Immunization therapies for CADASIL

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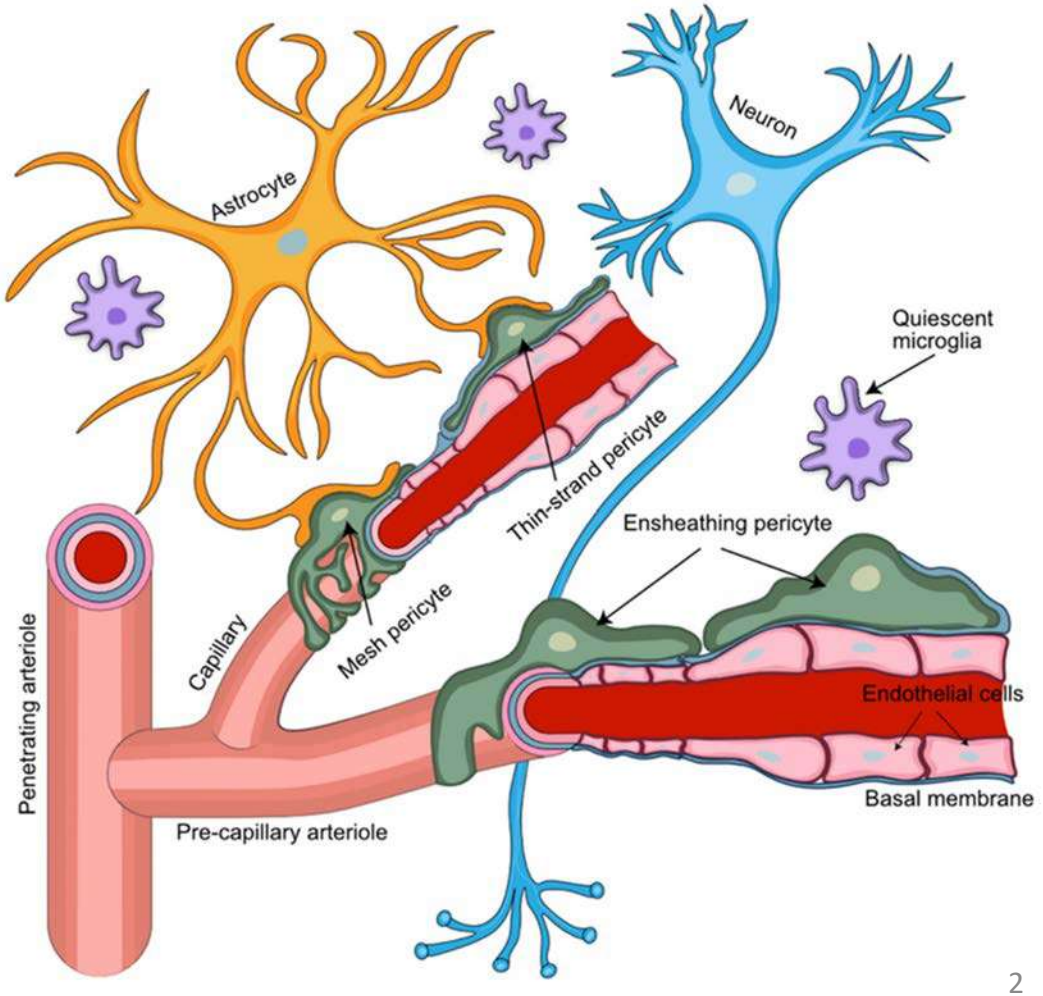
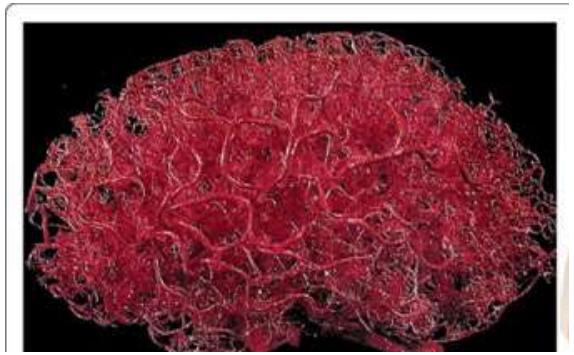
Brain cells

Neurons, Glia, vessels

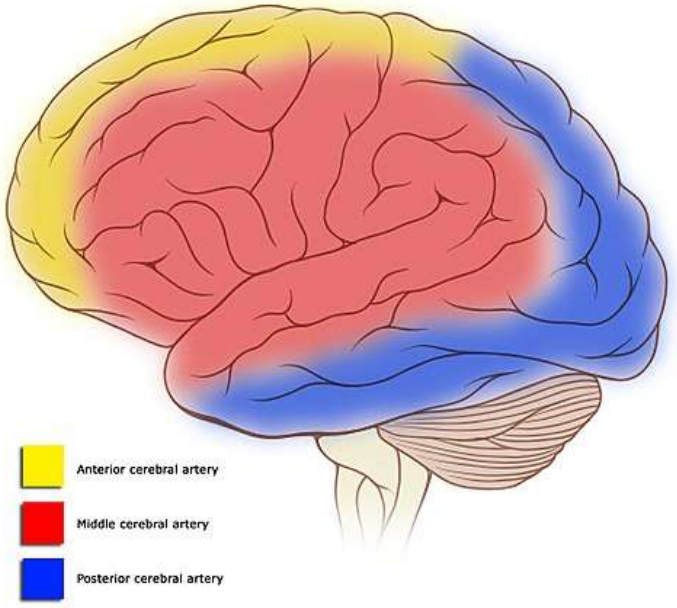


Brain & the vasculature

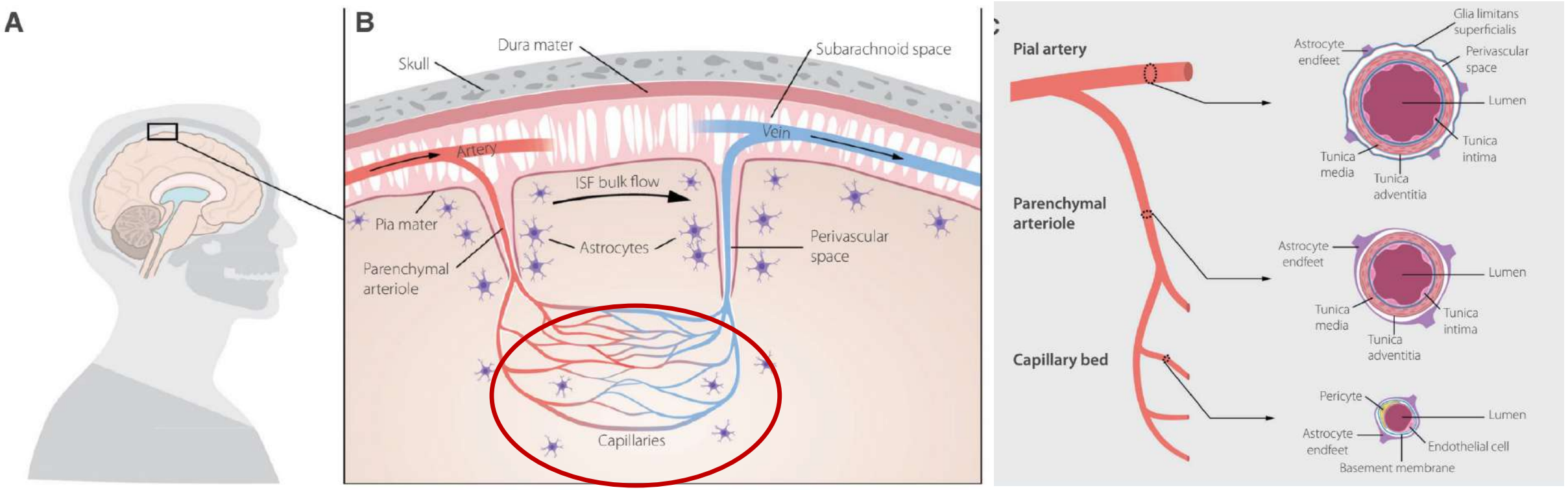
- 86 billion neurons
- 85 billion glia cells
- 2% body mass
- 20% energy consumption
- 644 km blood vessels



Cortical vascular territories



Vessels: artery, arteriole, and capillaries (also veins and venules)



