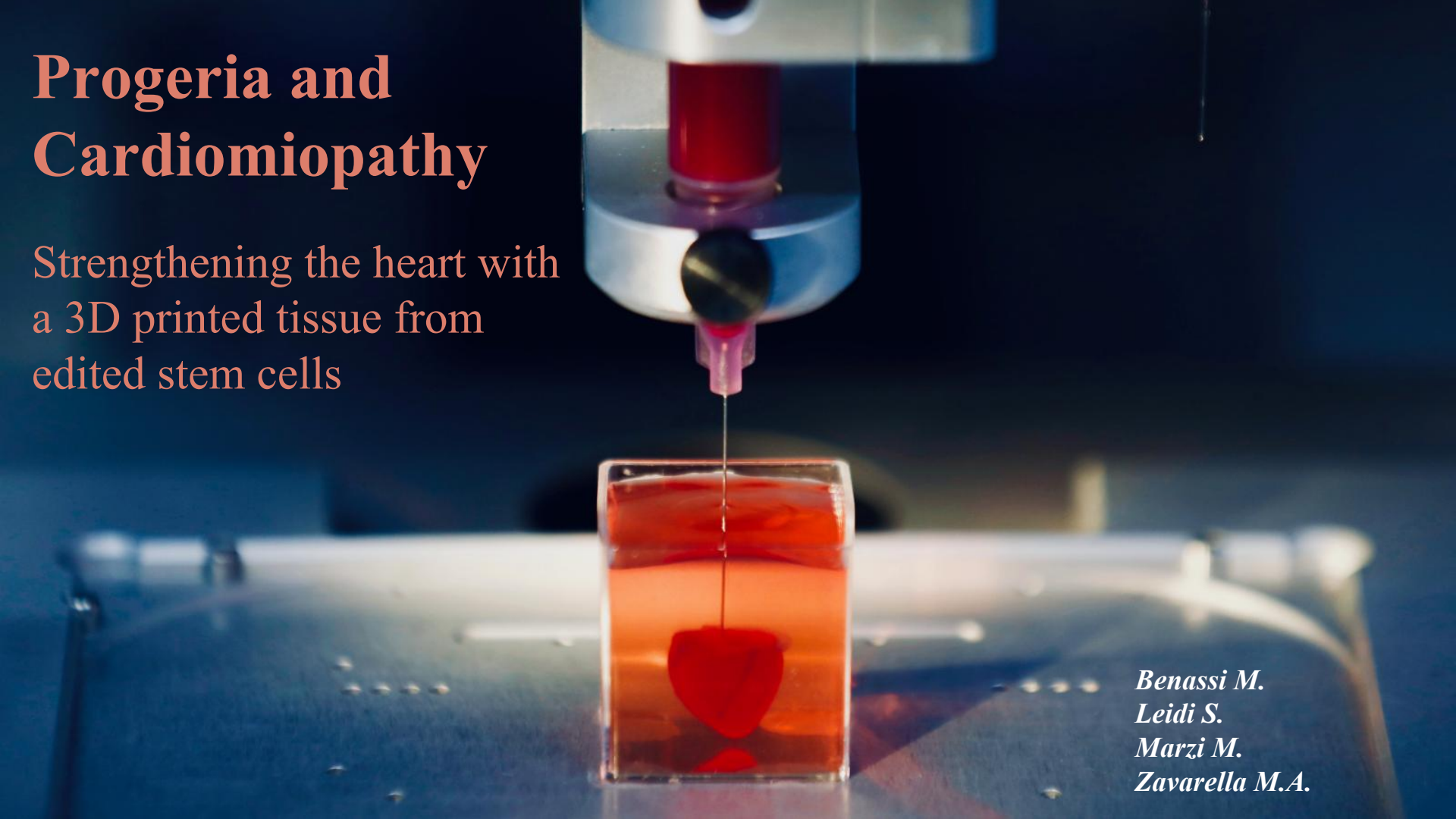
Progeria and Cardiomiopathy

Strengthening the heart with a 3D printed tissue from edited stem cells



Benassi M.
Leidi S.
Marzi M.
Zavarella M.A.

