



webinar

PATIENTS

Patients Webinar: Gene therapy for Pyruvate Kinase Deficiency and Congenital Dyserythropoietic Anemia Type II

J.C. Segovia, M. Dessy and O. Quintana
CIEMAT/CIBERER/IIS-FJD

15 September 2025



DISCLOSURES:

Jose-Carlos Segovia is Consultant of Rocket Pharmaceuticals, Co-Founder and Scientific Advisor of DanausGT Biotechnology

Oscar Quintana-Bustamante and Mercedes Dessy-Rodriguez, nothing to disclose



Summary

1

Survey: Expectations/requirements for a potential gene therapy for Pyruvate Kinase Deficiency (PKD) and Congenital Dyserythropoietic Anemia (CDA)

Speaker Oscar QUINTANA-BUSTAMANTE
CIEMAT/CIBERER/IIS-FJD

2

Gene Therapy for Pyruvate Kinase Deficiency (PKD)

Speaker José Carlos Segovia Sanz – Jose Luis López Lorenzo
CIEMAT/CIBERER/IIS-FJD IIS-FJD

3

Gene Therapy for Congenital Dyserythropoietic Anemia type II (CDAlI): Ple-clinical studies

Speaker Mercedes Dessy Rodríguez
CIEMAT/CIBERER/IIS-FJD

01

Survey:

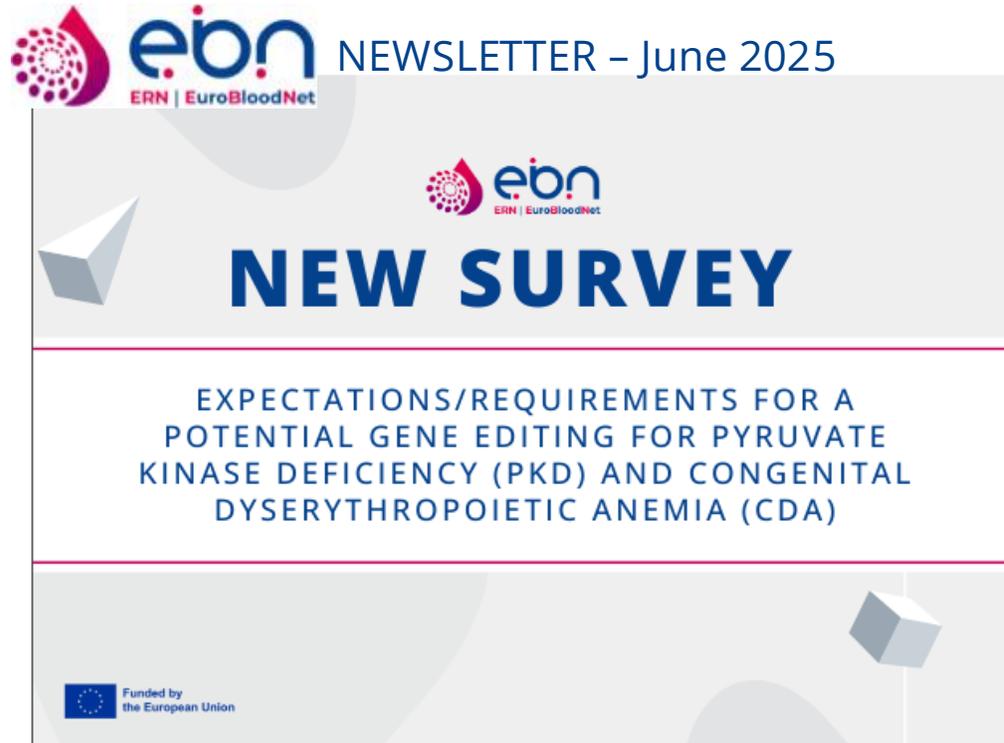
Expectations/requirements for a potential gene therapy for Pyruvate Kinase Deficiency (PKD) and Congenital Dyserythropoietic Anemia (CDA)

Speaker Oscar QUINTANA-BUSTAMANTE
CIEMAT/CIBERER/IIS-FJD

Aim



To better understand the expectations, concerns, and requirements of patients affected by Pyruvate Kinase Deficiency (PKD) and Congenital Dyserythropoietic Anemia (CDA) regarding gene therapy



t.ly/nbYe2



Open until: December 31, 2025.

