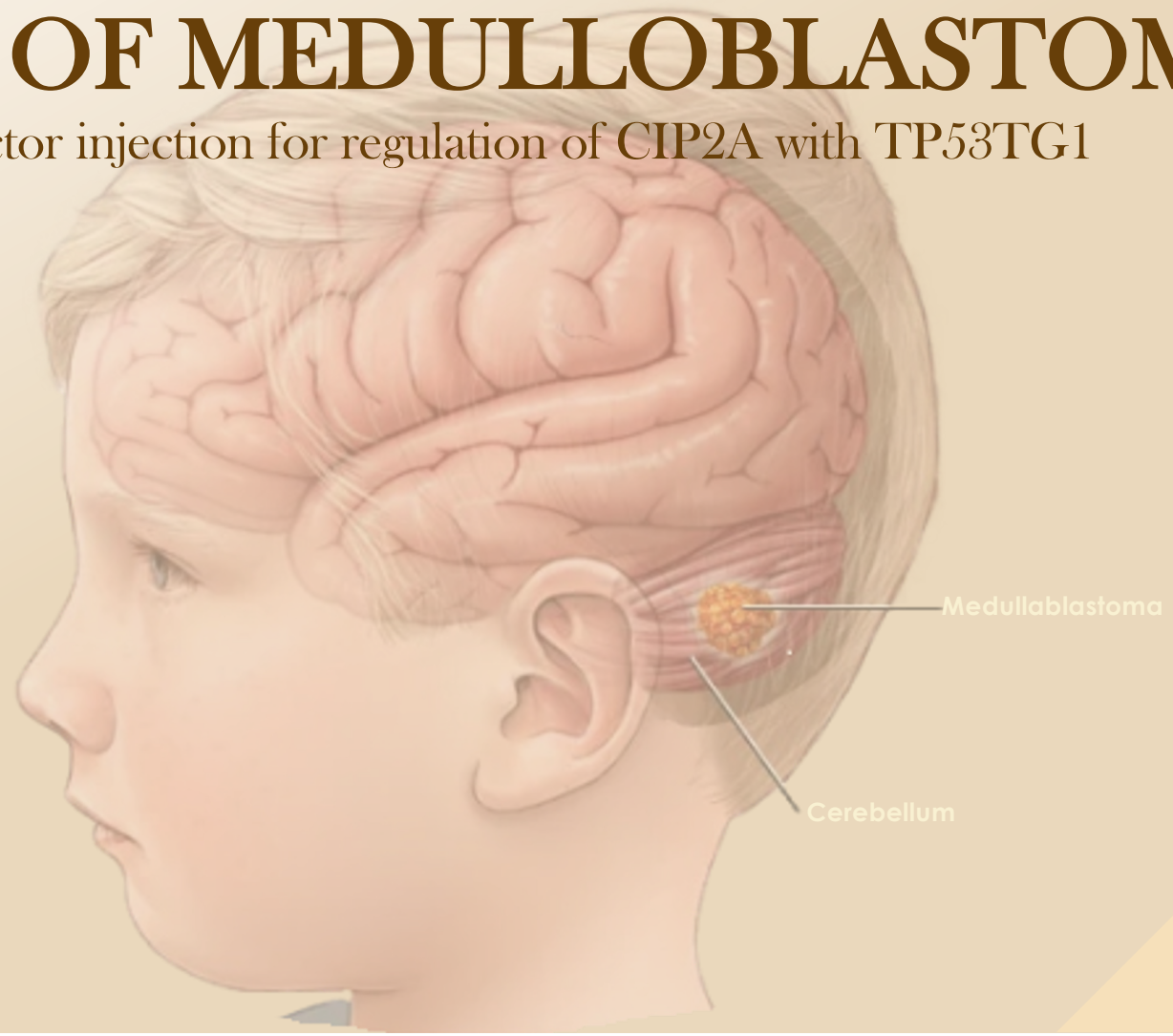
INTERACTION AMBRA1- c-MYC IN GROUP3 OF MEDULLOBLASTOMA

