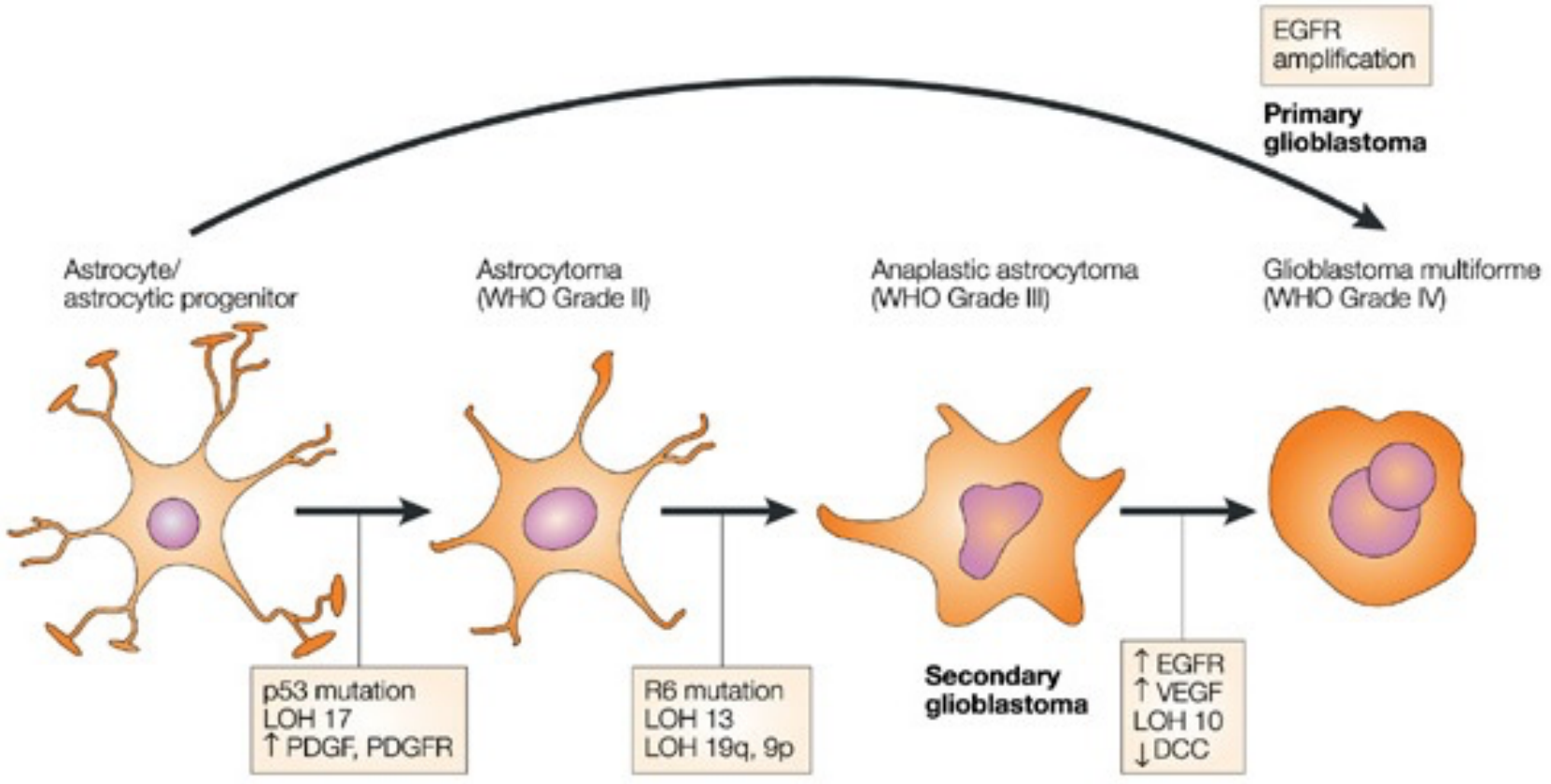
Synergic approach for Astrocytoma and Glioblastoma gene therapy

Boccuni Isabella
Cavalletti Alessandro
Porcheddu Giovanni Franc
Tortoriello Silvia

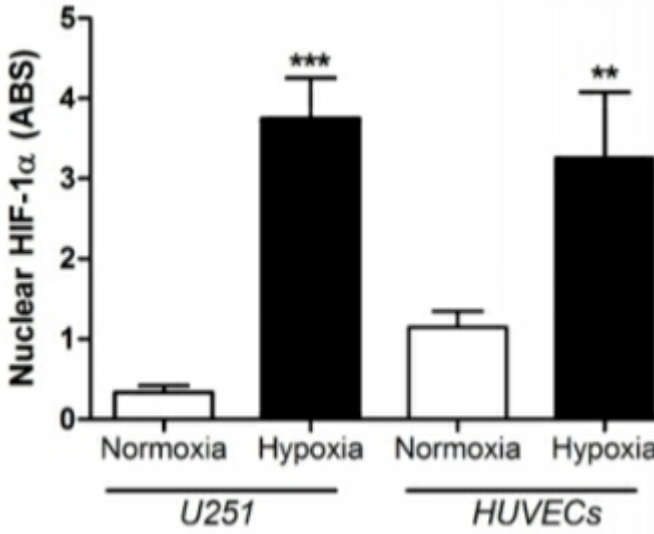
Corso di Laurea Magistrale
NEUROBIOLOGIA
SAPIENZA
Università di Roma

Astrocytoma and Glioblastoma



Background

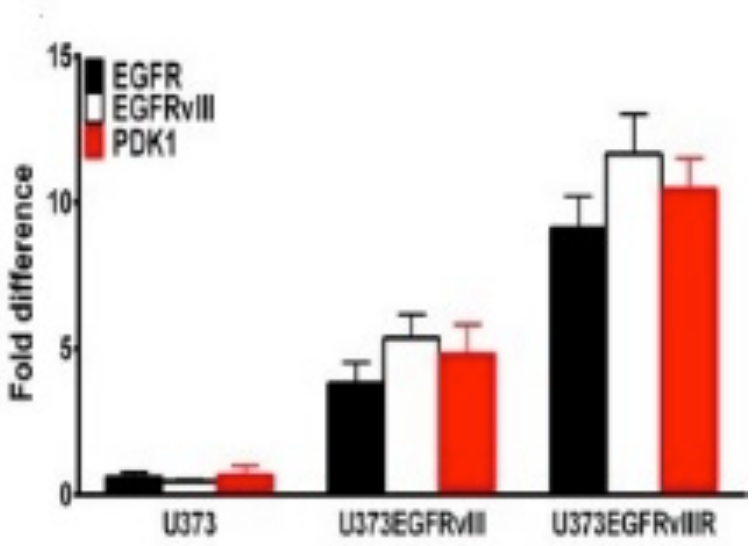
ne	Nucleotide change	Amino acid change	Total mutated cases	%	Reference
H1			1791		
	c.G395A	p.R132H	1655	92.4	28,30-39,41-48,56,59
	c.C394T	p.R132C	58	3.2	28,30-39,41-48,56,59
	c.C394G	p.R132G	38	2.1	28,30-39,41-48,56,59
	c.C394A	p.R132S	29	1.6	28,30-39,41-48,56,59
	c.G395T	p.R132L	11	0.6	28,30-39,41-48,56,59

