



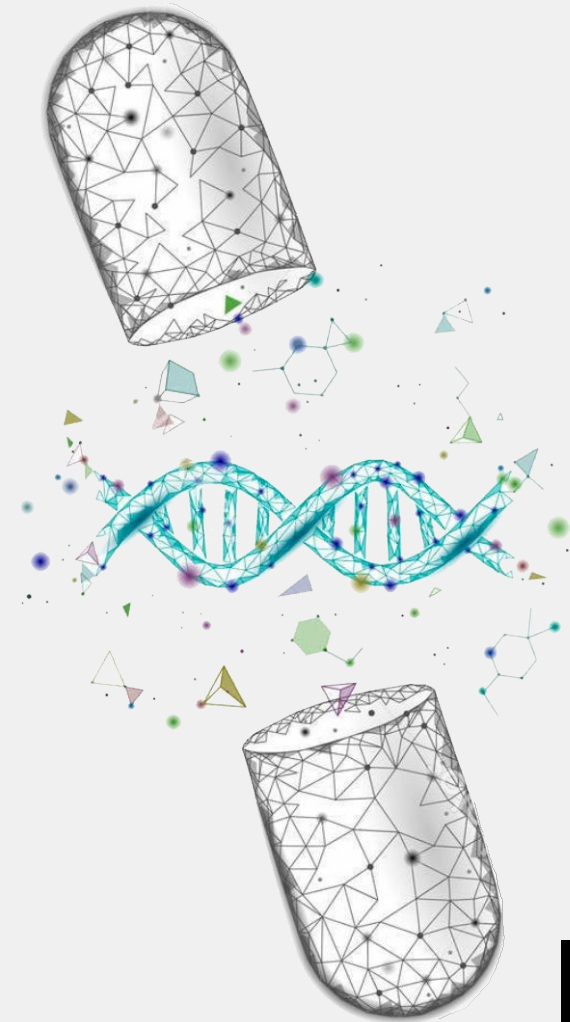
SAPIENZA
UNIVERSITÀ DI ROMA

Targeted gene therapy in Nasopharyngeal Carcinoma mediated by Epstein–Barr virus infection: EBNA–2 small interfering RNA

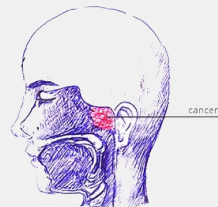
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A GENE THERAPY PROJECT
Prof.ssa I. Saggio
Tutor Dott.ssa La Torre

A.A. 2020/2021



BACKGROUND



What is Nasopharyngeal Carcinoma (NPC) ?

- NPC is a rare type of head and neck cancer that affects the epithelial cells of the nasopharynx.
- It is responsible for about 1/3 of childhood neoplasms of the nasopharynx.
- Symptoms related include lymphadenopathy, trism, pain, otitis, hearing loss, nasal blockage, nosebleeds.

