OLIGODENDROGLIOMA: COMBINED GENE THERAPY APPROACH

Corso di Laurea Magistrale
NEUROBIOLOGIA
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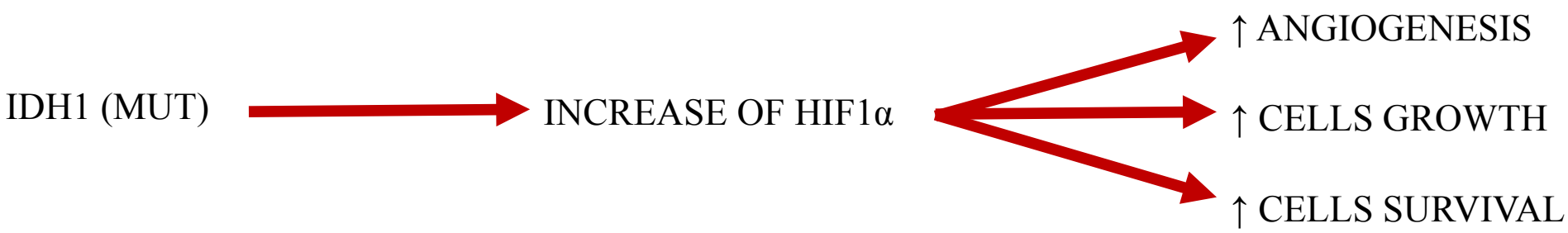
WHY OLIGODENDROGLIOMA?

- ✓ Predominantly adulthood tumor;
- ✓ Solid tumor hard to target;
- ✓ Actual Therapy: Surgery and Chemotherapy.

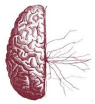
Van Den Bent J.M., et al., 2008

WHICH MUTATIONS?

Marker	Alteration	Tumor type	Comment
1p/19q	Deletion of short arm Ch. 1 and long arm of Ch. 19	Oligodendrogliomas	Never found in non<glial malignancies, often found with IDH mutations
Atrx	Mutation or deletion	Secondary GBM and Low Grade Glioma	Correlates with p53 expression, never found with 1p/19q deletions
BRAF	V600E or fusion gene KIAA1549:BRAF	Pilocytic Astrocytomas	
CDK4	Amplification	Proneural	
EGFR/EGFRvIII	Over amplification and mutation	Primary Glioma	Mutually Exclusive of p53 mutations
HIF1<a	Overexpressed	High Grade Gliomas	
IDH	Missense mutation at arginine 132 (1) or 172 (2)	Oligodendrogliomas and Secondary GBM	Associated with G<CIMP, precedes 1p/19q deletion or p53 alterations



Ludwig K. And Kornblum H., 2017



GOALS

1. Enhance tumoral cell apoptosis with:

- Temozolomide and Irinotecan chemoterapy;
- Tumoral cells targeted liposomes, carrying **HSV-TK1** construct under control of IDH1(R132H) Gene, overexpressed in oligodendroglioma cells.

2. Protect healthy cells with:

- Non-tumoral cells targeted liposomes carrying **artificial siRNA**;
- **Artificial siRNA** construct, under control of SDHB Gene, expressed on Chromosome 1p, to enhance off target protection.

