A Combined Approach To Treat NSCLC

SAPIENZA
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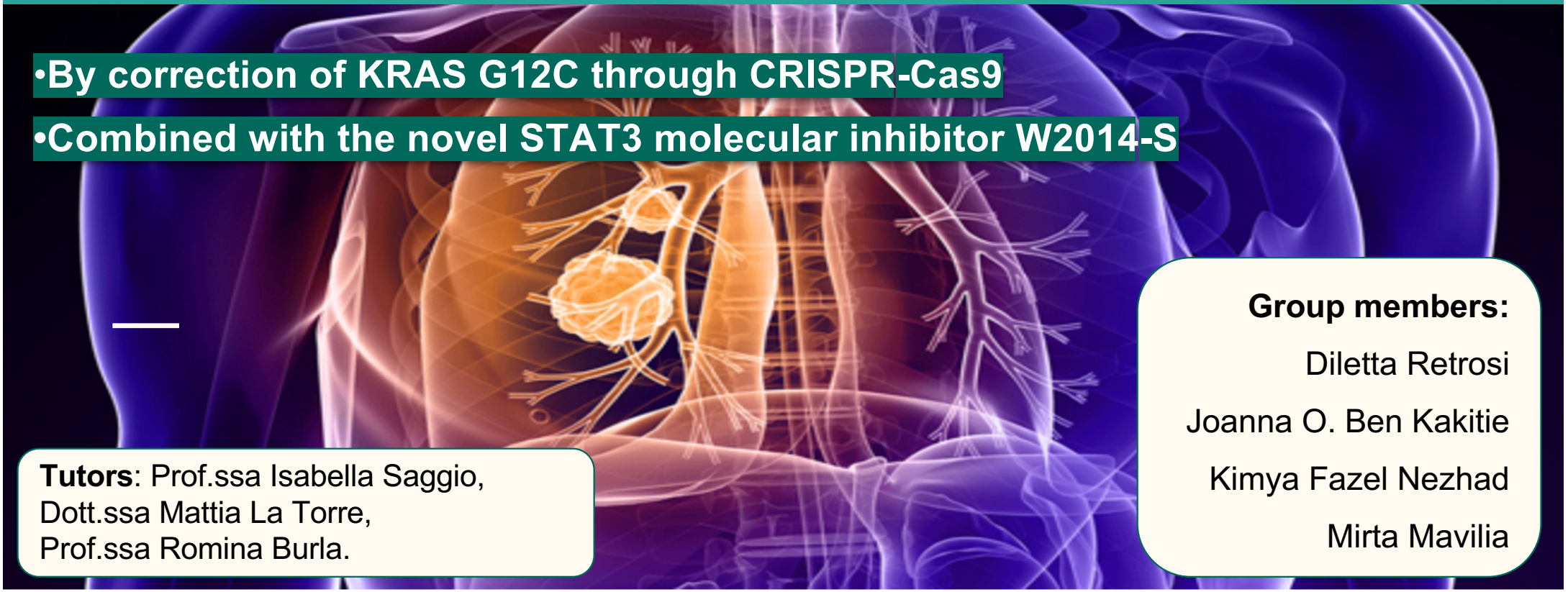

- By correction of KRAS G12C through CRISPR-Cas9
- Combined with the novel STAT3 molecular inhibitor W2014-S

