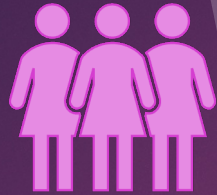


**A new approach against Breast
Cancer: Reversion of mutation at
exon 11 of BRCA1 using Prime
editing and a modified
Adenovirus**

Gruppo E: Chillura, Maccari, Radicioni, Scaramagli

Breast Cancer

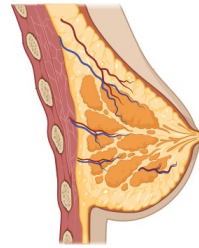


According to the presence or absence of molecular markers for ER, PR and ERBB2, BC is divided in 3 classes

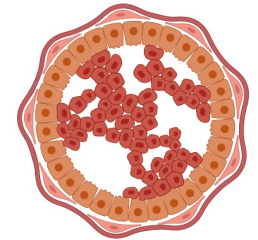
Hormone Receptor Positive/ ERBB2 Negative (70% Of Patients)

ERBB2 Positive (15-20% of patients)

Triple Negative (tumors that do not have all three markers, 15% of patients)



Background



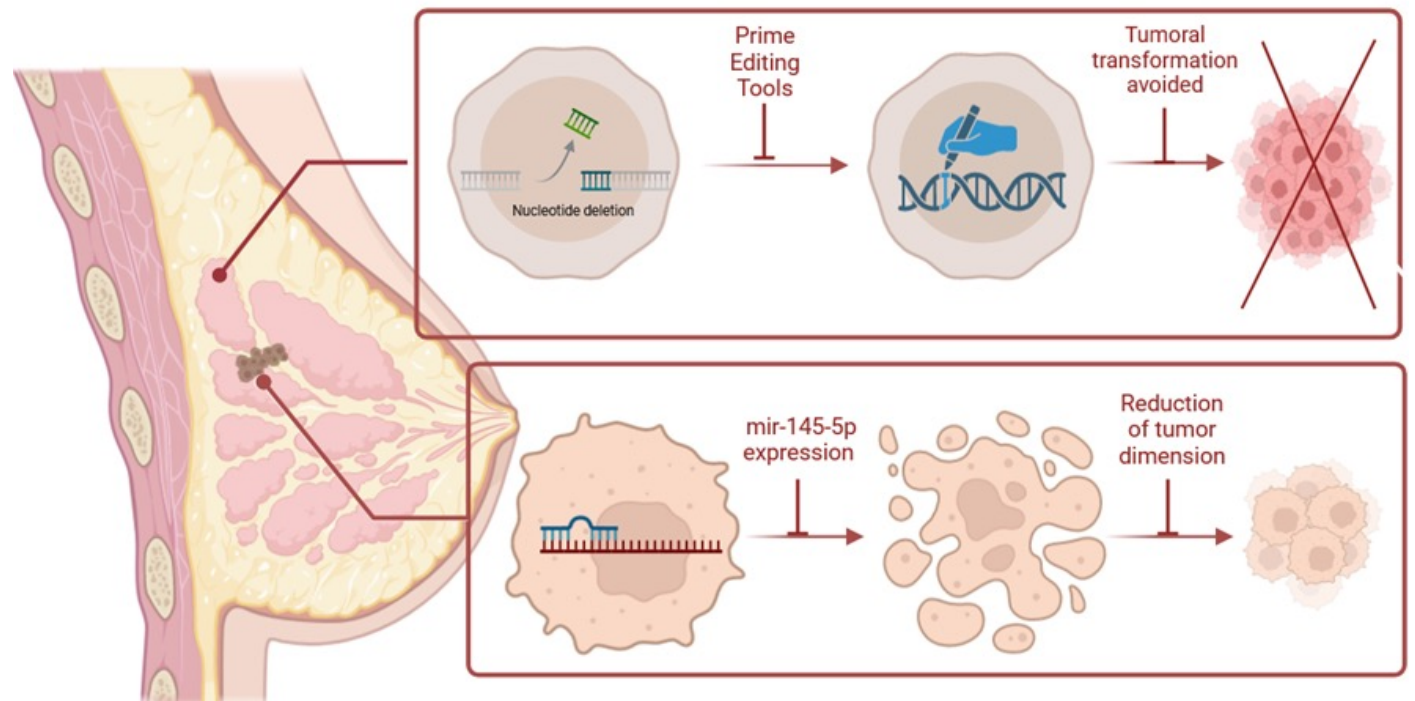
Breast cancer affects 1.7 million women worldwide each year and is the leading cause of cancer death in women