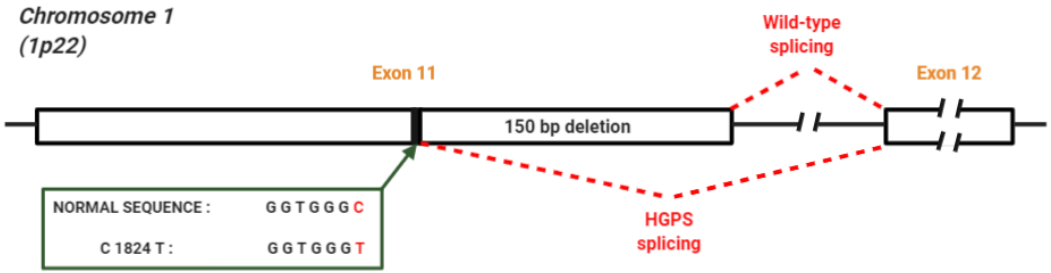
Hutchinson-Gilford Progeria Syndrome (HGPS)

HGPS is an extremely rare pathology characterized by premature aging with postnatal onset.

The main clinical features includes:

- alopecia
- loss of subcutaneous fat
- osteolysis
- cardiovascular conditions, like **cardiomyopathy**, which represents the major cause of premature death

Sammy Basso (26), founder of the Associazione Italiana Progeria Sammy Basso (A.I.Pro.Sa.B.)



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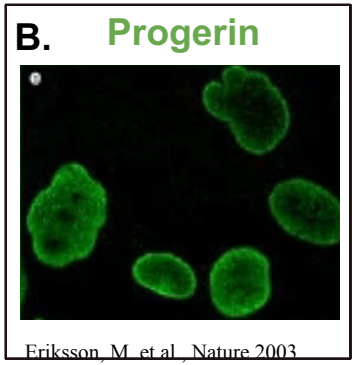
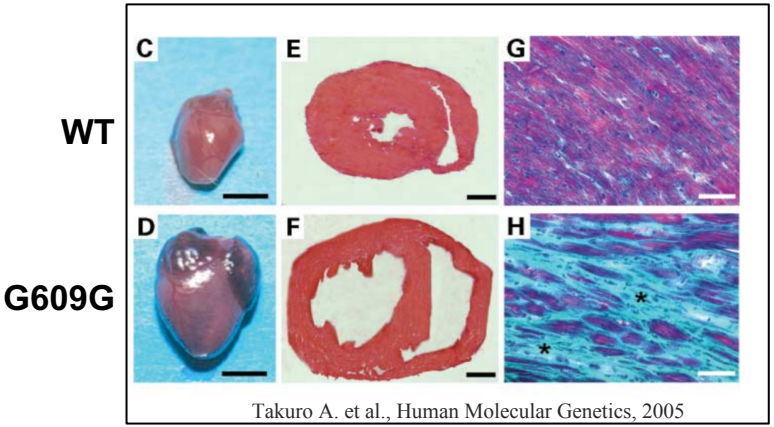
G609G mouse recapitulates Progeria phenotype

In *Mus musculus*, a **c.1827C>T;pGly609Gly** mutation in the LMNA gene produces **Progerin** as the c.1824C>T;pGly608Gly human mutation**.**

Osorio et al., Science Transl Med. 2011



Eriksson, M. et al., Nature 2003



Takuro A. et al., Human Molecular Genetics, 2005

A. 6 months G609G mouse with alopecia. **B.** G609G mouse cells show nuclear blebbing caused by Progerin. **C.** and **D.** WT and G609G mouse hearts. **E.** WT and **F.** G609G mice transversal section throught the hearts show both left and right ventricular dilatation; **H.** Histopathological anaylisis of G609G mouse heart with Gomori's trichrome stain reveals extensive degeneration of myocites and massive fibrosis (asterisk) compared to **G.** WT.