Intracerebellar lentivirus vector injection for regulation of CIP2A with TP53TG1



Corso di Terapia Genica e Neuroscienze

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A.A. 2023-2024

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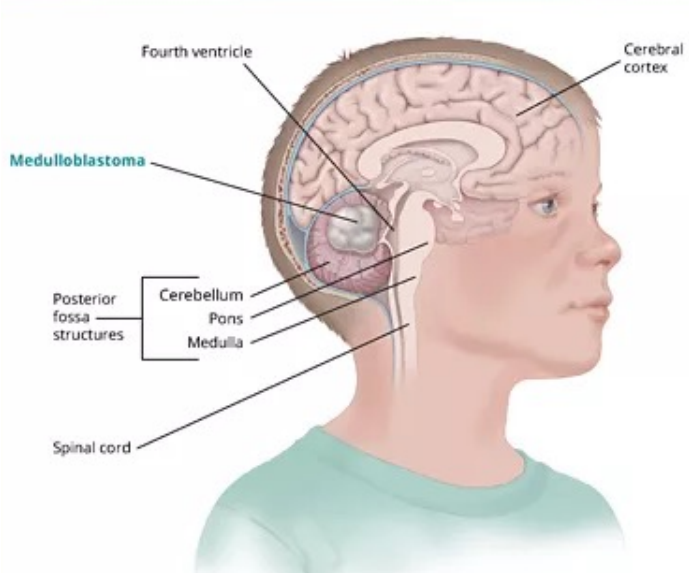
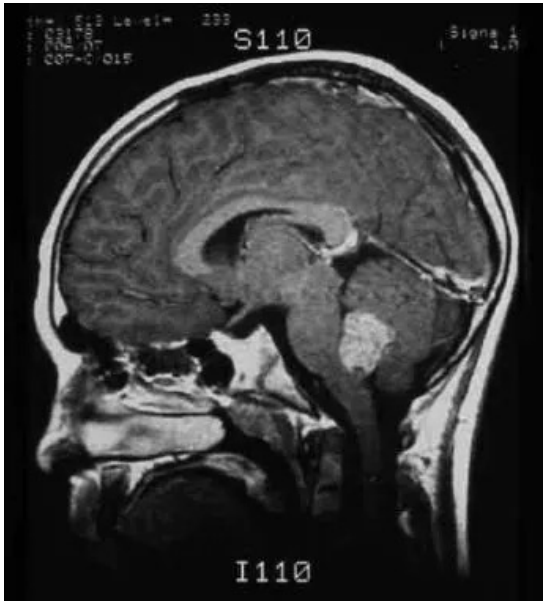
BACKGROUND



Diagnosis in childhood.
1st of the most common malignant brain tumors.
Current therapy: surgical export, chemotherapy and radiotherapy.



BUT THIS IS NOT ENOUGH!

Due to:

- Metastasis
- Resistance
- Recurrence
- Cognitive deficits

↑↑ c-MYC

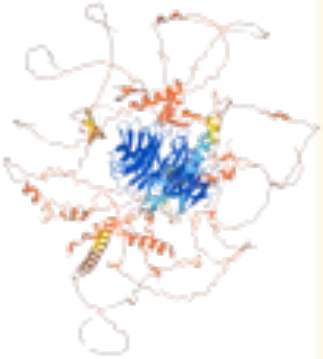
MBgroup3

c-MYC

MIZ-1

↑AMBRA1

↑↑AMBRA1



Physiological conditions

AMBRA1

PP2A

MBgroup3

↑CIP2A

c-MYC P

Stabilization c-Myc

Cell proliferation
Tumorigenesis

c-MYC P

Proteasomal Degradation

Cell proliferation
Tumorigenesis

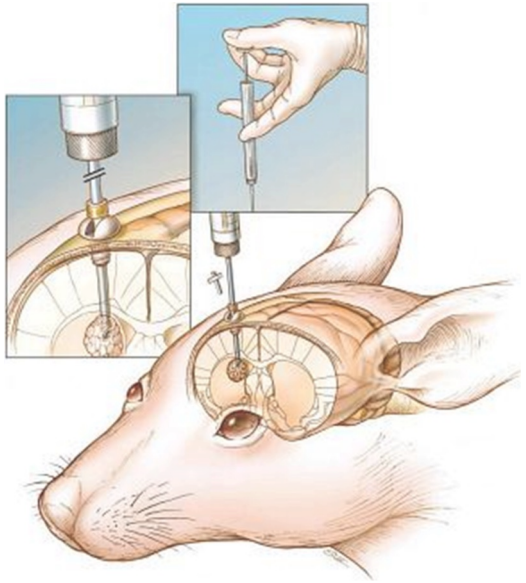
Inhibition

Interaction

AIM OF PROJECT

TP53TG1 —| CIP2A → ubiquitination-mediated degradation → Apoptosis

DELIVERY → intracerebellar injection



EXPERIMENTAL PLAN

LENTIVIRAL VECTOR ENGINEERING

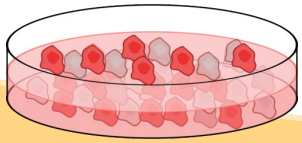
Vector plasmid: SIN 5' LTR — cPPT-CTS — Promoter P2 — TP53TG1 — SIN 3' LTR

Envelope plasmid: promotor — env (VSV-G) — polyA

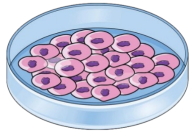
Packaging plasmid: promotor — gag-pol — polyA

promotor — rev — polyA

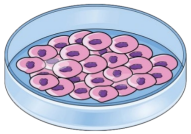
Transfection in HEK 293T Cells



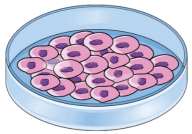
IN VITRO



Vector with P2 promoter



Vector with modified P2 promoter

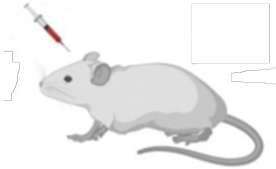


Control

Cell line D341, employed in many studies on Grop 3 of MB

IN VIVO

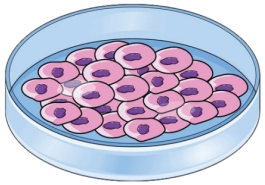
Modified P2 promoter



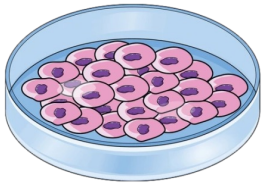
Control

Model mice employed in studies on Group 3 MB (Ballabio C et al., 2020)

