SAPIENZA
UNIVERSITÀ DI ROMA

AAV-mediated gene therapy for **Neuromyelitis Optica**

POLYPEPTIDE TRANSDUCTION TO INHIBIT IgG AUTOANTIBODIES-AQUAPORIN 4 BINDING IN SPINAL CORD ASTROCYTES

Terapia Genica e Neuroscienze, aa 2022/2023
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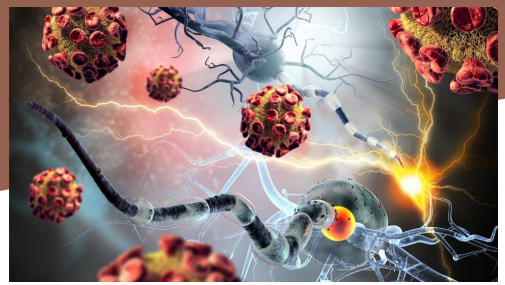
Background

NEUROMIYELITIS OPTICA (NMO)

WHAT IS IT?

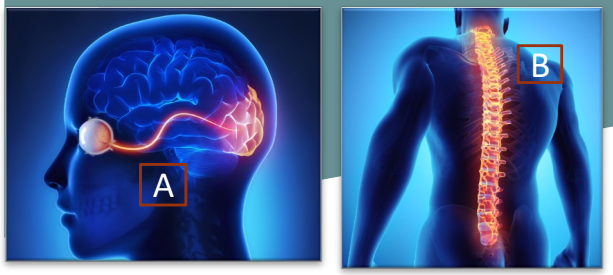
- **neurodegenerative autoimmune** disease of CNS.



https://iiman.co/terapias-naturales-para-las-enfermedades-autoinmunes/

WHAT CAUSES?

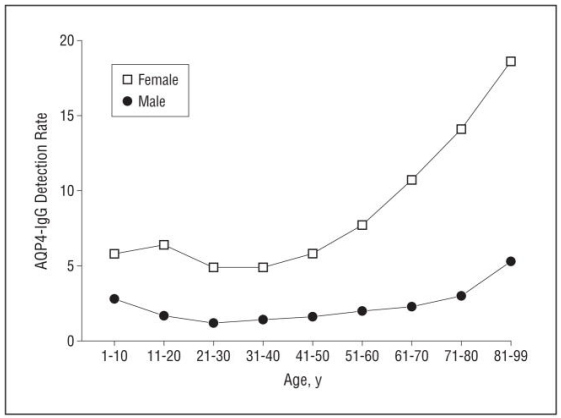
- **Optic neuritis** (A) and **spinal cord myelitis** (B)
- Course: blindness, paralysis and death for **acute neurogenic respiratory failure**.
- Disability associated with **relapses**



https://www.clinicabaviera.it/

EPIDEMIOLOGY

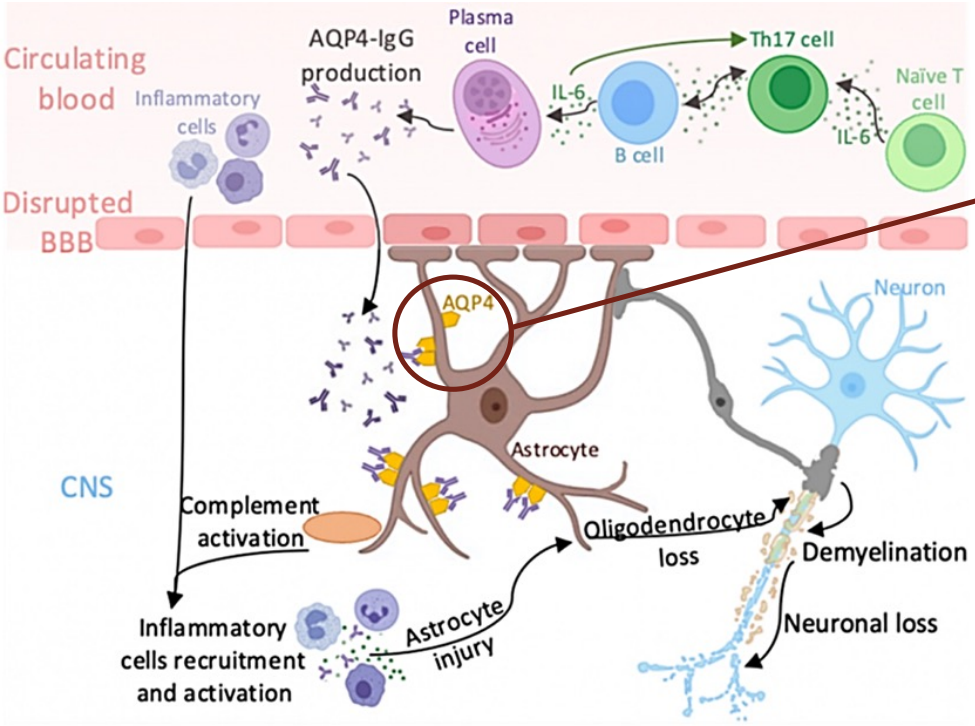
- Mean age : **40 years**
- Women > Men
- Prevalence ranges from **0.1–4.4 cases per 100,000**

