Targeting gene editing in pluripotent stem cells: X-SCID disease now in a dish

Jamal Alzubi, PhD

Institute for Cell & Gene Therapy

Freiburg, Germany

Outline

Immune system

- Intact vs. diseased immune system
- T cells function, Why they are important?
- T cells development in human and in cell culture
- Impaired T cells development (X-SCID, X-linked sever combined immunodeficiency)

Designer nucleases

- Types and mode of action
- Tools for gene editing

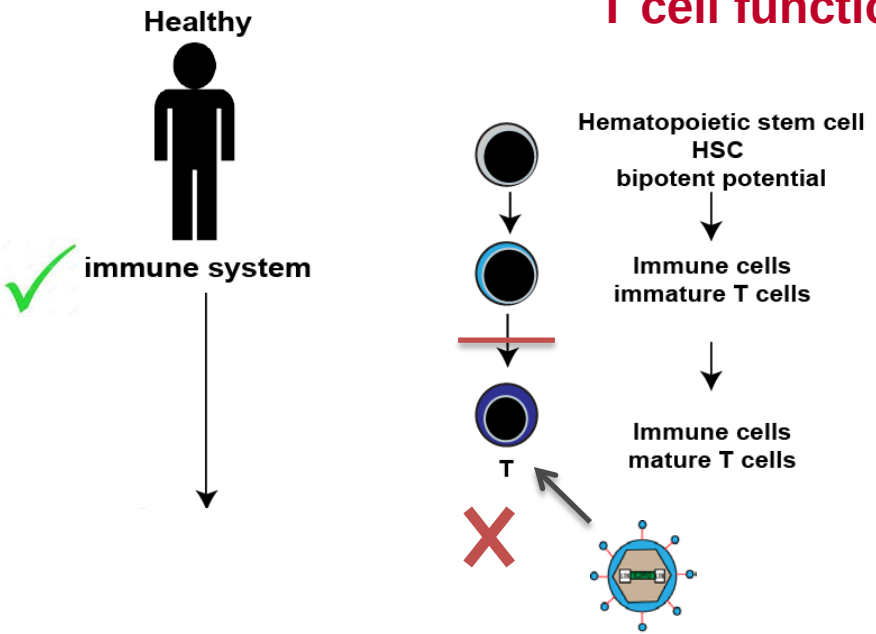
Pluripotent stem cells (PSCs)

- Types and potential
- Gene editing in PSCs
- Genetic screen for corrected cells
- Differentiation of PSCs to mature effector T cells

