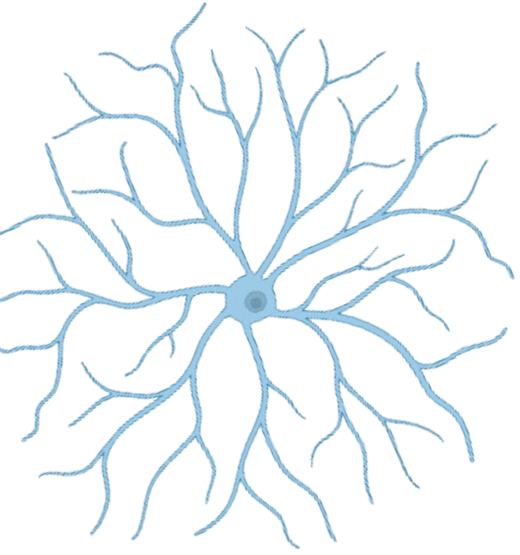
SAPIENZA
UNIVERSITÀ DI ROMA

FGFR3-TACC3 GENE FUSION IN HUMAN GLIOMA: A NEW COMBINED GENE THERAPY APPROACH USING CAS13A AND A GFAP-SELECTIVE ONCOLYTIC ADENOVIRUS



Class of Gene Therapy and Neuroscience A.A 2023/2024
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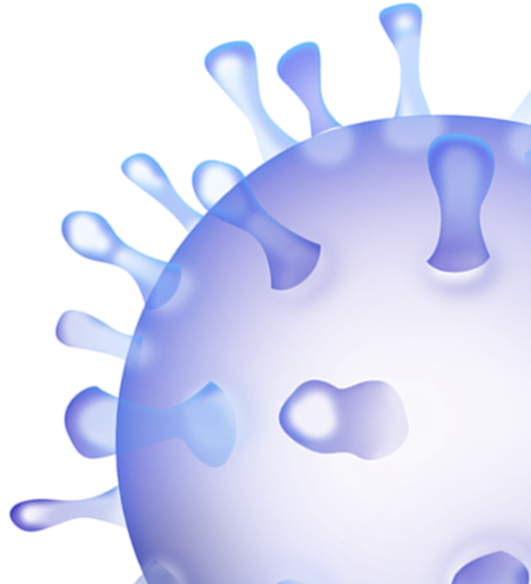

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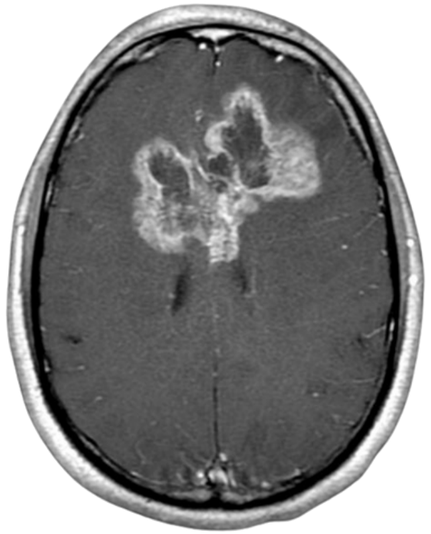
INTRODUCTION

WHAT IS GBM?

Glioblastoma multiforme (GBM), a type of central nervous system cancer, is the most common and most aggressive form of primary brain cancer