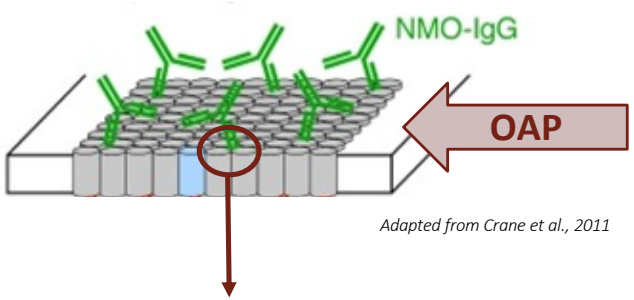


Quek et al., 2012

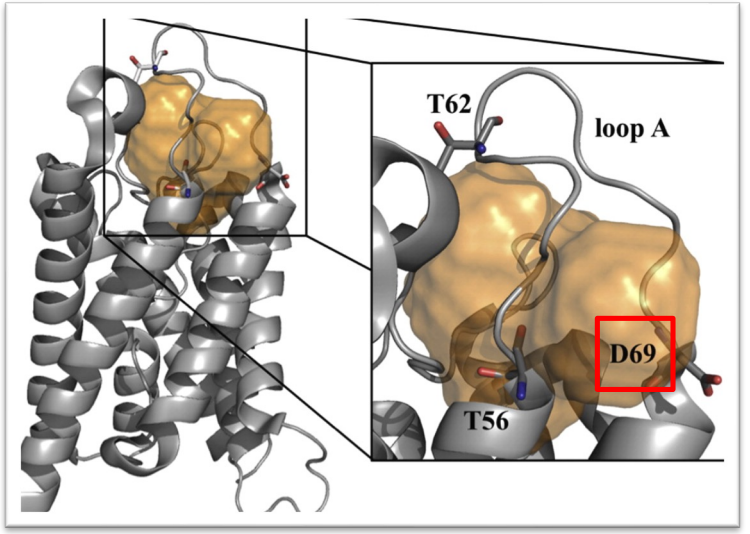
Background

MOLECULAR BASES





Adapted from Crane et al., 2011



https://www.researchgate.net/figure/Immunological-mechanism-of-MS-and-NMO-In-MS-T-helper-Th-17-and-Th1-cells_fig1_354042648

Mangiatordi et al., 2015

AIM OF THE PROJECT

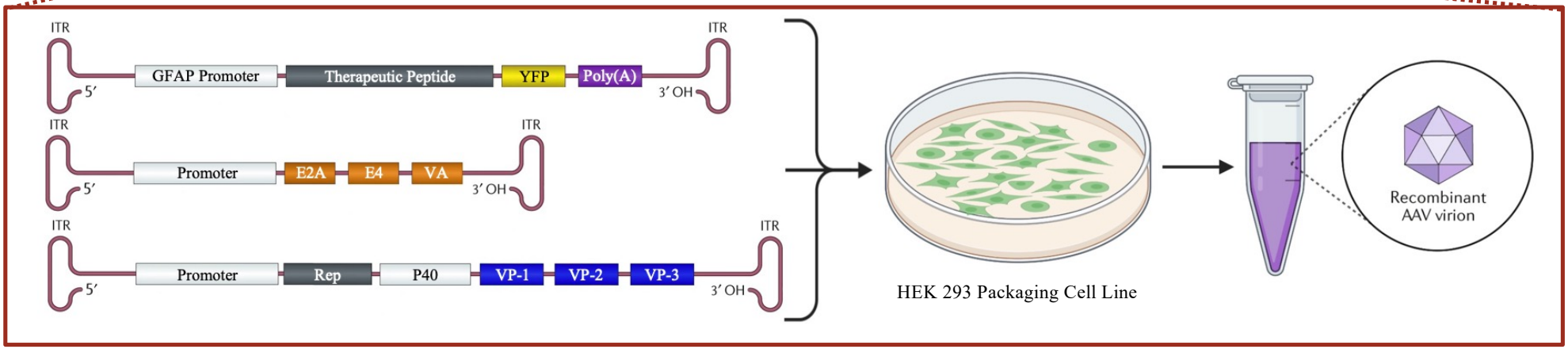
Reduction of NMO-IgG target recognition to decrease neurodegeneration

WHY? To reduce myelitis and avoid death

WHERE? In spinal cord astrocytes

HOW?

Insertion of a PEPTIDE by AAV-MEDIATED DELIVERY → MASKING OF ASP69 → PREVENTION OF AUTOANTIBODY RECOGNITION

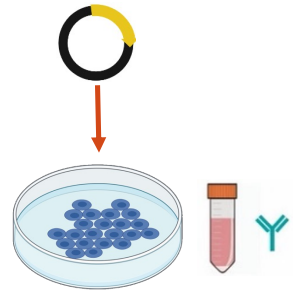


EXPERIMENTAL PLAN:

IN VITRO

- **Chinese hamster ovary (CHO) cells** expressing human **M23-AQP4** to test:
- → vector safety: toxicity's assessment
- → vector functionality: binding between antibody and channel

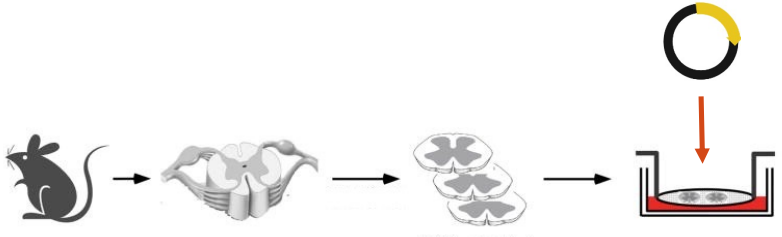
1.5 months



EX VIVO

- Transverse **slices of spinal cord from** CD1 **mice** to test:
- → AAV therapy blocks downstream cytotoxic effects of complement activation

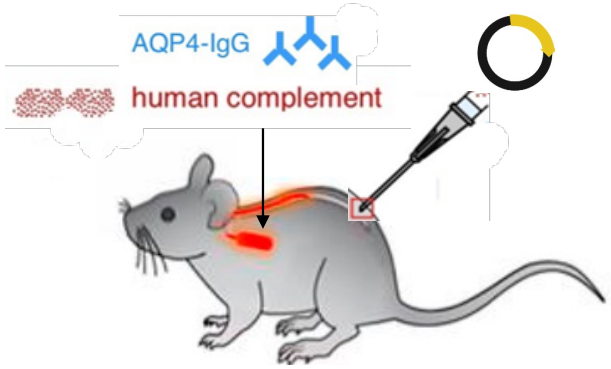
3 months



IN VIVO

- **Aldh1l1:GFP** transgenic mice to test vector functionality in:
- → limiting astrocytopathy (in vivo imaging)
- → preventing motor impairment

