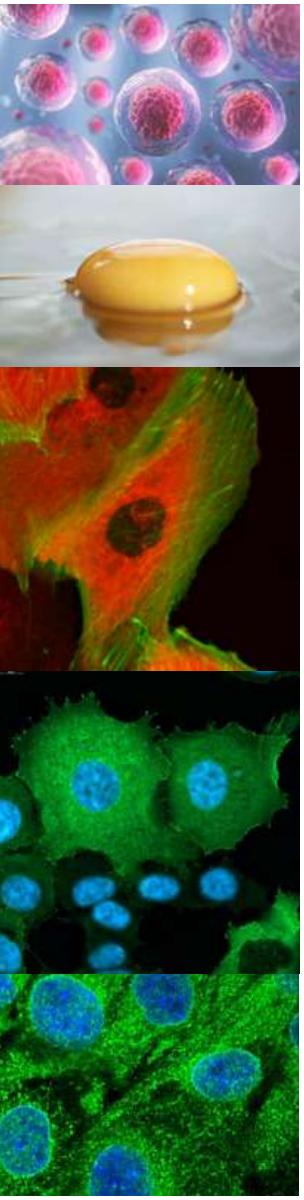
Gene Therapy

Definition:

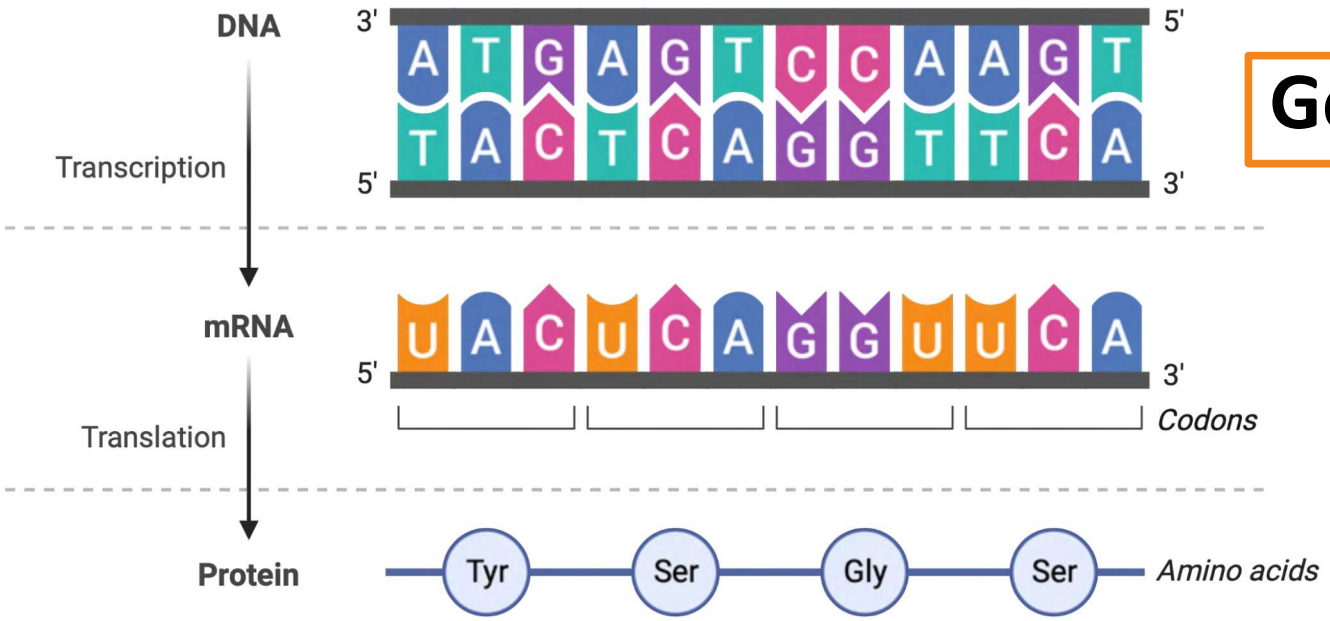
Transfer of DNA (one or more genes) to cells with the purpose of changing their functional properties and treatment of disease

Development of tools enabling efficacious delivery of genes to cells





The central dogma



Gen

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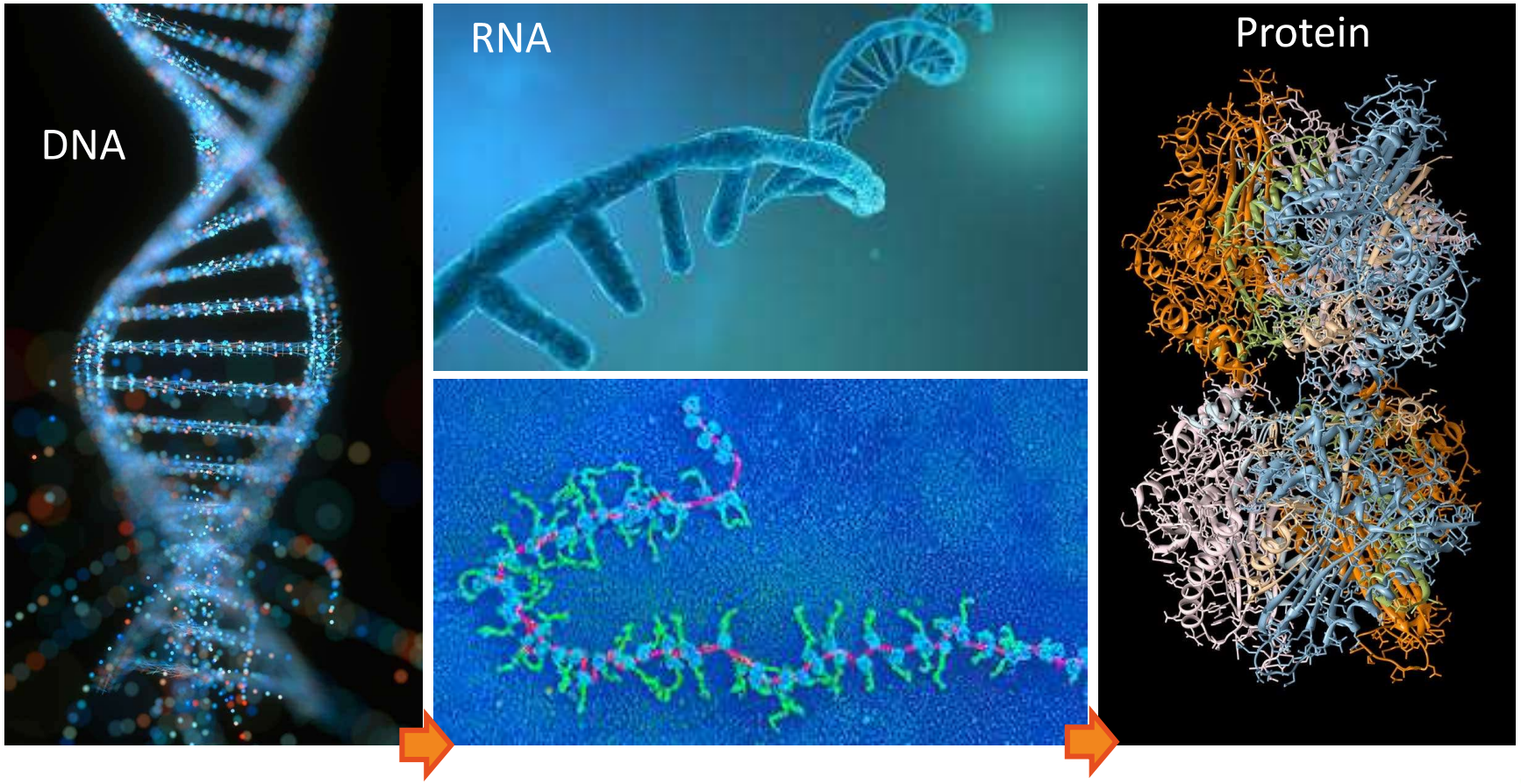
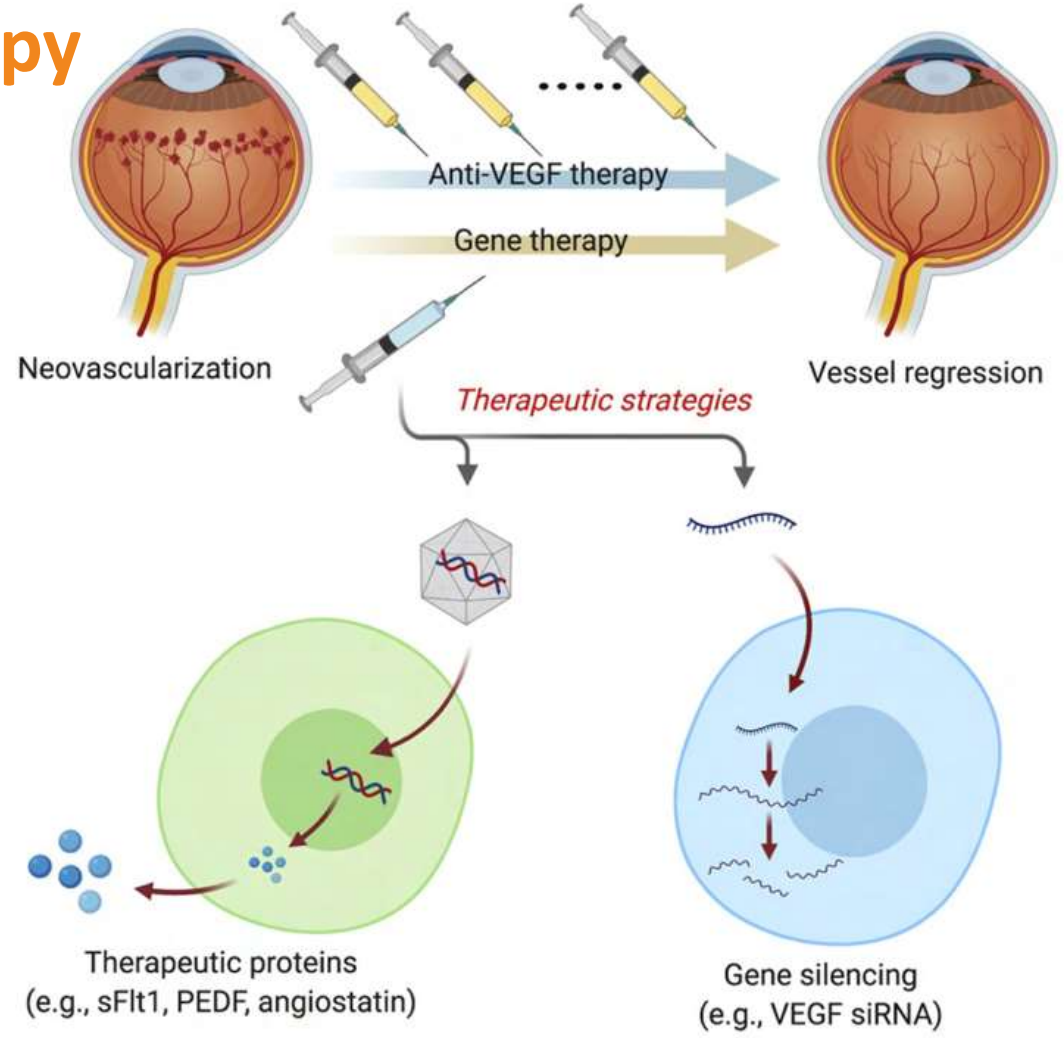
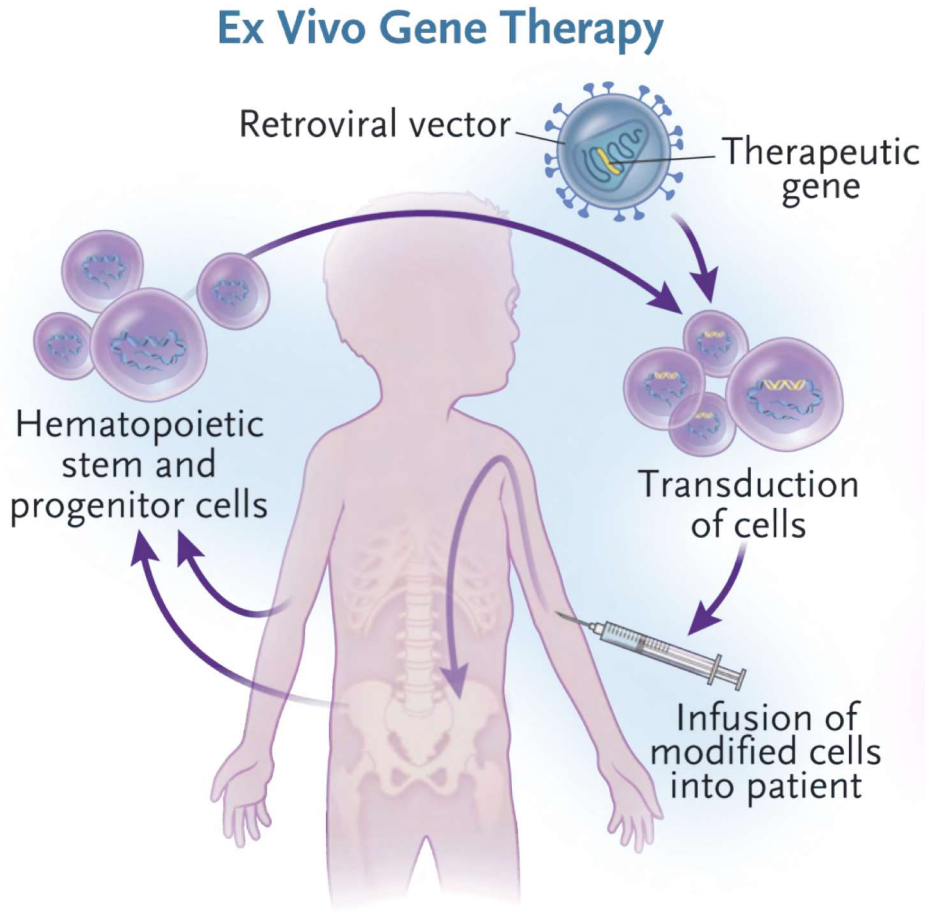
The central dogma and RNA interference

