DEVELOPMENT OF A CELL-BASED THERAPEUTIC STRATEGY FOR IDIOPATHIC PULMONARY FIBROSIS

L.M. in Biologia e Tecnologie Cellulari

Biology of Stem Cells and Applications

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IDIOPATHIC PULMONARY FIBROSIS

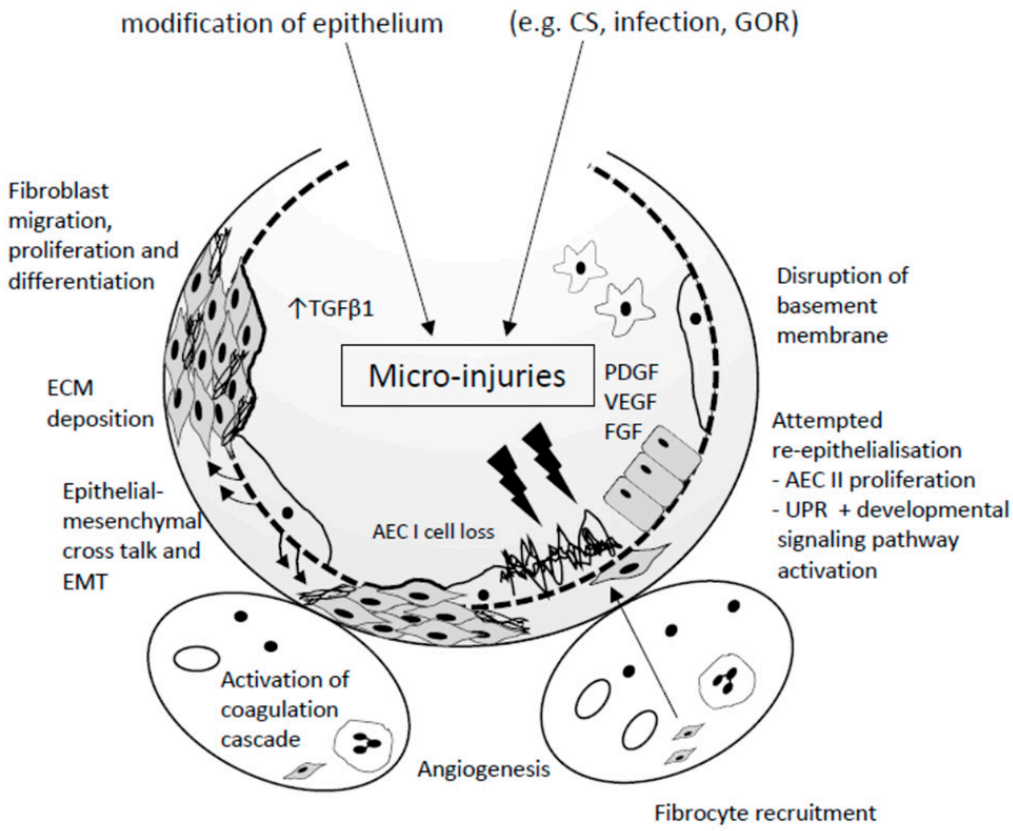
Idiopathic Pulmonary Fibrosis (IPF) is one of the most common and aggressive forms of Interstitial Lung Deseases (ILDs), with a reported incidence rates of 2,8-9,3 per 100.000 per year in North America and Europe

Clinical Features:

- Chronic and progressive fibrosis
- Progressive respiratory failure
- High mortality (survival of 2-3 years from diagnosis)

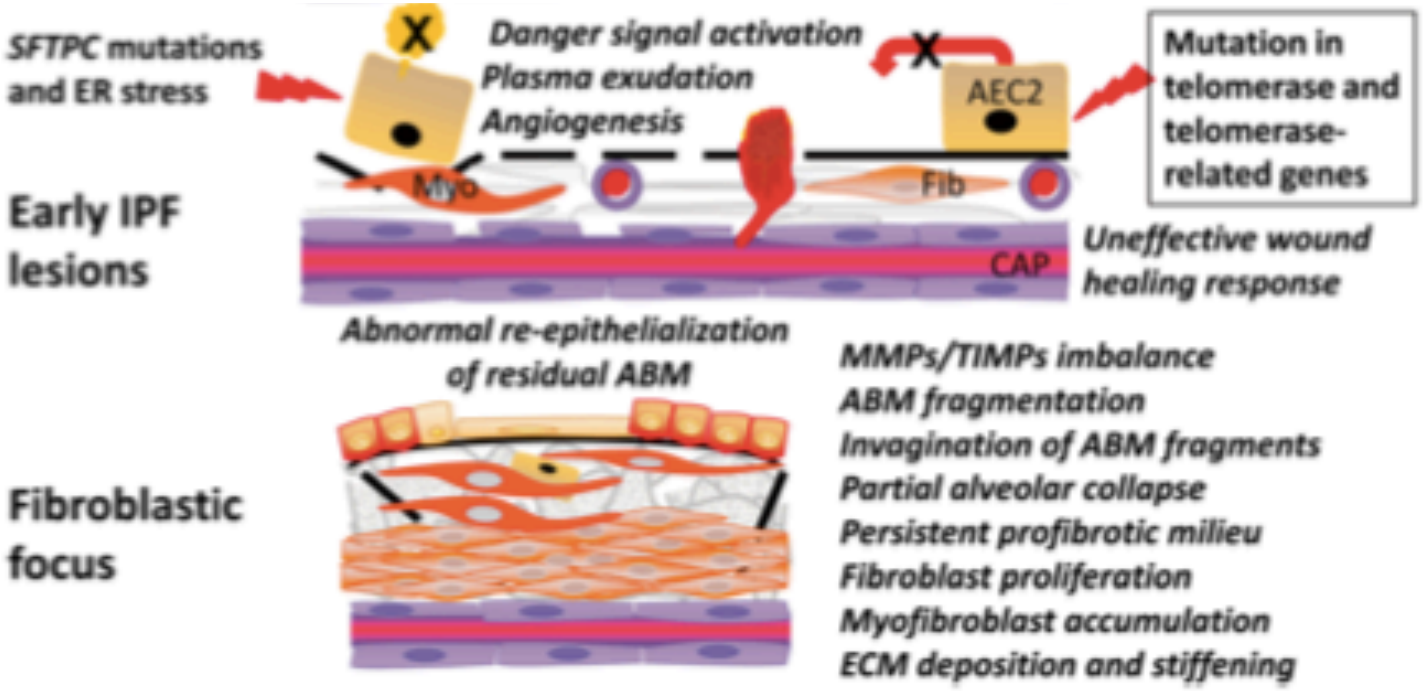
Actual therapy approaches:

- Lung transplantation
- Farmacologic therapy (Pirfenidone and Nintedanib)



IDIOPATHIC PULMONARY FIBROSIS

This desease causes the formation of clusters of fibroblasts and myofibroblasts (fibroblastic foci), and excessive deposition of disorganised collagen and extracellular matrix (ECM), due to regeneration failure of Alveolar Cells. In these cells a TERT gene mutation leads to premature telomeres shortening.

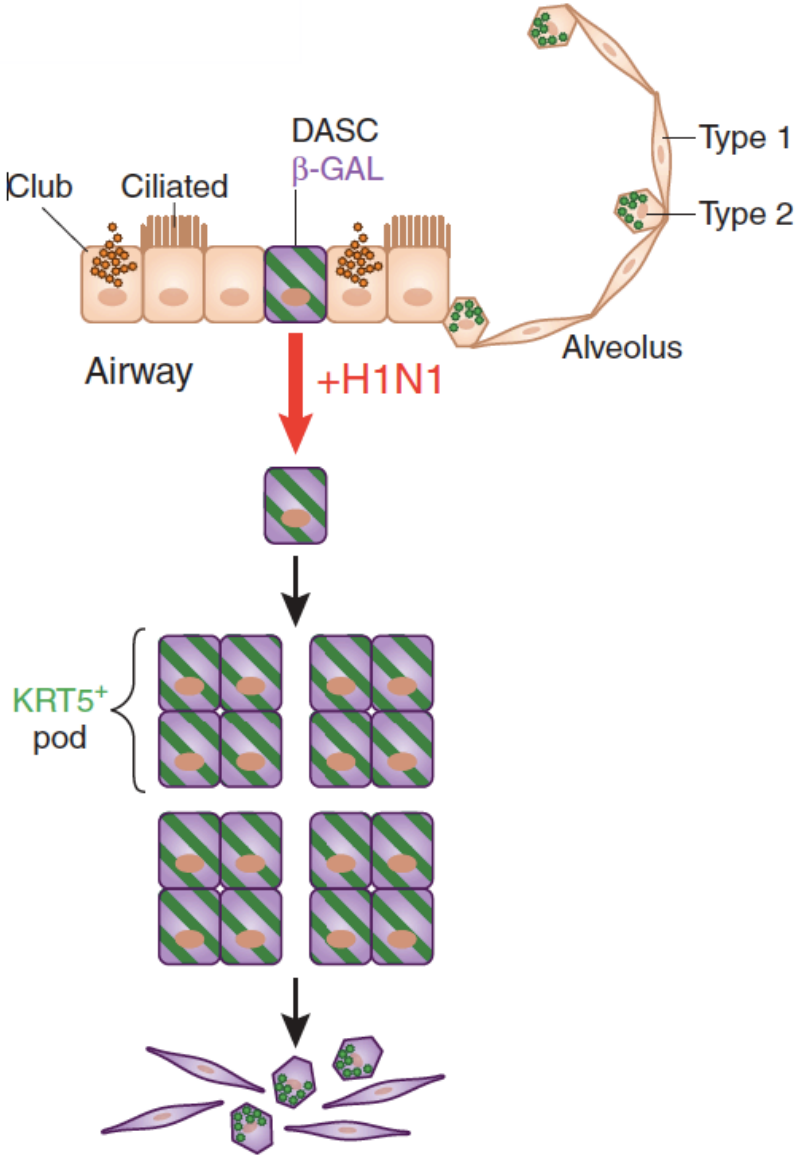


OUR CHOICE

DASC$^{p63+/krt5+}$ cells:

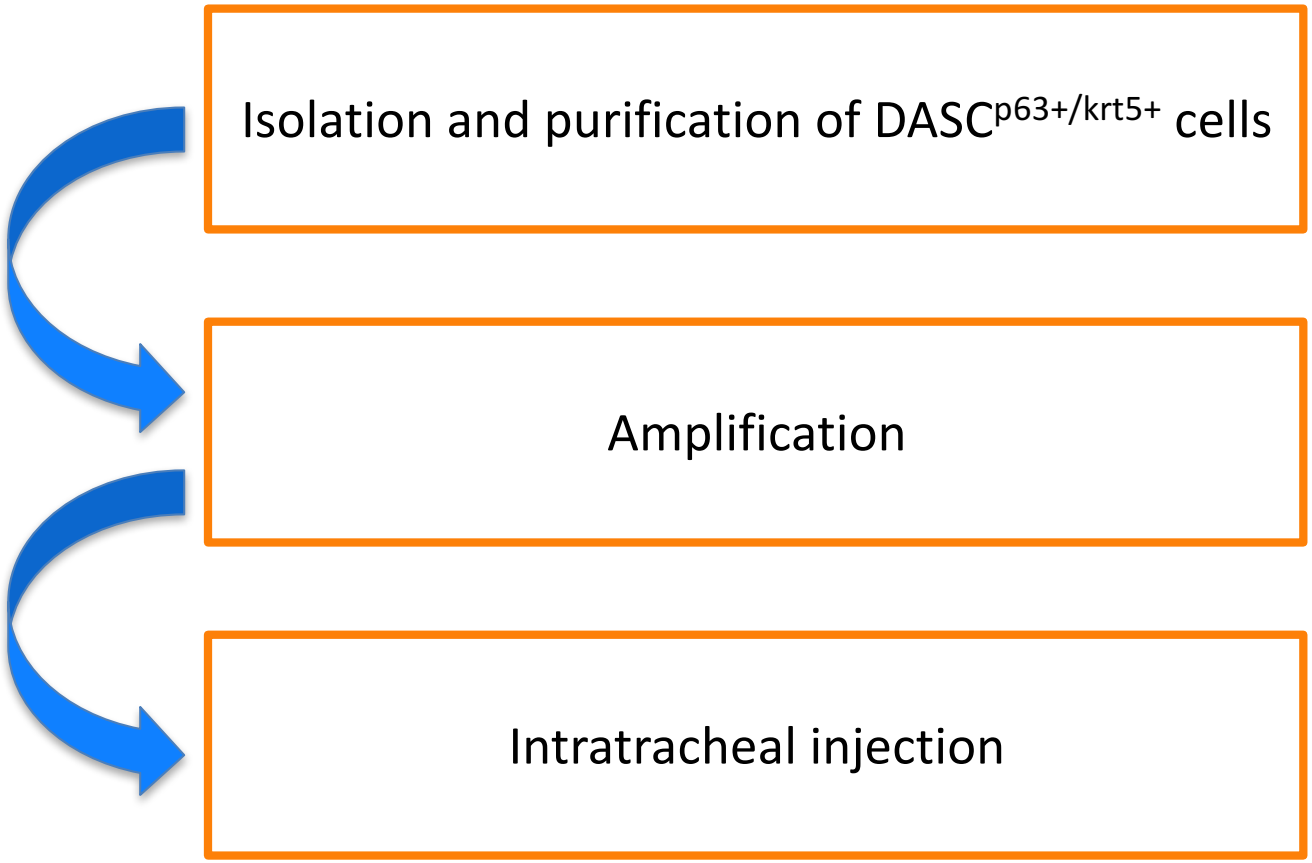
Arway epithelial basal cells present in the distal portion of the lung, which are capable of both self-renewal and generation of differentiated daughters.

DASCs respond to H1N1 infection by migrating to the interstitial space, proliferating into Krt5^{+} pods and then expressing markers of type 1 or type 2 cells.