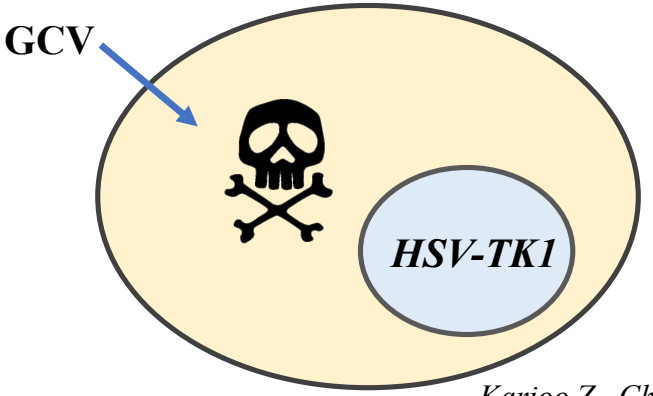
ANTITUMORAL STRATEGY

CHEMOTHERAPY
TEMOZOLOMIDE/IRINOTECAN

After 3 weeks

Igarashi K., Kawaguchi K. et al., 2017

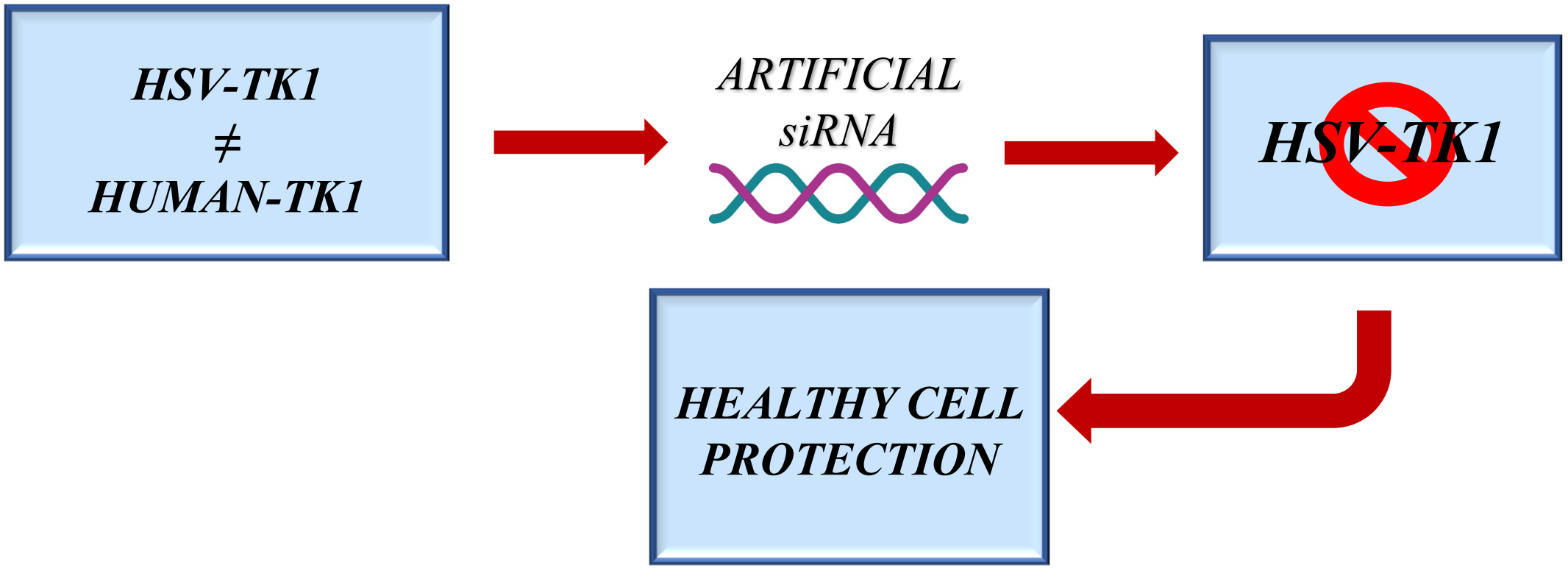
SUICIDE GENE
HSV-TK1 + GANCICLOVIR



Karjoo Z., Chen X. and Hatefi A., 2016

CELL APOPTOSIS

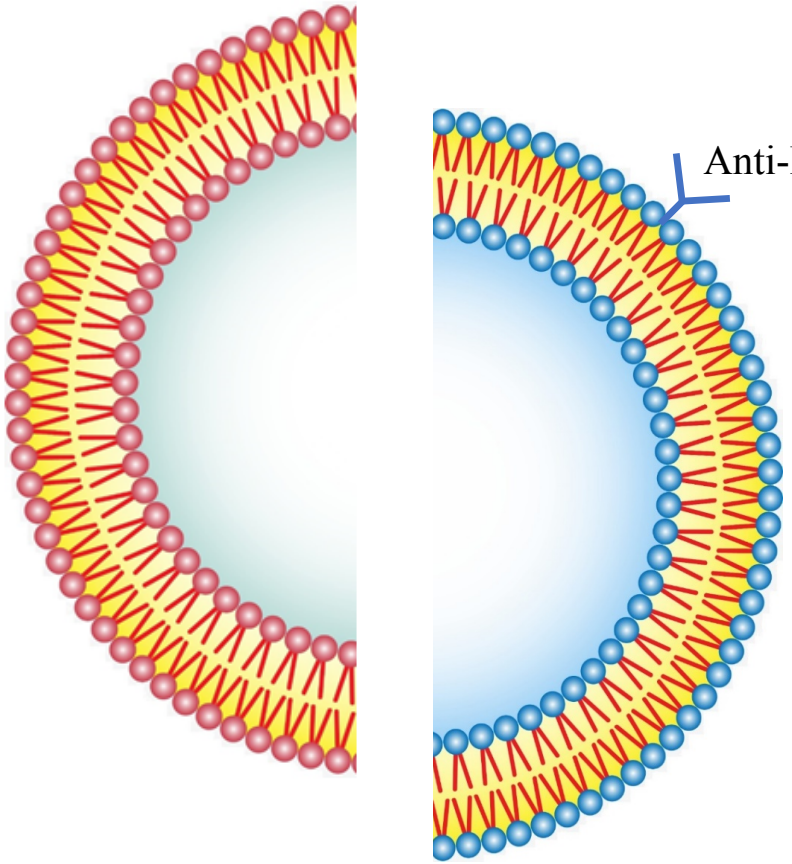
HOW TO PROTECT HEALTHY CELLS?



