

# IDH1 MUTATION IN GLIOMAS

## CRISPR/Cas9 gene therapy to restore the correct gene function

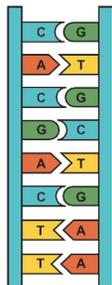
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2022/2023

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# BACKGROUND

GLIOMA IS A BENIGN OR MALIGNANT BRAIN AND SPINAL CORD TUMOR THAT ARISES FROM GLIAL CELLS (NCI)

SOMATIC HETEROZYGOTIC CHROMOSOME 2 MUTATION

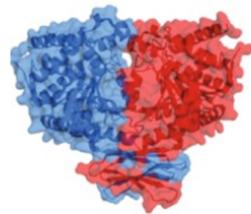


IDH1 c.395G>A

IDH1 p.R132H



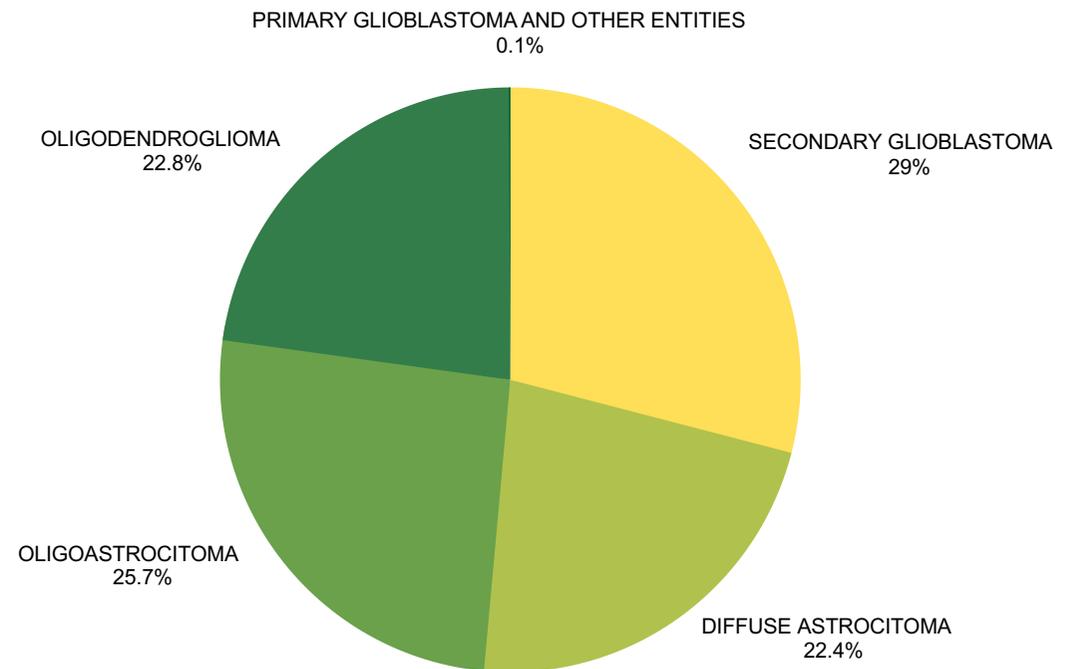