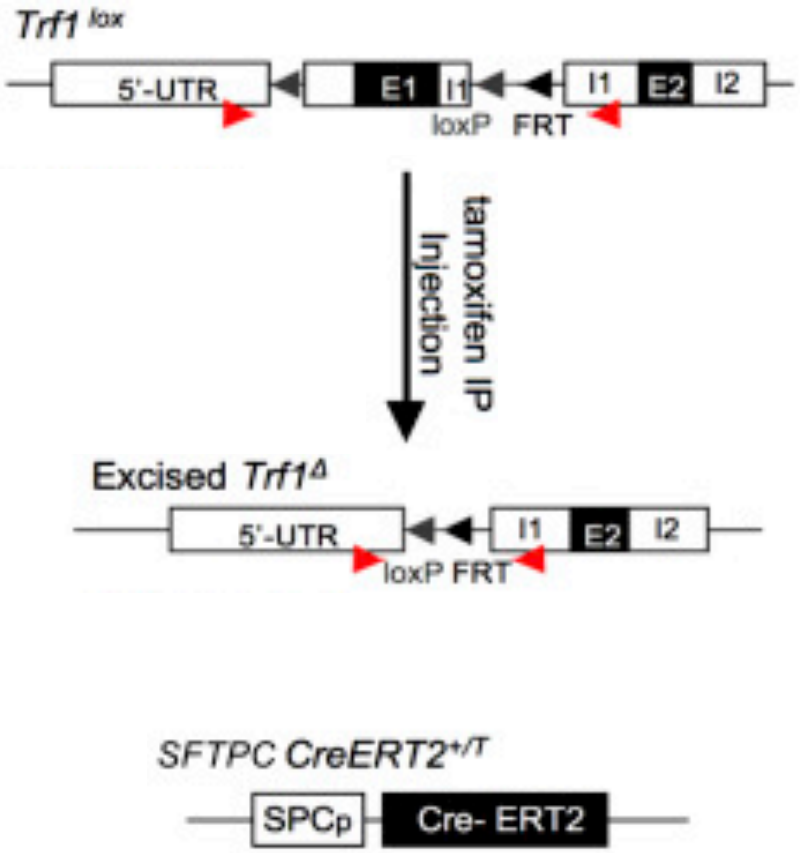


OUR PROPOSAL

GOAL → Regeneration of damaged alveolar tissue



MOUSE MODEL


Trf1 lox
5'-UTR
E1
I1
I1
E2
I2
loxP
FRT
tamoxifen IP
Injection
Excised Trf1Δ
5'-UTR
I1
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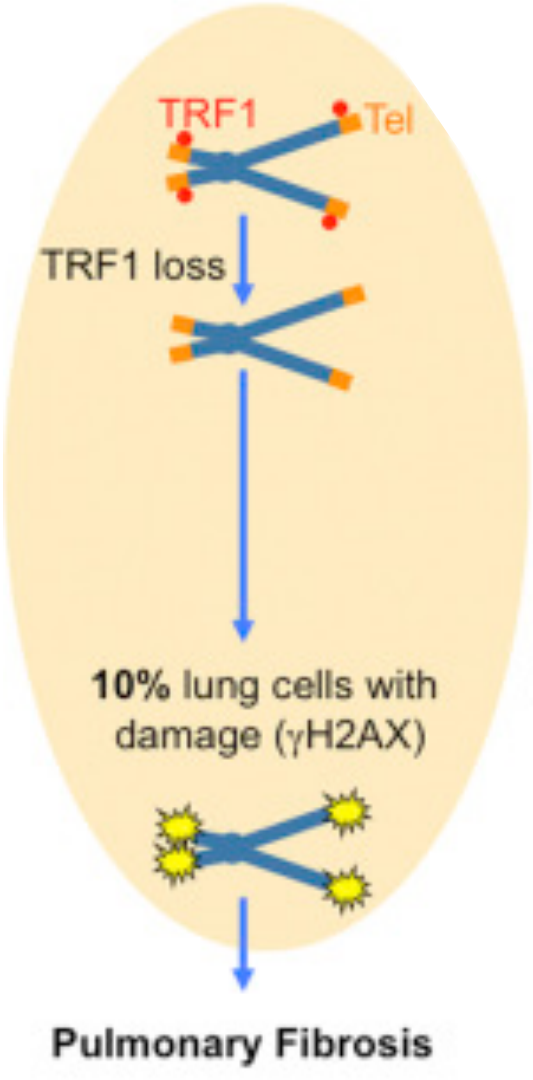
I2
loxP FRT
SFTPC CreERT2+/T
SPCp
Cre- ERT2

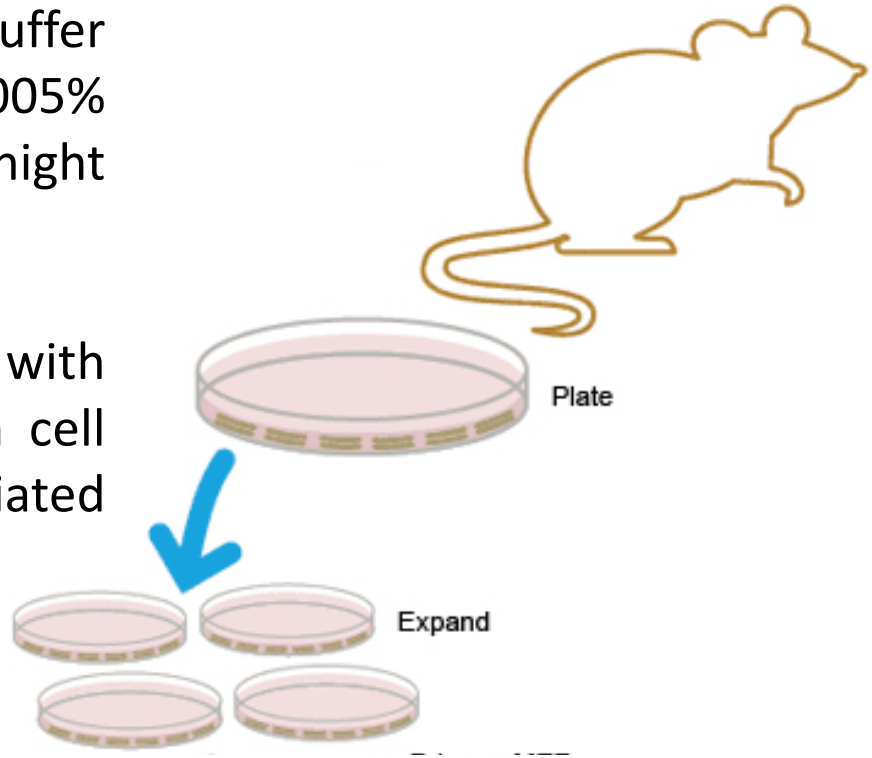

TRF1
Tel
TRF1 loss
10% lung cells with damage (γH2AX)
Pulmonary Fibrosis

ISOLATION AND AMPLIFICATION

- Lung tissue will be collected and immersed in cold wash buffer (F12 medium, 1% Pen/Strep, 5% FBS)