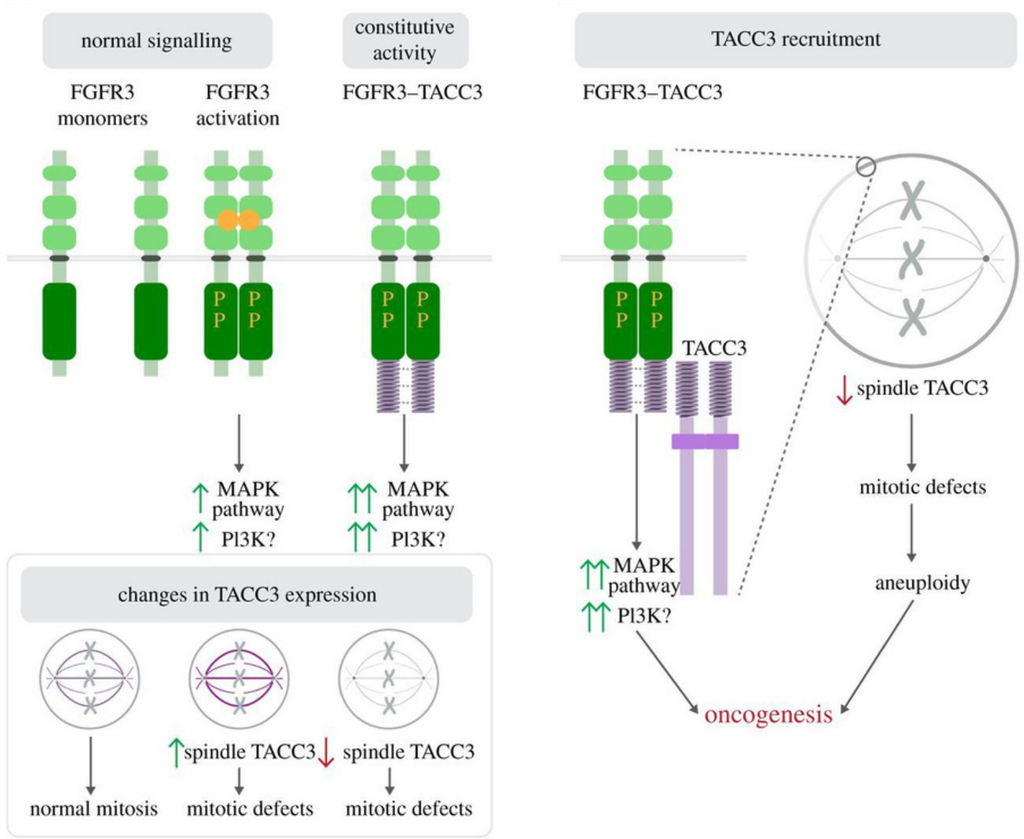


GBM RELATIVE SURVIVAL RATES

POTENTIAL & AVAILABLE TREATMENT OPTIONS

FGFR3-TACC3 FUSION IN GLIOBLASTOMA

A small subset of GBMs (3.1%) harbors oncogenic chromosomal translocations that fuse in-frame the tyrosine kinase coding domains of fibroblast growth factor receptor genes (FGFR1 or FGFR3) to the transforming acidic coiled-coil (TACC) coding domains of TACC1 or TACC3, respectively



WHAT ARE FGFR3 AND TACC3?

TACC3 is a cancer-associated protein belonging to the TACC family and is important for mitotic spindle stability

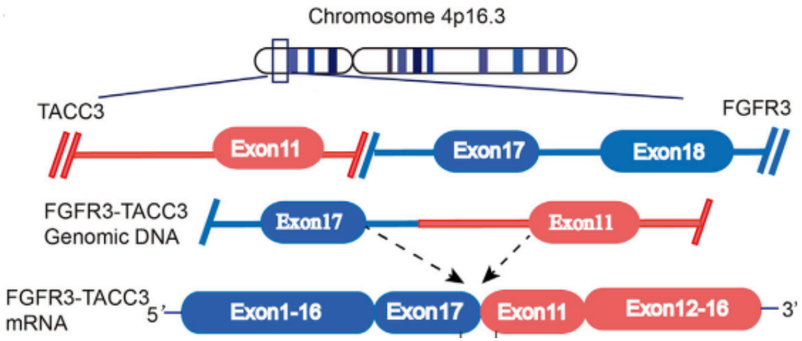
FGFR3 belongs to the FGFR family of receptor tyrosine kinases

SOME CHARACTERISTICS OF F3-T3 GBM CELLS

- No EGFR and EGFRvIII-amplifications
- Amplification of tyrosine kinase receptor such as PDGFR, KIT, MET
- No ATRX-loss and H3F3A mutations
- Higher expression of stemness markers such as OLIG2 and **GFAP**

AIM OF THE PROJECT

WHAT?



WHERE?

32 BALB/c-nu female mice

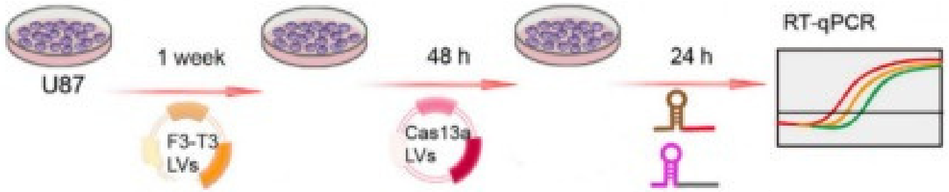
WHY?

FGFR3-TACC3 fusion protein has a constitutively active tyrosine kinase domain and promotes aneuploidy. This rearrangement represents a targetable molecular aberration in some patients with glioblastoma multiforme

HOW?