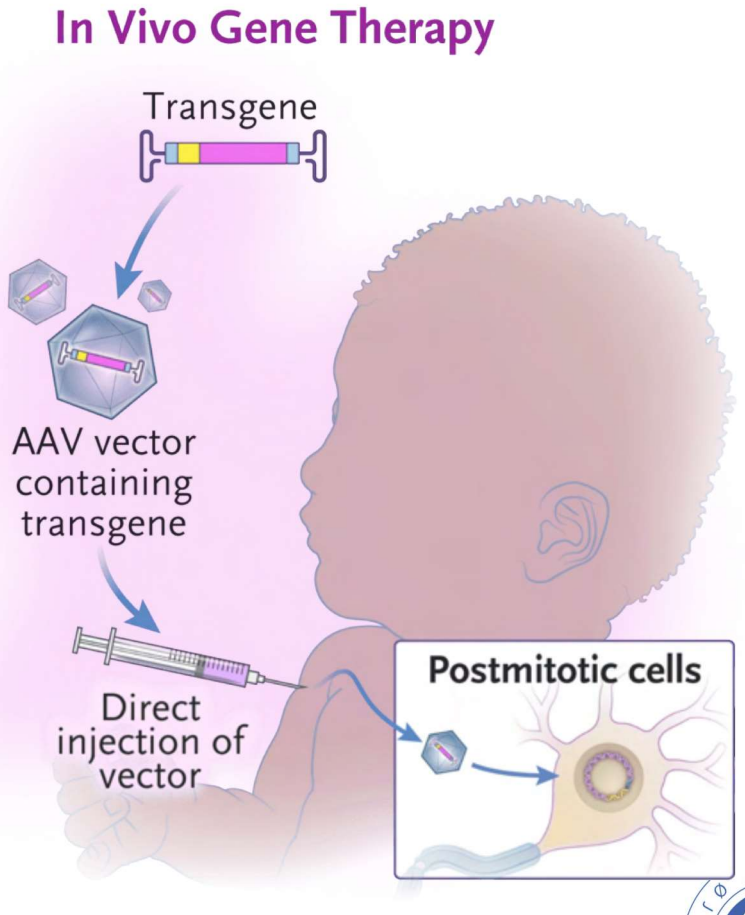


Gene Therapy



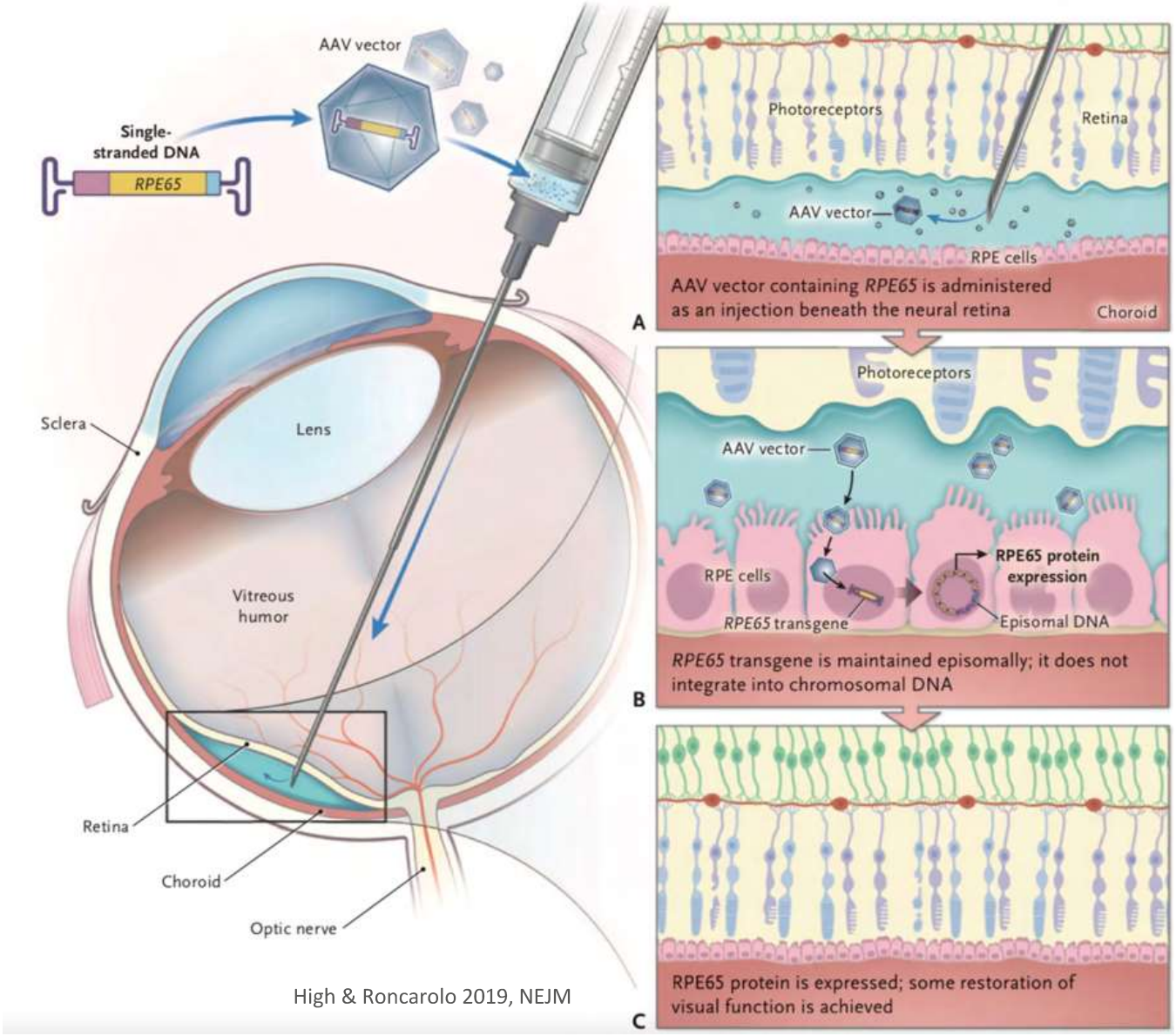
Gene Therapy





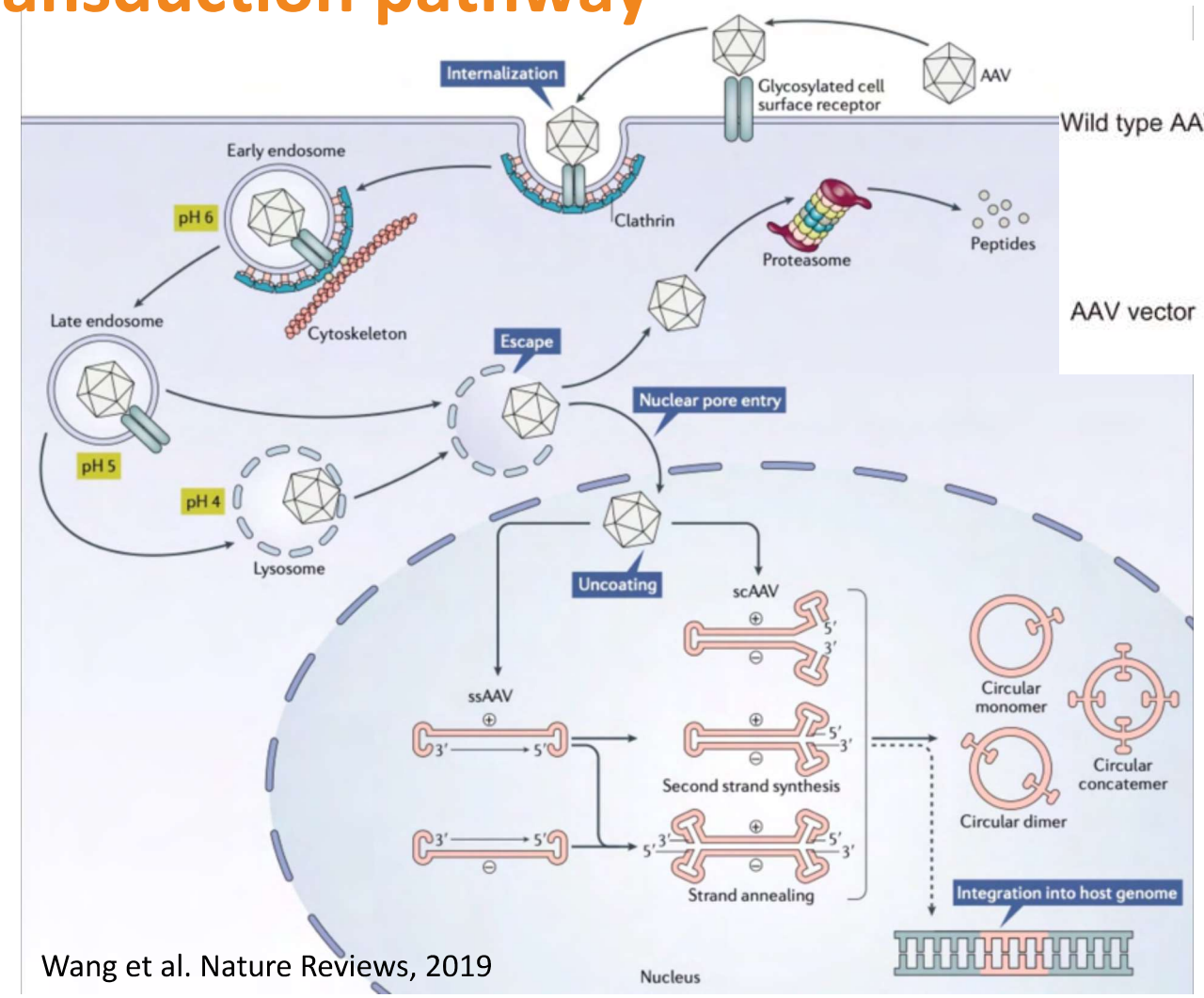
High & Roncarolo 2019, NEJM

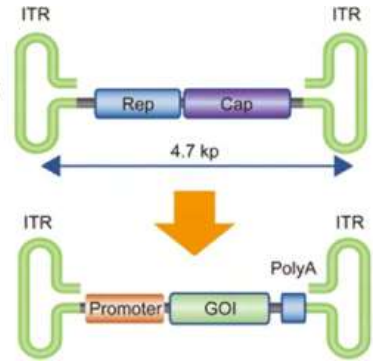
LCA2 Gene Therapy



High & Roncarolo 2019, NEJM

AAV transduction pathway

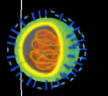


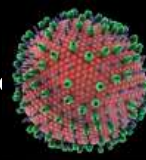


Wang et al. Nature Reviews, 2019

Vectors for Gene Therapy

	Virus	Expression	Genome	Packaging Capacity	Virus Size (nm)	Cells Infected	Target Cell Genome Integration	Immune Response
Bench & Clinic	Lentivirus	Stable	RNA	<8 kb	80-130	Dividing/Non-dividing	Yes	Low
Bench & Clinic	AAV	Transient or Stable*	Single stranded linear DNA**	≈4,5 kb	18-26	Dividing/Non-dividing	No*	Very Low
Bench	Adenovirus	Transient	Double stranded linear DNA*	>8 kb	105	Dividing/Non-dividing	No	High
Bench & Clinic	Gamma-Retrovirus	Stable	RNA	<8 kb	80-130	Dividing	Yes	Moderat

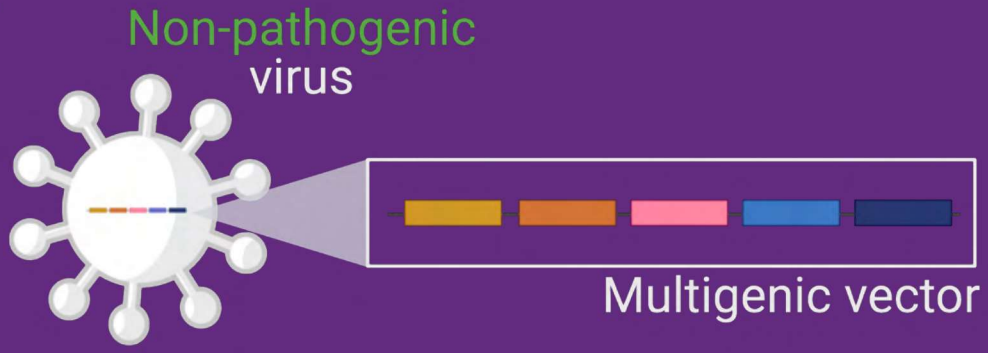


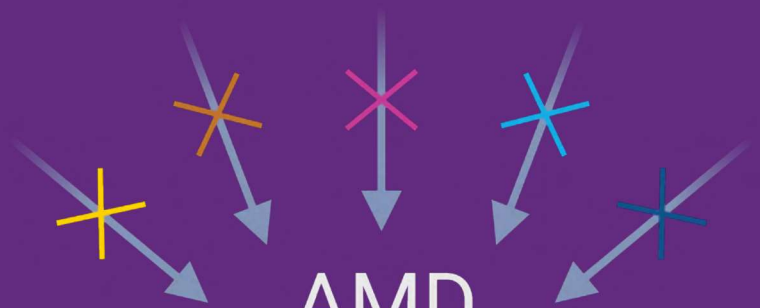


*Recombinant AAV has a low frequency of target cell genome integration
** AAV may also deliver double stranded DNA

addgene.org

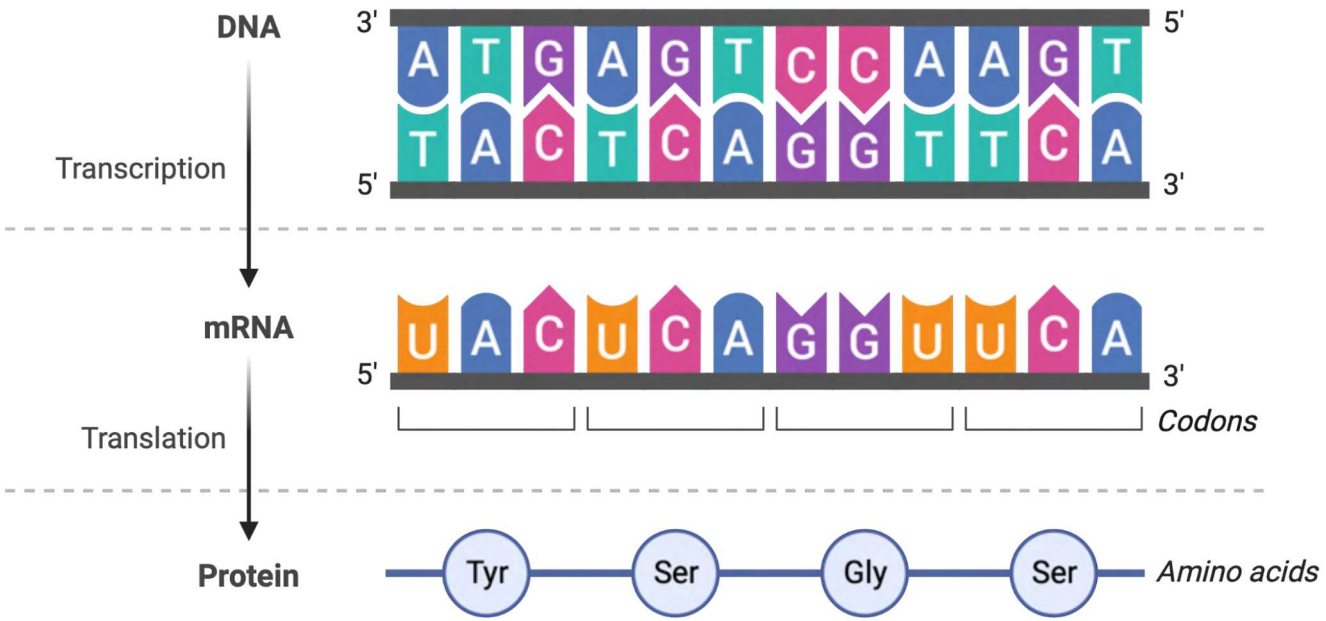
Our IDEA





... targets multiple pathways involved in AMD

RNA interference



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The central dogma and RNA interference

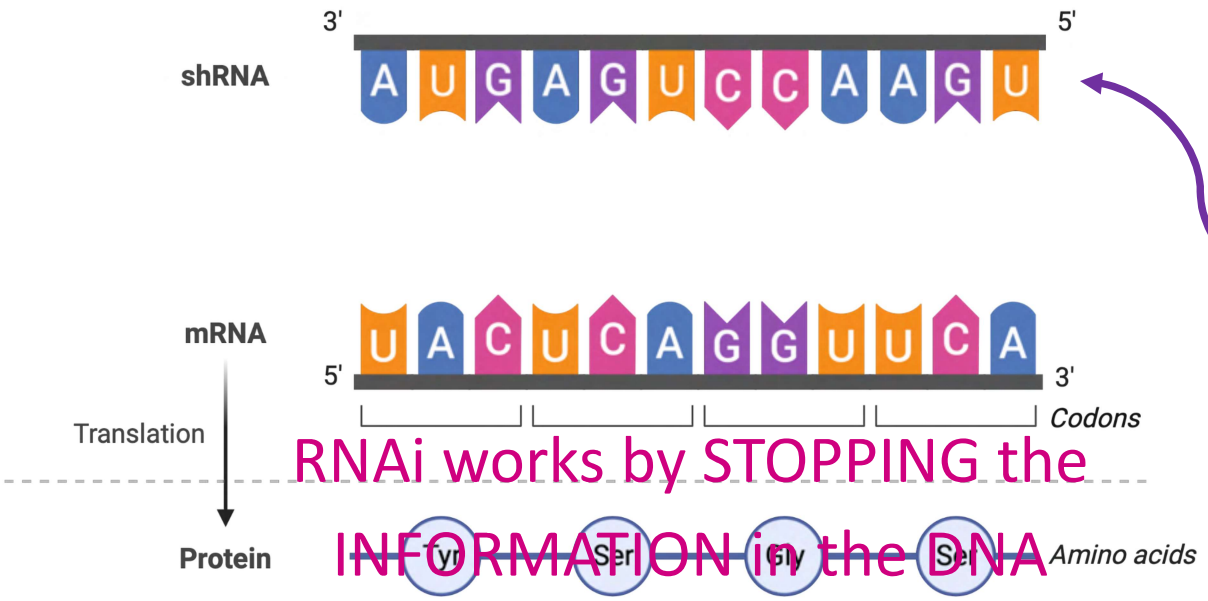
The Nobel Prize in Physiology or Medicine 2006