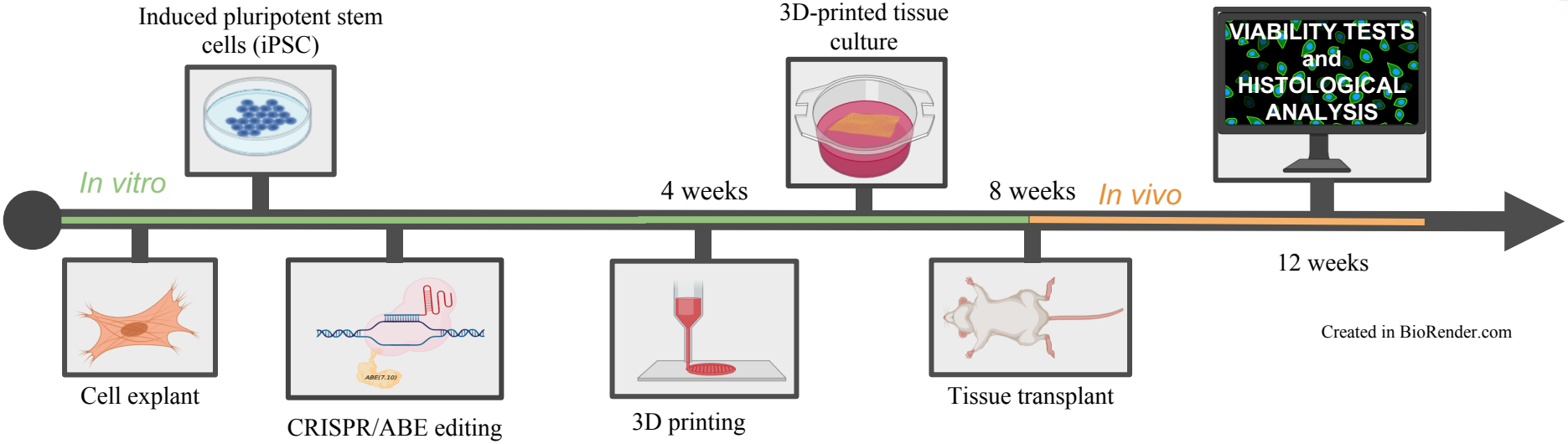
Aim

Extend life expectancy of progeroid mice by solving their early death caused by **cardiomyopathy**

How

Implanting a **3D printed** tissue made from CRISPR-ABE edited mice iPSC-CM to support the mices' heart

EXPERIMENTAL DESIGN



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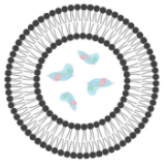
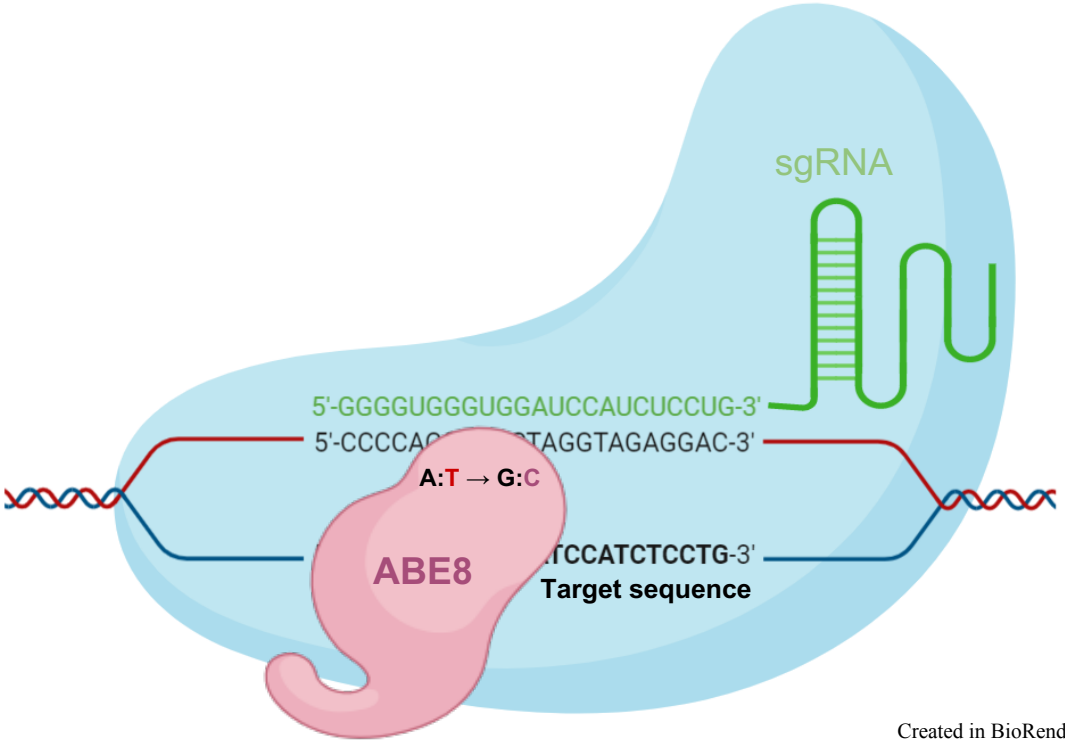
Step 1

How to “cure” HGPS affected cells?