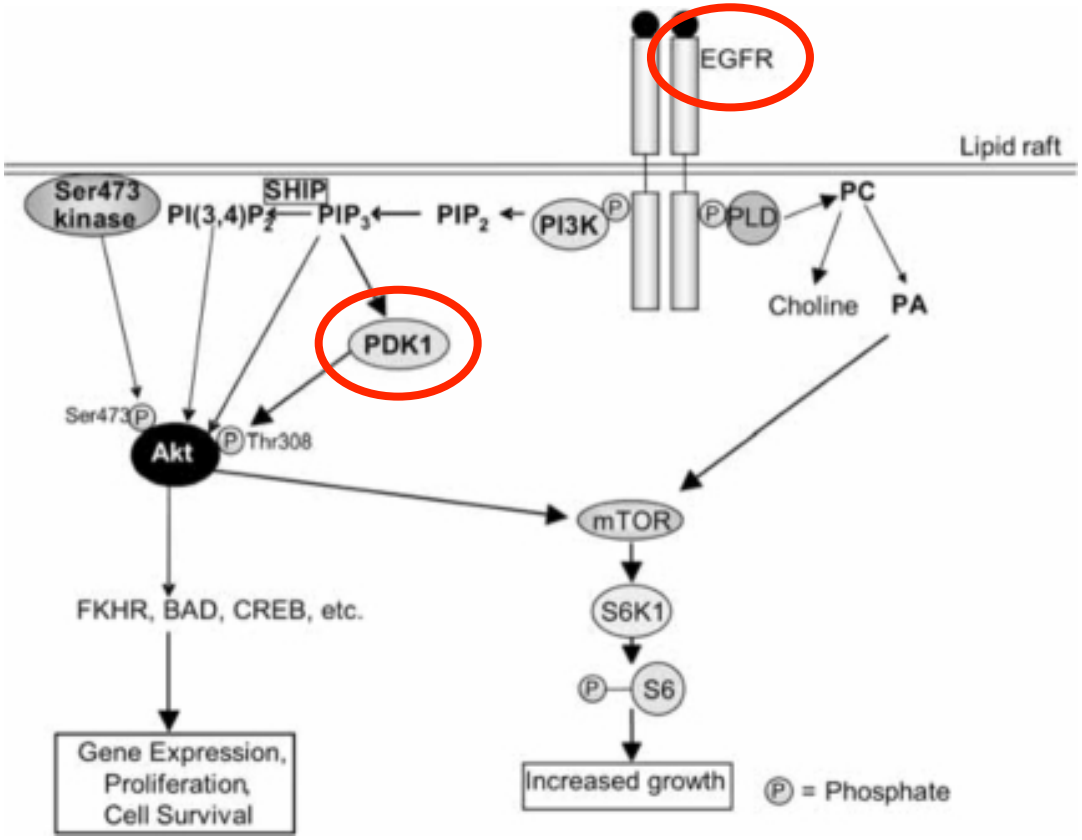


High levels of HIF-1α are common in human tumors and have been correlated with increased tumor vascularization, aggressive growth, resistance to radiation or chemotherapy, and overall poor clinical outcome (Gregg L.Semenza, 2007)

M. Mellai et al. (20

Background



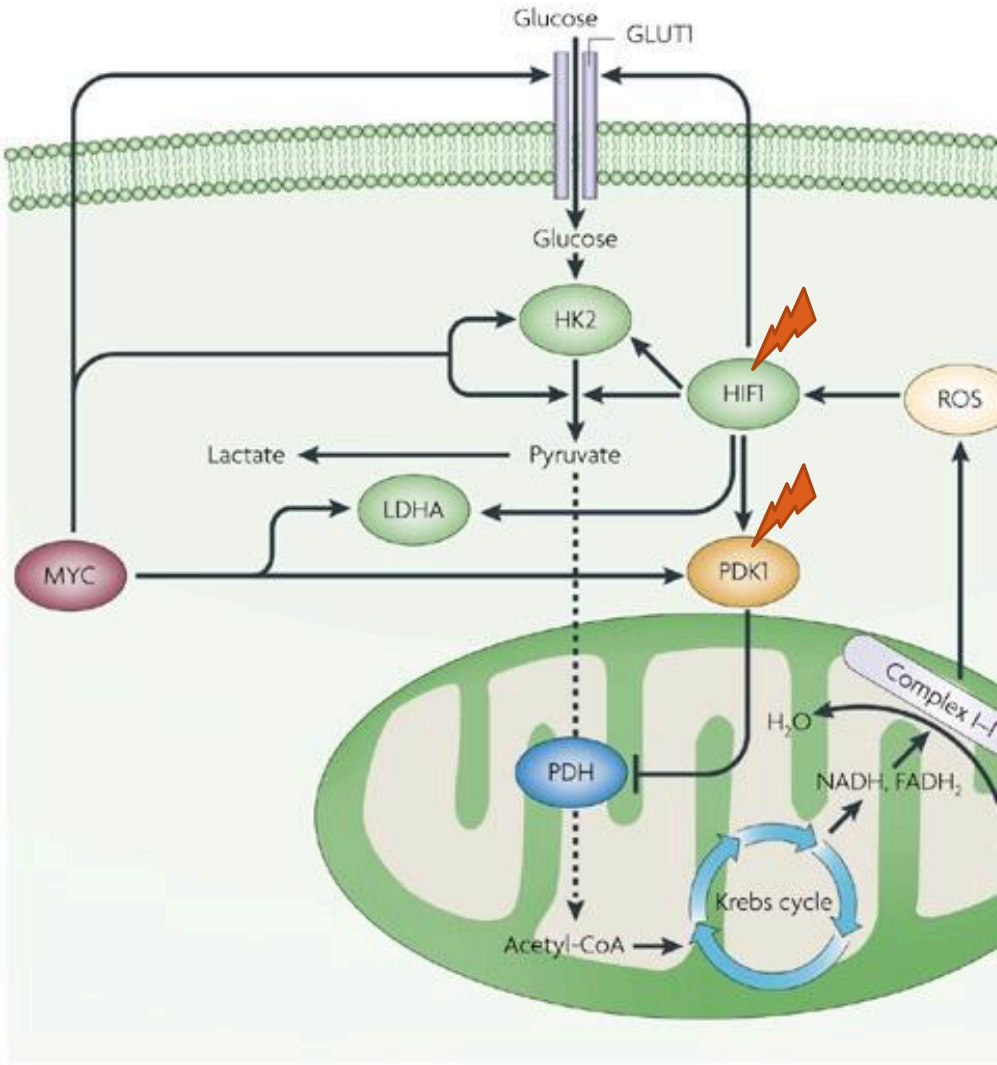


EGFRvIII → enhance PDK1 cascade
PDK1 → Anaerobic glycolysis
→ Cell survival and proliferation

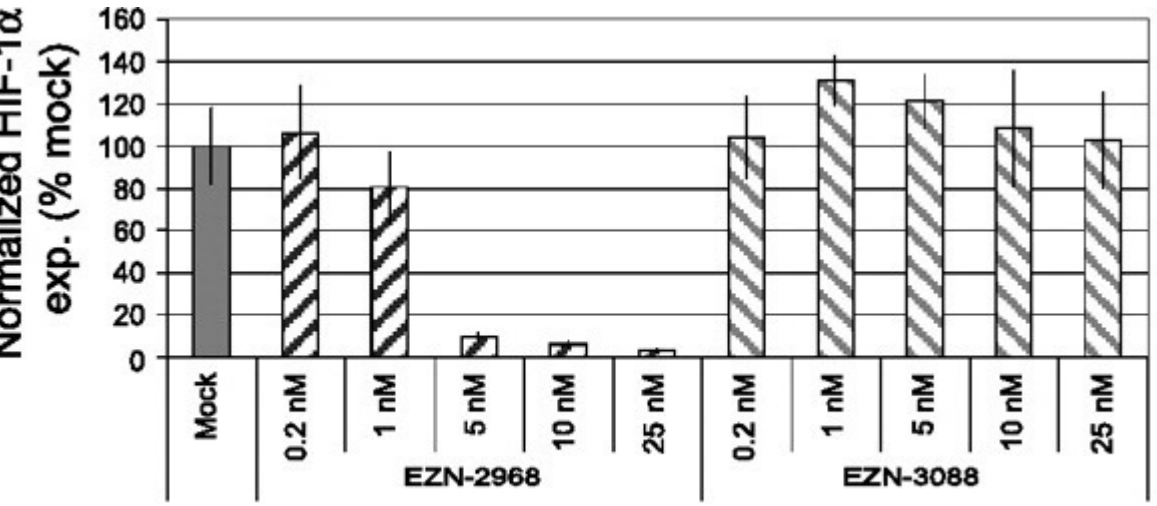
Therapeutic approach

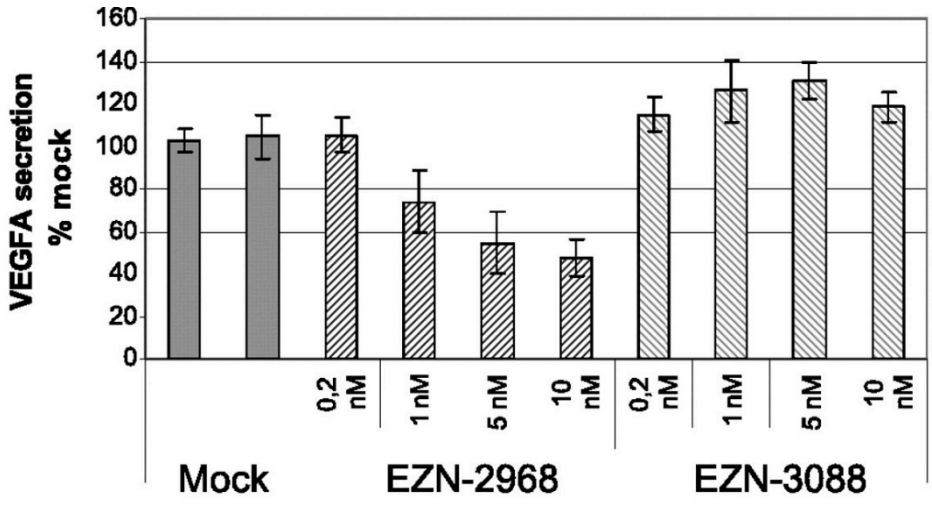
- EZN-2968 RNAinterference of HIF1a
- DCA inhibition of PDK1/EGFRvIII

