AIEOP in LAB
Bologna, 29 maggio 2018

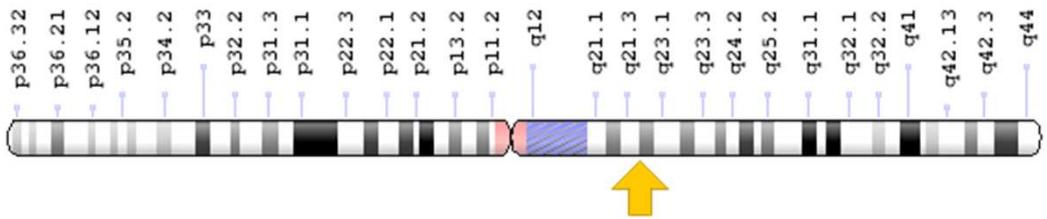
Messa a punto di modelli cellulari umani per lo studio della malattia di Gaucher

Daria Messelodi

Gaucher Disease

- the most common **lysosomal storage disorder**
- genetic autosomal recessive disease

Cause: **deficiency of the enzyme glucocerebrosidase,** required for the degradation of glycosphingolipids, due to **mutations in the GBA1** gene



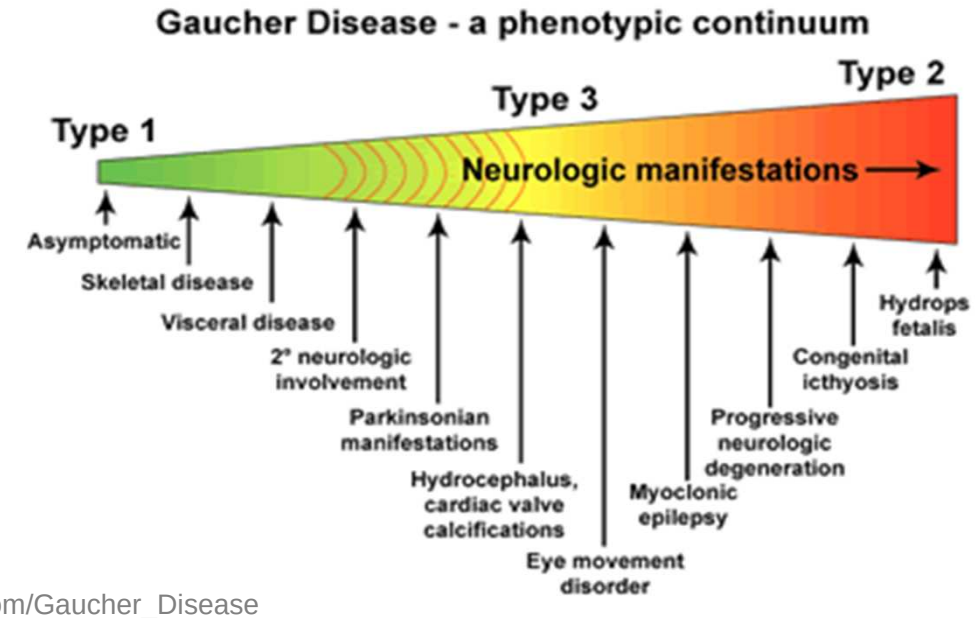
GBA1: chr 1q21-22, 11 exons encoding the glucocerebrosidase protein
Nearly **300 mutations** (N370S, L444P, IVS2+1, 84GG → the most frequent)

https://ghr.nlm.nih.gov/gene/GBA

Gaucher Disease
Phenotypic manifestations

Gaucher disease classification, depending on neurologic symptoms:
- **type 1:** non neuronopathic
- **type 2:** acute neuronopathic
- **type 3:** chronic neuronopathic

However, GD syntomatology represents a *continuum*



https://www.physio-pedia.com/Gaucher_Disease

Gaucher Disease
Search for new players

GD is a monogenic disease but there is a wide spectrum of phenotypic manifestations

Other genes are involved?

