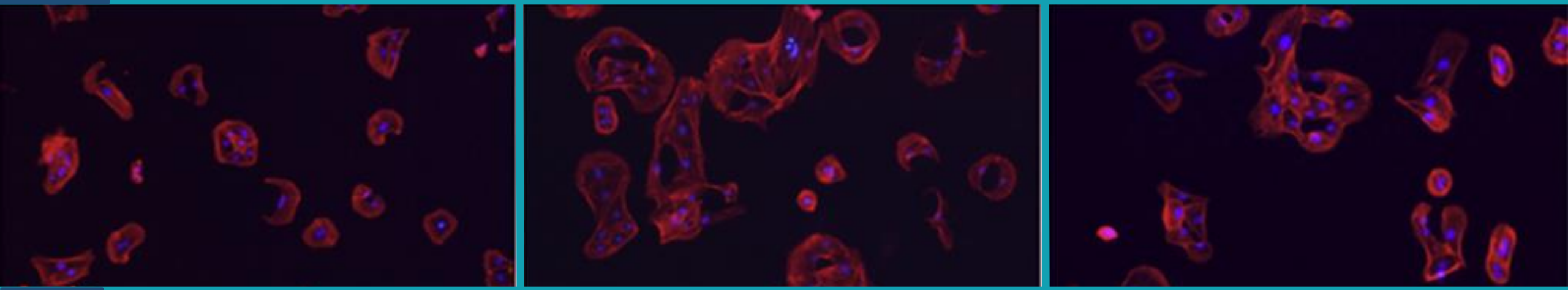
A novel combination therapy: NF2 gene addition and bevacizumab in vestibular schwannoma

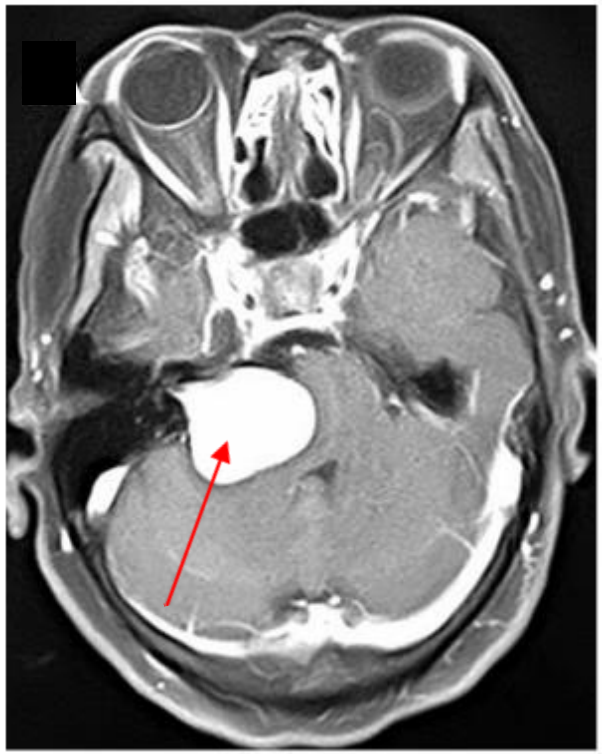


Theme II: Cancer
Group I: Addario Chieco Caterina Virginia, Furina Isabella, Meoni Martina, Pelli Fabrizio

1 Background

Vestibular Schwannoma is an autosomal dominantly inherited syndrome that predisposes individuals to multiple nervous tumors. Patients develop bilateral or unilateral schwannomas on the vestibular portion of the VIII cranial nerve and on other cranial nerves, spinal roots, or peripheral nerves.

This condition occurs in **1 in 25000** people. The actuarial survival after diagnosis is **15 years,** with an average age at death of 36 years and a 10-years survival rate of 67%.