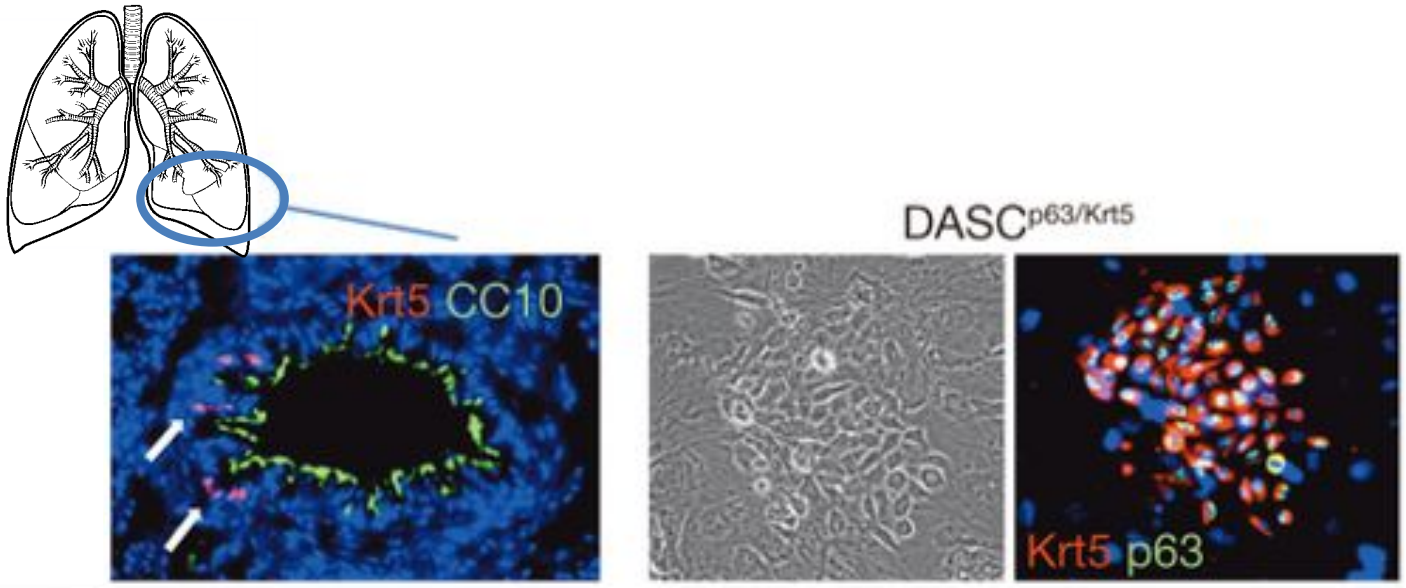
- The tissue will be cut into small pieces and digested with dissociation buffer (F12/DMEM, 1mg ml^{-1} protease, 0.005% trypsin and 10ng ml^{-1} DNase I) overnight with gentle rocking

- The dissociated cells will be washed with wash buffer, passed through a 40-µm cell strainer, counted and plated onto irradiated 3T3 feeder cells



ISOLATION AND AMPLIFICATION

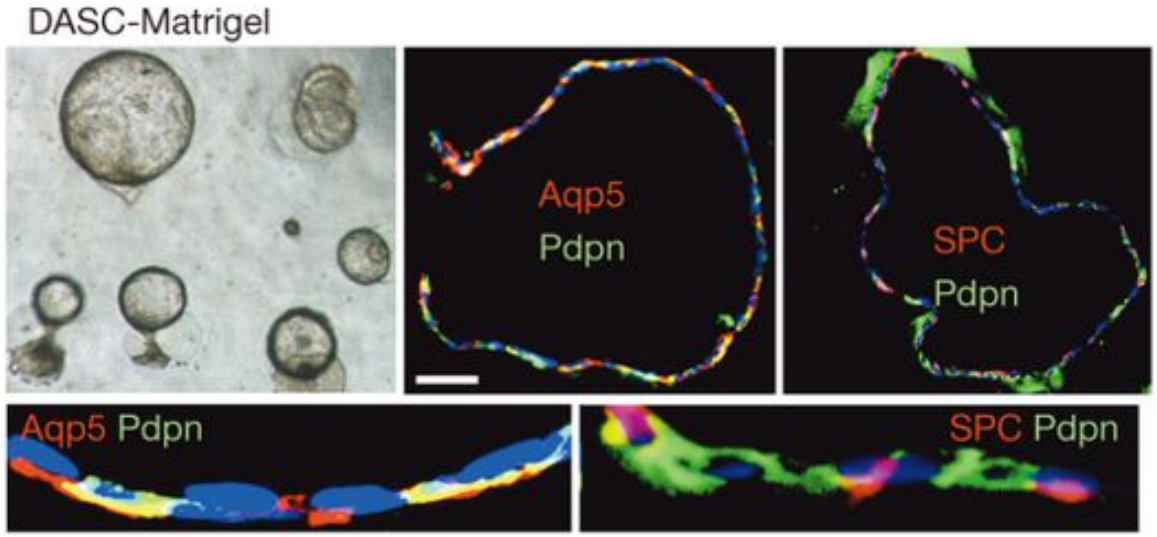
- Single cell colonies will be picked up by cloning ring and expanded
- Colonies will be characterized by immunofluorescence staining (E-cadherin^{+} Krt5^{+} p63^{+} Pdpn^{-} CC10^{-} SPC^{-})
- These colonies can be passaged up to 12 months with no observable phenotypic or chromosome count changes



in vitro DIFFERENTIATION

- The cell suspensions will be placed on Matrigel at $3x10^4$ cells/chamber and differentiated