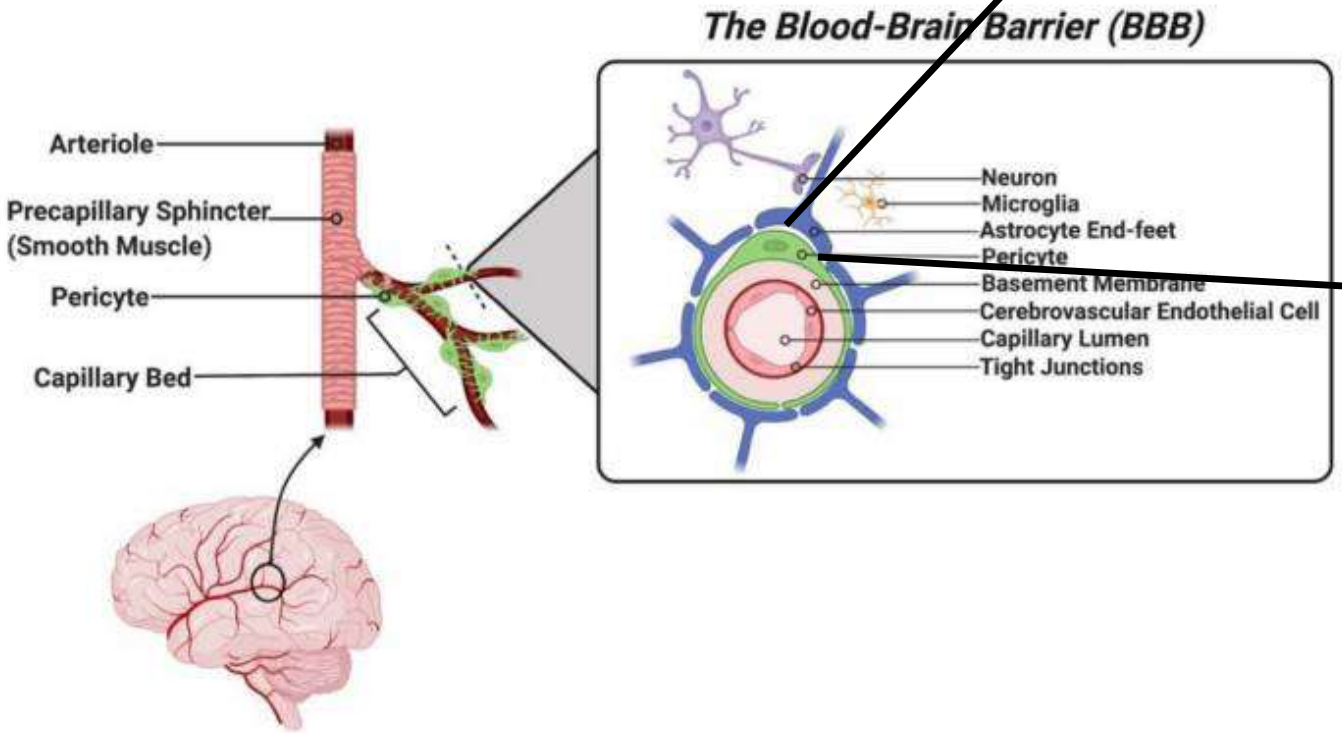
Coupland et al 2018, Stroke

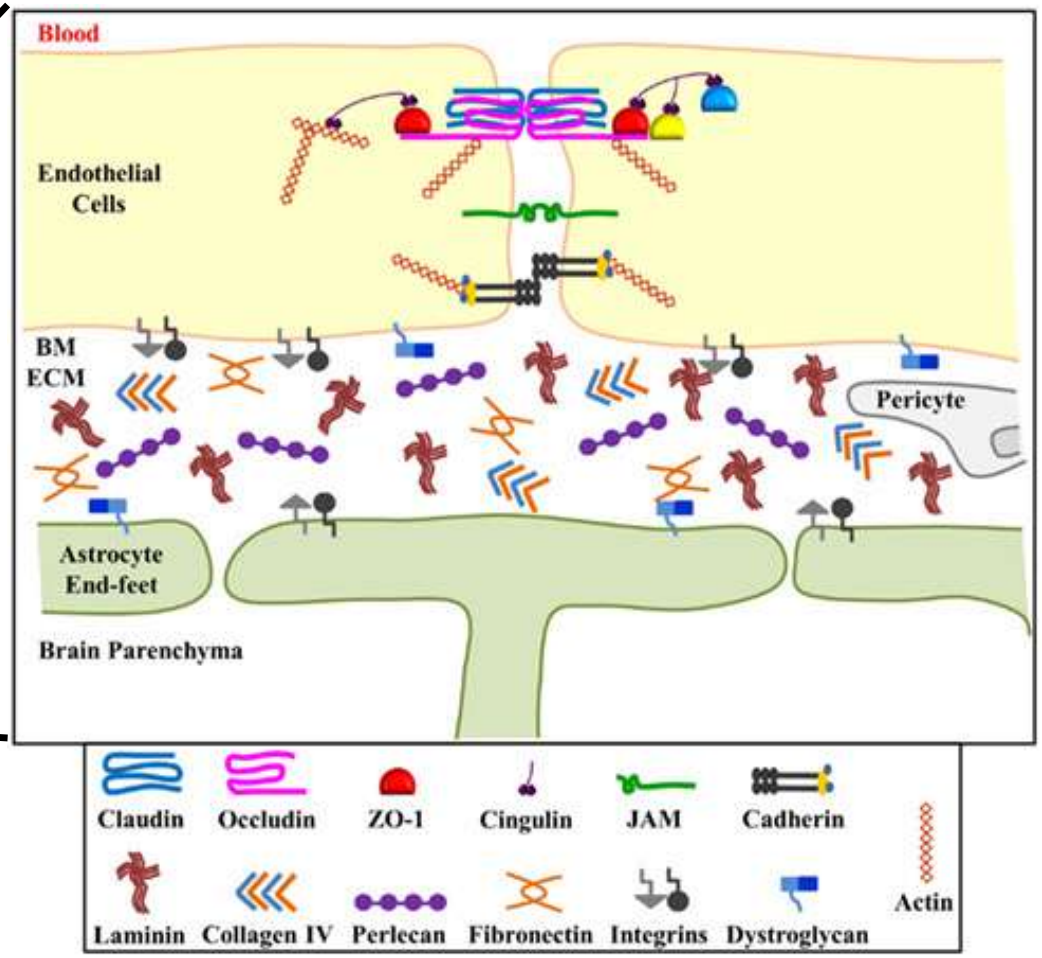
Blood brain barrier

Deliver from blood to brain:

- Oxygen, nutrients, ions, glucose

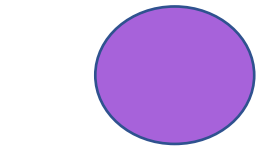
Prevent bacteria, pathogens etc to enter the brain





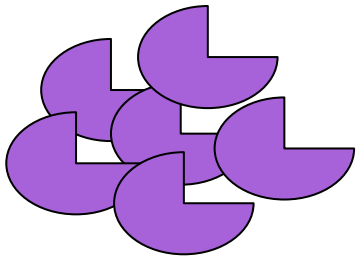
NOTCH3

NOTCH3 protein is needed for blood vessels to work properly (contraction/dilation etc)

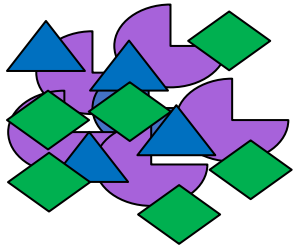


NOTCH3 protein

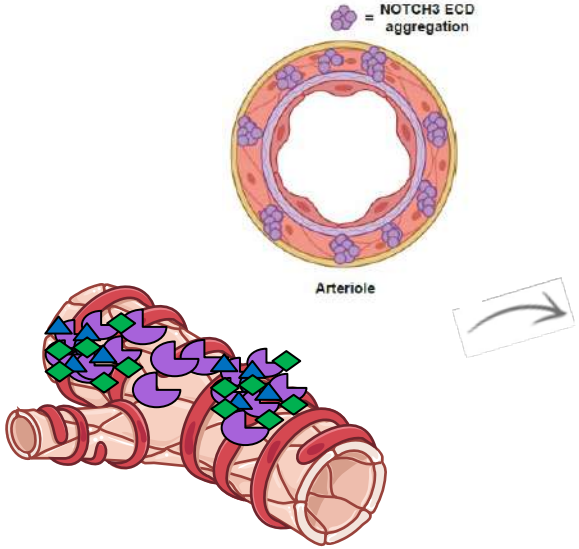
CADASIL-mutation



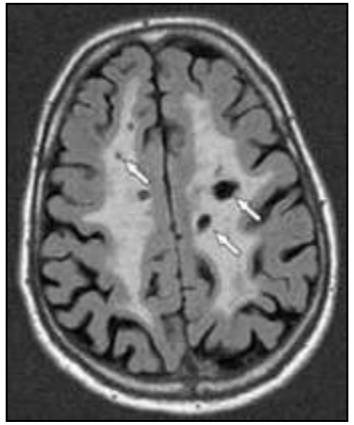
Mutant
NOTCH3 aggregation



Recruitment of
other proteins



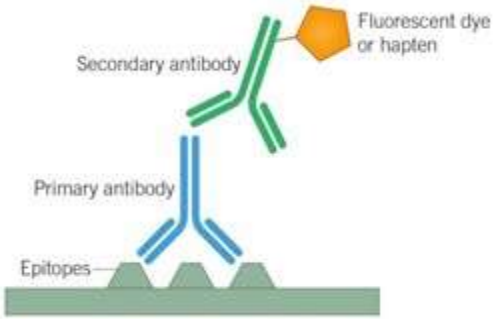
vessel wall
dysfunction



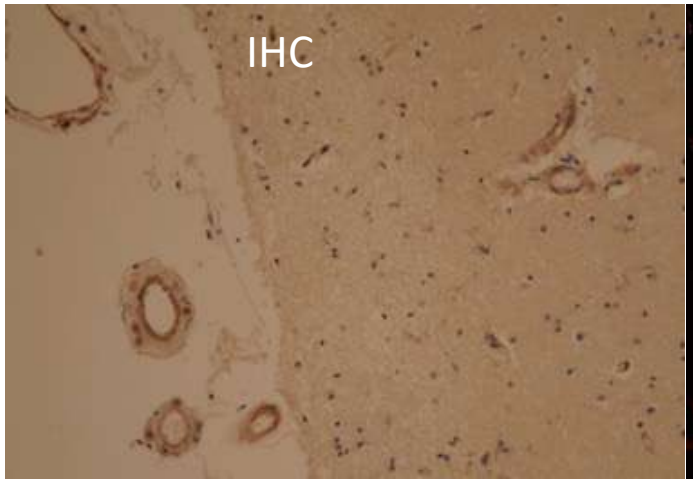
CADASIL brain MRI

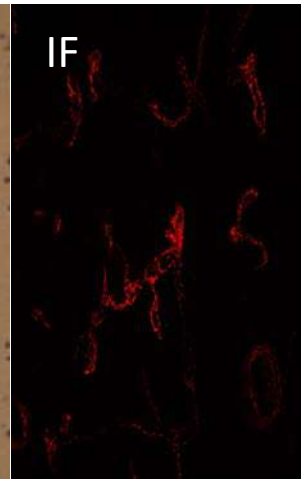
NOTCH3 aggregates in the vessel wall can be visualized