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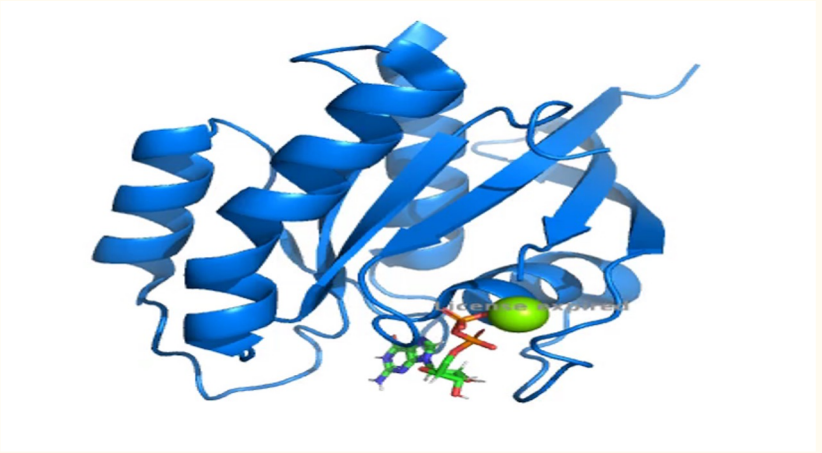
Background



Non-small cell lung cancer

- Most prevalent type of lung cancer (85% of all cases)
- Frequently diagnosed at an advanced stage, has a poor prognosis.
- 10–25% of patients with adenocarcinoma have KRAS mutation-associated (mostly G12C mutations) tumors

KRAS

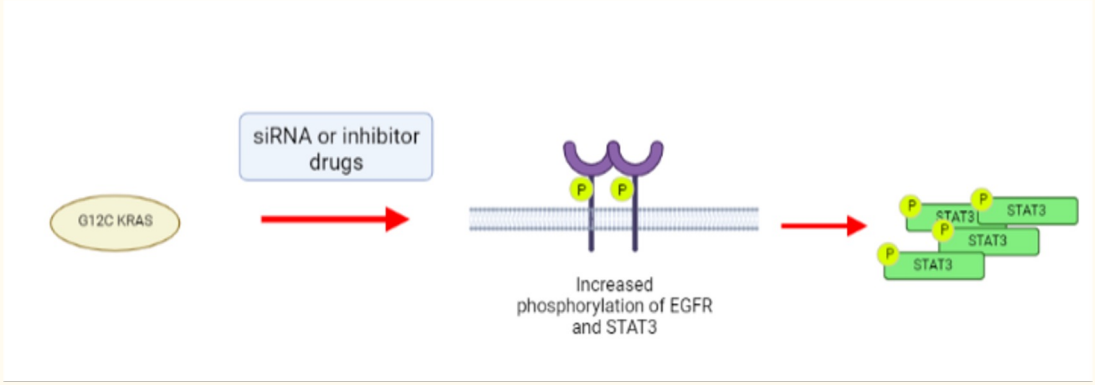
- Kirsten RAt Sarcoma virus oncogene homolog from the mammalian ras gene family
- Member of the small GTPase superfamily
- A single amino acid substitution, G12C, causes constant activation of the protein

Background

- **EGFR**: regulates epithelial development and homeostasis
- **STAT3**: activated by tyrosine phosphorylation by EGFR

Inhibition of **oncogenic KRAS**, by siRNA or drugs induces phosphorylation and activation of EGFR and STAT3

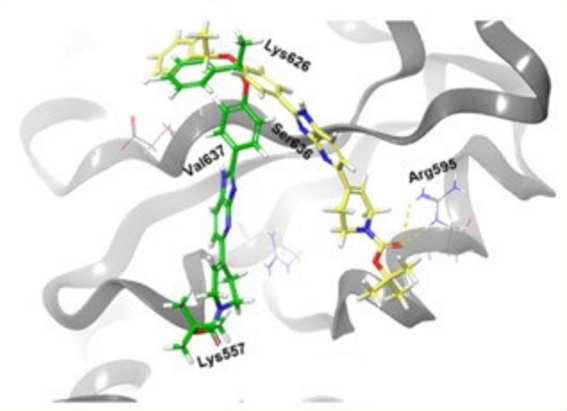
STAT3 & EGFR Correlation with KRAS



Accumulation of EGFR or STAT3 = **tumor formation**

W2014-S, STAT3 Inhibitor

- Derived from Imidazopyridine with one asymmetric center
- Occupies the phosphotyrosine-binding site of STAT3, inhibiting it.