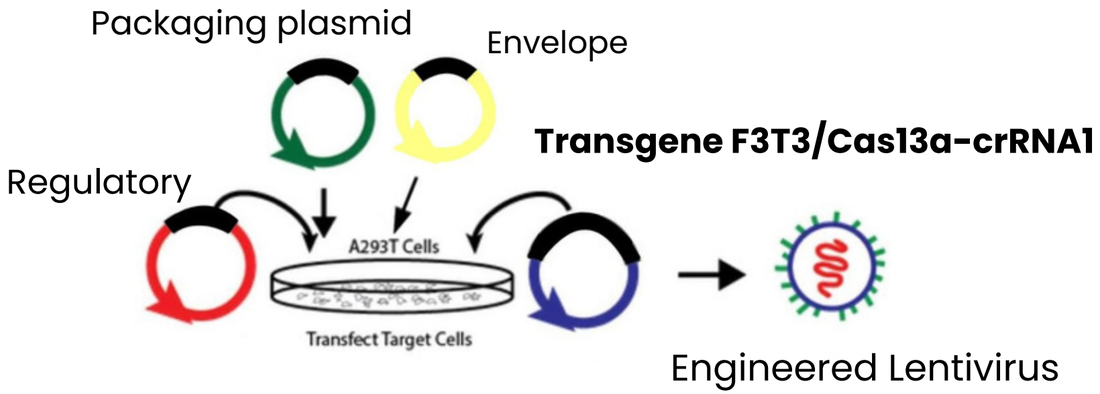
Precise editing of FGFR3-TACC3 fusion genes with CRISPR-Cas13a combined with a GFAP-selective oncolytic adenovirus reduces tumor mass expansion and extendes life

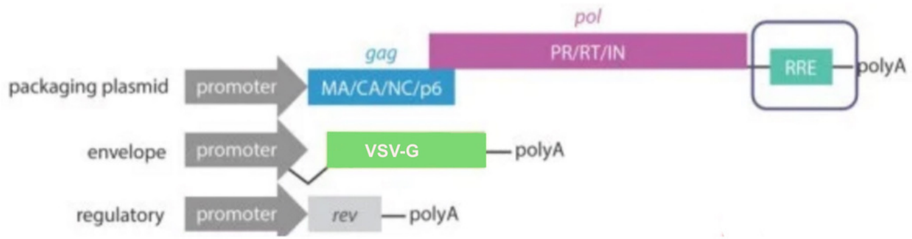
PRECISE EDITING WITH CRISPR-Cas13a SYSTEM

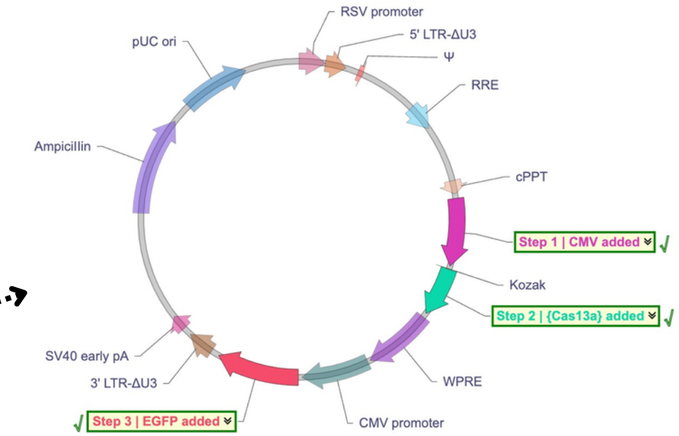


crRNA1 transfection
with Lipofectamina 3000

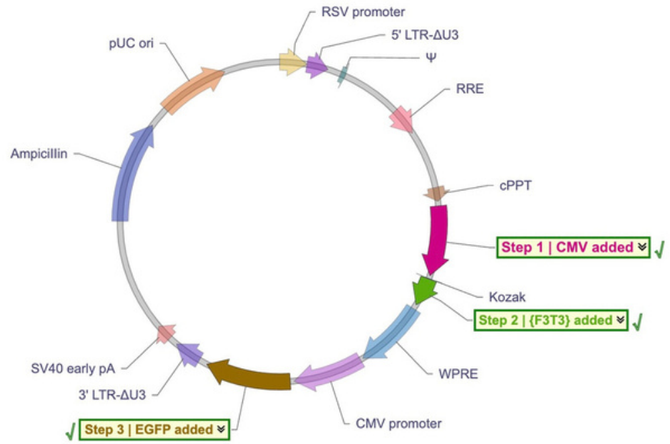
Schematic diagram of the trasfection process in U87 using an engineered Lentivirus of third generation
Fig. adapted from Wu et al. 2021







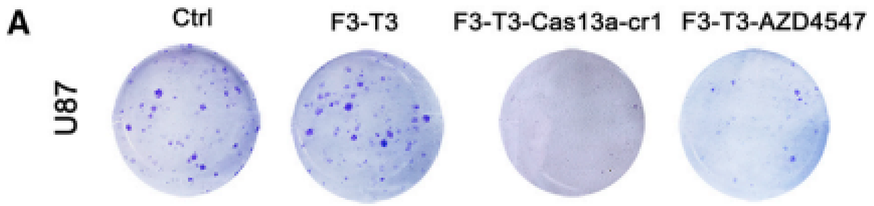
Plasmid expressing Cas13a created with VectorBuilder