Photo: L. Cicero
Andrew Z. Fire
Prize share: 1/2

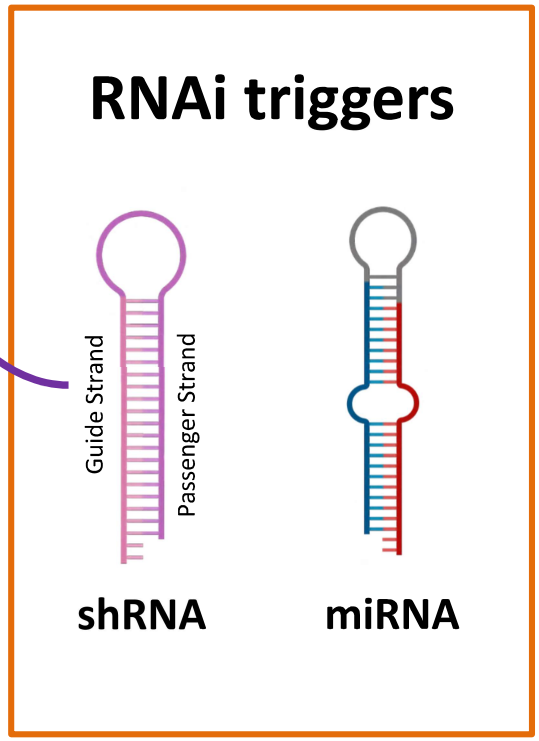
Photo: J. Mottern
Craig C. Mello
Prize share: 1/2

The Nobel Prize in Physiology or Medicine 2006 was awarded jointly to Andrew Z. Fire and Craig C. Mello *"for their discovery of RNA interference - gene silencing by double-stranded RNA"*

Effector Molecules of RNAi



RNAi works by STOPPING the INFORMATION in the DNA



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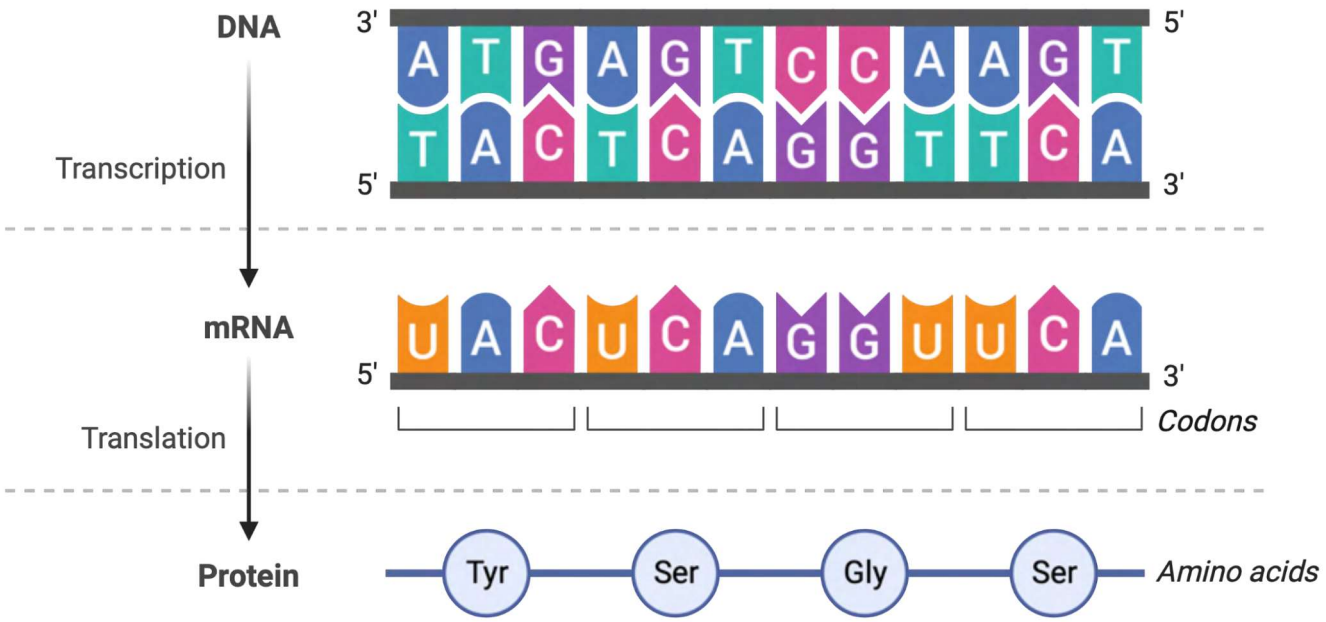
RNA interference



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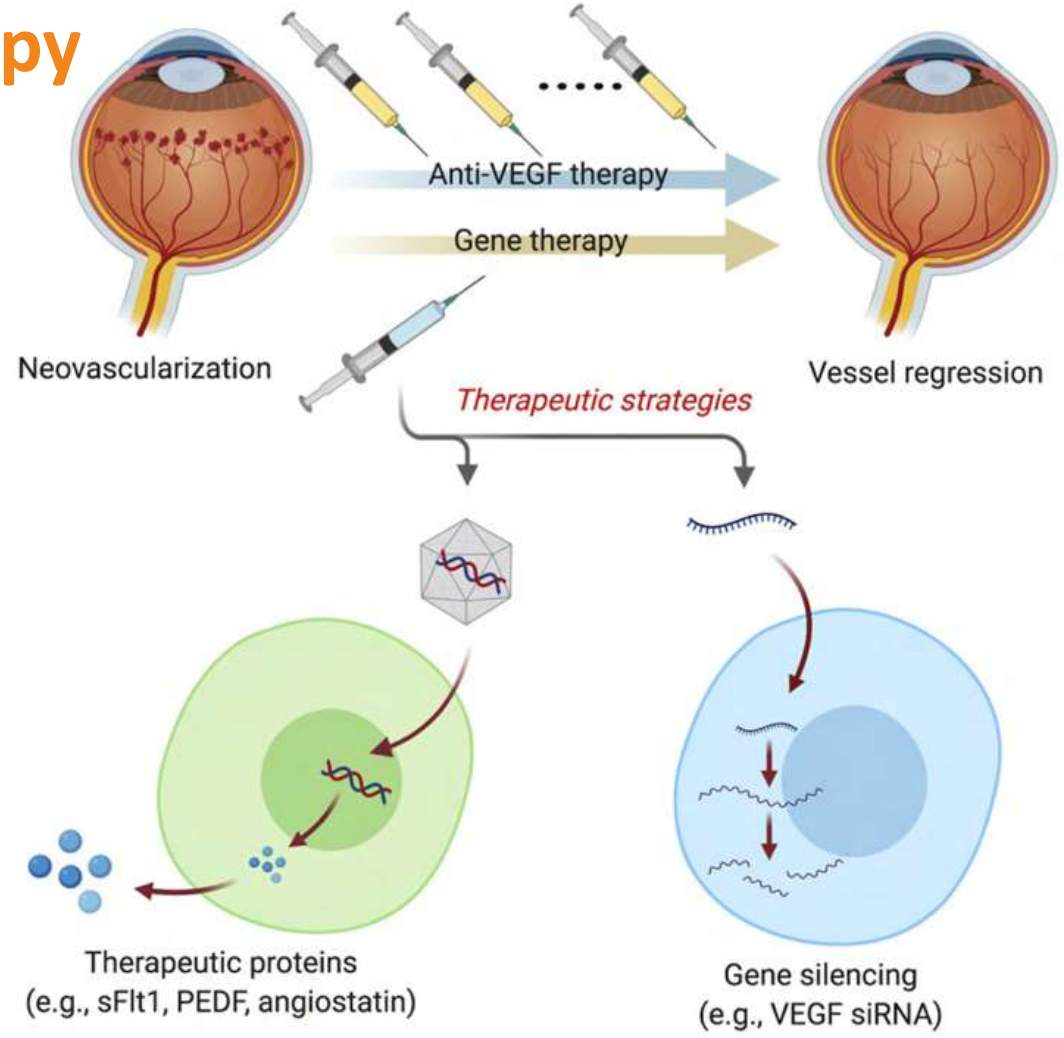
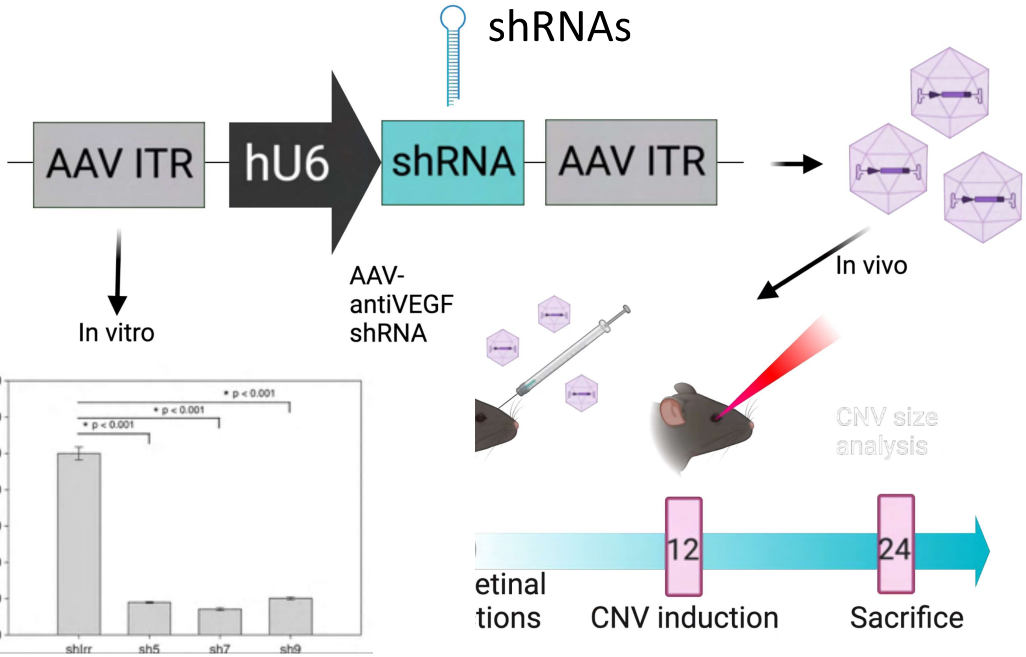
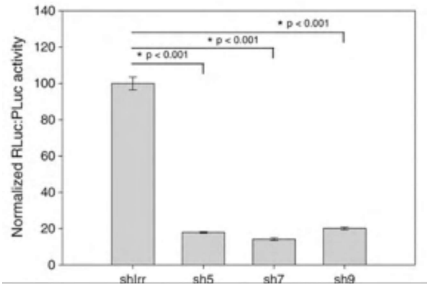
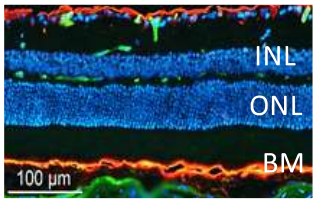
Gene Therapy



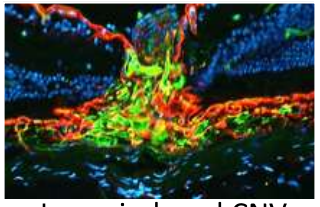
Anti-VEGF therapy by RNA interference



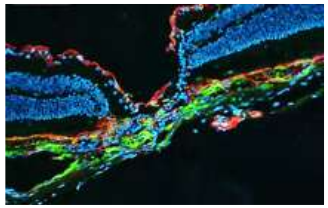




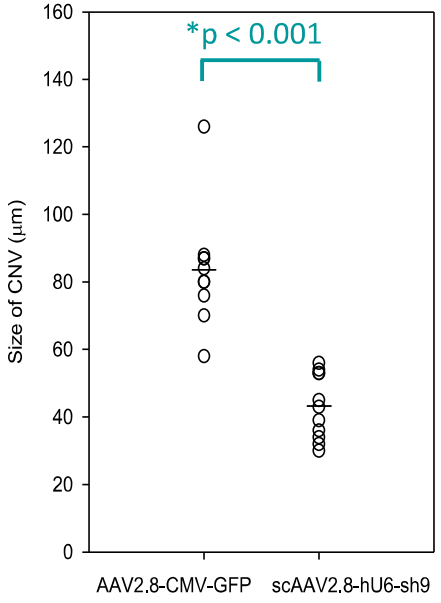
Normal retina



Laser-induced CNV



Laser-induced CNV
Anti-VEGF-sh9-treated



CONCLUSION

Reduction of CNV in mice by AAV-delivered anti-VEGF short hairpin RNA

Askou & Corydon
JGM

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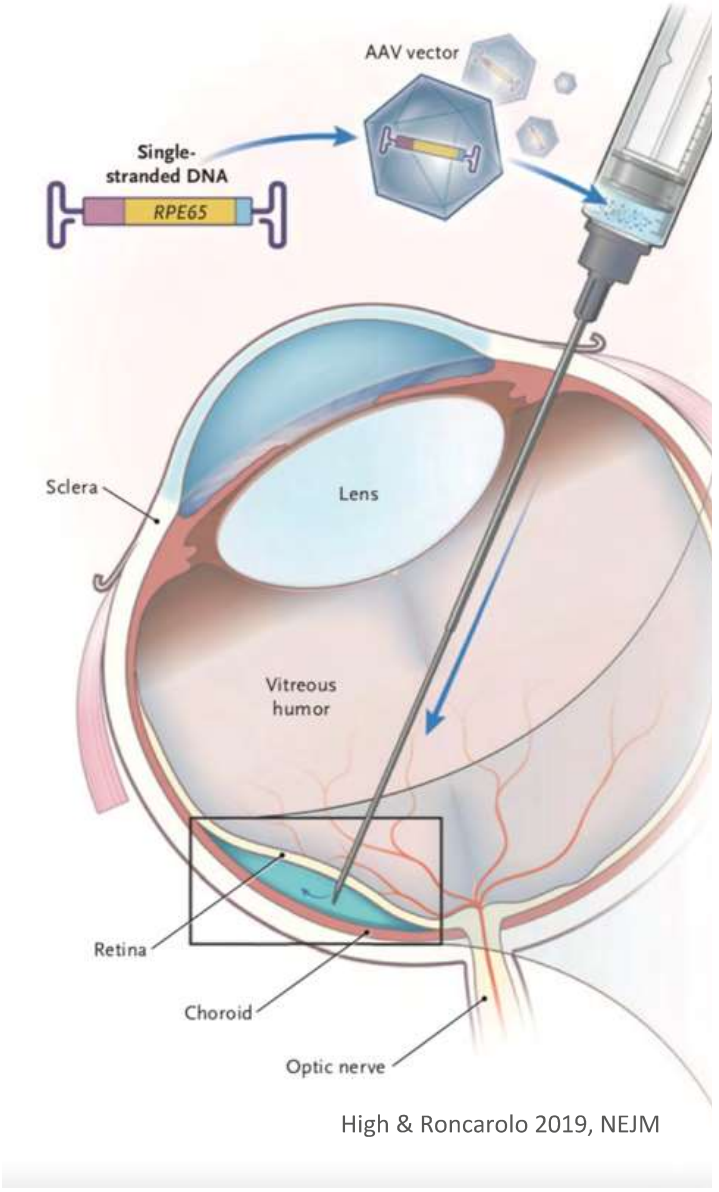
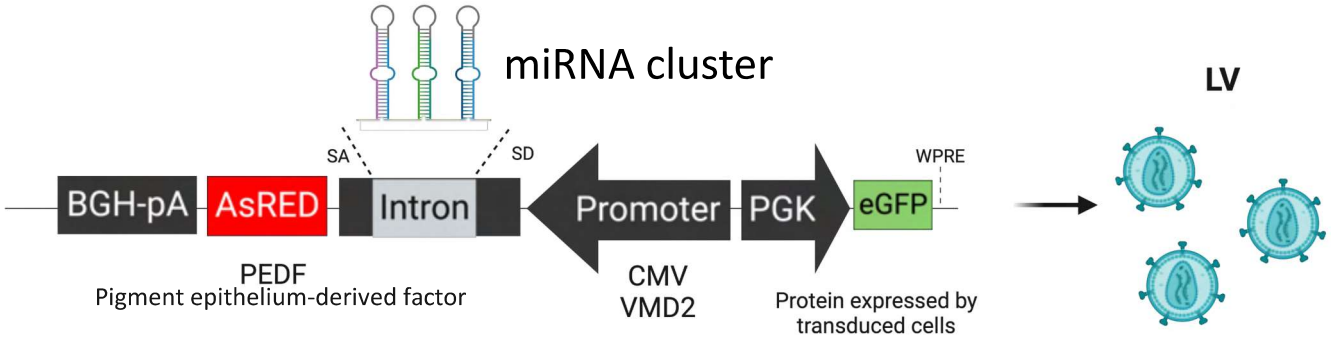
Delivery
Subretinal injection in mice