Wild type/R132H



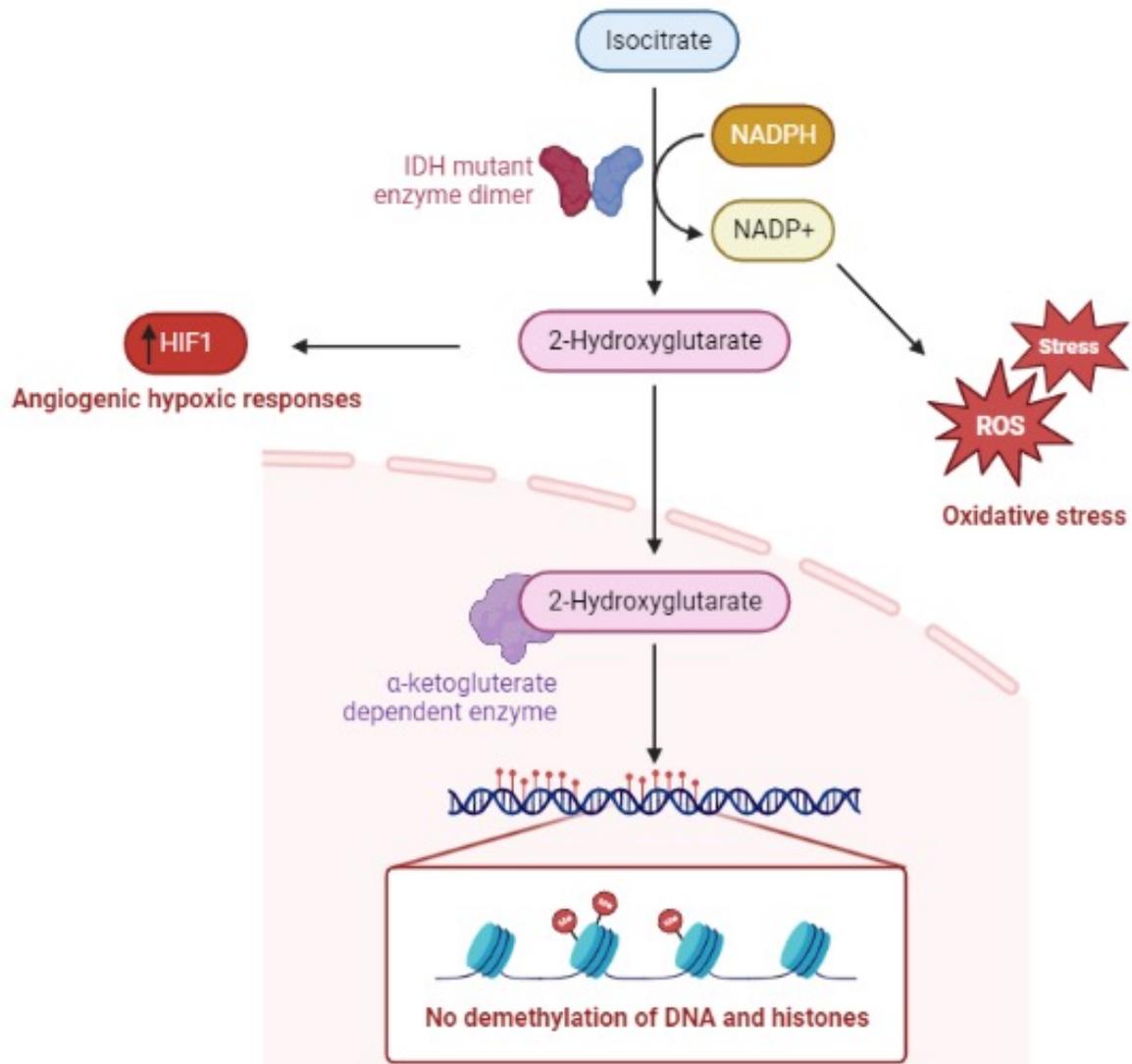
CURRENT TREATMENTS:

- Surgery
- Radiotherapy
- Chemotherapy (TMZ)

IDH1 MUTATION FREQUENCY IN GLIOMAS

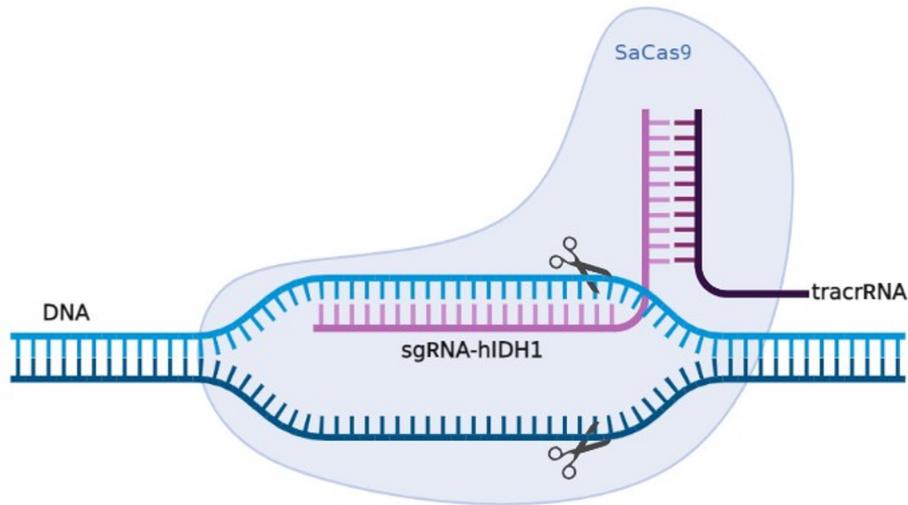


# IDH1 MUTANT PRODUCES THE ONCOMETABOLITE 2-HG



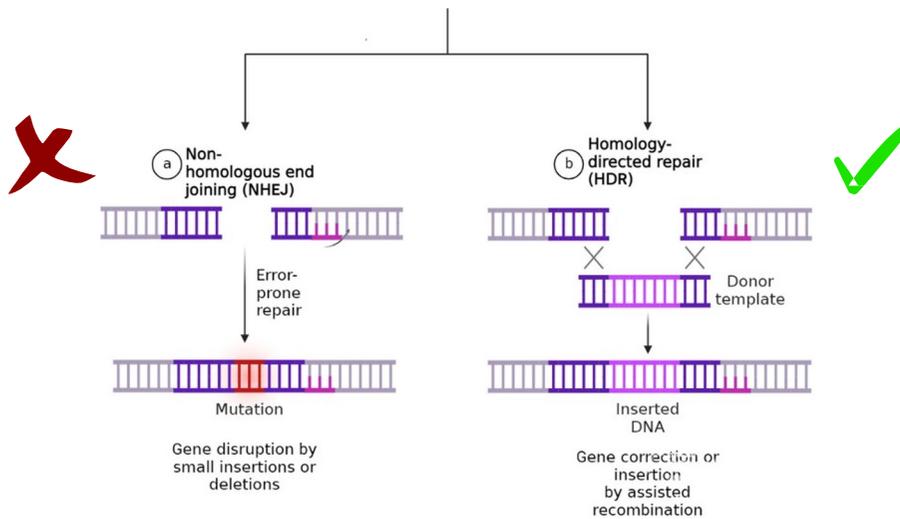
- increase in **oxidative stress** levels
- upregulation of the transcription factor HIF1 resulting in increased **angiogenesis**
- inhibition of demethylases resulting in **hypermethylation** of DNA and histones