WHAT IS THE DELIVERY SYSTEM?

Liposomes

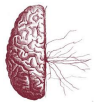
HEALTHY CELLS PROTECTION: NAKED LIPOSOME



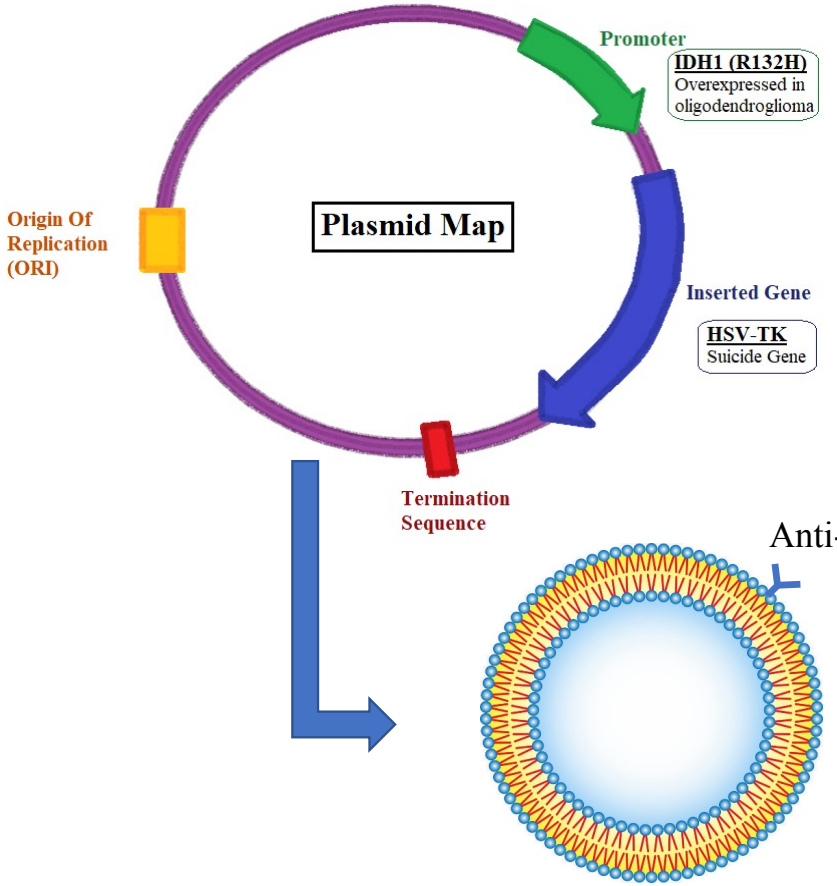
ANTITUMORAL TARGET: Directed against PDGFαR