5 to 10% of Breast Cancers are related to mutations in BRCA1, which is a gene implicated in numerous cellular functions critical for maintaining genomic stability

40% of BRCA1 mutations are located in the OCCR and BRCT domain encoded by exon 11

Aim of the project

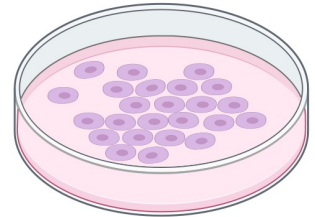
► Resolve the mutation in exon 11 of Brca1 by intraductal injection of Adenovirus5 into the mouse model bearing the same mutation and, in parallel, eliminate and slow down replication of tumor cells by the expression of mir-145-5p



Experimental Plan

In Vitro

- Verification of correct insertion of the mutation into the MCF-7/S.05 cell line;
- Evaluation of 4 different pegRNAs, with RT-PCR;
- Evaluation of off-target effects and validation with Microarray.

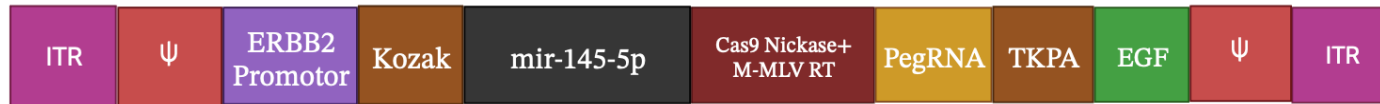


In Vivo

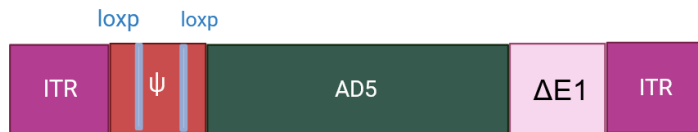
- Intraductal injection of the vector in the experimental groups;
- Evaluation of tumor progression by IHC toward ERBB2 receptor and FISH to determine the effectiveness of Prime Editing.



Design of the vector



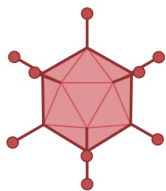
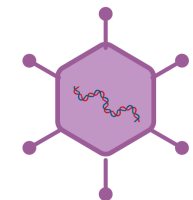
Vettore