# AIM OF THE PROJECT

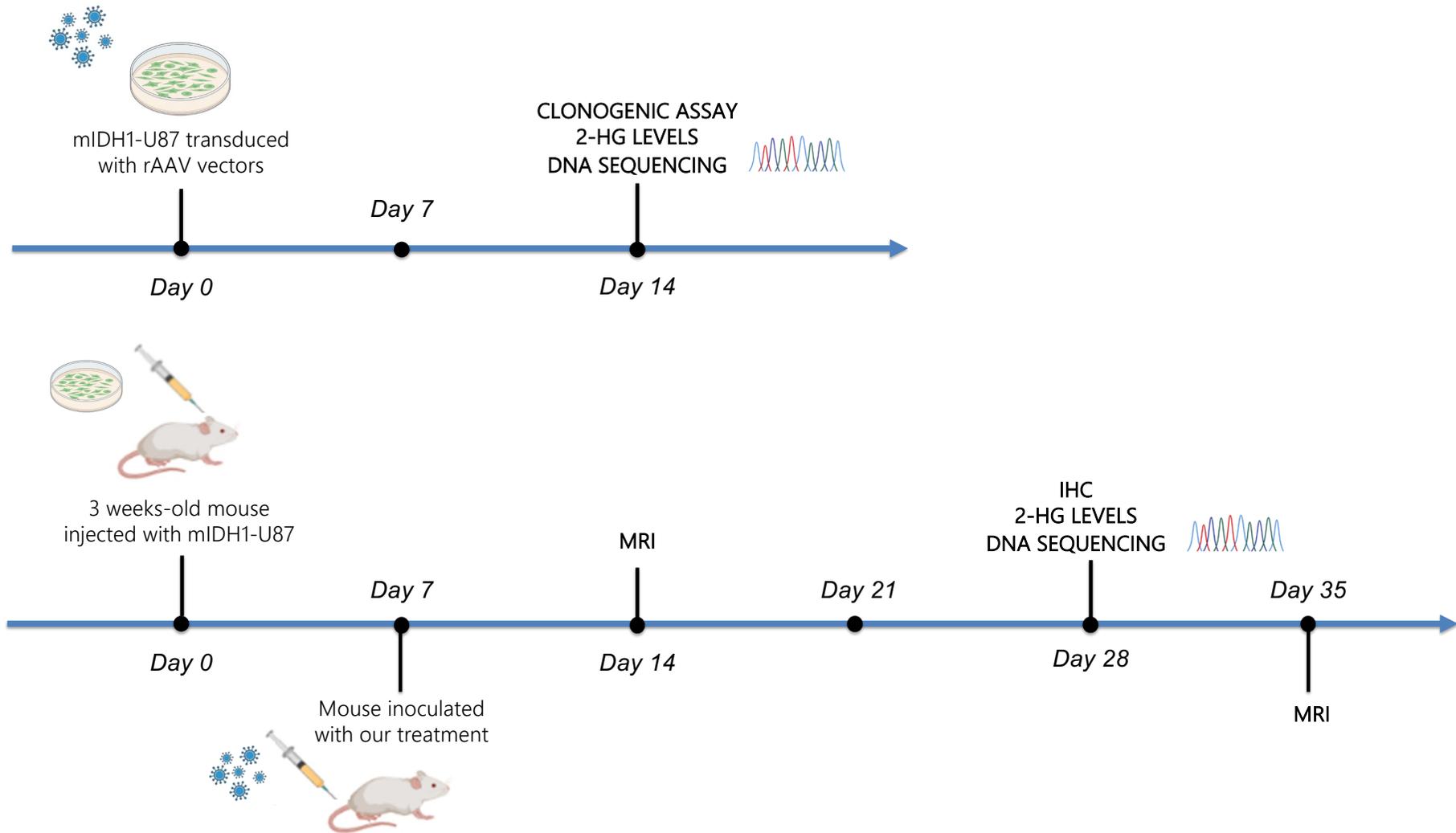


ATCATAGGTCGTCATGCTTAT  
ssDNA Template

Using CRISPR/Cas9 system to edit the c.395G>A mutation with the restoration of the correct aminoacid in the catalytic site of IDH1 enzyme leading to its proper functioning.