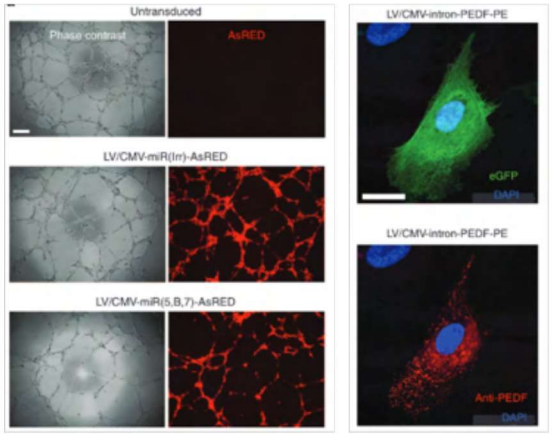


High & Roncarolo 2019, NEJM

Tissue-specific therapy using LVs

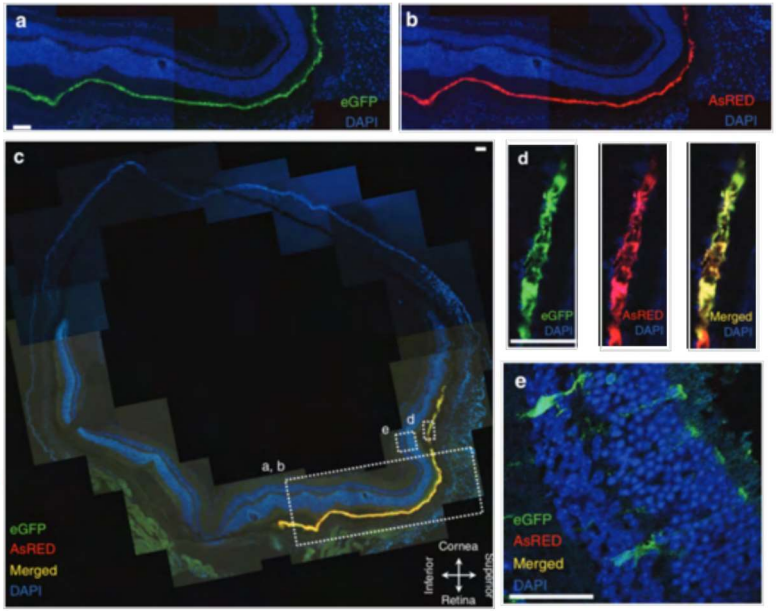


In vitro



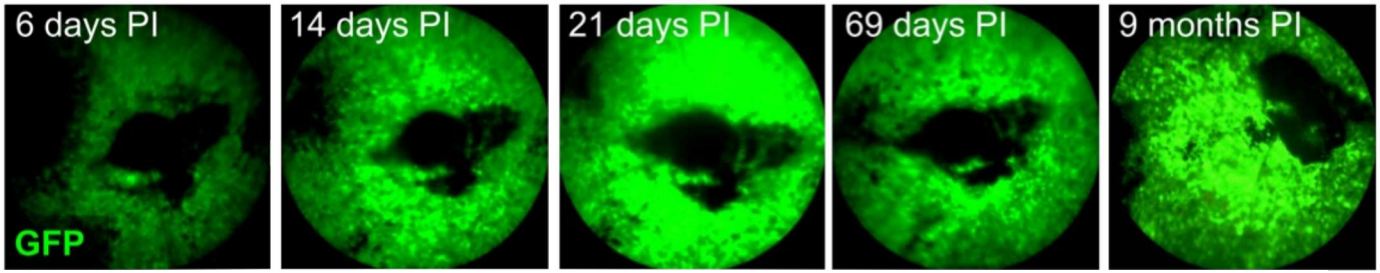
HUVECs

In vivo



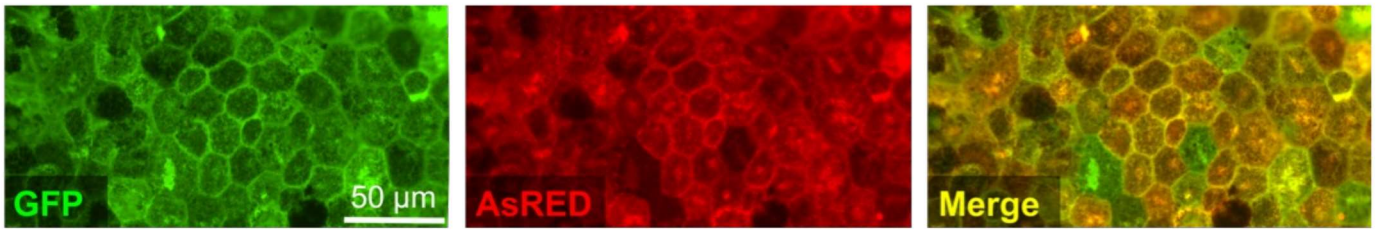
Askou and Corydon
Mol Ther Methods Clin Dev

Durability



Long-term

Widespread

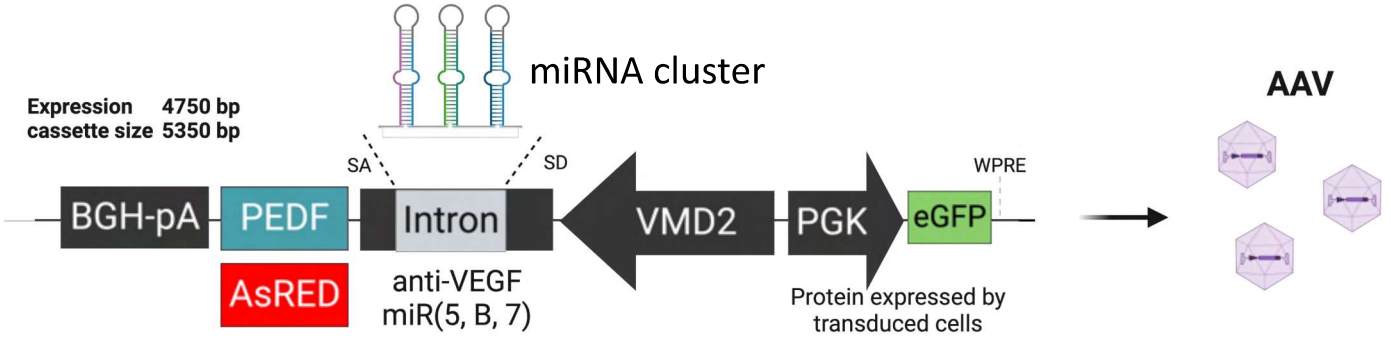


Specific

Askou and Corydon
HGT Methods

Like going to the Moon…

Multigenic AAV-based gene therapy



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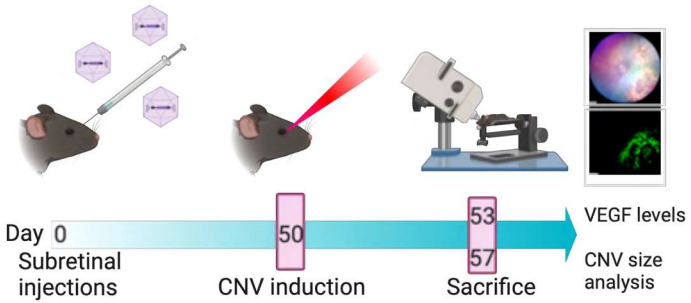
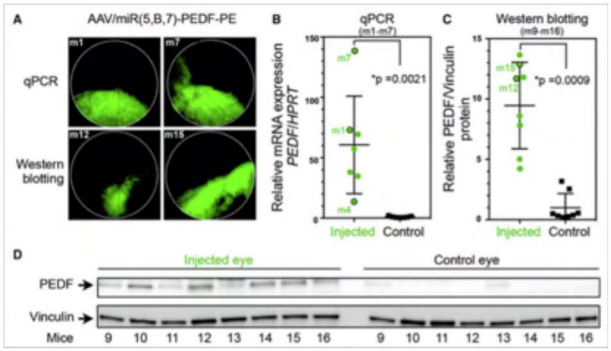
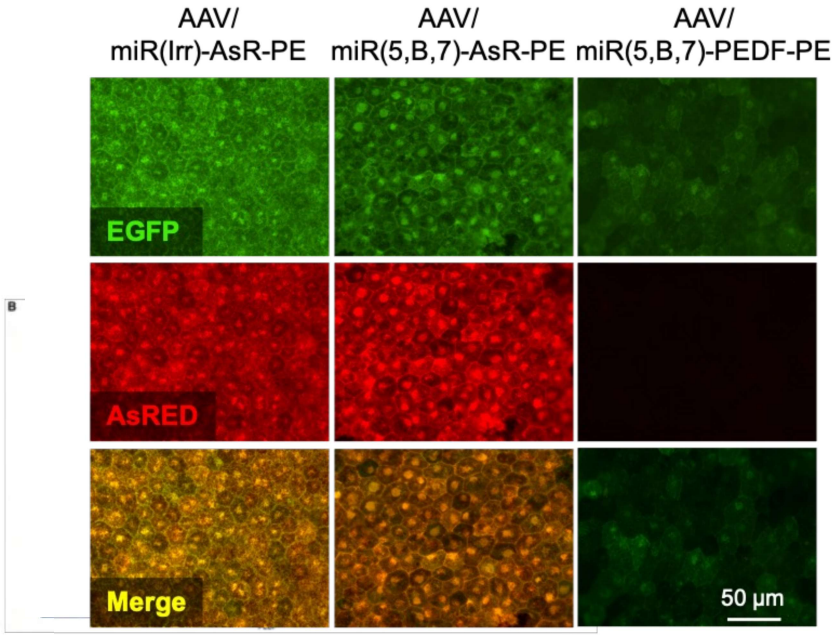






Askou and Corydon
MTNA 2019

Multigenic gene therapy

In vivo efficacy

Day 0
Subretinal injection of AAV/miR(Irr/5,B,7)-AsR/PEDF-PE

Day 50
Fundo-scopy

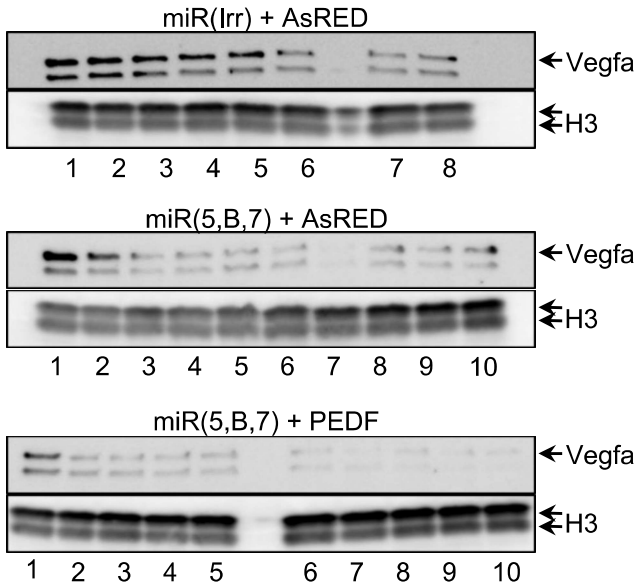
Day 50
Laser-Induced CNV

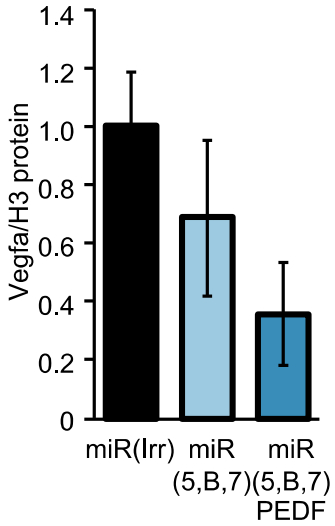
Day 53/57
WB/CNV analysis in RPE flat-mounts

CONCLUSION

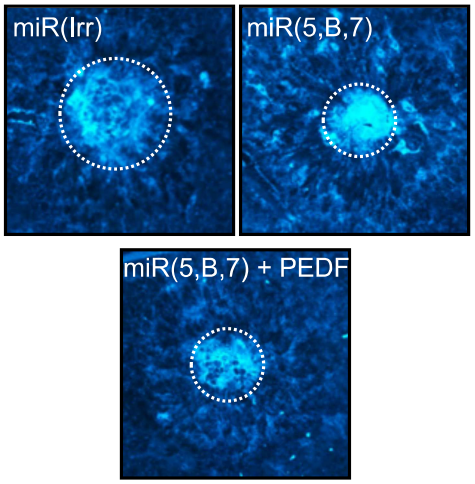
Gene therapy targeting VEGF via multigenic AAV vectors displays combined efficacy

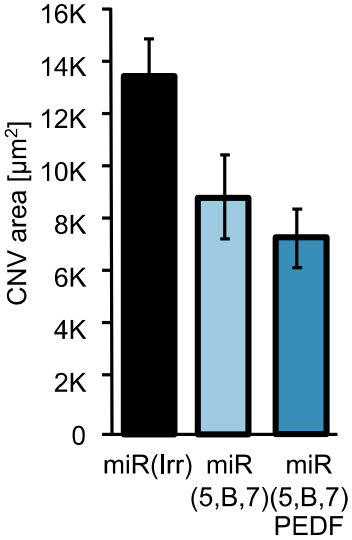
Vegfa KD





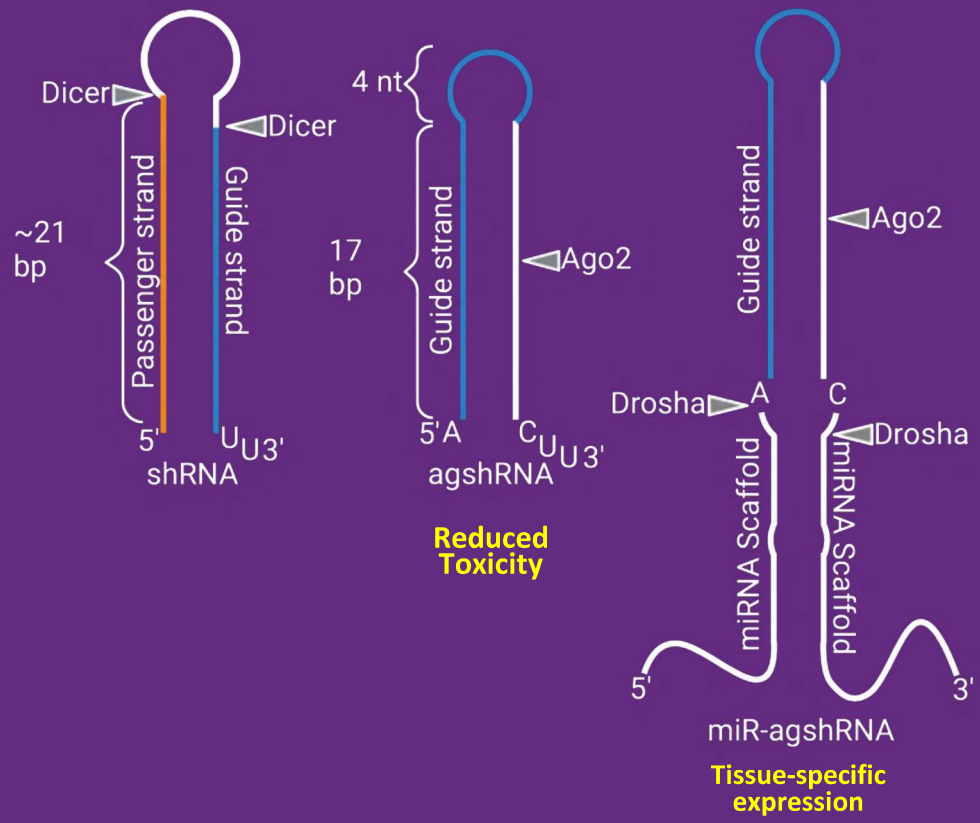
CNV reduction





Askou and Corydon
MTNA 2019

Right on target. The next class of efficient, safe, and specific RNAi triggers



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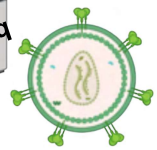
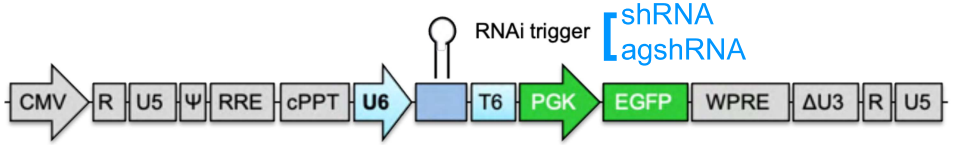
The next class of efficient, safe, and specific RNAi trigger