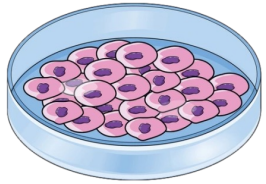
IN VITRO EXPERIMENT



Vector with P2 promoter



Vector with modified P2 promoter



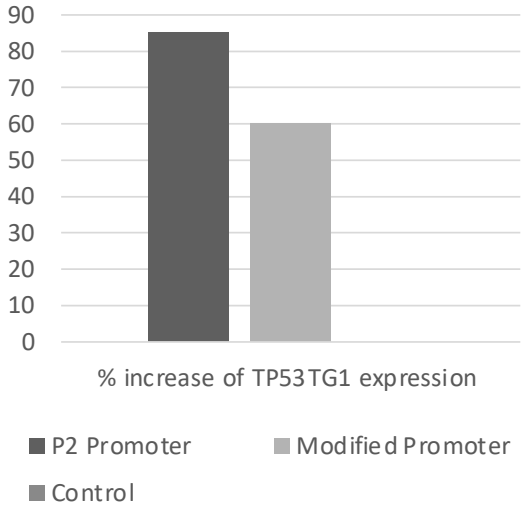
Treated with a control vector

→ Cells D341 Med

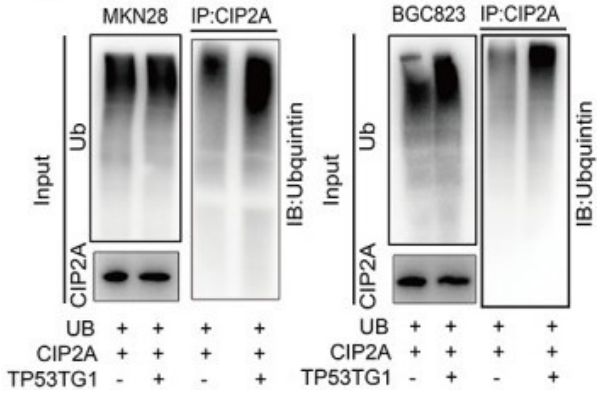
↓

deprived of the ME1a1 element

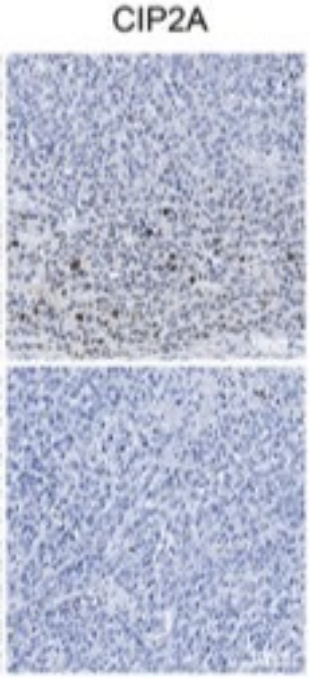
IN VITRO RESULTS



- High transduction efficiency in cells expressing the TP53TG1 gene.
- Transduction is lower for cells treated with the modified promoter, showing a reduced efficiency.
- No transcriptional increase is observed in the control.



- Proteomic screenings allow us to observe higher levels of ubiquitination in presence of TP53TG1 and lower levels in the control.
- Additional apoptotic assays show an optimal increase in apoptosis in cells treated with the modified P2 promoter.



(Fang D, et al., modification-mediated lncRNA TP53TG1 inhibits gastric cancer progression by regulating CIP2A stability. 2022)

A decrease in CIP2A levels concurrent with increasing apoptosis. This demonstrates the effectiveness of treatment against metastatic cells.

IN VIVO EXPERIMENT

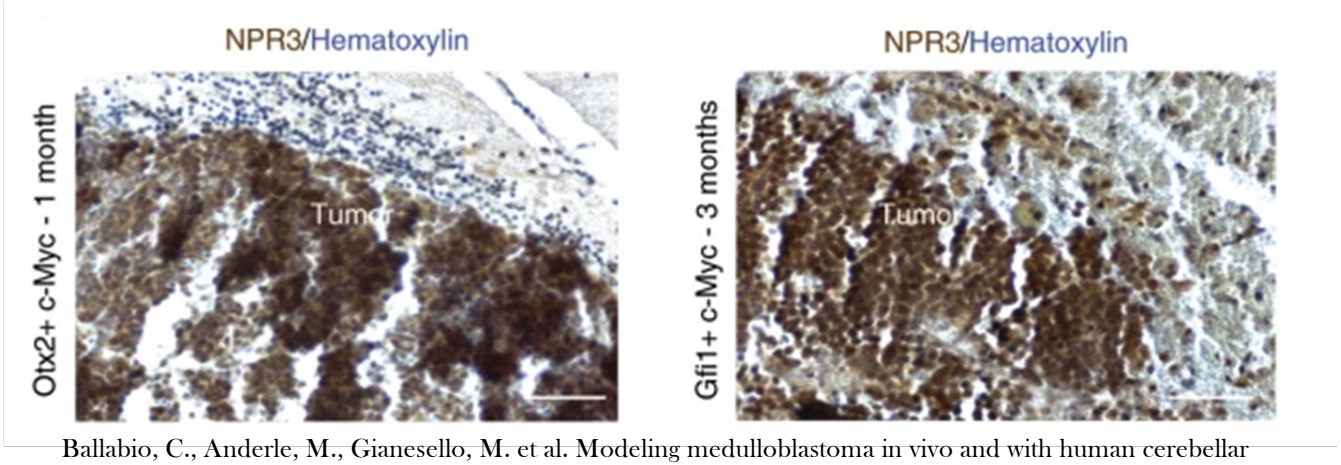
- Given the results of the in vitro experiments, we decide to adopt the modified P2 promoter, which showed a finer regulation of TP53TG1 expression.
- A model mice employed in other studies on Group 3 medulloblastoma (Ballabio C et al., 2020).
- Population of 30 mice: half of them will be administer with lentiviral vector and the other half with control vector.