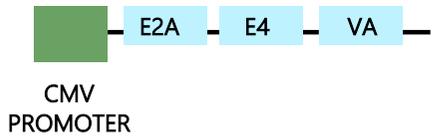
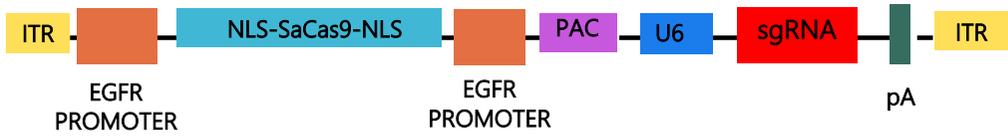


# EXPERIMENTAL PLAN

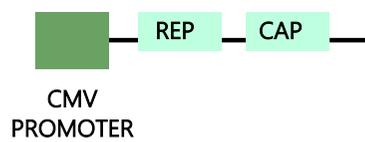


# VECTOR DESIGN

AAV-U87R7C5-Cas9+sgRNA

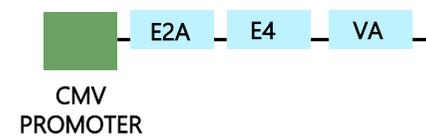
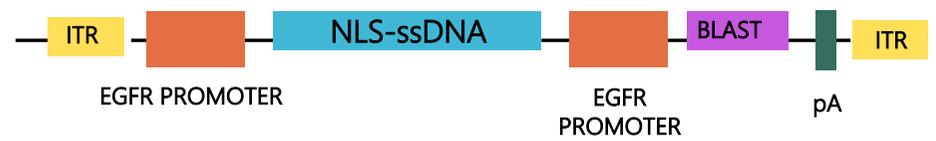


Helper plasmid

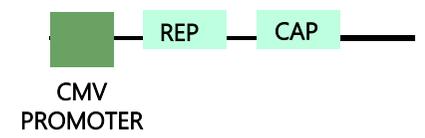


REP/CAP plasmid

AAV-U87R7C5-ssDNA Template



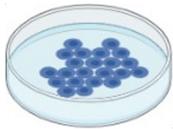
Helper plasmid



REP/CAP plasmid

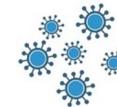
Transfection in HEK293

Selection with Puromycin



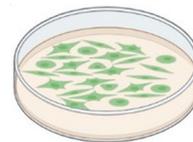
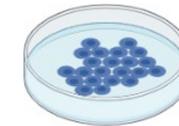
rAAV-U87R7C5-Cas9+sgRNA

Transfection in HEK293



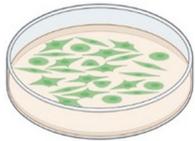
rAAV-U87R7C5-ssDNA Template

Selection with Blasticidin



Co-transduction of mIDH1-U87

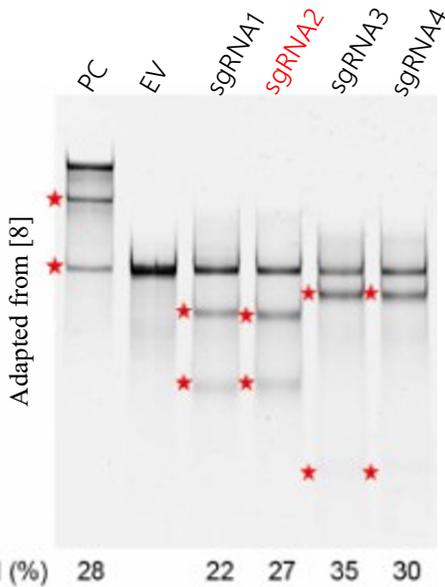
# TESTING THE BEST sgRNA AND OFF-TARGET EFFECTS



mIDH11-U87

Co-transfected with SaCas9 +

sgRNA1 5'-TACCCATCCACTCACAAGCCG-3'  
 sgRNA2 5'-ATCATAGGTCGTCATGCTTAT-3'  
 sgRNA3 5'-TATCCCCCGGCTTGTGAGTGG-3'  
 sgRNA4 5'-AAAATATCCCCCGGCTTGTGA-3'