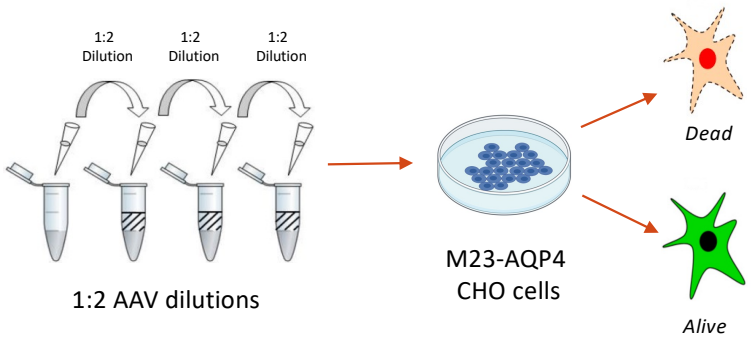
9 months

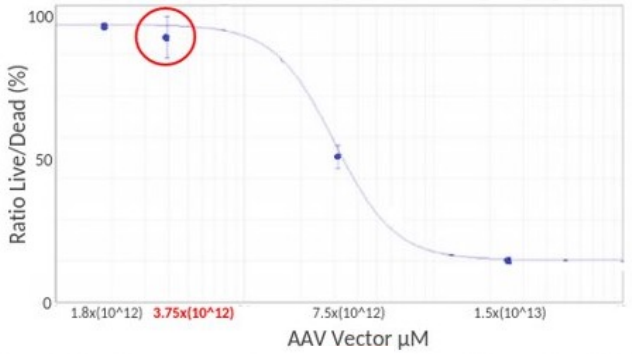


IN VITRO

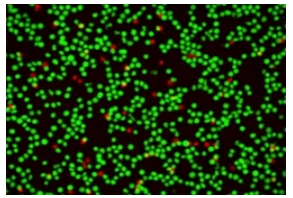
Adapted by: https://www.aatbio.com/products/live-or-dead-cell-viability-assay-kit

A **Vector's toxicity:** *Serial dilutions of AAV vector and Vitality assay (Live/Dead)*

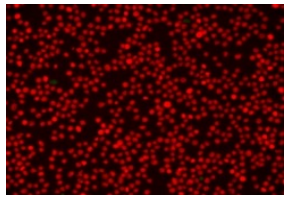




Adapted by: https://it.moleculardevices.com/applications/cell-viability-proliferation-cytotoxicity-assays

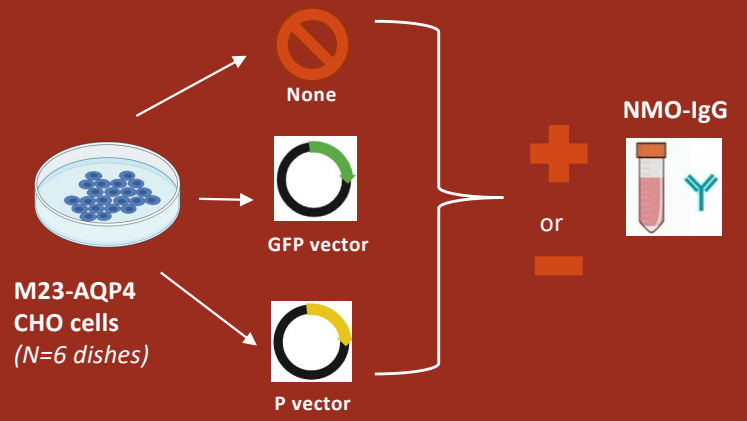


L/D at 3.75x10^12 vg/mL

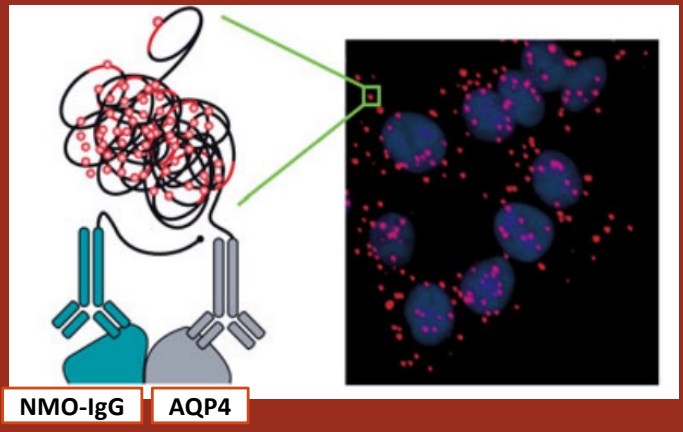


L/D at starting conc. of 1.5 x 10^13 vg/mL

B **Vector's functionality:** *Binding assay (PLA)*



Proximity Ligation Assay



Adapted by: Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1099

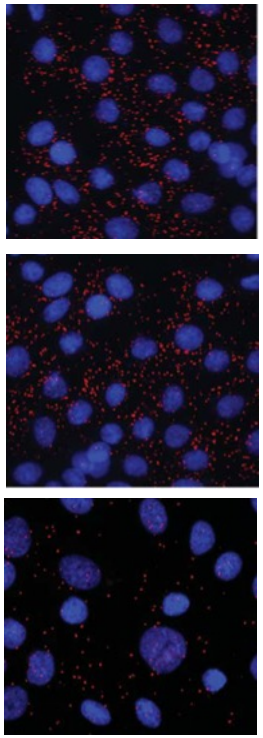
RESULTS: *in vitro*

Binding assay (PLA)