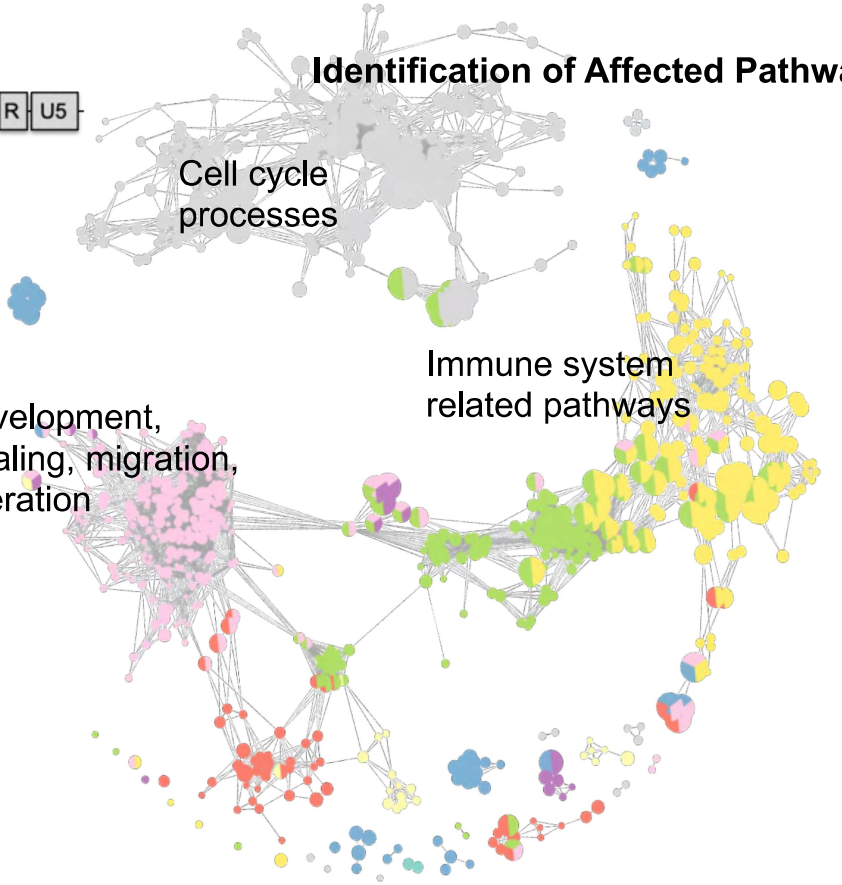




ARPE19

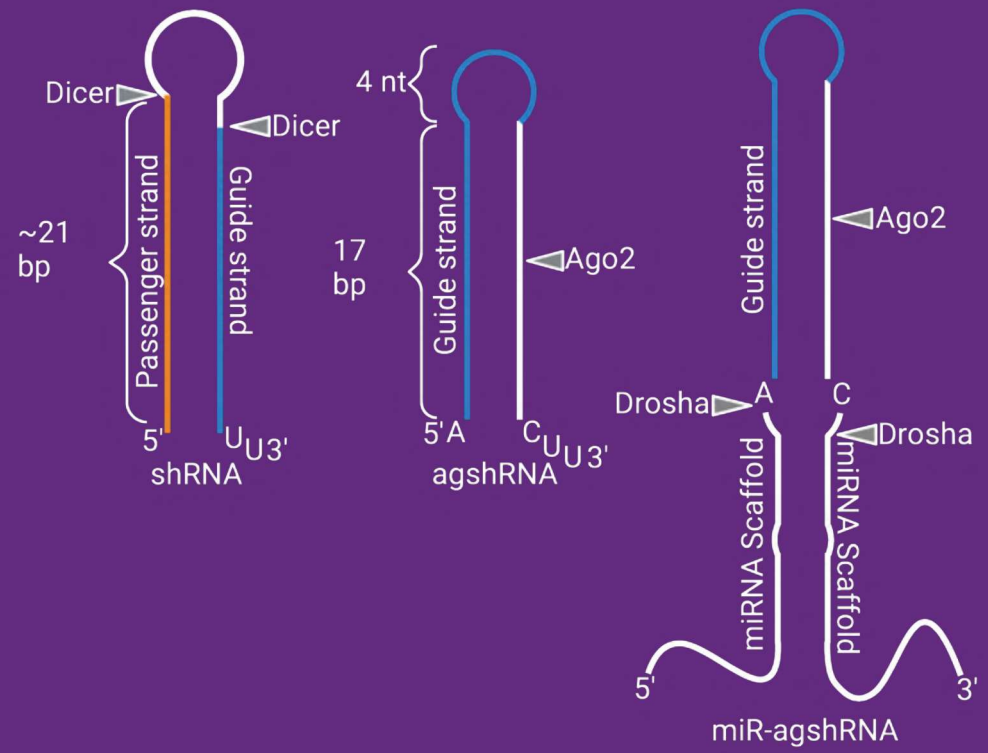
Self Organizing Maps

Identification of Affected Pathways



Alsing & Corydon 2022
MTNA

Right on target. The next class of efficient, safe, and specific RNAi triggers



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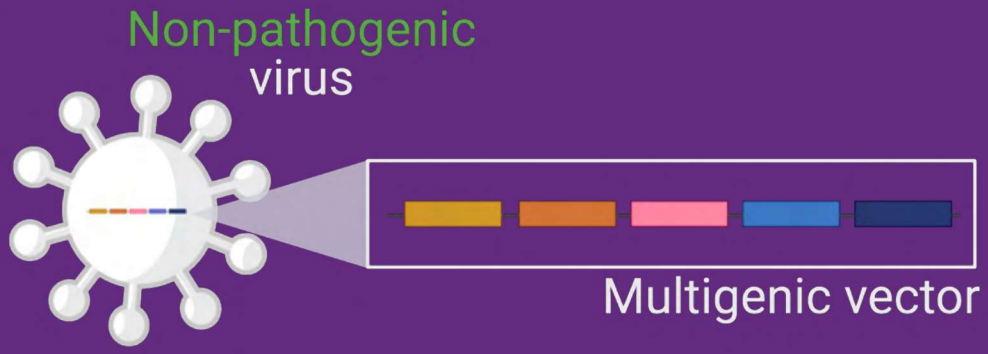
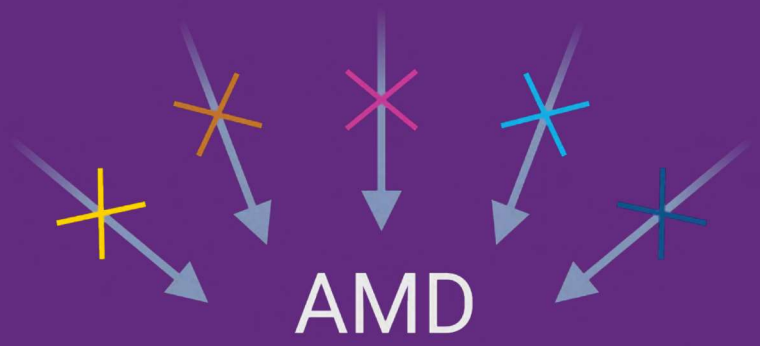
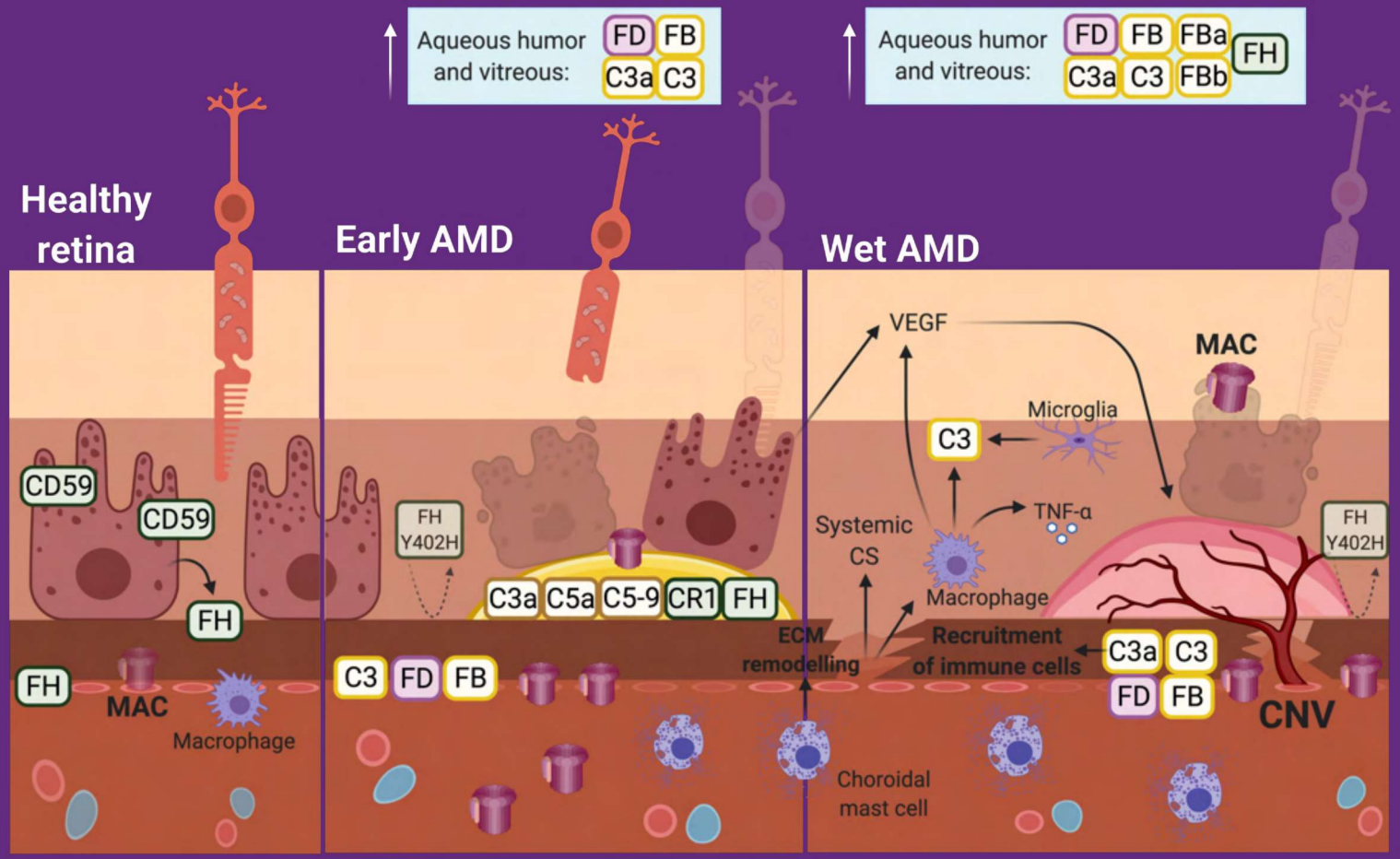
Our IDEA





... targets multiple pathways involved in AMD

Patient samples reveal increased complement deposition in age-related maculopathy and AMD eyes



Jensen…, & Corydon 2020 IJMS

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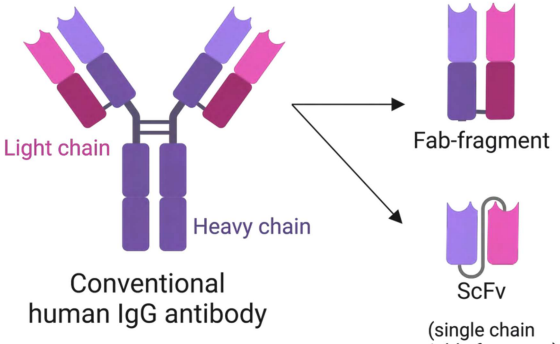
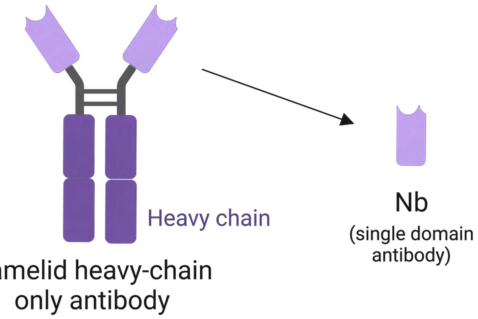
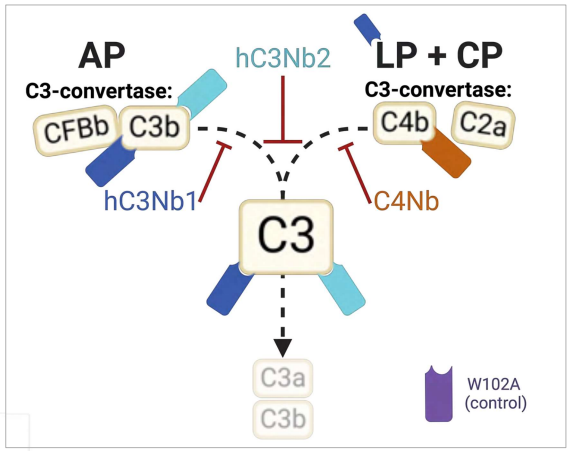
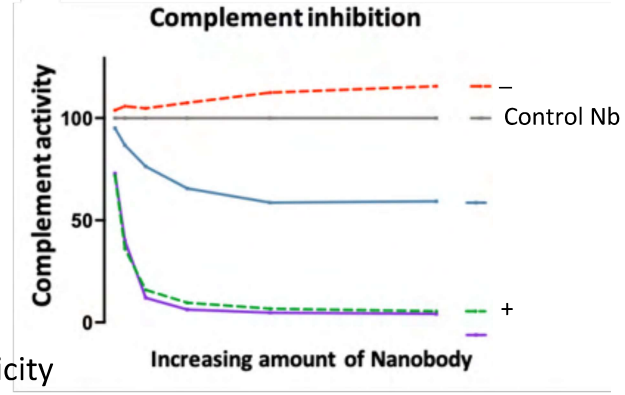
Novel Complement Inhibitors for suppression of VEGF





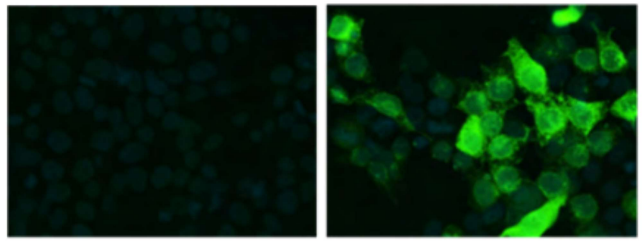
High affinity and specificity



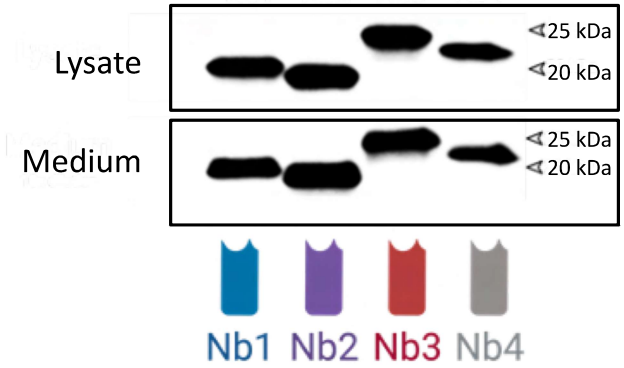


Jensen & Corydon
Unpublished

Nb1 expression in vitro



HEK293



Jensen…, & Corydon
2024 Unpublished

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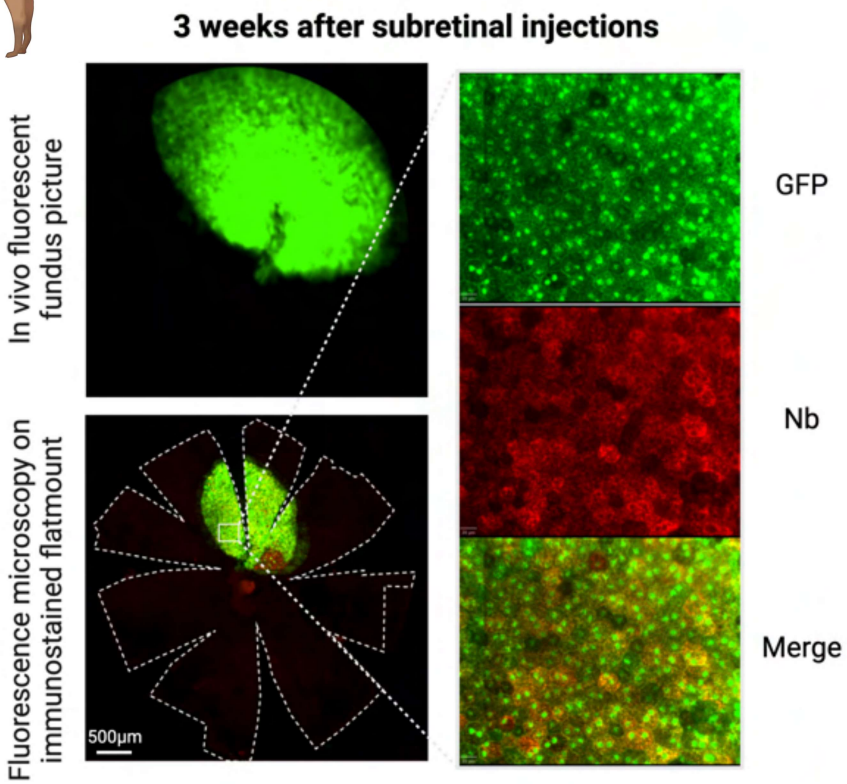
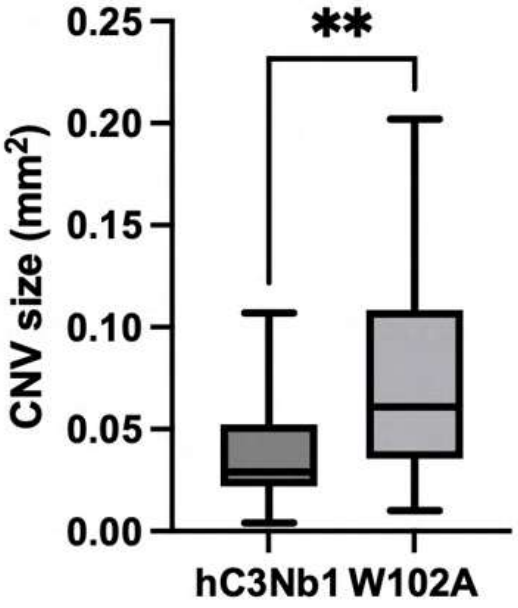
Novel Complement Inhibitors for suppression of VEGF