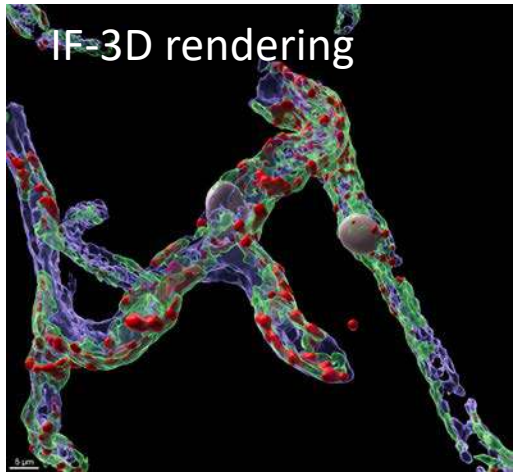
Immunostainings (IHC, IF)

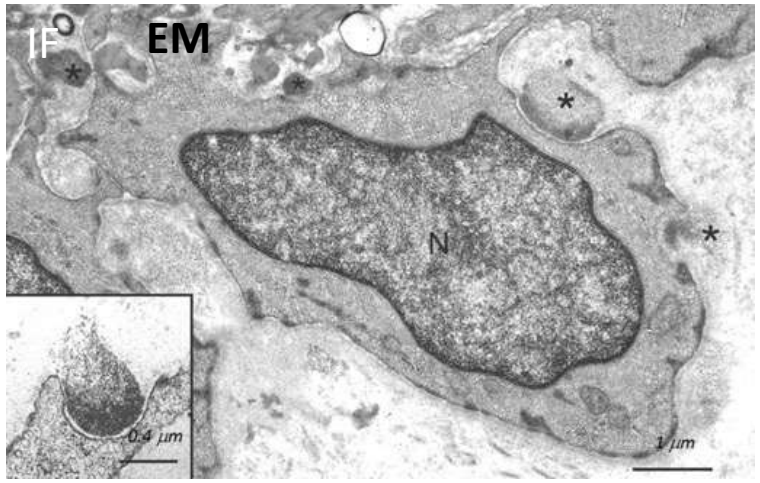


Electron Microscopy

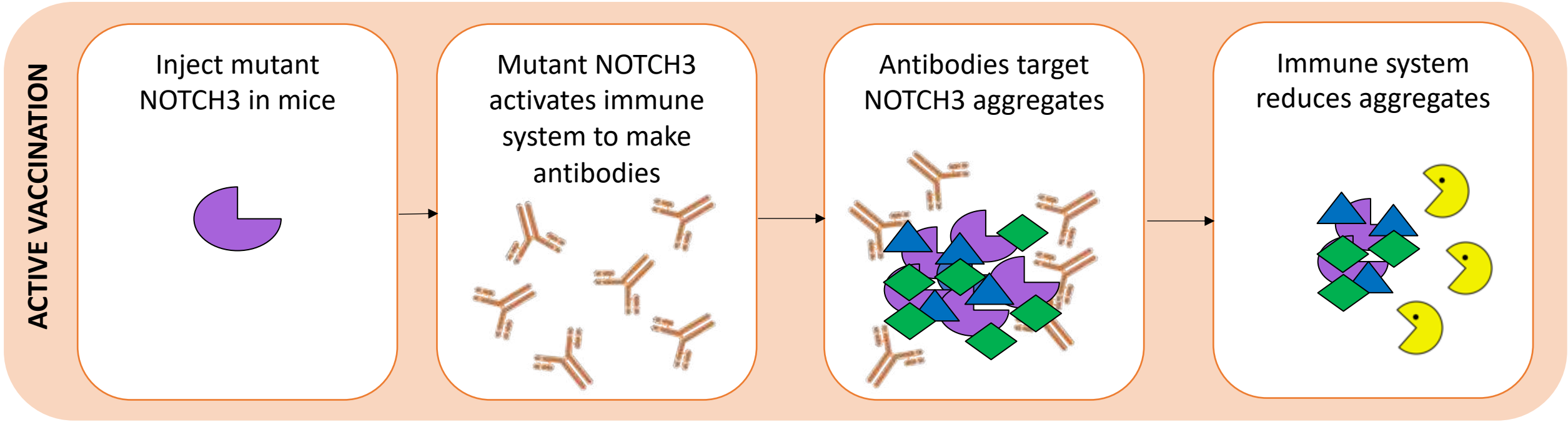








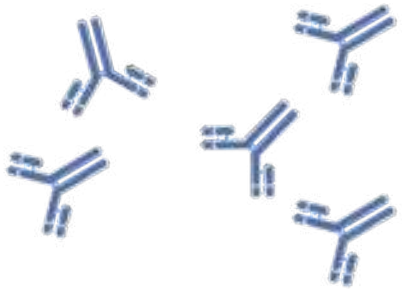
How can we get rid of these aggregates? Active vaccination



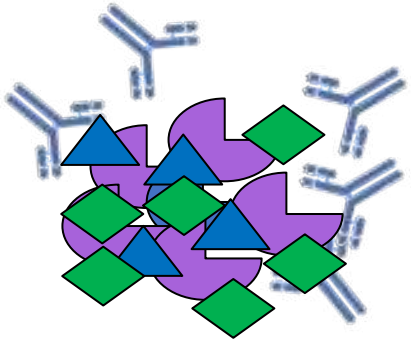
How can we get rid of these aggregates? Passive vaccination

PASSIVE VACCINATION

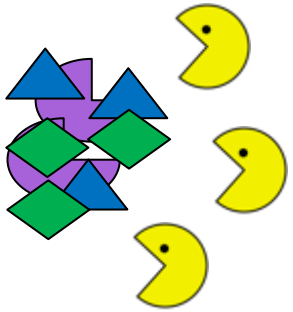
Antibodies against mutant NOTCH3 made in laboratory



Antibodies target NOTCH3 aggregates



Immune system reduces aggregates



Active vaccination

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- Use as preventive treatment before symptoms or at early stage
- Low frequency for dosing (e.g similar as Covid-19 vaccine-1 /year)
- Cost effective (low amount and frequency)

\-

- Unclear if it can be used in later stages of disease
- Dosage can be difficult to control – risk for some side effects.

Passive vaccination

\+

- Use at early-mid stage of the disesase
- Dosage can be controlled – less risk for some side effects.

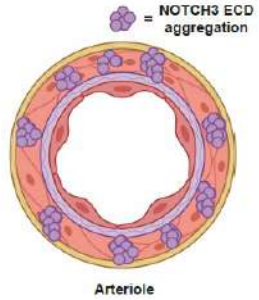
\-

- Unclear if it can be used for later stage of disease
- High frequency of dosing (1-2 times/ month)
- High cost due to amount and frequency
- Challenges to pass over the blood-brain-barrier?

Vaccination in clinical trials or approved for other diseases (brain-disorders)

Active vaccination	Passive vaccination
Alzheimers disease – target Tau-aggregation (phase II completed)	**Alzheimers disease – Aducanumab – target beta-amyloid aggregation (FDA approved)**
Alzheimers disease – target beta-amyloid - aggregation (phase II completed)	**Alzheimers disease – Lecanemab-target beta-amyloid aggregation (FDA approved)**
Parkinson's disease – target alpha-synuclein aggregation (phase I & II)	**Parkinson's disease – Target alpha-synuclein aggregation (phase 1 and II)**
Huntingtons disease – target huntingtin aggregates (pre-clinical phase)	Huntingtons disease – target huntingtin aggregates (preclinical phase)
ALS – target SOD aggregates (pre-clinical phase)	ALS – target SOD aggregates (pre-clinical phase)
Frontotemporal dementia – target C9orf72 polyQ repeats (pre-clinical phase)	Frontotemporal dementia – target C9orf72 polyQ repeats (pre-clinical phase)

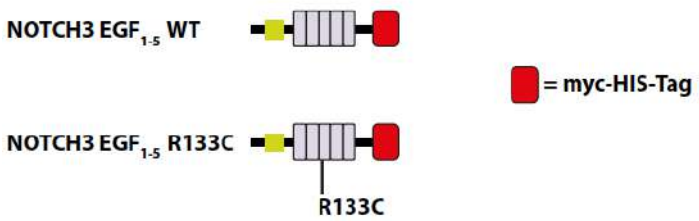
Active vaccination for CADASIL: why?