Sercombe L. and Hua S., et al. 2015

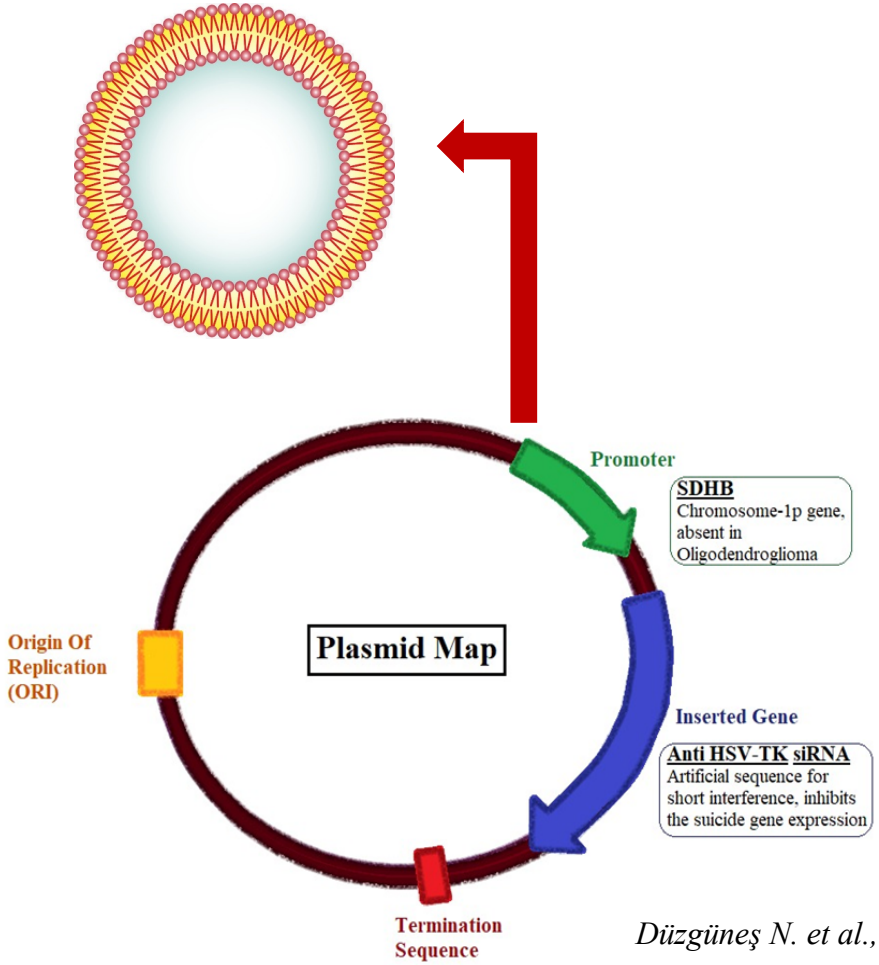
DNA CONSTRUCTS



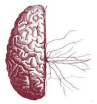
ANTITUMORAL TARGET



HEALTHY CELLS PROTECTION



Düzgüneş N. et al., 2018



EXPERIMENTAL PLAN

Cell lines used:

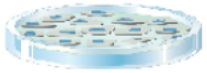
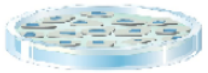
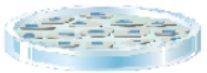
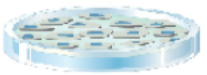
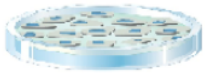
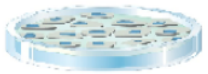
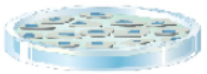
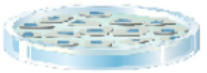
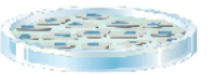
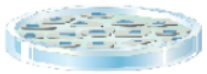
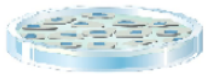
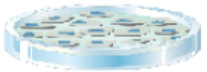
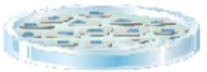
- Oligodendrocyte cell line ReNcell SCC007;
- Oligodendroglioma cell line HOG SCC163;
- CTR cell line.

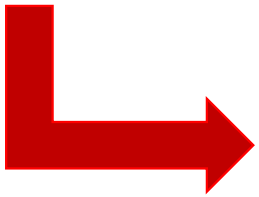
***In vitro conditions tested*:**

- Physiologic solution;
- Ganciclovir;
- Herpesvirus thymidine kinase 1;
- Ganciclovir and herpesvirus thymidine kinase 1;
- Ganciclovir, herpesvirus thymidine kinase 1 and siRNA.