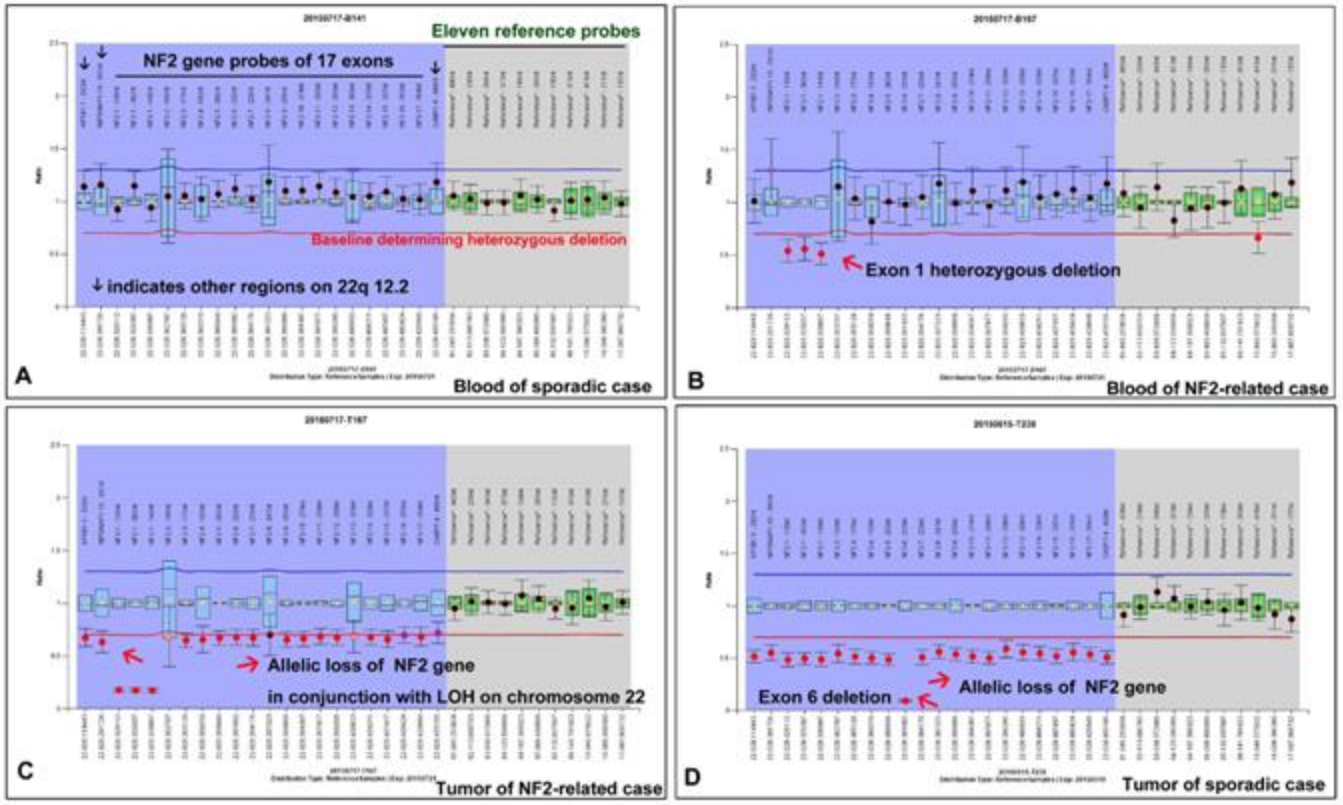
Li et al., 2021

There is **no established effective treatment** for schwannomas because these tumors are **highly likely to regrow** after surgical resection and there is also the risk that this can cause a malignant transformation.

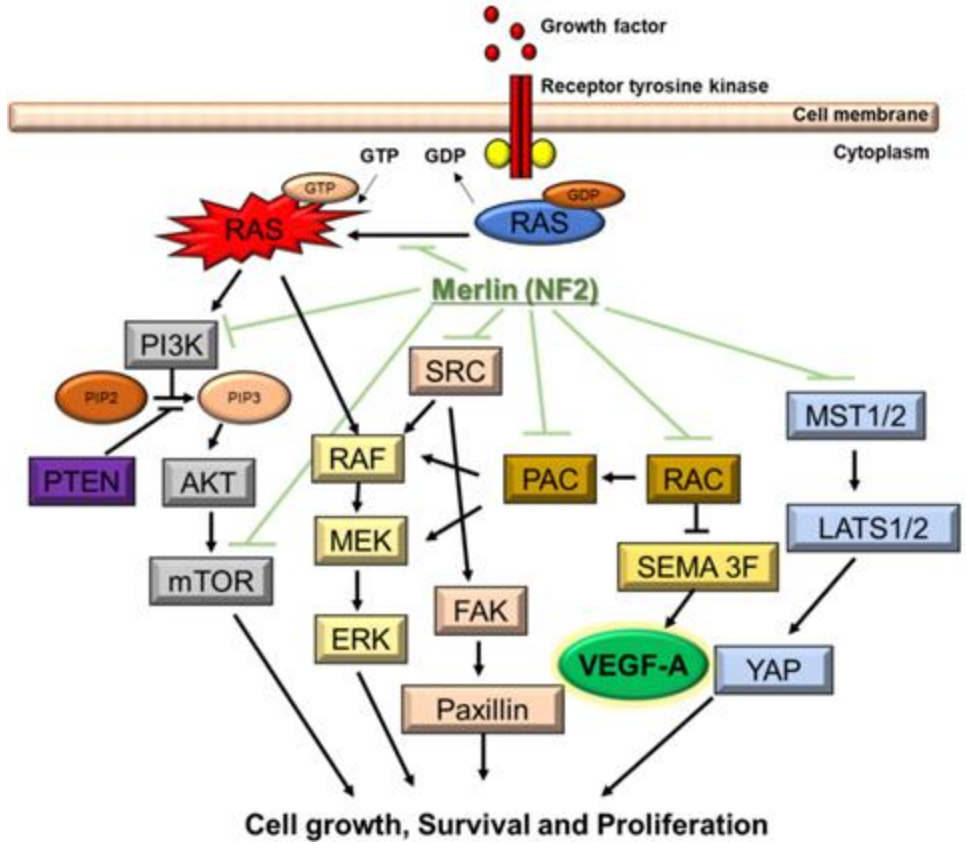
→ 122 clinical trials

Background

Vestibular Schwannoma is caused by a defect in the **NF2** gene that normally produces **Merlin**, located at 22q12.2 of chromosome 22, which regulates multiple proliferative signaling pathways.