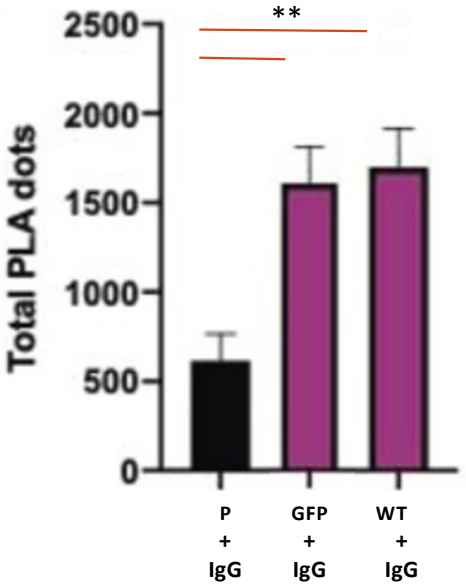
WT + IgG (BINDING CONTROL)

GFP vec + IgG (UNTREATED)

P vec + IgG (TREATED)

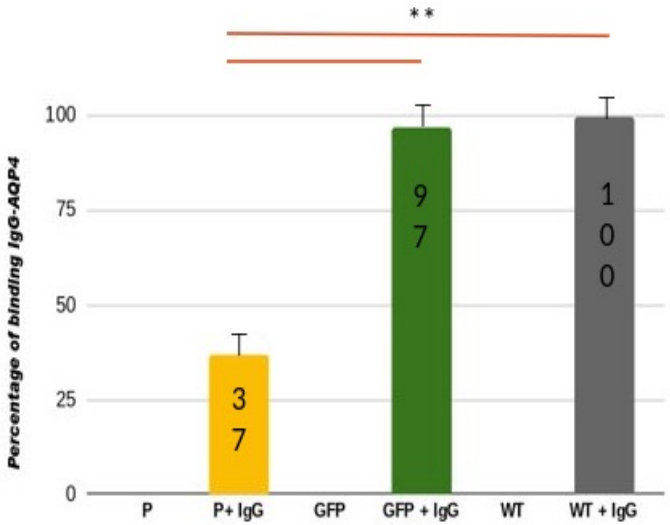


Adapted by: Hegazy et al., 2020



***= significant reduction of the PLA dots in P+IgG samples compared to controls*

Adapted by: Ching et al., 2021

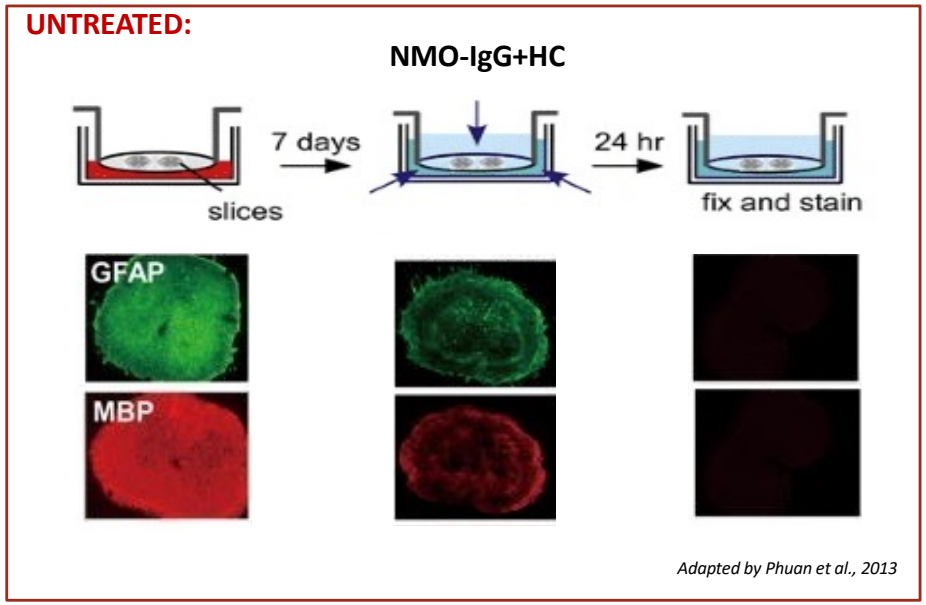


*** = significant reduction of the binding's percentage in P+IgG sample compared to controls*

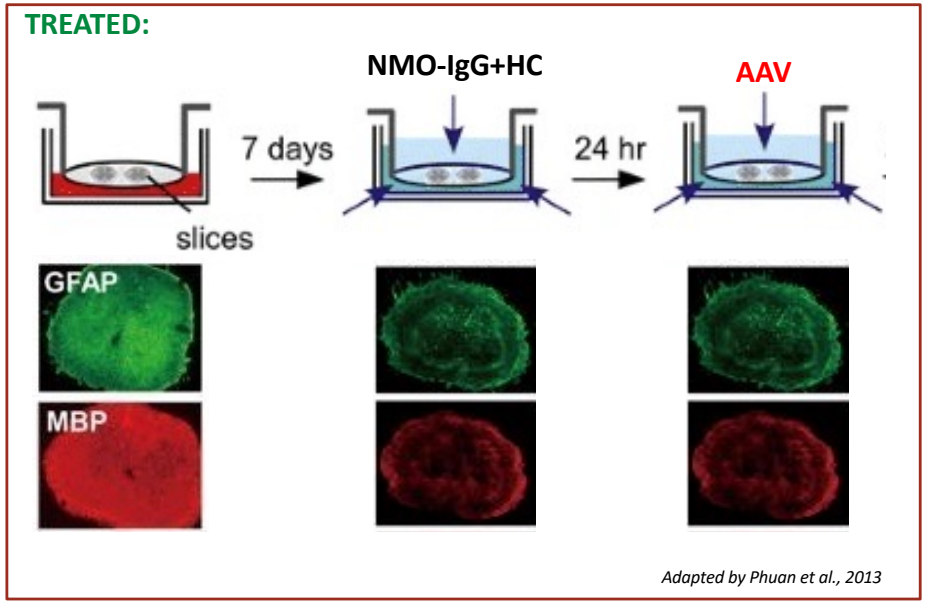
EX VIVO