Search for new:

- **modifier genes**
- **interacting pathways**

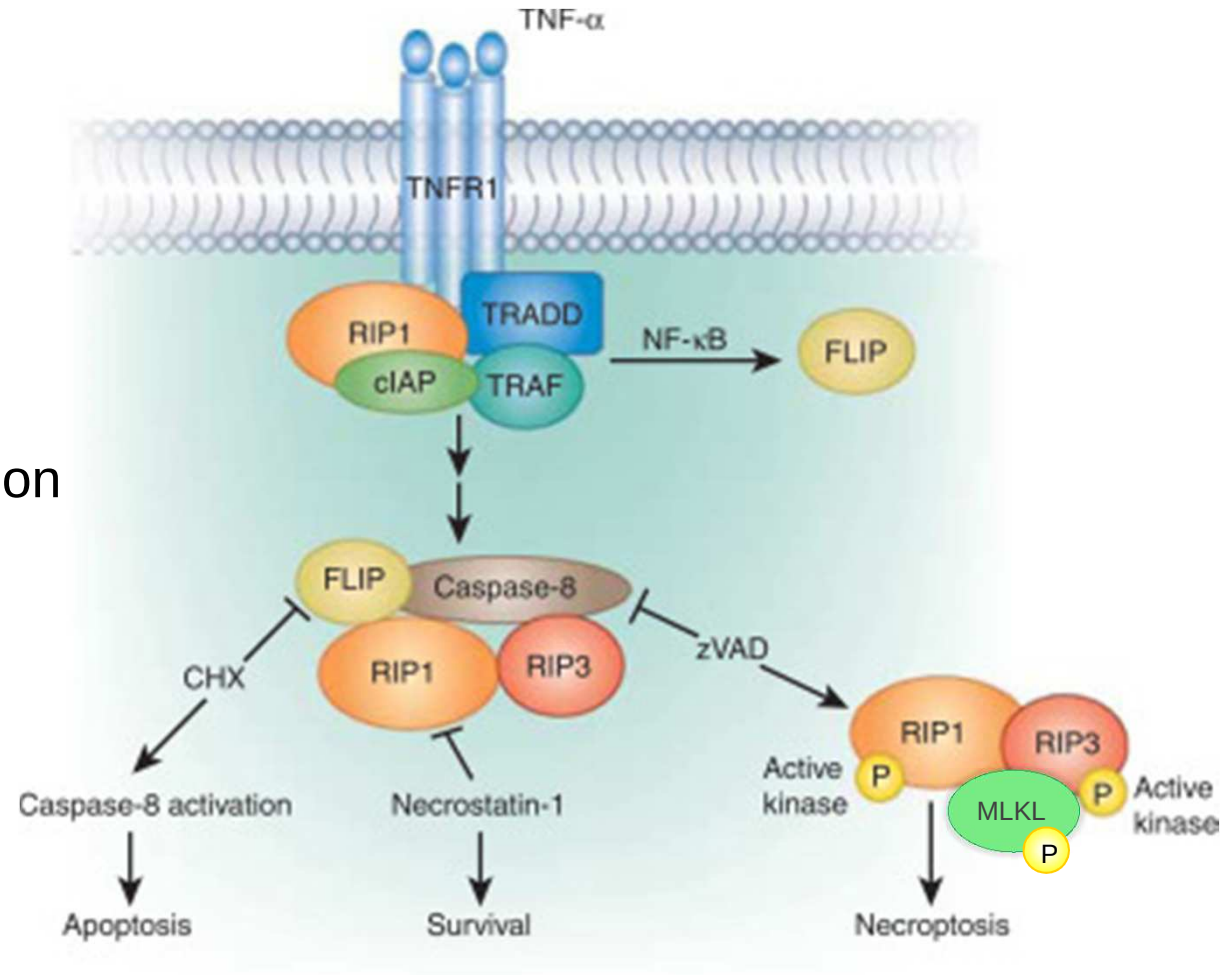
Necroptosis pathway

Necroptosis is a form of caspase independent regulated cell death

It is induced by inflammation

Kinase-regulated process with 3 key factors

- **RIP1/RIPK1**
- RIP3/RIPK3
- MLKL

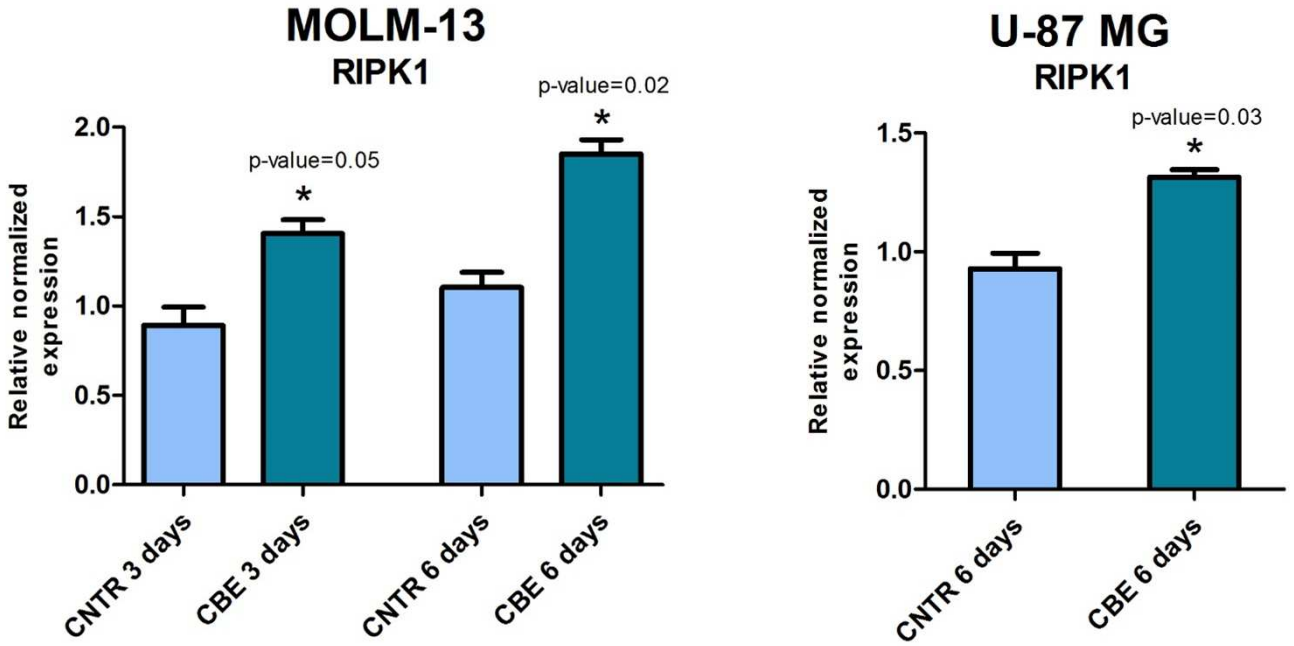


