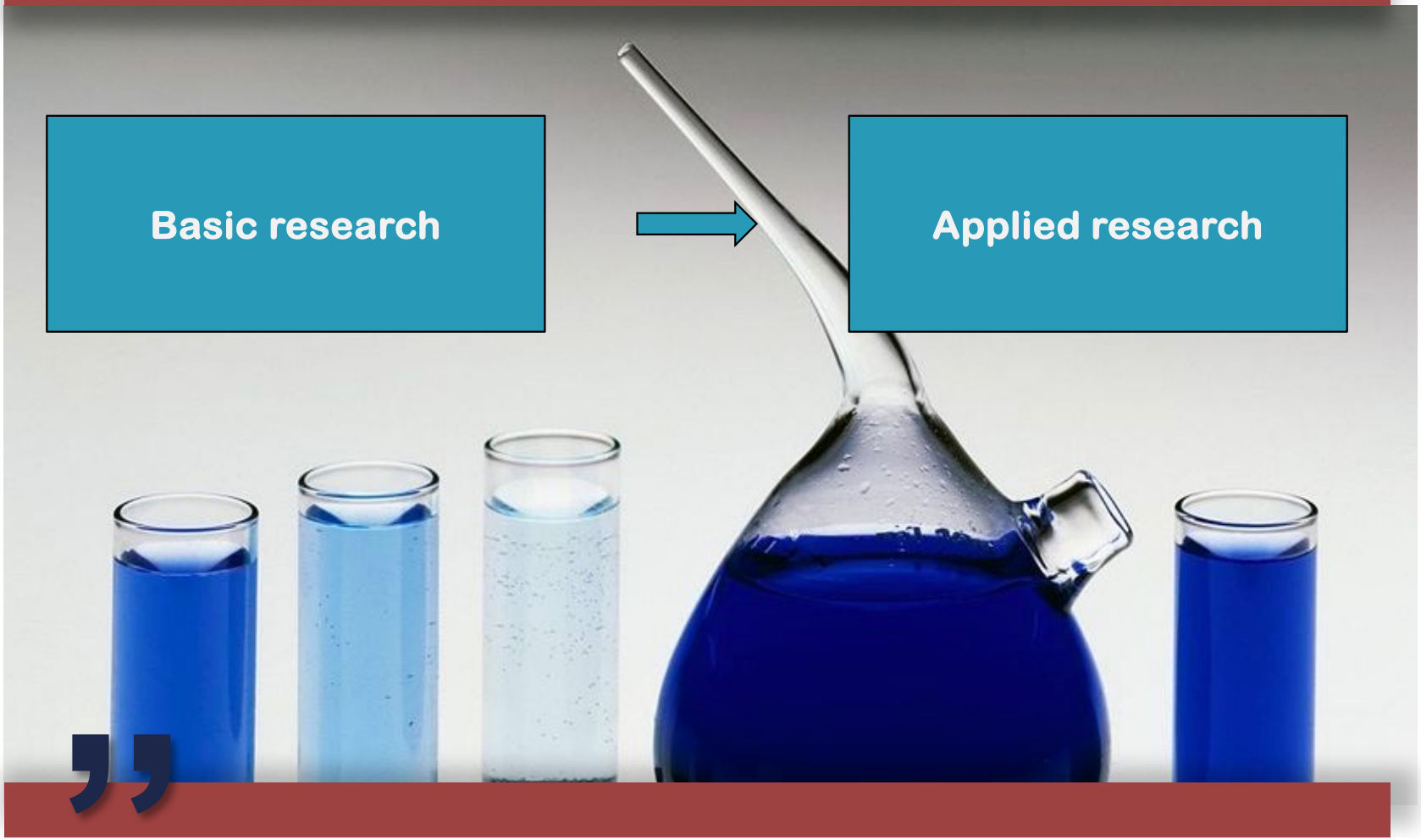
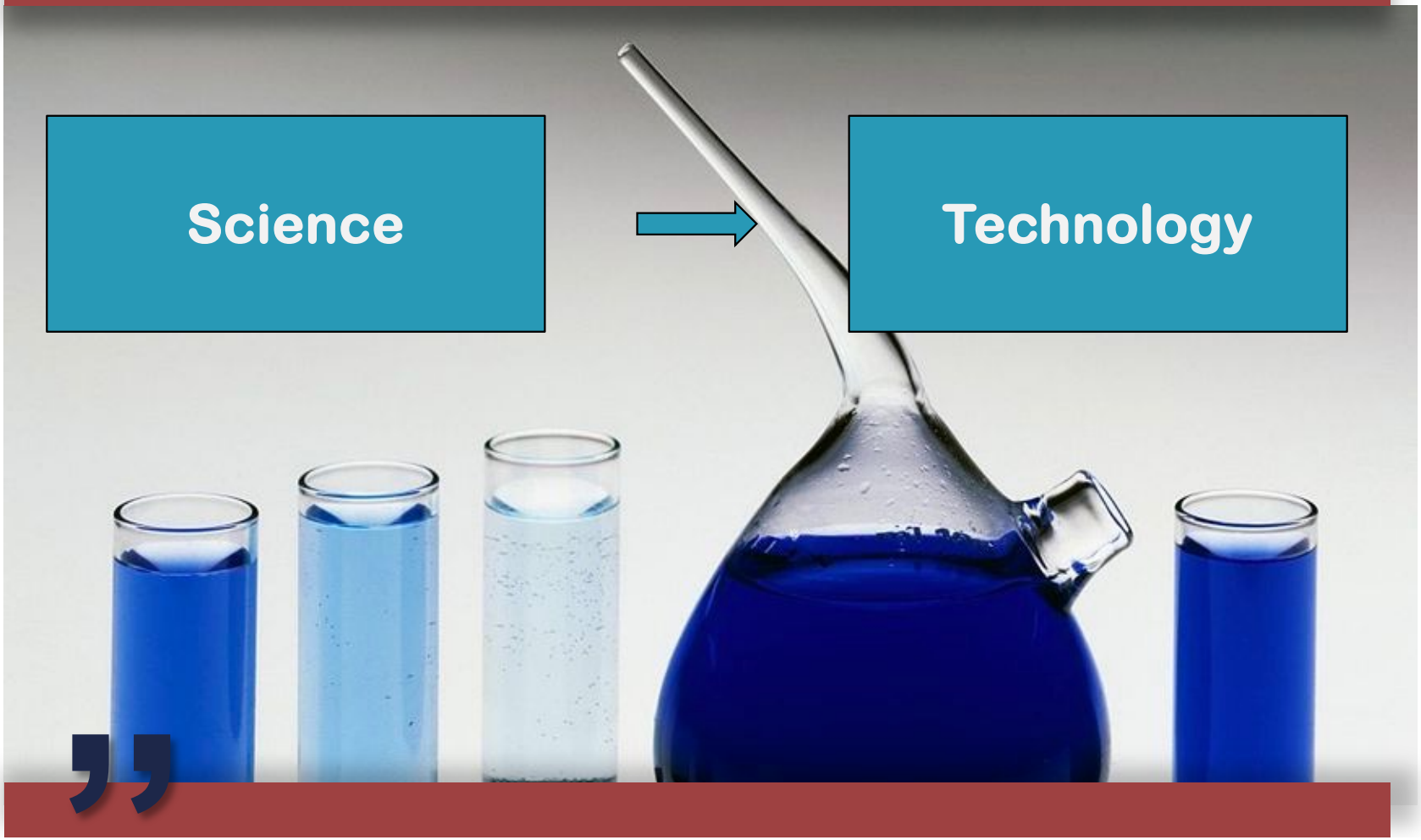
CELLULAR THERAPY IN PRIMARY CILIARY DYSKINESIA – TOWARDS A PROOF OF CONCEPT FOR CHRONIC RESPIRATORY DISEASES