EXPERIMENTAL PLAN AND RESULTS

A

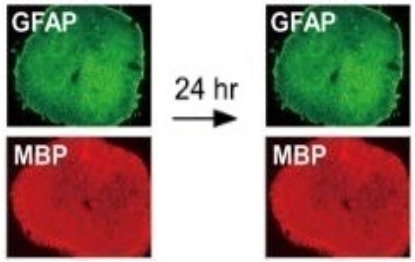


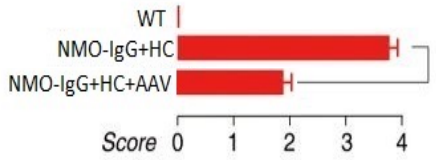
B



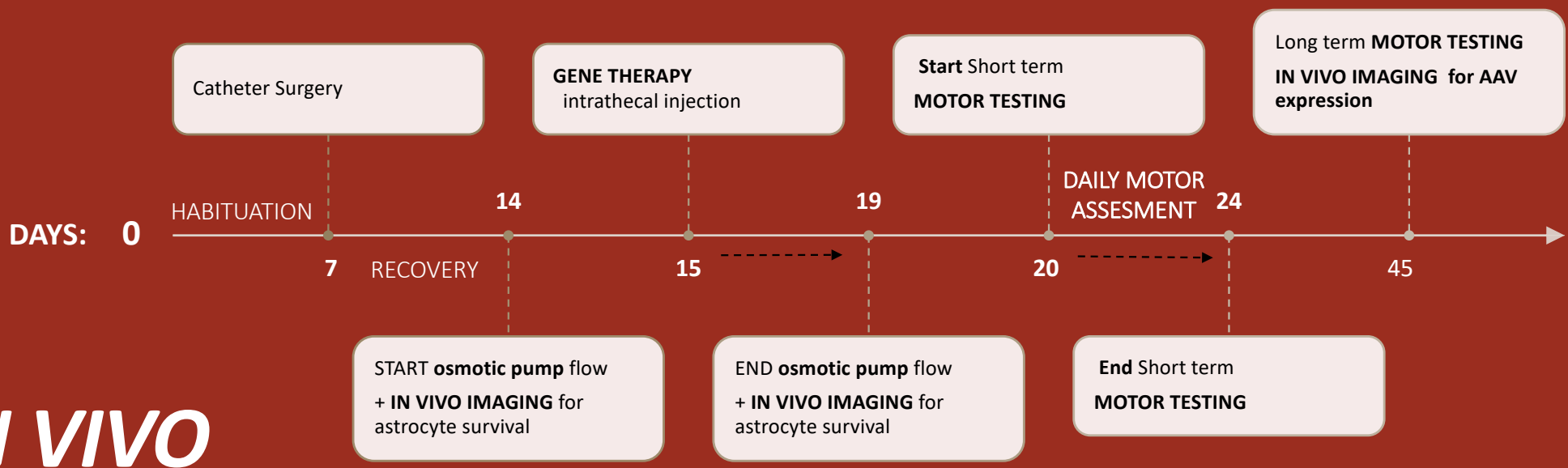
C **NOT-PATHOGENIC CONTROLS:**

1. **WT**
2. **HC**
3. **NMO-IgG**
4. **AAV**





IN VIVO



Strain
Aldh1l1:GFP
marked astrocytes

N=24
Male and female

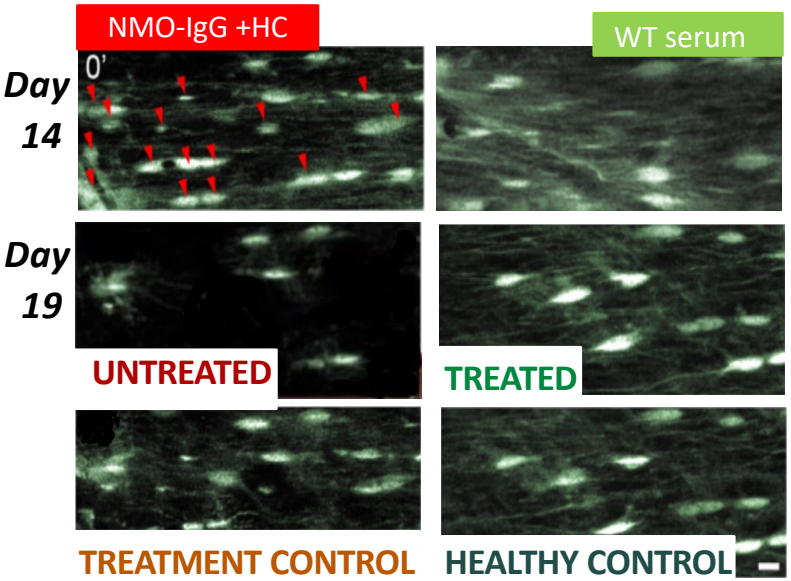
NMO-IgG +HC	AAV	**TREATED**
NMO-IgG +HC	ctrl	**UNTREATED**
WT serum+HC	AAV	**TREATMENT CONTROL**
WT serum+HC	ctrl	**HEALTHY CONTROL**