Risk Factors

GENETIC BACKGROUND



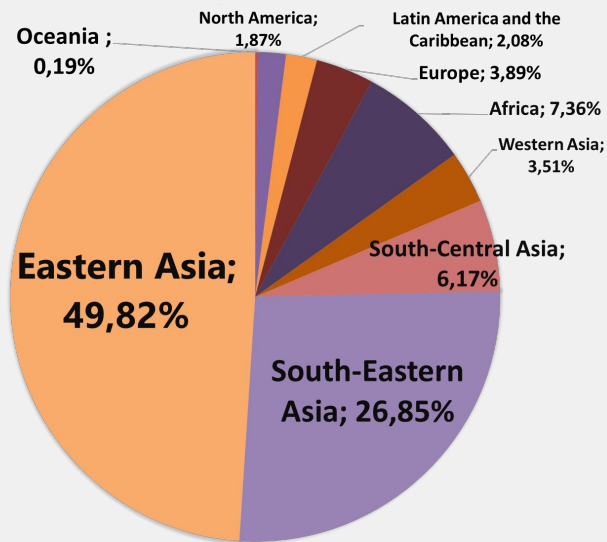
EPSTEIN-BARR VIRUS (EBV) INFECTION



ENVIRONMENT FACTORS AND LIFESTYLE



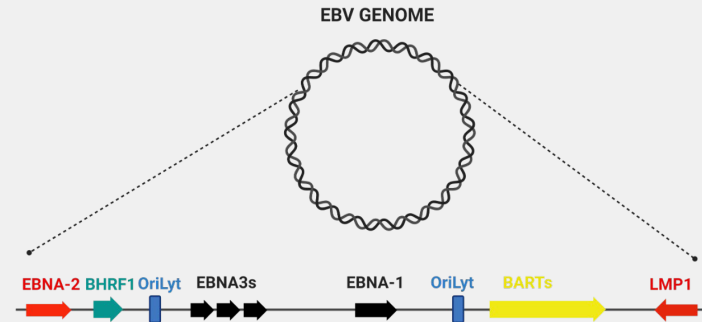
Worldwide Incidence (2018)



AIM OF THE PROJECT

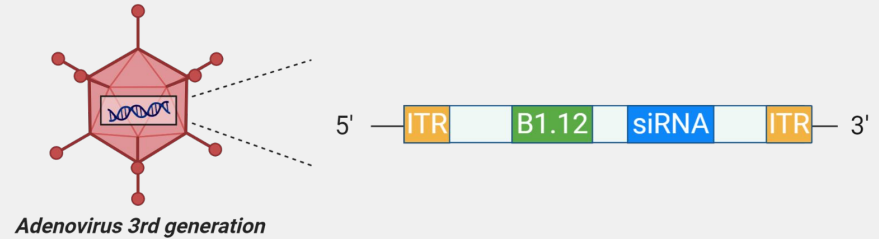
Why?

EBNA-2



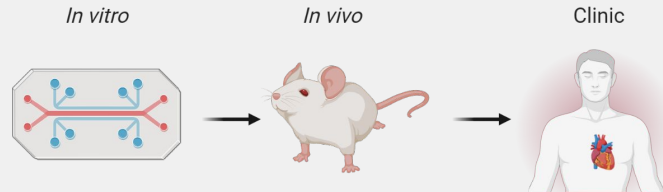
How?

siRNA silencing



For what?

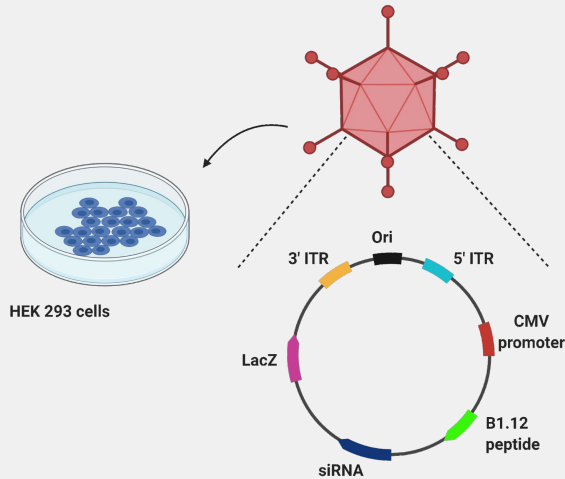
Therapy testing



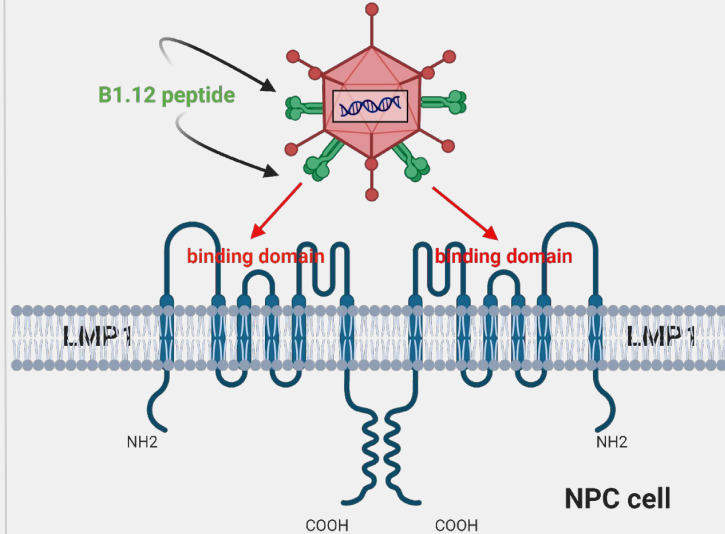
EXPERIMENTAL PLAN

In vitro

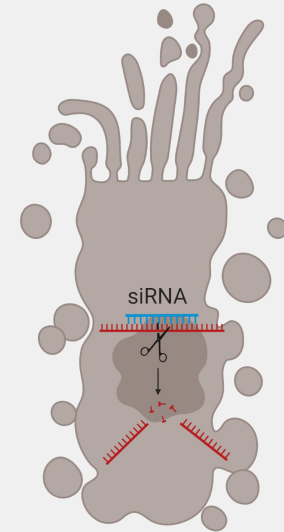
1 VECTOR DESIGN 3rd generation adenoviral vector