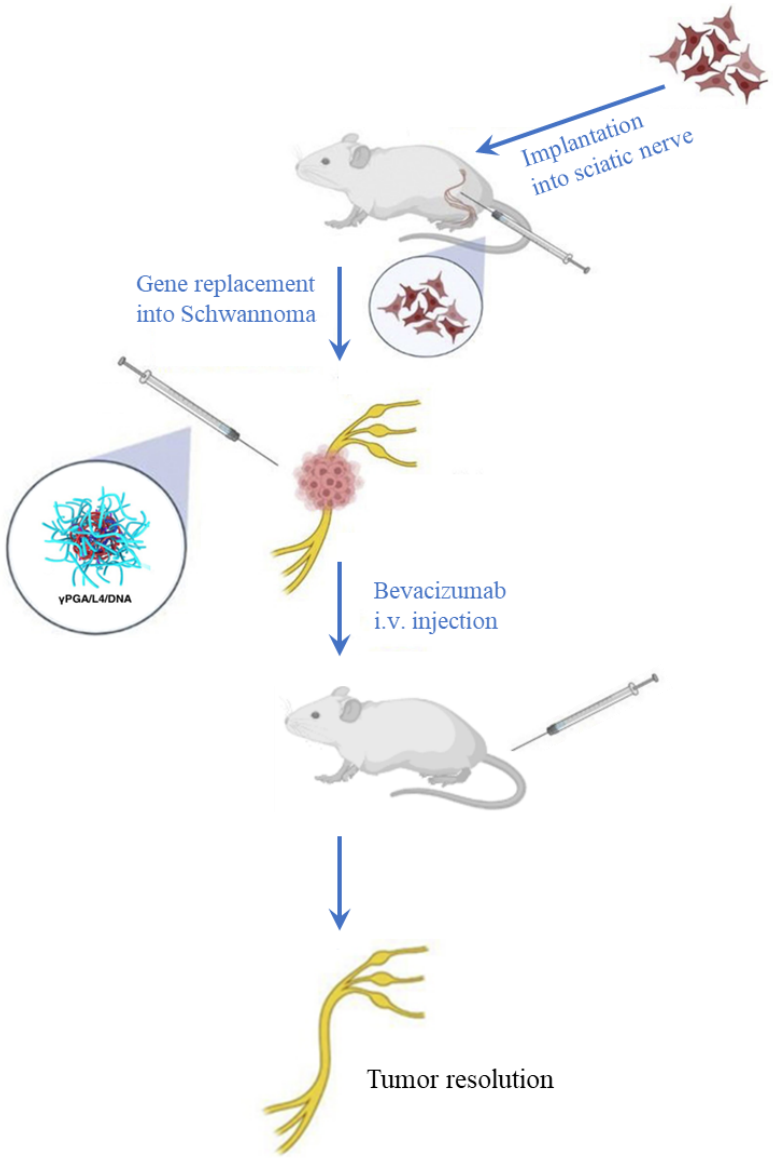


Chen et al., 2017. Nat. Sci. Rep.



Tamura et al., 2022

Aim of the project



NEW APPROACH THERAPY

↓

COMBINATION THERAPY

↓

Gene replacement *(NF2)*

+

Pharmacolagical treatment *(Bevacizumab)*

↓

COMPLETE REGRESSION OF THE TUMOR

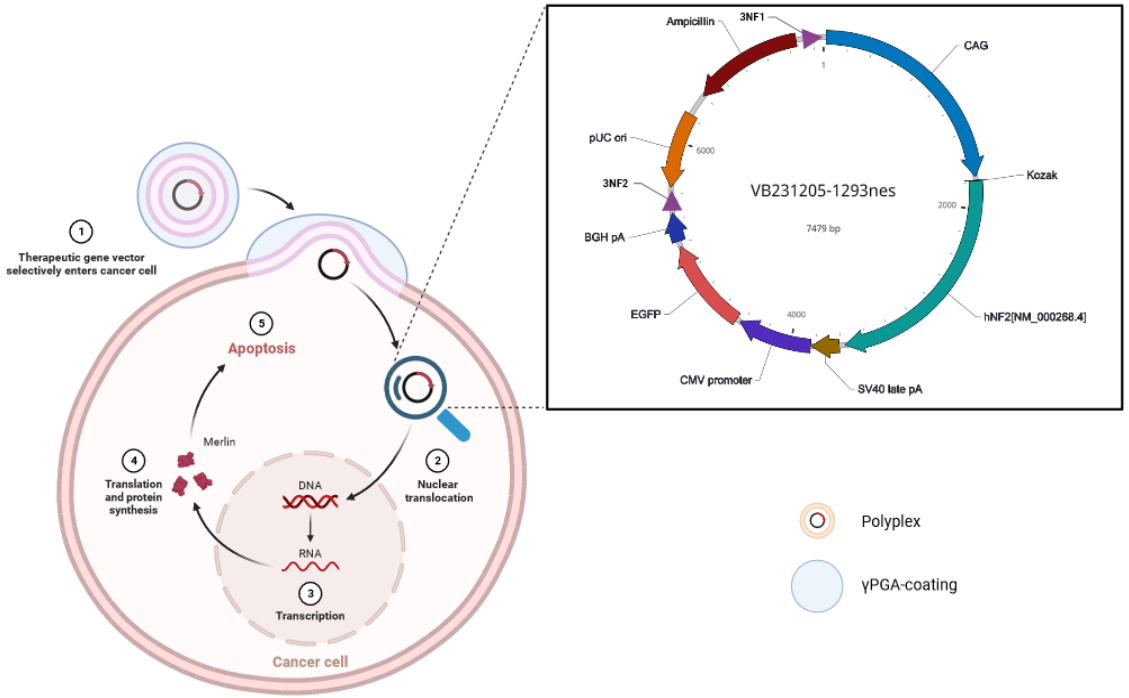
Treatments

Bevacizumab

- It is a monoclonal antibody directed against VEGF
- Induces 40% tumor regression
- Sadly, it causes severe dose-dependent side effects