http://www.kidney-international.org/article/S0085-2538(15)55392-1/fulltext

Necroptosis

Preliminary data on cell lines

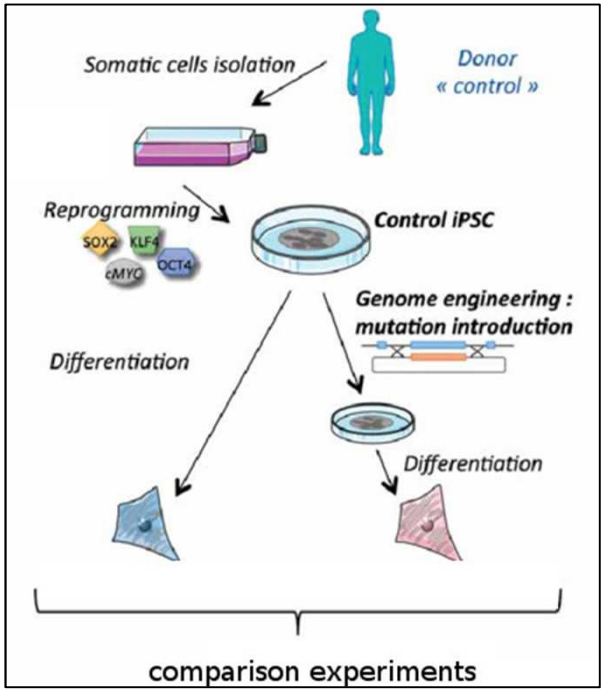
MOLM-13 and **U-87 MG** treated with **CBE**

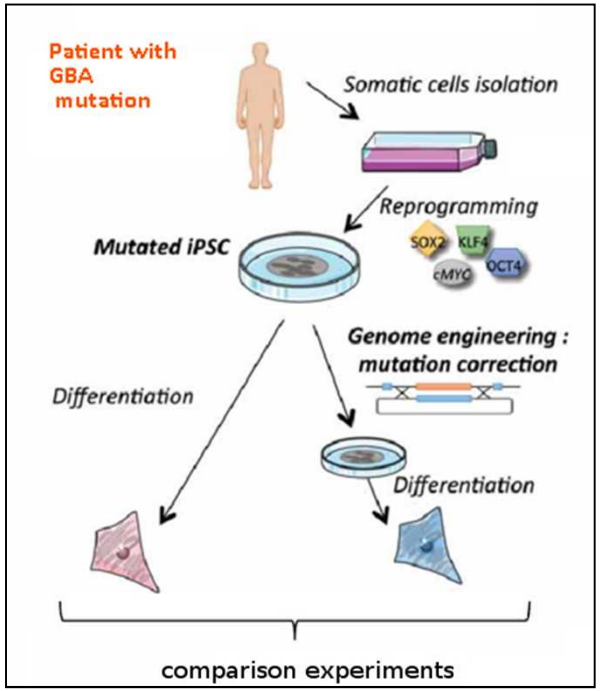


Increase in the RIPK1 gene expression after 3 and 6 days treatment with CBE (250µM)

iPSC model

Induced pluripotent stem cell (iPSC)