N=6 per group

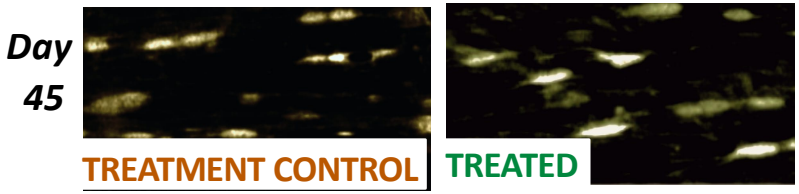
RESULTS: *in vivo*

in vivo Imaging

Astrocytopathy (GFP):

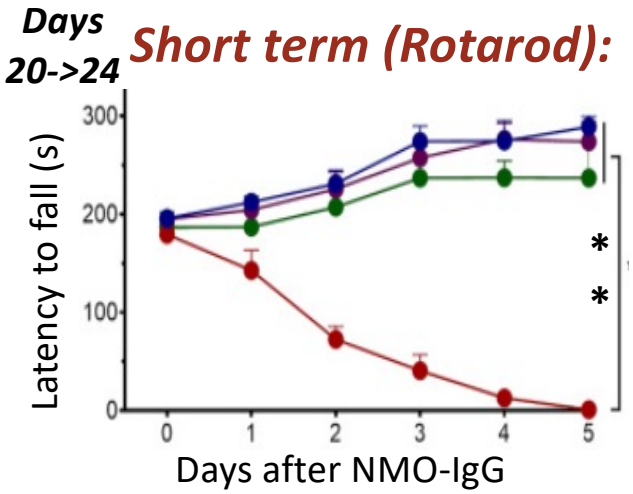


AAV Long term expression (YFP):



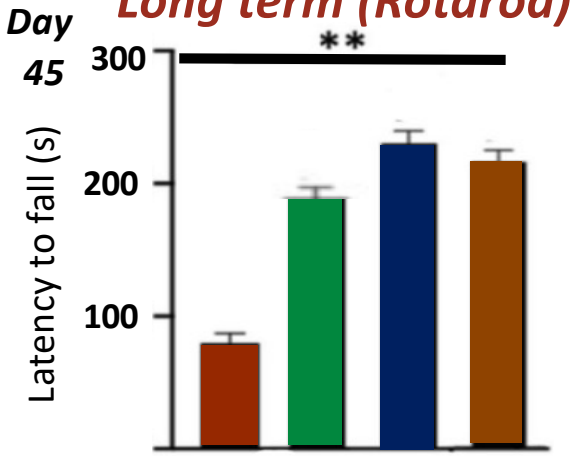
Motor Assessment

Short term (Rotarod):

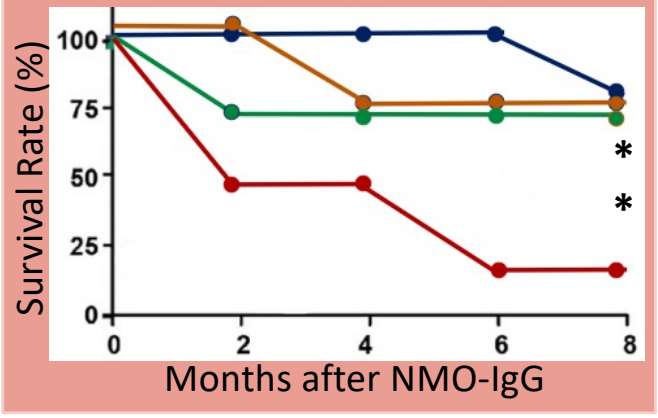


UNTREATED (NMO-IgG, HC + Ctrl)
TREATED (NMO-IgG, HC + AAV)
TREATMENT CONTROL (WT+ AAV)
HEALTHY CONTROL (WT+ Ctrl)

Long term (Rotarod):



Survival Rate



Pitfalls and Solutions

Gene therapy after late diagnosis:

progression of pathology with multiple lesions and invasive demyelination

Additional remyelinating treatment
(es. *Clobetasol*)

Safe dose of vector is shorter than circulant NMO-IgG:

fewer bonds locked than created

Preventive anti-inflammatory treatment to reduce NMO-IgG
(es. *azathioprine*, *mycophenolate* and *Rituximab)*

Conclusions and future perspectives

- ✓ AAV is not immunogenic.
- ✓ It reduces recognition and subsequent binding of the autoantibodies leaving AQP4 structure and functionality unaffected.
- ✓ Peptide is better tolerated by the immune system than a foreign protein.
- ✓ It prevent severe myelitis that is the main cause of death.

ABOUT OUR THERAPHY

- It could be a preventive therapy in presence of an early quick diagnosis.
- Masking epitope also in optic nerve to fully treat NMO.

OUR THERAPY IN THE FUTURE

Investigation of the molecular function of OAPs to consider dysregulation of their formation to further decrease the probability of autoantibody binding.

OTHER FUTURE THERAPIES

BUDGET

- HEK293 cell line - 3.500$
- AAV2, AAV.DJ/AAV8 vectors - 1.550$
- Immunofluorescence staining protocol - 250$
- IgG purification Kit - 365$
- Live/Dead Kit - 293$
- Machinery rent - 3.000$
- Transgenic mouse strain - 3.5000$
- Patients' serum and complement donated from red cross and hospitals - 0$
- Salary per year x 2 PhD students and 2 Post-doc - about 86.000$
- Duolink PLA Control Kit – PPI (Sigma-Aldrich, DUO92202-1KT) - 322$
- Renting Olympus FV1000 MPE Multiphoton Laser Scanning Microscope (With Multi-line Argon laser source for both GFP snd YFP.) - 600$
- Additional costs and supplies about - 5.500$