Dosage
10 mg/Kg

Functionalized polyplexes



Gene copies/mouse
4×10^8

Animal model

Animals will be injected with tumor cells at 4-6 weeks of age

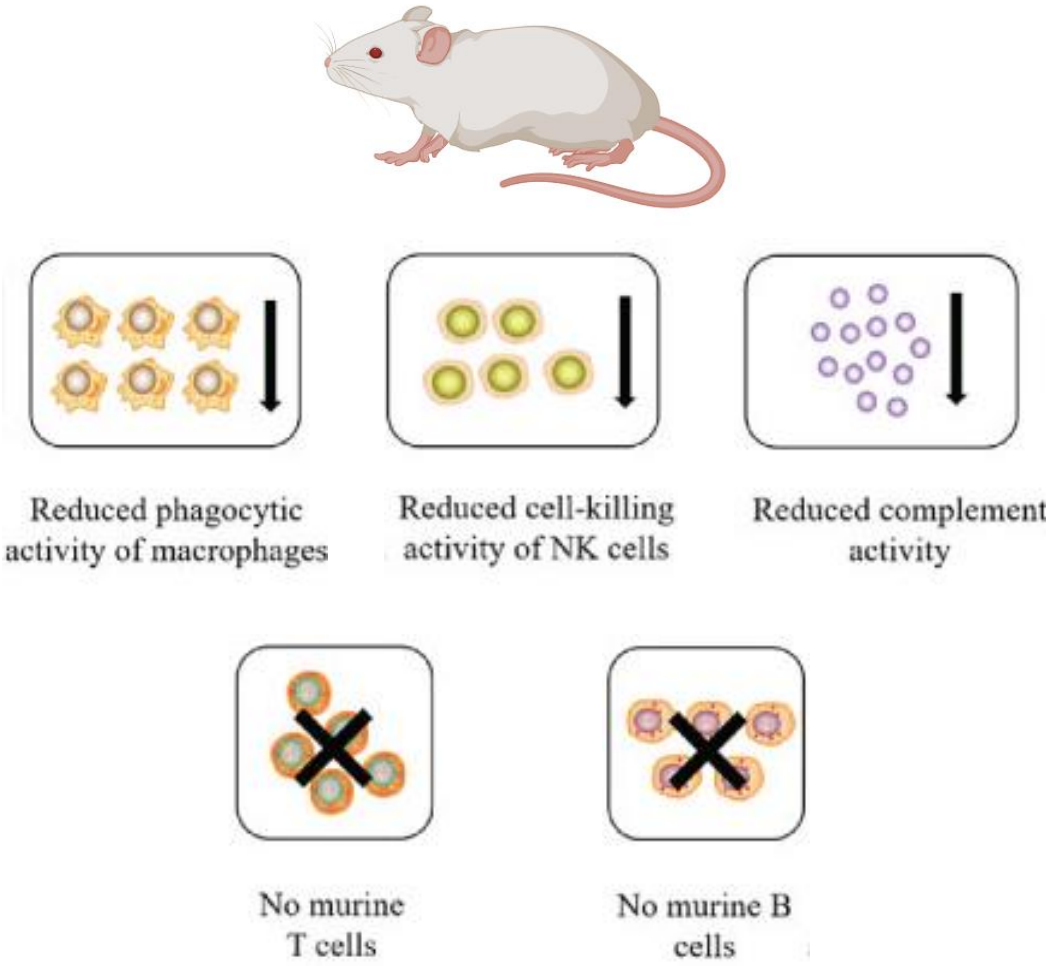
GENERAL DESCRIPTION

- Immunocompromised strain
- Spontaneous mutation of *Prkdc* gene
- Ideal model for xenograft human tumors