Healthy donor

• CRISPR-Cas9 to introduce GBA1 mutations;
• comparison with the wild type control.

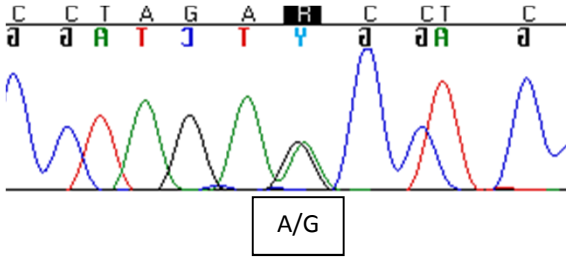
Patients

• reprogramming of peripheral blood mononuclear cells (PBMCs);
• correction of the mutation.

iPSC
Gene editing

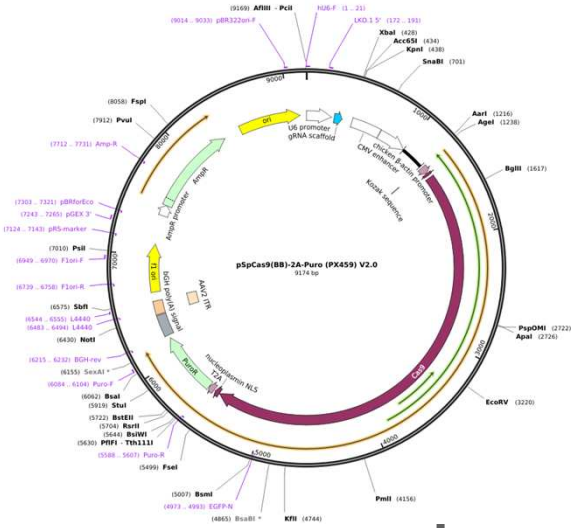
Gene editing → introduction of the proper GBA1 mutation into the cell genome

CRISPR-Cas9 system to introduce the GBA1 mutation N370S: A→G (ex 10)



Strategy:

- ssDNA donor including the mutation sequence
- Plasmid encoding for a gene specific RNA guide and Cas9 protein



https://www.addgene.org/62988/

Screening of:

- **38** iPSC colonies trasfected with guide 1
- **98** iPSC colonies transfected with guide 2

iPSC
GD patient

Reprogramming of mononuclear pheriperal blood cells of a **GD patient** with **N370S/L444P** mutations to iPSC