Pr. John DE VOS, University Hospital of Montpellier

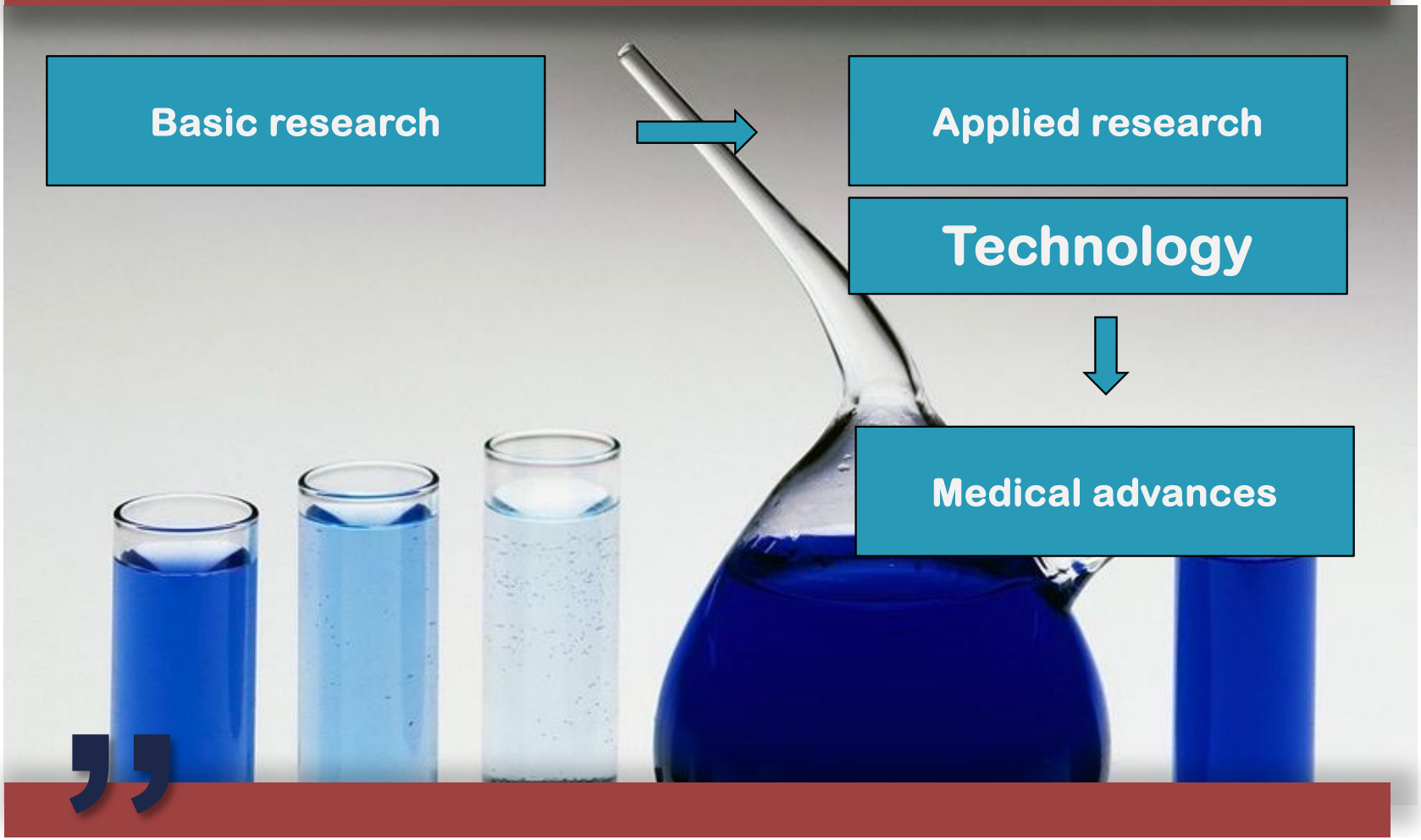
Basic and applied research



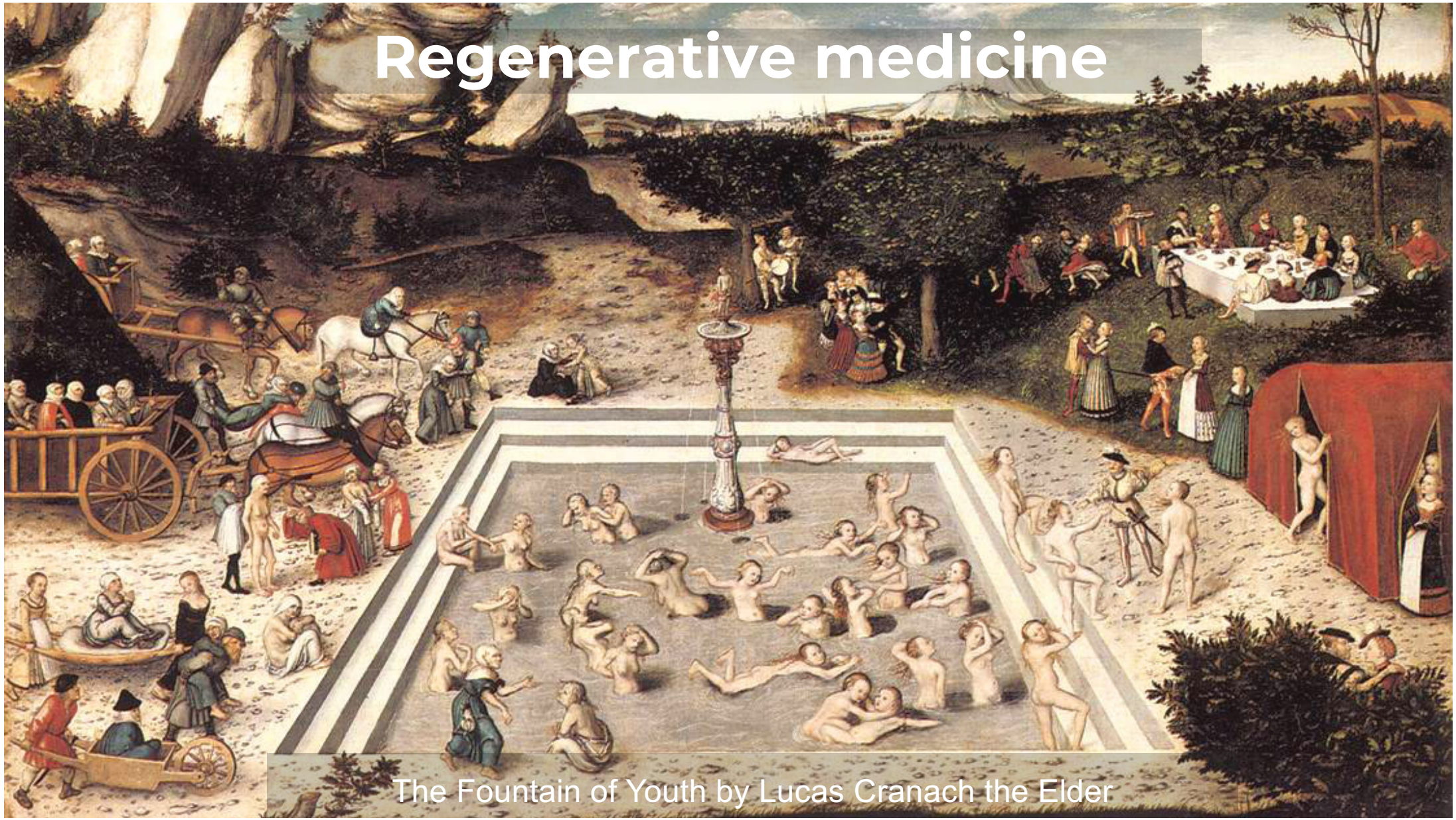
Science et technologie



Avancées médicales



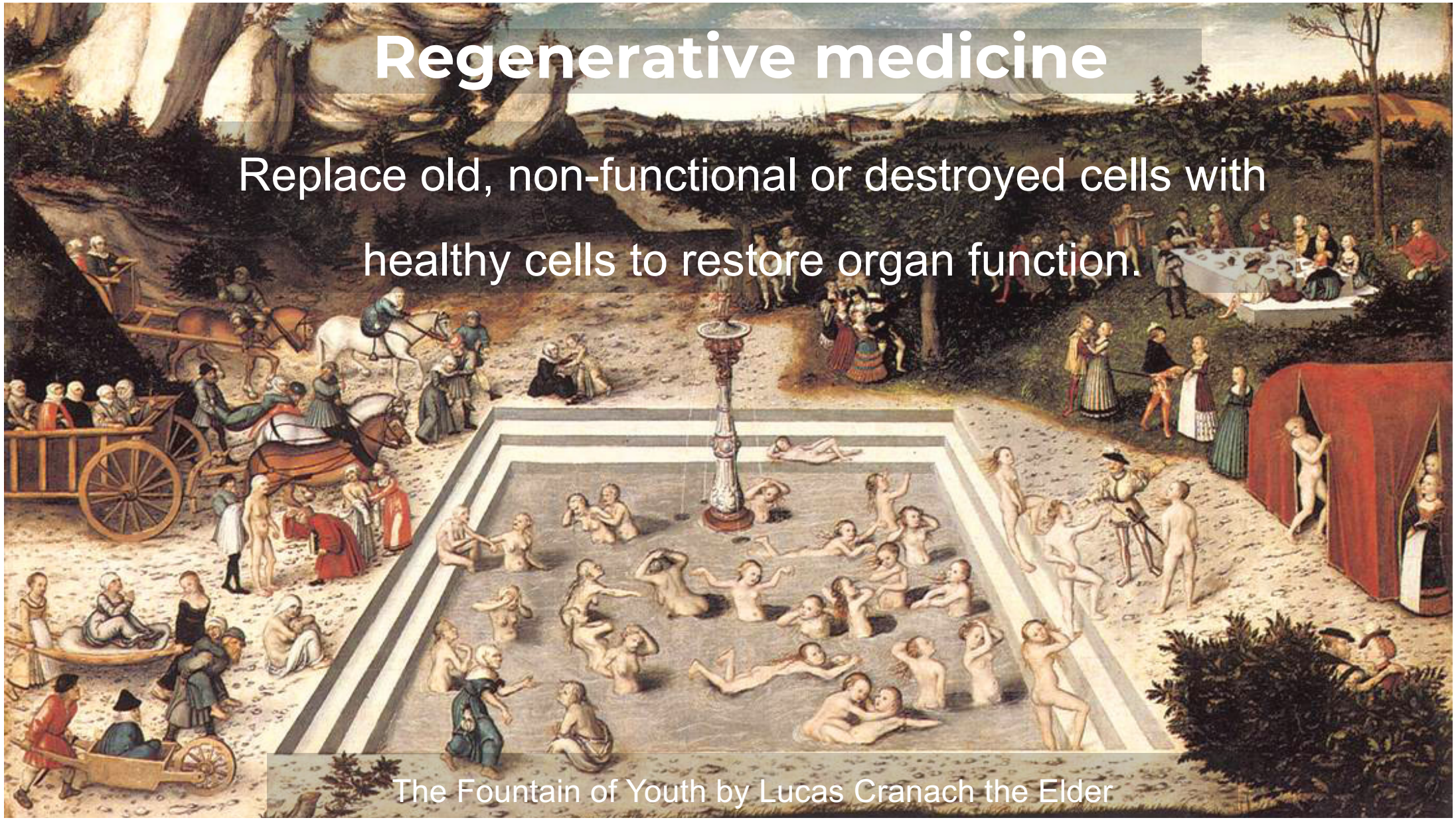
Regenerative medicine



The Fountain of Youth by Lucas Cranach the Elder

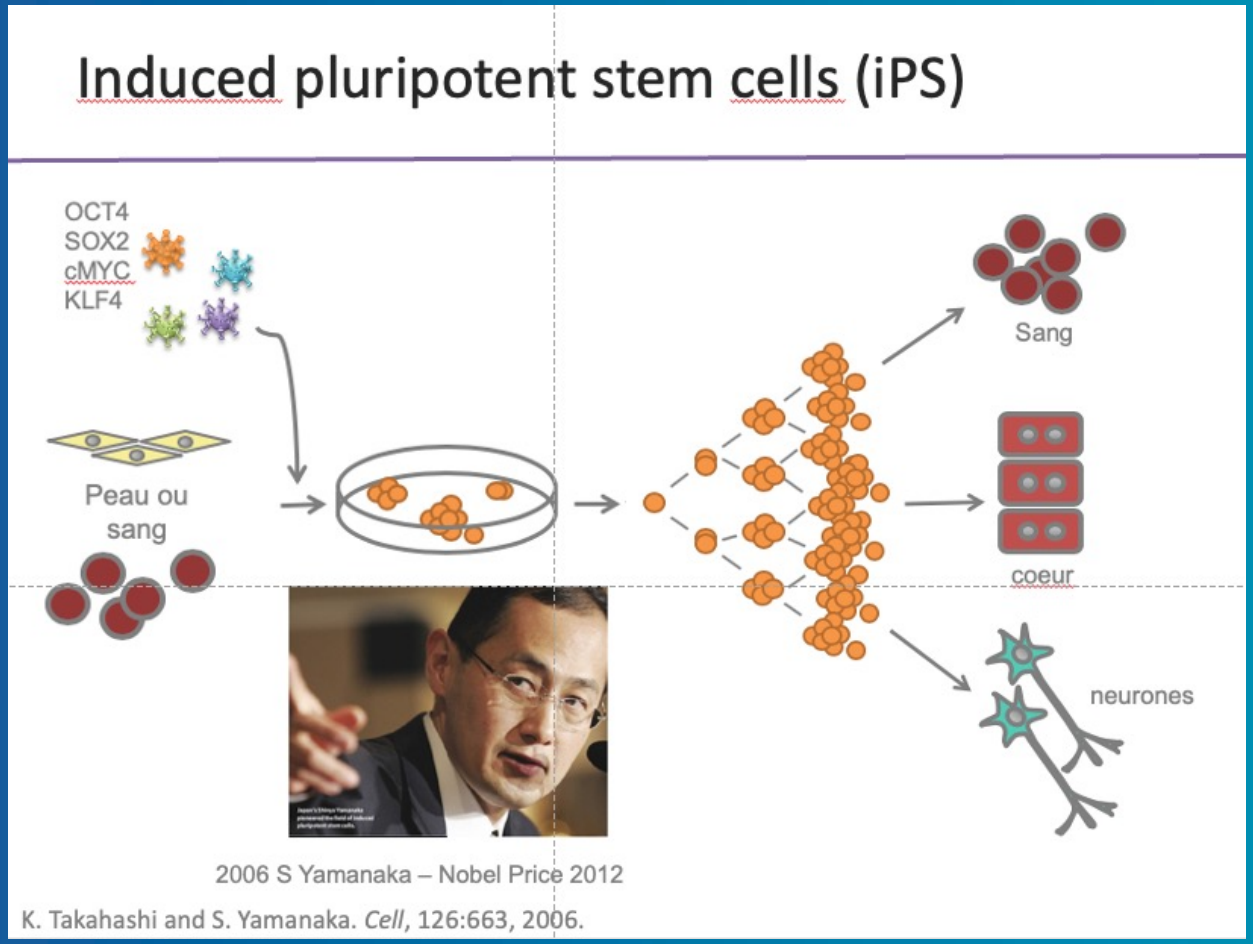
Regenerative medicine

Replace old, non-functional or destroyed cells with healthy cells to restore organ function.



The Fountain of Youth by Lucas Cranach the Elder

Technology: iPS cells



Cell-based regenerative medicine

What cell source?

Injection route?

The lung

What cell source?

- Hematology:
  - 1957: “Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy”

INTRAVENOUS INFUSION OF BONE MARROW IN PATIENTS RECEIVING RADIATION AND CHEMOTHERAPY*

E. Donnall Thomas, M.D.,† Harry L. Lochte, Jr., M.D.,‡ Wan Ching Lu, Ph.D.,§ and Joseph W. Ferrebee, M.D.¶

Cooperstown, New York, and Boston, Massachusetts

After a lethal dose of radiation in rodents,[1] canines[2] or primates,[3] the destroyed bone marrow may be repopulated by intravenous infusion of cellular suspensions of marrow taken from healthy isologous, homologous[4] and, in some cases, heterologous[5] donors. Effective cells for these infusions may be stored by the Polge technic of freezing to −80°C. in glycerol.[6] Hosts seeded with donor marrow have some of the immunologic characteristics of the donors, and in some circumstances will take and hold homografts of other organs from them.[7]

Since cases of radiation disaster may occur, and since bone-marrow deficiency from radiation or chemotherapy does occur in the normal course of clinical medicine, an effort has been made to determine the availability and usefulness of bone-marrow infusions for the treatment of these conditions in man.

Experimental Considerations

Bone marrow was collected from fetal and adult cadavers, from ribs removed at surgery and from aspiration biopsy of the ilium. Irrespective of source, it was passed repeatedly through a stainless-steel screen[8] and broken into a smooth cellular suspension, and the fat, as a rule, removed by centrifugation. The cells, resuspended in tissue-culture fluid and serum, were administered intravenously or frozen in glycerol and stored at −80°C.

One may assess permissible periods of post-mortem

*From the Mary Imogene Bassett Hospital (affiliated with Columbia University), Cooperstown, and the Children's Cancer Research Foundation, Children's Medical Center, Boston, and Harvard Medical School.
Supported by research grants (C-2643 and H-607) from the United States Public Health Service and by contract AT (30-1)-2005 from the United States Atomic Energy Commission.
†Associate clinical professor of medicine, Columbia University College of Physicians and Surgeons; physician-in-chief, Mary Imogene Bassett Hospital.

‡Public Health Service Research Fellow, National Heart Institute, National Institutes of Health, Bethesda, Maryland.

§Research assistant, Department of Pathology, Harvard Medical School; research assistant, Division of Laboratories and Research, Children's Medical Center.

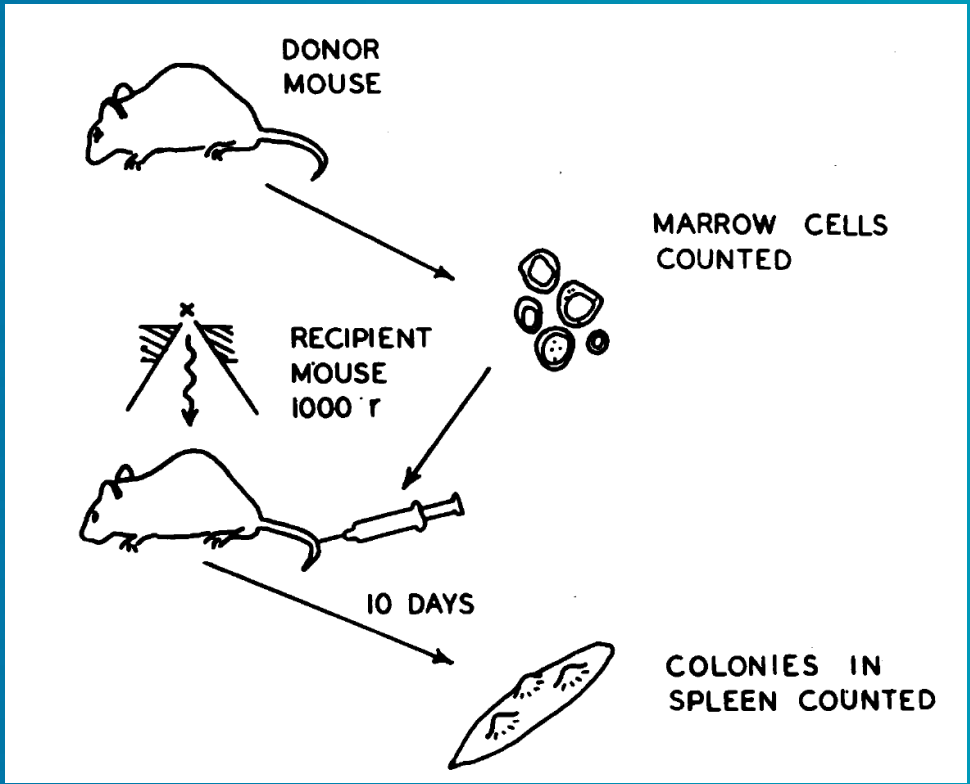
¶Associate clinical professor of medicine, Columbia University College of Physicians and Surgeons; research physician, Mary Imogene Bassett Hospital.

Thomas, E. D. et al. N Engl J Med 1957 257, 491-496.

E Donnall Thomas 1990

What cell source?

- Hematology:
  - 1957: “Intravenous infusion of bone marrow in patients receiving radiation and chemotherapy”
  - 1964: “A stochastic model of stem cell proliferation, based on the growth of spleen colony-forming cells”



Thomas, E. D. et al. N Engl J Med 1957 257, 491-496.
Till, J. E., McCulloch, E. A. et al. , 1964 PNAS 51, 29-36.

What cell source?

- Other organs
  - Skin: “Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells.”

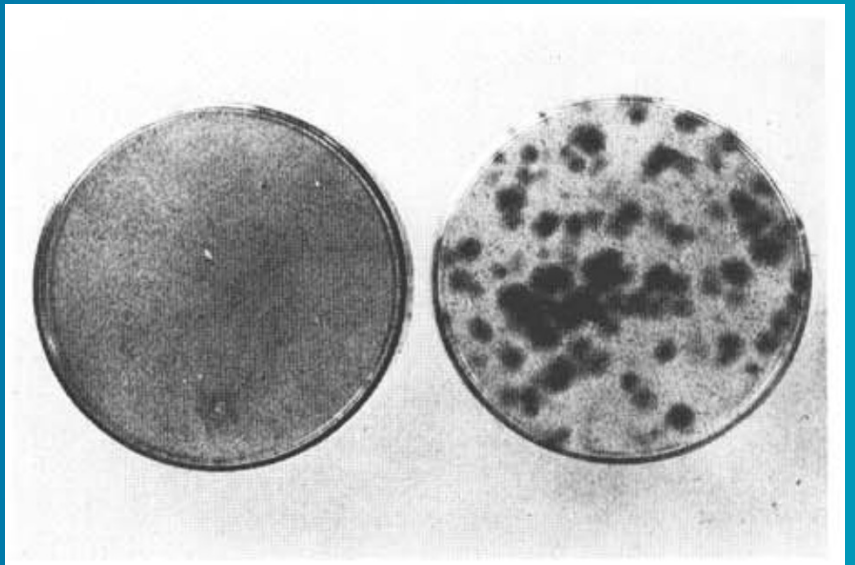


Figure 2. Inhibition of Colony Formation by Human Fibroblasts in the Presence of $\frac{n}{3}$ 3T3 Cells

Cultures were inoculated with 100 strain A human foreskin fibroblasts, together with either $\frac{n}{3}$ (left) or $\frac{n}{30}$ (right) lethally irradiated 3T3 cells. After 11 days, the cultures were fixed and stained with hematoxylin. Suppression of colony formation by the $\frac{n}{3}$ layer is evident.

Rheinwald, J. G., and Green, H. (1975). Cell 6, 331-343.

What cell source?

- Other organs
  - Skin: “Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells.”
  - 1997: Carticel®, Genzyme

Rheinwald, J. G., and Green, H. (1975). Cell 6, 331-343.

What cell source?

- But for most organs, cell therapy applications were blocked by scarce cell sources

Hematology is the exception rather than the rule!

What cell source?

- Pluripotent stem cells: give rise to any cell type

Mario R. Capecchi
Sir Martin J. Evans
Oliver Smithies
2007

- 1998 : Embryonic stem cells (but allogeneic)
- Therapeutical cloning
- 2006: Induced pluripotent stem cells (iPSC): give rise to any cell type

Shinya Yamanaka
John B Gurdon
2012

Cell-based regenerative medicine

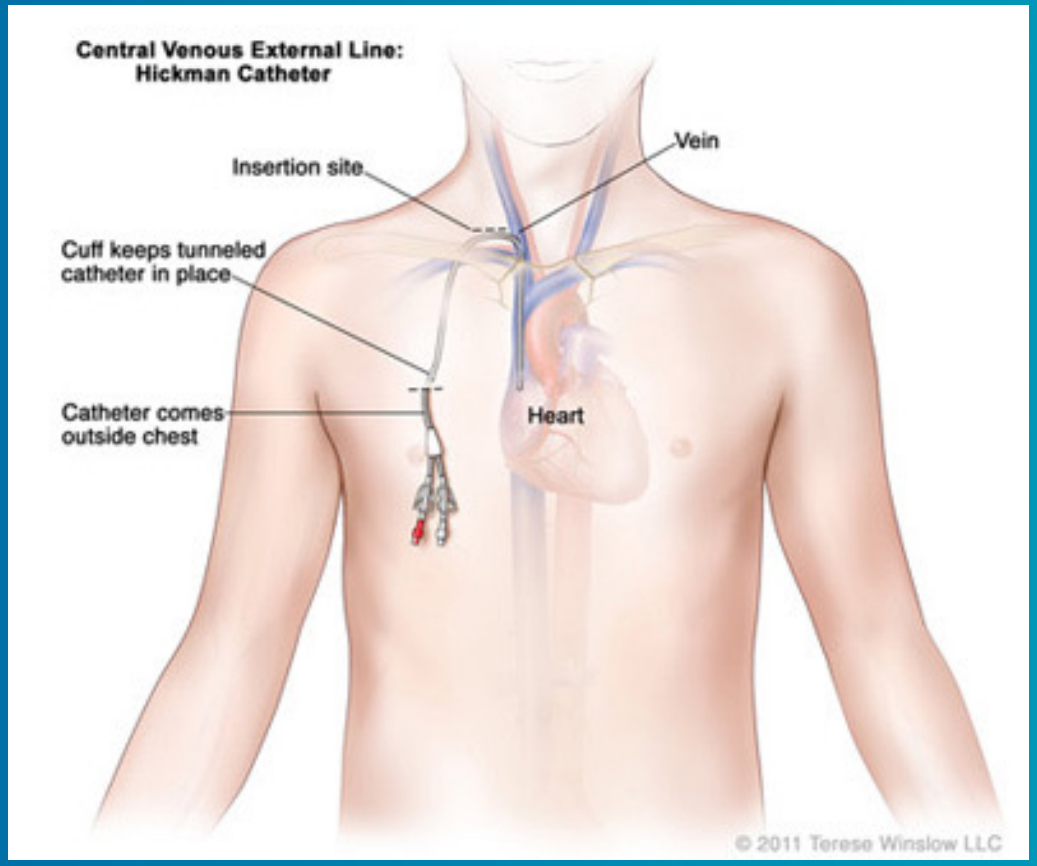
What cell source?