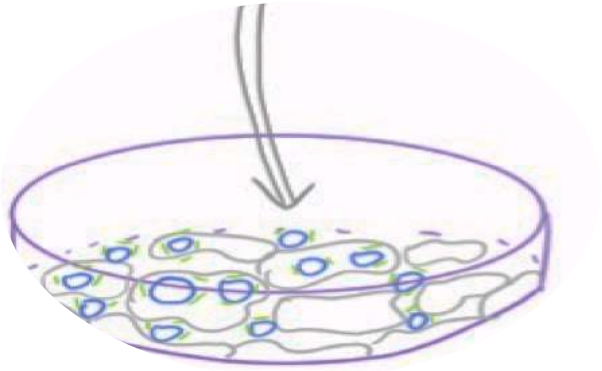


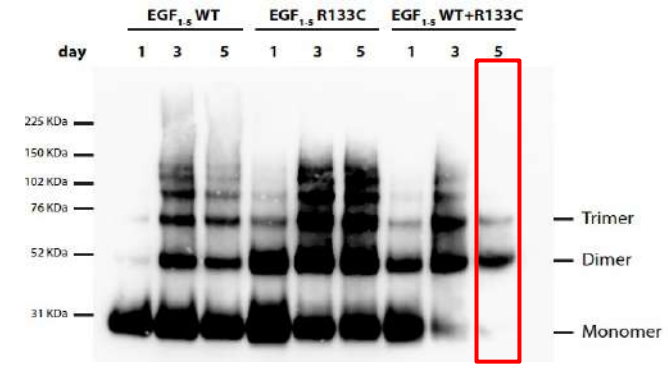
NOTCH3-ECD deposits are located in the vessel wall, thus accessible to the humoral immune response (antibody response)

Increase possibility of a greater immune response if administered before the onset
Impact on life quality

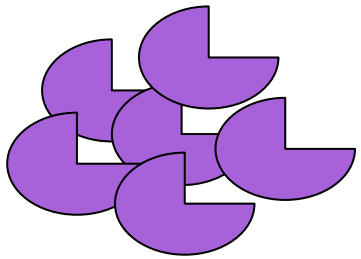
Production of antigen for active vaccination