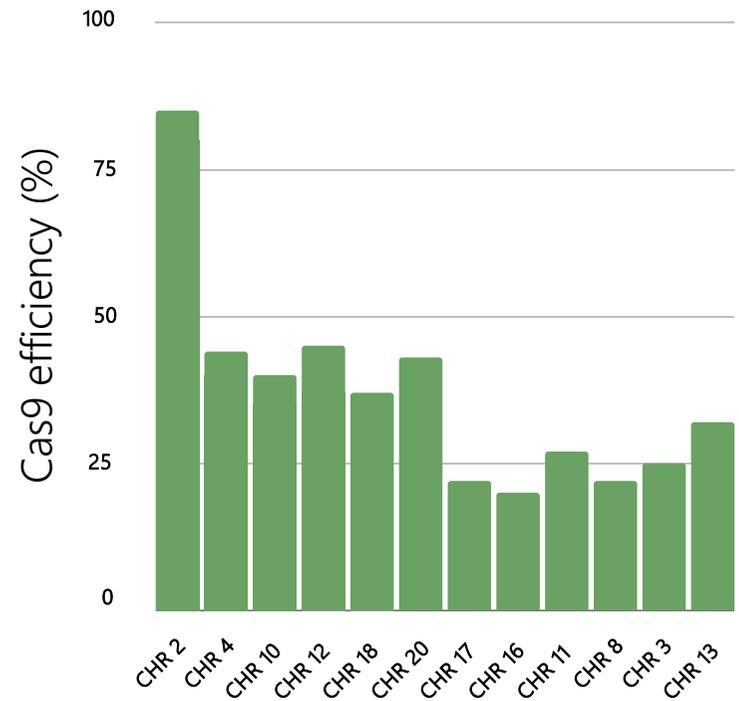


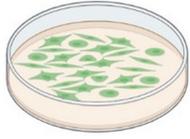
\*PC= POSITIVE CONTROL (SURVEYOR ASSAY KIT);  
 EV= EMPTY VECTOR

Online screening through  
casOFFinder



11 predicted off-targets





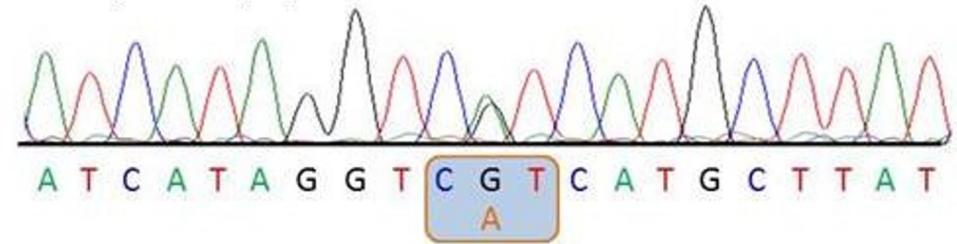
## EXPECTED RESULTS IN VITRO

Direct DNA Sequencing of IDH1 after PCR amplification:

mIDH1 GENE SEQUENCE BEFORE TREATMENT



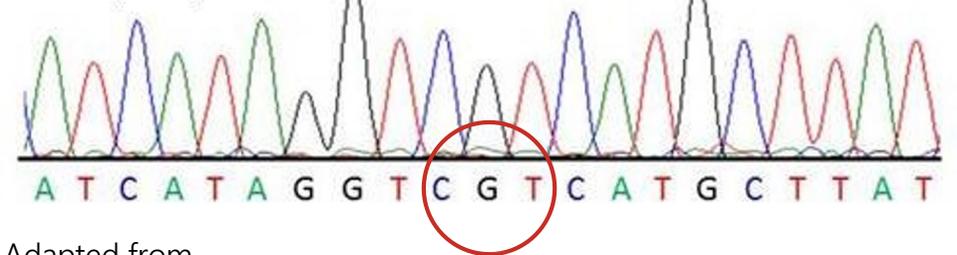
IDH1 (R132H/+)



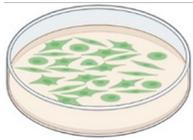
mIDH1 GENE SEQUENCE AFTER TREATMENT



IDH1 (+/+)



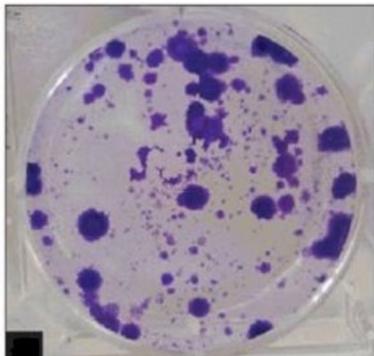
Adapted from  
[9]