- After 20 days culturing in the differentiation medium (CnT-PR-AD) + 1mM$CaCl_2$ with 1% Matrigel, the 3-D structures will be fixed for sectioning and staining

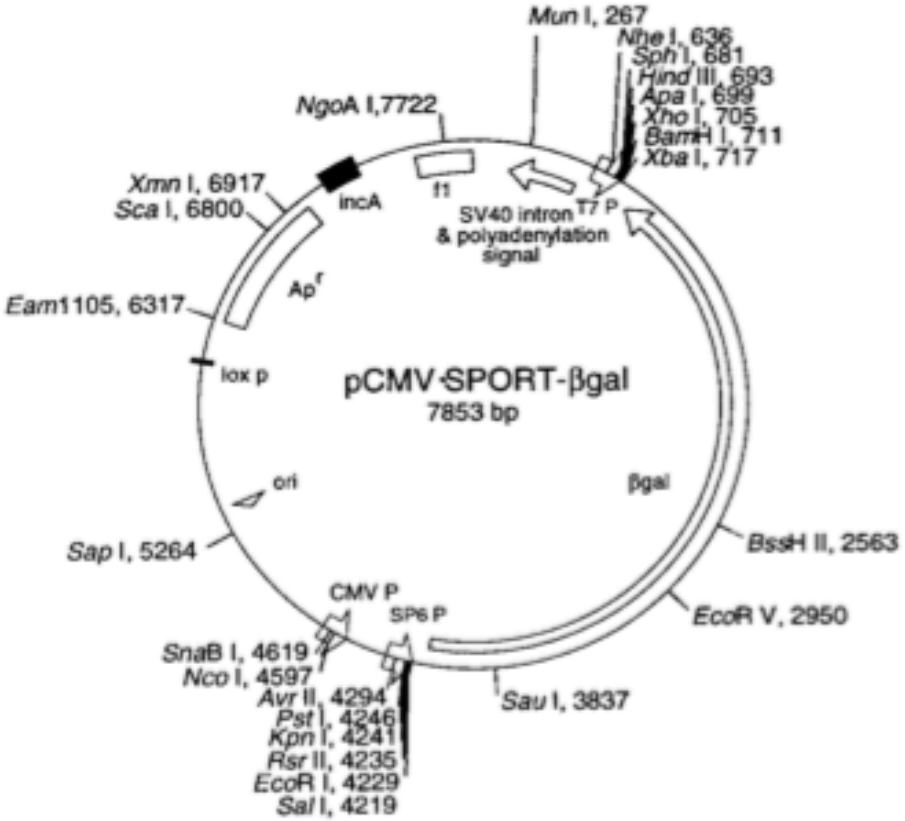


In three-dimensional Matrigel cultures these cells form unilaminar, alveolar-like spheres composed of cells expressing type I and type II pneumocyte markers

CELL ENGINEERING

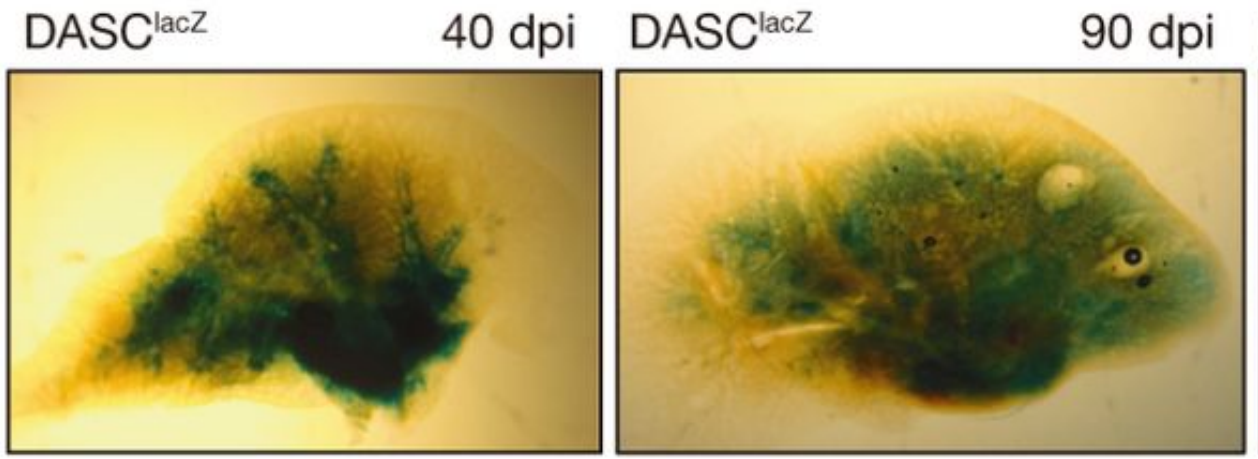
- Expanded cells will be harvested by differential trypsinization to remove feeder cells