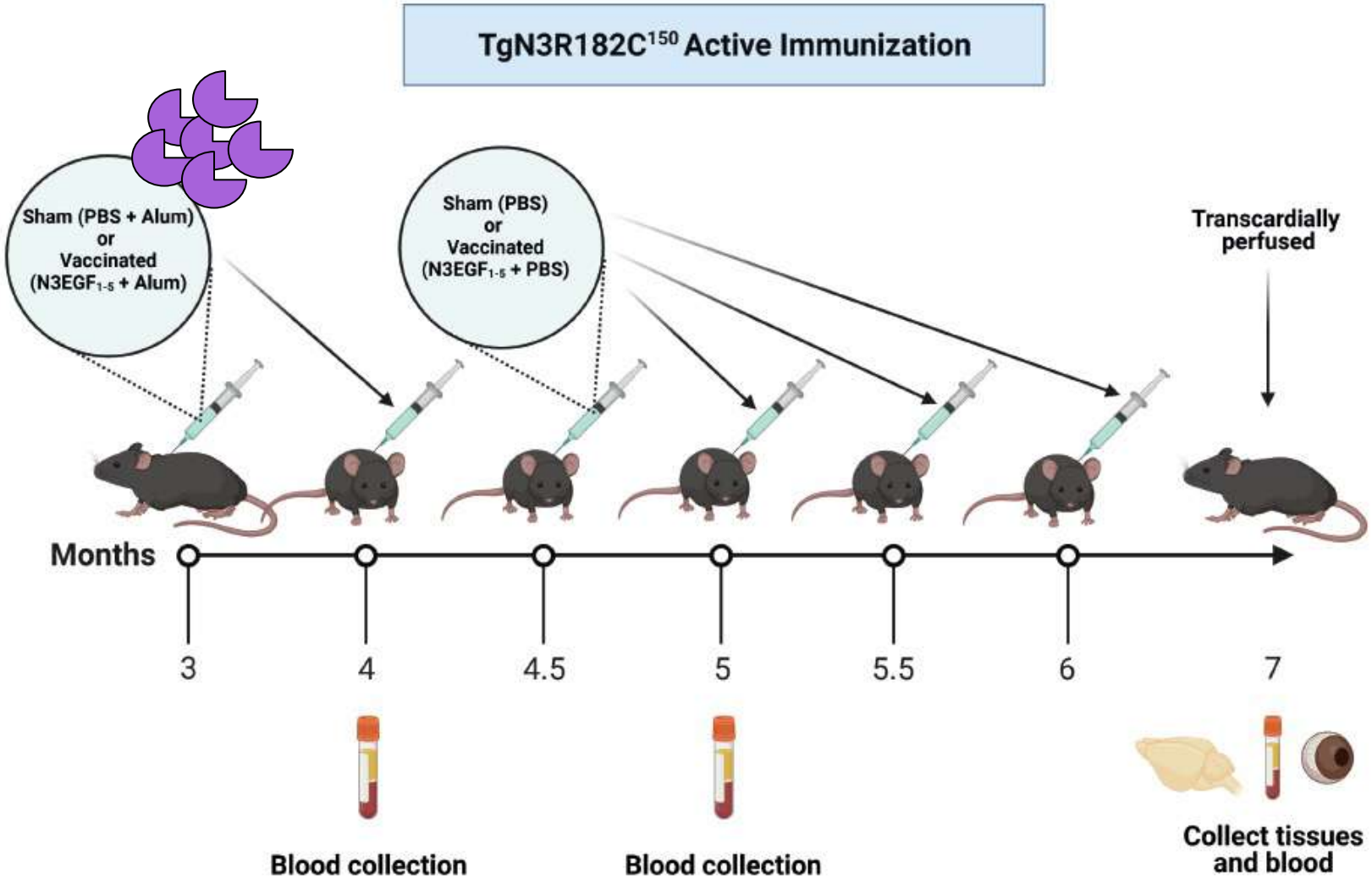




Selection of the aggregates favoring multimerization



Vaccination strategy



CADASIL mouse model – Tg150

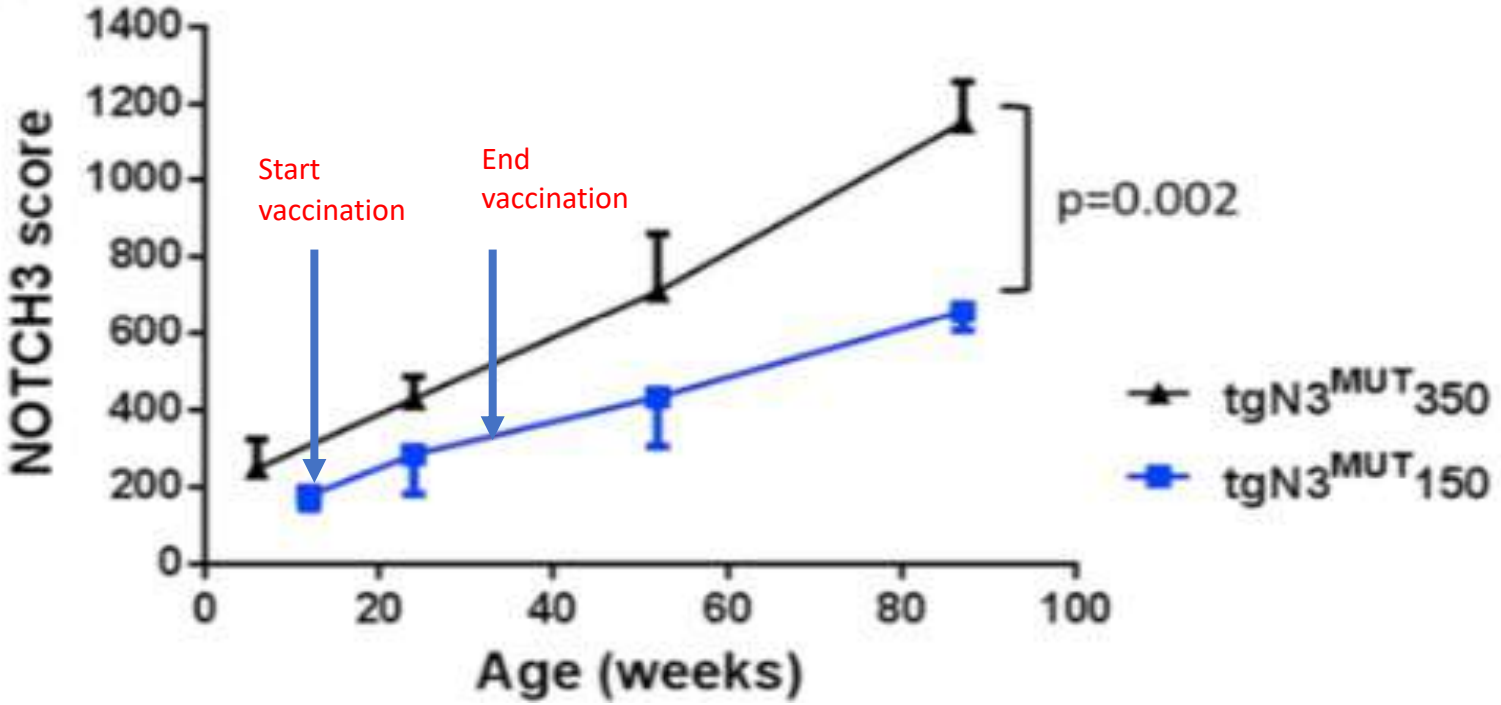


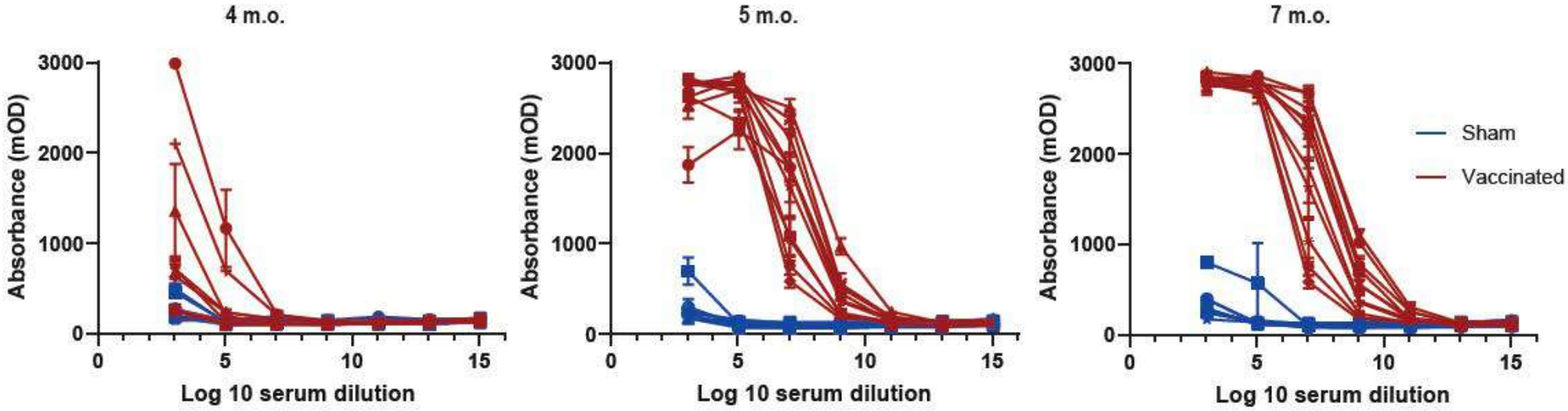
Table 1 The *NOTCH3* RNA expression level correlates with the age at onset of cerebrovascular NOTCH3 protein accumulation

Mouse strain	*NOTCH3* expression level[a]	Age at onset NOTCH3 accumulation[b]
tgN3^{MUT} 350	350 %	6 weeks
tgN3^{MUT} 200	200 %	3 months
tgN3^{MUT} 150	150 %	5 months
tgN3^{MUT} 100	100 %	12 months

[a] mRNA *NOTCH3* expression levels relative to endogenous mouse *Notch3* expression levels [b] first sign of positive, granular NOTCH3 staining in brain vessels, as determined by an experienced neuropathologist (S.v.D)

Rutten et al *Acta Neuropathologica Communications, 2015*

Vaccination evokes a robust antibody response in the Tg150 mice

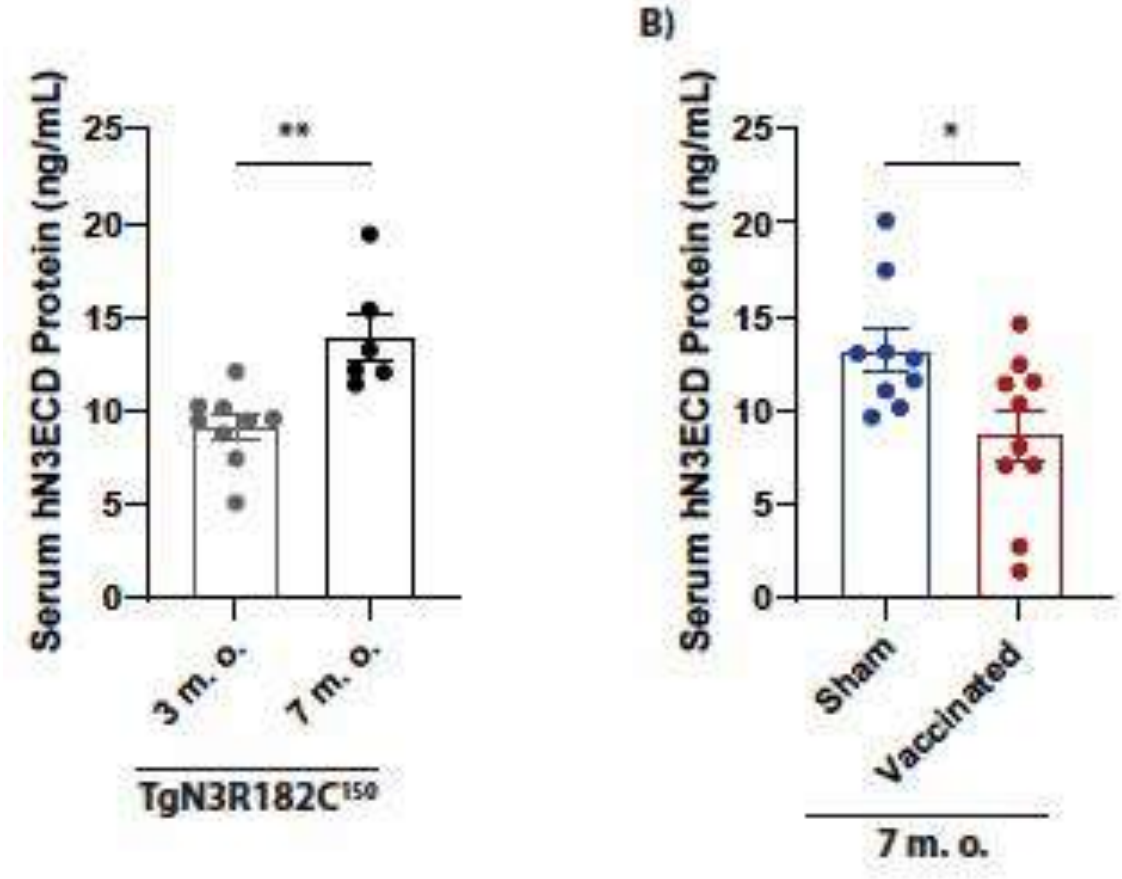


Vaccinated

Non-vaccinated

An immune response is mounted against the injected NOTCH3 EGF1-5 WT/R133C aggregates

The amount of NOTCH3 ECD in blood is incre with age but reduced after vaccination- used as a biomarker?

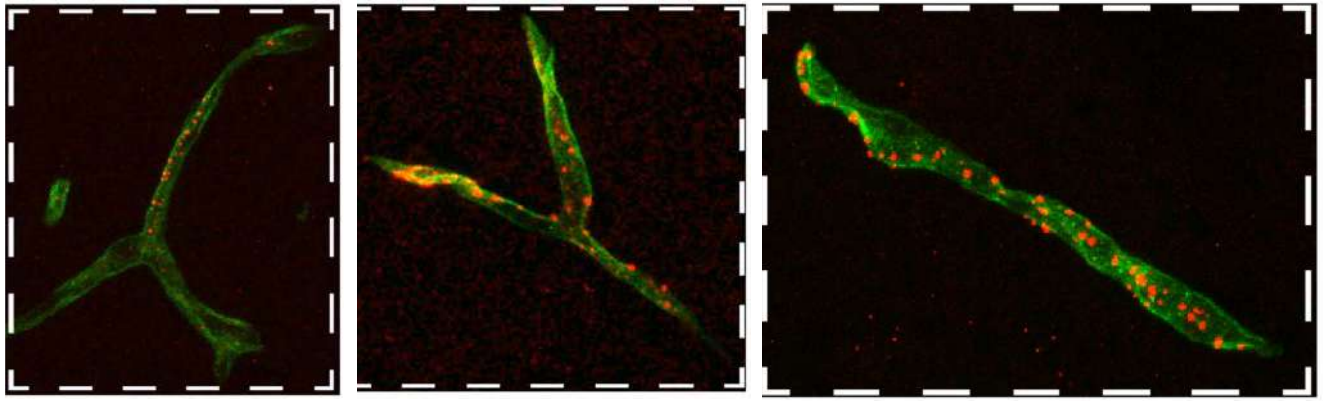


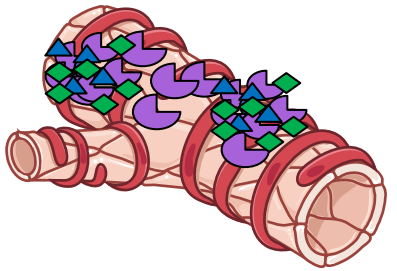
The numbers and size of NOTCH3 ECD deposits around capillaries are increased with age in non-vaccinated mice