Jensen…, & Corydon
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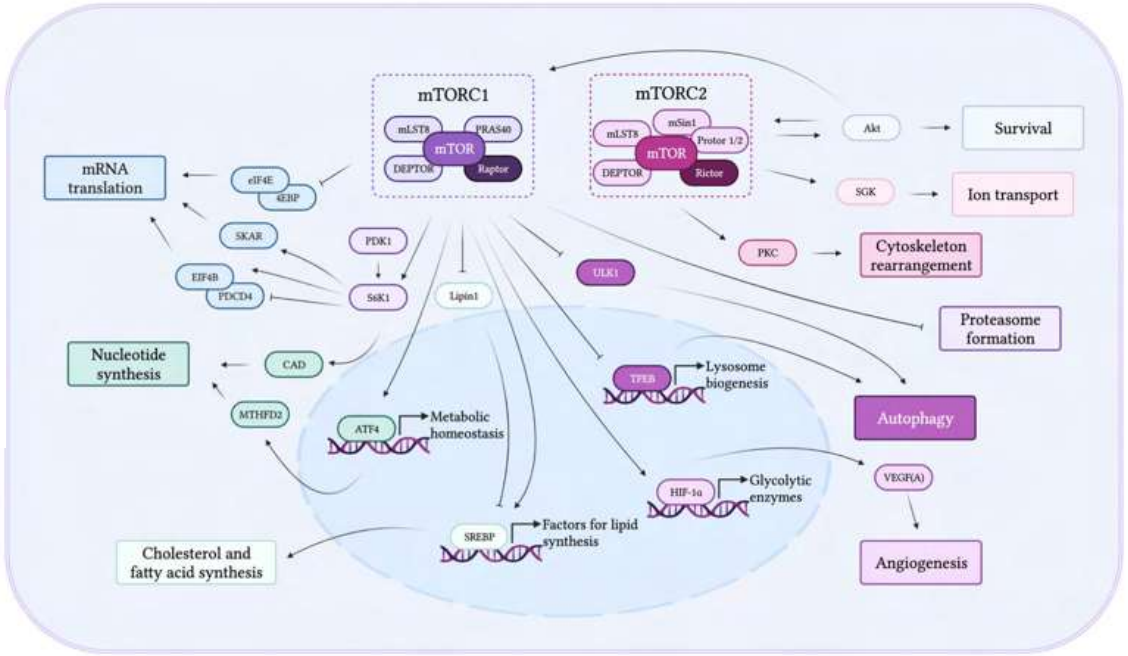
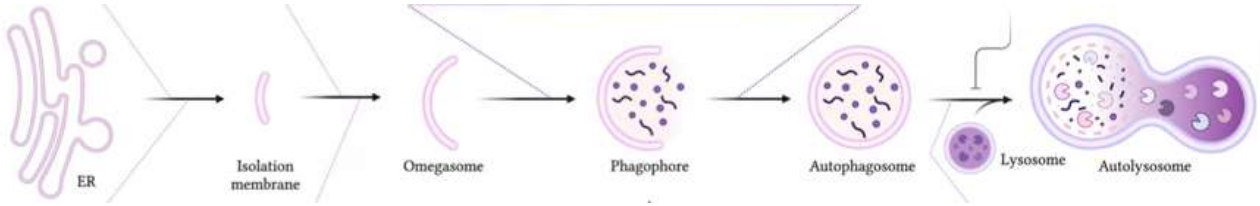
Autophagy





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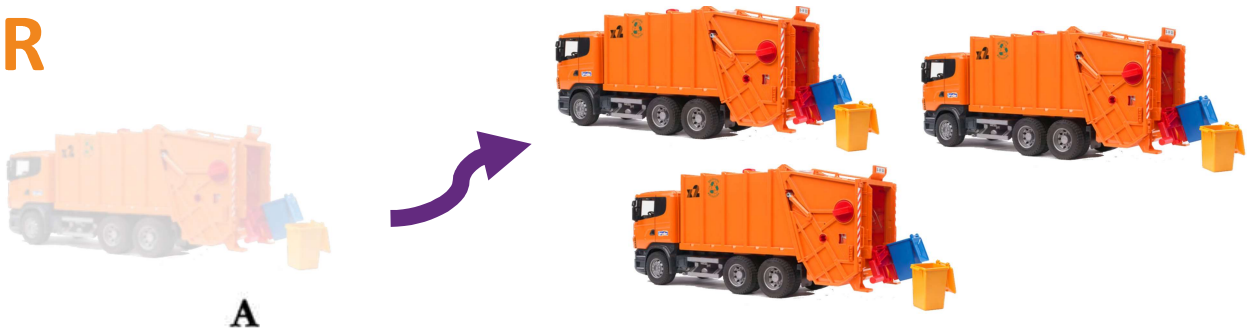
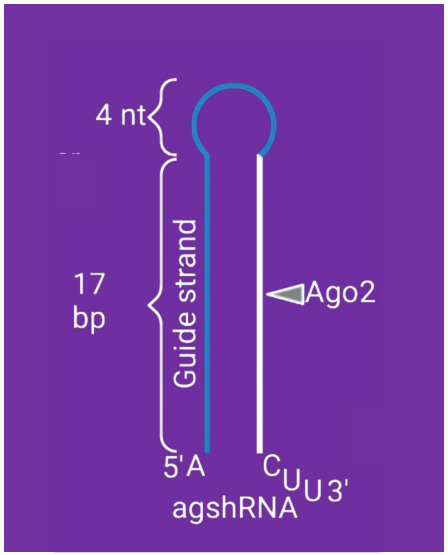
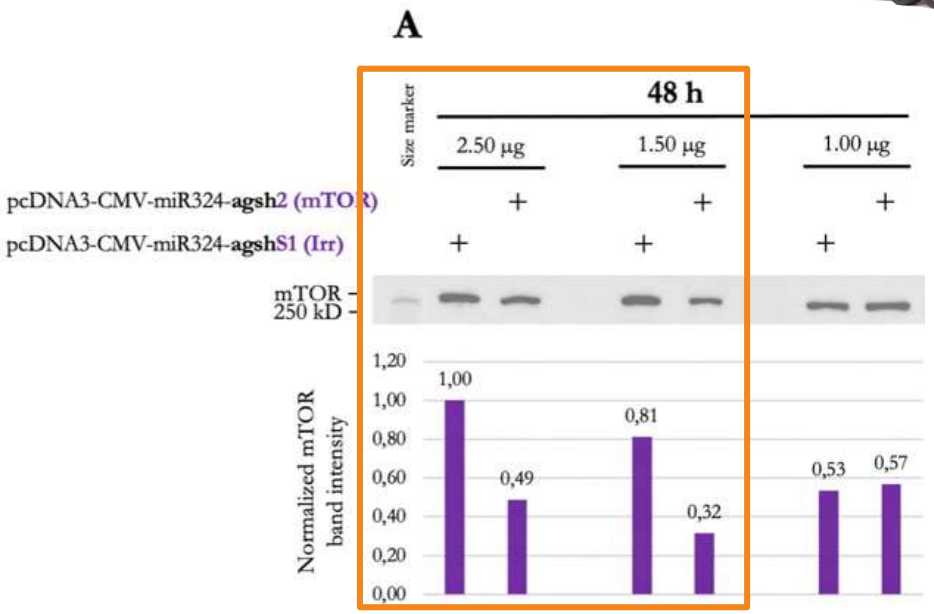
Knock-down of mTOR