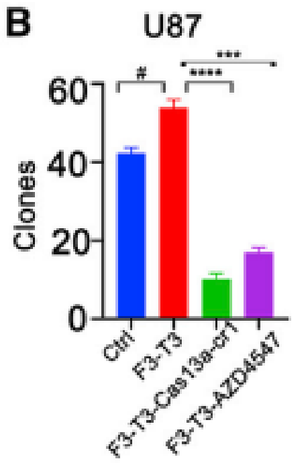
Plasmid expressing F3T3 created with VectorBuilder

IN VITRO

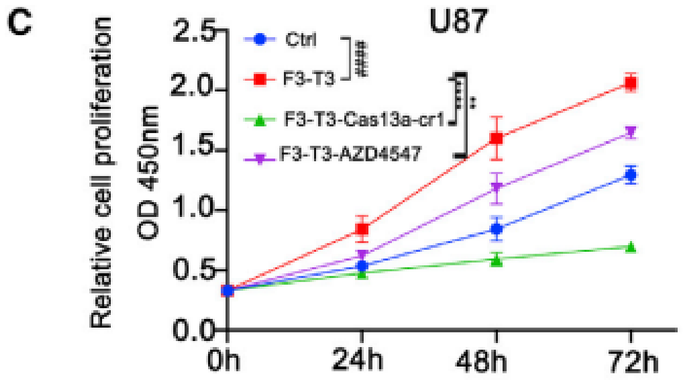
- GROUP 1: U87
- GROUP 2: U87 + F3T3
- GROUP 3: U87 + F3T3-Cas13a-cr1RNA
- GROUP 4: U87 + AZD4547



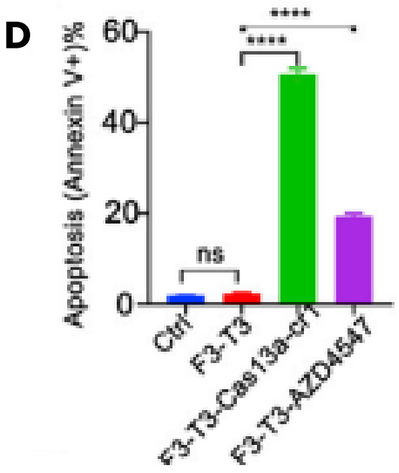
A. Images of colony formation assays performed in U87 cells



B. Quantification of colony numbers in (A)



C. Growth curves generated with CCK8 essays to analyze the proliferation of U87 cells

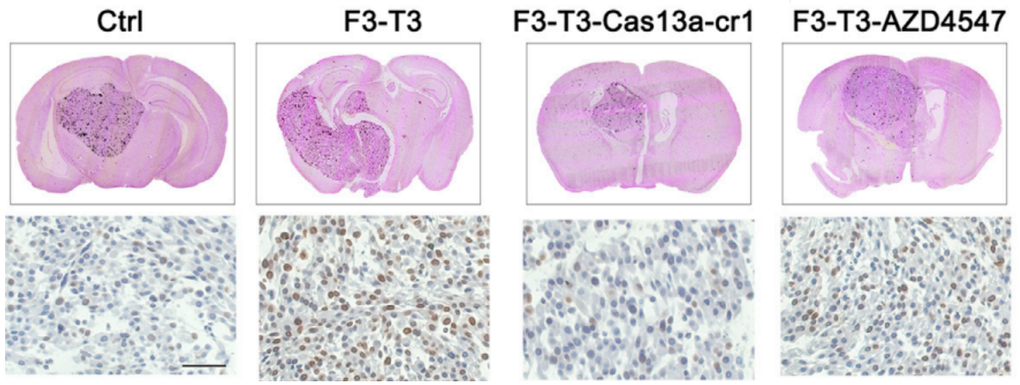


D. Apoptosis percentage

IN VIVO

32 4 week old female BALB/c-nu mice divided into four groups:

- GROUP 1: 8 MICE CTRL
- GROUP 2: 8 MICE F3T3
- GROUP 3: 8 MICE F3T3 + CAS13a-crRNA1
- GROUP 4: 8 MICE F3T3 + AZD4547

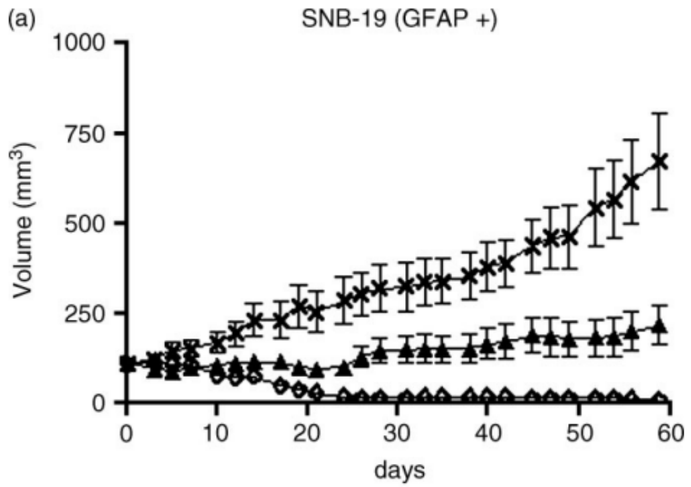


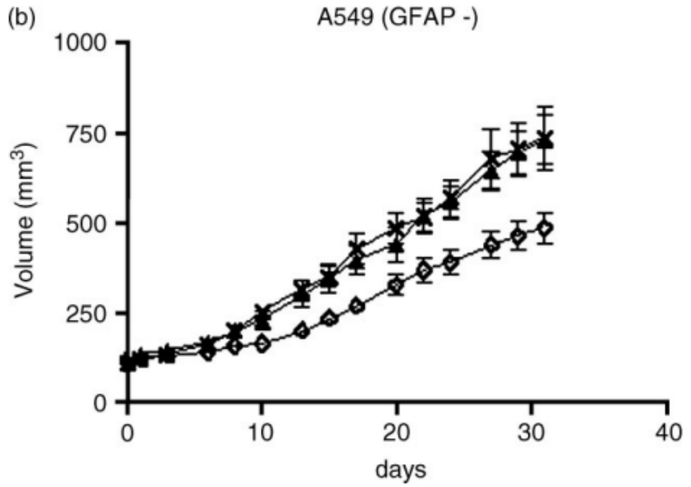
E. H&E staining of mouse cerebrum showing tumor volume
B.Immunohistochemical (IHC) staining for Ki67 in the samples

Cas13a-based tool is a novel strategy for the treatement of GBM harboring F3-T3 fusion genes because it inhibits cell proliferation and induces apoptosis in vitro, it reduces tumor growth and entends life in a mice model

Fig. A, B, C, D, E adapted from Wu et al., 2021

ONCOLYTIC ADENOVIRUS Ad5-gfa2(B)3-E1





PBS Ad5-gfa2(B)3-E1 wtAd5. Error bars indicate standard error of the mean (SEM)

Antitumor activity in subcutaneous xenografts. Tumor xenografts were established by injecting 1 × 107 SNB-19 (GFAP-positive; A) or A549 (GFAP-negative; B) cells suspended in 250 µl of HBSS in both flanks of nude mice. When tumor volumes reached about 100–150 mm3, animals were treated with a single injection of 4 × 107 pfu wtAd5 or Ad5-gfa2(B)3-E1 or PBS in a total volume of 50 µl. Each treatment group consisted of 8–12 tumors.

Ad5-gfa2(B)3-E1 injection resulted in a significant ($p < 0.05$) oncolytic effect in GFAP-positive SNB-19 xenografts (A) but not in GFAP-negative A549 xenografts (B). PBS Ad5-gfa2(B)3-E1 wtAd5. Error bars indicate standard error of the mean (SEM)

Fig. adapeted from ter Horst et al. 2007

Ad5gfa2(B)3-E1 is a conditional replication virus in which the E1A gene is under the control of the promoter (gfa2) of the glial-specific intermediate strand, Glial Fibrillary Acidic (GFAP) and three additional copies of the glial-specific enhancer "B"