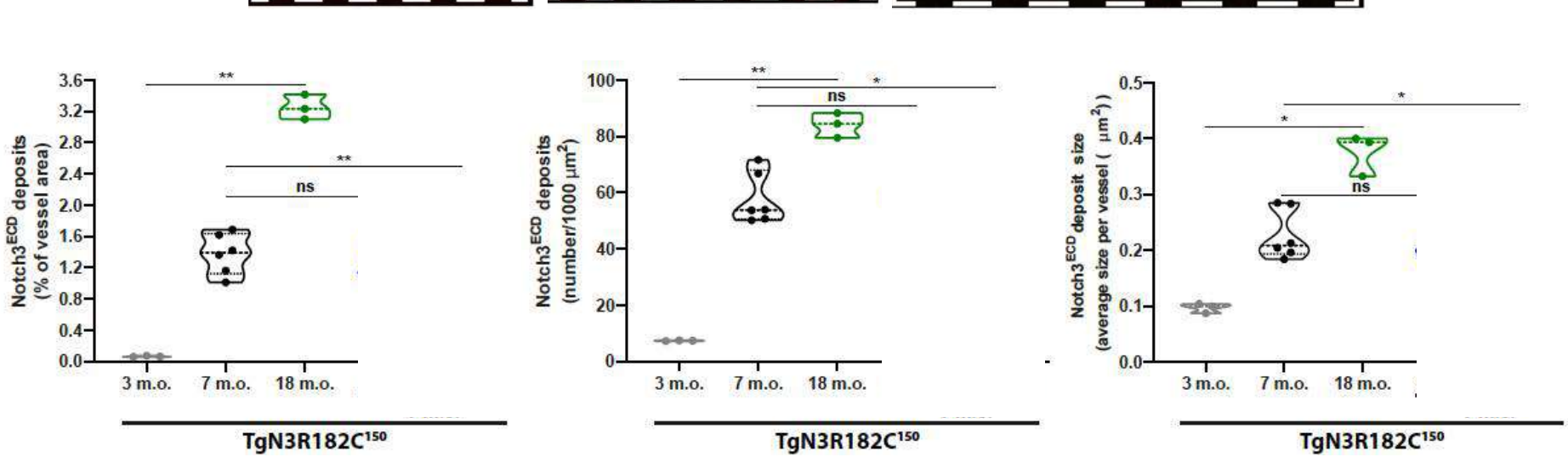
Non-vaccinated mice

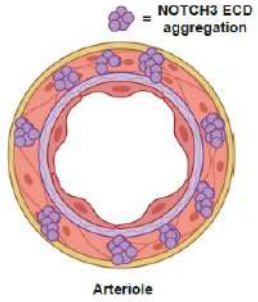
Capillaries/NOTCH3 ECD

3 months 7 months 18 months

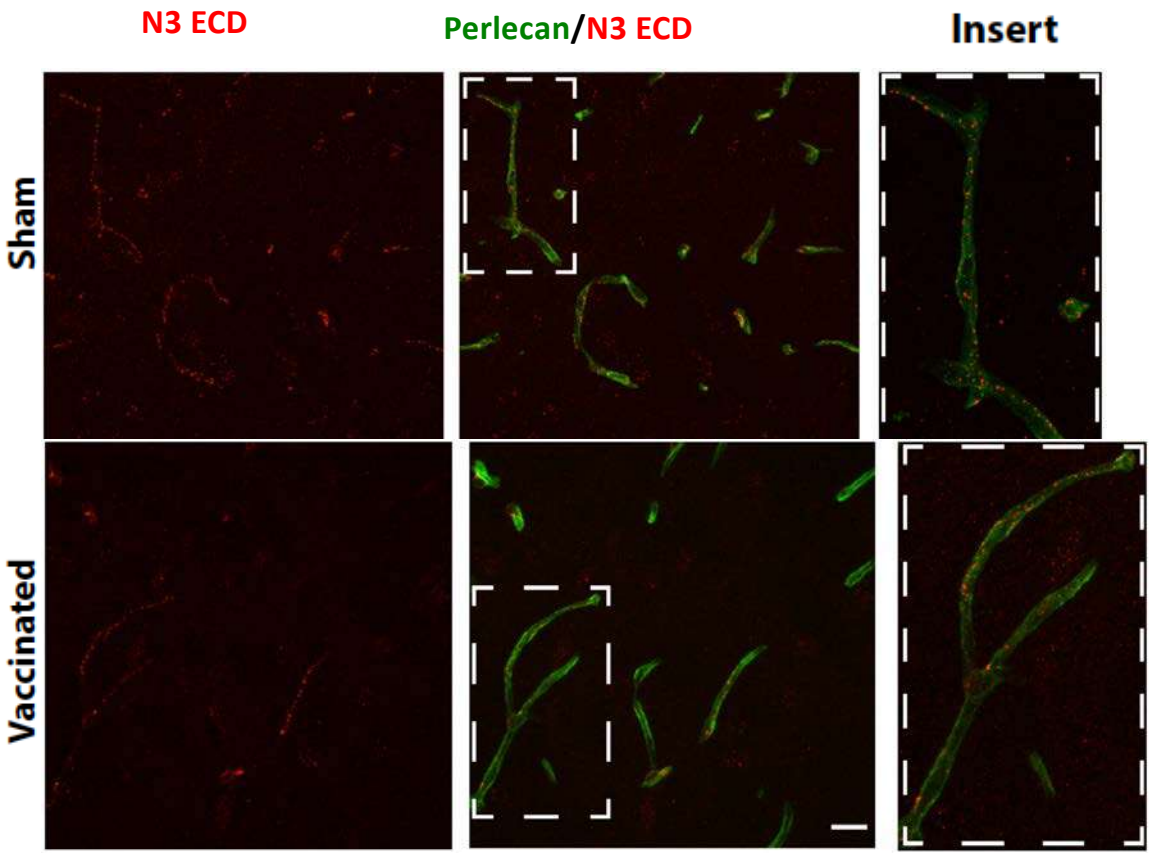




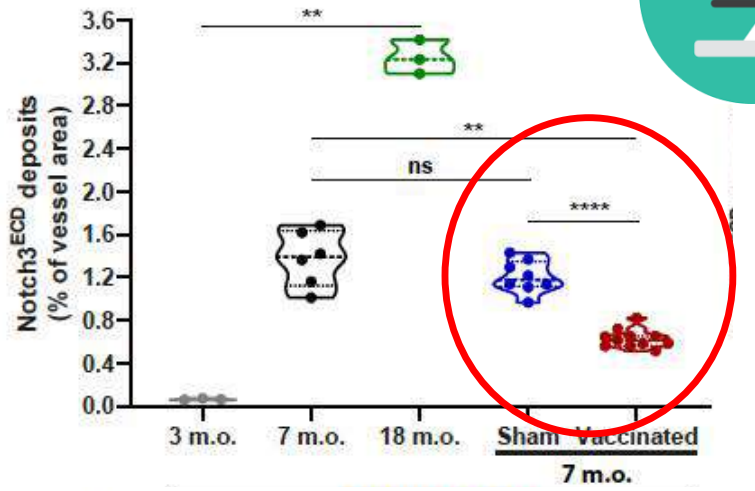




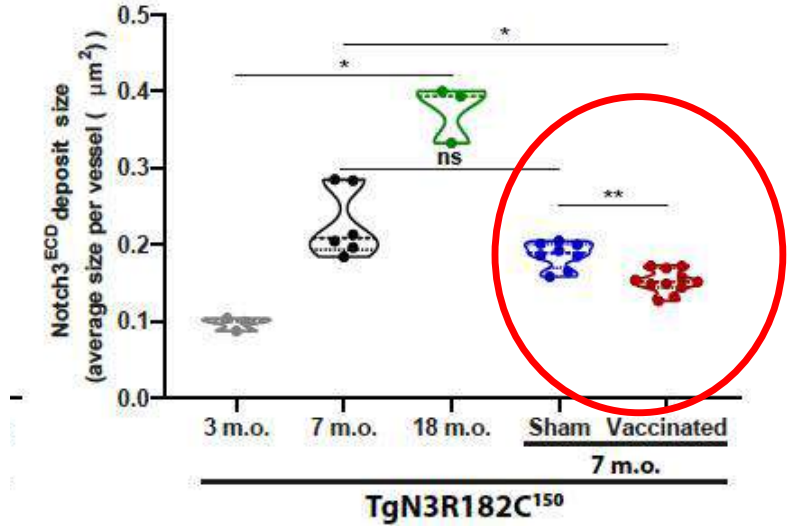
The numbers and size of NOTCH3 ECD deposits around capillaries are reduced after vaccination