Injection route?

The lung

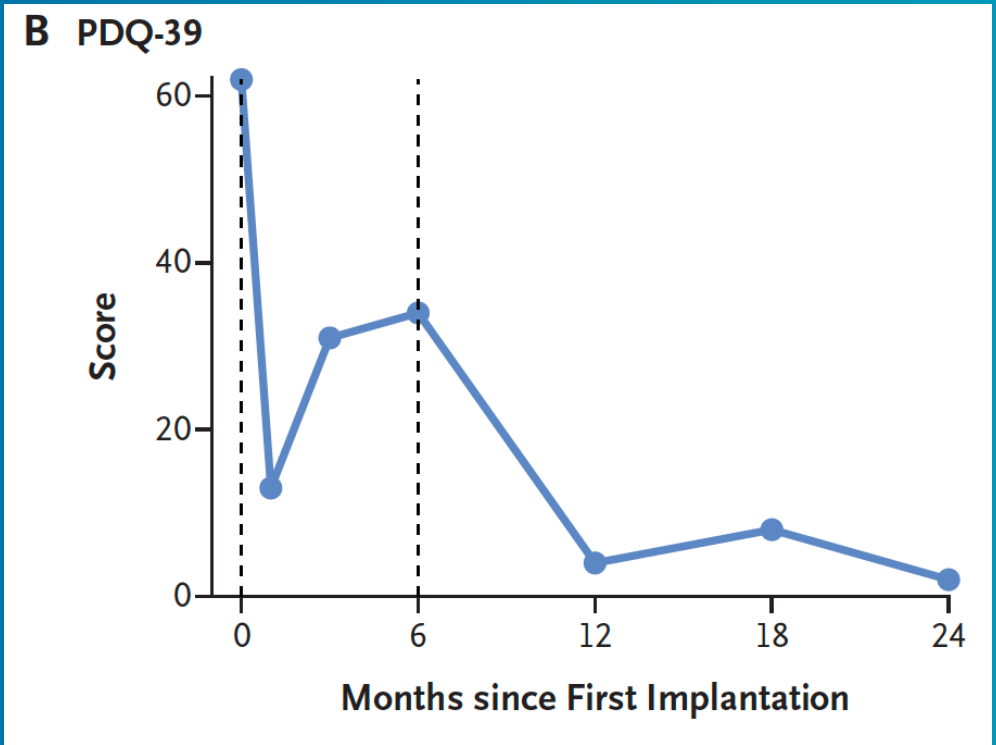
Injection route?

- Hematology:
  - IV!



Injection route?

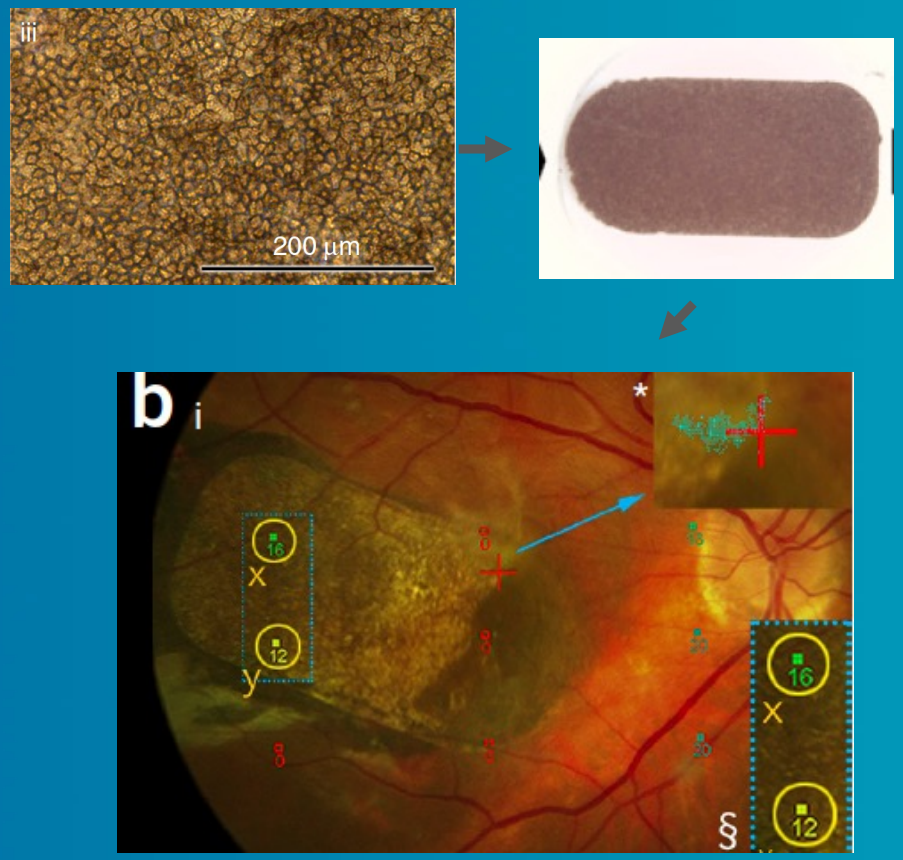
- Other organs
  - Parkinson's disease: iPSC-derived substantia nigra pars compacta neurons suspension injected into the putamen



Schweitzer, J. S. et al. Kim, K. S. (2020). Personalized iPSC-Derived Dopamine Progenitor Cells for Parkinson's Disease. N Engl J Med 382, 1926-1932.

Injection route?

- Other organs
  - Retina: ES-derived pigmented epithelium as a tissue sheet surgically implanted in situ



da Cruz, L. et al. Coffey, P. J. (2018). Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. Nat Biotechnol 36, 328-337.

Injection route?

- But for organs, the ideal injection route will be organ-specific, and for most organs, it still has to be defined (eg: heart? Lung? Etc.)

Hematology is the exception rather than the rule!

Cell-based regenerative medicine

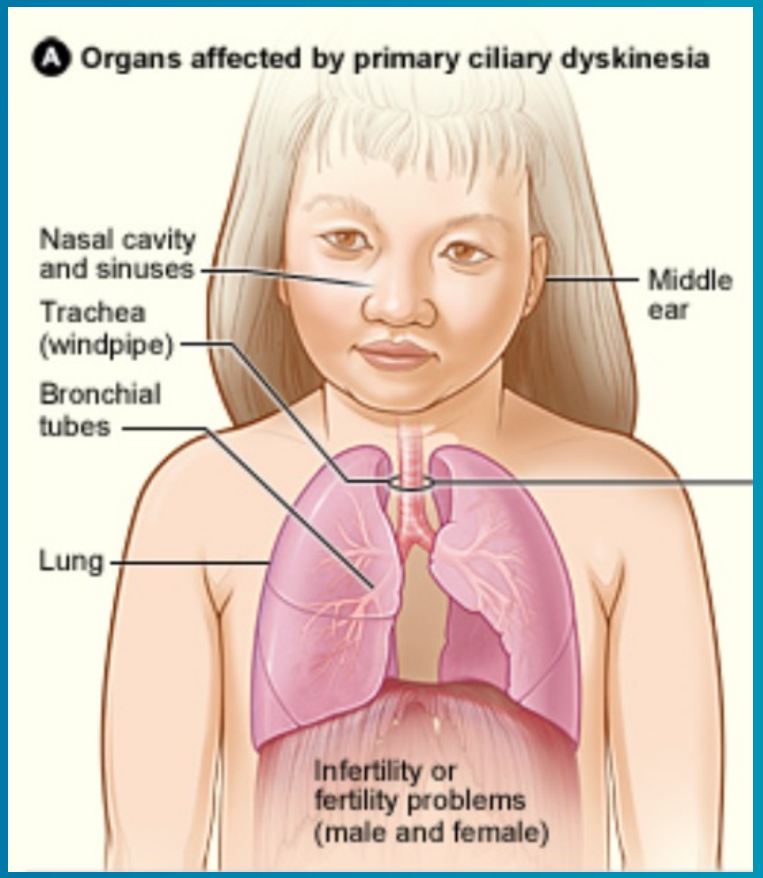
What cell source?

Injection route?

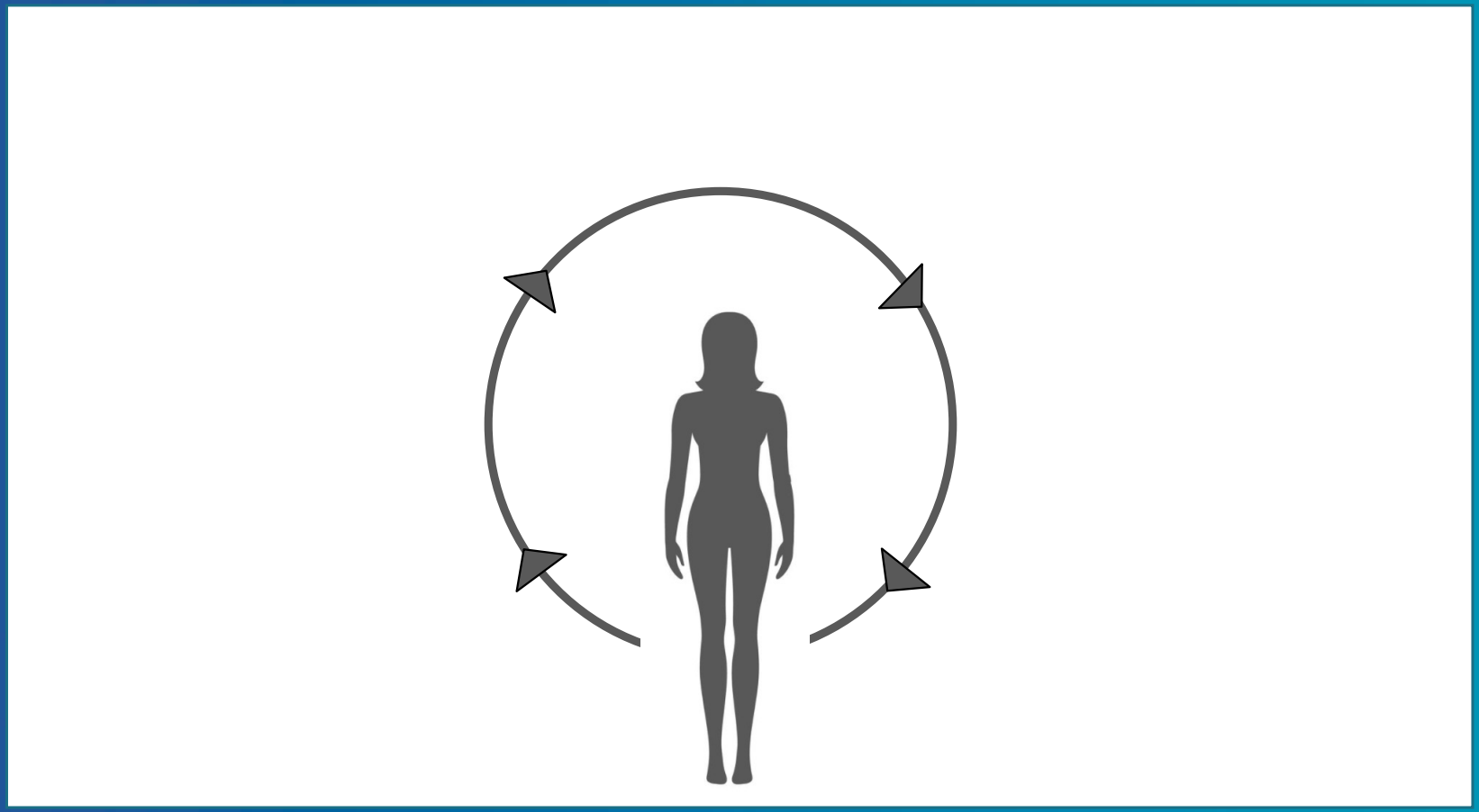
The lung

PRIMARY CILIARY DYSKINESIA

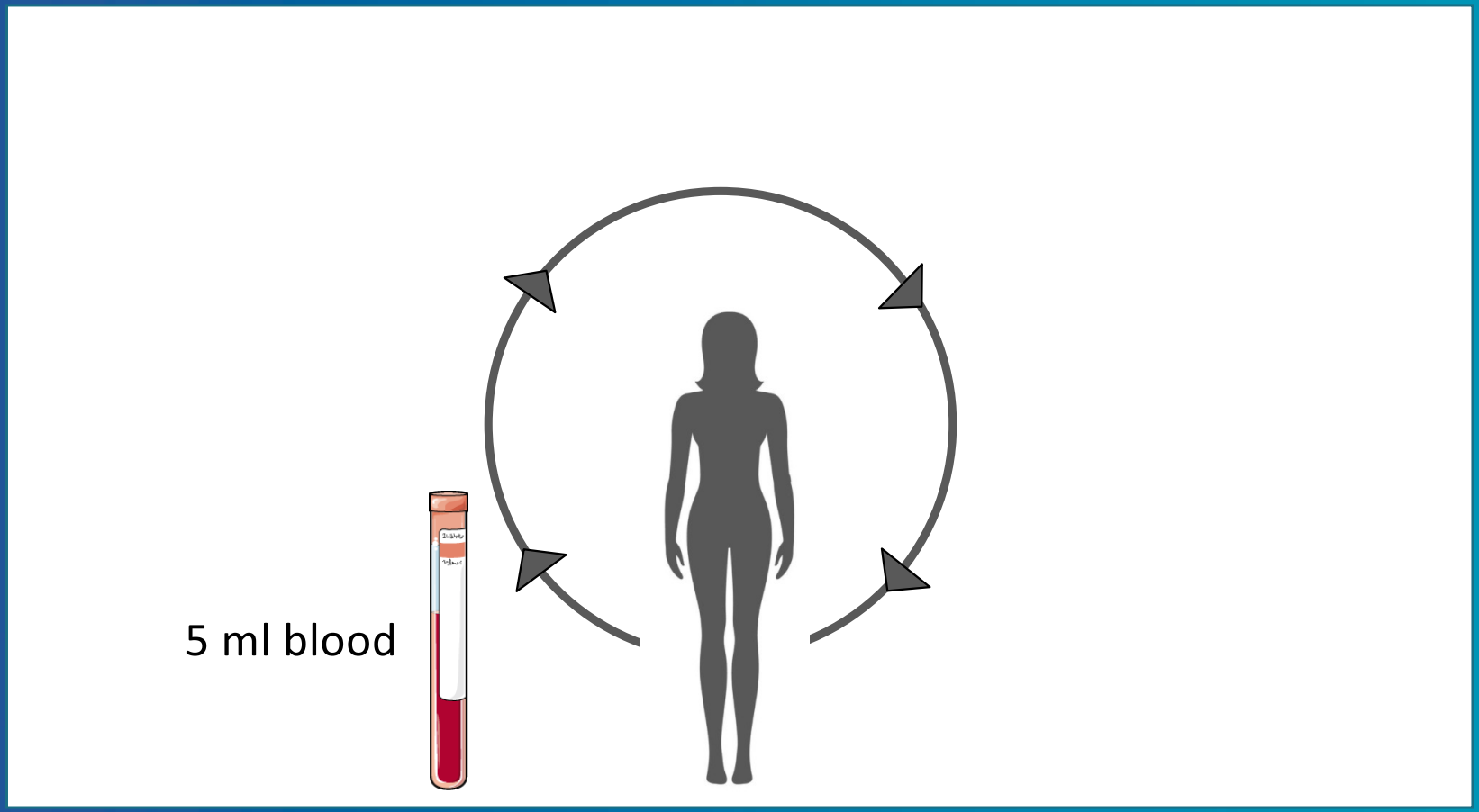
- Rare genetic condition (1/10,000)
- Ciliary dysfunction
- Heterogeneous: > 40 genes
- → mucus stasis, chronic respiratory infections, destruction of bronchi, death