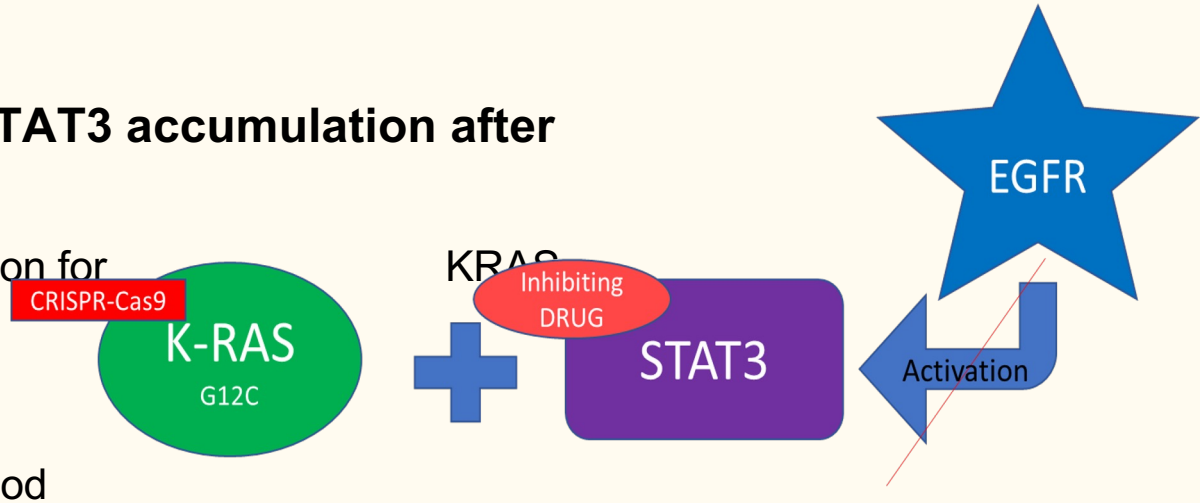


Zheng et al., 2021

Aim of the project

<u>Our strategy</u>: overcoming pEGFR and pSTAT3 accumulation after CRISPR-Cas9 correction

- **Combined therapy** = CRISPR-Cas9 correction for G12C + STAT3 drug inhibitor



Why CRISPR-Cas9?

- CRISPR-Cas9 is a long-term correction method
- KRAS G12C inhibition increases pEGFR and pSTAT3 the same is expected after CRISPR-Cas9 G12C correction

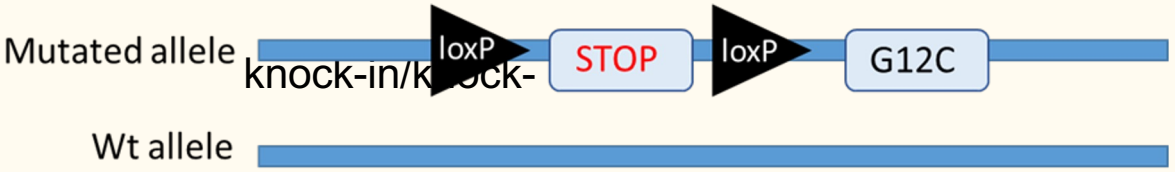
Cell line?

- HCC44, human G12C Lung Adenocarcinoma line

Animal model?