Modified P2 promoter

Control

Injection of $2x10^6$ cells per mice

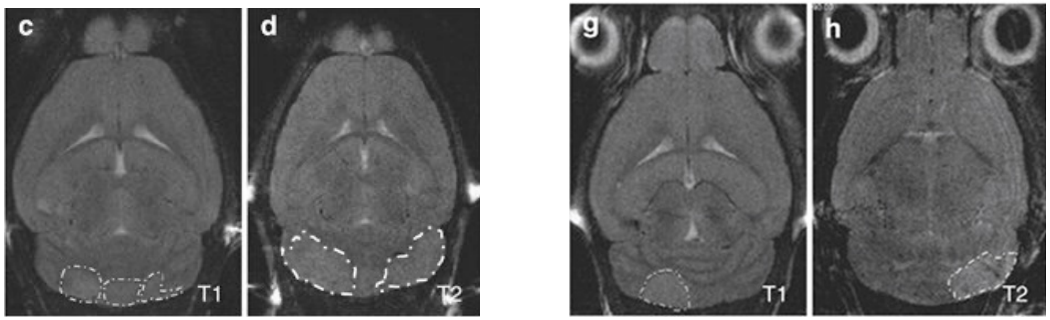
IN VIVO RESULTS



By comparing the mouse cerebellum one month and three months after treatment, we can observe a clear reduction in tumor mass.

Ballabio, C., Anderle, M., Gianesello, M. et al. Modeling medulloblastoma in vivo and with human cerebellar organoids. Nat Commun 11, 583 (2020). https://doi.org/10.1038/s41467-019-13989-3

MRI reveals tumor regression in 85% of treated mice.



CONCLUSIONS

Considering the results of the experiment in vitro and in vivo, the treatment guarantees:

1. apoptosis and reduction of tumor cells
2. reduction of tumor mass with increase survival
3. absence of relapses and extracranial metastases

PITFALLS

Death of cells that are not in metastasis and whose physiological state can be restored.

SOLUTIONS

Modification of the P2 promoter.

Intracerebellar injection is invasive. What if the vector was injected differently?

Use of the CACNA1A protein specific to some nervous cells and particularly present on the membranes of Purkinje cells.

MATERIALS AND BUDGET

Materials	Costs
Vector development and optimization	$10.000,00
Cell line HEK 293T	$400,00
Cell line D341 in EMEM	100 x $54,00
Raw materials	$4.500,00
Mice tumor models	30 x $56,29

Research teams	Salary
Lead Researcher	$100.000,00 per year
Ph.D Researchers	2 x $60.000,00 per year
Junior Researcher (Master's level)	$17.000,00 per year
DURATION	3 YEARS
TOTAL	**$732.988,70**

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