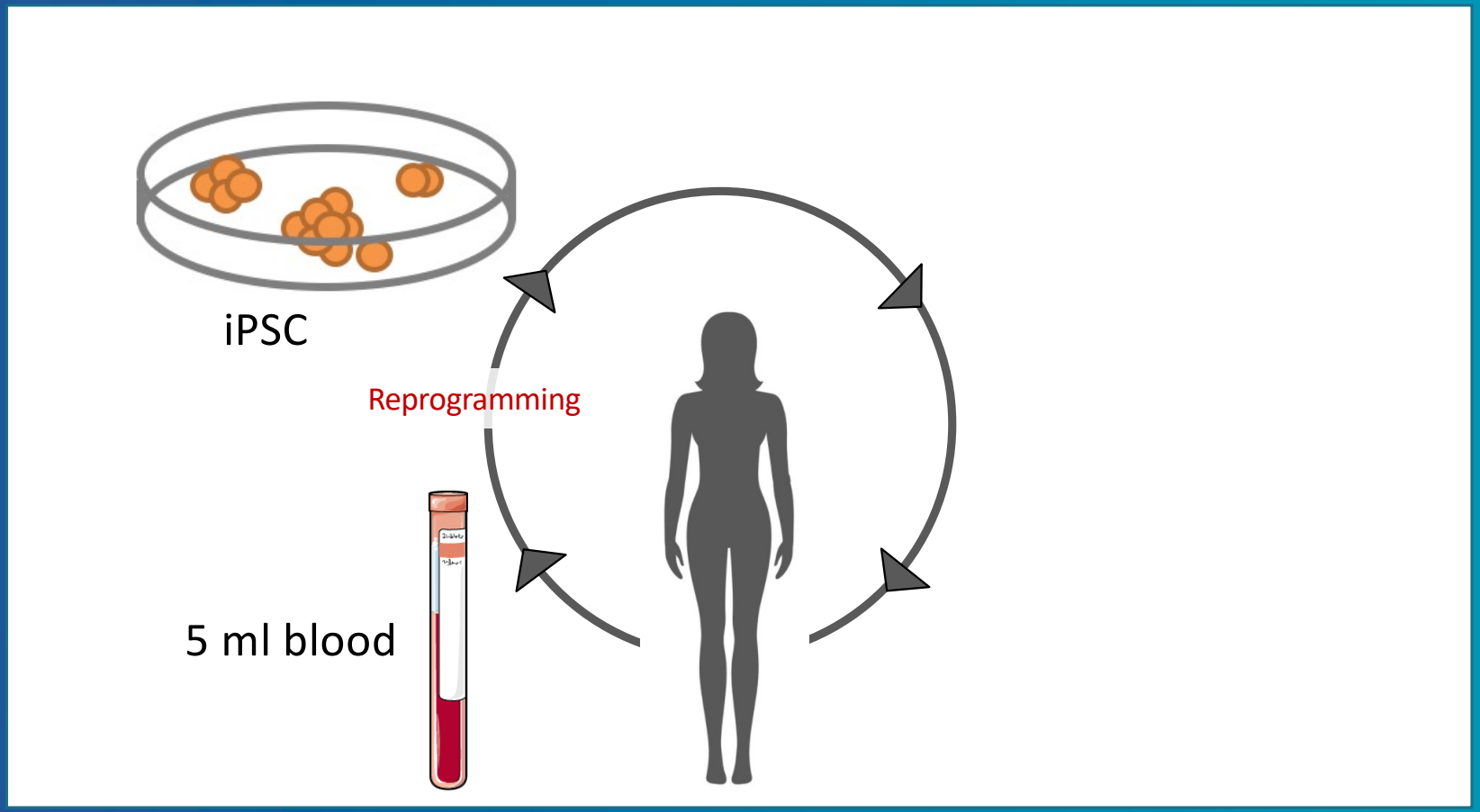
Gene and cell therapy for PCD



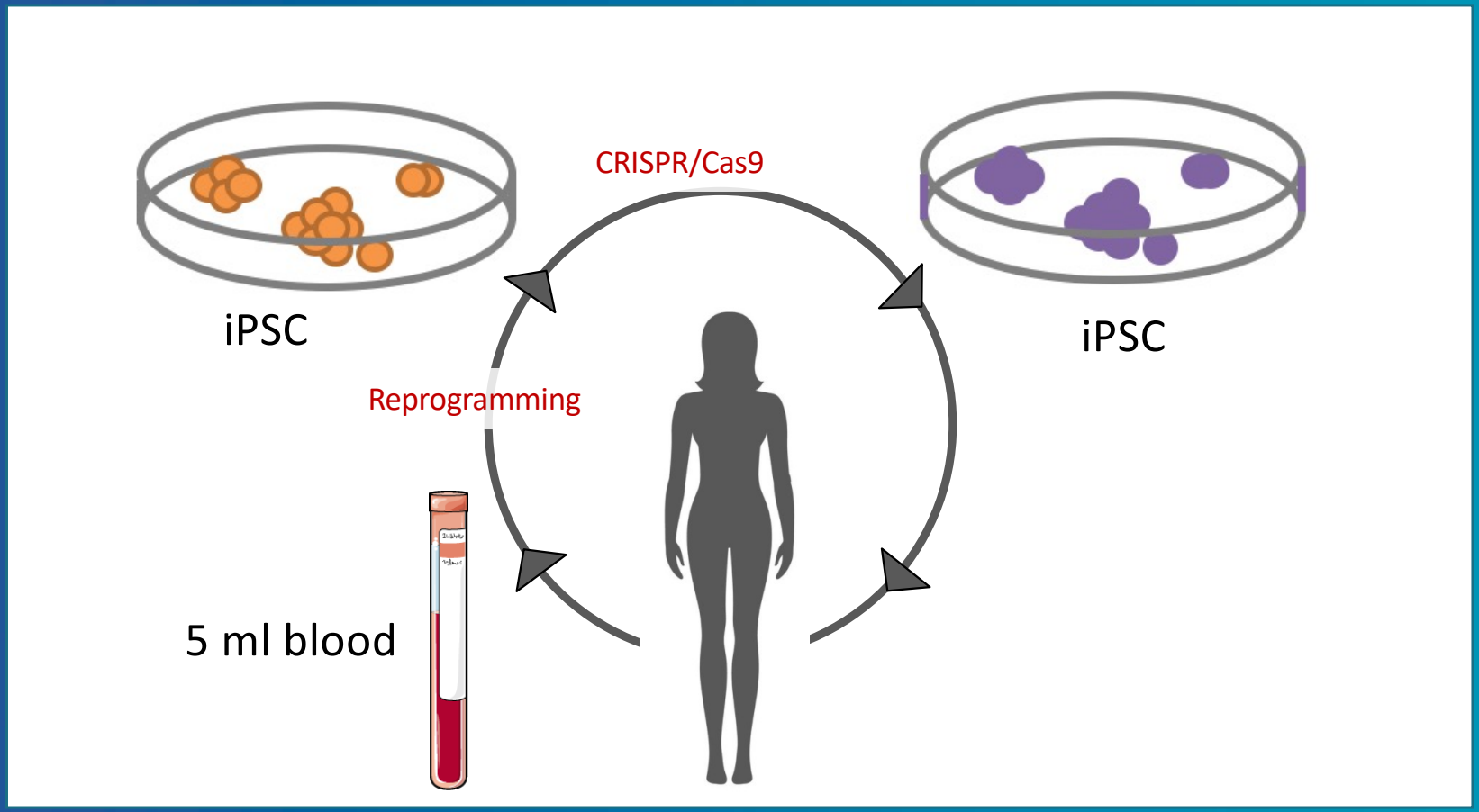
Gene and cell therapy for PCD



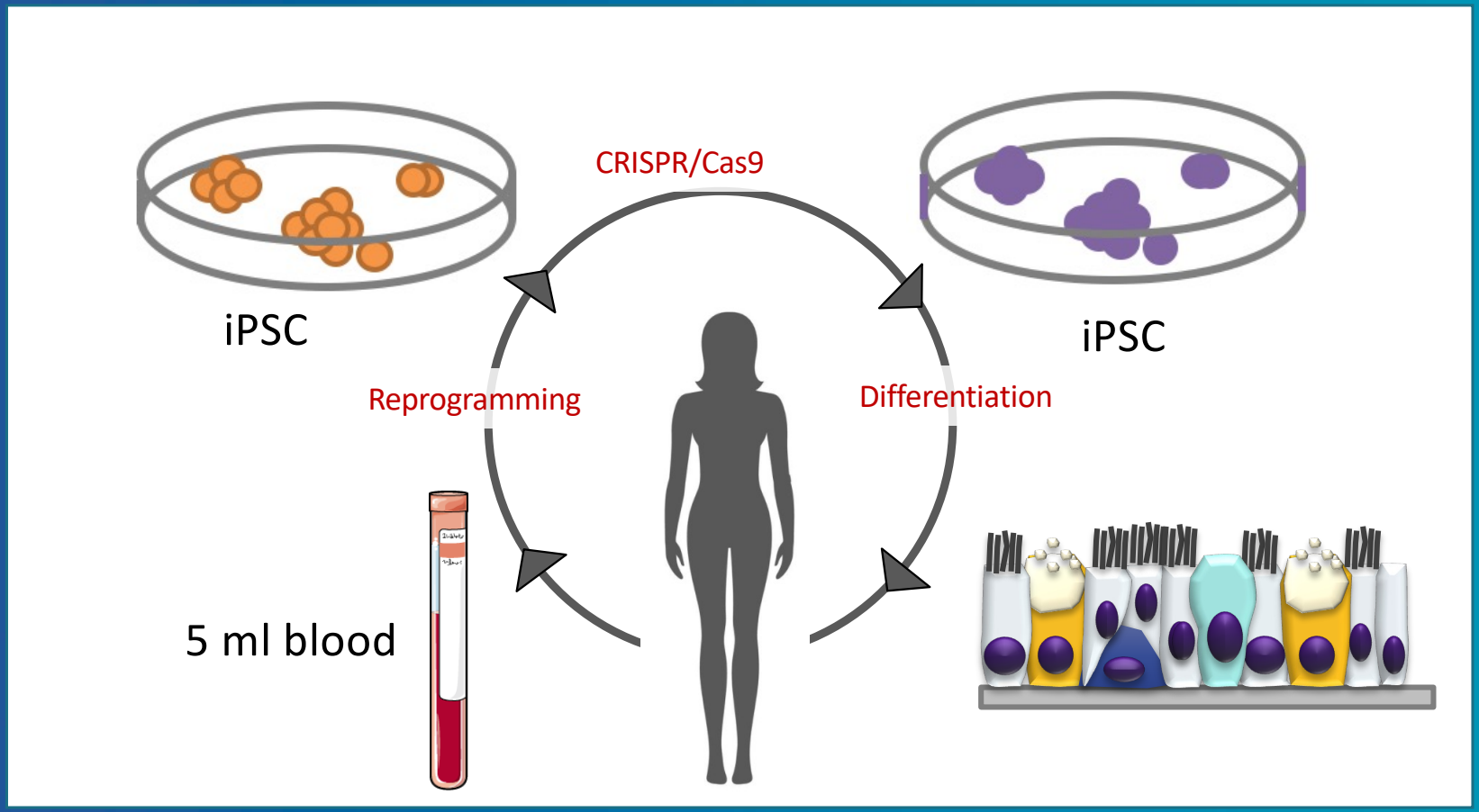
Gene and cell therapy for PCD



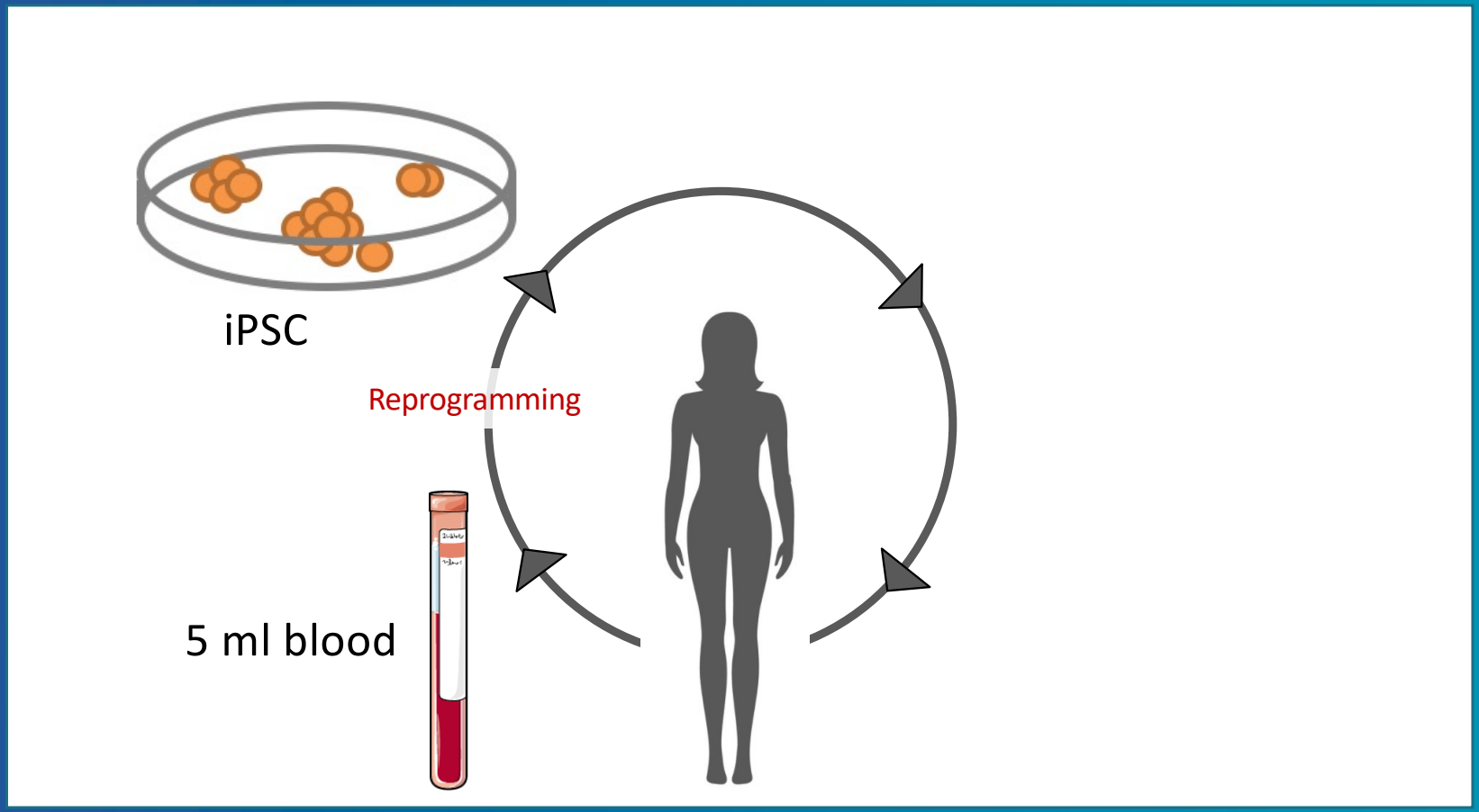
Gene and cell therapy for PCD



Gene and cell therapy for PCD

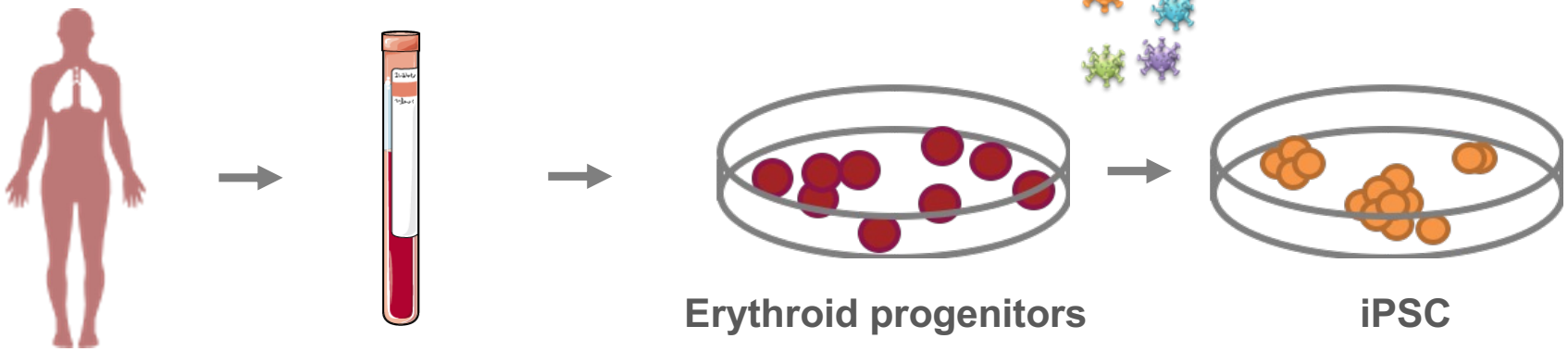


Gene and cell therapy for PCD



TURNING BLOOD INTO LUNG

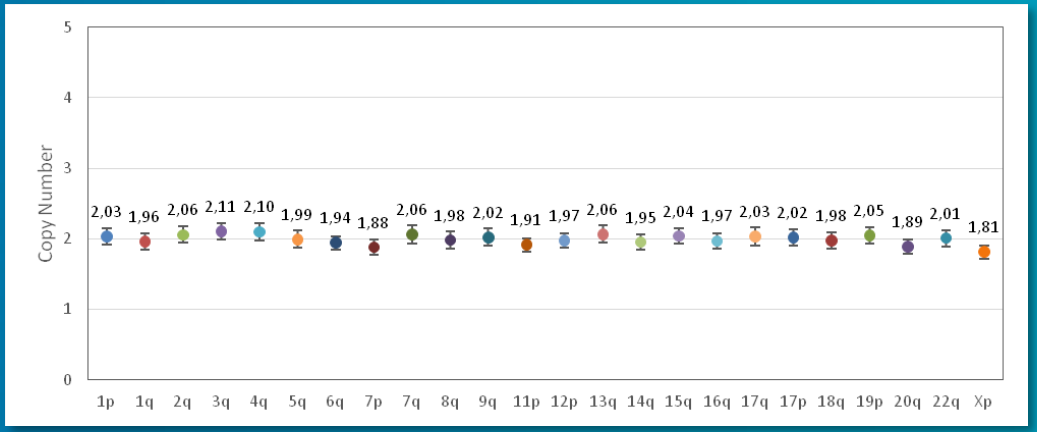
Engi Ahmed



Ahmed, E et al. 2021 Stem Cell Res
Fieldes, M et al. 2020 Stem Cell Res
Ahmed, E et al. 2018 Stem Cell Res