Conclusions and perspectives

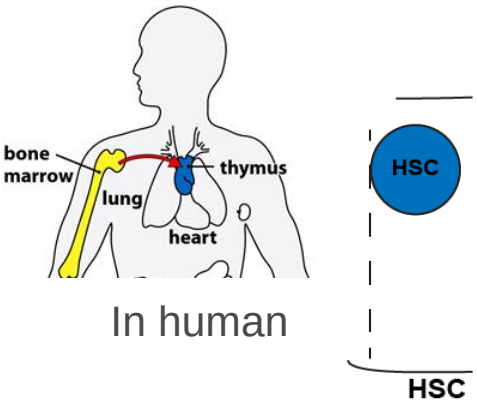
Immune system

T cell function



Immune system

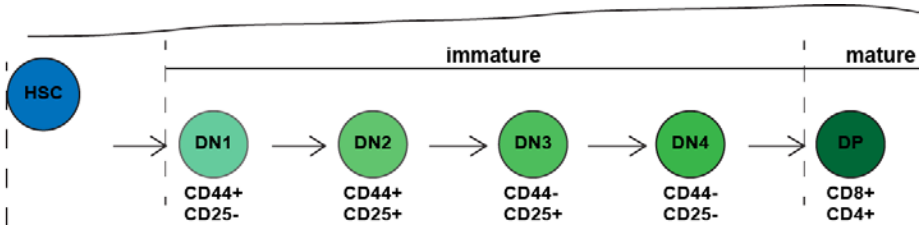
T cell development



In human

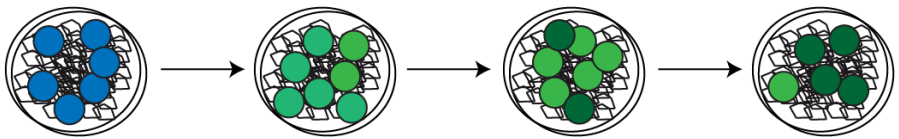
Immune system

visualization of T cell development

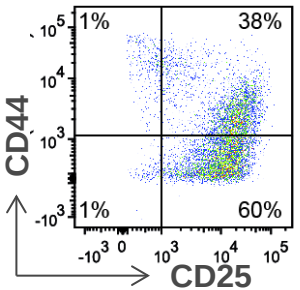


DN, double negative

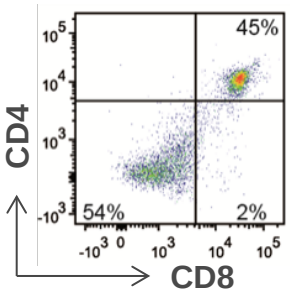
DP, double positive



immature

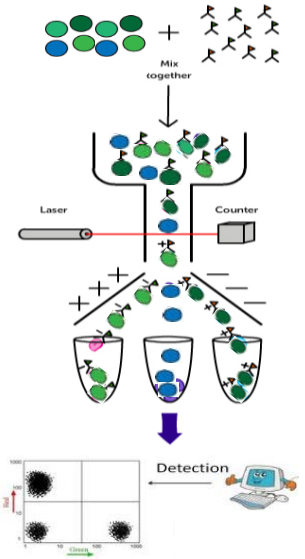


mature



Fluoresence-Activated Cell Sorting

FACS



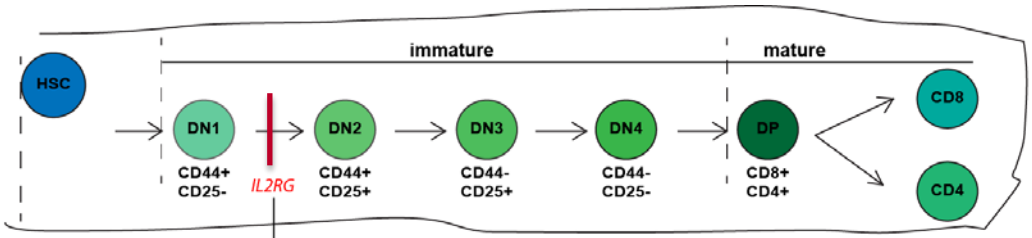
CD, cluster of differentiation

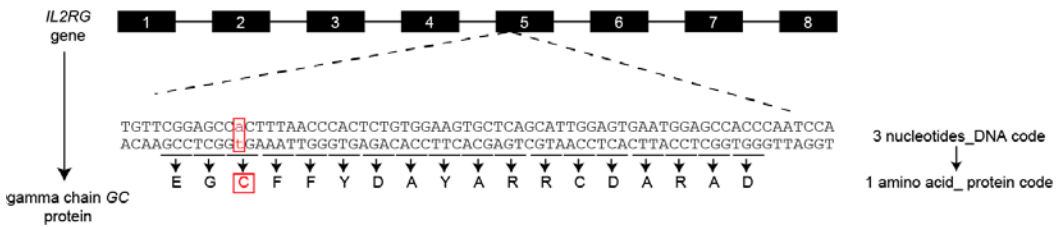
Impaired T cell development

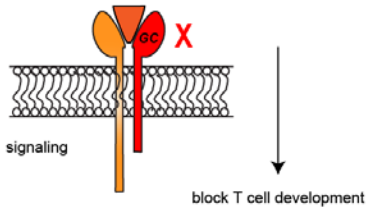
primary immune deficiency

Healthy

Patient





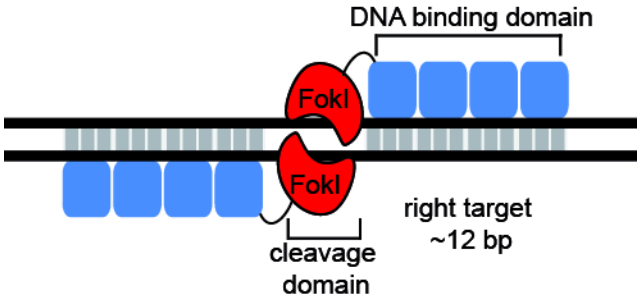


How to correct this mutation?