TOT. about $110.000/year

Experimentation time: 2.5 years

References

1. Quek AM, McKeon A, Lennon VA, Mandrekar JN, Iorio R, Jiao Y, Costanzi C, Weinshenker BG, Wingerchuk DM, Lucchinetti CF, Shuster EA, Pittock SJ. Effects of age and sex on aquaporin-4 autoimmunity. Arch Neurol. 2012 Aug;69(8):1039-43. doi: 10.1001/archneurol.2012.249. PMID: 22507888; PMCID: PMC3746965
2. Wu, Y., Zhong, L., & Geng, J. (2019). Neuromyelitis optica spectrum disorder: Pathogenesis, treatment, and experimental models. *Multiple sclerosis and related disorders*, *27*, 412–418.
3. Papadopoulos, M., Bennett, J. & Verkman, A. Treatment of neuromyelitis optica: state-of-the-art and emerging therapies. *Nat Rev Neurol* **10**, 493–506 (2014). https://doi.org/10.1038/nrneurol.2014.141
4. Ratelade, J., & Verkman, A. S. (2012). Neuromyelitis optica: aquaporin-4 based pathogenesis mechanisms and new therapies. *The international journal of biochemistry & cell biology*, *44*(9), 1519–1530.
5. Verkman, A. S., Ratelade, J., Rossi, A., Zhang, H., & Tradtrantip, L. Aquaporin-4: orthogonal array assembly, CNS functions, and role in neuromyelitis optica(2011). . *Acta pharmacologica Sinica*, *32*(6), 702–710.
6. D.M. Wingerchuk, V.A. Lennon, C.F. Lucchinetti, S.J. Pittock, B.G. Weinshenker The spectrum of neuromyelitis optica Lancet Neurol, 6 (2007), pp. 805-815
7. Nicchia GP, Mastrototaro M, Rossi A, Pisani F, Tortorella C, Ruggieri M, Lia A, Trojano M, Frigeri A, Svelto M. Aquaporin-4 orthogonal arrays of particles are the target for neuromyelitis optica autoantibodies. Glia. 2009 Oct;57(13):1363-73
8. Pisani, Francesco et al., Identification of a Point Mutation Impairing the Binding between Aquaporin-4 and Neuromyelitis Optica AutoantibodiesJournal of Biological Chemistry, Volume 289, Issue 44, 30578 – 30589
9. Mangiatordi GF, Alberga D, Siragusa L, Goracci L, Lattanzi G, Nicolotti O. Challenging AQP4 druggability for NMO-IgG antibody binding using molecular dynamics and molecular interaction fields. Biochim Biophys Acta. 2015 Jul;1848(7):1462-71
10. Duan, T. and Verkman, A.S. (2020), Experimental animal models of aquaporin-4-IgG-seropositive neuromyelitis optica spectrum disorders: progress and shortcomings. Brain Pathol, 30: 13-25.
11. B. Yang, D. Brown, A.S. Verkman The mercurial insensitive water channel (AQP-4) forms orthogonal arrays in stably transfected Chinese hamster ovary cells J. Biol. Chem., 271 (9) (1996), pp. 4577-4580
12. Yang P, Li J, Peng C, Tan Y, Chen R, Peng W, Gu Q, Zhou J, Wang L, Tang J, Feng Y, Sun Y. TCONS_00012883 promotes proliferation and metastasis via DDX3/YY1/MMP1/PI3K-AKT axis in colorectal cancer. Clin Transl Med. 2020 Oct;10(6):e211
13. Phuan, PW., Zhang, H., Asavapanumas, N. *et al.* C1q-targeted monoclonal antibody prevents complement-dependent cytotoxicity and neuropathology in in vitro and mouse models of neuromyelitis optica. *Acta Neuropathol* **125**, 829–840 (2013).
14. Verkman AS, Phuan PW, Asavapanumas N, Tradtrantip L. Biology of AQP4 and Anti-AQP4 Antibody: Therapeutic Implications for NMO. Brain Pathol. 2013;23:684–95.
15. Chen, T.; Lennon, V.A.; Liu, Y.U.; Bosco, D.B.; Li, Y.; Yi, M.H.; Zhu, J.; Wei, S.; Wu, L.J. Astrocyte-microglia interaction drives evolving neuromyelitis optica lesion . *J. Clin. Investig.* **2020**, *130*, 4025–4038
16. Herwerth M, Kalluri SR, Srivastava R, et al. In vivo imaging reveals rapid astrocyte depletion and axon damage in a model of neuromyelitis optica-related pathology. Ann Neurol. 2016;79:794–805.
17. Elabi, O., Gaceb, A., Carlsson, R. *et al.* Human α-synuclein overexpression in a mouse model of Parkinson's disease leads to vascular pathology, blood brain barrier leakage and pericyte activation. *Sci Rep* **11**, 1120 (2021).
18. Rajiv R. Mohan, Jonathan C. K. Tovey, Ajay Sharma, Gregory S. Schultz, John W. Cowden, Ashish Tandon, Targeted Decorin Gene Therapy Delivered with Adeno-Associated Virus Effectively Retards Corneal Neovascularization *In Vivo, Plos One, (Oct 2011)*
19. Jared Ching, Andrew Osborne, Richard Eva, Julien Prudent, Patrick Yu-Wai-Man, "Quantifying inter-organelle membrane contact sites using proximity ligation assay in fixed optic nerve sections", *Exp Eye Res*, (Dec 2021)
20. Marihan Hegazy,Eran Cohen-Barak,Jennifer L. Koetsier,Nicole A. Najor,Constadina Arvanitis,Eli Sprecher,Kathleen J. Green,Lisa M. Godsel, "Proximity Ligation Assay for Detecting Protein-Protein Interactions and Protein Modifications in Cells and Tissues in Situ, *Current Protocols in Cell Biology*, (Oct 2020)
21. Acta Universitatis Upsaliensis (AUU), Digital comprehensive summaries of Uppsala dissertations from the Faculty of Medicine, 2005
22. Yao, X., Su, T. & Verkman, A.S. Clobetasol promotes remyelination in a mouse model of neuromyelitis optica. *acta neuropathol commun* **4**, 42 (2016).
23. Nicchia, G. P., Rossi, A., Mola, M. G., Pisani, F., Stigliano, C., Basco, D., Mastrototaro, M., Svelto, M., & Frigeri, A. (2010). Higher order structure of aquaporin-4. *Neuroscience*, *168*(4), 903–914.
24. Furman, C. S., Gorelick-Feldman, D. A., Davidson, K. G., Yasumura, T., Neely, J. D., Agre, P., & Rash, J. E. (2003). Aquaporin-4 square array assembly: opposing actions of M1 and M23 isoforms. *Proceedings of the National Academy of Sciences of the United States of America*, *100*(23), 13609–13614.
25. Jin, B. J., Rossi, A., & Verkman, A. S. (2011). Model of aquaporin-4 supramolecular assembly in orthogonal arrays based on heterotetrameric association of M1-M23 isoforms. *Biophysical journal*, *100*(12), 2936–2945.
26. Rossi, A., Moritz, T. J., Ratelade, J., & Verkman, A. S. (2012). Super-resolution imaging of aquaporin-4 orthogonal arrays of particles in cell membranes. *Journal of cell science*, *125*(Pt 18), 4405–4412.
27. Crane, J. M., Lam, C., Rossi, A., Gupta, T., Bennett, J. L., & Verkman, A. S. (2011). Binding affinity and specificity of neuromyelitis optica autoantibodies to aquaporin-4 M1/M23 isoforms and orthogonal arrays. *The Journal of biological chemistry*, *286*(18), 16516–16524.