- ❖ Inhibit the oxidative stress response of the glioma cells
- ❖ Increase ROS production in glioma cells



EZN-2968

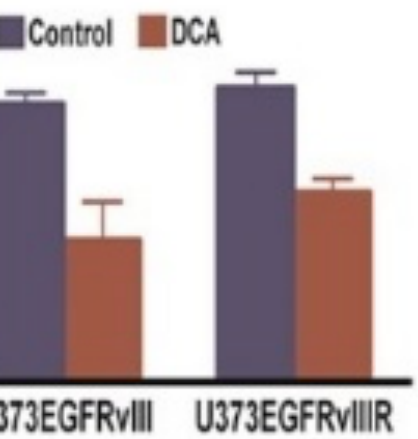


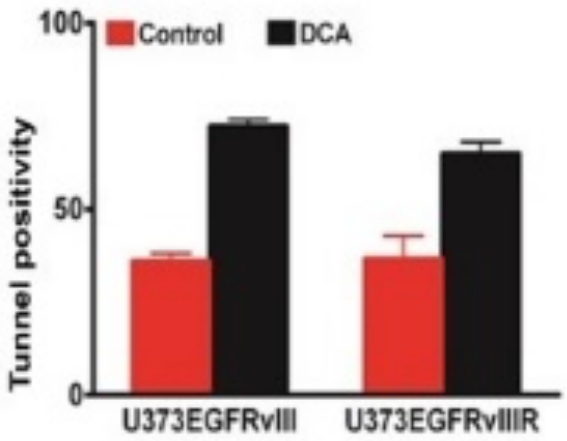


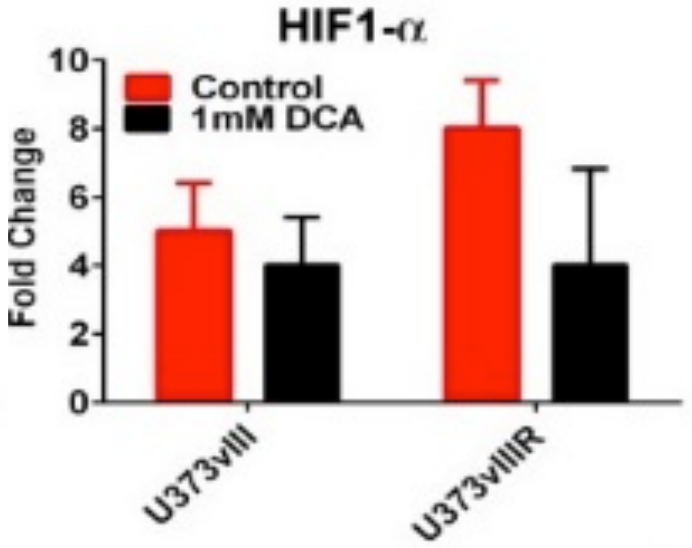
U373 glioma cell line

DCA treatment

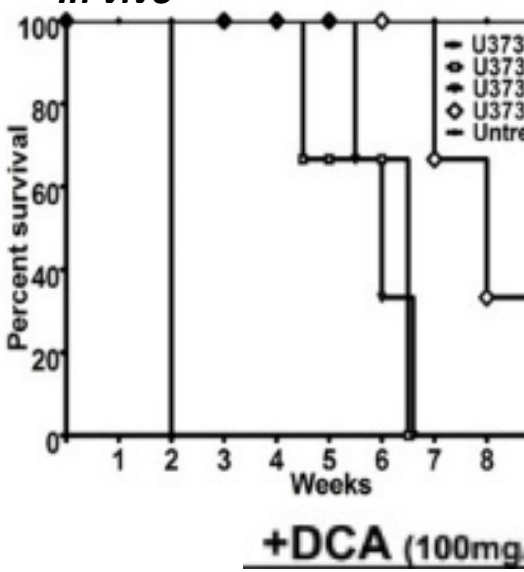
vitro







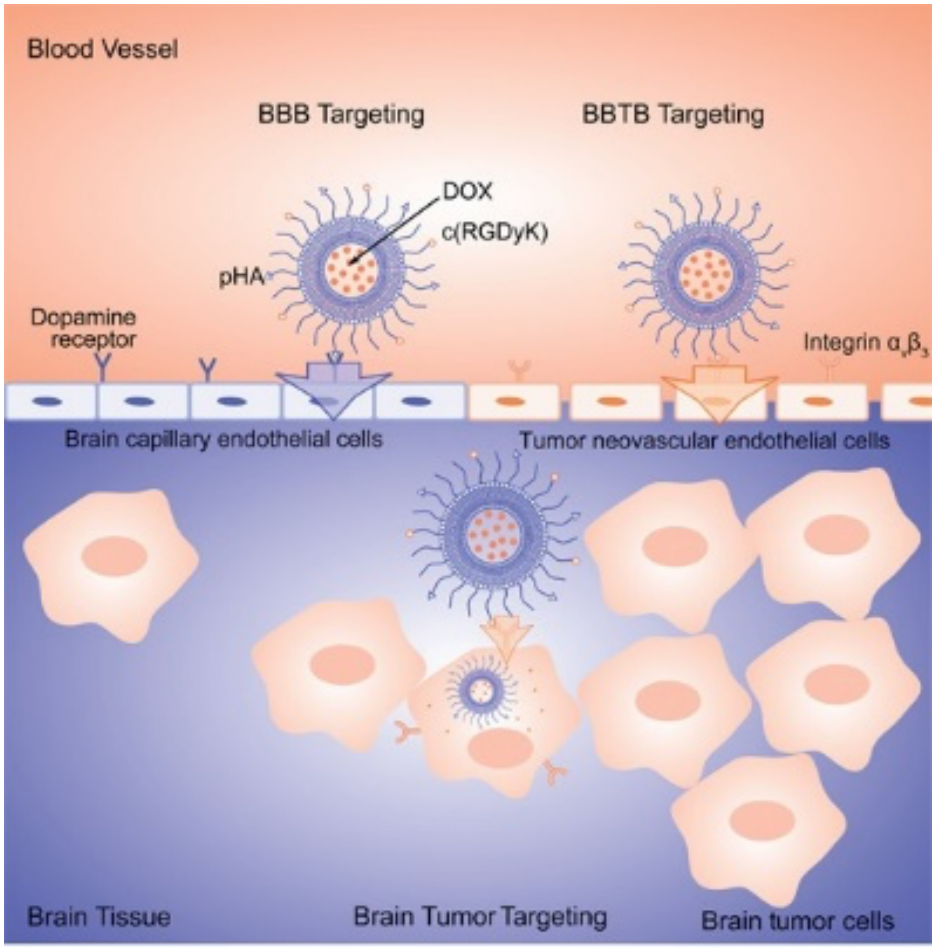
In vivo



U373 glioma cell line

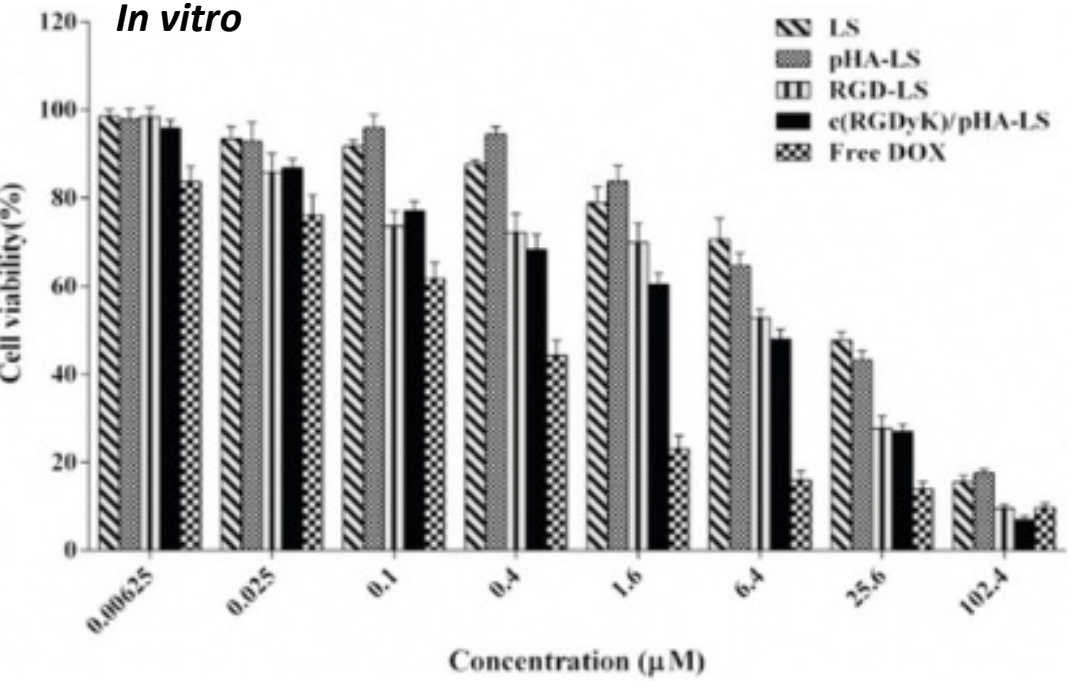
Delivery system: Liposomes

U87 glioma cell line

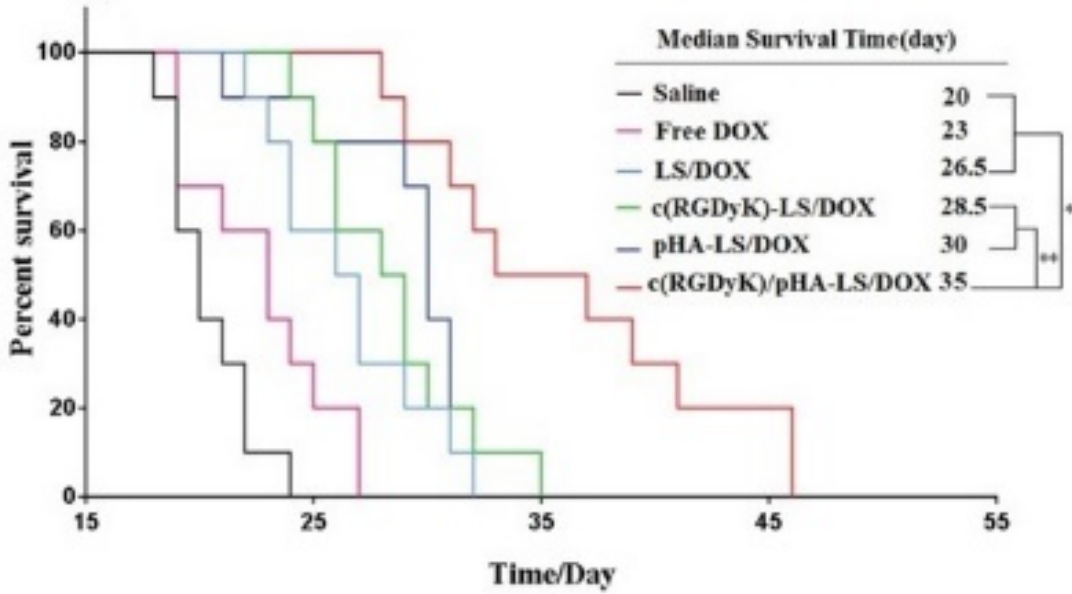


Effectiveness

In vitro



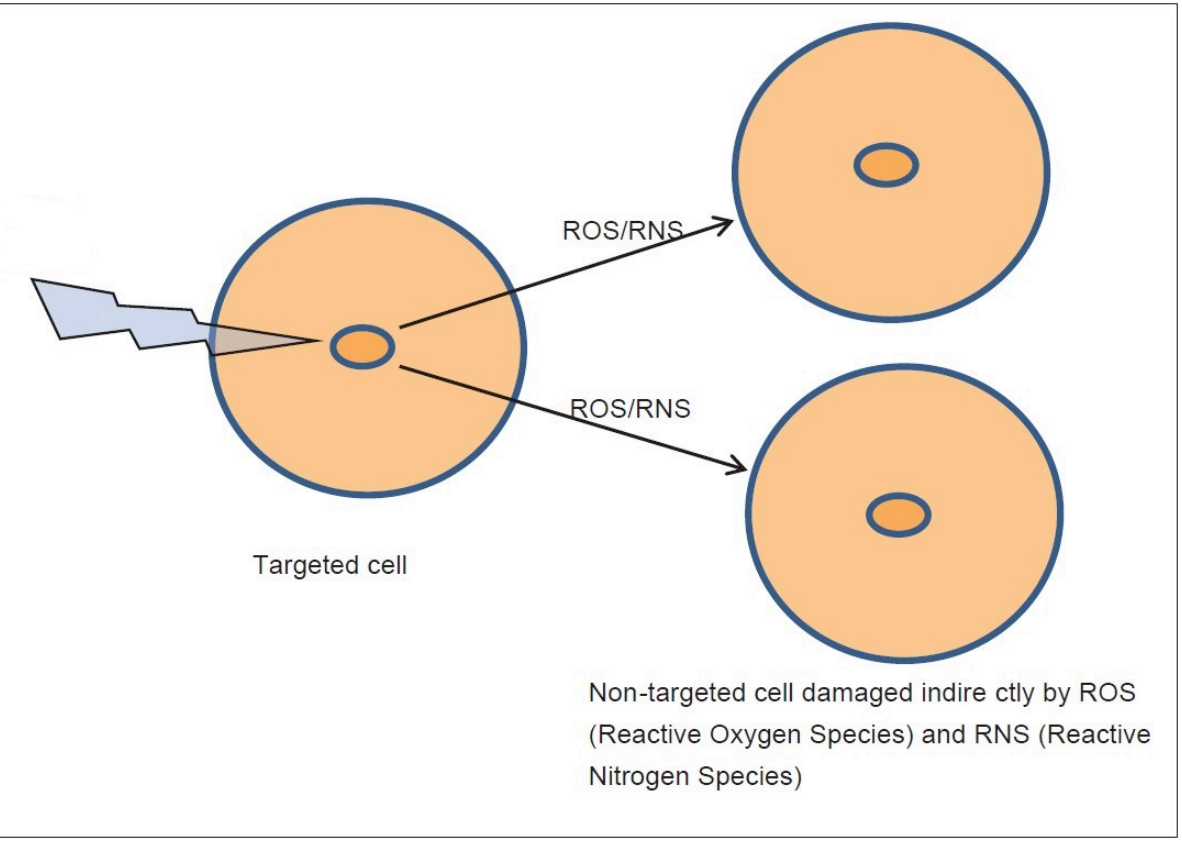
In vivo

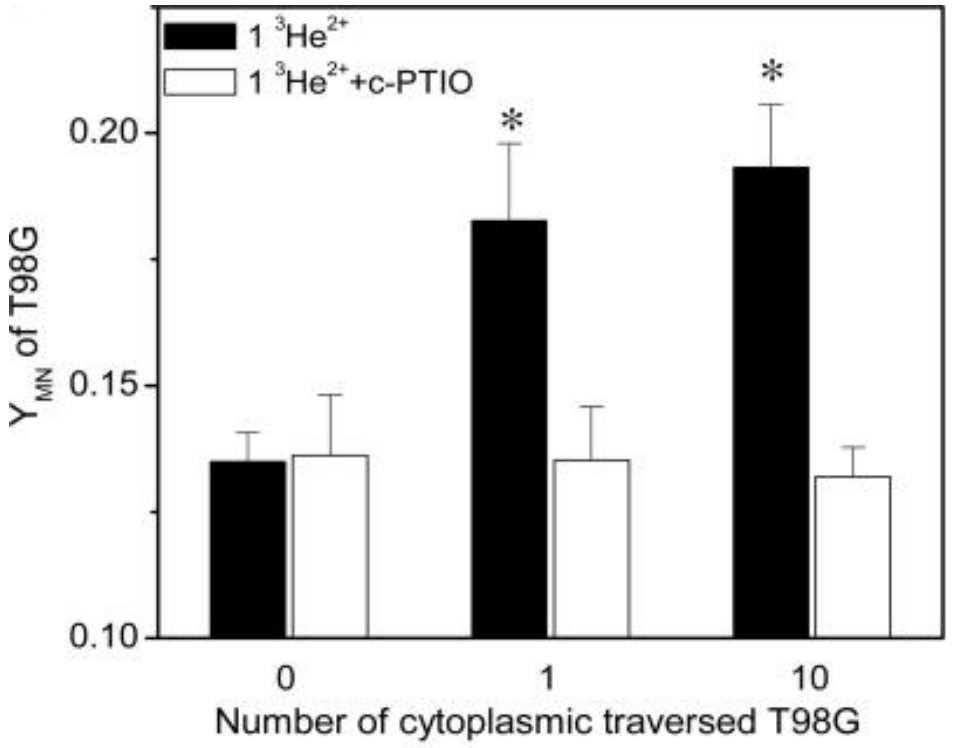


IC_{50} value of c(RGDyK)/pHA-LS/DOX was 4.79μM

Bystander effect

Free radical overproduction liberation in the tumor microenvironment





Human glioblastoma T98G cells

al N et al. J Can Res (2014)

Shao C et al. Proc Natl Acad Sci U S A. 2

Gene therapy strategy

1. First intravenous injection to enhance oxidative stress in glioma cells containing:

➢Glioma cells targeted liposomes

➢DCA targeting of EGFRvIII overexpressed in glioma cells

➢**Cre/STOP-loxP** under control of IDH1(R132H) gene overexpressed in glioma cells

2. Second intravenous injection to enhance antioxidant system in non-tumoral cells containing:

➢Non-tumoral cells targeted liposomes carrying ARE binding protein-1

➢DCA targeting of EGFRvIII overexpressed in glioma cells

➢**RNA detargeting** construct to avoid ARE binding protein-1 expression in glioma cells

Experimental plan

- Packaging of the DNA construct into the liposomes
- Physiologic solution of liposomes and DCA

Cell lines:

U373 glioma cell lines and U87 glioma cell line.