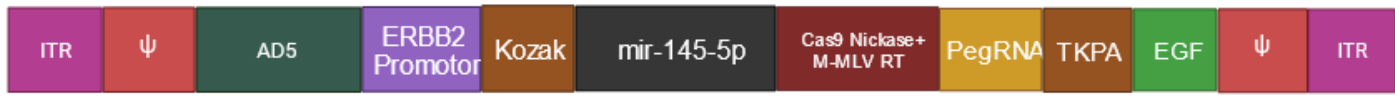
Virus Helper

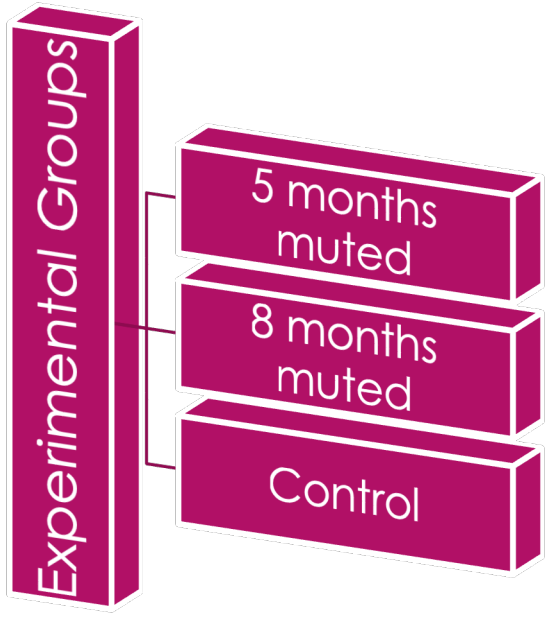
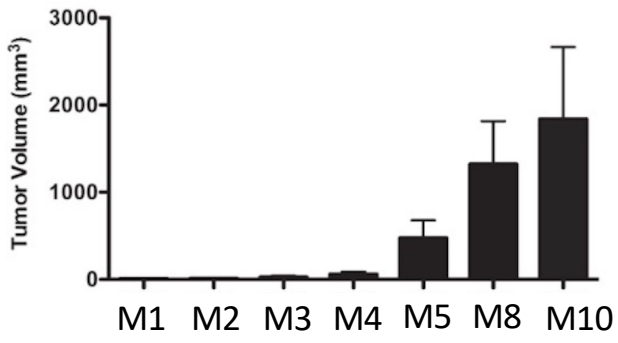
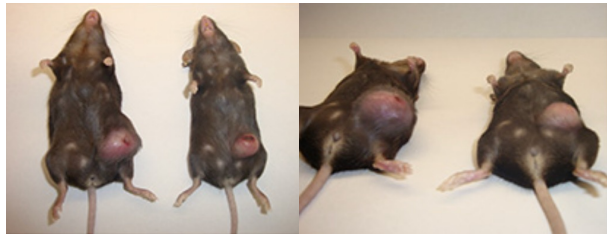
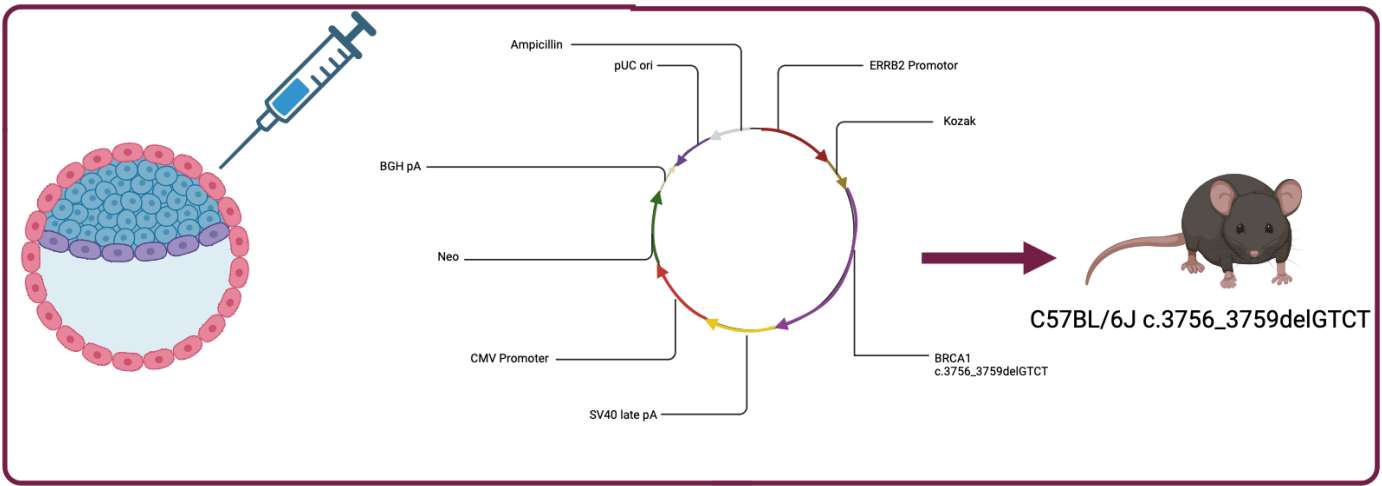