NUMBER
40%



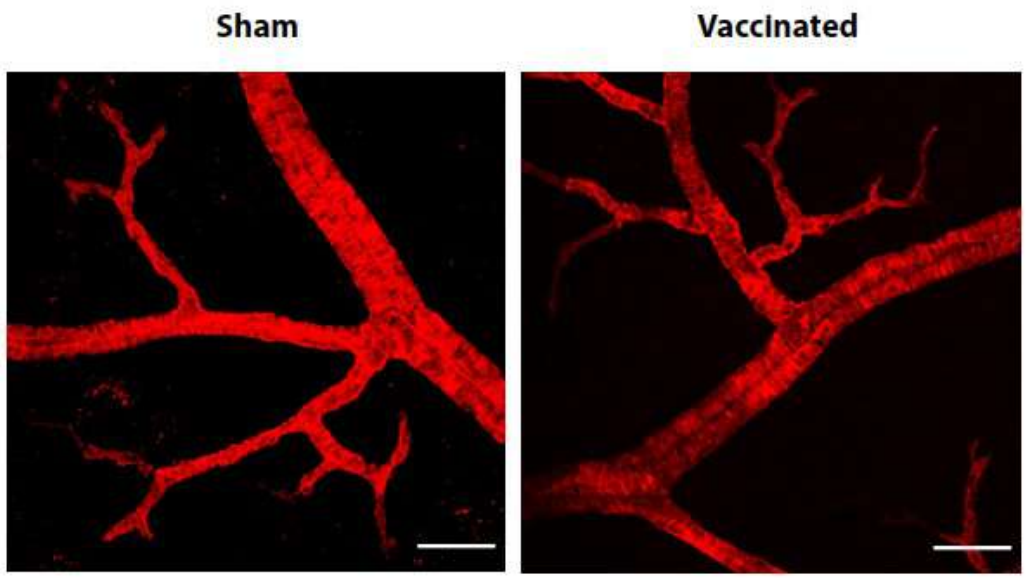
SIZE
15%

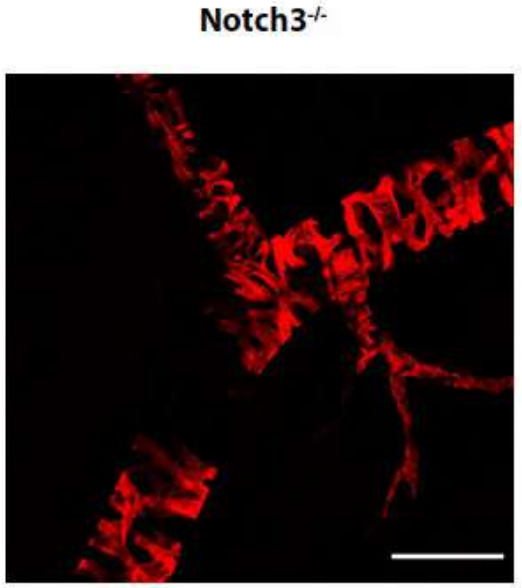


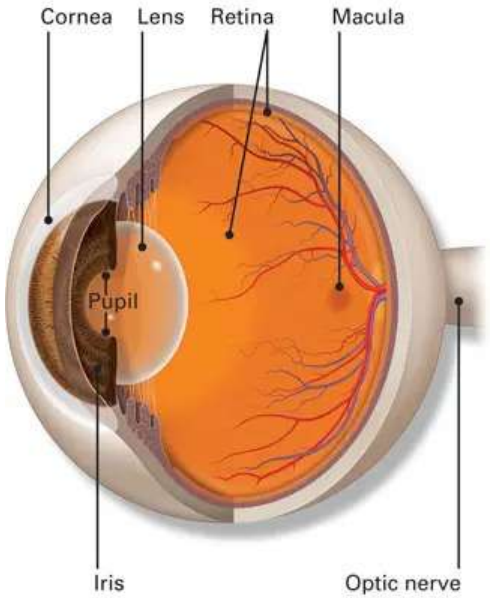
Active immunization specifically reduces the amount of NOTCH3 aggregates around cerebral capillaries.

What about side effects?

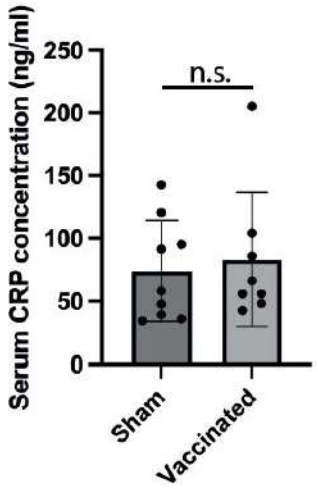
The number of retinal vessel cells is not reduced by active immunization

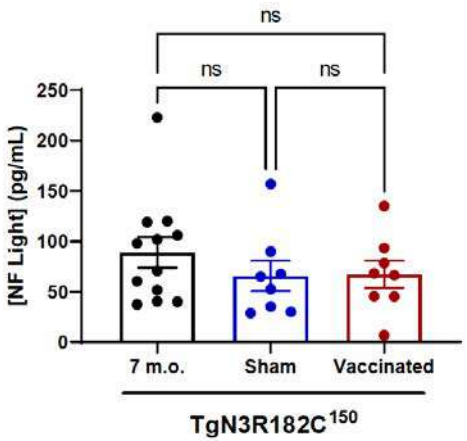


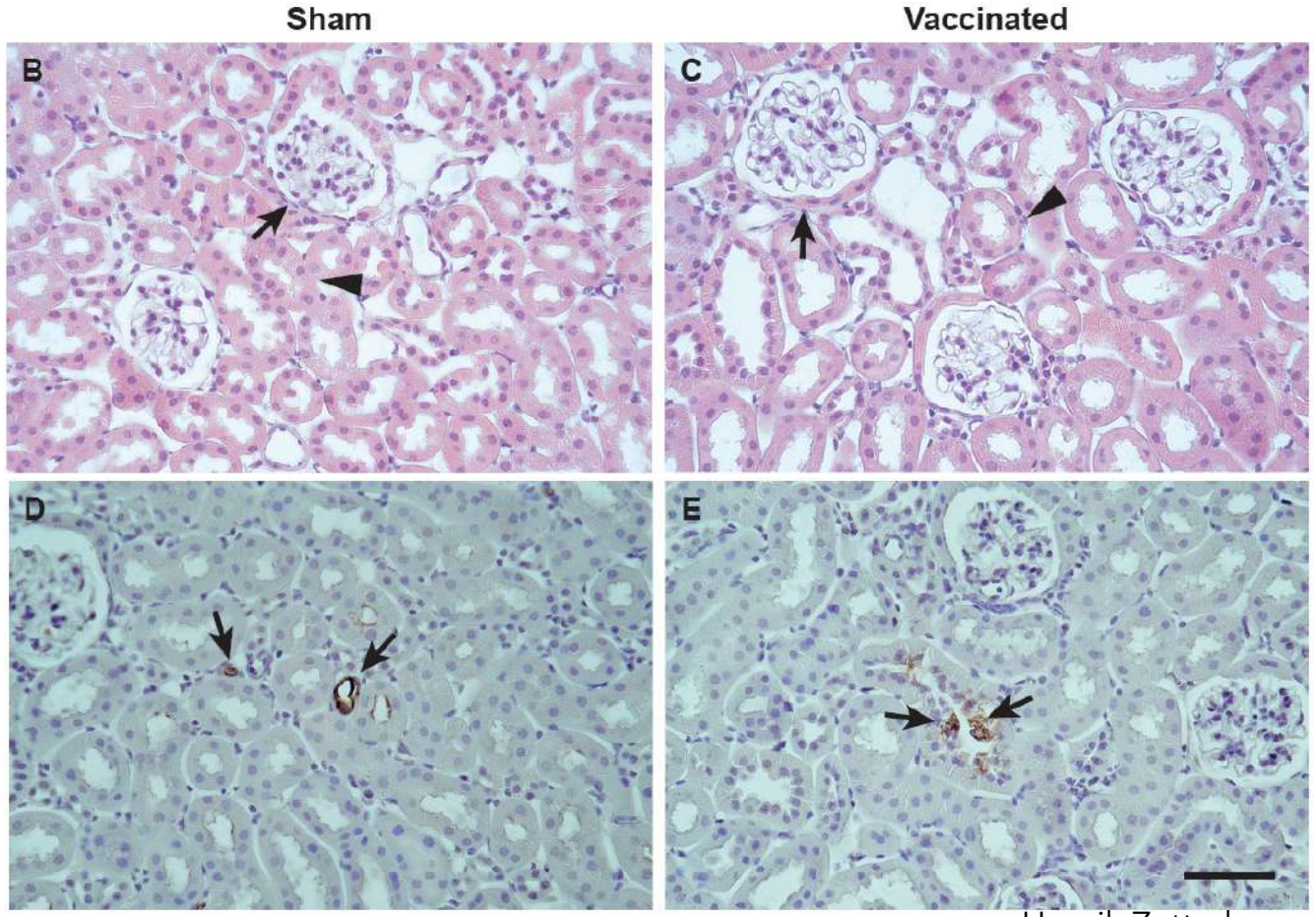




Vaccination is safe and tolerable – no inflammation, neurodegeneration or effect on kidney