In vitro: Culture and co-culture of glioma cell lines with fibroblasts A0

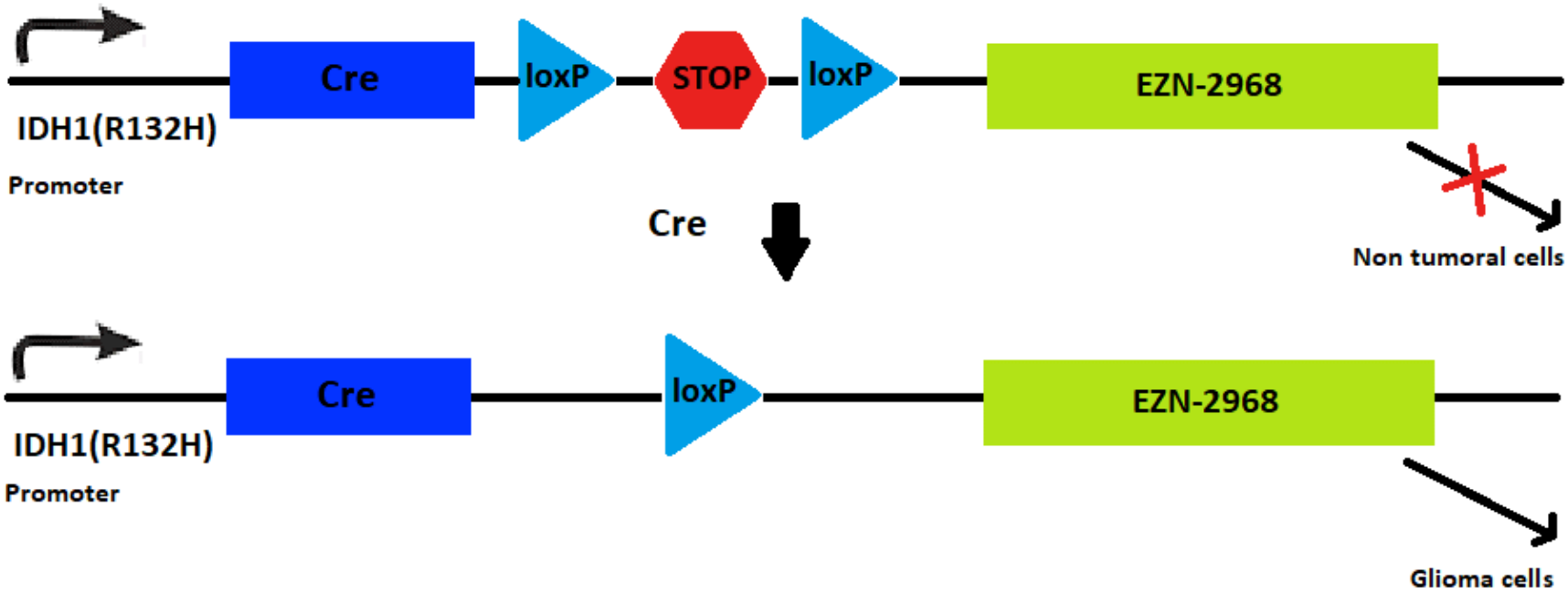
In vivo: Genetically engineered mice

Mouse model

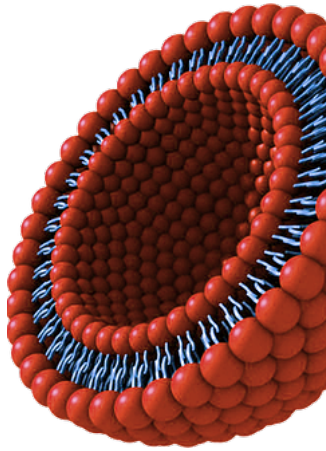
C57BL/6N mice CRISPR/Cas9 engineered

DNA construct and packaging

1. I DNA construct: **Cre/loxP system**



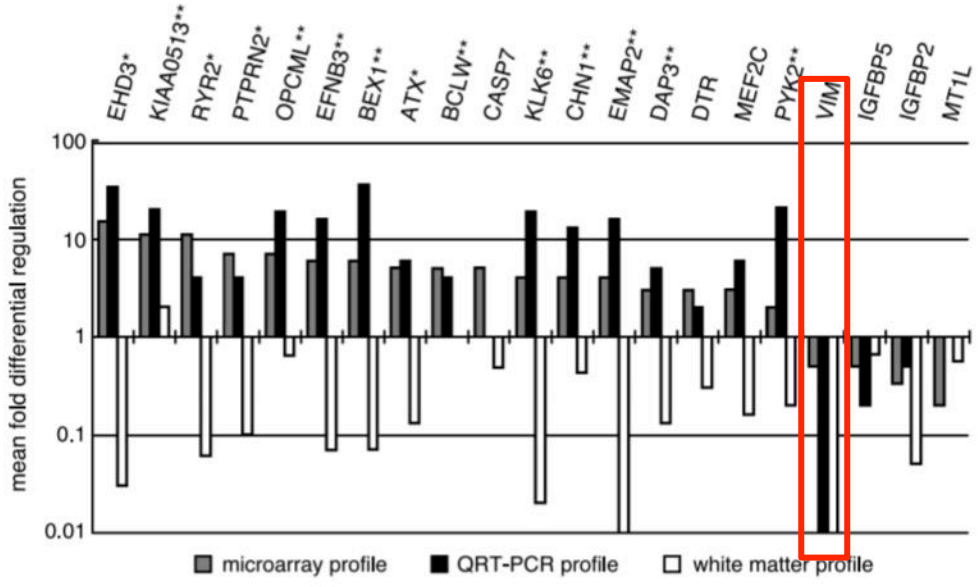
PACKAGING in
Liposomes
c(RGDyK)/pH



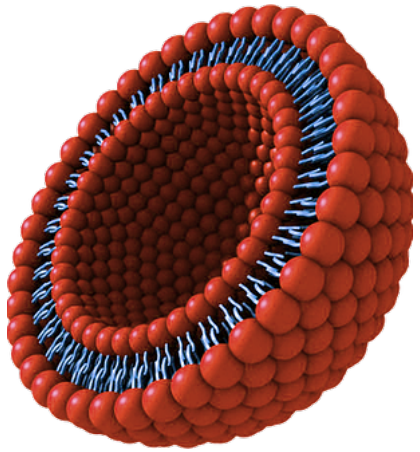