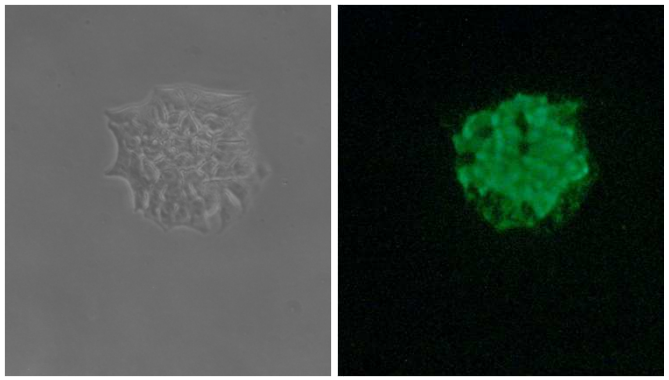
Strategy

Sendai vectors: KOS, c-myc, Klf4

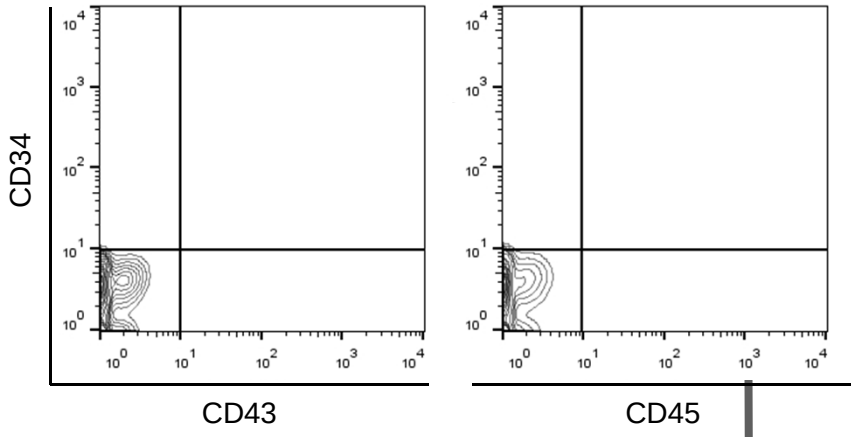
⇩

✓ Obtainment of iPSC

✓ Validation of the model

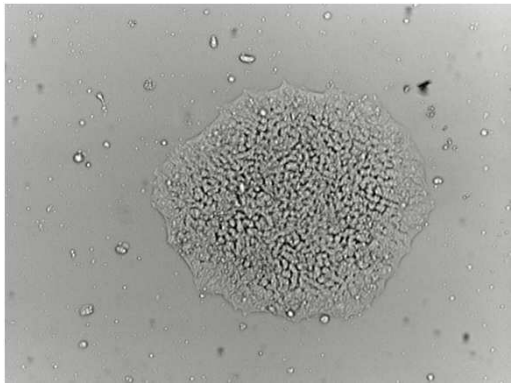


No staining | TRA 1-60

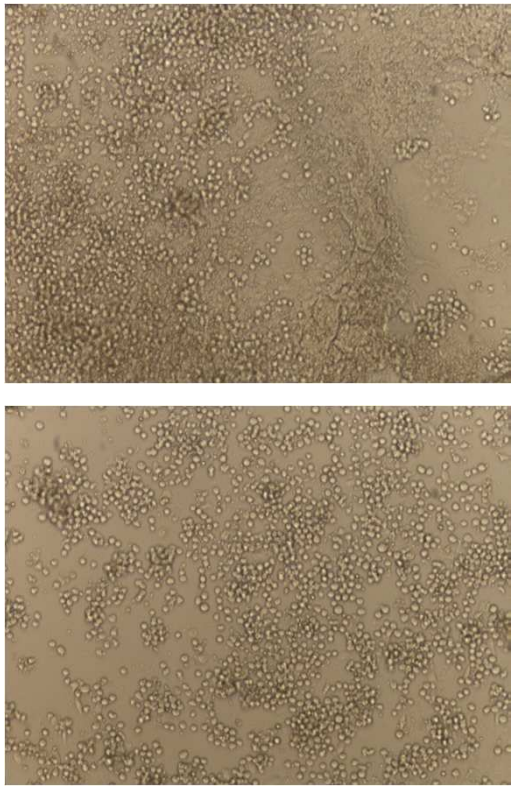


Differentiation
GD - iPSC vs CNTR iPSC

Hematopoietic differentiation →12 days protocol to obtain hematopoietic progenitor cells