***In vivo*:** SCID mouse as oligodendroglioma model, obtained by xenotranspalntation of SCC 163 cell line.

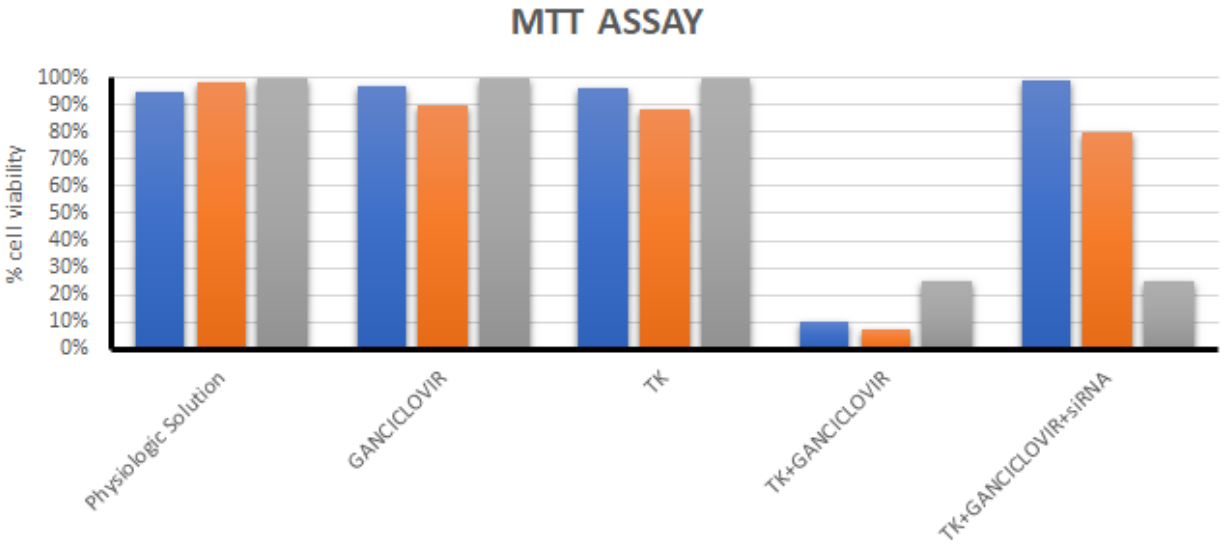
IN VITRO MODEL

	PHYSIOLOGIC SOLUTION	HSV-TK	GCV	GCV + HSV-TK	GCV + HSV-TK + siRNA
RENCELL SCC007 (WT)					
HOG SCC163 (MUT)					
CTR					



MTT assay (cell viability %)
TUNEL assay (cell apoptosis %)

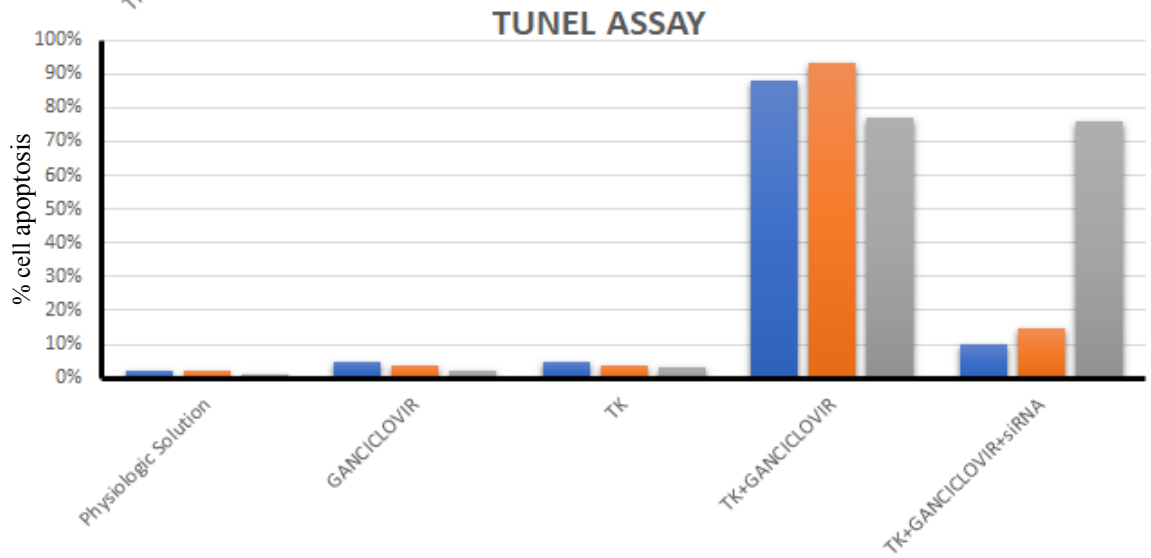
WHAT TO EXPECT?