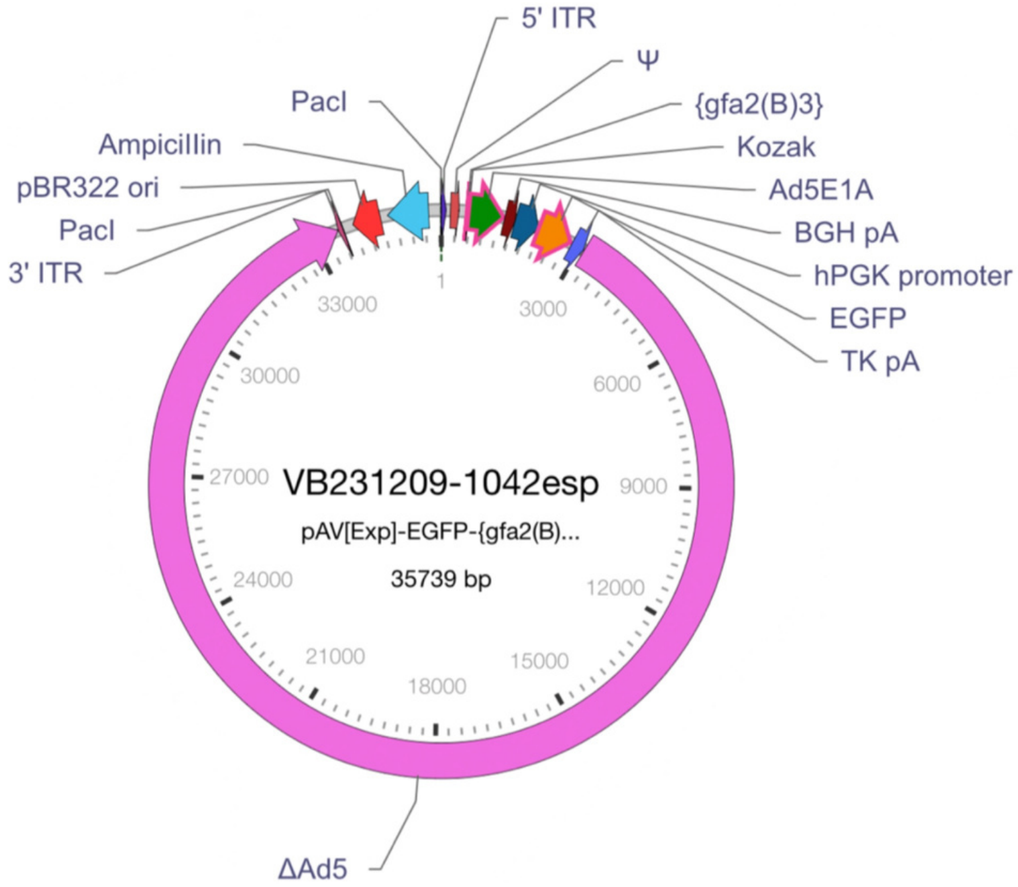
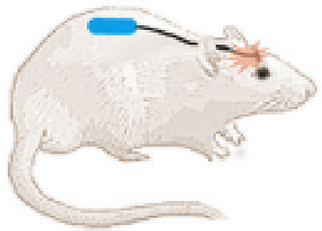


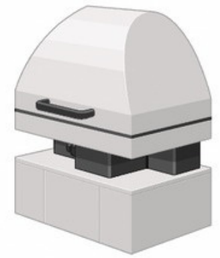
Image created with VectorBuilder

EXPERIMENTAL PLAN



- Mini osmotic pump implantation in each group
- Injection of Cas13a-crRNA1 lentivirus into the right hemisphere of the thrid group
- Oral administration of AZD4547 (50 mg/kg) is performed daily in the fourth group

▲ Bioluminescence imaging



DAY 7

DAY 21

DAY 0

U87 or U87 with F3T3 cells (5x105) are stereotactically injected into the right hemisphere of the female mice:

- GROUP 1: Ctrl U87
- GROUP 2: U87 with F3T3
- GROUP 3: U87 with F3T3
- GROUP 4: U87 with F3T3

DAY 14

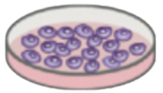
4 F3T3-Cas13a-crRNA1 mice of the third are injected with **Ad5-gfa2(B)3-E1 oncolytic adenovirus** (4x107 pfu) with the mini osmotic pump

DAY 28

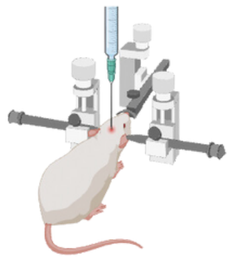
- 4 mice of the first group are sacrified
- 4 mice of the second group are sacrified
- 2 mice of the third group F3T3-Cas13a-crRNA1 are sacrified
- 2 mice F3T3-Cas13a-crRNA1Ad5-gfa2(B)3-E1 are sacrified
- 4 mice F3T3-AZD4547 of the fourth group are sacrified