Cell 293 Cre+ with E1 in culture



Ad5-mir145p-Cas9nRT-pegRNA-EGF



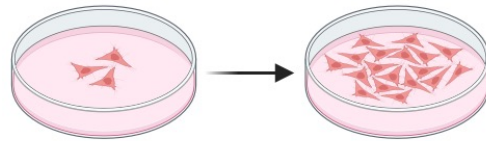


Murine Model

In Vitro Experiments

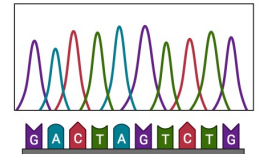
1

MCF-7/S0.5



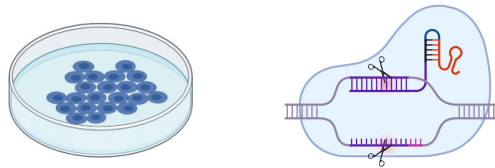
4

Sanger's sequencing



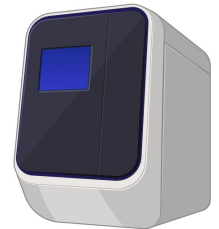
2

Modification of MCF-7/S0.5 line with CRISPR-CaS9



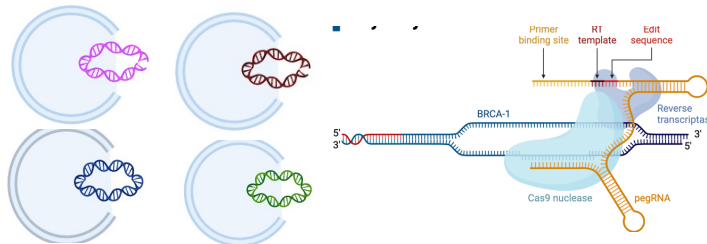
5

RT-PCR



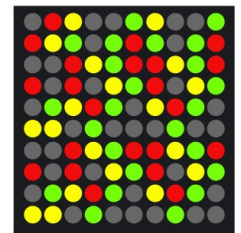
3

Insertion of 4 plasmids with different pegRNAs



6

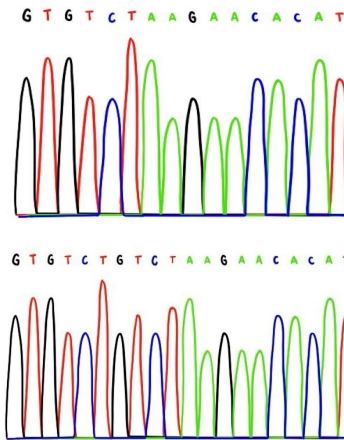
Microarray



In Vitro Expected Results

4

Verification of correct insertion and reversion of the mutation into the MCF-7/S.05 cell line.



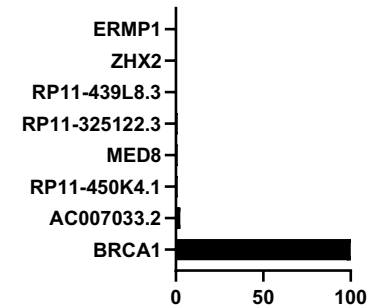
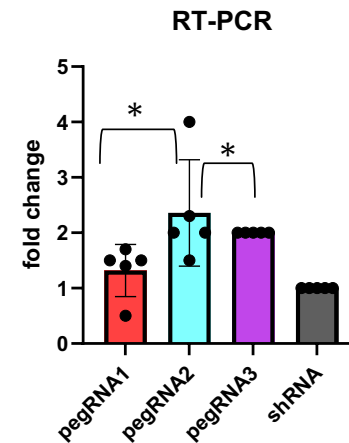
5

Evaluation of 4 different pegRNAs, with RT-PCR;