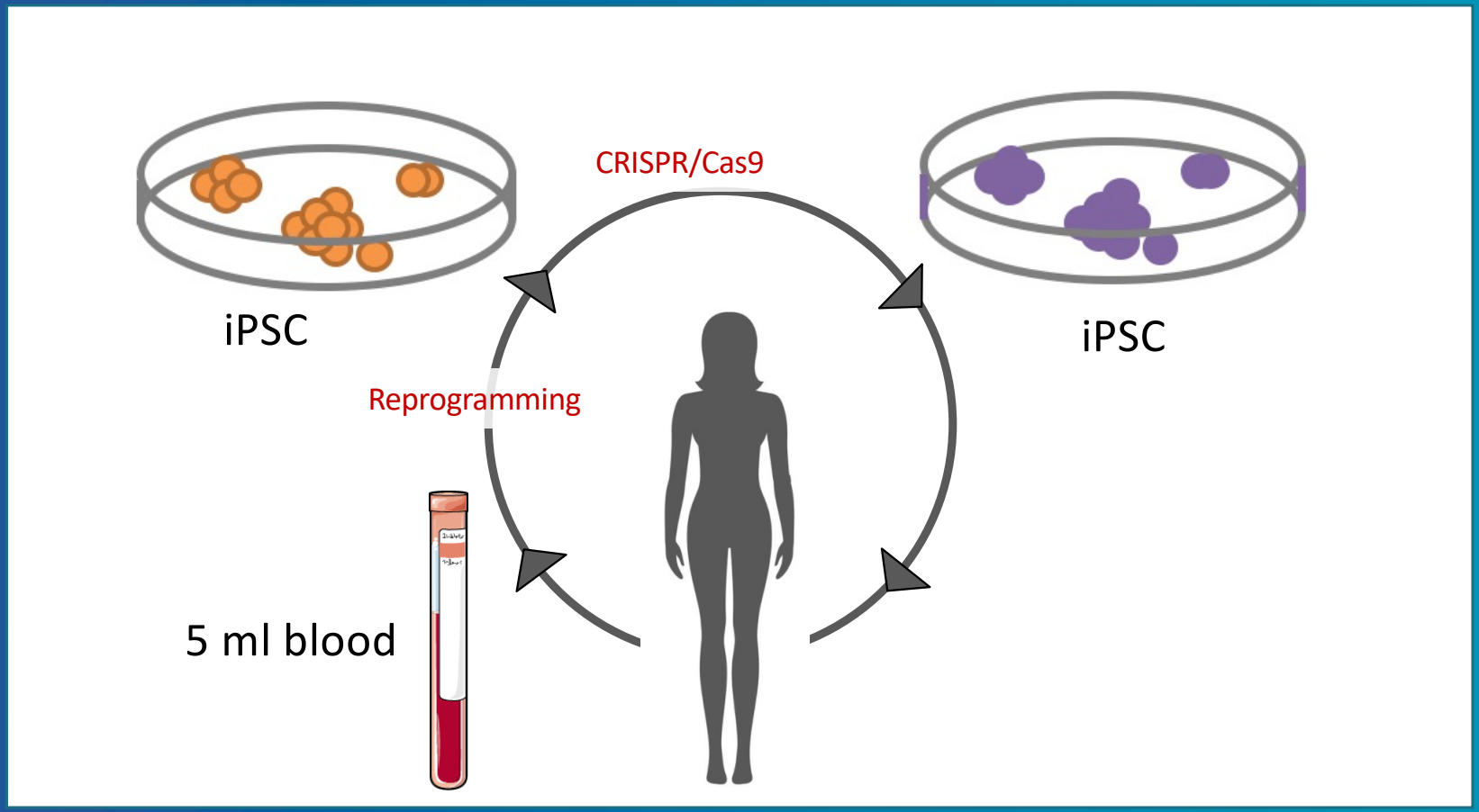
iPSC DERIVATION

- 7 lines of which:
  - 1 healthy donor
  - 4 COPD, 1 heavy smoker without COPD
  - 1 primary ciliary dyskinesia (CCDC40)
- Quality controls including genetic integrity



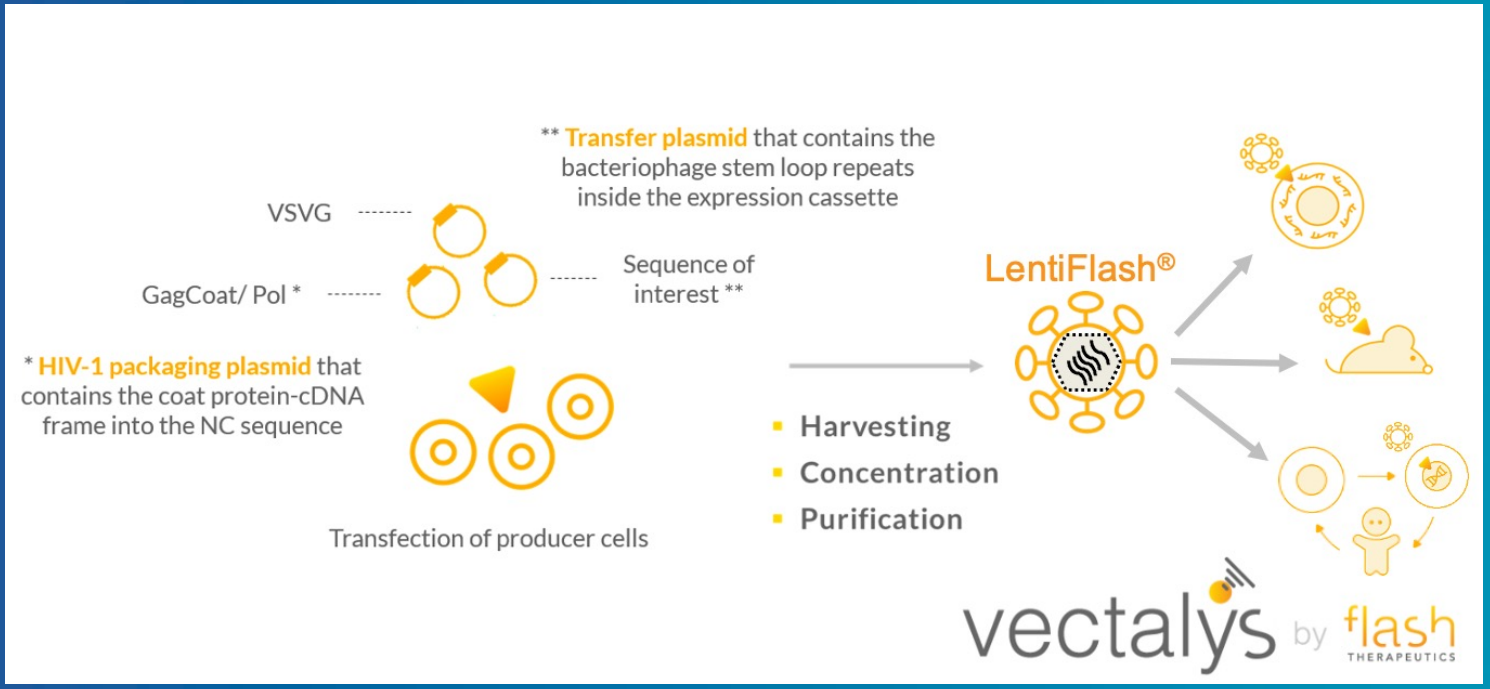
stemgenomics cell integrity

Gene and cell therapy for PCD



Genetic engineering of iPSC

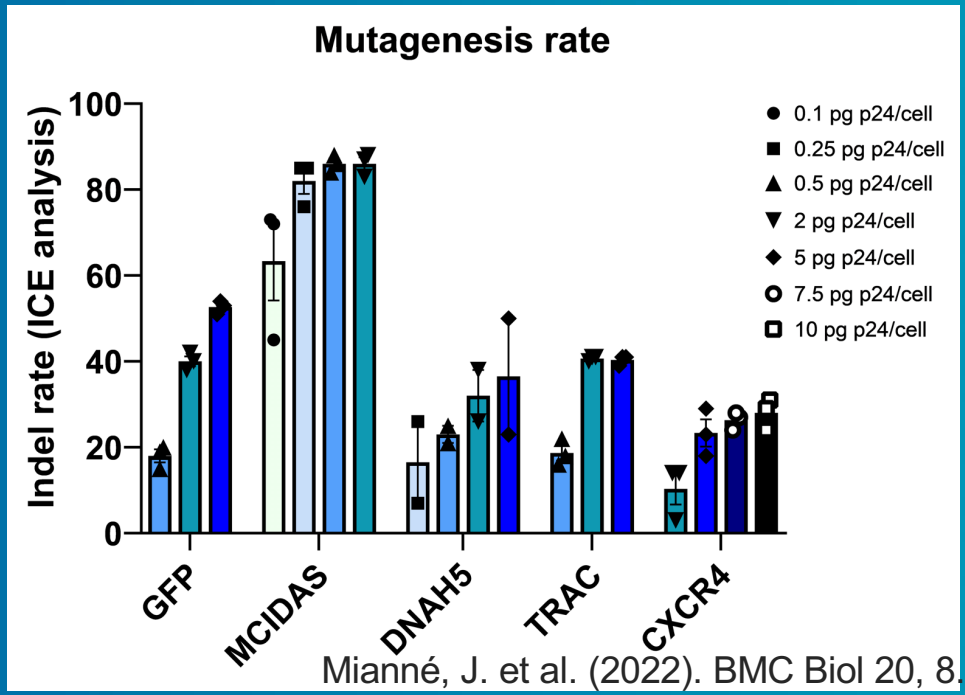
- Use of nonintegrative bacteriophage-chimeric retrovirus-like particles (LentiFlash®) for RNA delivery



Joffrey Mianné

Gene KO

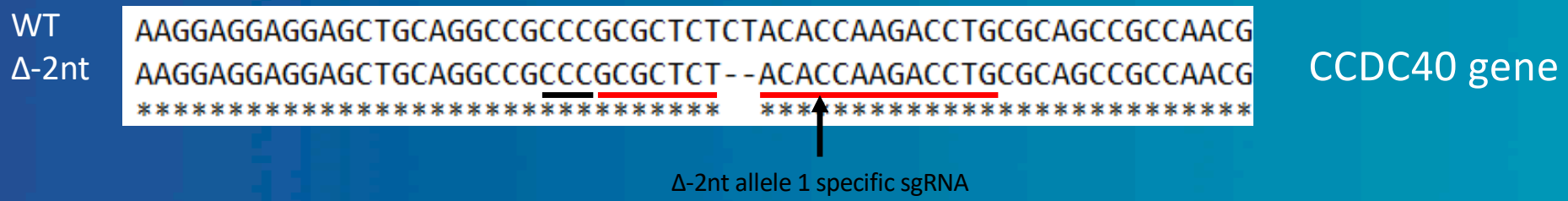
- A healthy iPS cell line (HY03)
- LentiFlash® particles that carry a sgRNA targeting two genes involved in motile ciliary biology (MCIDAS and DNAH5) and two endogenous genes implicated in T-cell biology (TRAC and CXCR4).



Mianné, J. et al. (2022). BMC Biol 20, 8.

- Similar data for two other iPSC lines (PCD_02:30 and iCODP9_B27)

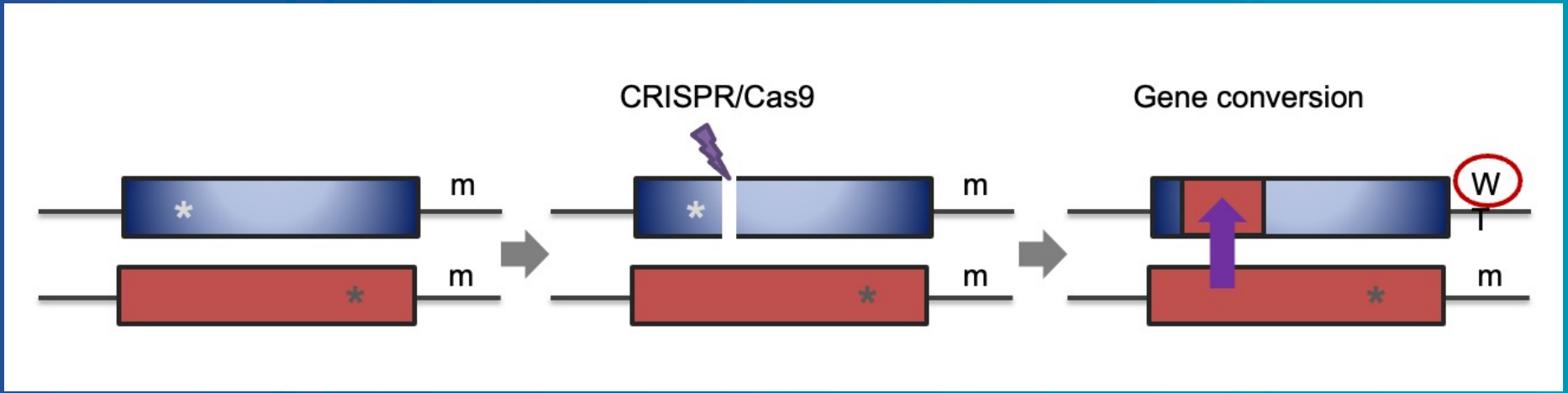
Allele-specific gene editing induces interallelic gene conversion in hiPSC



CCDC40 gene

Mianné, J. et al. (2022). BMC Biol 20, 8.

Interallelic gene conversion in hiPSC



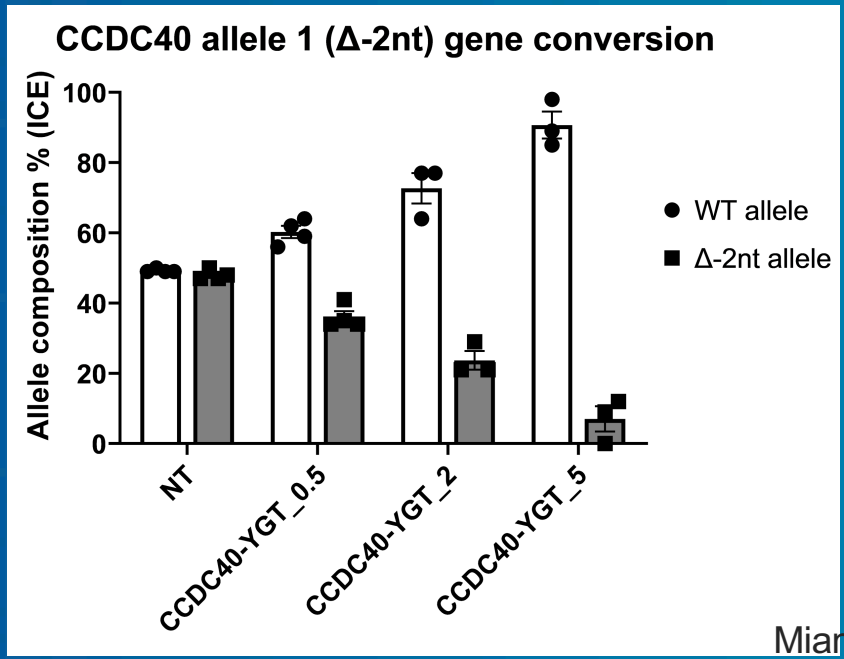
Allele-specific gene editing induces interallelic gene conversion in hiPSC

WT
Δ-2nt

```
AAGGAGGAGGAGCTGCAGGCCGCCCGCGCTCTCTACACCAAGACCTGCGCAGCCGCCAACG
AAGGAGGAGGAGCTGCAGGCCGCCCGCGCTCT--ACACCAAGACCTGCGCAGCCGCCAACG
**********************************  ****************************
```

