SAPIENZA
UNIVERSITÀ DI ROMA

STIM1 siRNA-Neutral-DOPC liposome-mediated knockdown in Liver Metastatic Uveal Melanoma

Class of Gene therapy and Neuroscience
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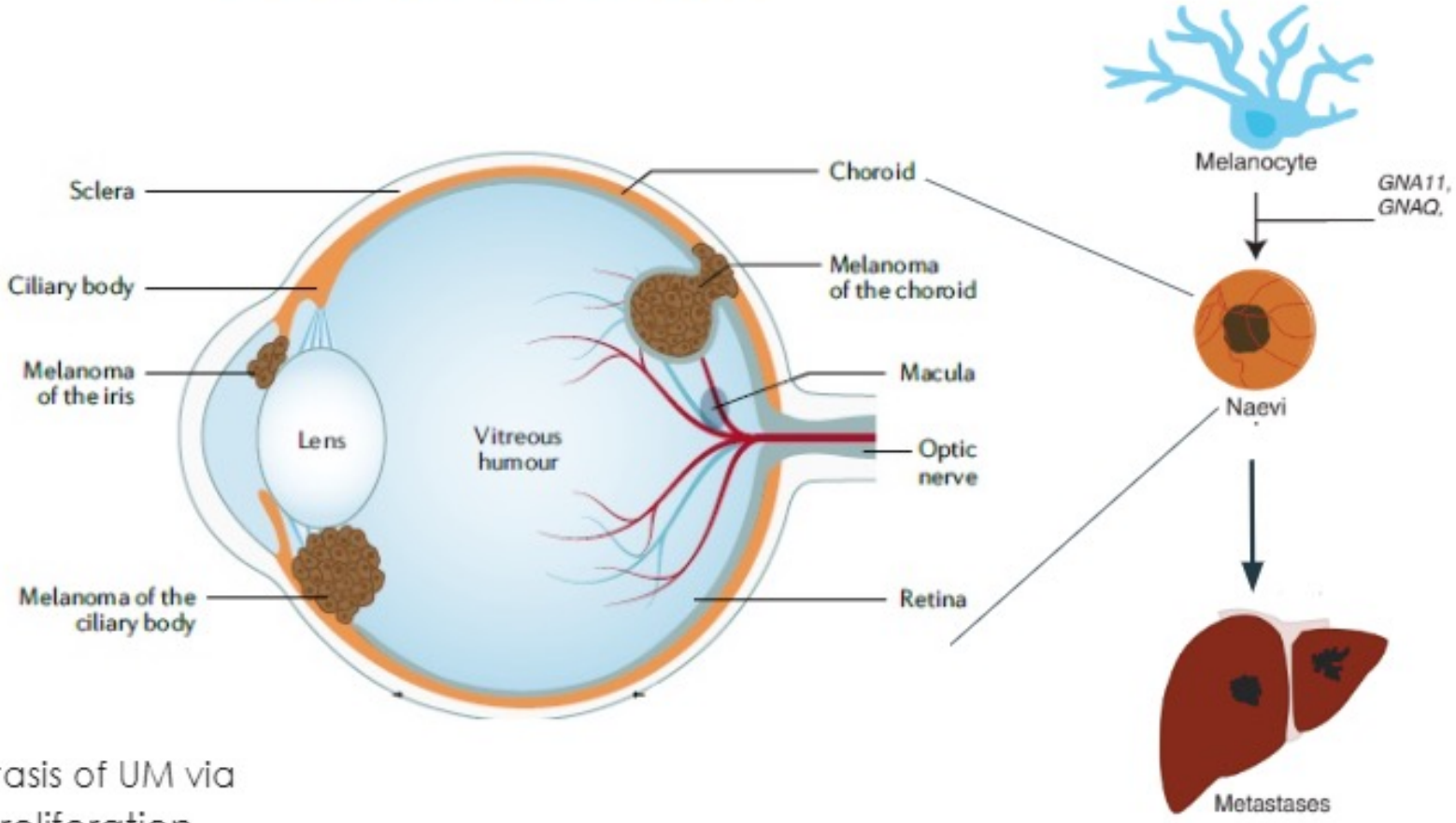
BACKGROUND

- UMs arise from **melanocytes** in the uvea.
- The most common primary intraocular malignancy in adul with a strong tendency to **metastasize in liver**.