Designer nucleases

mode of action

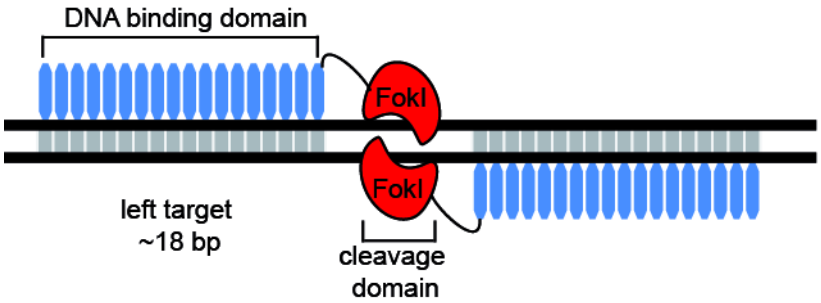
Zinc Finger Nuclease (ZFN)



1:3 code

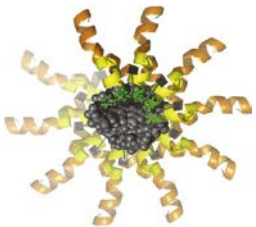
1 ZF ≈ 3 bp

Transcription Activator-Like Effector Nucleases (TALEN)



1:1 code

1 repeat = 1 bp

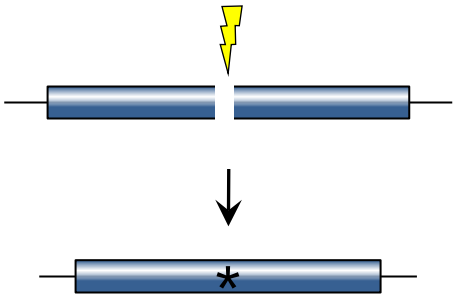


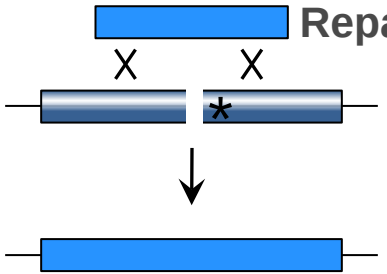
Designer nucleases

tools for gene editing

Non-**H**omologous **E**nd **J**oining
(NHEJ) (Error-prone)

Homologous **D**irected **R**epair
(HDR) (Accurate)