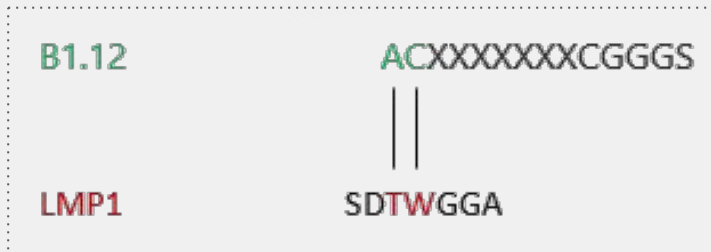
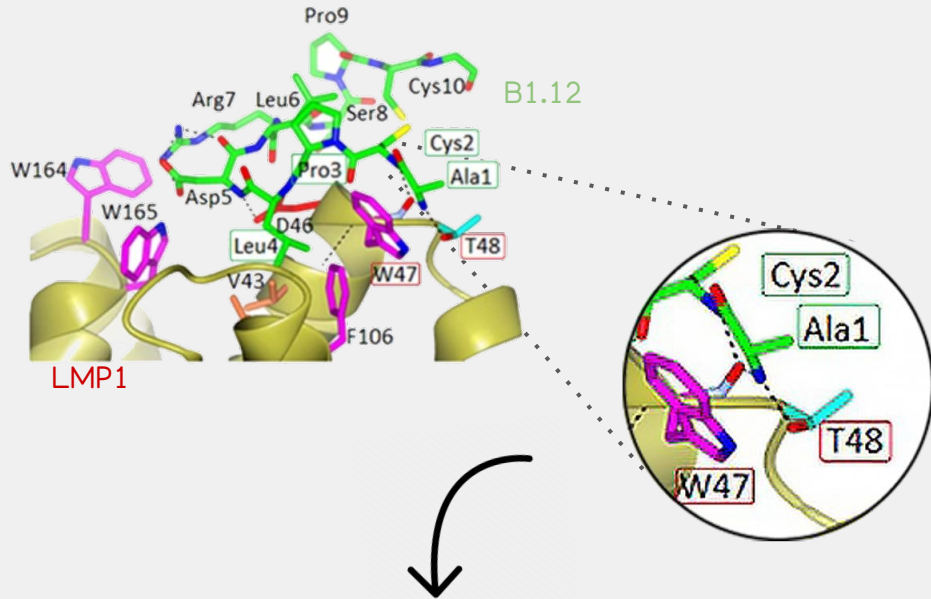
2 DOCKING LMP1-B1.12