- Cells will be transfected with pCMV-SPORT-βgal to trace them after transplantation

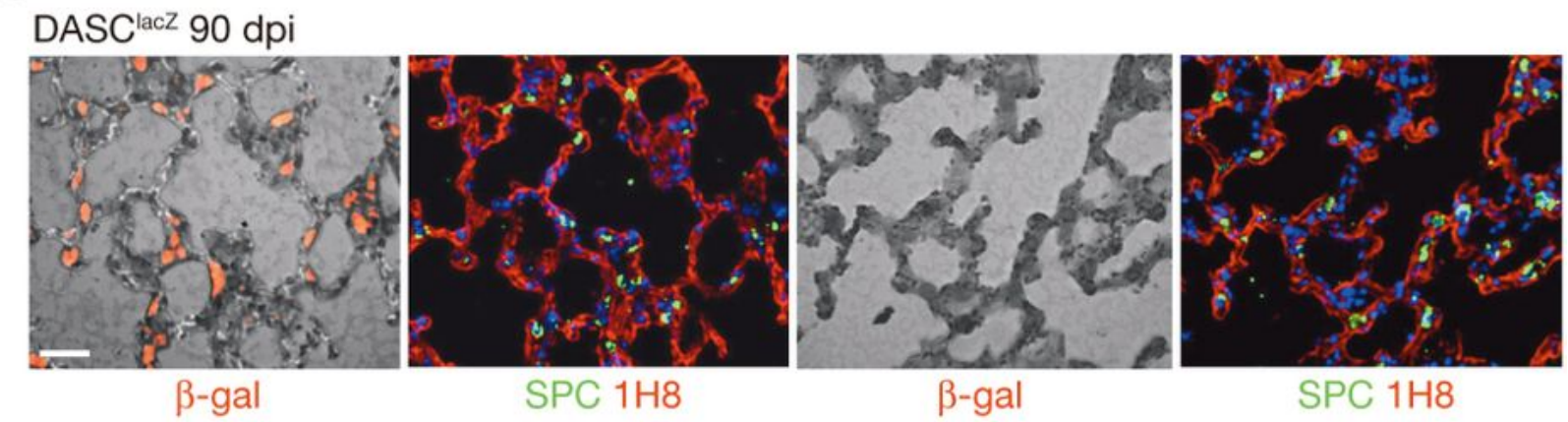


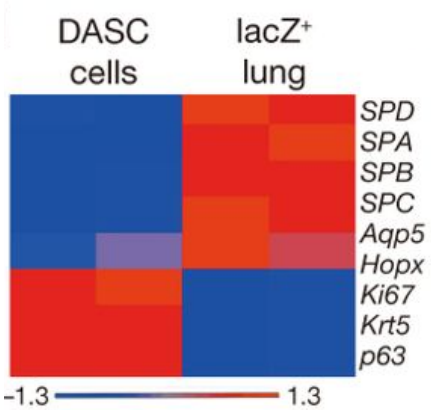
INTRATRACHEAL INJECTION

- One million cells will be diluted in 50 ml DMEM/F12 medium for transplantation into each mouse



IS THE STRATEGY WORKING?





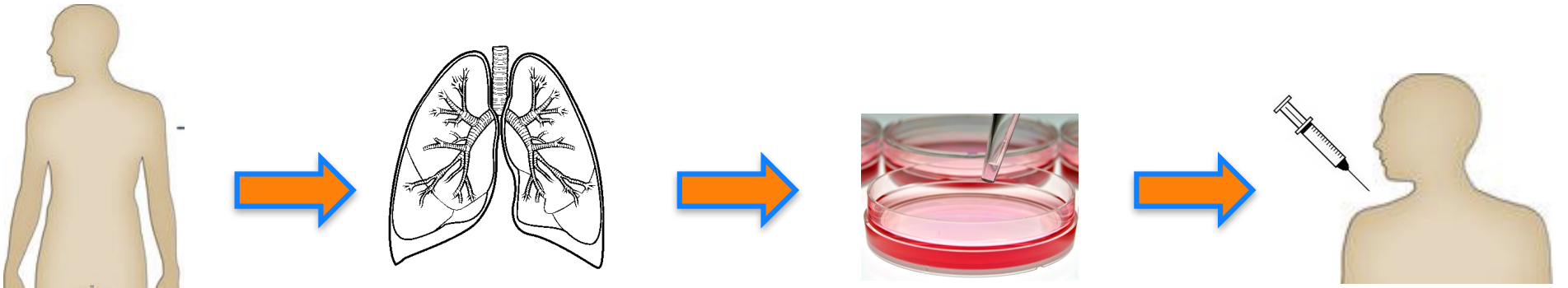
p63+/krt5+ cells differentiate in Alveolar type II (SPC) and Alveolar type I cells (1H8)