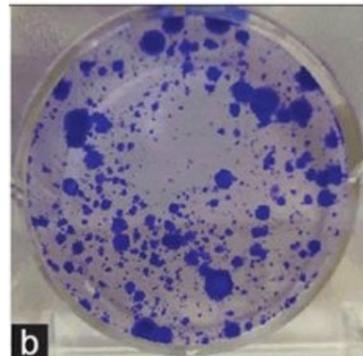
## EXPECTED RESULTS IN VITRO

### CLONOGENIC ASSAY AT DAY 14

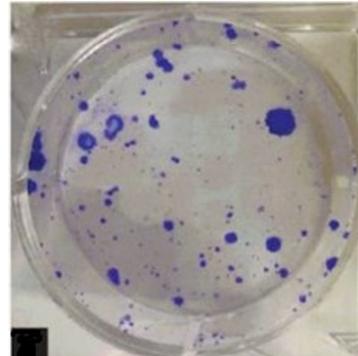
mIDH1-U87  
CTRL



mIDH1-U87 +  
EMPTY VECTOR

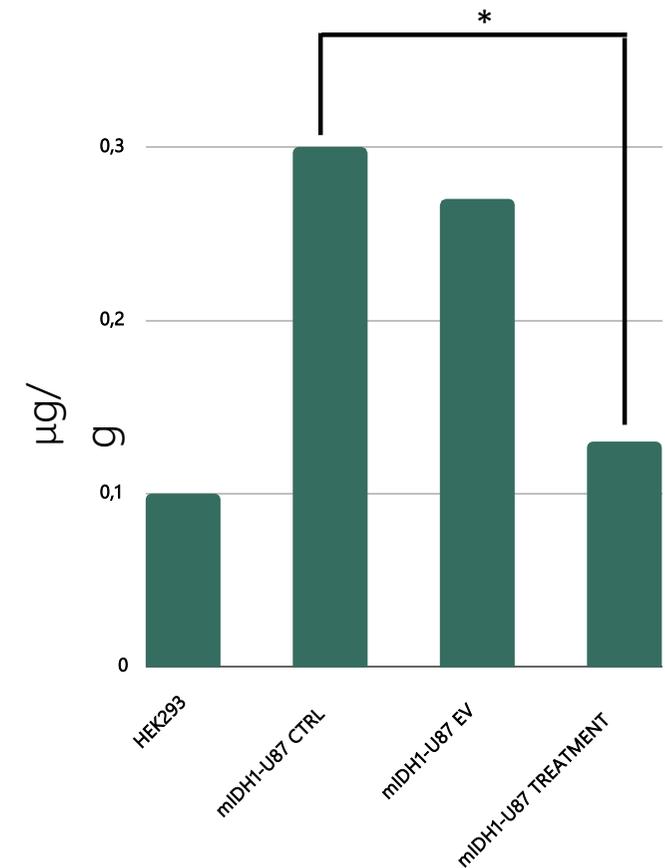


mIDH1-U87 +  
TREATMENT



Adapted from Journal of medical Signals and Sensor

### 2-HG LEVELS AT DAY 14

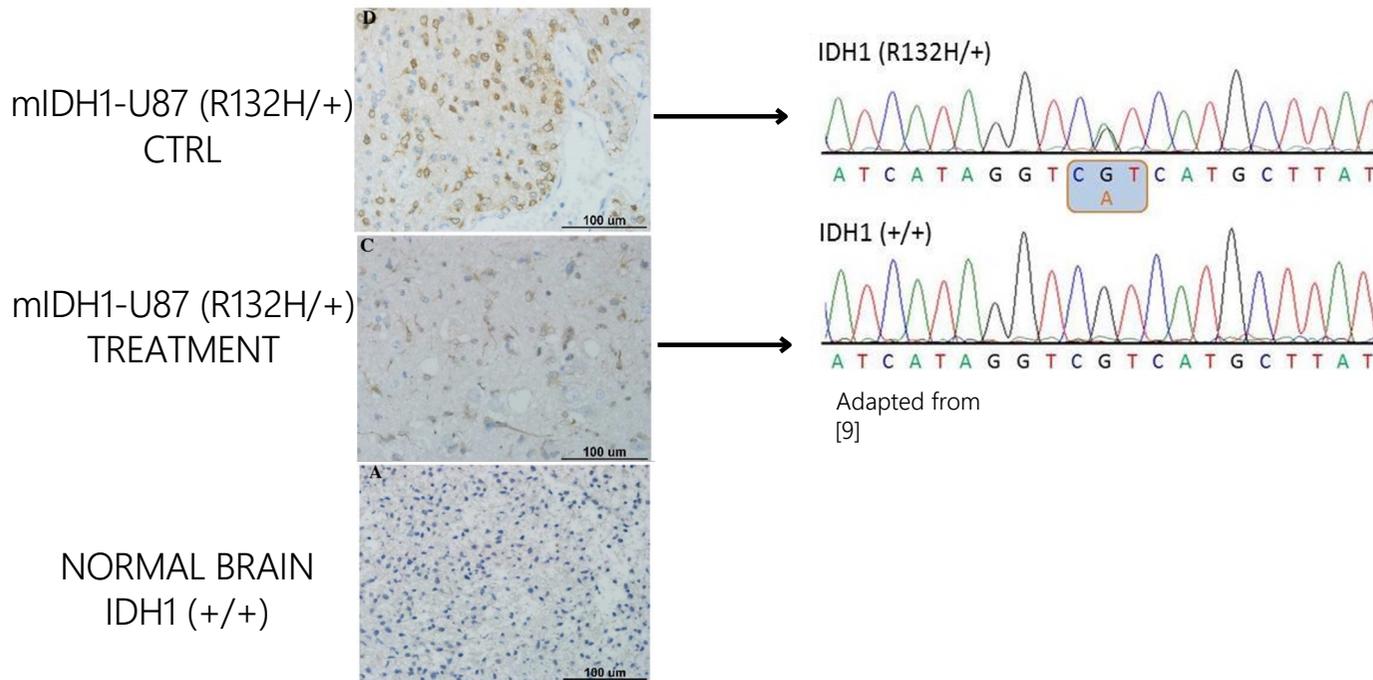


After AAV-U87R7C5 transduction in cell culture there is both decrease in cell proliferation and 2-HG levels