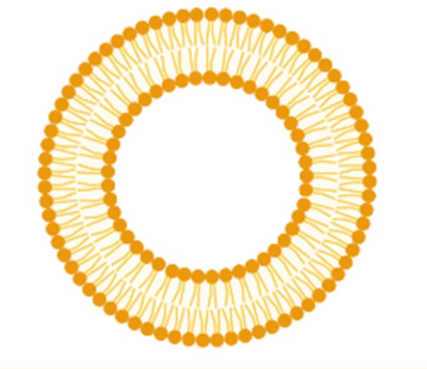
- Mouse model: Kras LSL-G12C out mice

KRAS alleles in the LSL-G12C mouse



Materials and Methods

Delivery method: by liposomes through inhalation

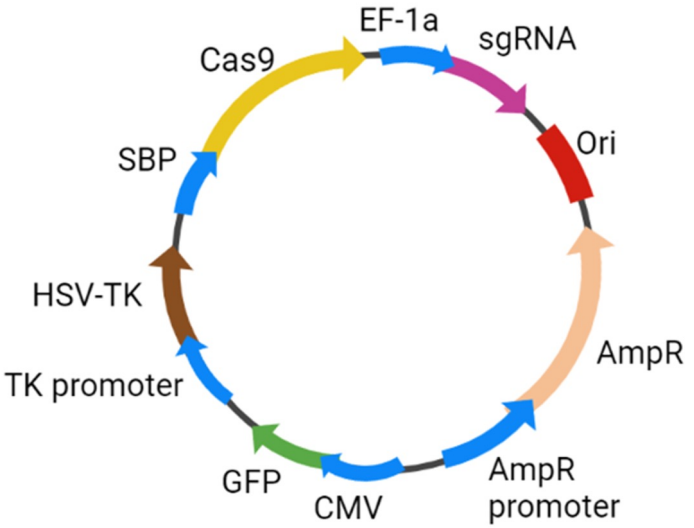


Formulation GL67A:

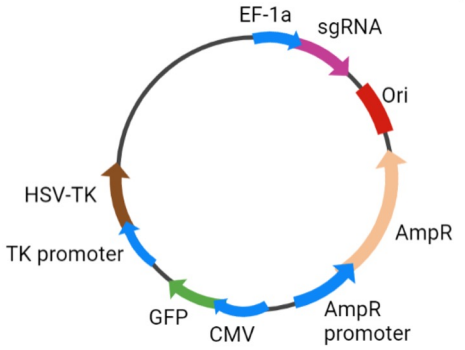
- GL67 cationic lipid
- Zwitterionic colipid DOPE
- Steric stabilizer DP5K

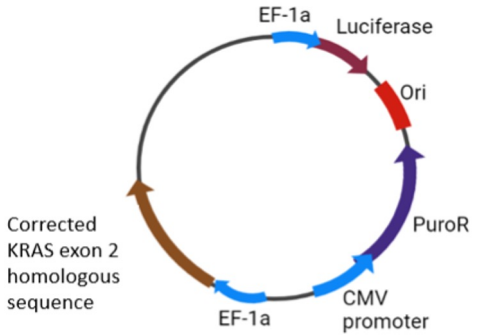
CRISPR-Cas9 system → DSBs

- DSBs repaired with HDR



SB-P (surfactant protein B): promoter specific for epithelial lung cells

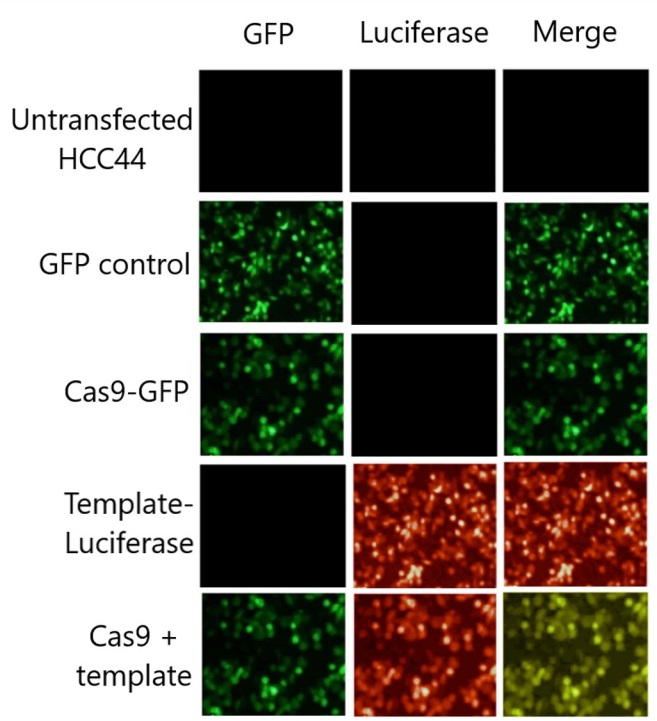




In vitro experiments:

	HCC44 cells	
	Cleavage at KRAS exon2/ Total	HDR-mediated repair/ Total
sgRNA1	26/30	15/30
sgRNA2	3/30	1/30
sgRNA3	0/30	0/30
sgRNA4	25/30	7/30

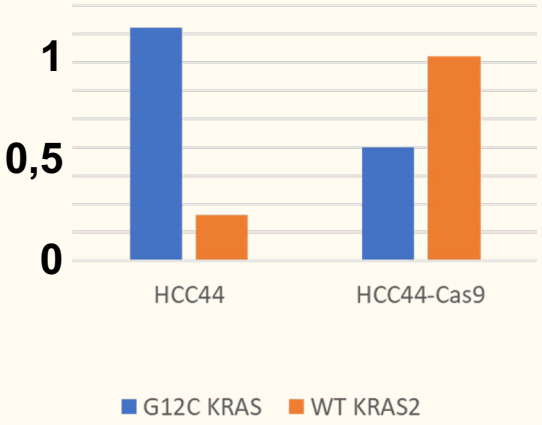
Cleavage and HDR-mediated repair efficiency in different gRNAs



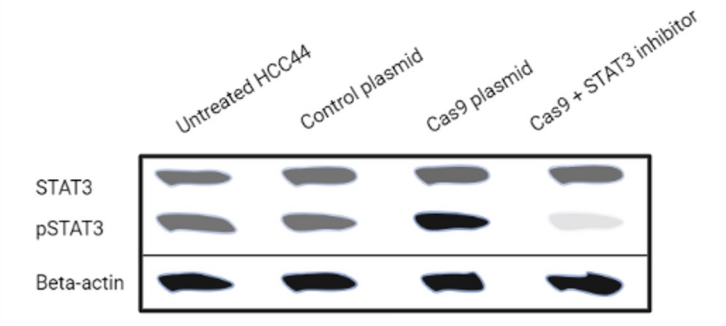
GFP and Luciferase transfection efficiency check

Adapted from https://genome.ucsc.edu/ ,Xu et al., 2017

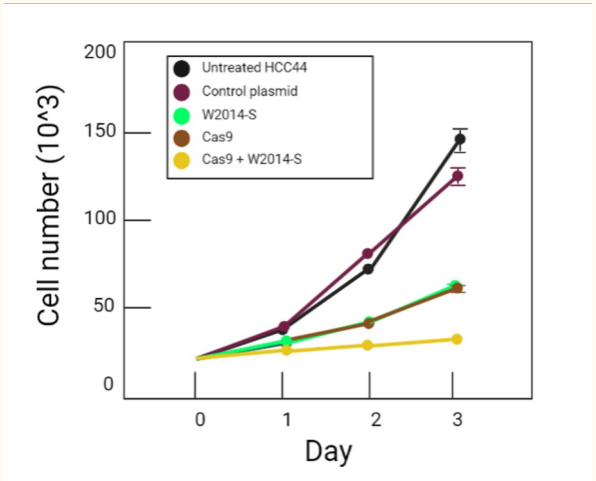
RT-PCR (KRAS/Beta-actin mRNA expression)