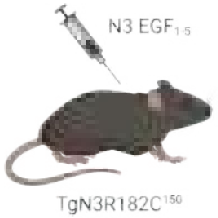


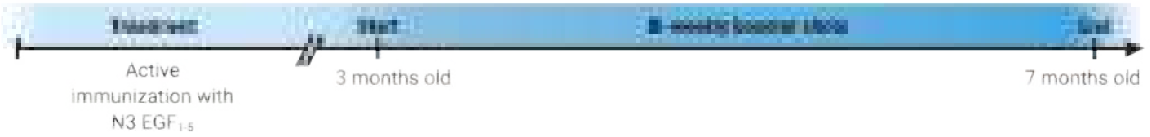




Henrik Zetterberg

SUMMARY



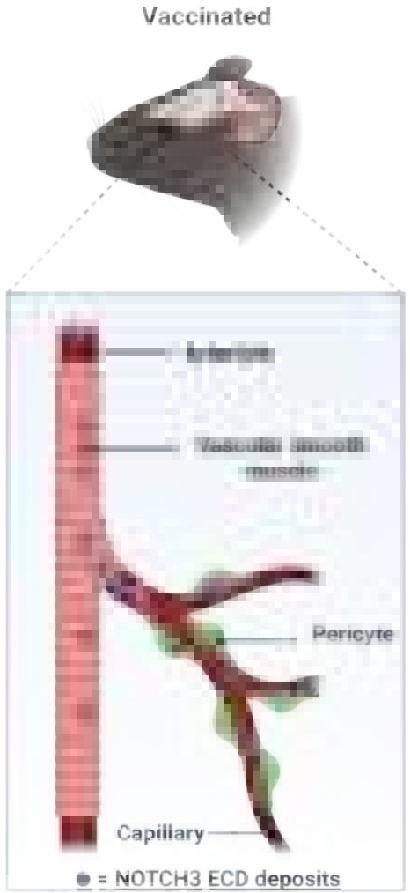


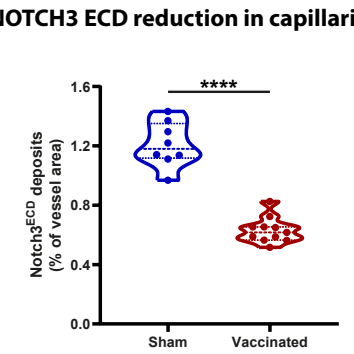
1. Vaccination give rise to a high antibody response in mice

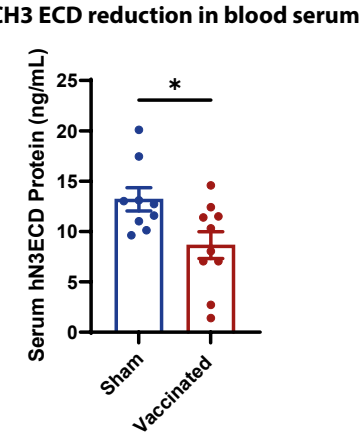
2. Vaccination reduces NOTCH3 aggregates in brain vessels and blood

3. Vaccination is activating the cleaning cells microglia to clear away the NOTCH3 aggregates

4. Vaccination is safe and tolerable in a pre-clinical mouse model



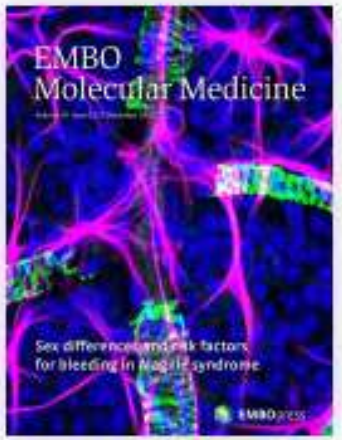




Article | 16 December 2022 | OPEN ACCESS SOURCE DATA | TRANSPARENT PROCESS

Active immunotherapy reduces NOTCH3 deposition in brain capillaries in a CADASIL mouse model

Daniel V Oliveira, Kirsten G Coupland, Wenchao Shao, Shaobo Jin, Francesca Del Gaudio, Sailan Wang, Rhys Fox, Julie W Rutten, Johan Sandin, Henrik Zetterberg, Johan Lundkvist, Saskia AJ Lesnik Oberstein, Urban Lendahl, Helena Karlström



About the cover

Volgende stappen

- Actieve vaccinatie in een ernstiger CADASIL-muismodel - aanvangsleeftijd ongeveer 2 maanden. Begin op de leeftijd van 3 maanden tot 12 maanden.

Vraag: Kan actieve vaccinatie worden gebruikt als therapeutische behandeling – wanneer de symptomen al zijn begonnen?

- Maken van een antilichaam dat bindt aan geaggregeerde NOTCH3 - test op weefsel van verschillende CADASIL-patiënten en mutaties

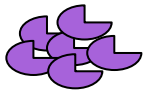
Vraag: Kan het geselecteerde antilichaam binden aan NOTCH3-aggregaten met verschillende mutaties en dus worden gebruikt voor passieve vaccinatie?

- Passieve vaccinatie in een CADASIL-muismodel - begin bij een leeftijd van 2 maanden tot 12 maanden

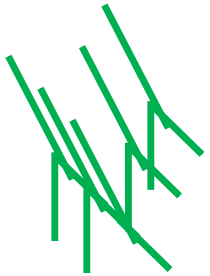
Vraag: Kan passieve vaccinatie worden gebruikt als therapeutische behandeling – wanneer de symptomen zijn begonnen?

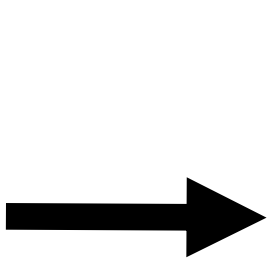
Van muis naar patient: wat is er nodig?
Pre-klinische ontwikkeling (veiligheid en dosering)
Klinische trials: fase I, II, III, IV → goedkeuring voor gebruik

NOTCH3 eiwit

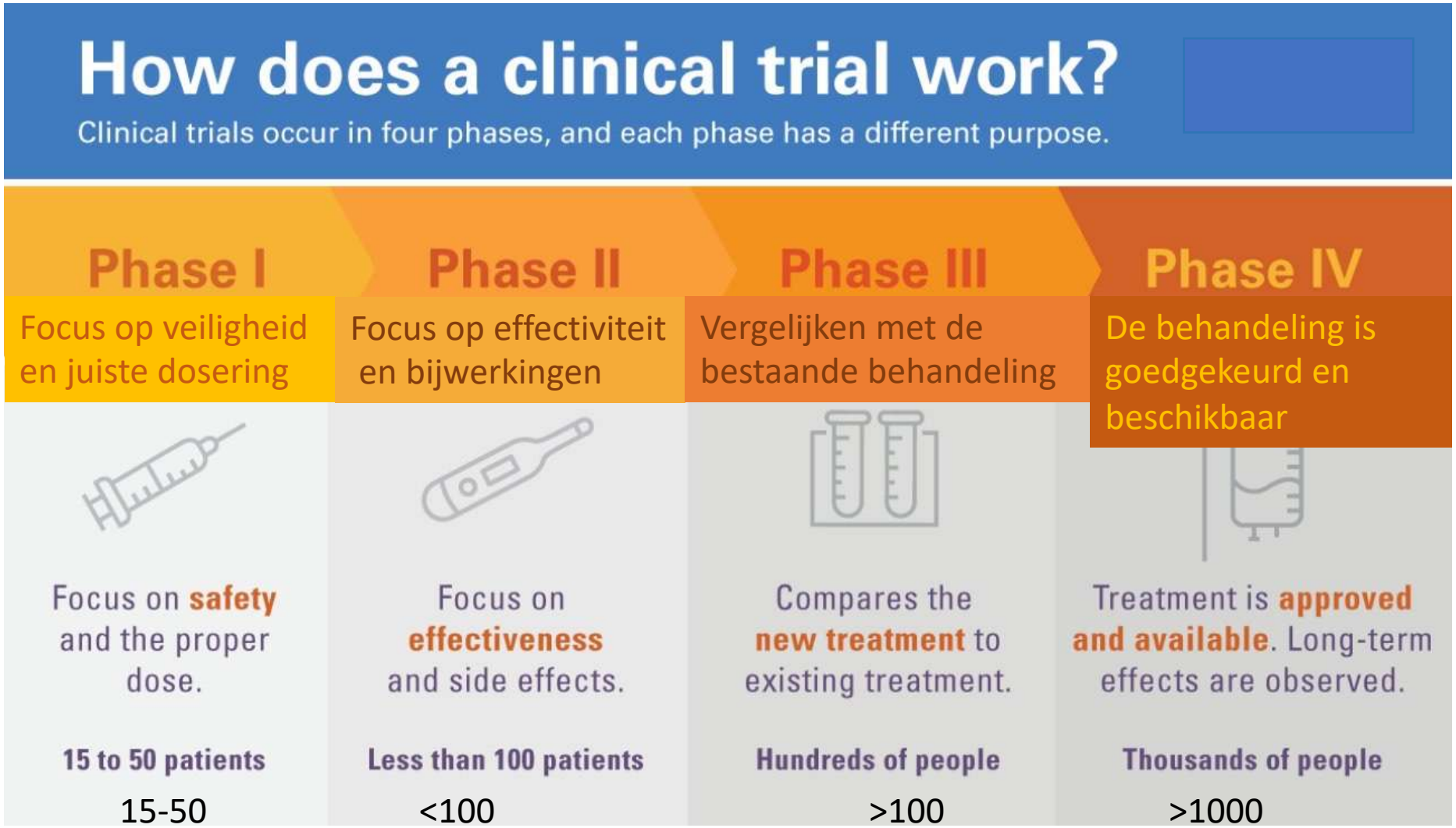


NOTCH3 antilichaam





Hoe werkt een klinische proef?



Next steps

- Perform active vaccination in a more severe CADASIL mouse model – age of onset around 2 months. Start at 3 months to 12 months of age.

Q: Can active vaccination be used as a therapeutic treatment – when symptoms have started?

- Generate a monoclonal antibody that binds to aggregated NOTCH3 – test on tissue from different CADASIL patients and mutations

Q: Can the selected antibody bind to NOTCH3 aggregates with different mutations and thus be used for passive vaccination?

- Perform passive vaccination in a CADASIL mouse model – start at 2 months to 12 months of age

Q: Can passive vaccination be used as a therapeutic treatment – when symptoms have started?

Thank you!

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