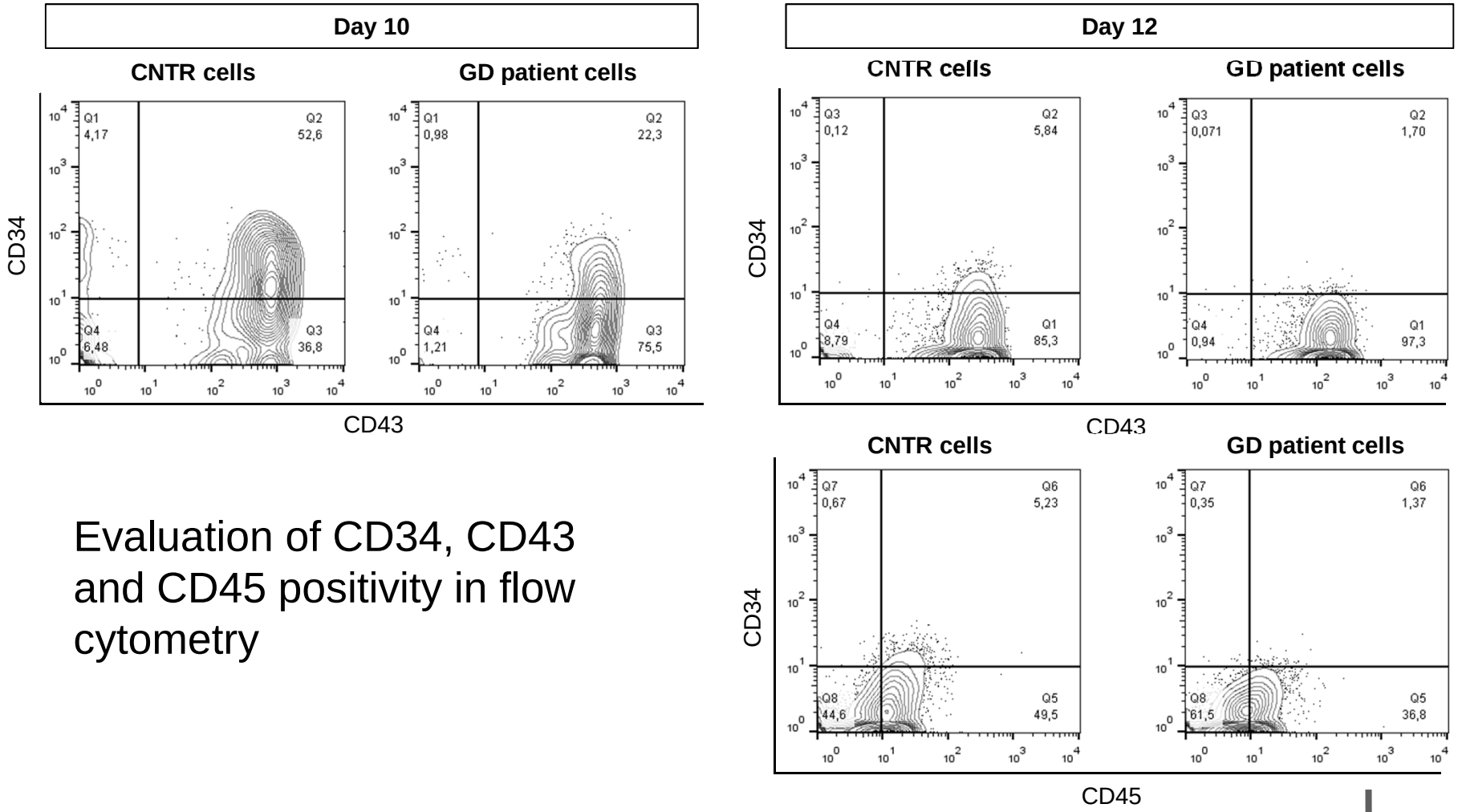
GD - iPSC colony



GD cells after 12 days of differentiation

Differentiation

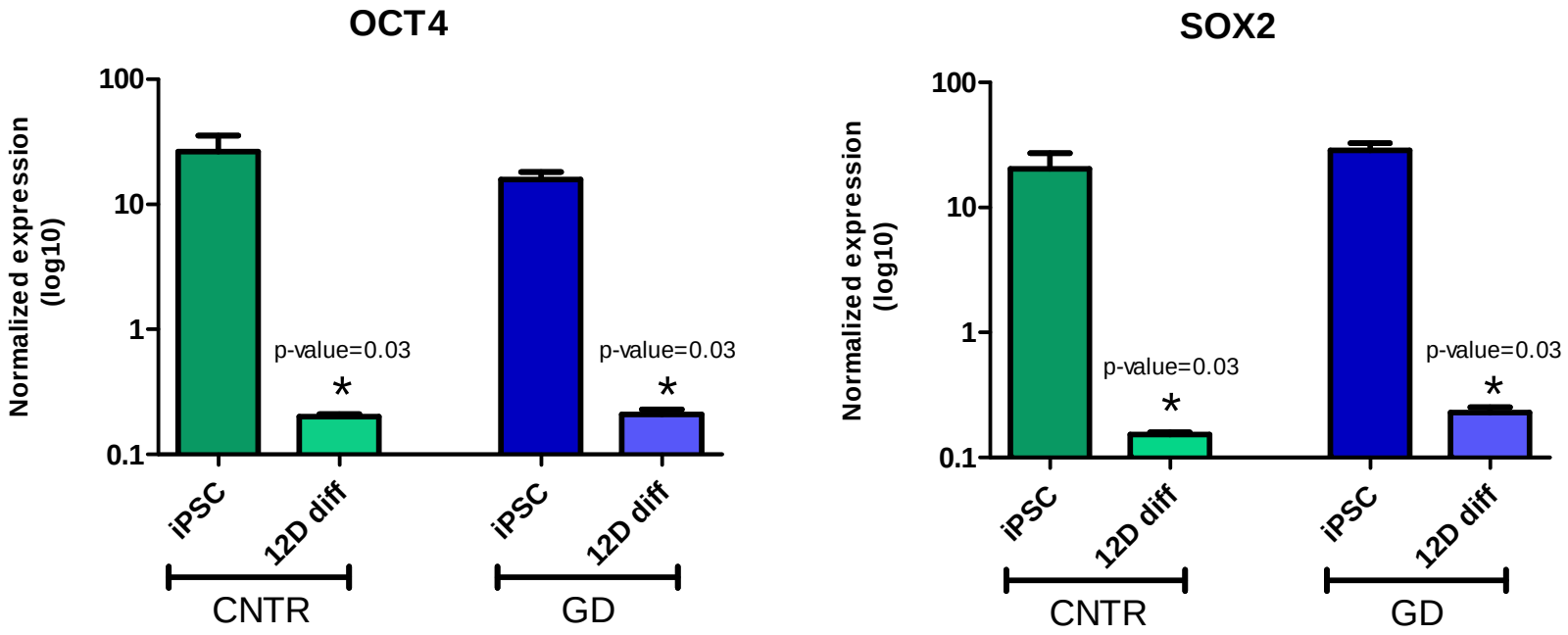
GD - iPSC vs CNTR iPSC



Evaluation of CD34, CD43 and CD45 positivity in flow cytometry

Differentiation

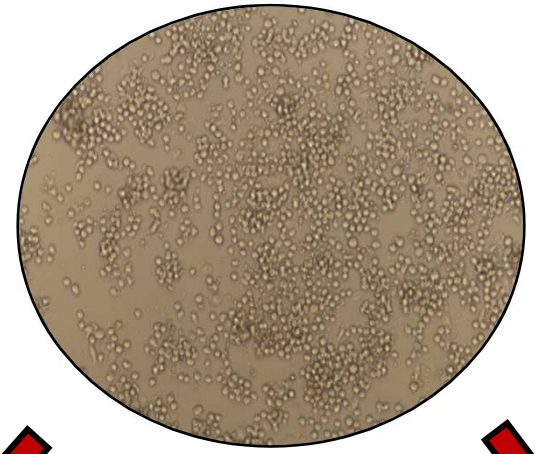