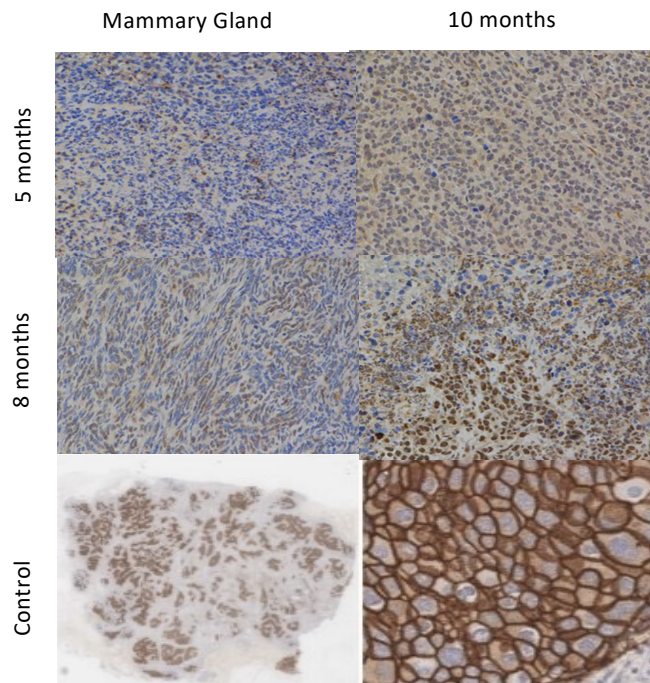
pegRNA1	GTGTCTGTCTAAGAACACAG CTATGCCTAGTAGACTGAGA
pegRNA2	GTGTCTGTCTAAGAACACAG AGACAGACTCGGTAGCAA
pegRNA3	CTTAATGACTGCAGTAACC TGTGTTCTTAGACAGACT
shRNA	

6

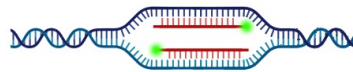
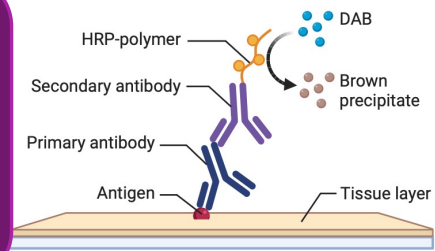
Evaluation of off-target effects and validation with microarray



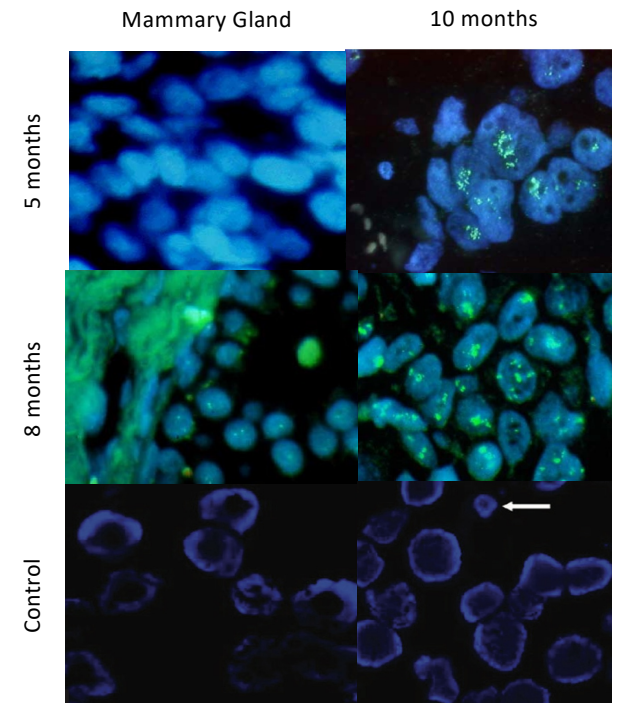
In Vivo Results



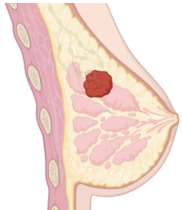
Assessment of ERBB2 protein overexpression at the tumor cell membrane by IHC



Examination of the correct occurrence of the modification implemented by Prime Editing due to FISH



Pitfalls And Solutions



The effect of increasing miR-145-5p expression in healthy mammary gland cells is not known