3 EXPECTED OUTCOME EBNA-2 mRNA degradation and HONE1 cells apoptosis



2 Peptide–oncoprotein interaction



3 siRNA sequences

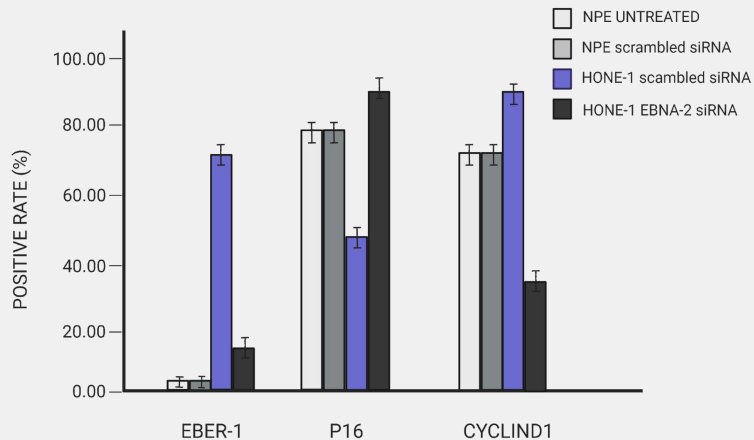
4 different siRNAs + 1 scrambled siRNA



IN VITRO RESULTS

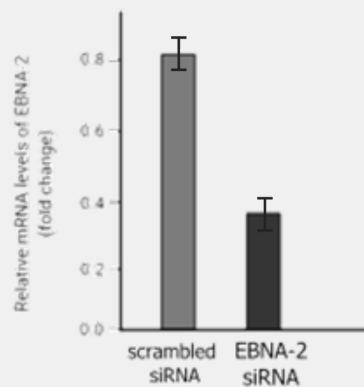
1

ISH, ICH

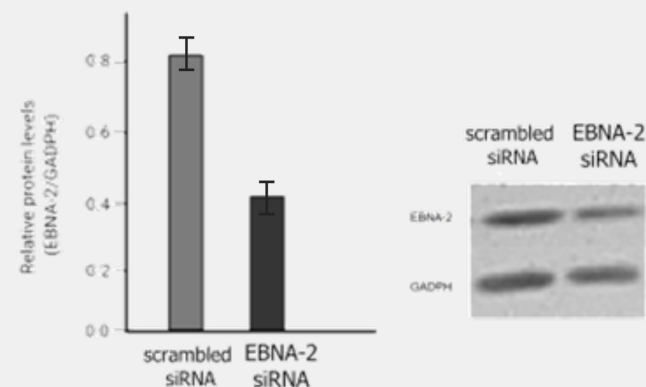


2

qPCR

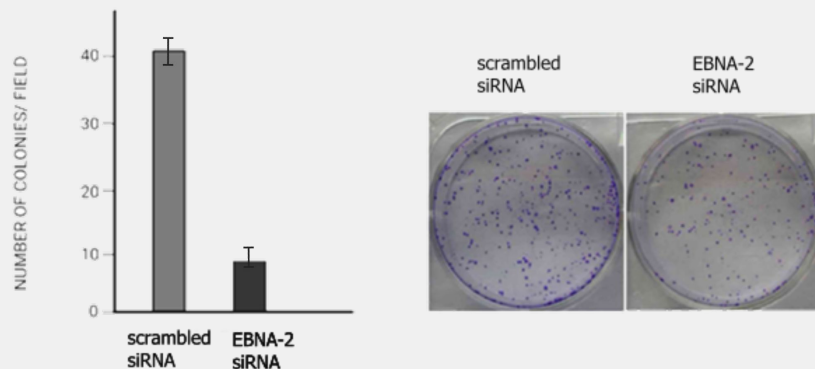


WESTERN BLOT



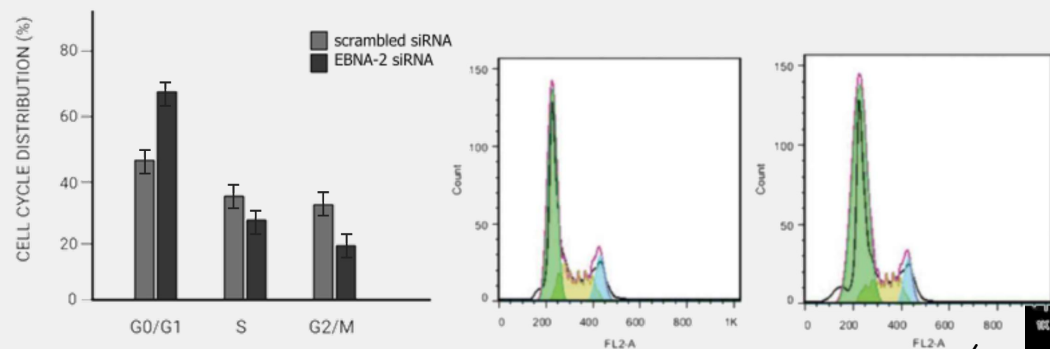
3

CLONOGENIC ASSAY



4

FLOW CYTOMETRY



Figure(1) adapted from Zhang et al. (2009)