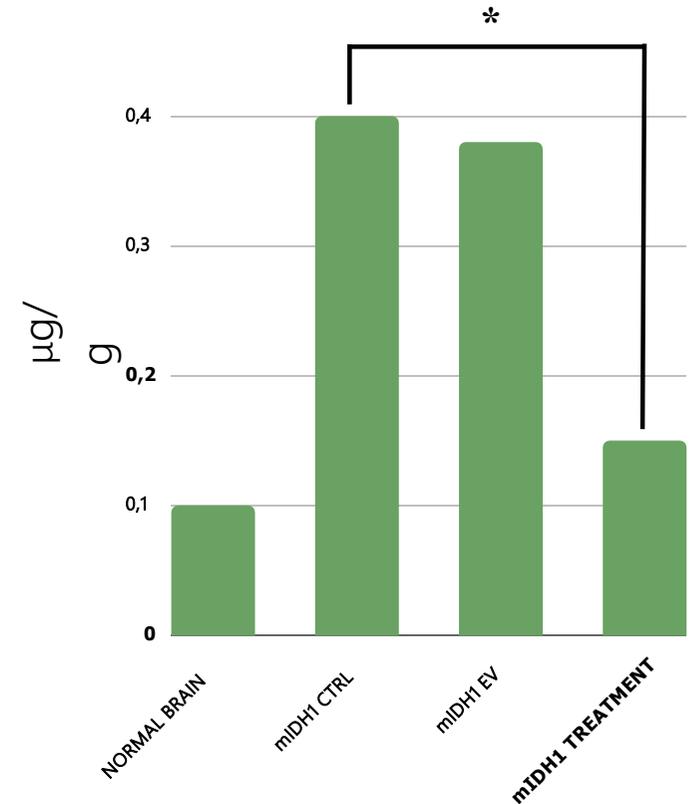


## EXPECTED RESULTS IN VIVO

### IHC AT DAY 28



### 2-HG LEVELS AT DAY 28



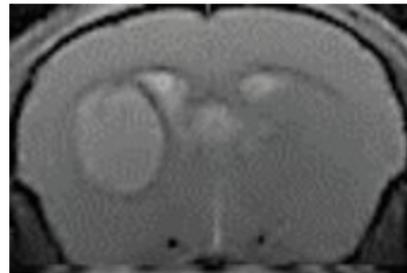
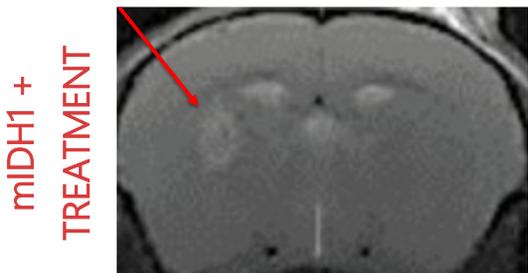
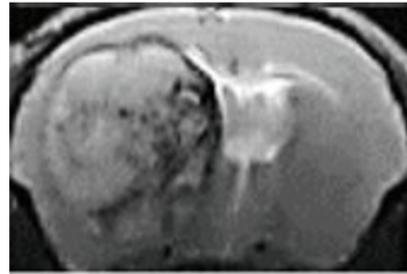
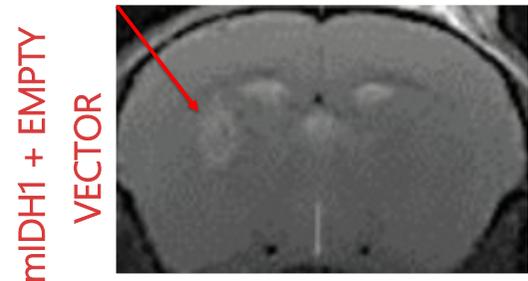
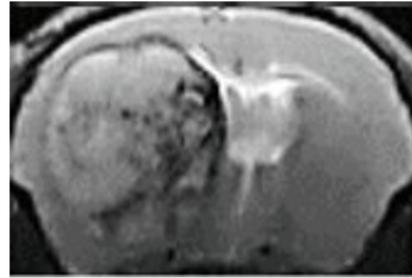
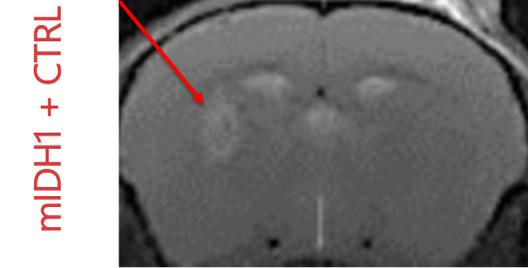
IHC confirmed a decrease U87 cells positive to mIDH1-R132H ONLY in mIDH1- treatment. Liquid chromatography and mass spectrometry confirmed a decrease in 2-HG levels ONLY in mIDH1- treatment