ABE8 is a newly developed adenine base editor with a 98-99% efficiency, making it the perfect tool for the job. The system is carried by **lipofectamine**





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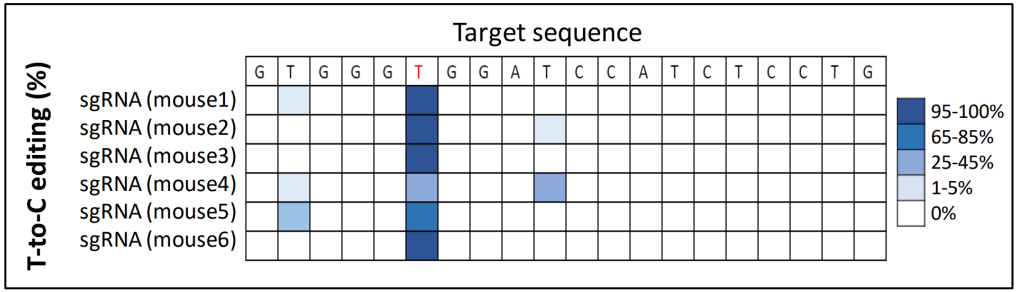
Step 2

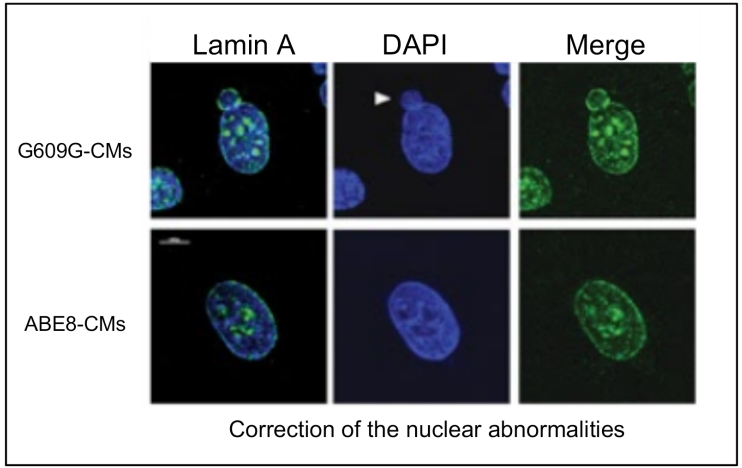
Was the editing successful?

To check if the editing was successfully we sampled cells from each colture, extracted genomic DNA, amplified fragments from LMNA exon 11 and sequenced with the Sanger method. To confirm that the editing reverted the nucleus phenotype to WT we used fluorescent microscopy.

Sanger sequencing



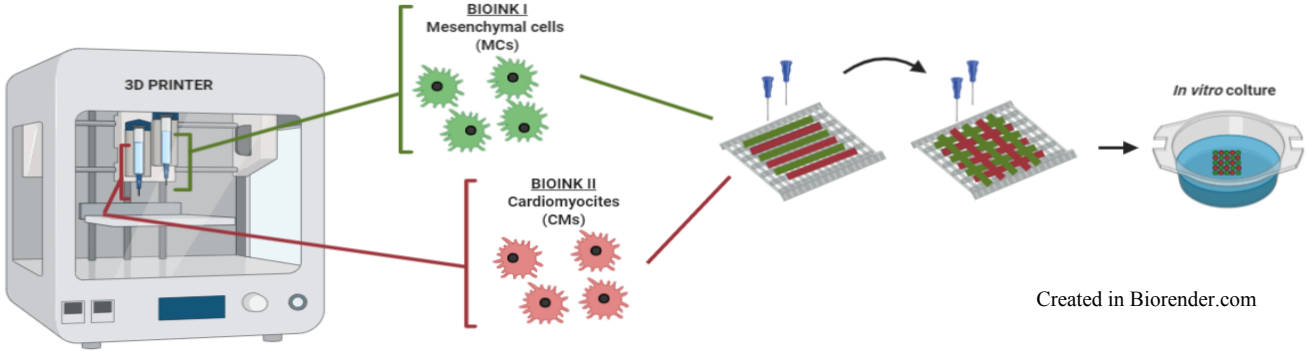
Phenotype analysis



Osorio et al., Science Transl. Med. 2011

Step 3

3D printing the Edited Patch Implant (EPI)