GD - iPSC vs CNTR iPSC



Decrease of the expression of pluripotency marker genes after hematopoietic differentiation

Differentiation
GD - iPSC vs CNTR iPSC

iPSC-derived hematopoietic precursors

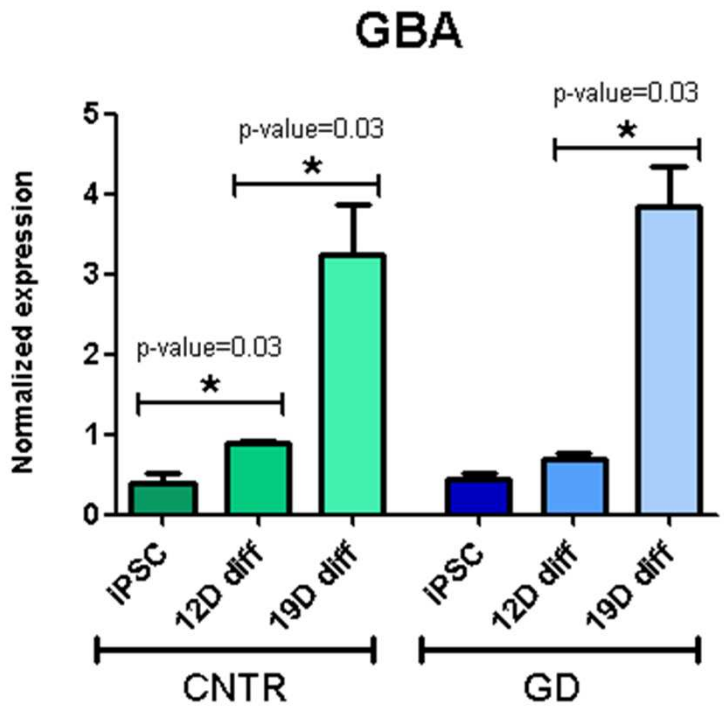


CNTR and GD cells
N=375000
in **liquid colture** with StemPro34 and myeloid cytokines