Adapted from Martine J. Jager1 et al (2020)

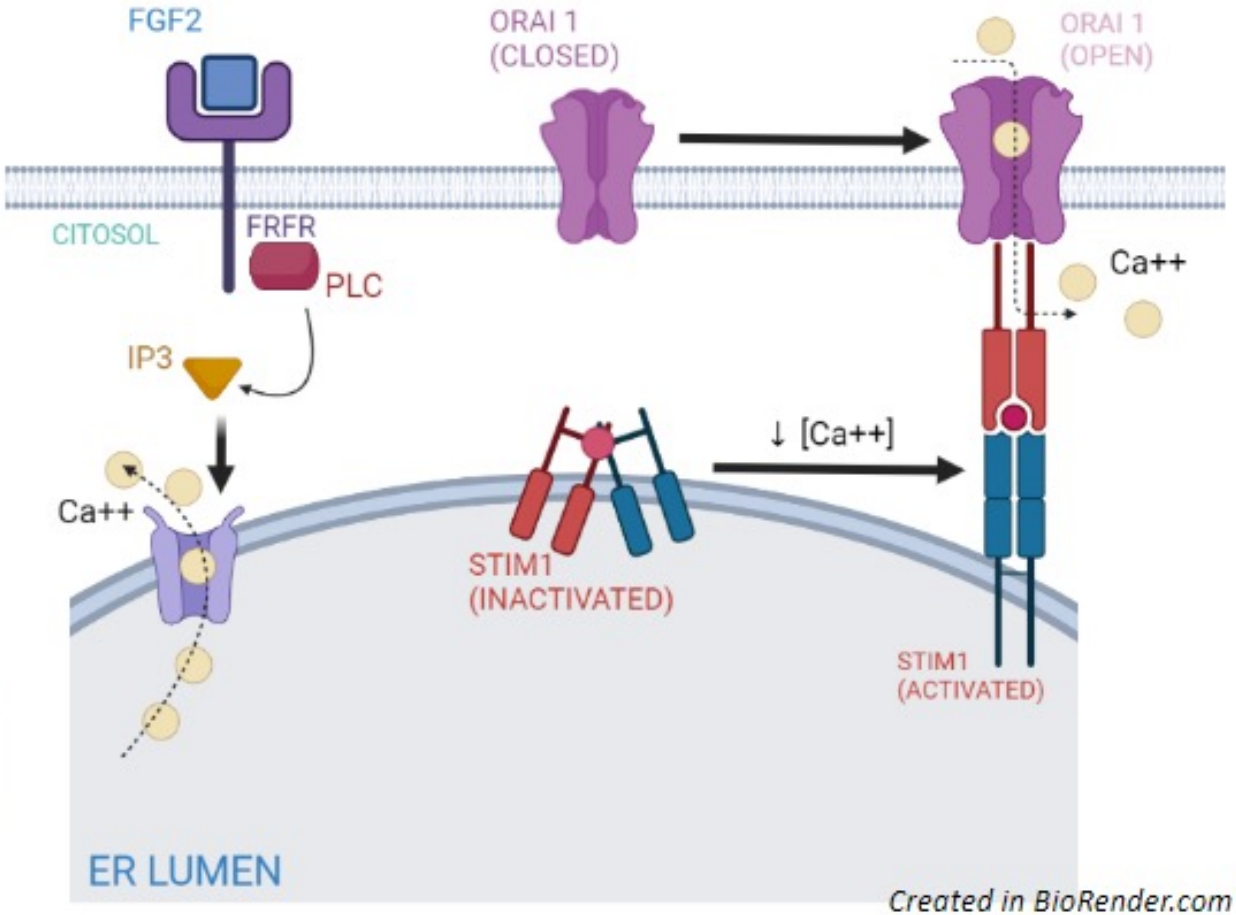
- **FGF2** promotes metastasis of UM via SOCE, inducing cell proliferation and angiogenesis.

FGF2 through its overexpressed binding to the FGFR receptor causes an increased intracellular calcium concentration and expression of **ORAI1 and STIM1** – two key regulatory proteins of store-operated calcium entry.