DNA construct and packaging

2. II DNA construct: **RNA detargeting**

Glioma gene expression profile

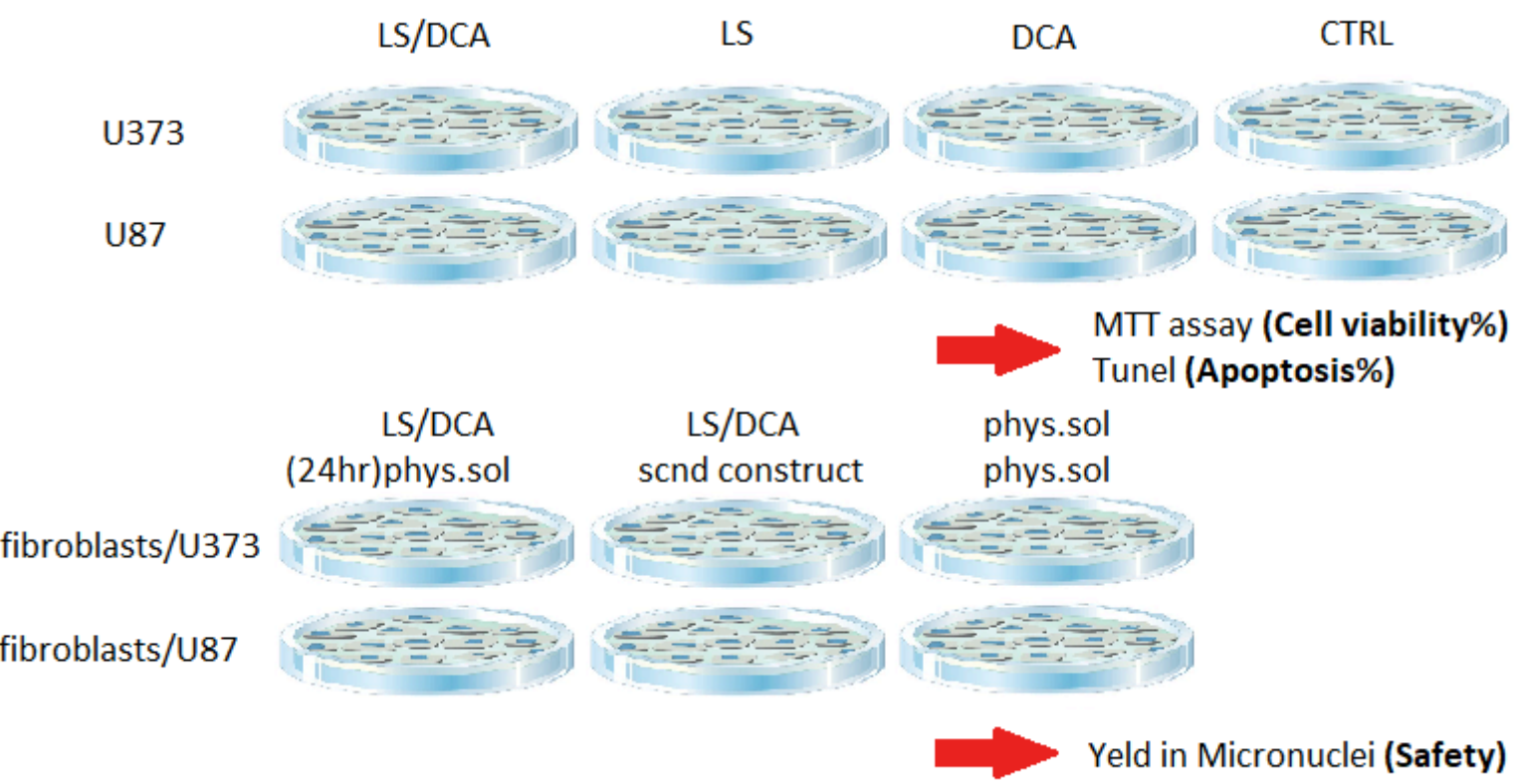


PACKAGING into
Liposomes
pHA



Dominique B. Hoelzinger et al. Neoplasia

In vitro

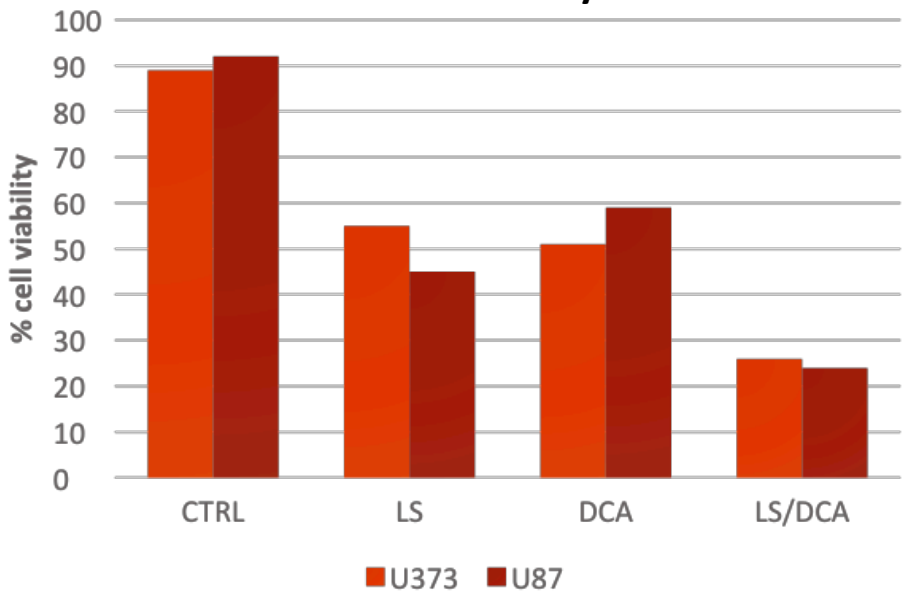


Physiologic solution with

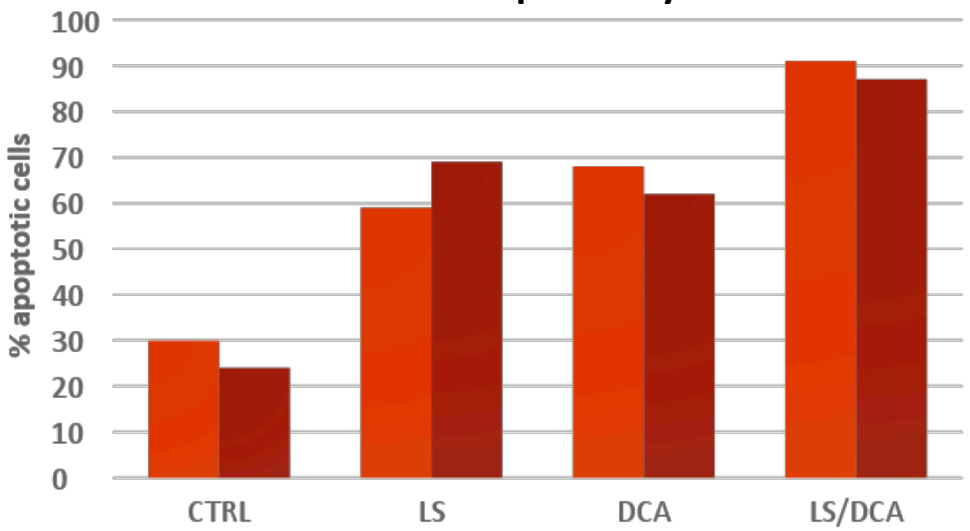
- 5uM c(RGDyK)/pHA-Liposome with 5nM/L of DNA construct I
- 1mM DCA