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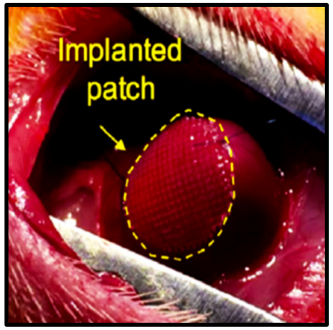
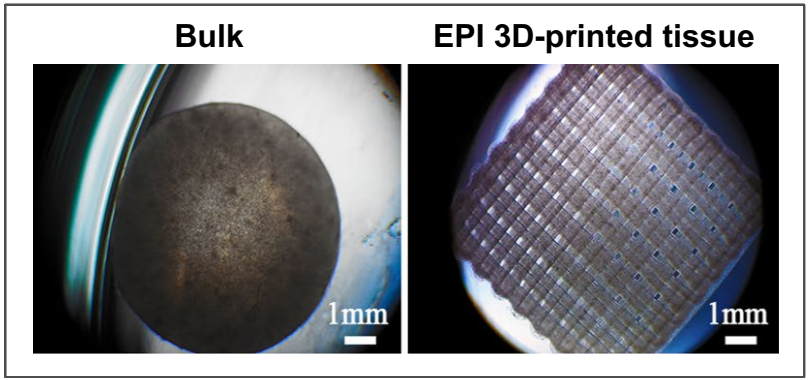


Cui H et al., Adv Drug Deliv Rev. 2018

The Bulk control: *does the correct spatial cellular disposition really give an effective enhancement to the G609G mouse heart?*



Maiullari F et al., Sci Rep. 2018

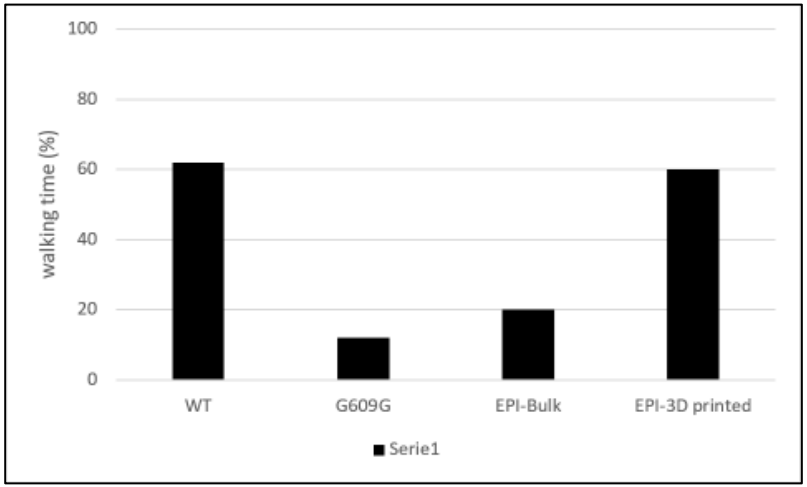
(yes!) Results: physiological assessment

Table1. Echocardiographic data for WT, G609G, Bulk and 3D printed EPI mice at 2 and 4 months after the transplantation