Why STIM1?

1. **Calcium sensor protein** in the ER membrane
2. SOCE process, following ER store depletion caused by FGF2.

Inhibiting the expression of STIM 1
all of these effects of FGF2 decrease.



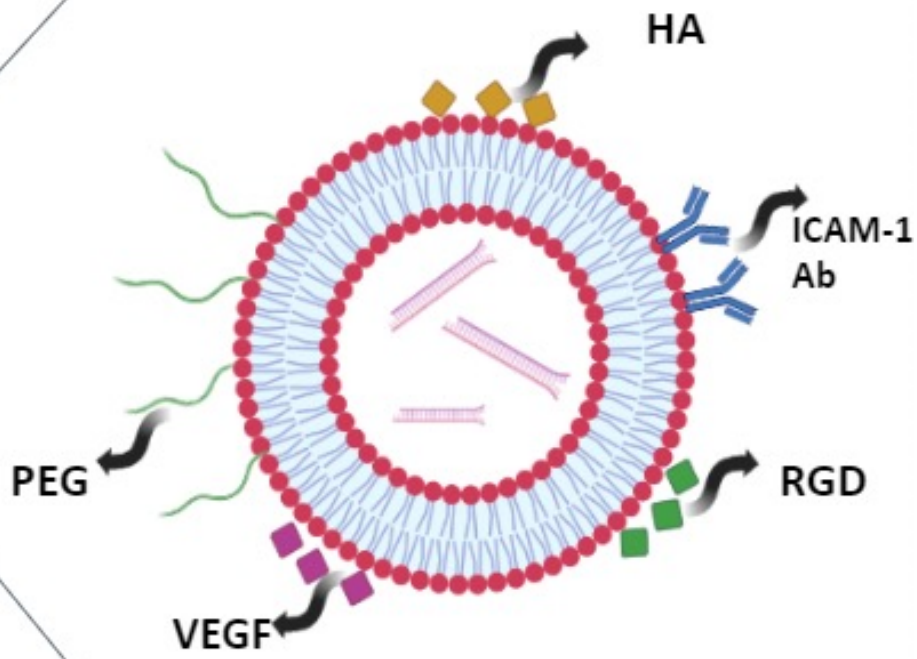
AIM OF THE PROJECT

WHAT? STIM1 knockdown via siRNA

HOW? Neutral 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC)-based nanoliposome

WHERE? Liver implanted UMM cells

Neutral-DOPC-liposome: Surface modifications in UM treatment

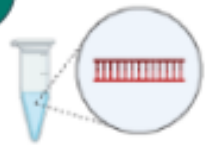


≃ 65nm liposome

Created in BioRender.com

EXPERIMENTAL PLAN

1



Best siRNA selection

siRNA SELECTION

$1x10^6$ OMM2.3 cell colture

Best siRNA?

Different siRNAs transfection

Analysis

-qPCR
-Western Blot

2

$1x10^6$ OMM2.3 cell colture

IN VITRO

Best siRNA+ Liposome DOPC transfection

To monitor duration R

5' RACE-PCR

Analysis

-Western Blot
-Intracellular calcium measurement
-Clonogenic assay

3

NSG mouse
(Injected with1x10^6 OMM2.3 cells)

IN VIVO

Injection with OMM2.3 cells → (8 weeks later)

Treatment

Injection of siRNA/DOPC liposome → (Analysis two times for weeks for 10 weeks)

Analysis

-IVIS imaging
-Size tumor analysis

Created in BioRender.com

siRNA selection

1 **Online tools** to find candidate siRNA sequences and scumble sequence.
Thermo Fischer to buy them.