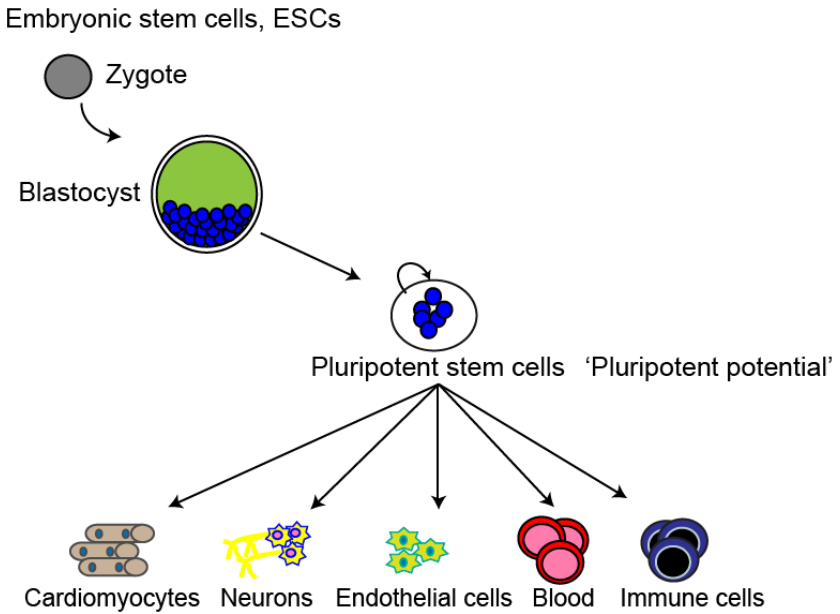
gene disruption

What are the suitable cells for correction?

gene correction

Pluripotent stem cells

types & potential

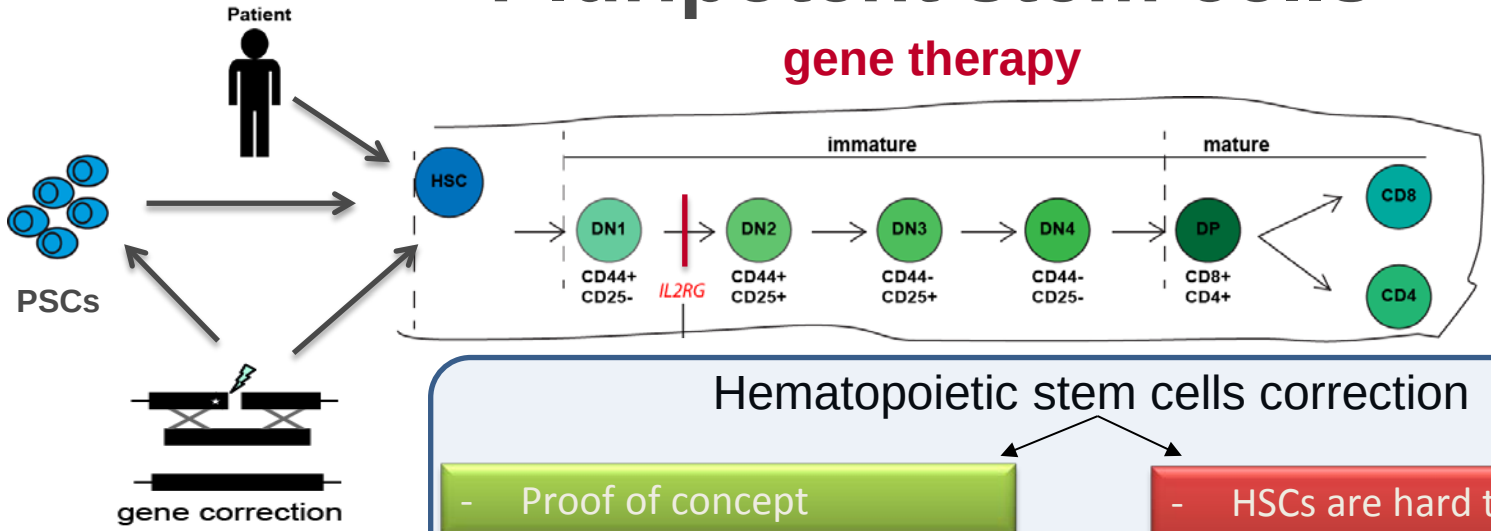


Limitations:
- Ethical concerns
- Immune rejection

1) Proof of concept for gene therapy
2) Disease modeling
3) Regenerative medicine applications

Pluripotent stem cells

gene therapy



Hematopoietic stem cells correction

- Proof of concept

Genovese P, et al. 2014. Nature.