Results

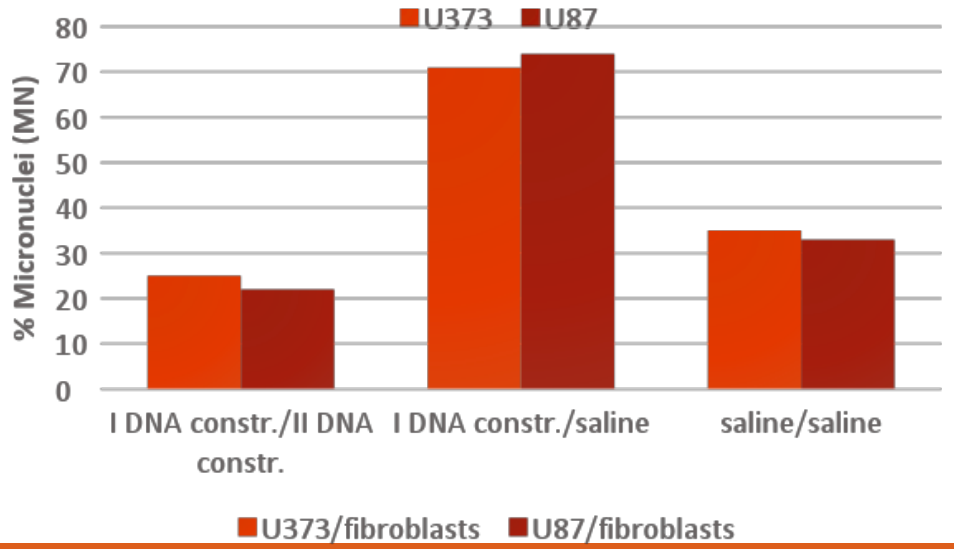
MTT assay



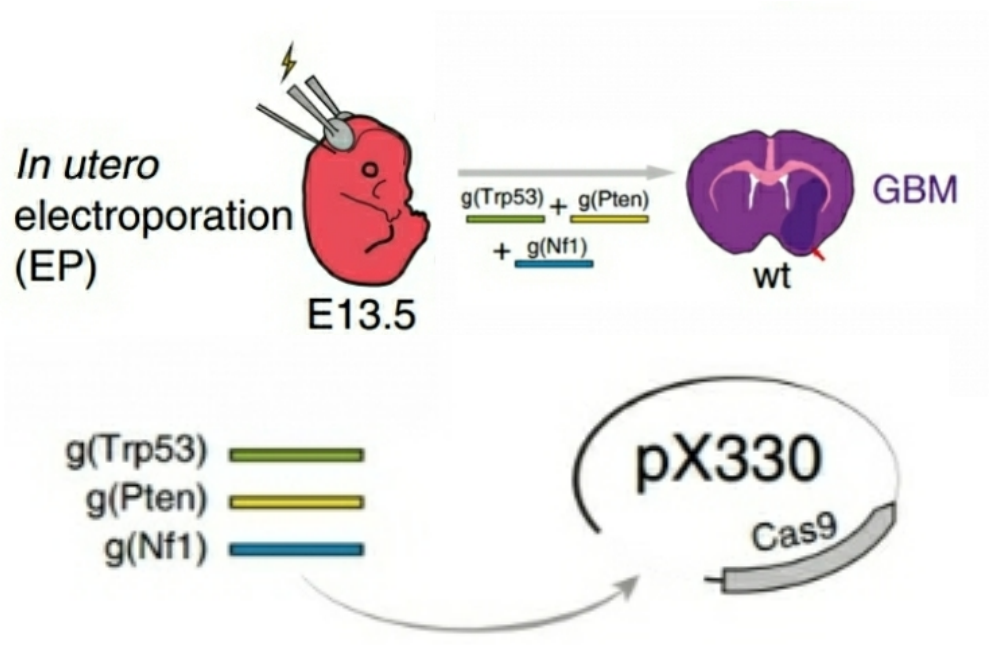
TUNEL positivity



Yield in Micronuclei (MN)



In vivo: Genetic model



Simultaneous disruption of multiple TSGs (Tumor Suppressor Genes) by using in utero electroporation with CRISPR/Cas9 system to induce Glioblastoma

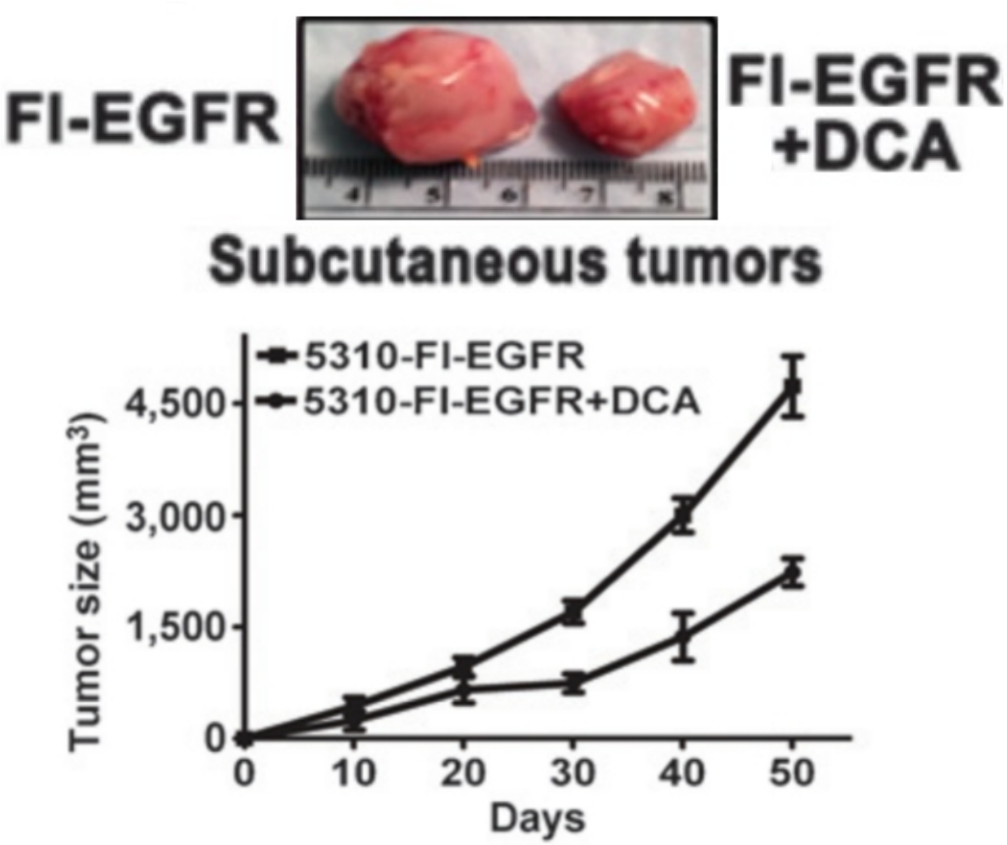
In vivo test survival of genetic mouse model of glioblastoma.