1

Future experiments could try to target in a more specific way the mutation



There is no comprehensive characterization of the phenotype of the proposed mouse model

2

Further research is needed to demonstrate the face and construct validity of the model, to verify the safety of the treatment and not to damage healthy cells that are not yet cancerous



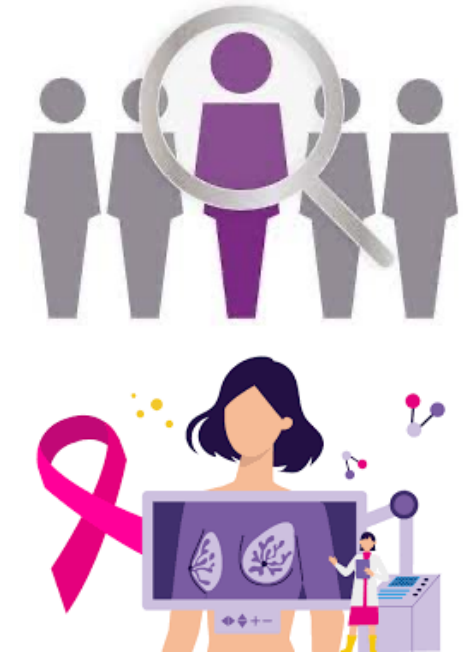
Off target activity of CRISPR/Cas9 could negatively affect the experiment and the vector may not be efficient in targeting and there is still the possibility of Helper Virus contamination.

3

The improvement of the vector and a stable transfection method should be used in future, paying attention to insertional mutagenesis

Conclusions and Future perspectives

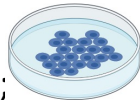
- ▶ According to the results obtained, we can say that the combined action of prime editing with the injection of the viral vector derived from serotype 5 adenovirus is effective in trying to fight breast cancer, showing significant reduction over months, in the animal model, so the clinical trial phase can be started.
- ▶ As for the future, it would also be possible to think of modifying this project to obtain a tool useful only for breast cancer prevention, thinking of using the viral vector alone with prime editing to reverse the mutation in the BRCA1 gene early and this would replace the preventive breast removal surgery; this would be even more efficient if combined with a dense screening program for all women whose age start from 18 years old.



Budget and timing



In Vitro



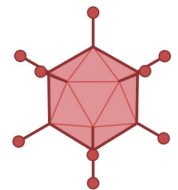
- ☆ CMF-7/S0.5 cell line: 1.600 € (Merck);
- ☆ Cell line 293: 400 € (Merck);
- ☆ CRISPR CaS9 kit: 1.648 € (Invitrogen);
- ☆ Helper virus: 2.750 € (Creative Biogene)
- ☆ Plasmids with pegRNA: 120 € (Vector Builder)
- ☆ Sanger's Sequencing kit: 505 € (NimaGen);
- ☆ Microarray kit: 456 € (NimaGen);
- ☆ RT-PCR kit: 500 € (Qiacen)
- ☆ Construct plasmid: 240 € (Sigma Aldrich);
- ☆ Lab material: 10.000 (1y) €

Estimated Budget:
430.000€

Estimated Time: 3
Years

In Vivo

- ☆ Plasmid to be injected into the Blastocyst: 960 € (Vector Builder);
- ☆ 4 Females CD-1 mice: 33,48 € (Charles River);
- ☆ 2 Males CD-1 mouse: 16,74(Charles River);
- ☆ 7 Females C57BL/6J mice: 269,15 € (Charles River);
- ☆ Kit for IHC: 558 € (Merck);
- ☆ Ab ERBB2: 890 € (Merck);
- ☆ Anti-ab ERBB2 Alexa Fluor: 580 €(Merck);
- ☆ Kit FISH: 418 € (Merck);
- ☆ Fluorescent Probe: 2.100 €(Abnova)
- ☆ Housing expenses: 10.000 (1y) €
- ☆ 1 PI, 2 Phd students, 1 Lab Technician: 120.000 € (1y)



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