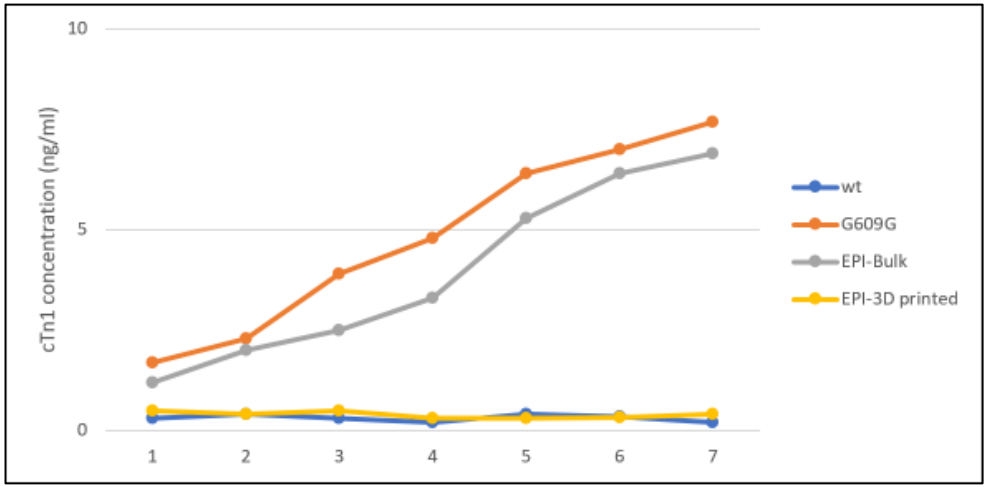
	2 months				4 months			
	WT	**G609G**	**EPI-Bulk**	**EPI-3D printed**	**WT**	**G609G**	**EPI-Bulk**	**EPI-3D printed**
LVM (mg)	46.3±3.5	54±1.4	50±1.7	47.5±3.7	48.7±1.8	61.8±2.7	63±2.3	49±1.3
LVFS (%)	46.9±2.6	38.2± 2.6	42.4±2.6	46.2±2.3	37.2±1.6	27.3±2.4	28.2±2.4	37.4±1.6
Hearth rate (bpm)	574±24	524±21	527±23	574±21	515±27	498±23	502±25	515±22

Cardiac function was evaluated by transthoracic echocardiographic analyses of the left ventricle (LV): LVM = LV mass; LVFS = LV fractional shortening