InvivoGen siRNA Wizard Software 3.1

ThermoFisher SCIENTIFIC

siRNA A (STIM1_696): AUAAGCUUGUCCUCACCAUGGAAGG

siRNA B (STIM1_664): UUUCACUGUAGGGUCAUGGUAAUUG

siRNA C (STIM1_547): AAAGCUGAGCUUCUCAUCUUCACUG

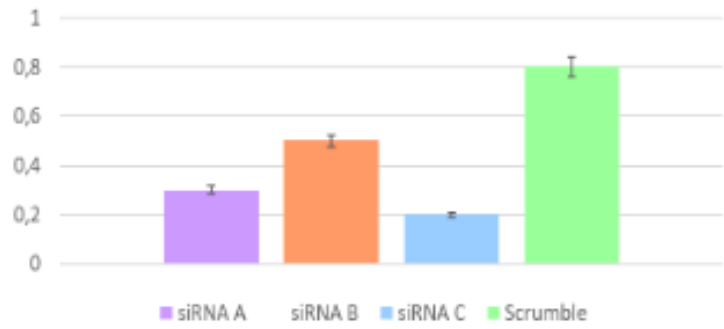
Scrumble GGTACATCCACTTCGTTAGCTATCA

2 **In vitro** evalutation of best siRNA

OMM2.3 cells colture

Which one colud be the best siRNA?

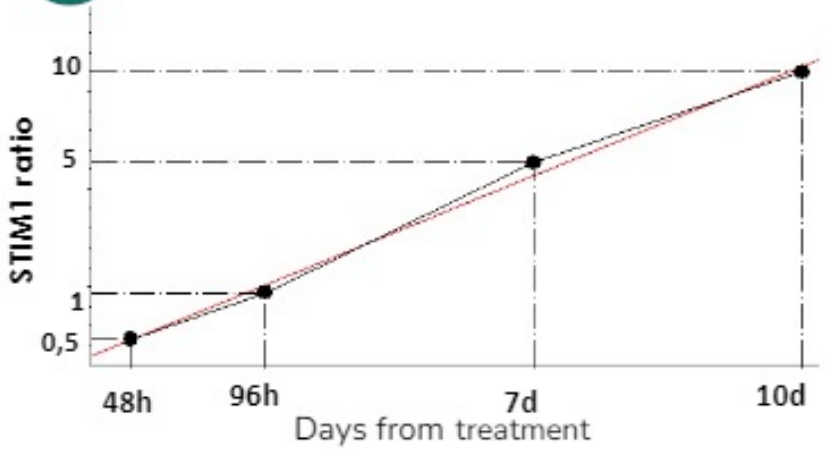
qPCR



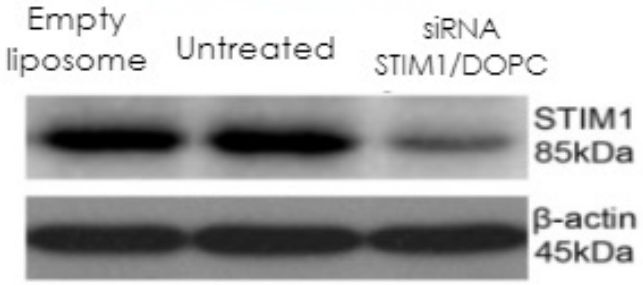
Western Blot

EXPECTED RESULTS (IN VITRO)

1 **5' RACE-PCR: STIM1 mRNA recover**



2 **Western Blot: STIM1 expression**



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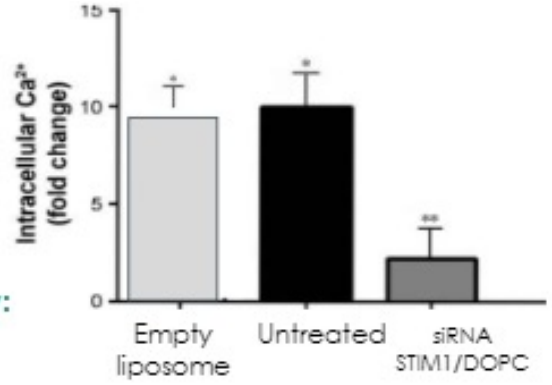
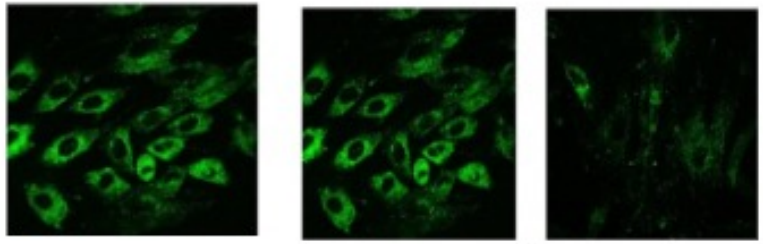
Confocal microscopy: Ca2+ concentration