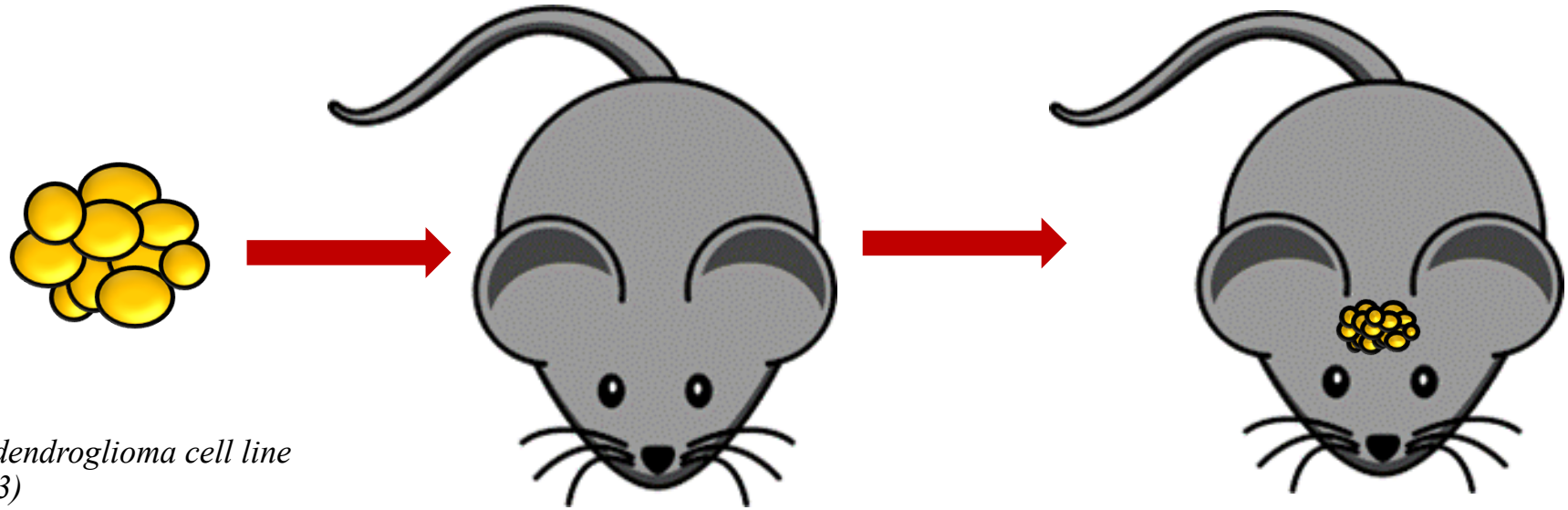
CTR

RENCELL SCC007 (WT)

HOG SCC163 (MUT)



…AND IN VIVO?



Human Oligodendroglioma cell line (HOG SCC163)