- HSCs are hard to culture
- HDR is low in these cells

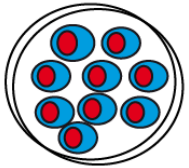
Pluripotent stem cells correction

- Disease modelling
- Regenerative medicine application

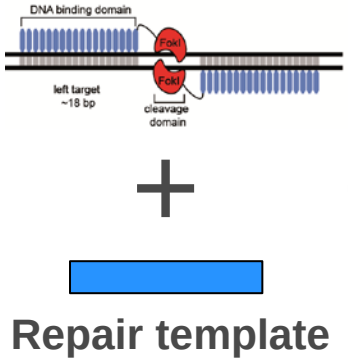
- Efficient differentiation protocols

Pluripotent stem cells

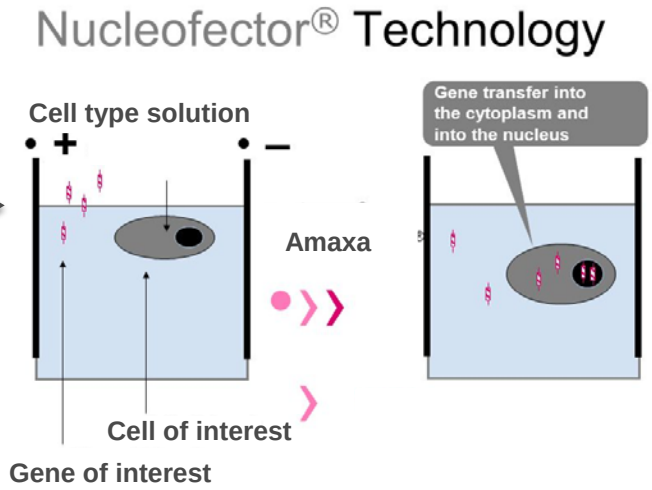
gene editing

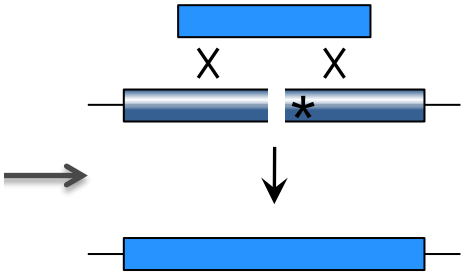