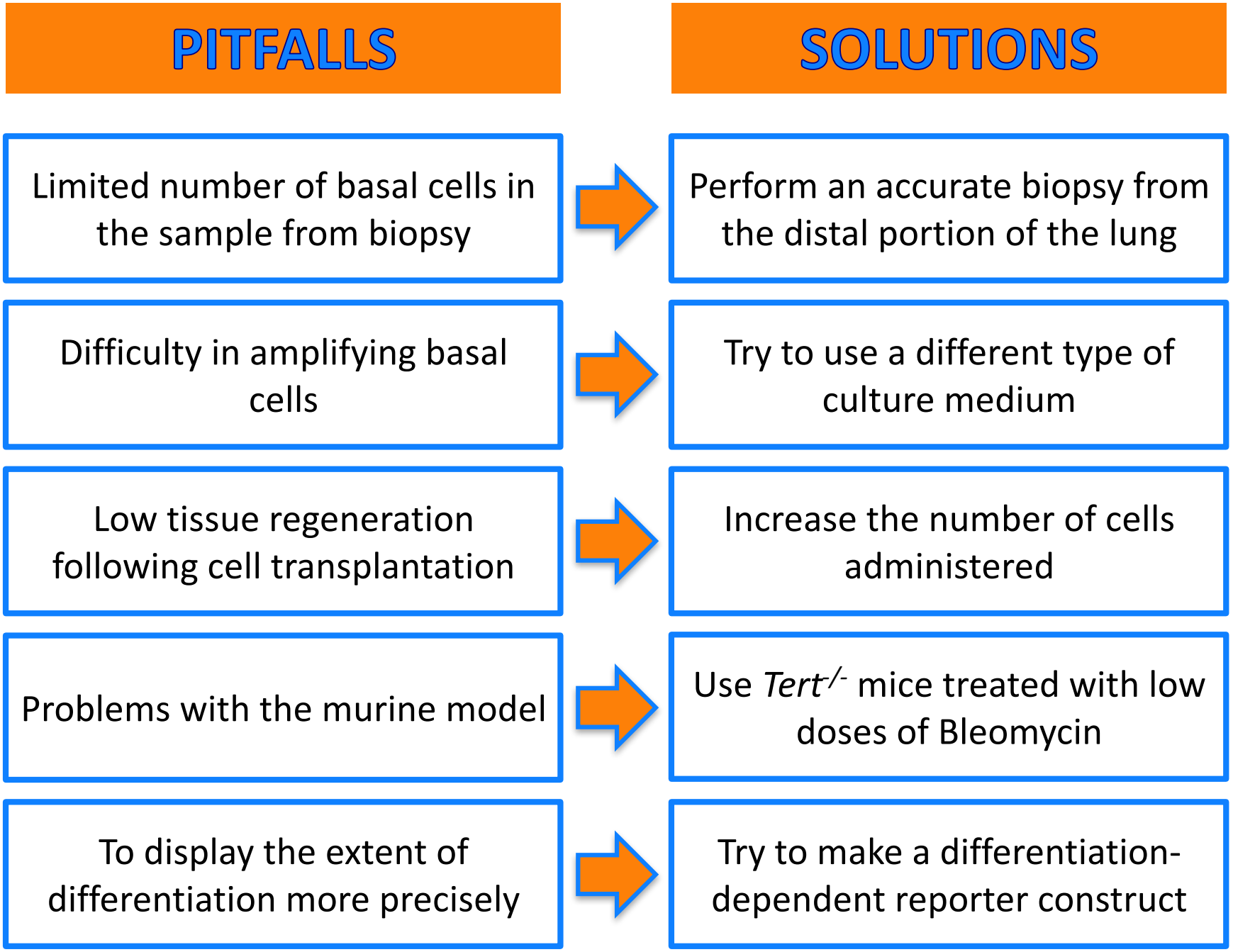
FUTURE PERSPECTIVES

- If experiments in mouse model will be successful, we will try to isolate and *in vitro* differentiate DASC$^{p63+/krt5+}$ cells from tissue samples derived from pulmonary biopsy of IPF patients

- If human DASC$^{p63+/krt5+}$ cells will show the same behaviour of their mouse counterpart, we will try to reproduce this protocol on an another animal model more similar to human

- Final perspective will be to try to design a terapeutic approach for future clinical trials




PITFALLS
SOLUTIONS
Limited number of basal cells in the sample from biopsy
Perform an accurate biopsy from the distal portion of the lung
Difficulty in amplifying basal cells
Try to use a different type of culture medium
Low tissue regeneration following cell transplantation
Increase the number of cells administered
Problems with the murine model
Use Tert-/- mice treated with low doses of Bleomycin
To display the extent of differentiation more precisely
Try to make a differentiation-dependent reporter construct

MATHERIALS AND COSTS

Construction of mouse model	24.233,96 €	www.cyagen.com
3T3 Feeder Cells	300,50 €	www.kerafast.com
Antibodies (E-cadherin, Krt5, p63, Pdpn, CC10, SPC, Aqp5)	2321,45 €	www.biorbyt.com
Matrigel	225,72 €	www.corning.com
Differentiation medium (CnT-PR-AD)	160,00 €	www.cellntec.com
Plasmid pCMV-SPORT-βgal	247,00 €	www.termofisher.com
B-Gal staining kit	554,00 €	www.termofisher.com
Ovation RNA Amplification System V2	1345,65 €	www.nugen.com
GeneChip Mouse Exon 1.0 ST Array	612,46 €	www.affymetrix.com

+ Additional costs from basic lab manteinance and materials

Timescale of the project: 3 years

Total cost: 40.000 €

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