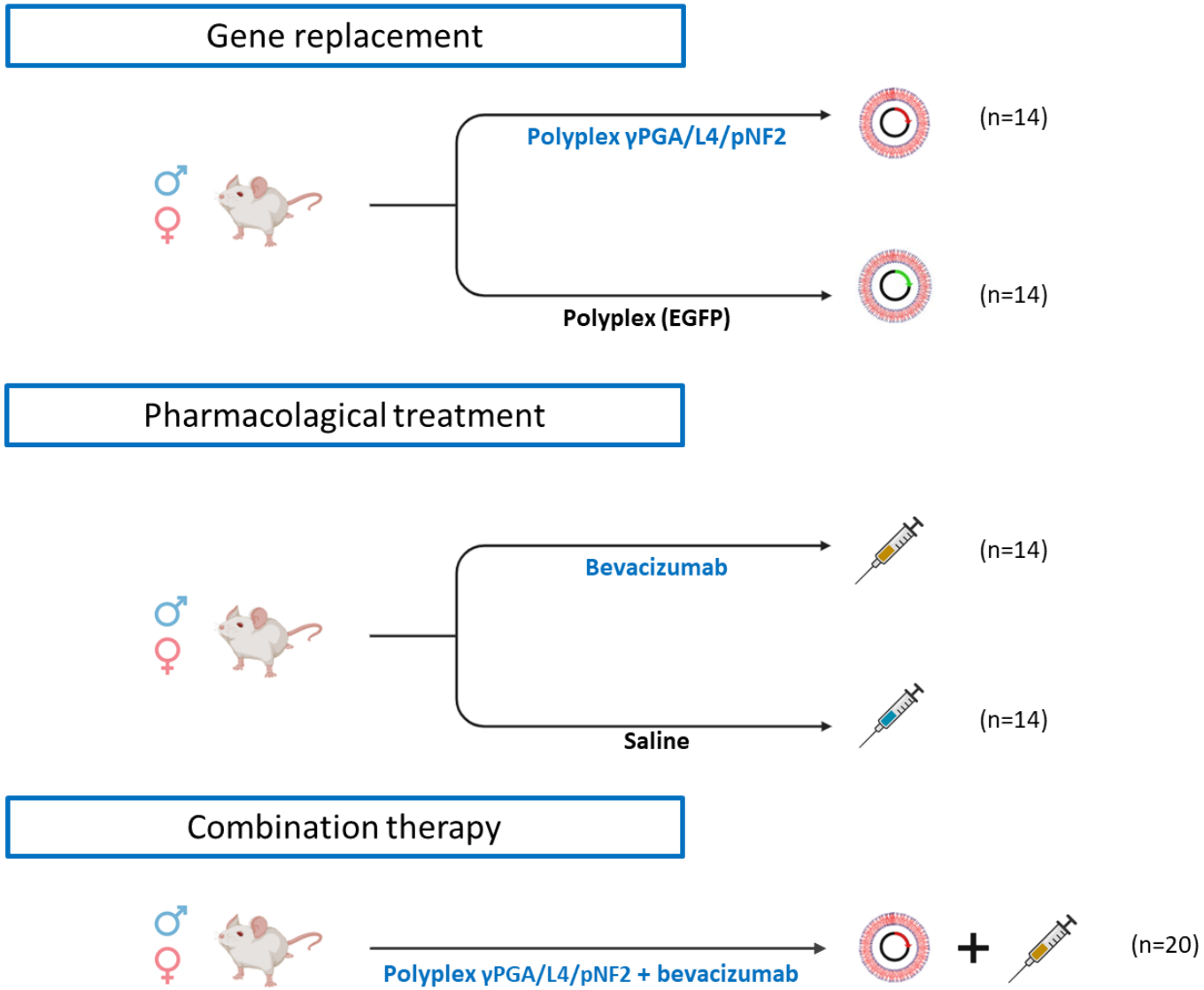
MAINTENANCE

- Specific pathogens free (SPF)
- Sterilised material in autoclave
- Forced-ventilated racks (IVC)
- Staff must wear sterile clothes