pluripotent stem cells



Repair template

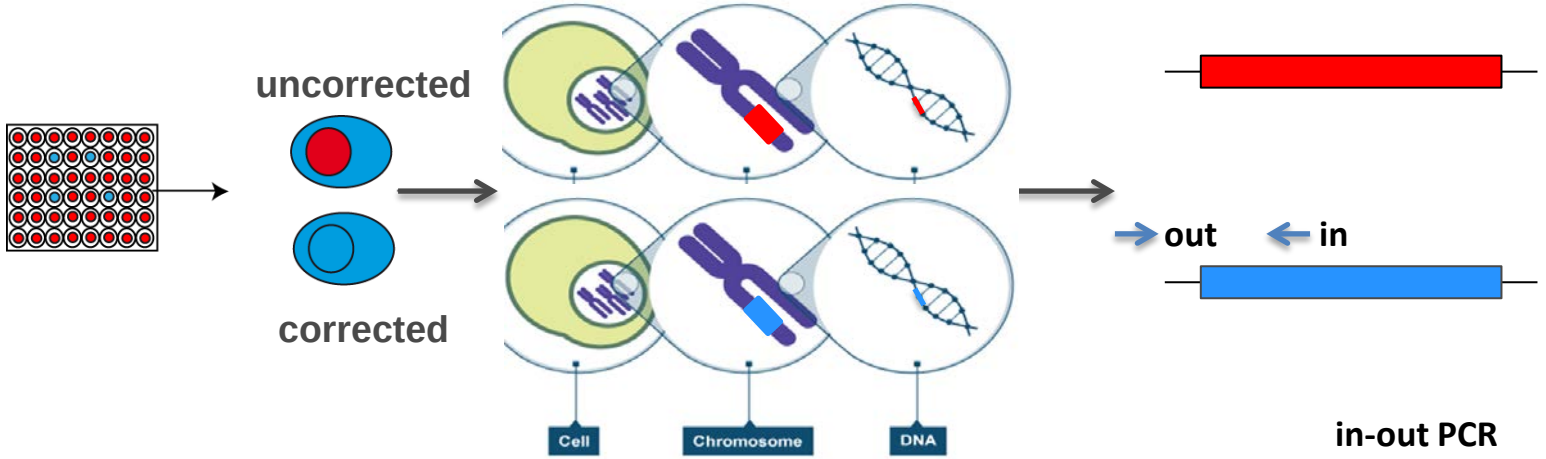




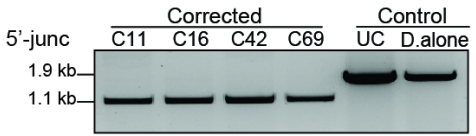
gene correction

Gene editing

Genetic screen



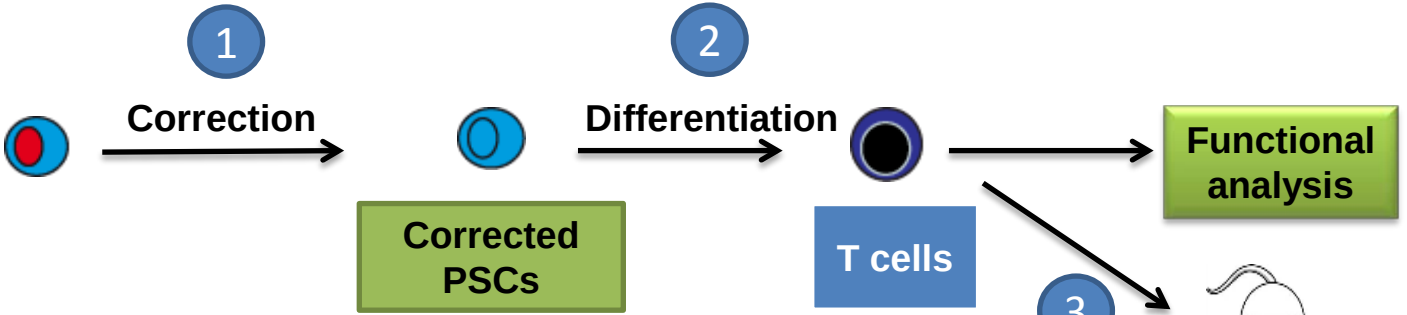
in-out PCR

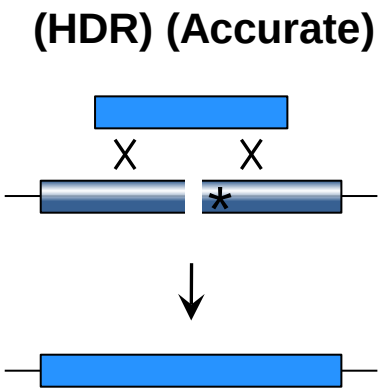


Successful genetic correction
GT: 4 out of 100

Gene editing

disease model of immunodeficiency



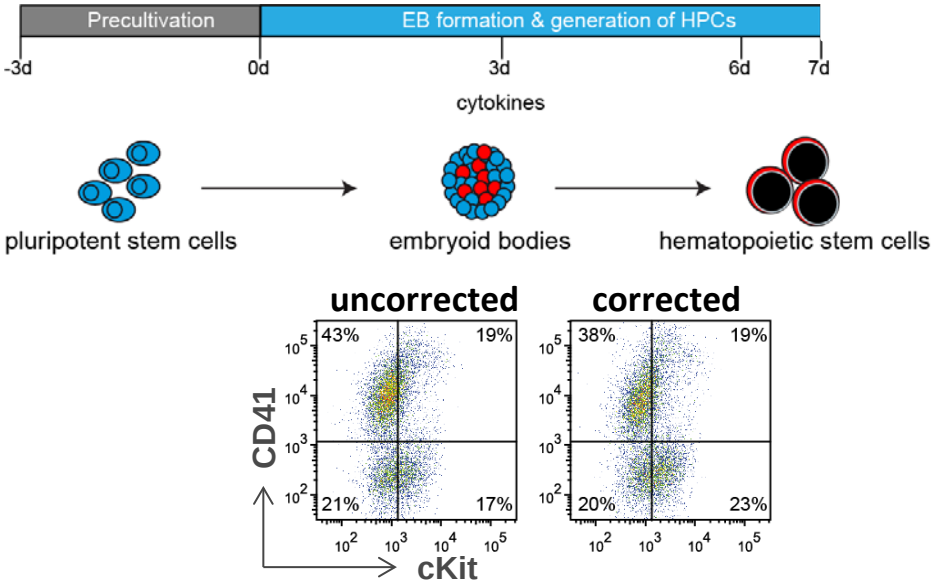