EXPERIMENTS

DAY 0



U87



DAY 7

Mini osmotic pump implantation

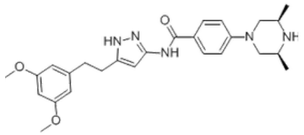
GROUP 1
8 mice Ctrl U87

GROUP 2
8 mice U87 with F3T3

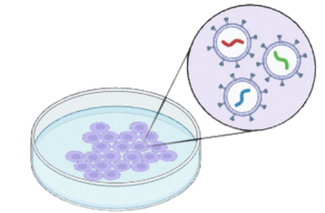
GROUP 3
8 mice U87 with F3T3

GROUP 4
8 mice U87 with F3T3

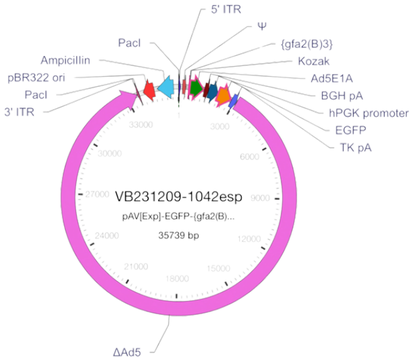
Injection of Cas13a-crRNA1



Oral administration of AZD4547



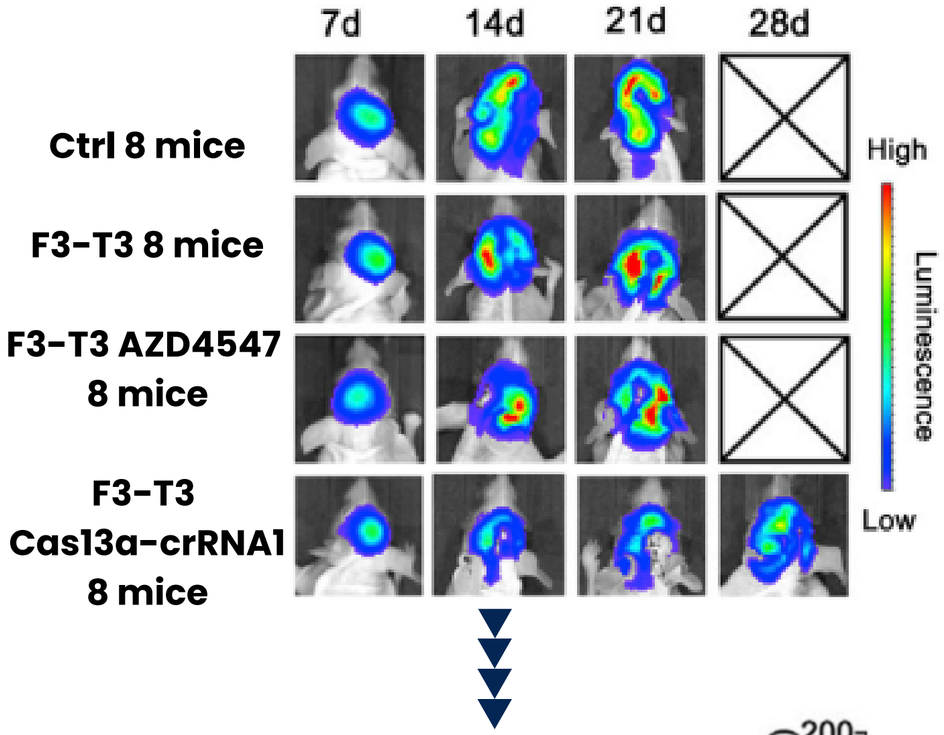
DAY 14



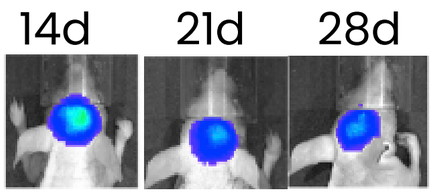
4 mice with Ad5-gfa2(B)3-E1 oncolytic adenovirus