JAX® Mice: NOD SCID

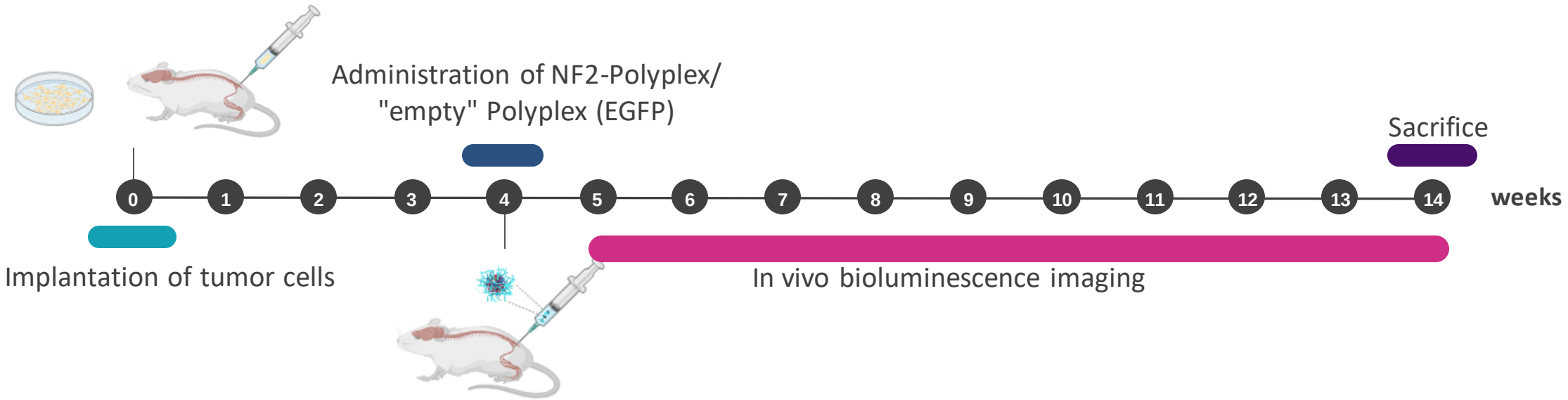


Experimental groups

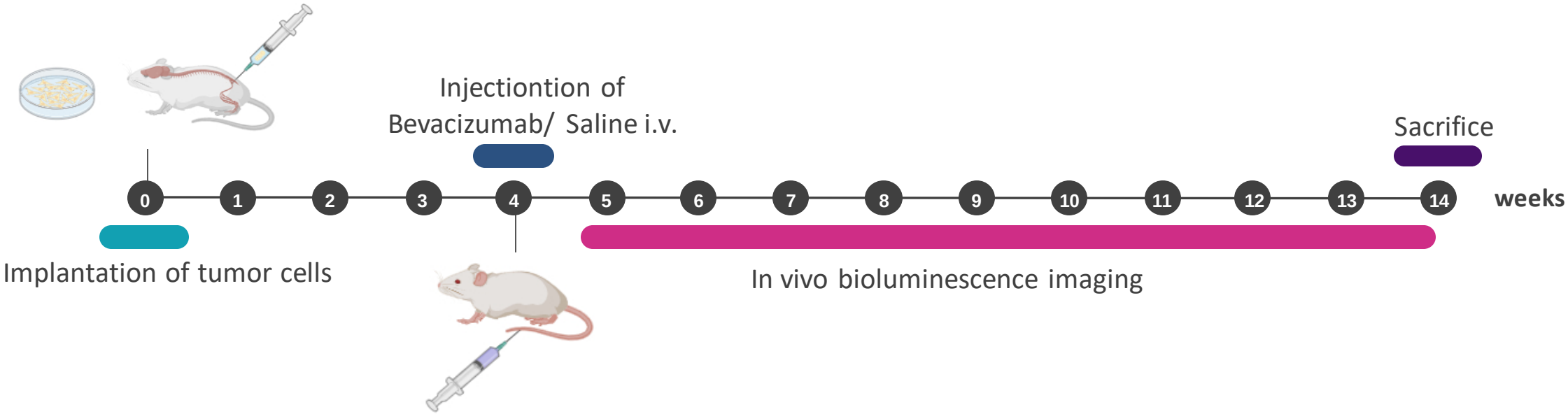


Experimental plan

Gene replacement

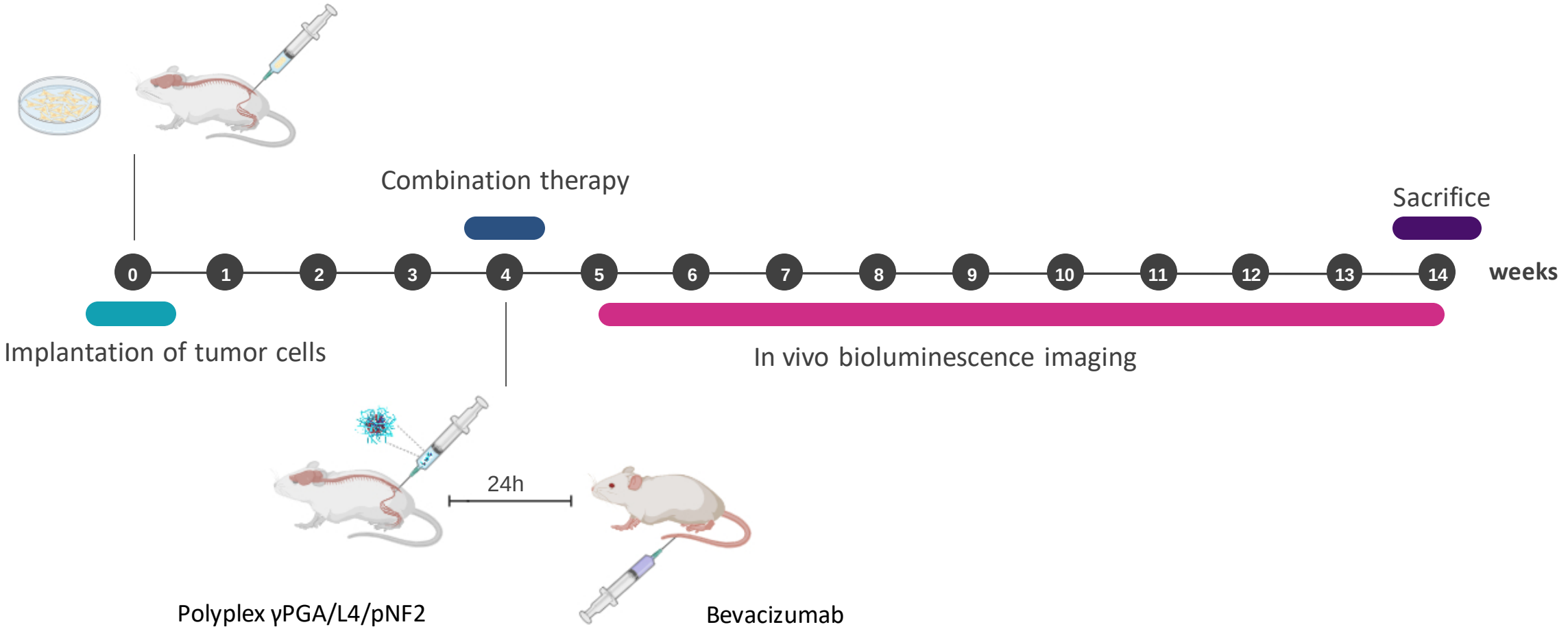


Pharmacolagical treatment



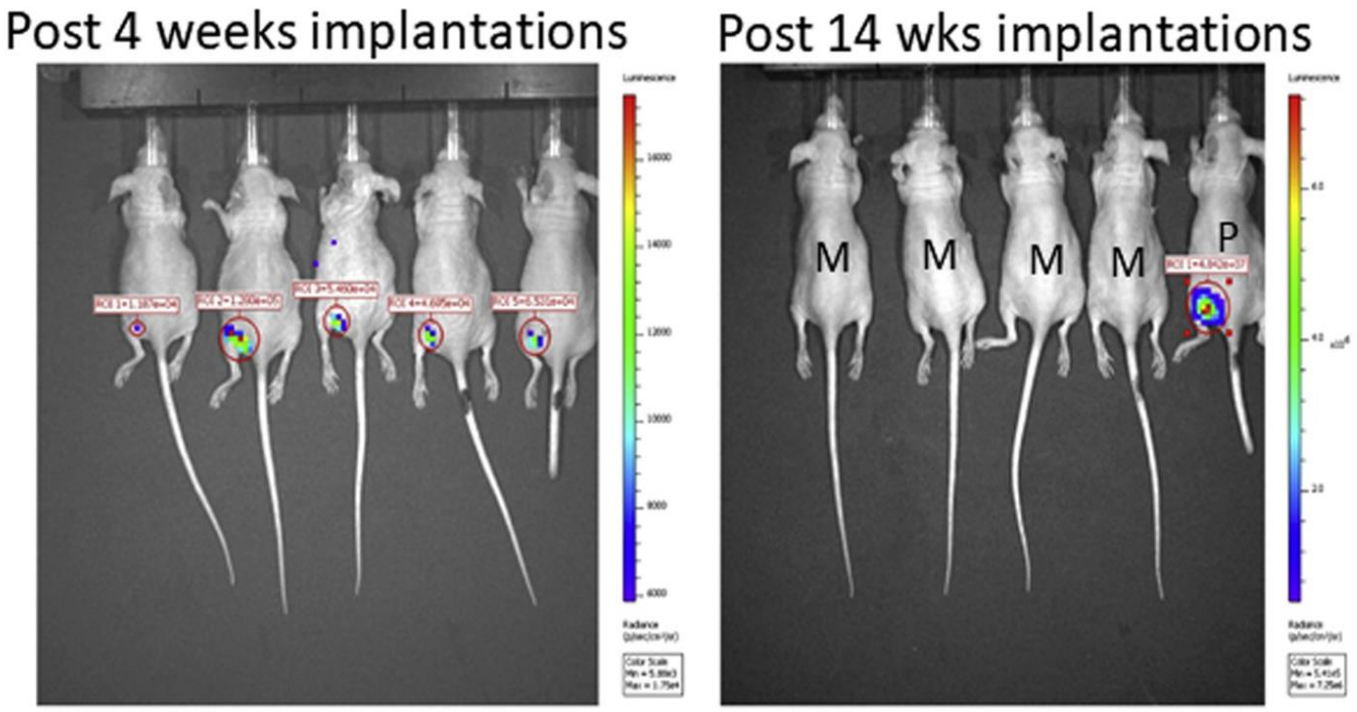
Experimental plan

Combination therapy



Expected results

A)



Prabhakar et al., 2022. Molecular Therapy.