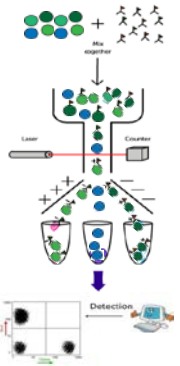
1. Proof of concept for gene therapy
2. Disease modeling
3. Outlook
   Regenerative medicine applications ?

Pluripotent stem cells

differentiation

Hematopoietic stem cells generation

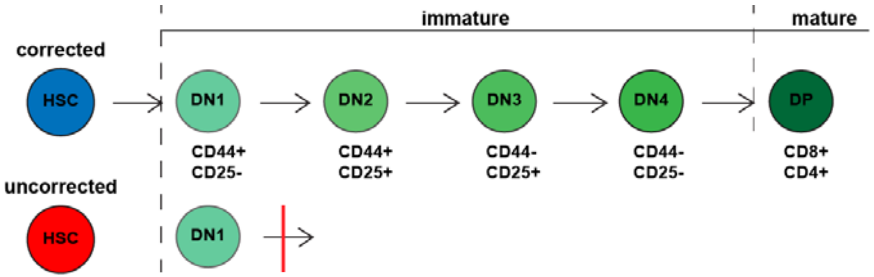


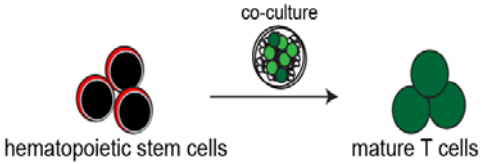


Comparable levels of hematopoietic stem cells from both uncorrected and corrected clones