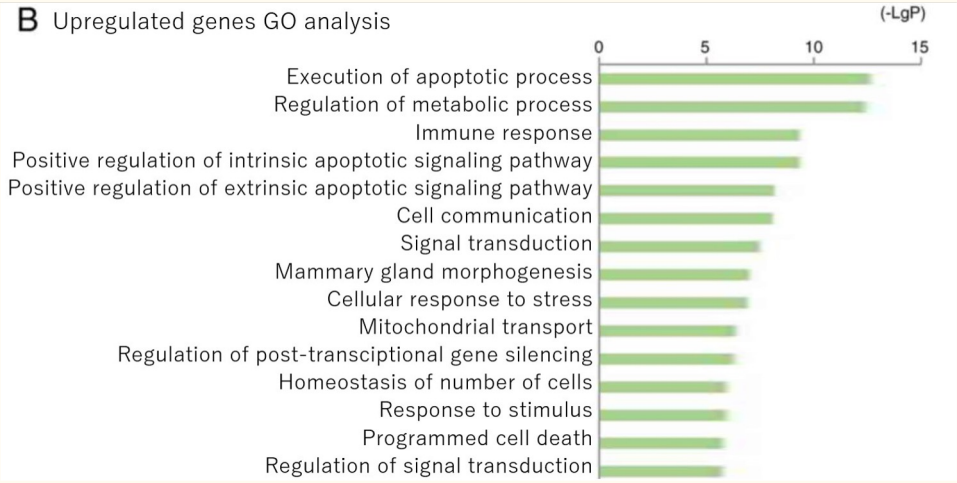
pSTAT3 western blot

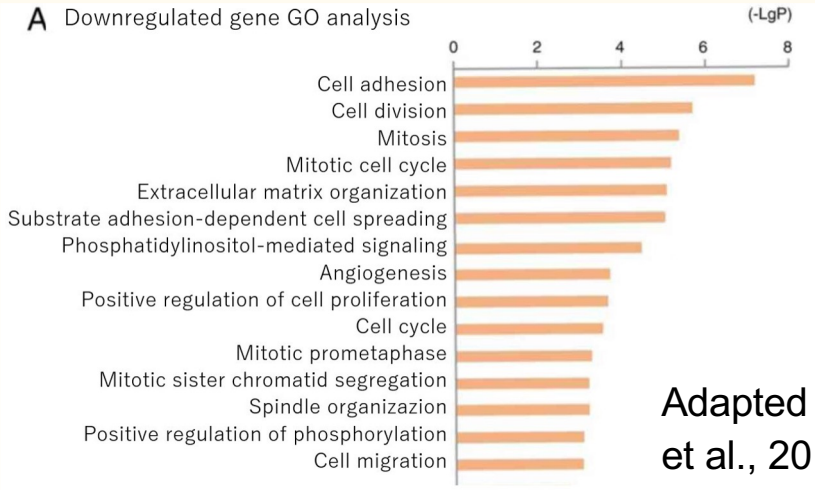


Cell count



RNA-seq analysis after Cas9 + W2014-S

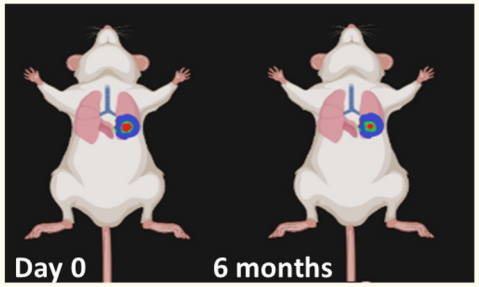




Adapted from Zhu et al., 2019

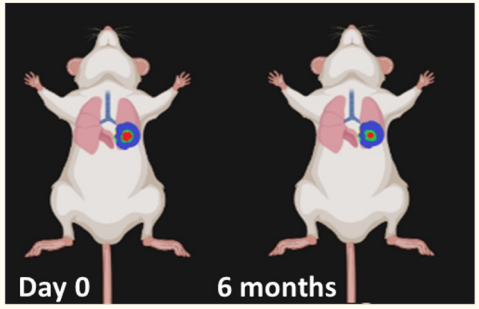
In vivo experiments:

Untreated mouse



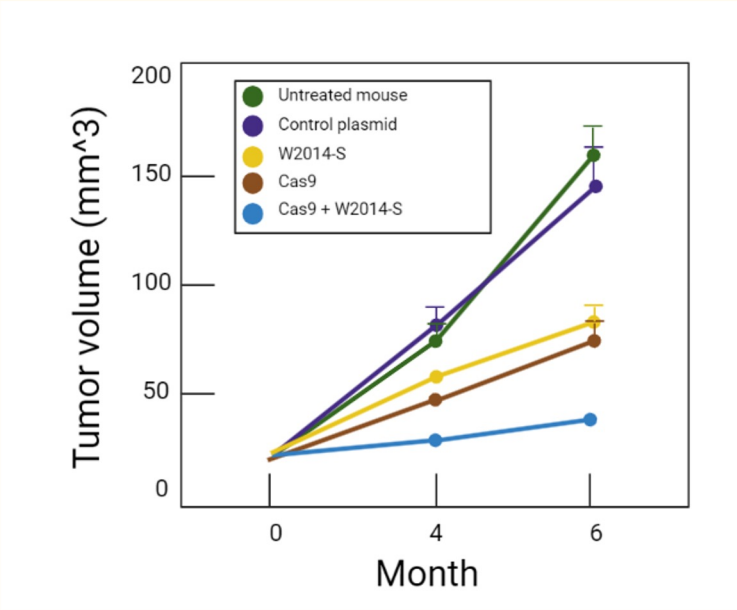
Mouse treated with Cas9 plasmid only

Mouse treated with control plasmid

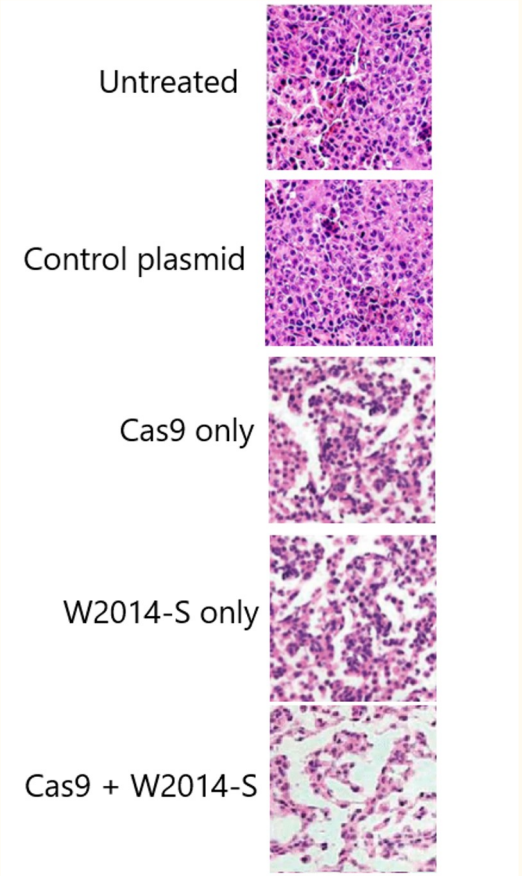


Mouse treated with W2014-S only

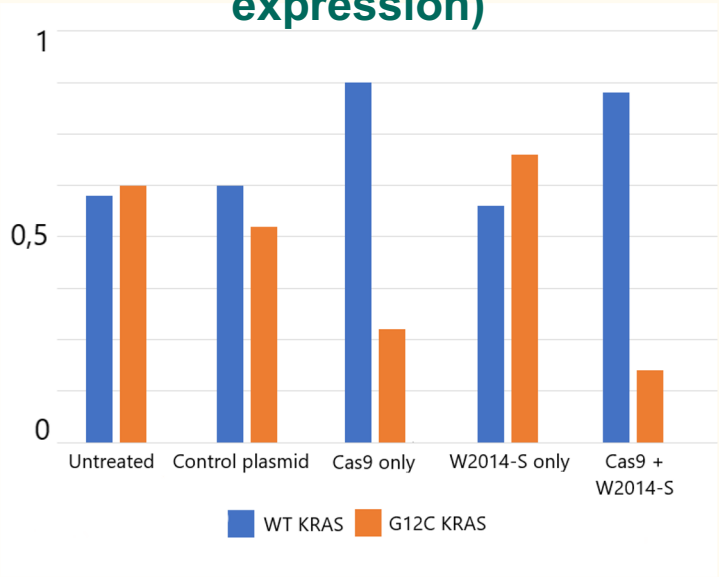
Mouse treated with combination of W2014-S and KRAS G12C correction by CRISPR-Cas9