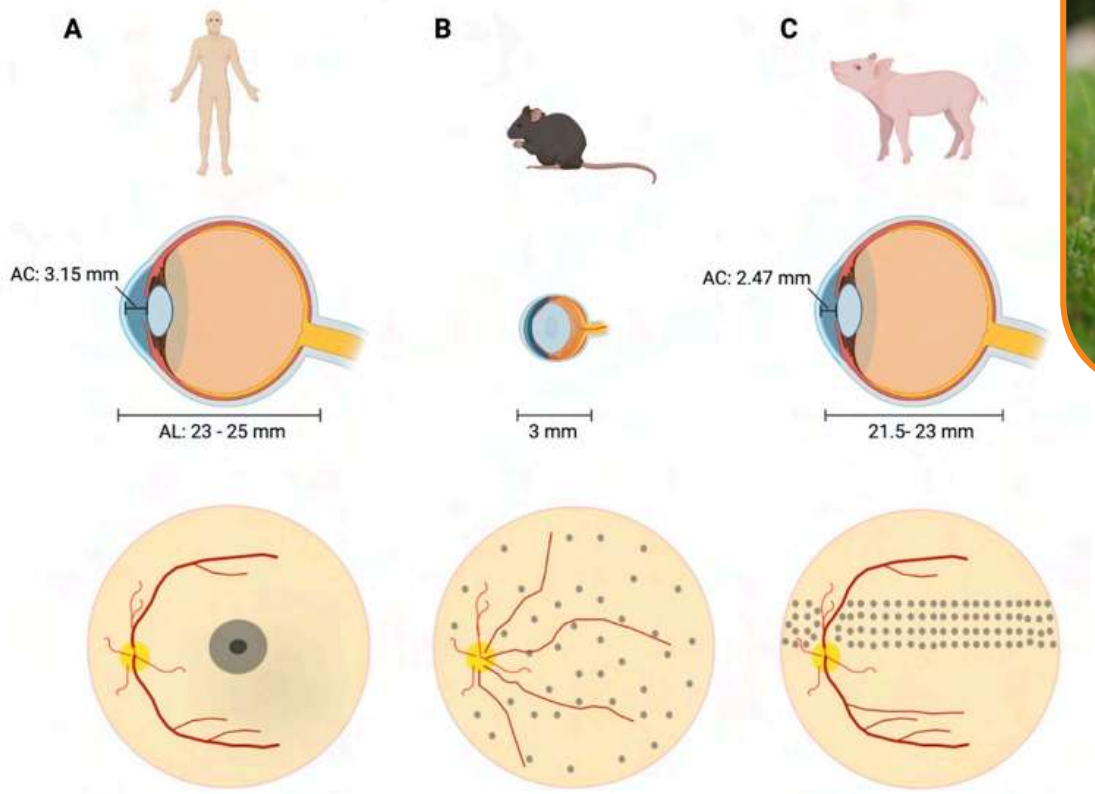


Køllner Bjerre & Corydon, unpublished 2024

Gene Therapy in CNV Pig Model

Hansen…Corydon, Unpublished 2024

Gene Therapy in CNV Pig Model



MD Silja Hansen, PhD dissertation 2023

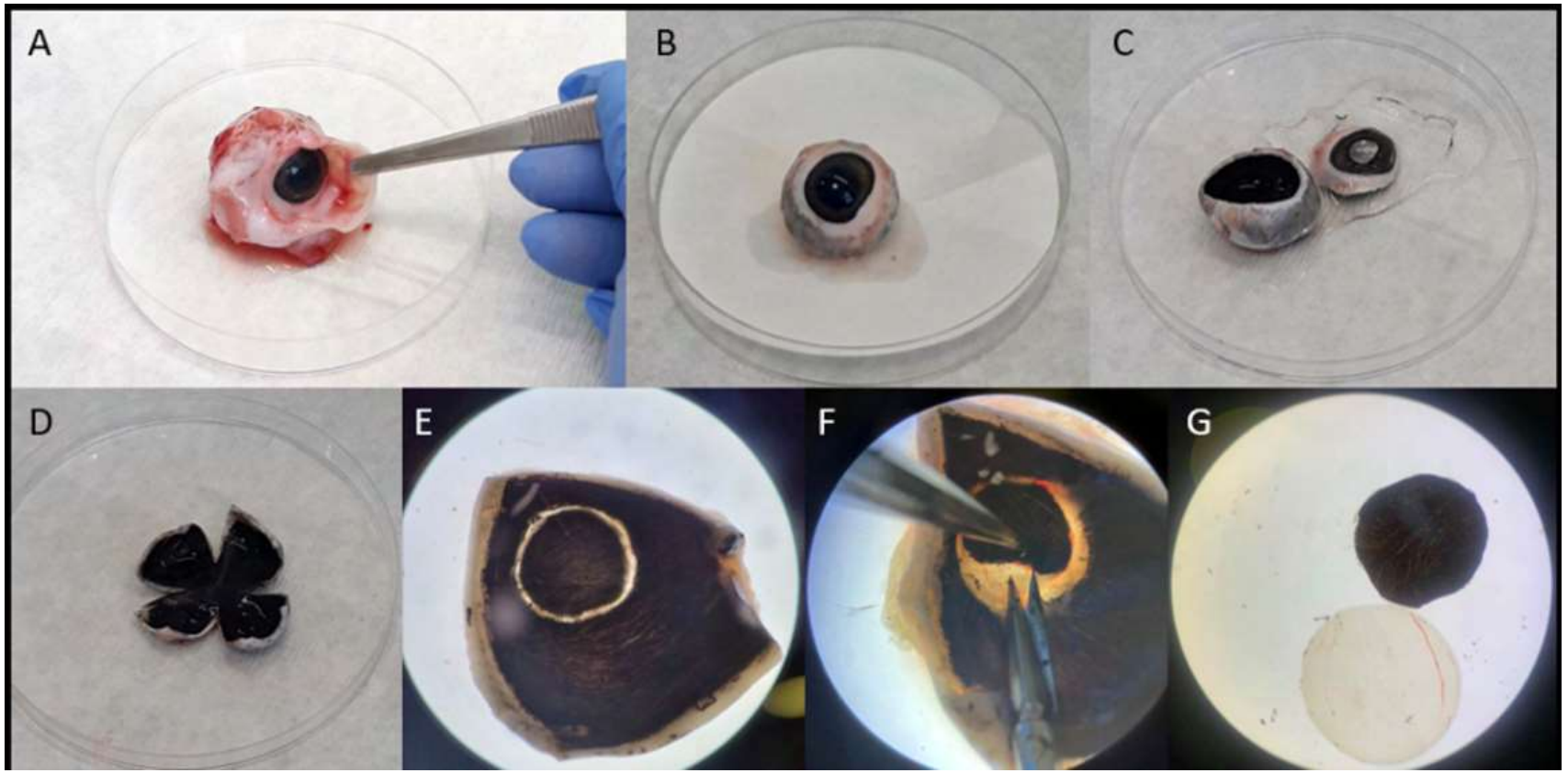
Gene Therapy in CNV Pig Model

Hansen…Corydon, Unpublished 2024

TV2: Vores vilde hospital

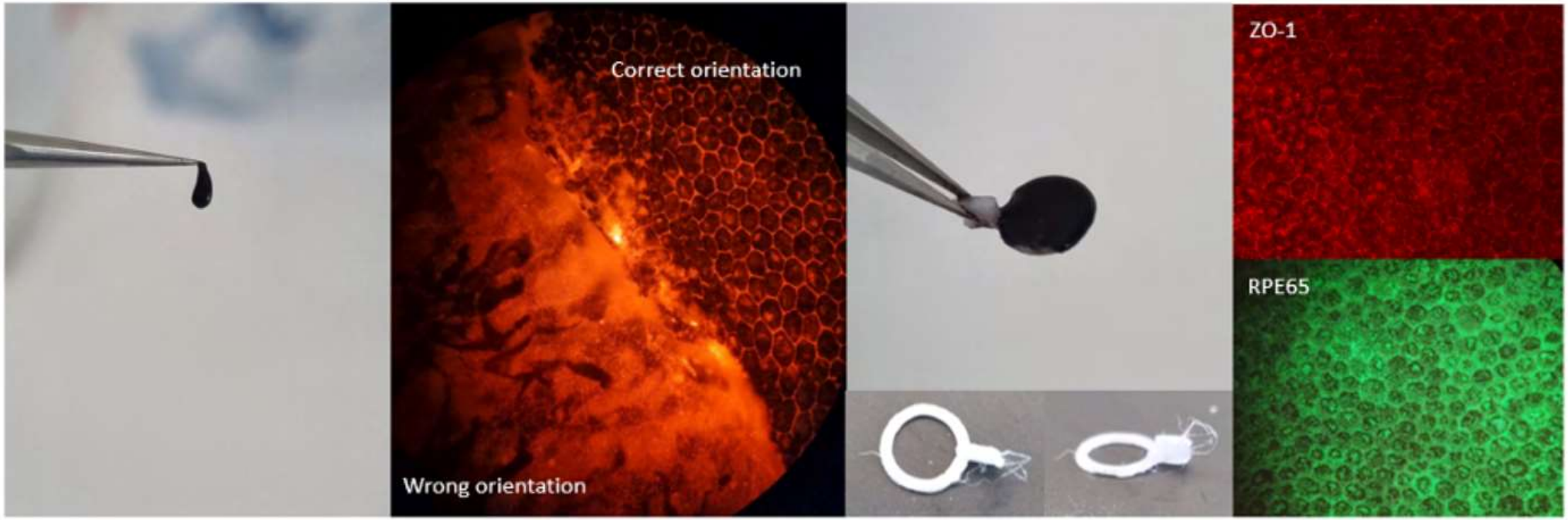
Validation in Human Retinal Explants

Training in progress using pig eyes



Bøgh, Askou, and Corydon
Unpublished 2024

Pig Retinal Explants



Bøgh, Askou, and Corydon
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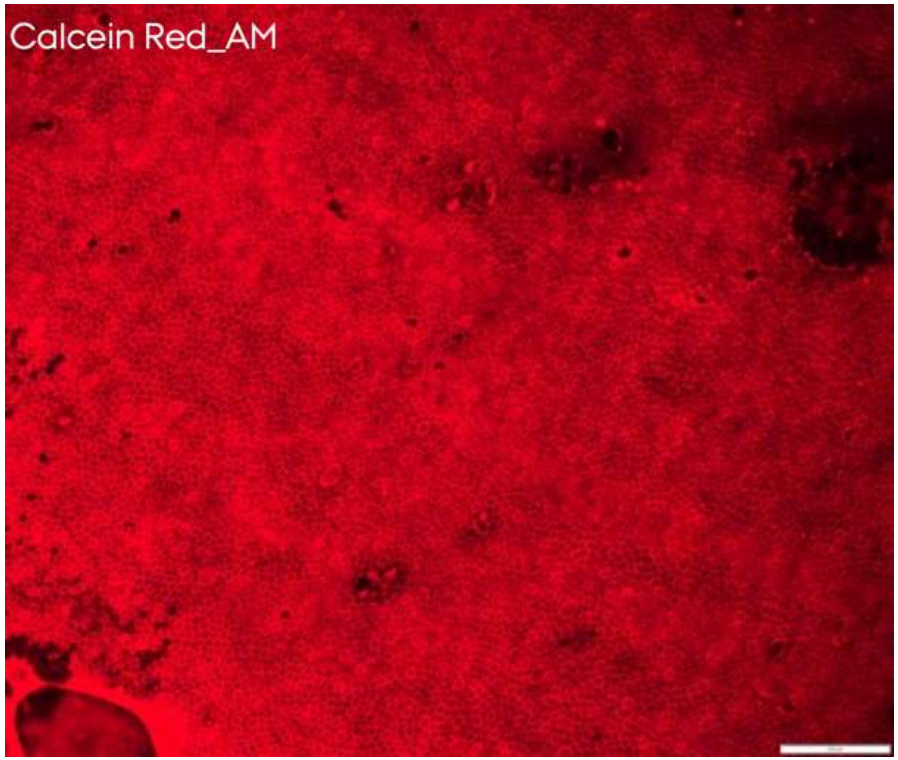
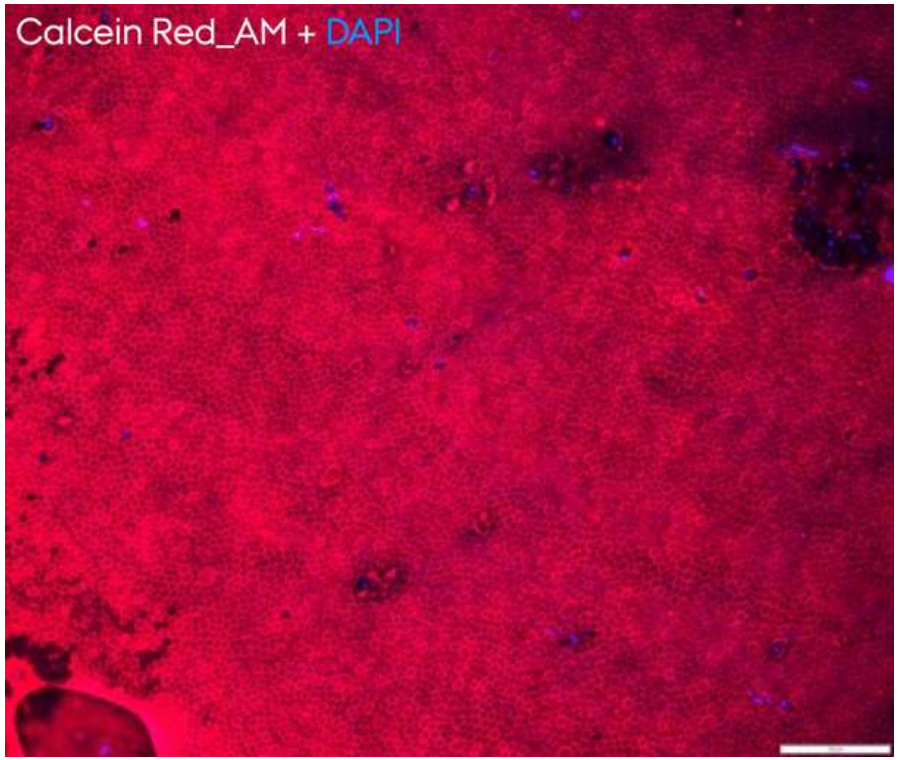
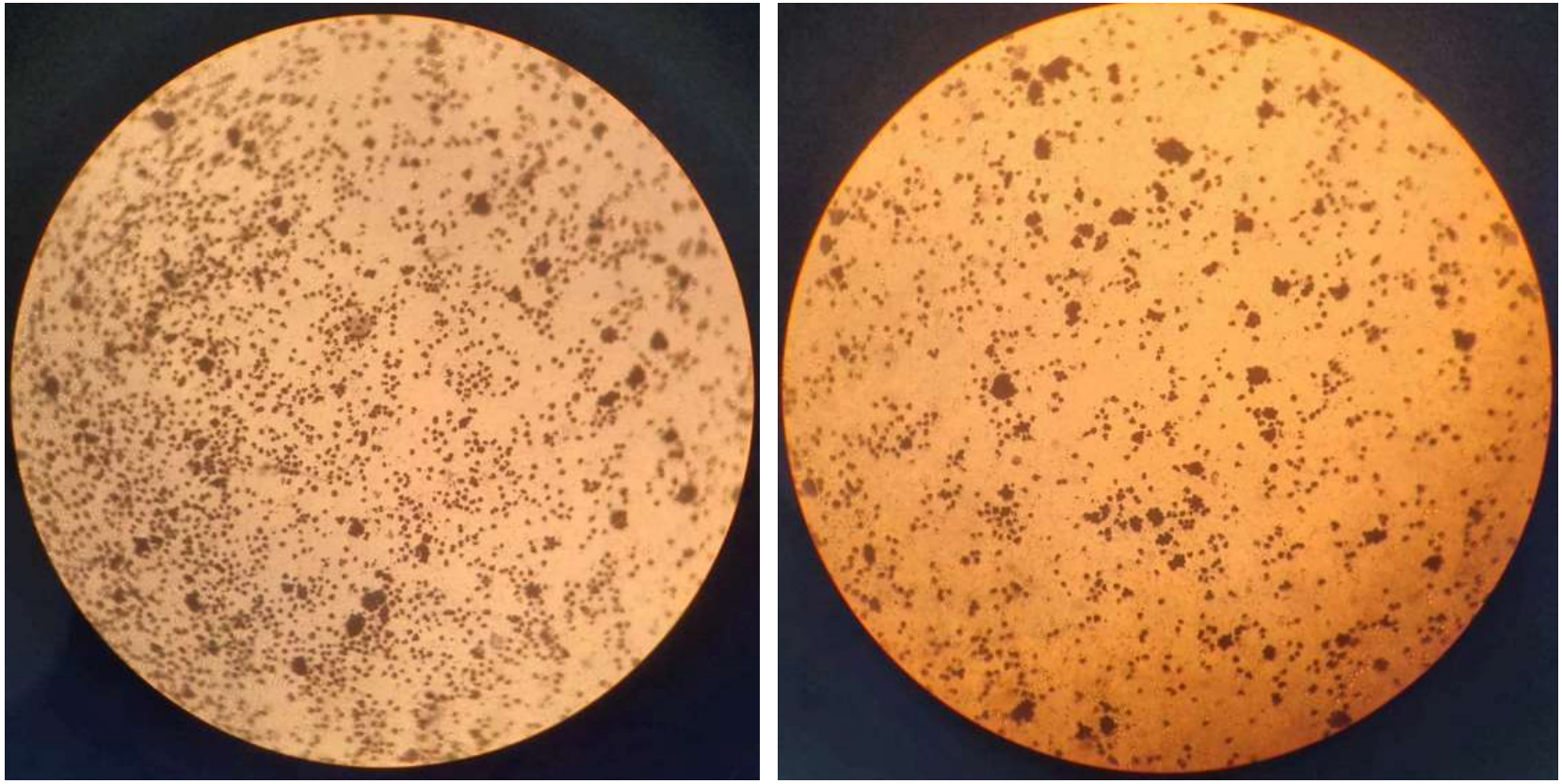


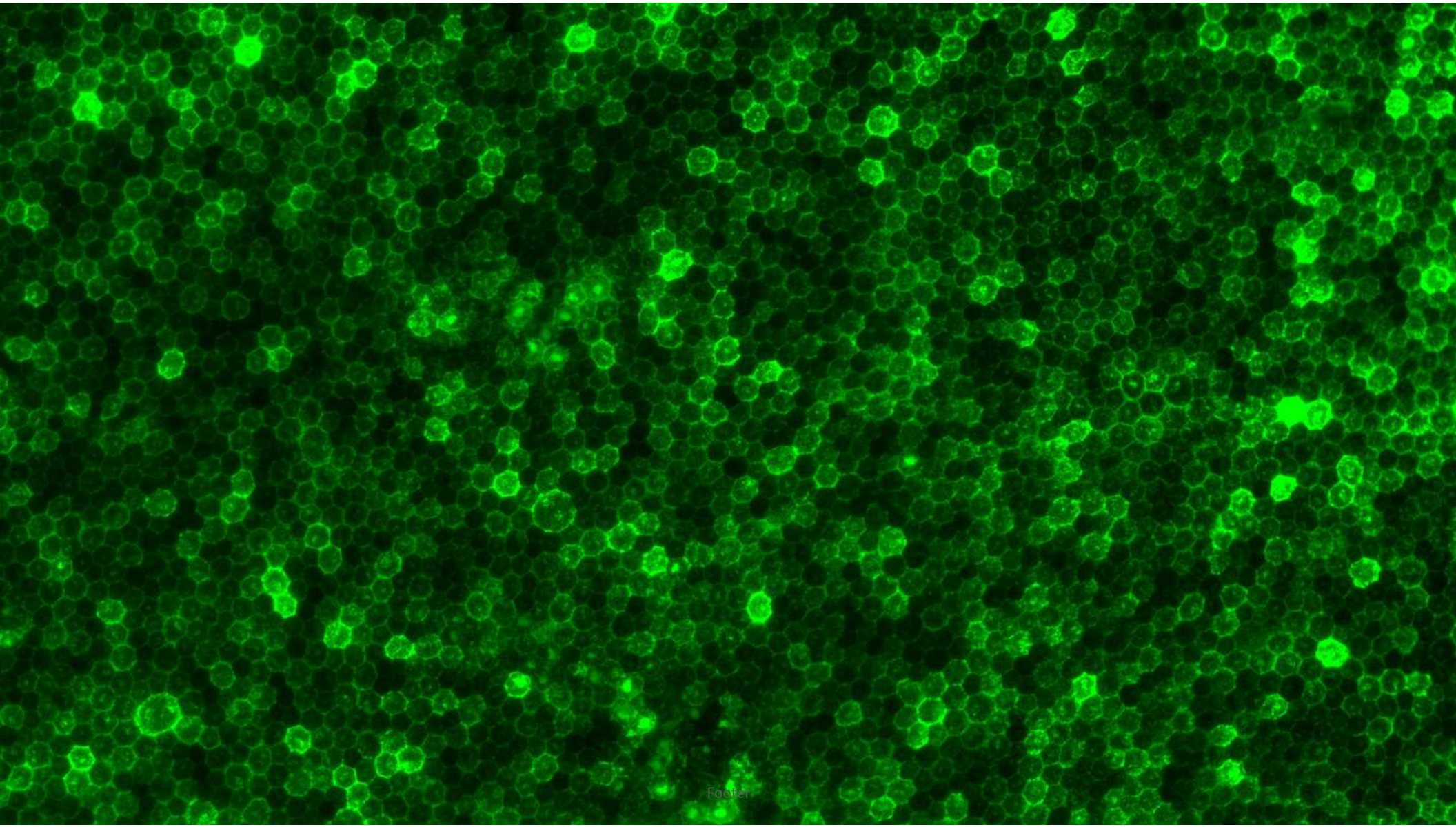


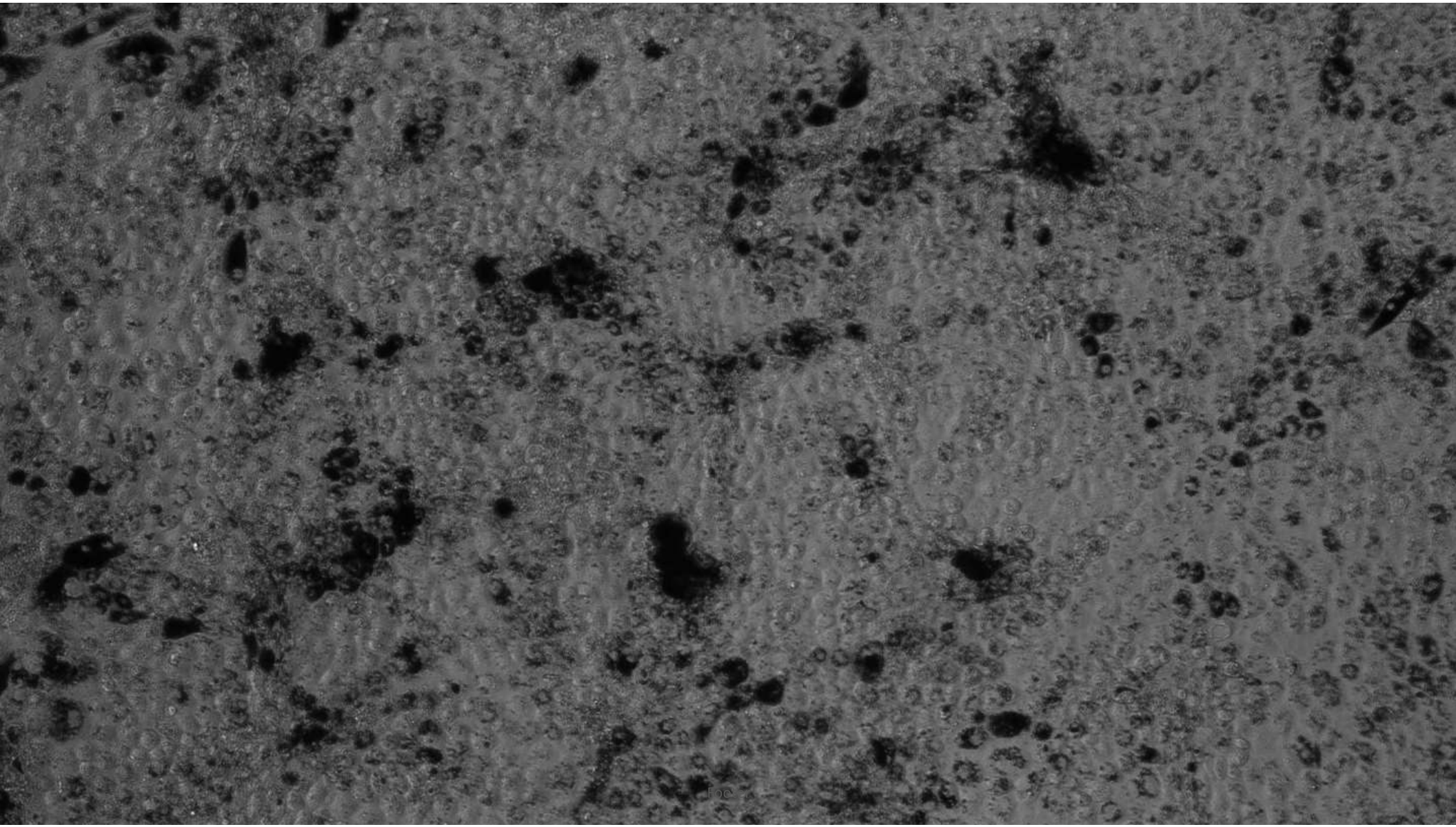
Bøgh, Askou, and Corydon
Unpublished 2024