B)

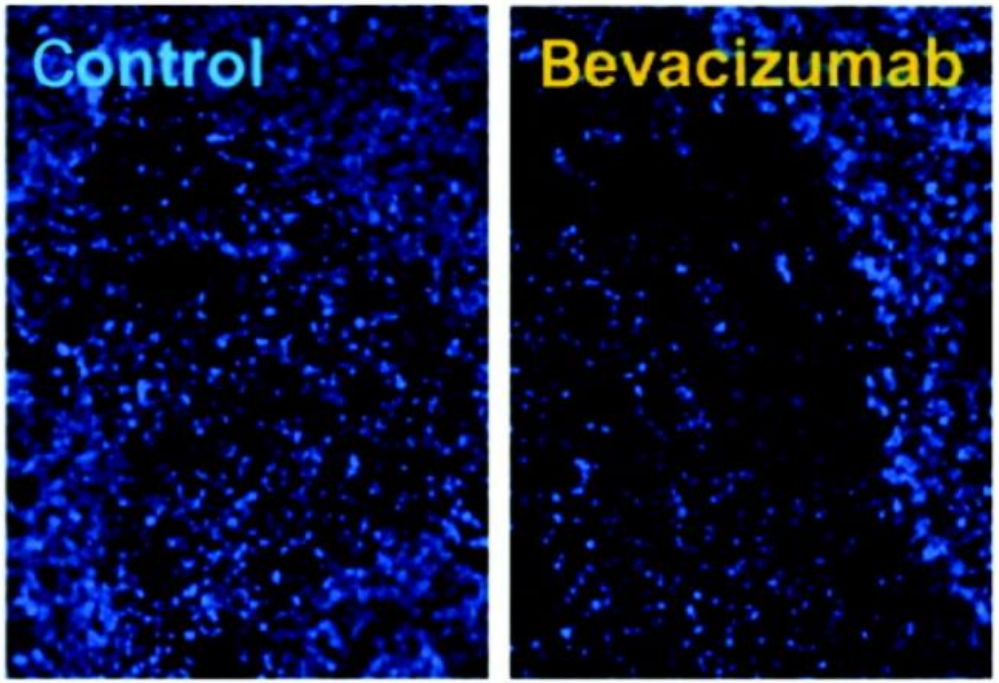
Post injected	NF2	PBS
1 week	1.14E+04	1.08E+04
2 weeks	1.19E+04	6.55E+04
3 weeks	0.00E+00	7.24E+04
4 weeks	3.81E+04	8.02E+04
5 weeks	7.71E+04	8.32E+04
6 weeks	8.63E+04	9.91E+04
7 weeks	5.59E+04	1.66E+05
8 weeks	0.00E+00	1.79E+05
9 weeks	0.00E+00	1.81E+05
10 weeks	0.00E+00	1.98E+05
11 weeks	0.00E+00	2.70E+05
12 weeks	0.00E+00	4.70E+05
13 weeks	0.00E+00	5.72E+05
14 weeks	0.00E+00	6.62E+05
Vector was injected at post 4 weeks.		

edit from Prabhakar et al., 2022. Molecular Therapy.

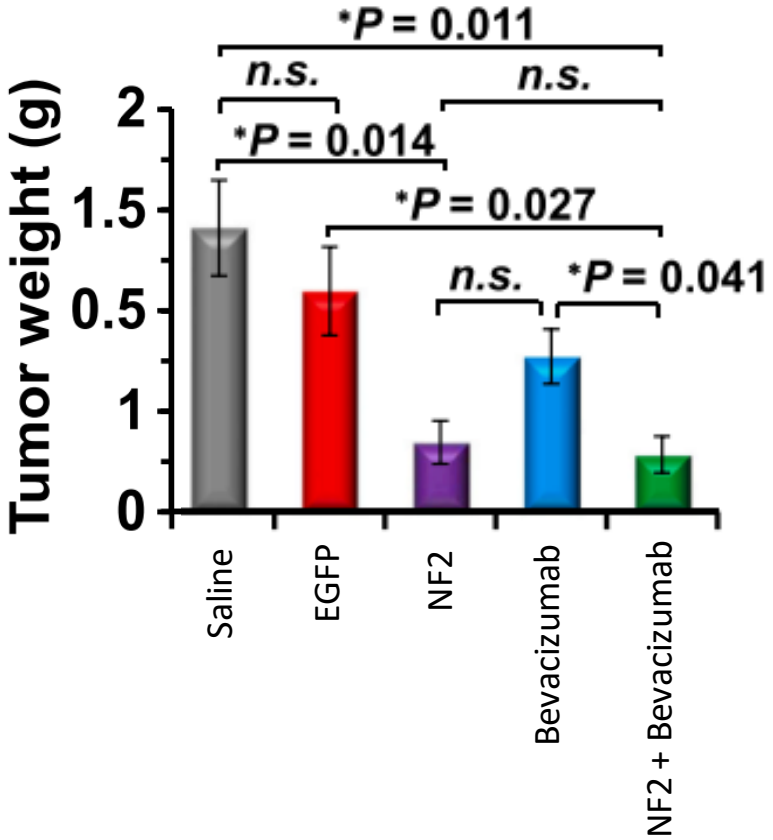
A) Bioluminescence imaging (BLI) of the mice with tumor signals. Four weeks after implantation of tumor cells in the sciatic nerve (left, before vector injection) and 14 weeks after NF2 replacement (right, after vector injection at week 4–5).

B) Complete tumor regression is observed in 77% of the mice after 14 weeks monitoring.

Expected results



Wong et al., 2010. Cancer Res.