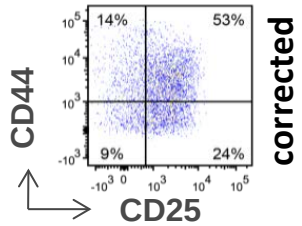
Pluripotent stem cells

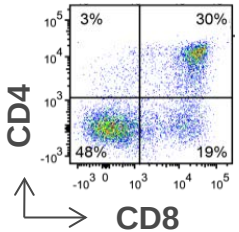
differentiation to T cells

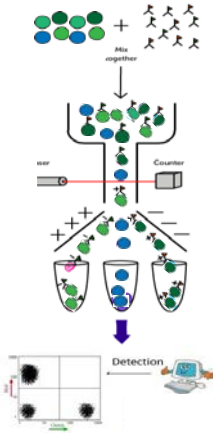




restoration of T-cell differentiation
X-SCID disease in a dish

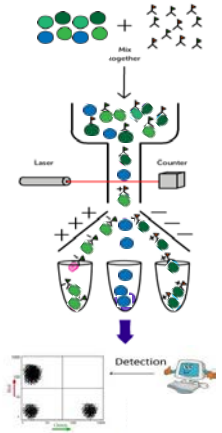


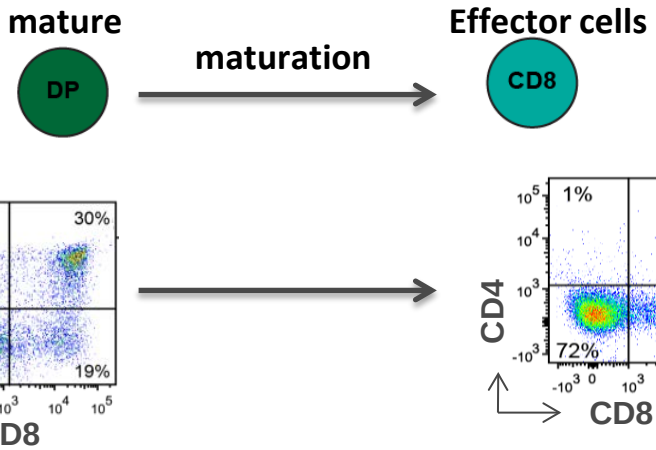


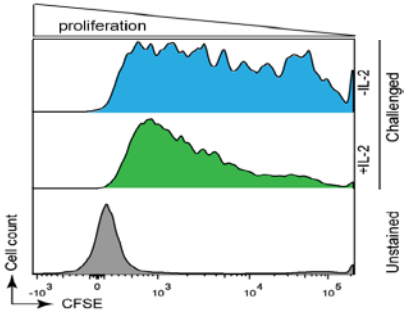


Pluripotent stem cells

maturation of T cells

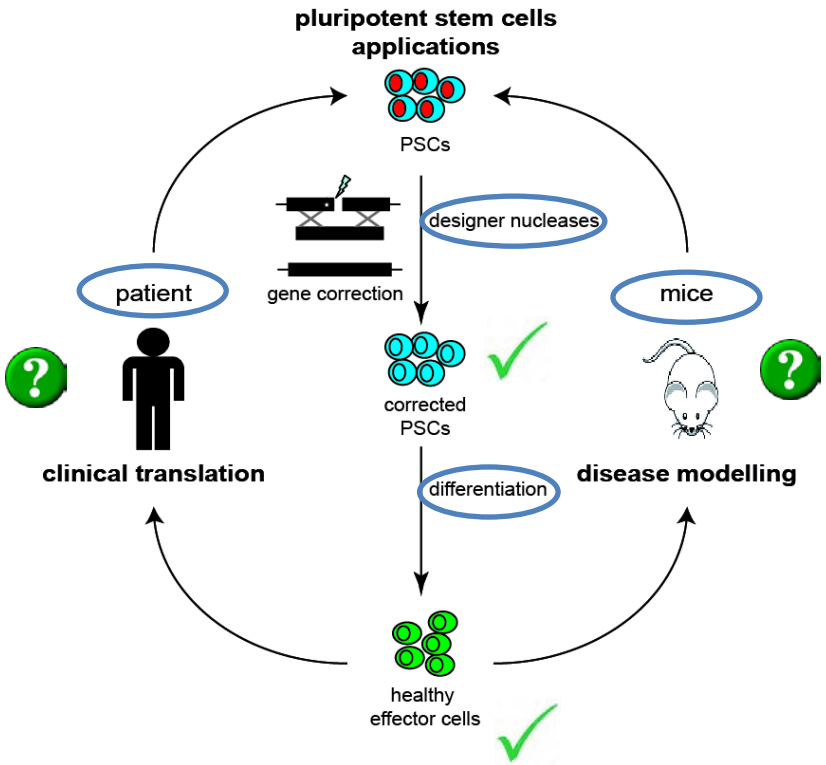






T cells can mature in a dish to effector T cells

Conclusion & perspectives



Technology platform development

→ Designer nuclease can correct pluripotent stem cells of an immunodeficiency (**X-SCID**).

Effector T cells can be generated from PSCs

→ X-SCID disease model in a dish

Transplantation of effector T cells to mice

→ Check functionality and side effects

Transplantation of effector T cells to human?

→ Stabilize the immune system and cure patients

Toni Cathomen
Christien Bednarski
Laura Mosti
Nicola Bundschuh
Nils Craig-Muller
Viviane Dettmer
Simone Haas
Ruba Hammad
Markus Hildenbeutel
Beate vom Hövel
Valentina Pennucci
Christine Reichenbach
Giandomenico Turchiano

Tatjana Cornu
Marianna Romito
Petra Scheliga-Marschner
Ilona Skatulla

Claudio Mussolino
Melina el Gaz
Saskia König
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