Supplementary: OAPs

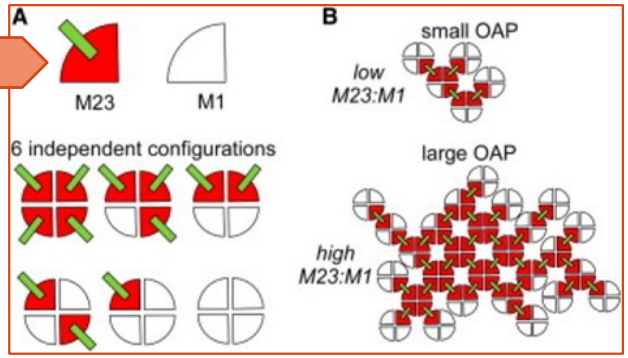
- AQP4-M23 alone forms stable OAPs (*fig. 2*).
- M1 is unable to form on its own OAPs (*fig 2*).
- When M1-M23 are co-expressed, they form OAPs of intermediate size because M1 blocks intertetrameric M23 associations (*fig. 1-2*)

Many OAP configurations are possible in order to concentration M23/M1 ratio *(fig. 1-3-4*).

The greater the M23 isoform expression, the larger the pool and the OAPs'size (*fig. 3-4*).

1,3: Jin et al., 2011

1



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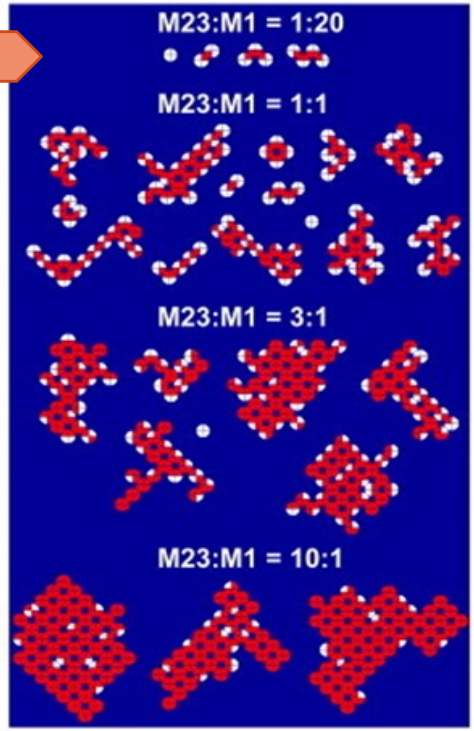
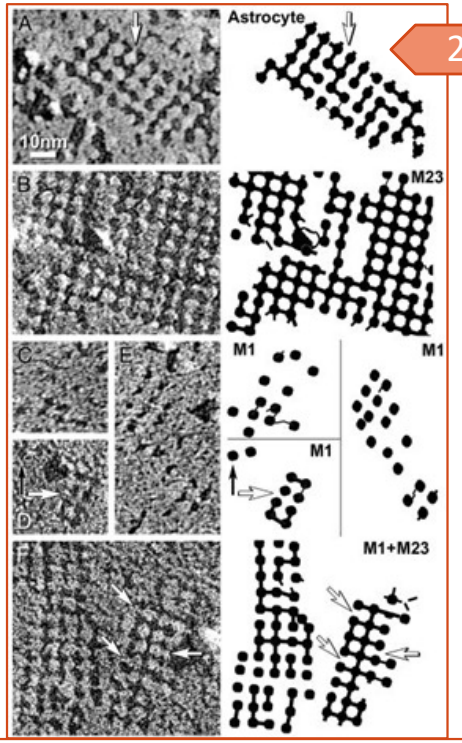


Furman et al., 2003

2



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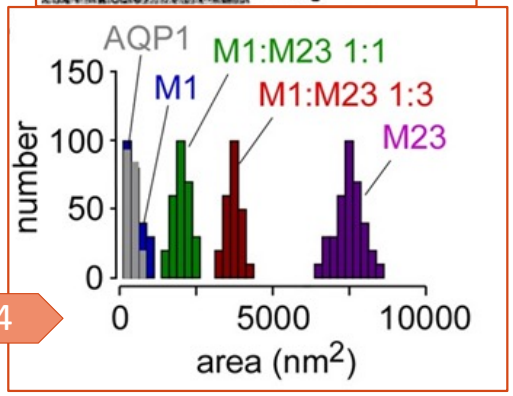

Rossi et al., 2012