Schwannomas are vascularized: anti-VEGF therapy decrease vessel size and number

↓

Anti-VEGF therapy delays tumor growth and improves overall survival in mice

This graph is only predictive of the possible outcome of our combination approach, but it is based on real data from previous studies that use similar treatments.

Conclusion

- Bevacizumab is a first-choice drug for schwannoma treatment, but it has a short-term effect, for this reason we speculate that a combination therapy with the reintroduction of NF2 gene can provide a definitive tumor resolution

- Schwannoma gene therapy for NF2 reintroduction it is not a popular approach yet, and when it is used, it is carried out with AAV vectors: our innovation consists into using **Polyplexes**, a family of non-viral vectors made of self-assembly engineered nanoparticles

- Polyplex-mediated delivery provide comparable efficiency to traditional viral vectors, but they are **cheaper** and **less immunogenic**

- Our pursuit is to lay out a definitive therapy that aims to completely eliminate the tumor, but also to minimize the risk of tumor recurrence that is high even after surgical removal

Budget & Timing

	Cost per unit (€)	Cumulative cost (€)
Model		
JAX® Mice: NOD SCID	177,48	177,48 x 21 = 3727,08
JEI-001 Luc2-expressing Schwannoma cells	6.000 + 800*	6.800 x 1 = 6.800
Treatments		
Avastin® (bevacizumab) 400 mg	1.289	1.289 x 1 = 1.289
NF2-containing plasmid 25 ul, >100 ng/ul	239	239 x 1 = 239
PEI Prime linear polyetilenimine 100 mg	240	240 x 1 = 240
γ-PGA (100 mg)	302	302 x 1 = 302
Others expenses		
Luciferin 5 mg (x 5)	66	66 x 5 = 330
SuperCult® Schwann Cell Medium 500 ml	750	750 x 1 = 750
Plasmid control (GFP expressing)	189	189 x 1 = 189
Total		13.866,08 €

Timing

Step	Time (months)
Colony engraphment	4-5
Experimental plan	5
Analysis (immunohistochemistry for Ki67, imaging acquisition & quantification)	2
Analysis (statistical)	2
Total	13-14

Bibliography

- Tamura, R., & Toda, M. (2022). A Critical Overview of Targeted Therapies for Vestibular Schwannoma. *International journal of molecular sciences*, *23*(10), 5462. https://doi.org/10.3390/ijms23105462
- Tamura, R., Fujioka, M., Morimoto, Y., Ohara, K., Kosugi, K., Oishi, Y., Sato, M., Ueda, R., Fujiwara, H., Hikichi, T., Noji, S., Oishi, N., Ogawa, K., Kawakami, Y., Ohira, T., Yoshida, K., & Toda, M. (2019). A VEGF receptor vaccine demonstrates preliminary efficacy in neurofibromatosis type 2. *Nature communications*, *10*(1), 5758. https://doi.org/10.1038/s41467-019-13640-1
- Wong, H. K., Lahdenranta, J., Kamoun, W. S., Chan, A. W., McClatchey, A. I., Plotkin, S. R., Jain, R. K., & di Tomaso, E. (2010). Anti-vascular endothelial growth factor therapies as a novel therapeutic approach to treating neurofibromatosis-related tumors. *Cancer research*, *70*(9), 3483–3493. https://doi.org/10.1158/0008-5472.CAN-09-3107
- Tamura R. (2021). Current Understanding of Neurofibromatosis Type 1, 2, and Schwannomatosis. *International journal of molecular sciences*, *22*(11), 5850. https://doi.org/10.3390/ijms22115850
- Kulkarni, J. A., Myhre, J. L., Chen, S., Tam, Y. Y. C., Danescu, A., Richman, J. M., & Cullis, P. R. (2017). Design of lipid nanoparticles for in vitro and in vivo delivery of plasmid DNA. *Nanomedicine : nanotechnology, biology, and medicine*, *13*(4), 1377–1387. https://doi.org/10.1016/j.nano.2016.12.014
- Tros de Ilarduya, C., Sun, Y., & Düzgüneş, N. (2010). Gene delivery by lipoplexes and polyplexes. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences*, *40*(3), 159–170. https://doi.org/10.1016/j.ejps.2010.03.019
- Prabhakar, S., Beauchamp, R. L., Cheah, P. S., Yoshinaga, A., Haidar, E. A., Lule, S., Mani, G., Maalouf, K., Stemmer-Rachamimov, A., Jung, D. H., Welling, D. B., Giovannini, M., Plotkin, S. R., Maguire, C. A., Ramesh, V., & Breakefield, X. O. (2022). Gene replacement therapy in a schwannoma mouse model of neurofibromatosis type 2. *Molecular therapy. Methods & clinical development*, *26*, 169–180. https://doi.org/10.1016/j.omtm.2022.06.012
- Li, J., Wang, Q., Zhang, M., Zhang, G., Zhang, S., & Hui, X. (2021). Malignant Transformation in Vestibular Schwannoma: Clinical Study With Survival Analysis. *Frontiers in oncology*, *11*, 655260. https://doi.org/10.3389/fonc.2021.655260
- Guowei Wang, Siqin Chen, Nasha Qiu, Bihan Wu, Dingcheng Zhu, Zhuxian Zhou, Ying Piao, Jianbin Tang, Youqing Shen. (2021)Virus-mimetic DNA-ejecting polyplexes for efficient intracellular cancer gene delivery.Nano Today,Volume 39,2021,101215, https://doi.org/10.1016/j.nantod.2021.101215
- Chen, H., Xue, L., Wang, H., Wang, Z., & Wu, H. (2017). Differential NF2 Gene Status in Sporadic Vestibular Schwannomas and its Prognostic Impact on Tumour Growth Patterns. *Scientific reports*, *7*(1), 5470. https://doi.org/10.1038/s41598-017-05769-0