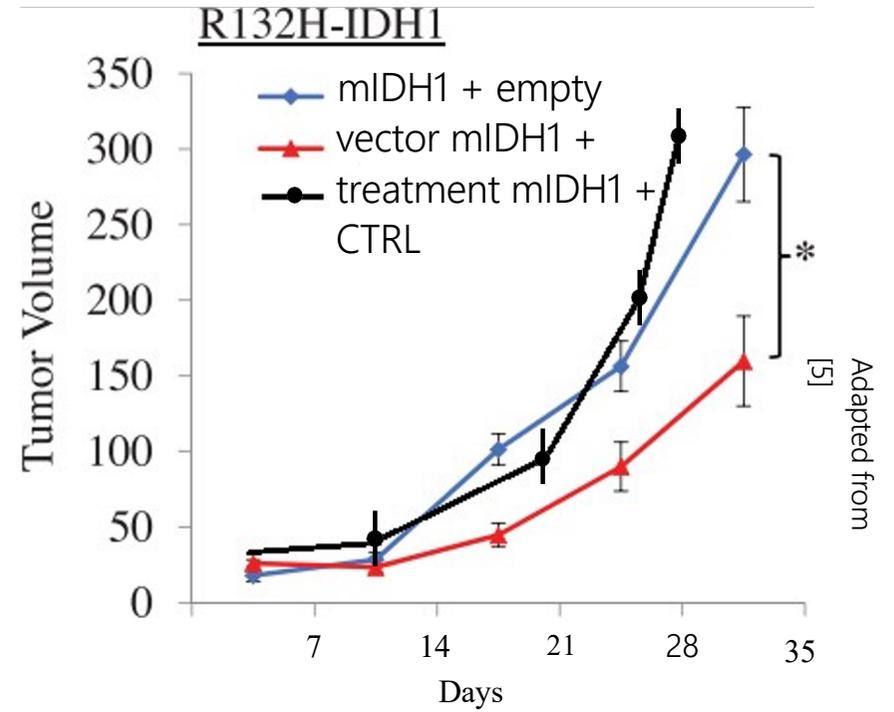
# EXPECTED RESULTS IN VIVO

Day 14

Day 35



Adapted from [15]



TUMOR PROGRESSION DECREASES IN TREATED MICE COMPARED TO CTRLs

# CONCLUSIONS

Thanks to the use of CRISPR/Cas9 tool transfected through the AAV vector, it was possible to restore the correct IDH1 functioning leading to:

- decrease in n. of U87 cells positive to mIDH1
- decrease in 2-HG LEVELS
- decrease in cell proliferation rate
- decrease in tumor progression

# FUTURE PERSPECTIVES

Due to these encouraging findings, there are concrete possibilities to use our CRISPR/Cas9 technique as a therapy in conjunction with chemotherapy and radiotherapy.

Further studies are surely needed to verify the efficacy and safety of our therapy on human clinical trials.



## PITFALLS

- Off-target effects
- CRISPR/Cas9 specificity
- Unwanted targeting of other CNS cells



## SOLUTIONS

- Alternative CRISPR/Cas approaches (e.g. ABE, Cas13, Cas9 nickases)
- Addition of 2 sgRNAs
- Use a promoter only express in our target cells

# COSTS AND MATERIALS

- GBM MURINE CELLS: 541\$
- AAV VECTOR: 200\$X2
- MRI: 6.000\$/YEAR
- NOD/SCID MICE: 156.67\$X20 MICE (~3.000\$)
- MICE STABULATION: 10.000\$
- RESEARCH TEAM: ~100.000\$
- CRISPR-CAS9 KIT: 700\$
- CLONOGENIC ASSAY KIT: 190 \$
- LAB EQUIPMENTS BASICS: ~10.000\$
- MAB-0662: 150 \$
- IHC KIT: 300 \$
- PCR KIT: 500 \$
- HEK 293 CELL LINE: 341 \$



TOTAL COST OF  
~ 150.000\$  
FOR A TOTAL OF  
1.5 YEARS OF  
RESEARCH

# REFERENCES:

- <https://doi.org/10.3892/mmr.2015.3987> [1]
- [10.1158/1541-7786.MCR-19-0309](https://doi.org/10.1158/1541-7786.MCR-19-0309) [2]
- <https://doi.org/10.1158/2326-6066.CIR-15-0151> [3]
- [10.7150/jca.20665](https://doi.org/10.7150/jca.20665) [4]
- [10.1126/science.1236062](https://doi.org/10.1126/science.1236062) [5]
- <https://doi.org/10.1172/JCI139542>. [6]
- [10.1016/j.jconrel.2016.01.001](https://doi.org/10.1016/j.jconrel.2016.01.001) [7]
- [10.1038/srep15587](https://doi.org/10.1038/srep15587) [8]
- <https://horizondiscovery.com/en/reference-standards/products/idh1-r132h-reference-standard-50> [9]
- <https://en.vectorbuilder.com/> [10]
- <http://www.rgenome.net/cas-offinder/> [11]
- <https://pubmed.ncbi.nlm.nih.gov/28281173/> [12]
- <https://www.criver.com/products-services/find-model/nod-scid-mouse?region=27>
- [13]
- <https://www.atcc.org/products/htb-14ig> [14]
- <https://www.molcells.org/journal/view.html?doi=10.14348/molcells.2020.0098> [15]