4 mice with no virus

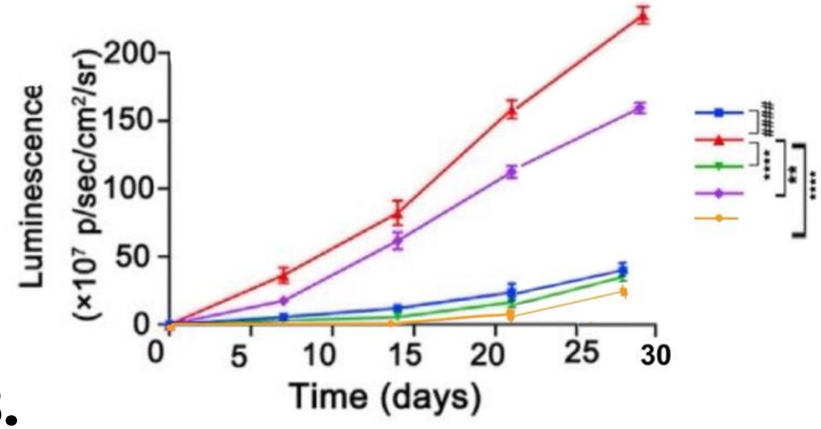
EXPECTED RESULTS



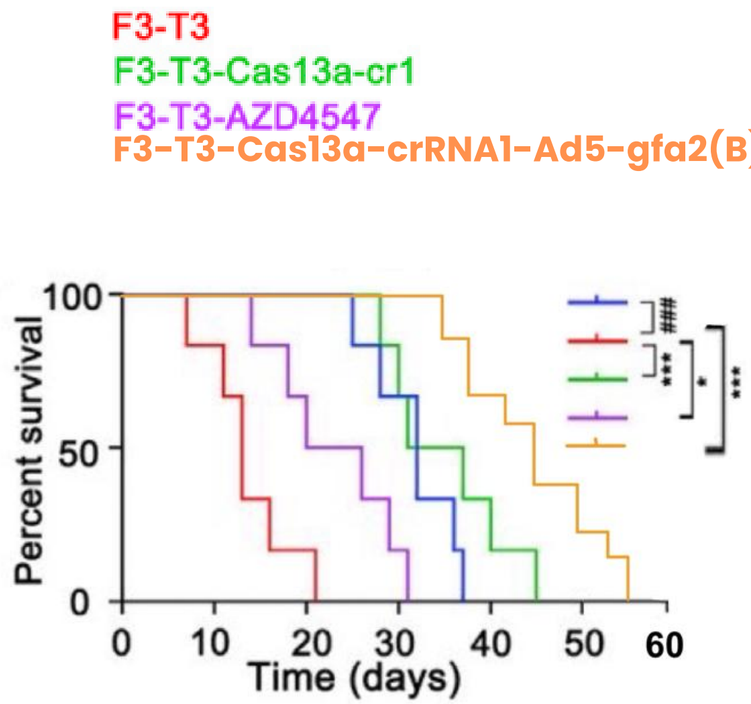
At day 14, 4 F3T3 Cas13a crRNA1 mice were injected with Ad5-gfa2(B)3-E1 using a mini osmotic pump



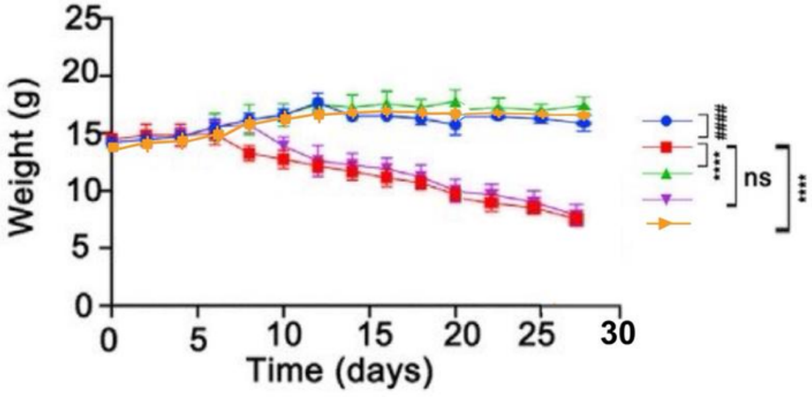
A.



B.



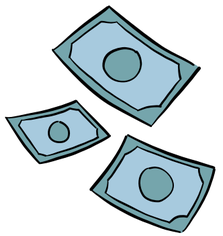
C.



D.

COSTS AND MATERIALS → 2 years

Mouse BALB/c (F) 3/4 week	$581 (x50) = $30.000
Mice stabulation	$12.000 (per year)
AZD4547 (200 mg)	$377
Lentivirus	$2.250
Adenovirus	$395
Cell U87	$1200
Research team	$216.000 (per year)
Lab equipments basics	$3.000
Scientific machinery (mini osmotic pump and bioluminesce imagind machinery)	Offered by the laboratory



TOT: $272.000 per year

PITFALLS

1. Potential long-term side effects of the oncolytic virus therapy

2. Oncolytic virus effect translatability between pre-clinical models and human patients

3. Lentivirus-CRISPR toolbox creation faces high production costs

4. Oncolytic virus efficacy could be reduced due to immune responses

5. The replicative potential of Ad5gfa2(B)3-E1 in non-malignant GFAP-positive astrocytes surrounding the tumor

SOLUTIONS

1. Performance of long-term studies investigating side effects deriving from the induction of systemic antitumor immunity

2. The therapy should subsequently be tested in human clinical trials

3. The predicted costs of the study should be put into perspective by considering the benefits deriving from the results that will emerge

4. The oncolytic virus should be administered in rapid, repeated, high doses within the first week of treatment before the rise of serum neutralizing antibodies

5. The use of mini osmotic pump is an efficient approach for intratumoral adiminstration circumventing the BBB

CONCLUSIONS

Using a combined gene therapy with the oncolytic adenovirus (Ad5-gfa2(B)3-E1) and Cas-13a, we can see a further slowing of tumor mass growth and an increase in life span in an vivo model

FUTURE PERSPECTIVES

With these encouraging results, there are actual possibilities for using our technique as a therapy in combination with chemotherapy and radiotherapy. Further studies are definitely needed to verify the efficacy and safety of our therapy in human clinical trials.

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