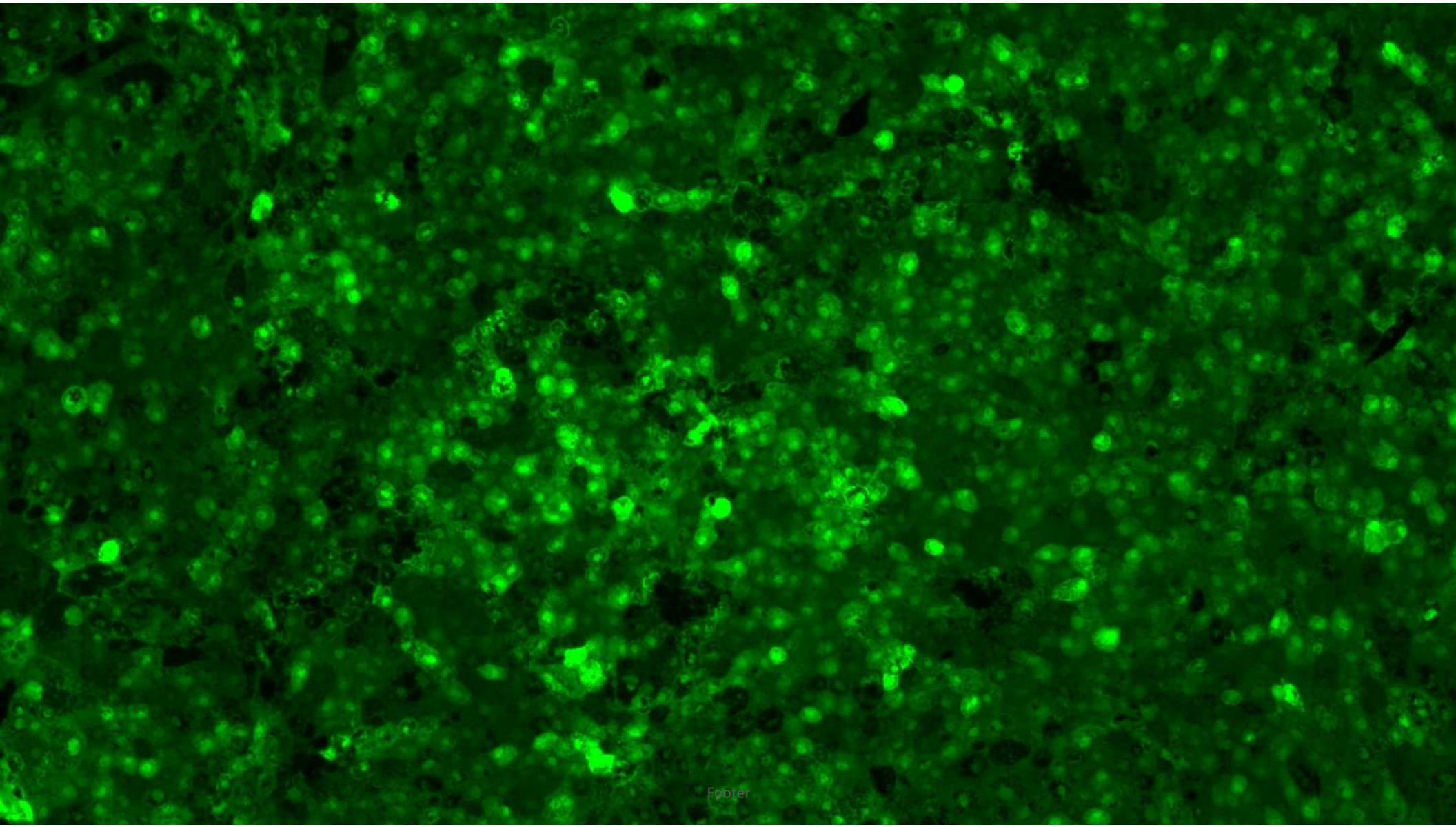
Pig Retinal Explants Single cells

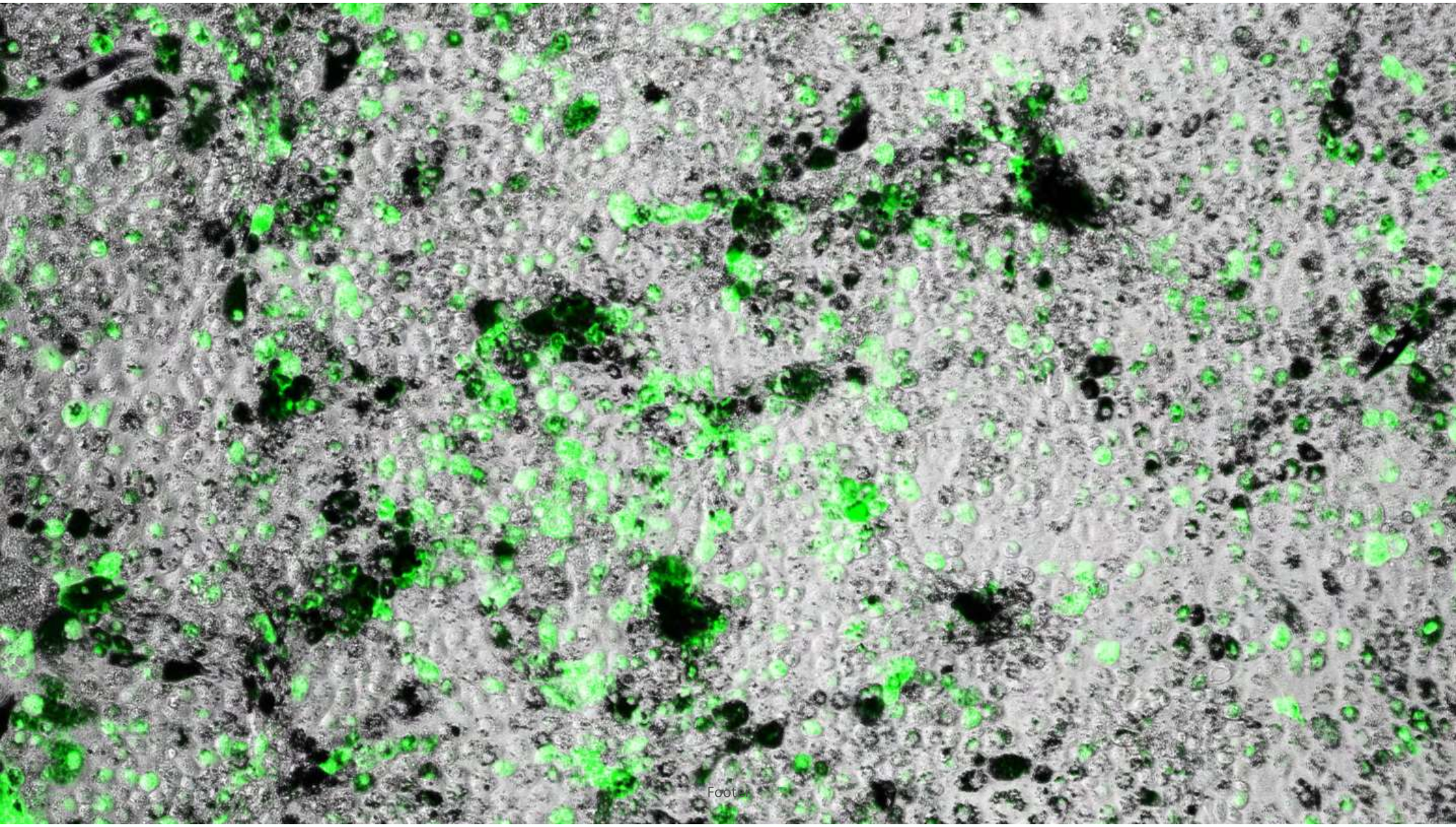


Bøgh, Askou, and Corydon
Unpublished 2024

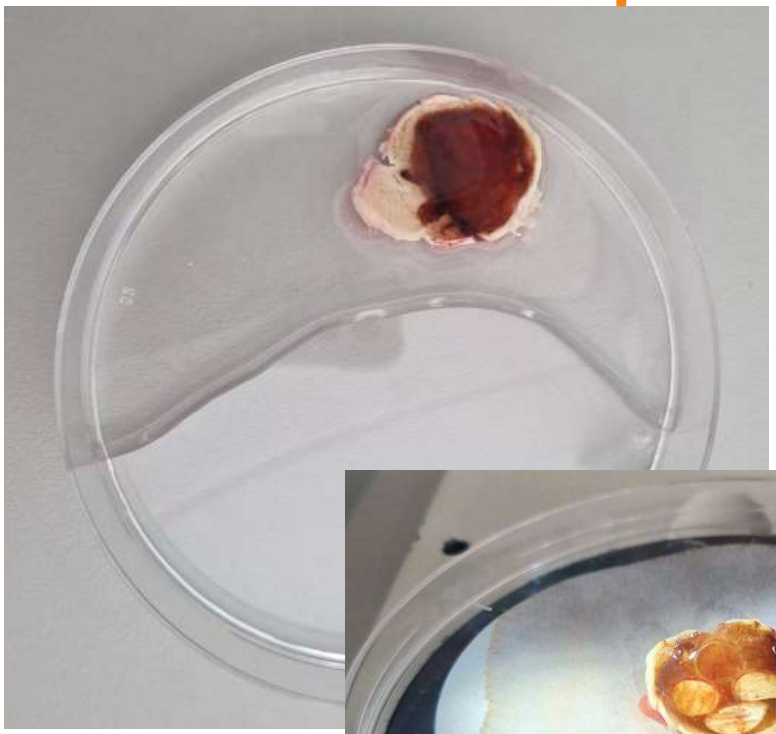


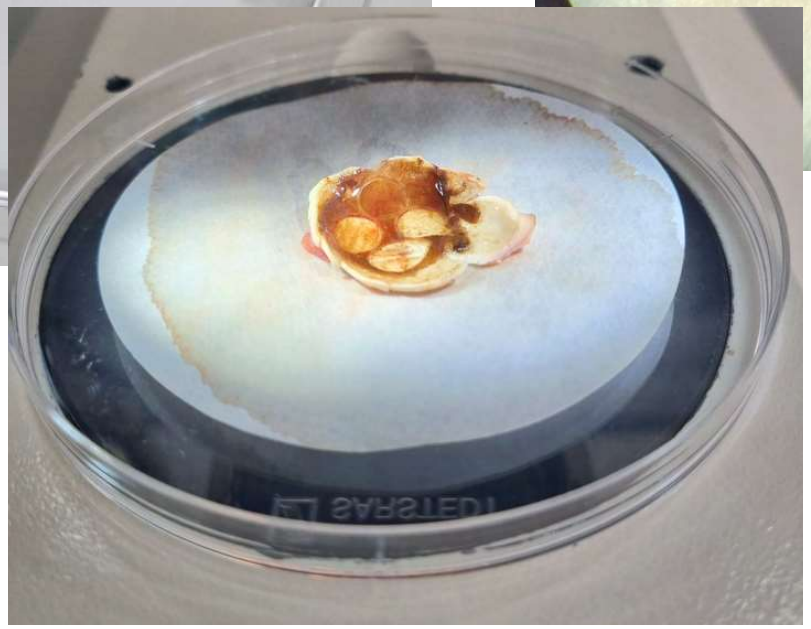






Human retinal explants

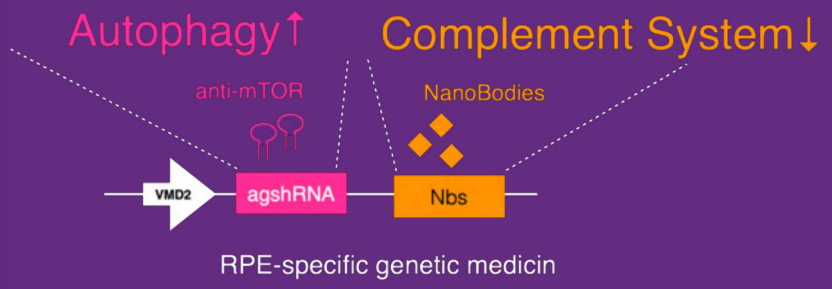




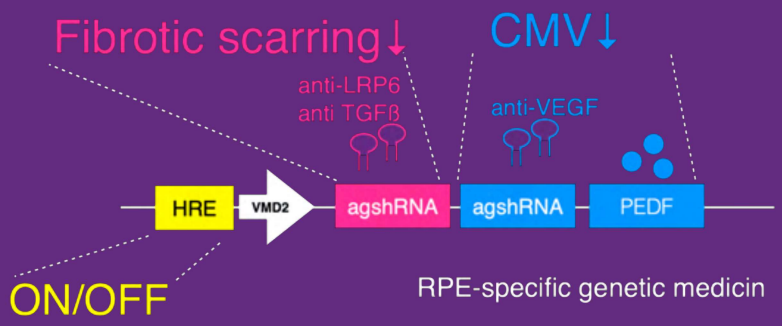
Human retinal explants

AMD Gene Therapy: Targeting pathogenic pathways in wAMD

Dry AMD



Wet AMD



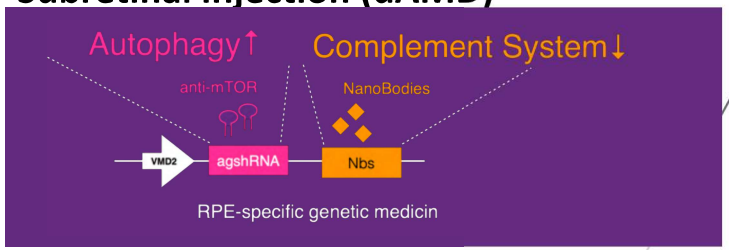
Avoid repeated injections
Treatment of PT which do not benefit of the current treatment
Treatment at the time and in the ocular <u>space where it arises</u>
Regulated RNAi-based therapy => less atrophy?

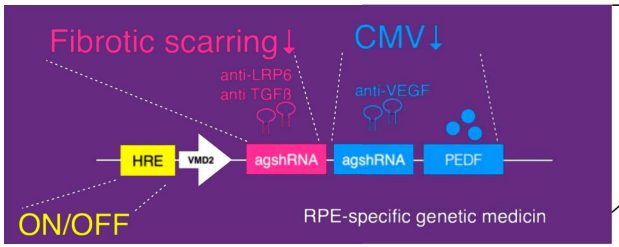
AMD Gene Therapy: Prediction rule

Scenario 1: Anti-VEGF (aflibercept)
Intravitreal injection

VEGF

Scenario 3: Therapeutic rAAV-based (multigenic)
Subretinal injection (dAMD)





Scenario 2: Therapeutic rAAV-based (multigenic)
Subretinal injection (wAMD)

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CONCLUSIONS

- Improved and safe suppression of CNV by combination anti-angiogenic GT
- CONCEPT: Targeting several pathogenic pathways in wAMD at the time and in the ocular space where it arises
- Developed a novel experimental CNV a el
- Validation in human retinal explants

Performed GT in pigs

Financial support

OC

ULA

RGEN

ETHER

APY

- DFF
- Insusense
- GTi Aarhus
- Øjenfonden
- Riisfort Fonden
- Danielsens Fond
- Synoptik-Fonden
- Aarhus Universitet
- Lundbeck Fonden
- The VELUX Foundation
- Knud og Edith Eriksens Fond
- Bagenkop-Nielsens Myopifond
- Købmand Marie Kirstine Jensens Fond
- Svend Helge Schrøder og hustru Ketty Lydia Larsen Schøders Fond
- Maskinfabrikant Jochum Jensen og hustru Mette Jensens Mindelegat

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