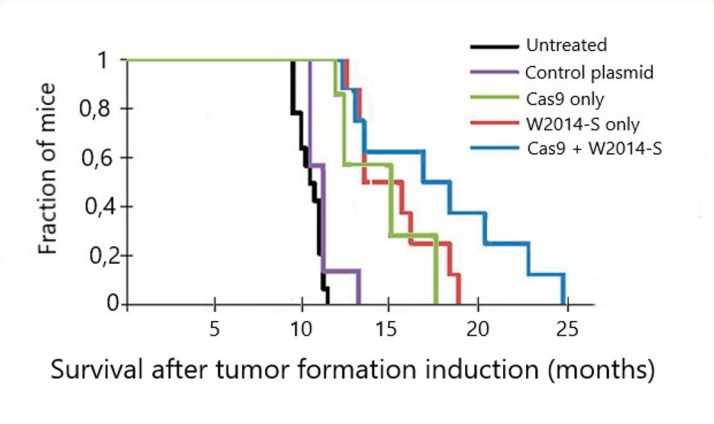
Histopathological analysis



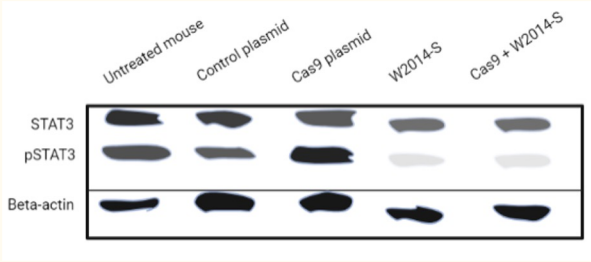
RT-PCR (KRAS/Beta-actin mRNA expression)



Survival curve



Western blot



Guide-seq analysis

Potential off target sites	Mutated mice
intron CYTH4	0/12
intron TEX14	0/12
intergenic RNF38-MELK	0/12
exon F11R	0/12
intron GTF2F1	0/12
intergenic CCDC162P-C6orf185	0/12
intergenic PCSK6-TM2D3	0/12
intergenic LOC401324-HERPUD2	1/12
intergenic MIR551A-MEGF6	0/12

Adapted from Zhou et al. 2008, Ramelow et al. 2020, Edrei et al. 2012

Budget

Costs/3 years	Budget (€)
Lab equipment	Donated by Sapienza University
LSL-KrasG12C Mouse model (excluding maintenance)	10 000
Researchers' salary (2 Ph students + 1 Post-Doc)	240 000
Liposome Vector	8 400
RT-PCR Kit	600
Western Blot Kit	400
Crispr-Cas9 Kit	1 280
HCC44 Cell Line	400
RNA-Seq	3 500
GUIDE-Seq	4 000
Transfection Reagents	280
Total	**268 860**

Pitfalls and Solutions

Effects of long-term administration of W2014-S in CRISPR-Cas9 corrected cells haven't been studied yet.	Regular monitoring of STAT3 levels and mice health conditions is necessary.
CRISPR-Cas9 can cause off-target cleavage, leading to mutations.	A modified and more efficient Cas9, like the Sniper-Cas9, could be used instead.
Liposome delivery efficiency is lower than other vectors.	We can add surface molecules like IL4RPep-1 to increase the targeting to lung cancer cells.

Timeline

1st year	Preparation of vectors In vitro experiments Data collection
2nd year	In vivo experiments In vitro replicates Data collection
3rd year	In vivo replicates Post-mortem analysis Survival rate analysis

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