CCDC40 gene

Δ-2nt allele 1 specific sgRNA



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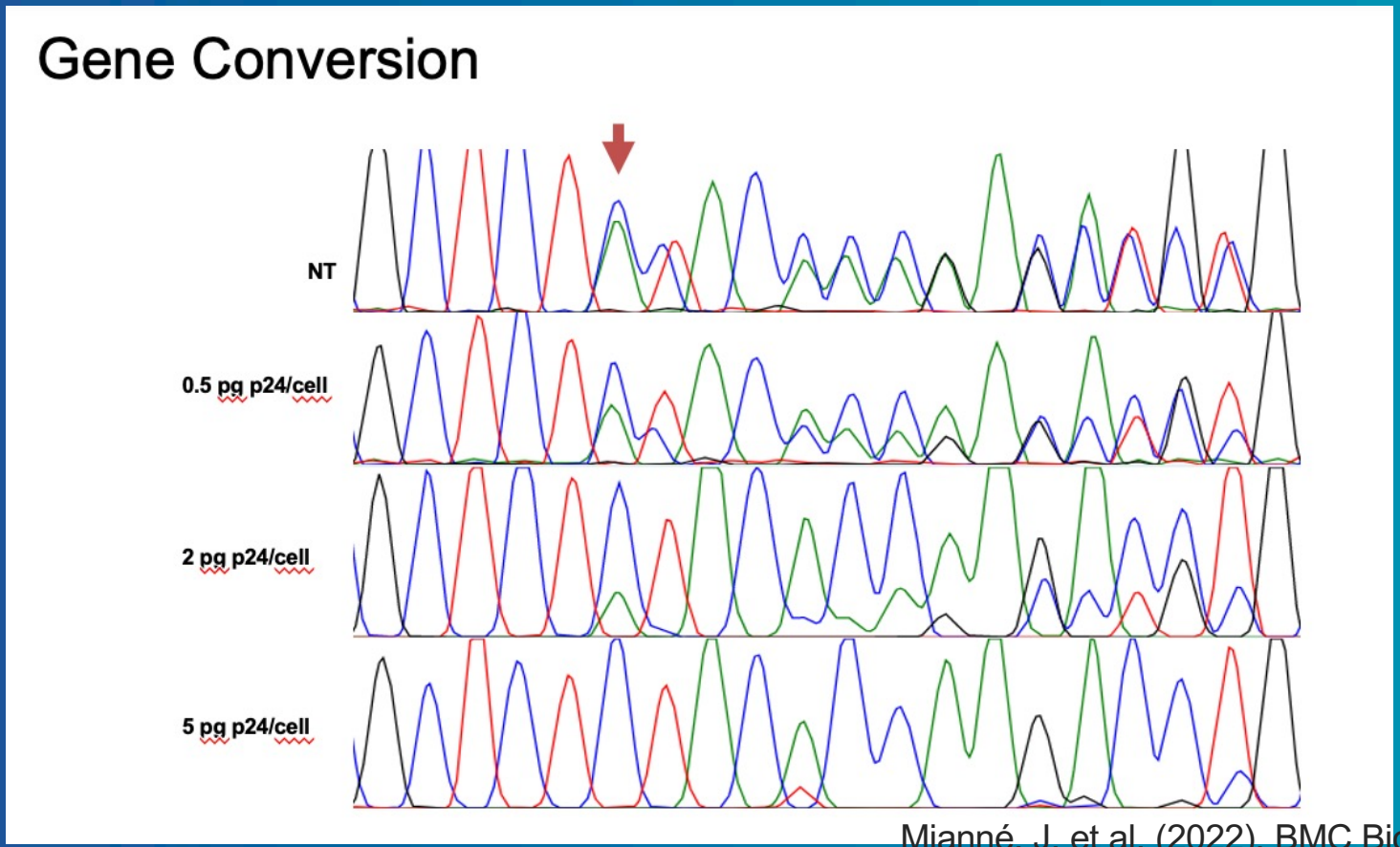
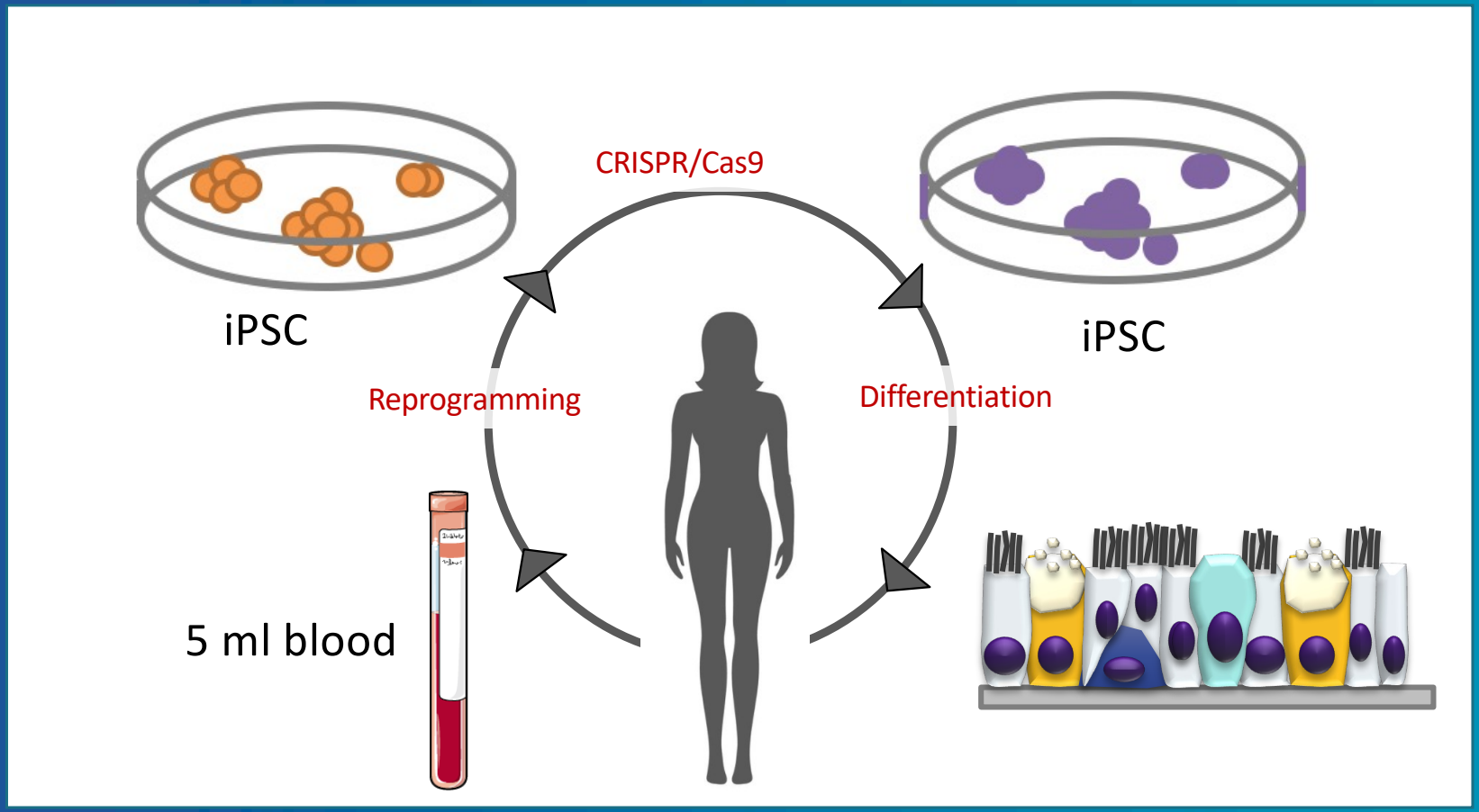
Allele-specific gene editing induces interallelic gene conversion in hiPSC



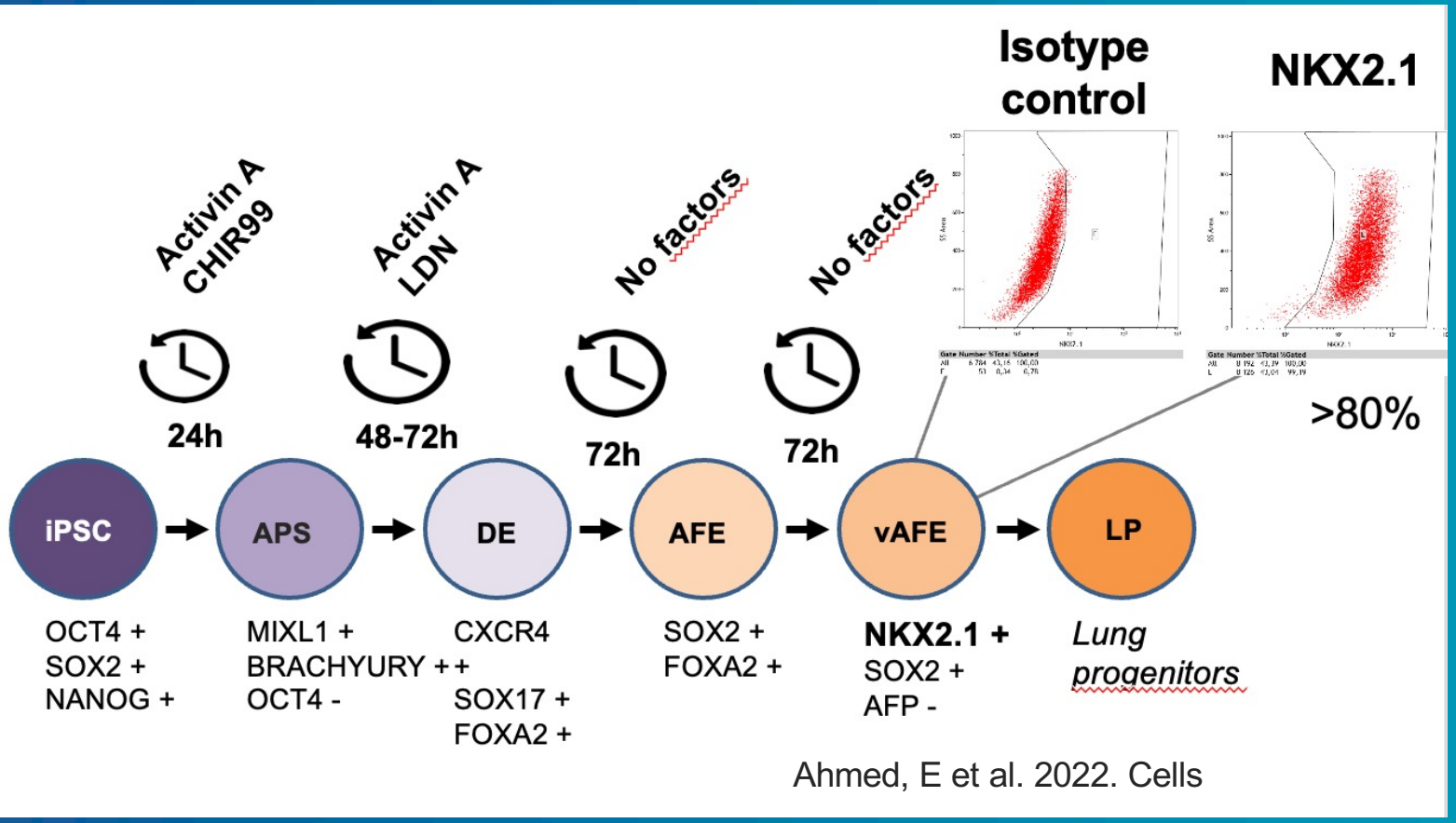
Mianné, J. et al. (2022). BMC Biol 20, 8.