SCID Mouse

This mouse model is obtained by xenotranspalntation of SCC 163 cell line

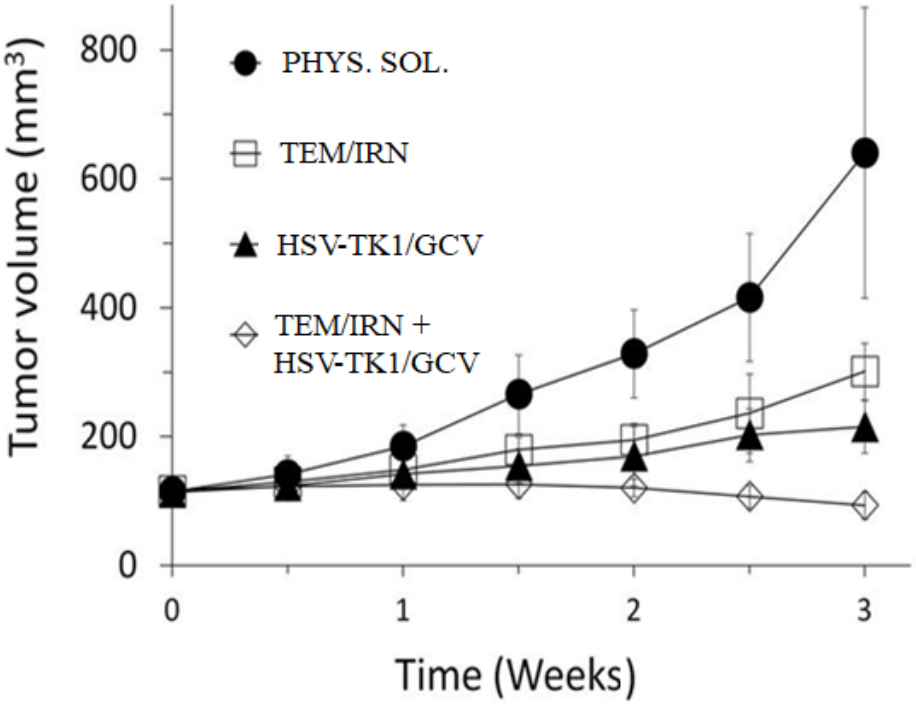
Xu H., Shen B. et al., 2018

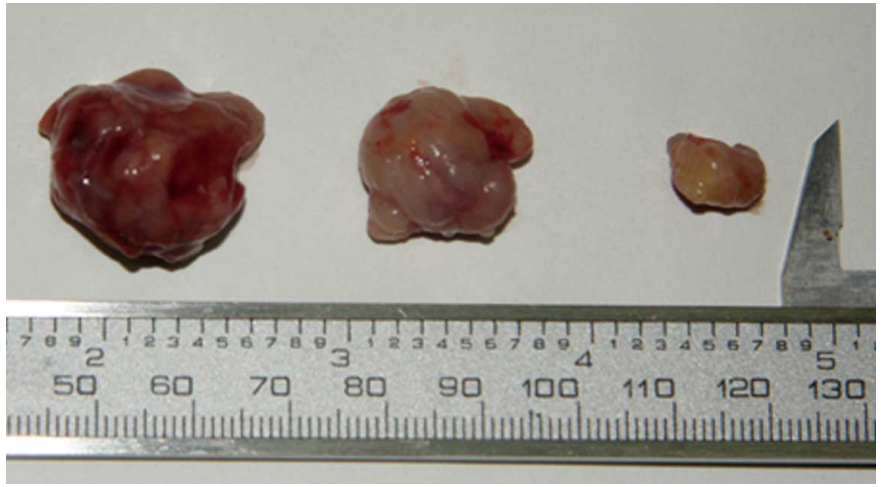
EXPERIMENTAL PLAN
… 14 days later

Phys Sol.	●	●	●	●
TEM/IRN	●	●	●	●
Liposome Anti-PDGFαR	●	●	●	●
Naked Liposome	●	●	●	●
Ganciclovir	●	●	●	●

- ✓ Phys Sol., TEM/IRN and Ganciclovir are given by intravenous injections into the tail vein;
- ✓ Anti-PDGFαR Liposomes and Naked Liposomes are given by intraperitoneal injections into the tumoral mass;
- ✓ Ganciclovir is given 2-3 days after liposomes administration.