Results: viability tests

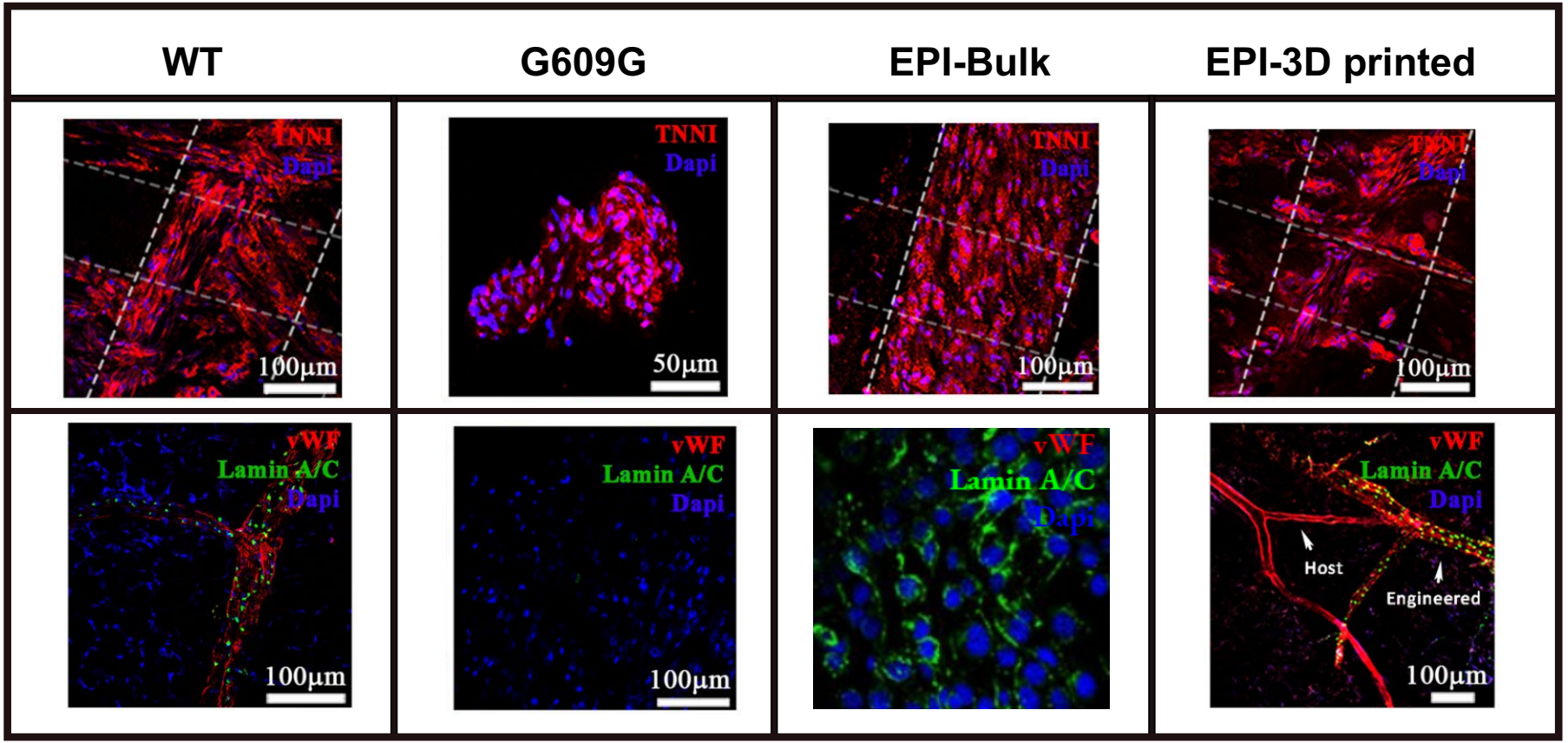


Locomotion activities of the mice at 2 weeks from transplant. Exploratory locomotion activity of WT, G609G, EPI-Bulk and EPI-3D printed implant mices in open field test for 5 min (n=3 for each group).



Cardiac troponin concentration evaluated 2 weeks from transplant (TnI concentration in serum from WT, G609G, EPI-Bulk and EPI-3D printed implant mices (n=5 for each group).

Immunohistochemical analysis

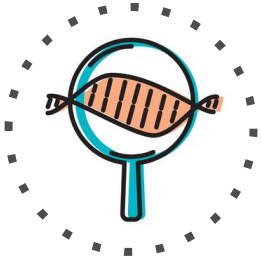


Implant are analized 4 weeks after transplant

Maiullari F et al., Sci Rep. 2018

Pitfalls & Solutions

Not all progeria cases are caused by the same mutation



A genetic screen is needed in order to identify the disease causing mutation, develop and apply the right gene therapy needed to generate the corrected cells for the EPI

EPIs, as we show, are effective, but an optimal solution would be an edited organ transplant

Advancements in 3D bioprinting are needed in order to open the possibility of whole organ printing and transplant

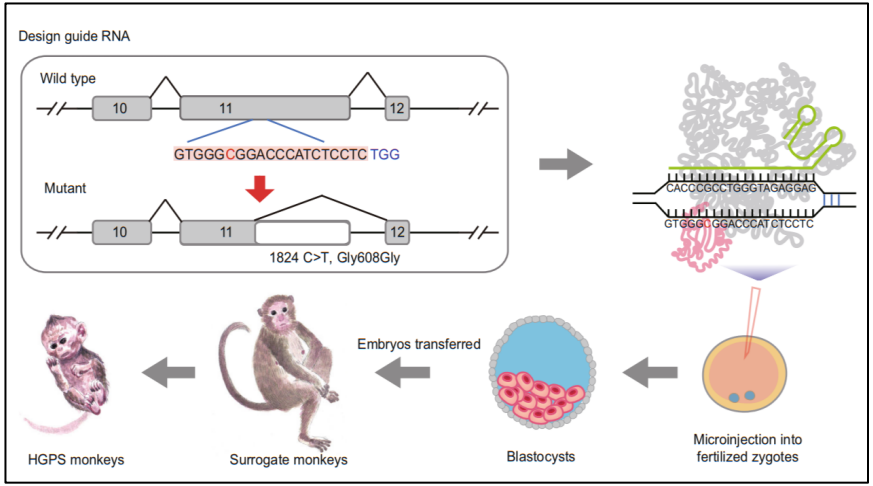
Conclusion and future perspectives:

Advantages:

- Avoids rejection of the implant by using the patient's own cells.
- Avoids ethical concerns of organ transplant
- Combines three recent advances in the biomedical field

Next steps:

Higher organism trials to further validate our results and drive the research towards the development of similar techniques.



Wang F et sl., Protein Cell. 2020

Materials :

- HGPS mice (x 20) courtesy of Osorio's Lab
- sgRNA + ABE kit + lipofectamine (ABE/Cas9) from Synthego
- From ThermoFisher:

  - Yamanaka factors + Lin28 + lipofectamine vector
  - Sanger Sequencing Kit
  - Polycaprolactone
  - CMs/MCs growth factors + Gibco™ medium
  - Lamin A, vWF and Progerin Antibodies

- Stabulation and standard laboratory equipment

Budget: 200.000$

(excluding researchers salaries)

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