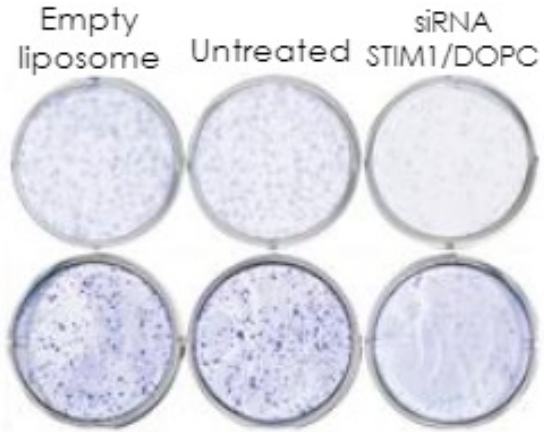


Intracellular Ca2+ levels



4

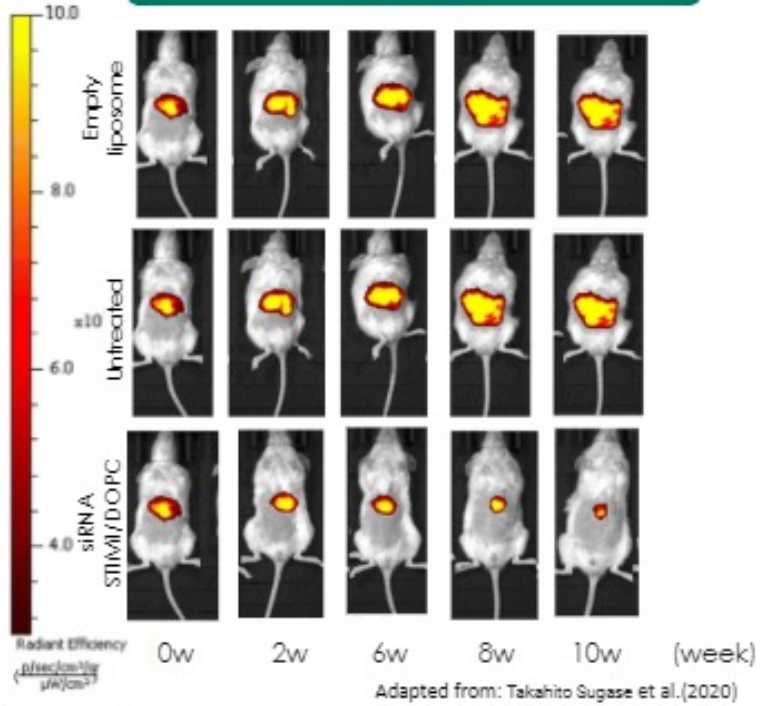
Clonogenic assay: cell proliferation



Adapted from Cheng et al.

EXPECTED RESULTS (IN VIVO)

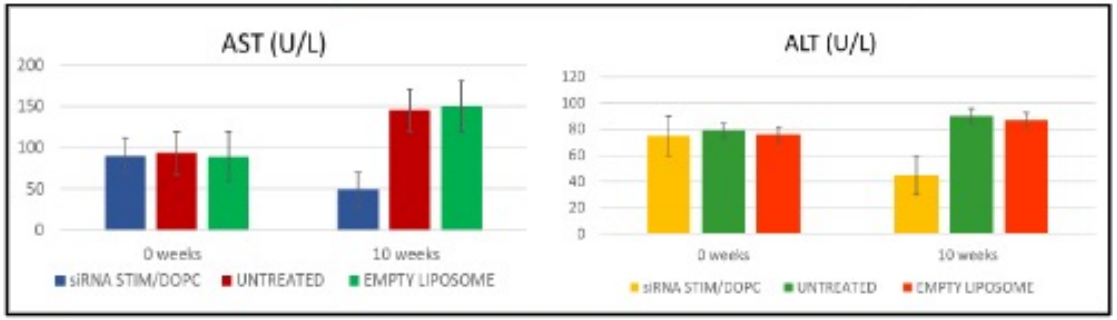
IVIS Images at 2,4,6,8,and 10 weeks after treatment