Gene and cell therapy for PCD



DIFFERENTIATION: MIMIC HUMAN DEVELOPPEMENT

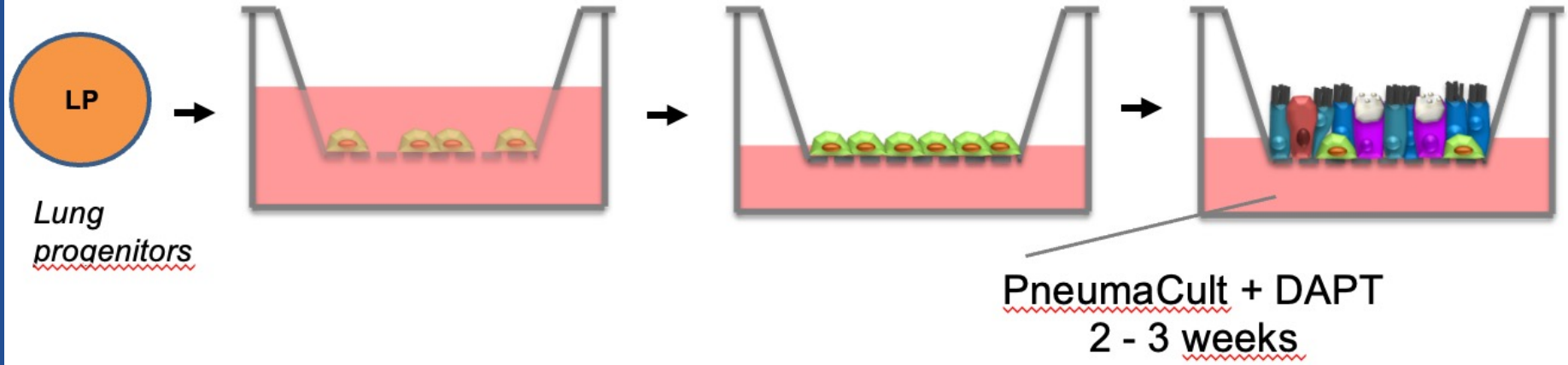
Engi Ahmed



Ahmed, E et al. 2022. Cells

iPSC-derived bronchial epithelium at air/liquid interface: iALI

Mathieu Fieldès



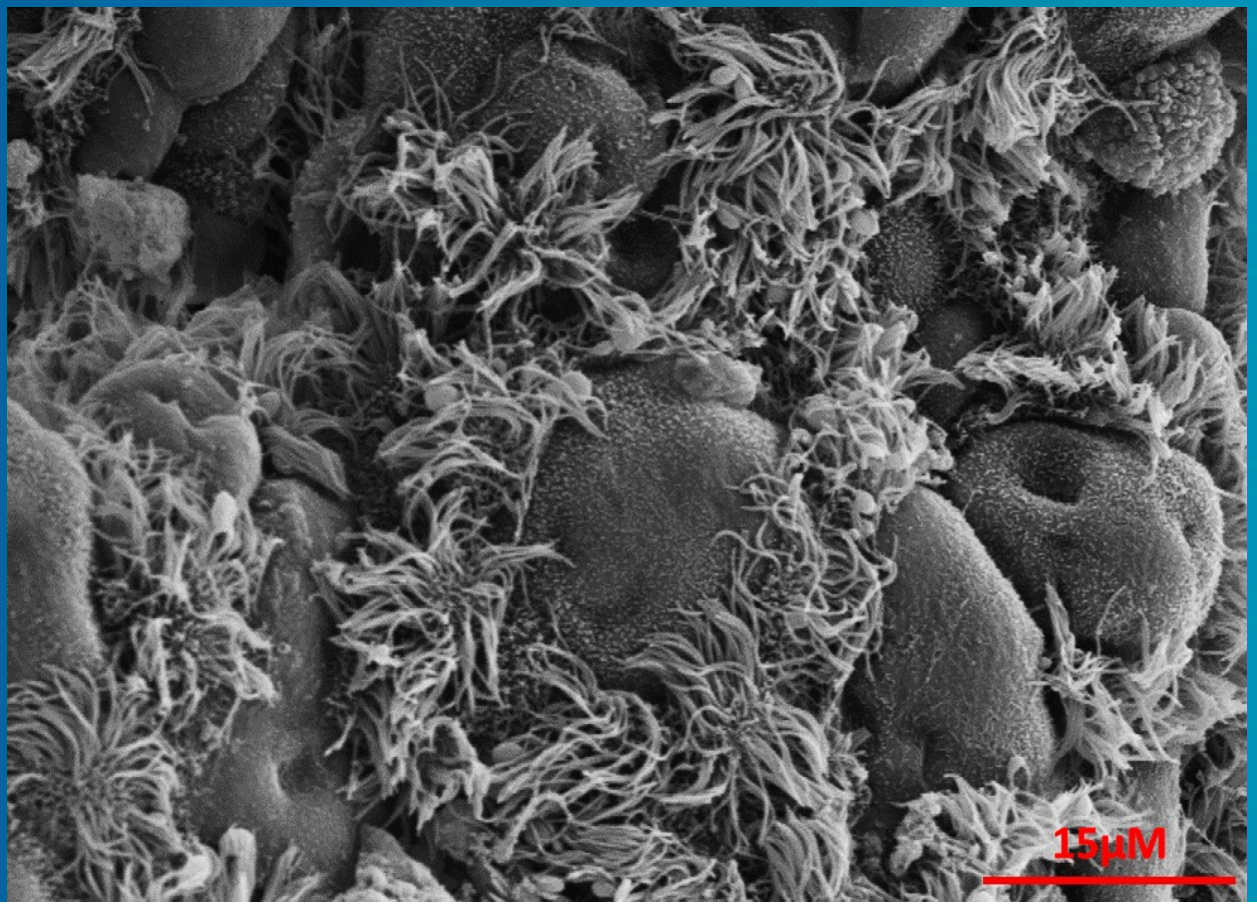
Ahmed, E et al. 2022. Cells


TURNING BLOOD INTO LUNG
SPL

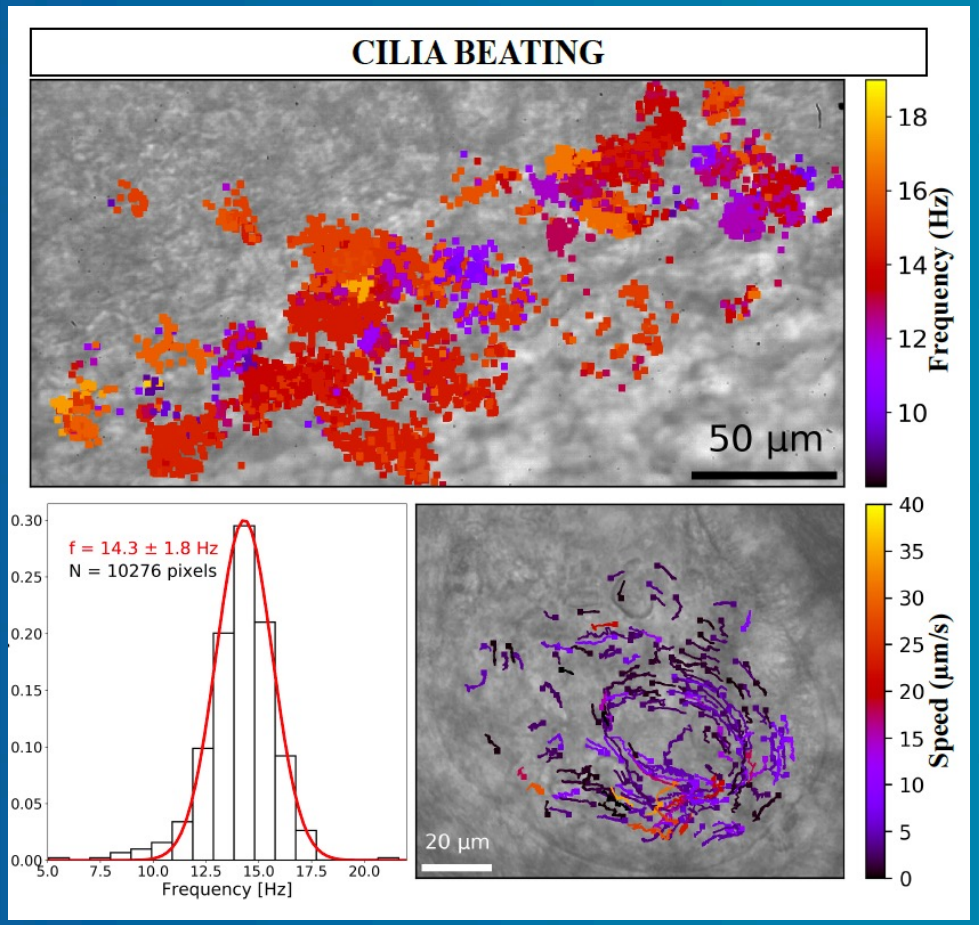
iALI

Ciliated cells

- Scanning electron microscopy

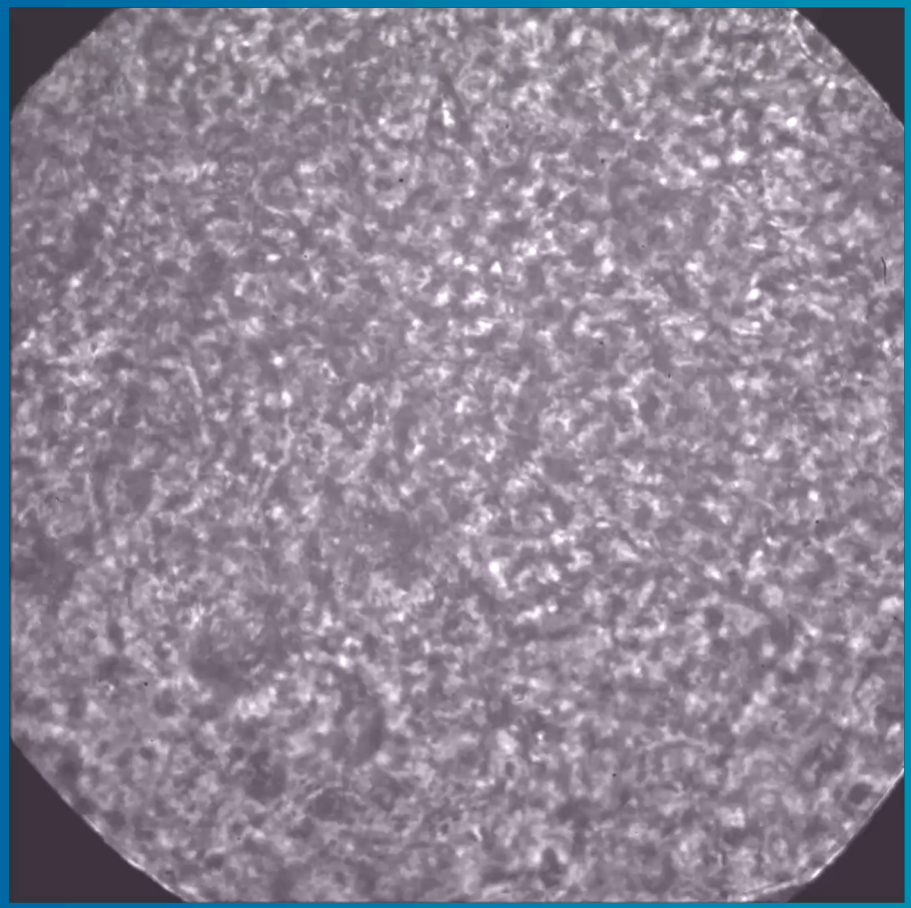


Ciliated cells

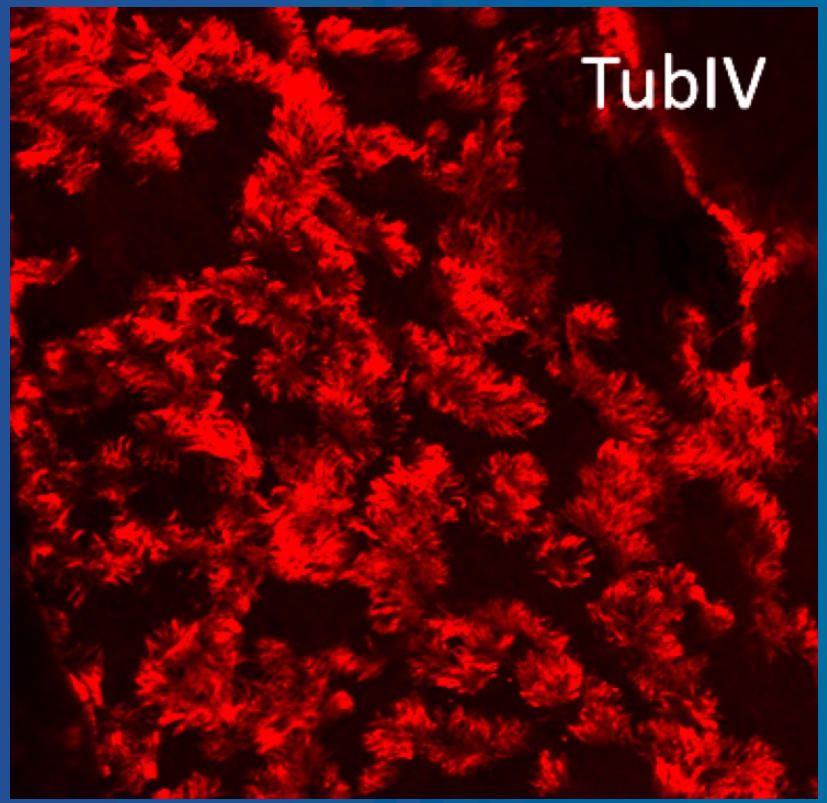


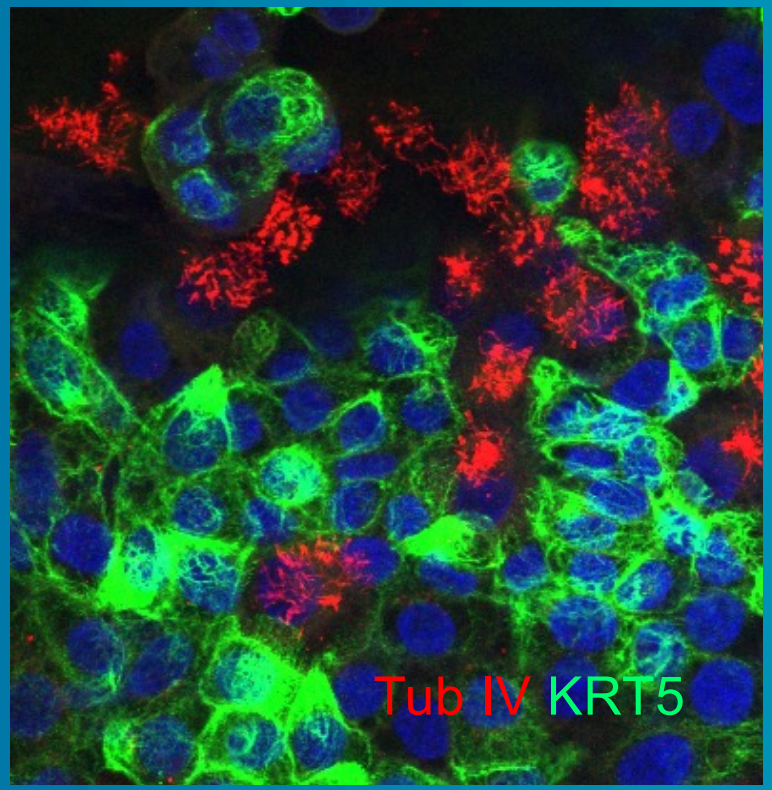
Ciliated cells

Digital high-speed camera footage



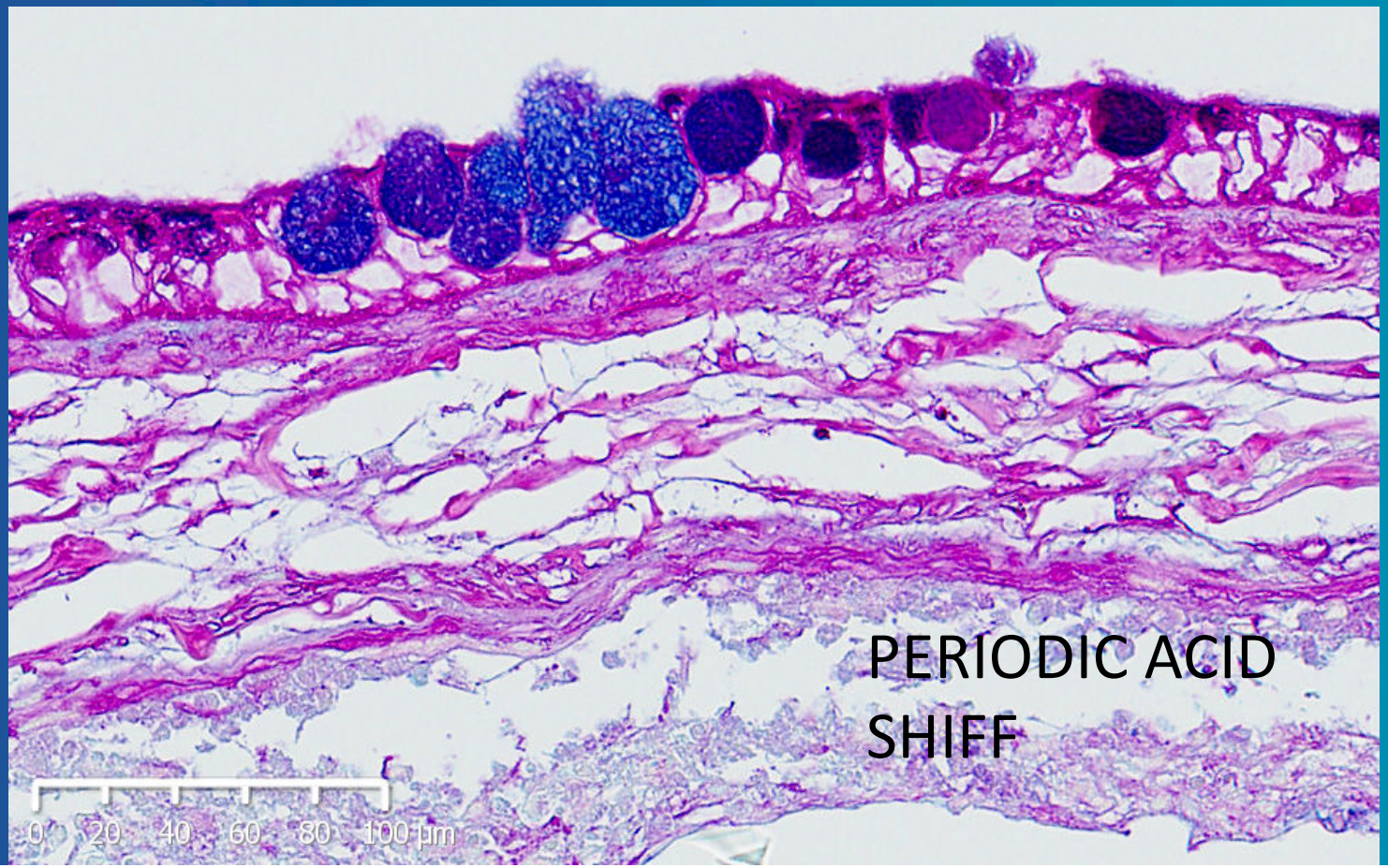
Ciliated cells and basal cells



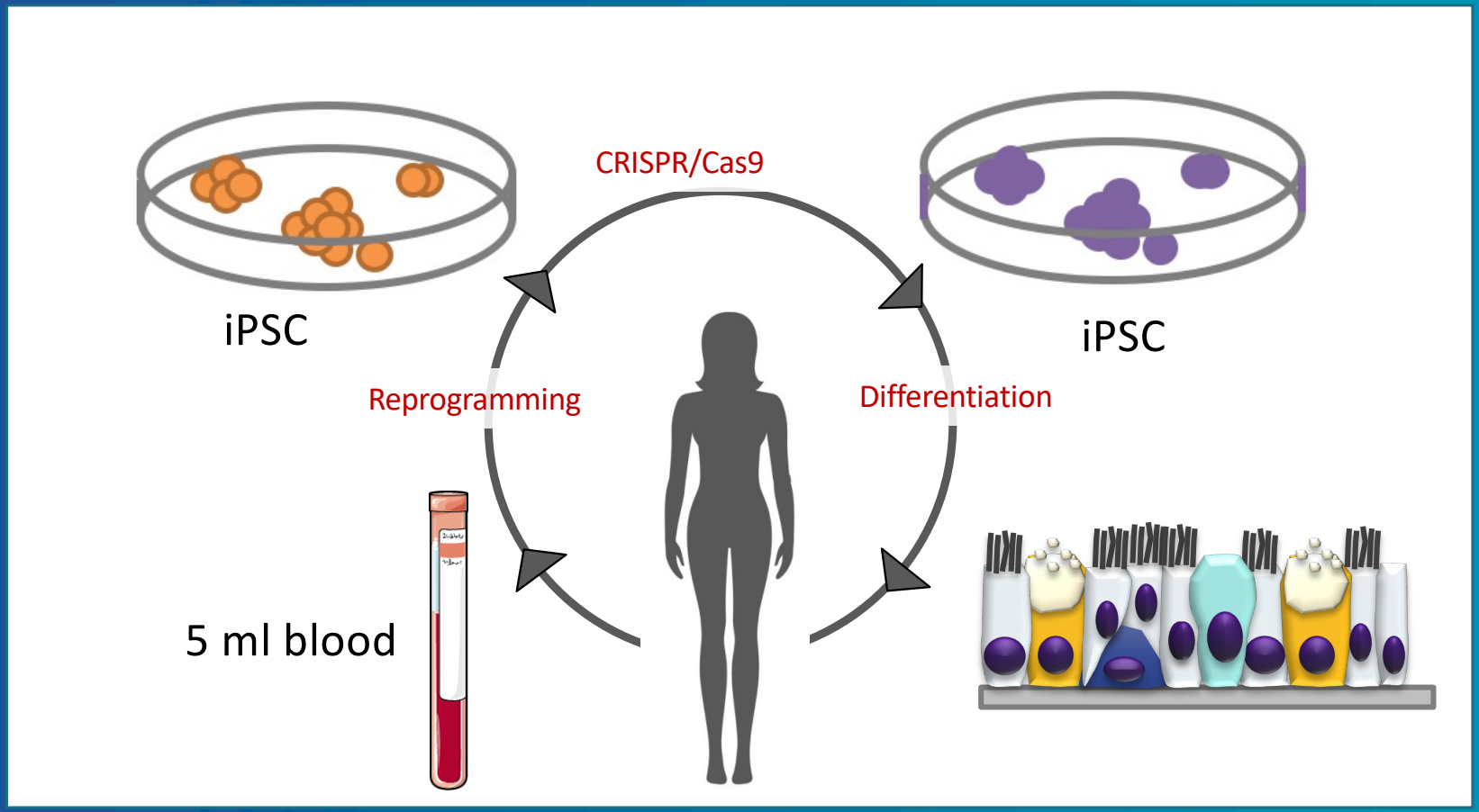


Goblet cells

Florent Foisset



Gene and cell therapy for PCD



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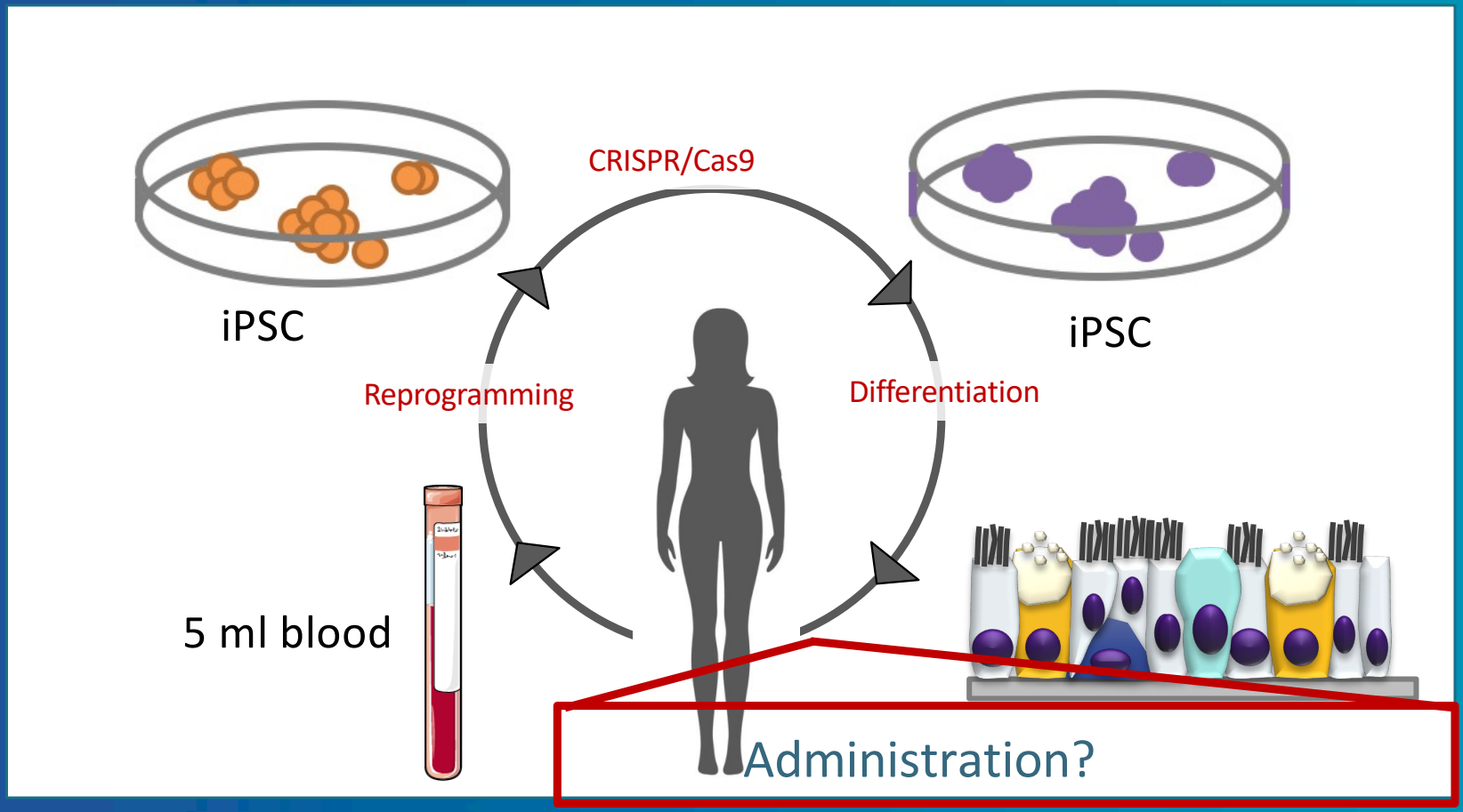