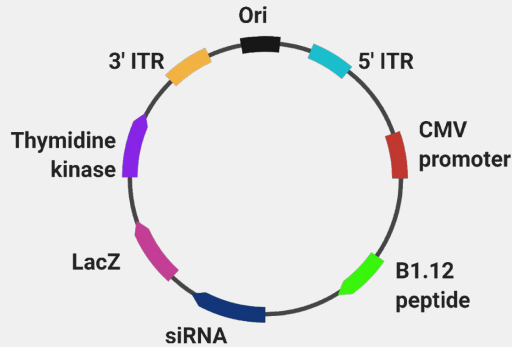
Figure (2) (3) (4) adapted from Wang et al. (2019)

EXPERIMENTAL PLAN AND TIMELINE

In vivo

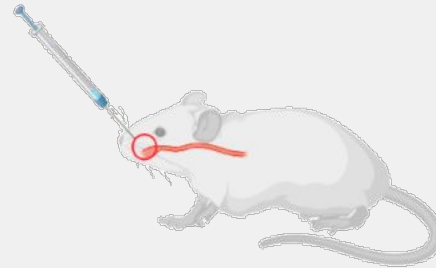
DAY 7

1 Same adenoviral vector + thymidine kinase



DAY 7

2 Localized injection

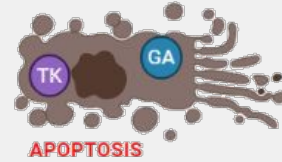


129/J mice

DAY 15

3 Safety protocol: Ganciclovir

Nasopharyngeal cell EBV +
(Ganciclovir + Thymidine Kinase)



Sistemic cell EBV --
(Ganciclovir)



Injection
HONE-1

Adenovirus
localized
injection

[Ganciclovir
Administration]

Sacrifice

Day 0

Tumor monitoring

Day 7

Volume measurement
(PET)

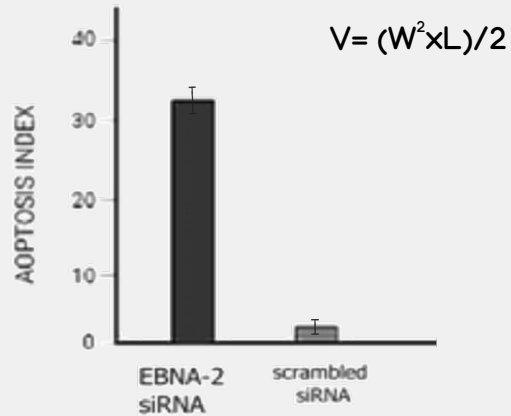
Day 15

Day 25

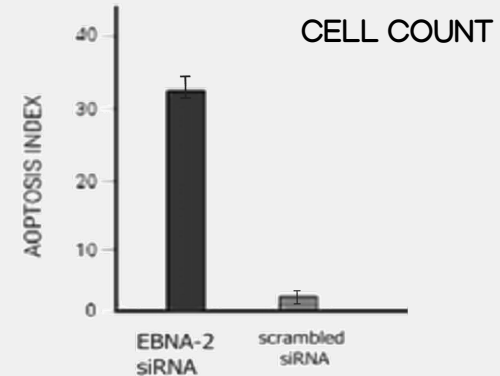
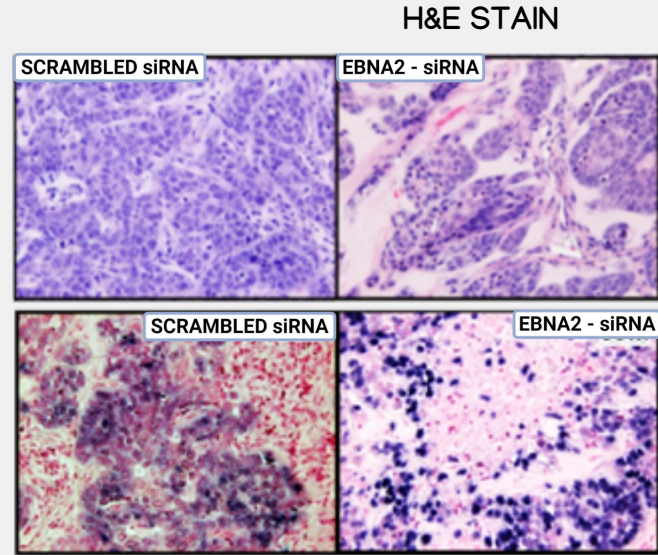
Behavioral tests