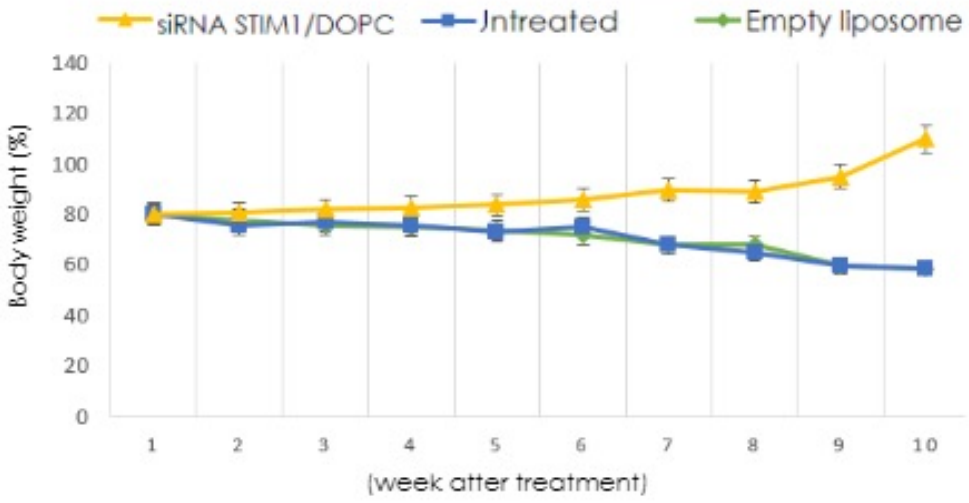
Adapted from: Takahito Sugase et al.(2020)

3 group NGS mice with different treatment
5 males and 5 females (of 6 weeks) for each group
(total n=30)

Hepatic function



Body weight



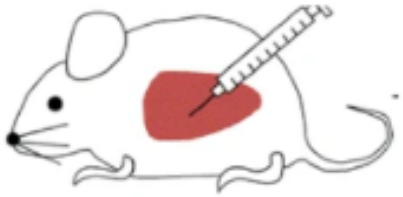
CONCLUSIONS

IN VITRO

According to the expected results, a decreasing intracellular Ca^{2+} levels mediated by STIM1 downregulation is correlated with a decrease in proliferation of OMM2.3 metastatic tumor cells.

IN VIVO

A lower proliferation in vivo determines a decrease in tumor mass and a recovery of body weight of the treated mice, compared to those treated with vehicle or not treated. This is also associated with an improvement in liver function, which is an indication of the effectiveness of the treatment.



INNOVATION

Actually, there are no standard therapies approved by FDA and EMA for UMM, due to the complex etiology causing the disease. Our therapy is proposed as a wider perspective to treat metastatic liver cancer cause ninety-five percent of metastatic uveal melanomas involve the liver.

PITFALLS

Transient structure forces an increase in number administrations.

Using immunocompromised mice.

Liver injection does not take into account all aspects of metastatic etiology and pathophysiology

SOLUTIONS

Neutral DOPC-siRNA-based therapy can be effectively combined with other anti-cancer therapies, such as chemotherapy, to enhance the efficacy of conventional drugs.

Mice with humanized immune systems would be ideal recipients for xenograft models of all tumor types.

Improving implantation capabilities in the suprachoroidal area in order to assist in the spontaneous onset of liver metastases.

BUDGET

WHAT	HOW MUCH	WHERE
Neutral-DOPC-liposome (2.4 g)	€ 1081,96	BroadPharm® A Worldwide Leading PEG Supplier
siRNA and control siRNA	€ 2700	ThermoFisher Scientific
OMM2.3 cells	//	Donated by Leiden University Medical Center
Western Blot Kit and PCR-Kit	€ 3000	LSBio LifeSpan BioSciences, Inc.
NOD-SCID Mouse	€ 5300	The Jackson Laboratory
Mice Stabulation	€ 10.000/year	
Clonogenic Assay Kit, 100 assay	€ 180,33	BioPioneer
Visual analysis	≈ € 2000	
Research Team	€ 96.000/year	

TOTAL: 120.262,29 €

ESTIMATED TIME: 1 YEAR

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