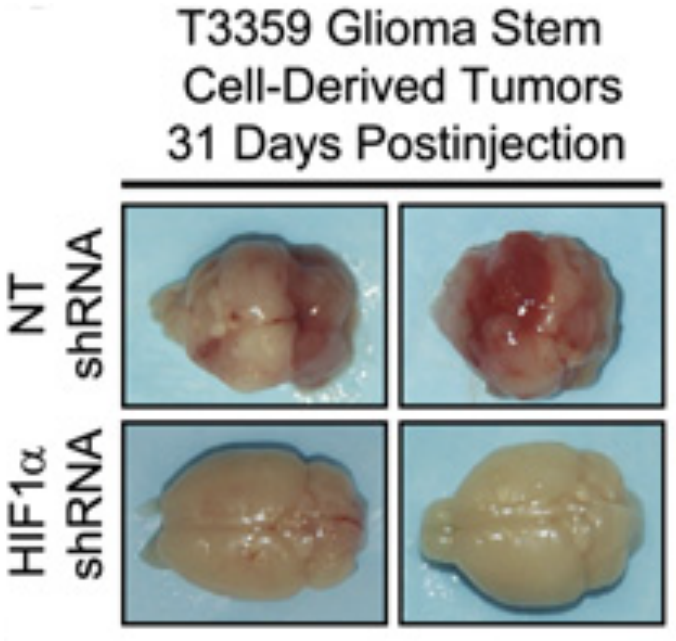
Intravenous injection into the tail vein of:

- 1° injection LS/DCA; 2° injection (24hr) phys sol (15 subjects)
- 1° injection LS/DCA; 2° injection (24hr) second construct (15 subjects)
- 1° injection phys sol; 2° injection (24hr) phys sol (15 subjects)

→ **Tumor Size measurement**

Results

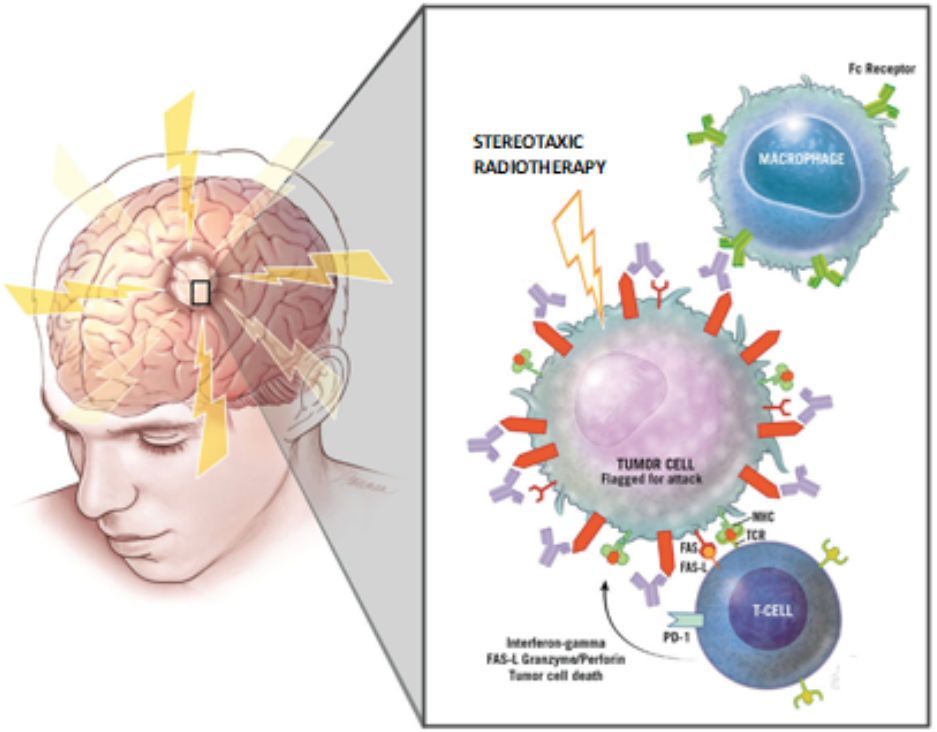




We expect a tumor regression and a reduced vascularization

Pitfalls and Possible improvement

Inflammation spreading from the tumor site to the cerebral parenchyma could dangerously affect the viability of health cells
→ Stereotaxic radiotherapy may define the site of oxidative damage exclusively in the tumoral mass.



Costs and time

3 (Uppsala) cell line	447.50EUR (ECACC)
cell line	346.50EUR (ECACC)
(3-(4,5-Dimethylthiazol-2-yl)-2,5-henyltetrazolium Bromide)	146EUR (Thermo Fisher Scientific)
EL In Situ Cell Death Detection Kit	682EUR (Sigma-Aldrich)
philized powder	89.20EUR (Sigma-Aldrich)
loroacetate	246.50EUR (Sigma-Aldrich)
ulation	9000EUR/year
BL/6N male-female mice (breeding)	20.32EUR male + 22.22EUR female (The Jackson Laboratory)
PR/CAS9 system	745EUR (ZhangLab)

ECAC
European Collec
of Authenticated
Cell Cultures
Operated by Public Health Engla

ThermoFisher
SCIENTIFIC

SIGMA-ALDRIC

The Jackso
Laboratory

References

Hoelzinger DB, Mariani L, Weis J, Woyke T, Berens TJ, McDonough WS, Sloan A, Coons SW, Berens ME (2005), *Gene expression profile of glioblastoma multiforme invasive phenotype points to new therapeutic targets*, Neoplasia, **7**, pp. 7-16

Kiran K. Velpula, Maheedhara R. Guda, Kamlesh Sahu, Jack Tuszynski, Swapna Asuthkar, Sarah E. Bach, Justin D. Lathia, and Andrew J. Tsung (2017) *Metabolic targeting of EGFRvIII/PDK1 axis in temozolomide resistant glioblastoma,* Oncotarget 8(22), pp. 35639–35655

Kyeung Min Joo, Jinkuk Kim, Juyoun Jin, Misuk Kim, Ho Jun Seol, Johongir Muradov, Heekyoung Yang, Yoon-La Choi, Woong-Yang Park, Doo-Sik Kong, Jung-Il Lee, Young-Hyeh Ko, Hyun Goo Woo, Jeongwu Lee, Sunghoon Kim, Do-Hyun Nam (2013) *Patient-Specific Orthotopic Glioblastoma Xenograft Models Recapitulate the Histopathology and Biology of Human Glioblastomas In Situ*, Cell Press

Lee M. Greenberger, Ivan D. Horak, David Filpula, Puja Sapra, Majken Westergaard, Henrik F. Frydenlund, Charlotte Albæk, Henrik Schrøder and Henrik Ørum (2008) *A RNA antagonist of hypoxia-inducible factor-1α, EZN-2968, inhibits tumor cell growth,* Molecular Cancer Therapeutics, vol.**7**

Lesniak M.S. & Brem H., (2004) *Targeted therapy for brain tumours,* Nature Reviews Drug Discovery **3**, pp. 499–508

Mellai M., Caldera V., Annovazzi L. , Schiffer D. (2013) *The Distribution and Significance of IDH Mutations in Gliomas*, Evolution of the Molecular Biology of Brain Tumors and the Therapeutic Implications

Nasir Jalal, Saba Haq, Namrah Anwar, Saadiya Nazeer, Umar Saeed (2014) *Radiation induced bystander effect and DNA damage,* Journal of Cancer Research and Therapeutics, **10**, pp.819-833

References

Semenza GL., (2007) *Evaluation of HIF-1 inhibitors as anticancer agents*, Drug Discov. Today,**12**, pp.853–859.

Shao C, Folkard M, Barry DM, Prise K (2004) *Targeted cytoplasmic irradiation induces bystander responses*, Proc Natl Acad Sci U S A, **101**, pp.13495–13500

Sharabi AB, Tran PT, Lim M, Drake CG, Deweese TL (2015), *Stereotactic radiation therapy combined with immunotherapy: augmenting the role of radiation in local and systemic treatment*, Oncology (Williston Park), **29**, pp. 331-340

Zakia Belhadj, Changyou Zhan, Man Ying, Xiaoli Wei, Cao Xie, Zhiqiang Yan, and Weiyue Lu (2017) *Multifunctional targeted liposomal drug delivery for efficient glioblastoma treatment*, Oncotarget **8**, pp. 66889–66900.

Zuckermann M, Hovestadt V, Knobbe-Thomsen CB, Zapatka M, Northcott PA, Schramm K, Belic J, Jones DT, Tschida B, Moriarity B, Largaespada D, Roussel MF, Korshunov A, Reifenberger G, Pfister SM, Lichter P, Kawauchi D, Gronych J (2015) *Somatic CRISPR/Cas9-mediated tumour suppressor disruption enables versatile brain tumour modelling*, Nature Communications

Velpula KK, Bhasin A, Asuthkar S, Tsung AjJ. (2013) *Combined targeting of PDK1 and EGFR triggers regression of Glioblastoma by reversing the Warburg effect*. Cancer Research

Zhizhong L et al. (2009) *Hypoxia-Inducible factors regulate Tumorigenic capacity of glioma stem cells.* Cancer Cell