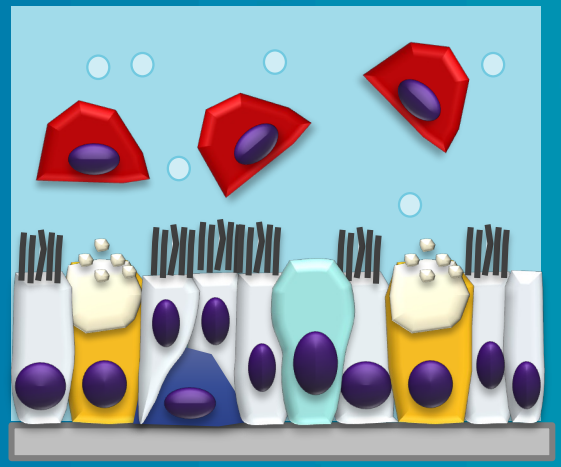


Administration

- Cell type: NKX2.1lung progenitors
- Administration?

→ Flooding, one bronchi at a time



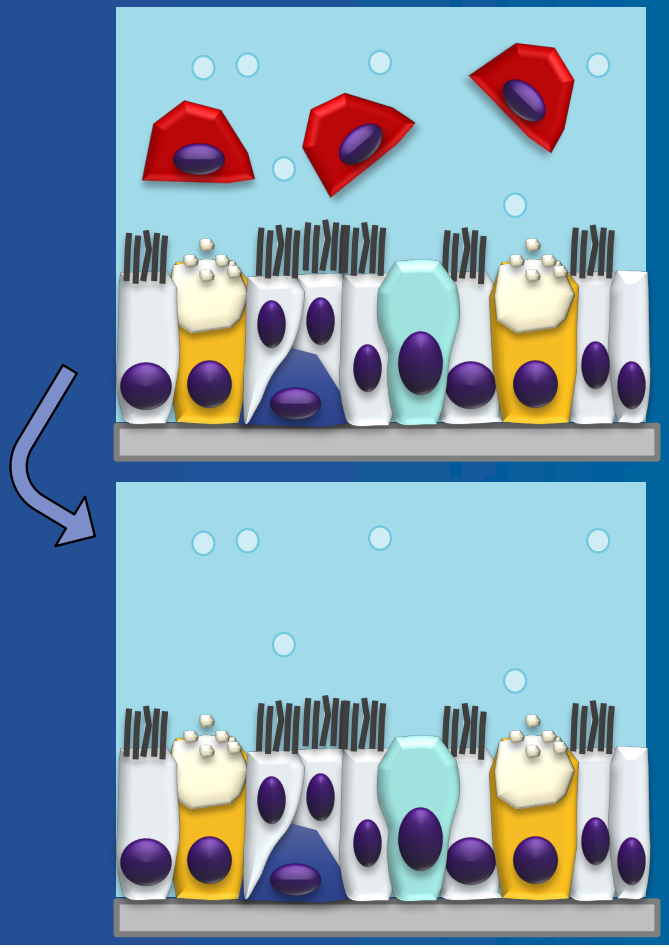
Cell-based regenerative medicine

What cell source?

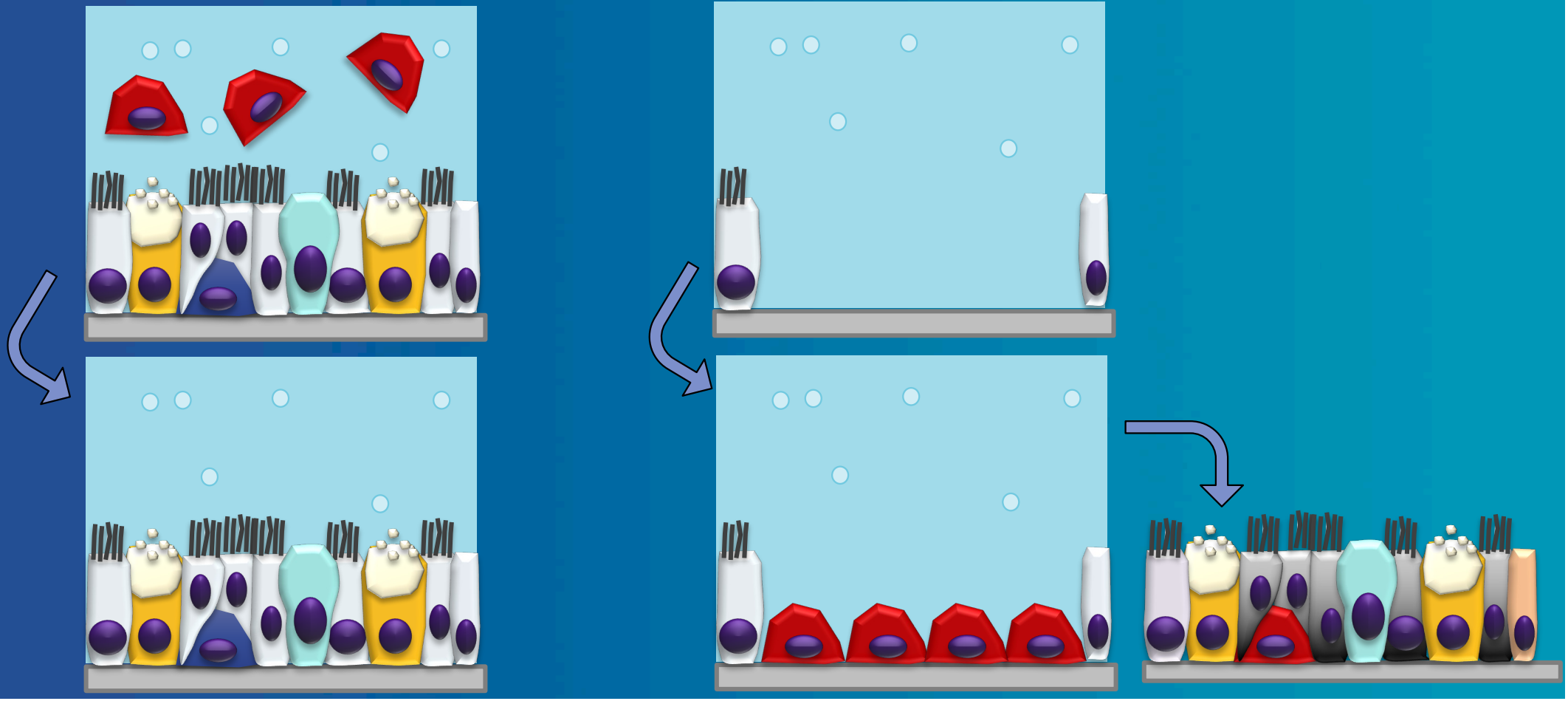
Injection route?

Conditioning!

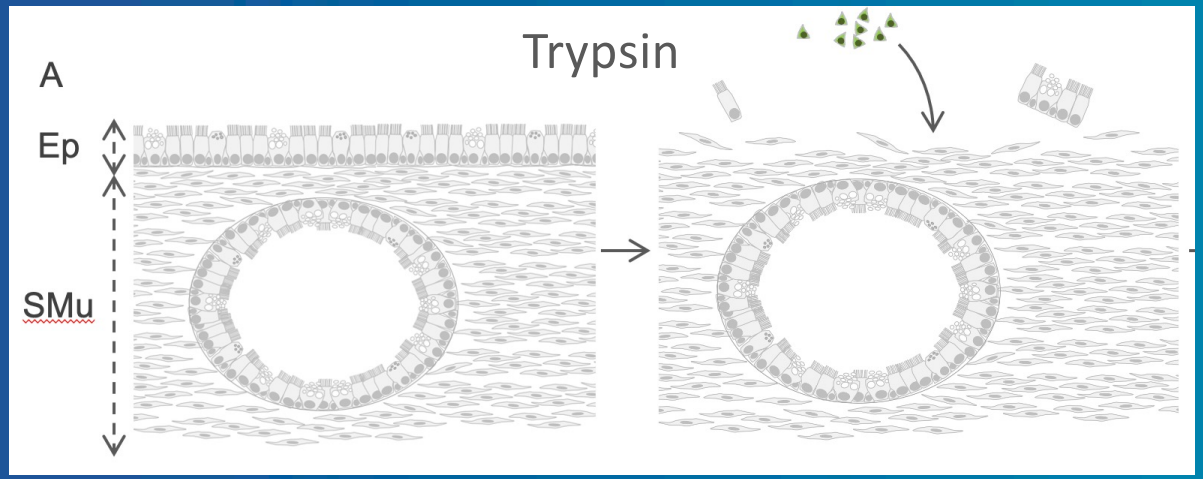
Why conditioning?



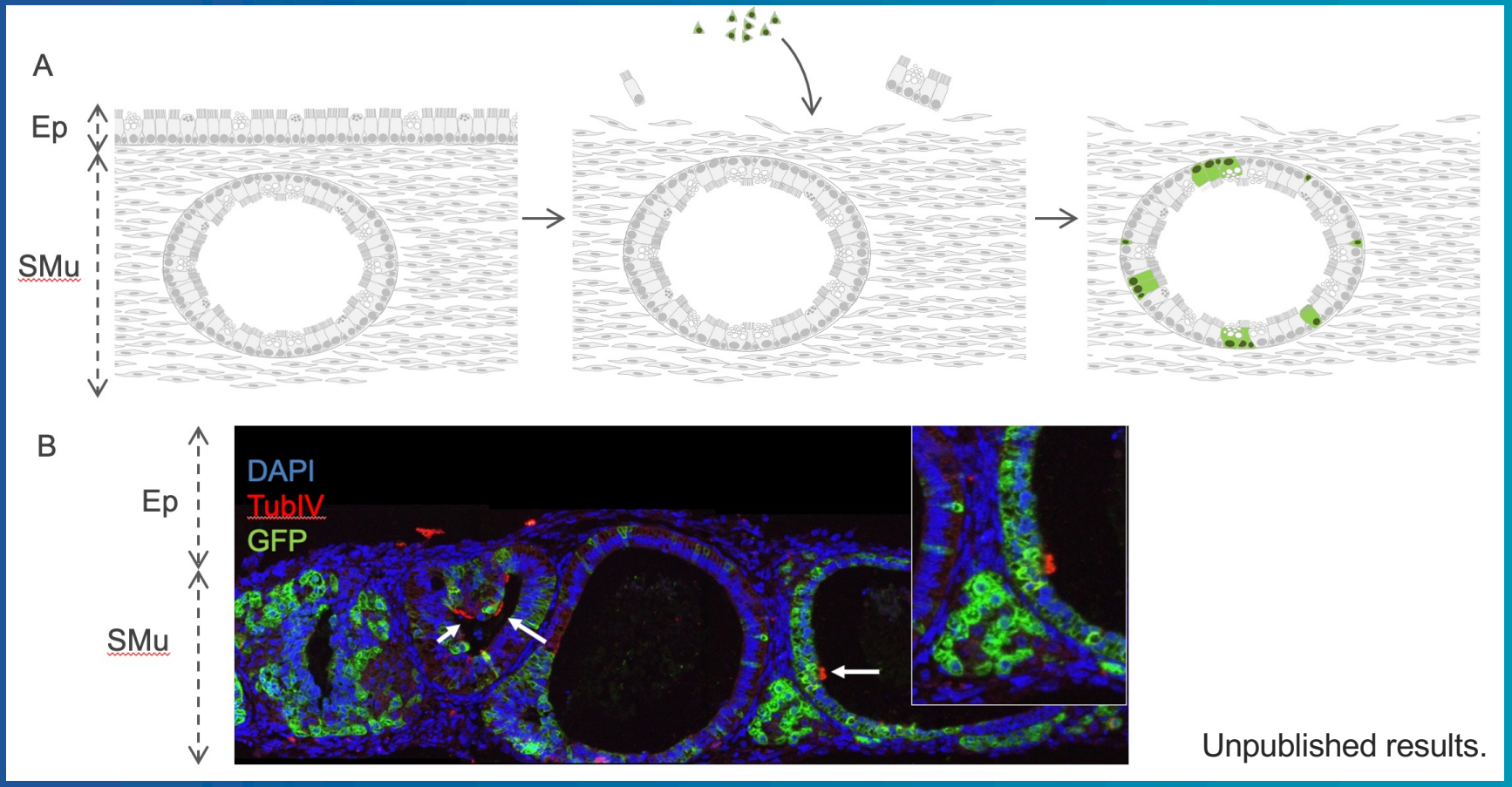
Why conditioning?



Conditioning + graft



Conditioning + graft



Unpublished results.

Take home message

- Regenerative medicine for non-hematopoietic organs (The hematopoietic tissue is the exception, rather than the rule!):
  - Source of cells is solved: iPSC
  - But administration in the case of the lung?
  - Conditioning will be mandatory
  - Towards personnalized autologous cell & gene therapy

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