…AND 3 WEEKS LATER





We expect a tumor regression and a reduced vascularization

Adapted from Igarashi K., Kawaguchi K. et al., 2017

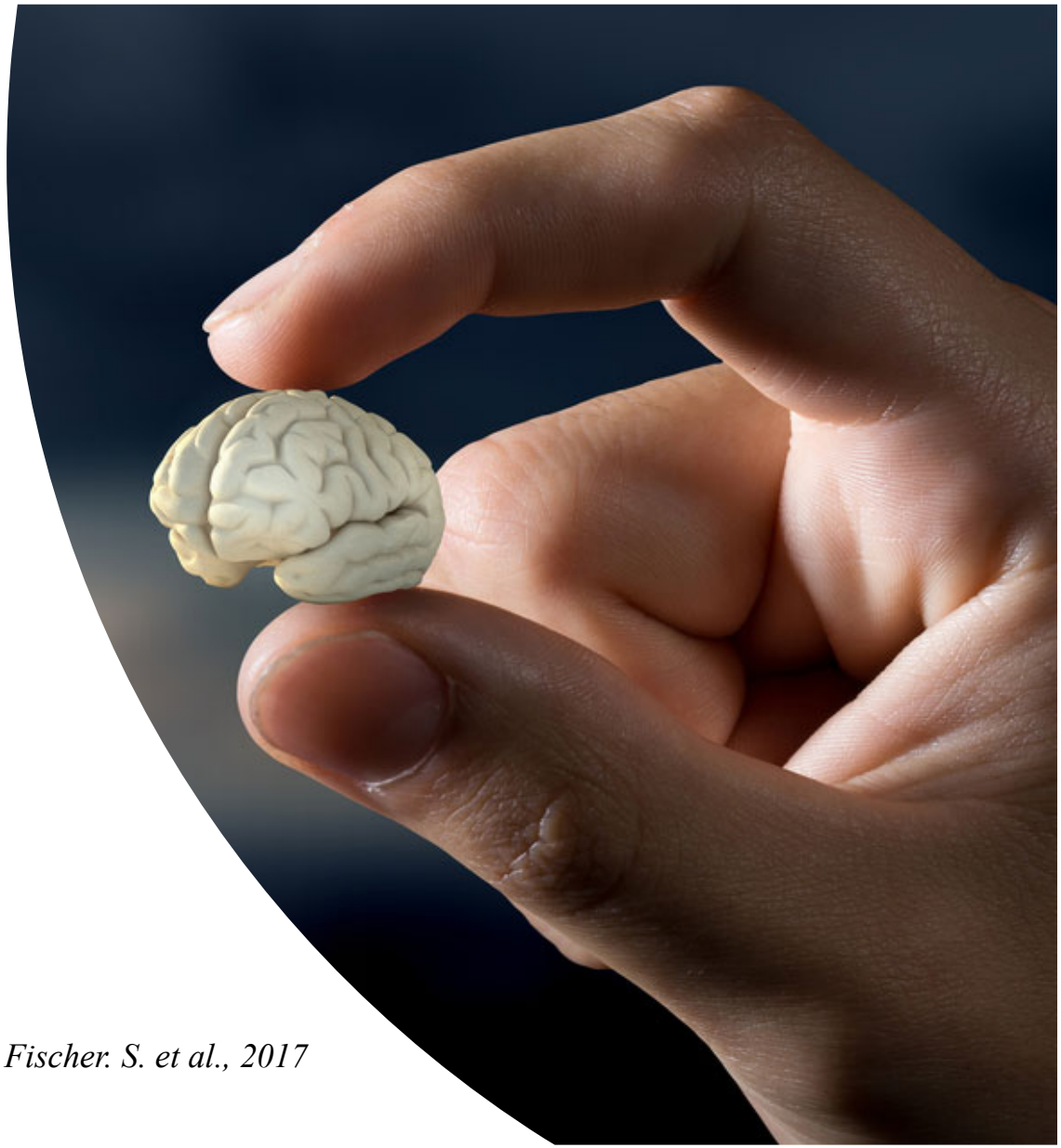
PITFALL AND SOLUTION

PITFALL

Xenotranslplantation doesn't mimic what happen into the brain, perfectly.

POSSIBLE IMPROVEMENT

Minibrain can be used.



Fischer. S. et al., 2017

MATERIALS AND COSTS

TOT. 21.678,66€ (Without laboratory materials and researchers salaries)

MATERIALS	COSTS	TIME/QUANTITY	SUPPLIERS
ReNCell CX Human Neural Progenitor Cell Line (*SCC007*)	2960 €	$1x10^6$	SIGMA-ALDRICH
Human Oligodendroglioma Cells (*SCC163*)	853 €	$1x10^6$	SIGMA-ALDRICH
Liposomes Kit (*Cationic Liposomes with Transfection Reagents*)	400 €	25g	ThermoFisher SCIENTIFIC
Anti-PDGFα Antibody	322 €	50µg	SIGMA-ALDRICH
Ganciclovir (*GCV*)	319 €	500mL	AdooQ BIOSCIENCE
HSV-TK1 Plasmid	405 €	20µg	InvivoGen
Homozygous for $Prdk^{scid}$	179,66 €	Fertile Couple 3 weeks old	The Jackson Laboratory Leading the search for tomorrow's cures
Gene Designed Construct for Short Interference	7240 €	Replicable Strand	GenScript Make Research Easy
Stabulation	9000 €	Yearly	

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