IN VIVO RESULTS

1



2

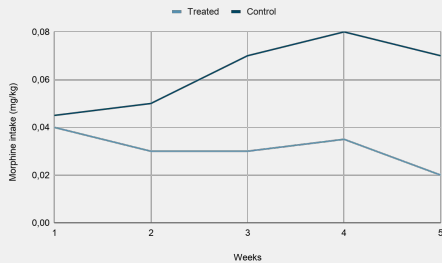


BEHAVIOURAL TESTS and SYMPTOMS MEASUREMENTS

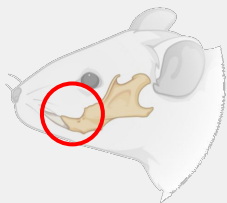
Evaluation of perceived pain



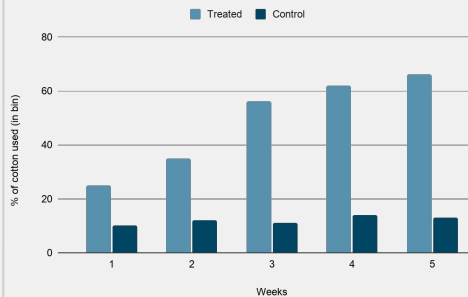
Self-administration of painkiller medicine



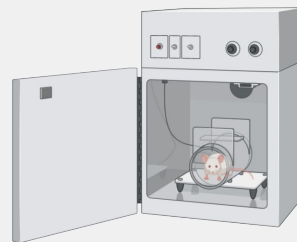
Evaluation of fine motor skills



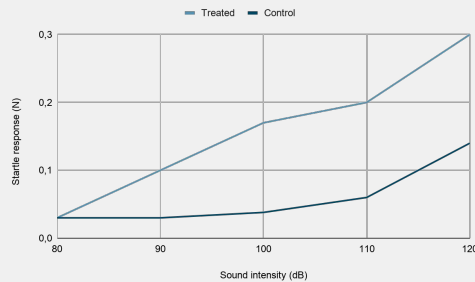
Cotton use test



Hearing evaluation



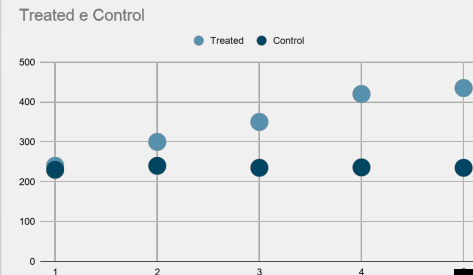
Startle response



Quantitative measurements

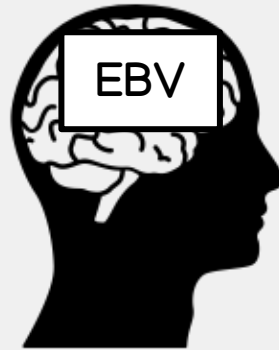
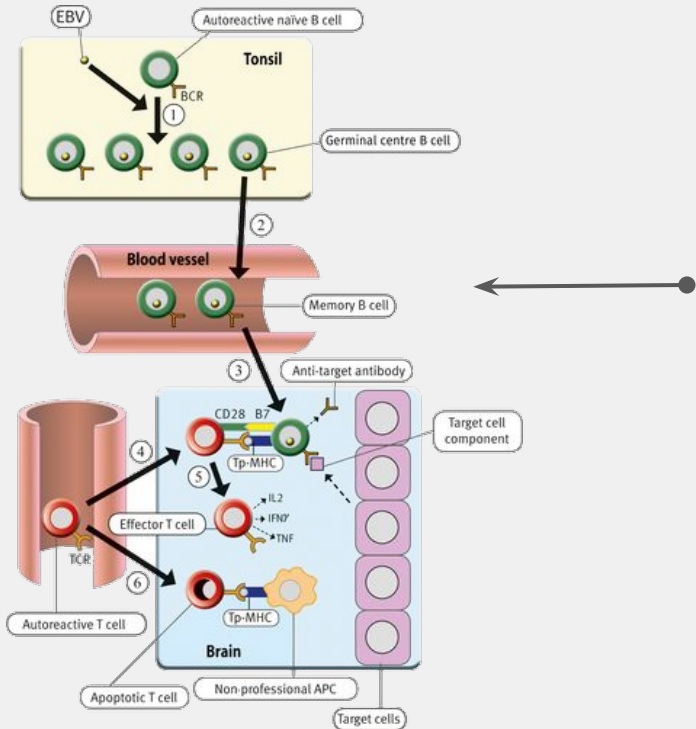