CNTR and GD cells
N=25000
in **methylcellulose** for Colony-Forming Unit assay

Differentiation

GD - iPSC vs CNTR iPSC

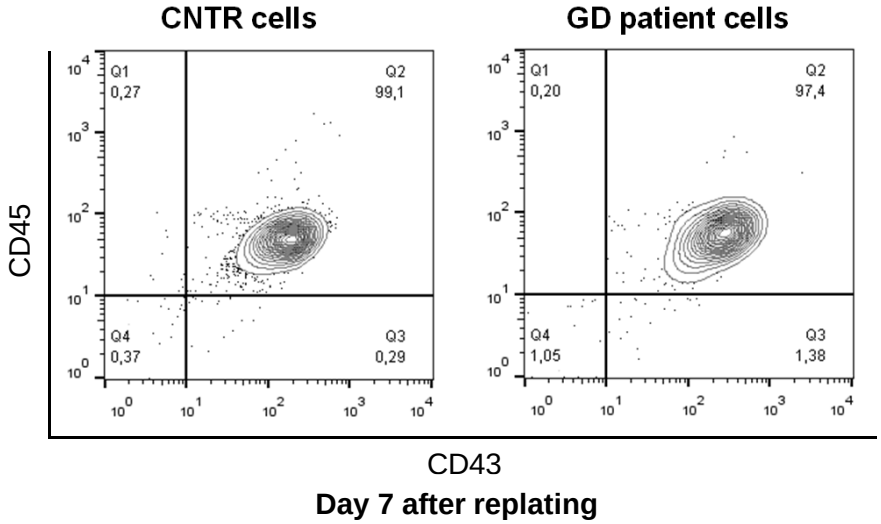


Increase of the expression of GBA after differentiation towards the myeloid fate

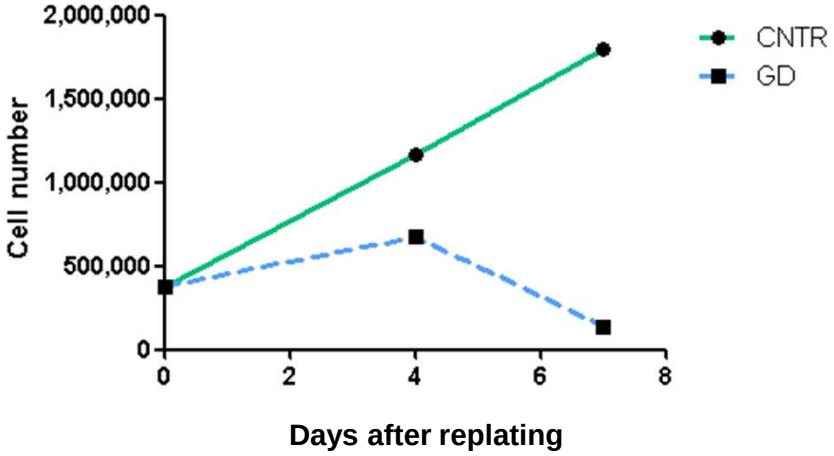
Differentiation

GD - iPSC vs CNTR iPSC

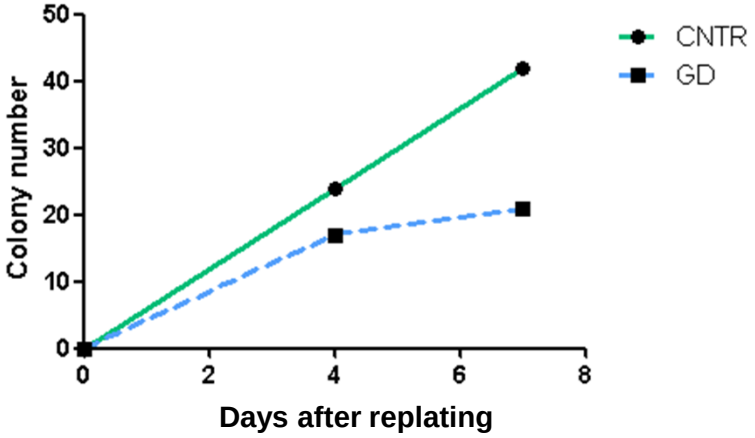
Liquid colture



Day 7 after replating



Methycellulose colture



Next steps…

- Differentiation of iPSCs towards the monocytic/macrophagic fate
  → cells mainly involved in GD
- Analysis of the necroptosis pathway effectors on iPSC model
- Development of a new gene editing strategy to correct the mutation

Gaucher Disease

Study group

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Dott.ssa Jessica Bandini
Dott. Salvatore Serravalle

Cancer Revolution Lab
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Thank you for your attention!