Food intake

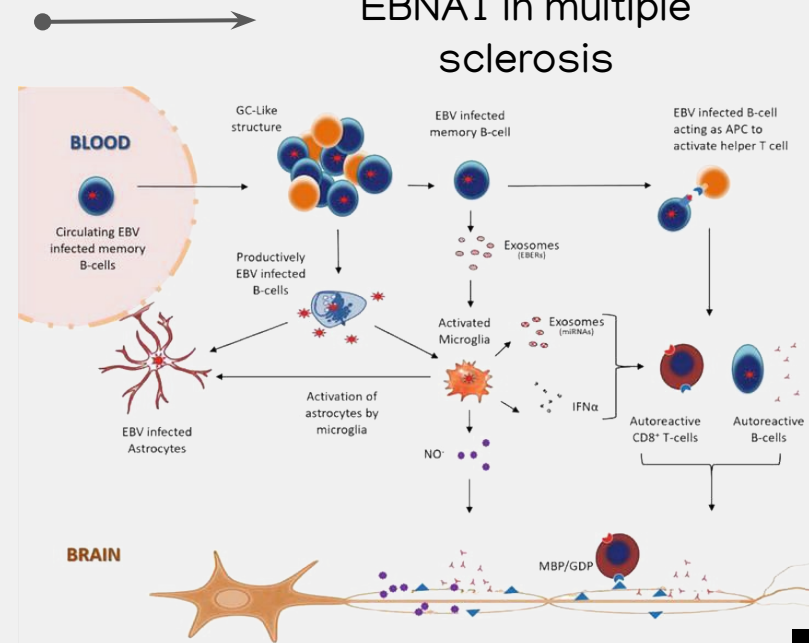


EBV-ASSOCIATED NEUROLOGICAL COMPLICATIONS

Hypothesis of Bipolar Disorder
as an EBV-driven chronic
autoimmune disease



Emerging role of
EBNA1 in multiple
sclerosis



COSTS & MATERIALS

Plasmide	Adenoviral Vector Herpes simplex	€1300
	Timidina Kinase gene - Thermofisher	€600
	siRNA - Thermofisher	€1800
	B1.12 peptide - Proteogenix	€ 2300
	LacZ kit expression kit - Thermofisher	€900
PCR kit	DNA ligase - Thermofisher	€80
	Taq Polimerase - Thermofisher	€339
	Kit DNA extraction - Eppendorf	€790
	Rat recombinase - Eppendorf	€200
Cell Cultures	Mice lines (model 129) - The Jackson Laboratory	€1200
	Cell culture dishes 70mm - Eppendorf	€700
	Bovine Serum for cellular culture - Lonza	€500
	Behavioural test equipment - mazeengineers	€10000

APPROXIMATE COST FOR EACH EXPERIMENT
(without salary costs of researchers)

€ 20.000

CONCLUSIONS

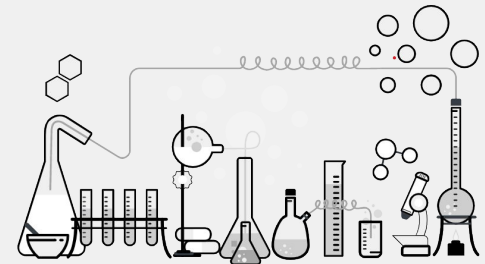
- In vitro, transfection with EBNA2-siRNA downregulated EBNA-2 expression by 42.7– 50%. Proliferation was decreased by 65%
- In vivo, EBNA2-siRNA reduced tumor weight by > 79,7%
- The therapy may also be effective in case of lymph nodes metastasis derived from NPC

PITFALLS

- EBV remains largely latent in the cells for the host's lifetime. Continuous administration is a requirement for the therapy in order to guarantee its efficiency
- Adeno is a non-integrating vector. There is limited transmission to daughter cells
- The therapy may not be effective on RB2 gene mutation NPC (30% of NPC cases) or in cases where NPC arises from a combination of EBV infection and RB2 mutation

FUTURE PERSPECTIVES

Engineering of immune system cells, aimed to a finer antigen recognition (through use of Crispr/Cas9 technology)



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