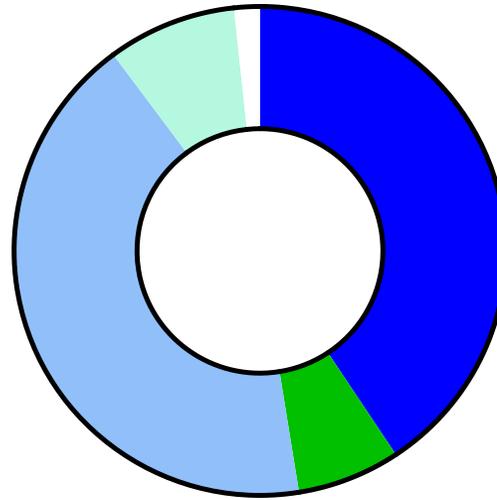
Full responses 59

Incomplete responses 435

Total responses 494

Demographics

Total=59



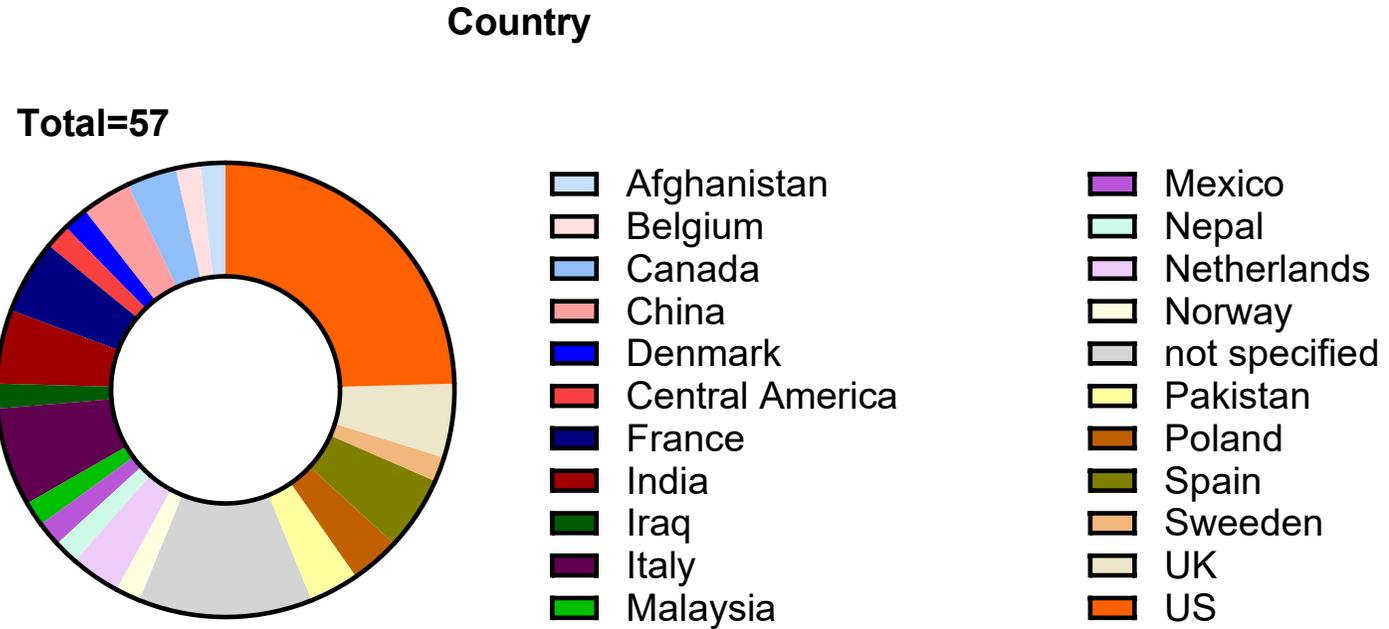
- 40.68% PKD patient
- 6.78% CDA patient
- 42.37% Relative/friend of PKD patient
- 8.47% Relative/friend of CDA patient
- 1.69% No answer

78.9% of the relatives/friends are the patient's parents





Demographics



Answers from more than 21 countries

Impact of the disease: How does PKD or CDAlI affect the following activities in your life?



English



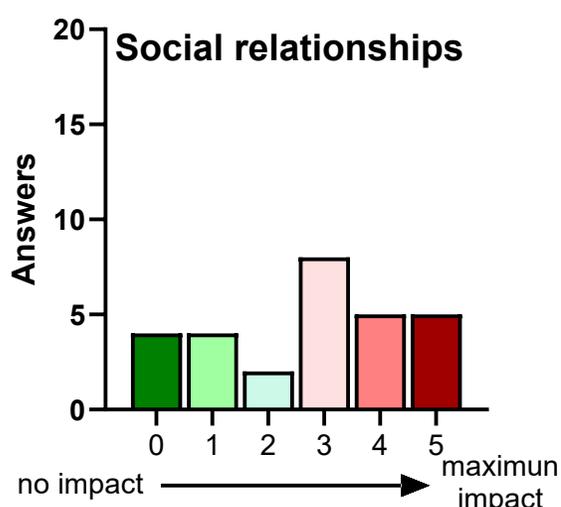
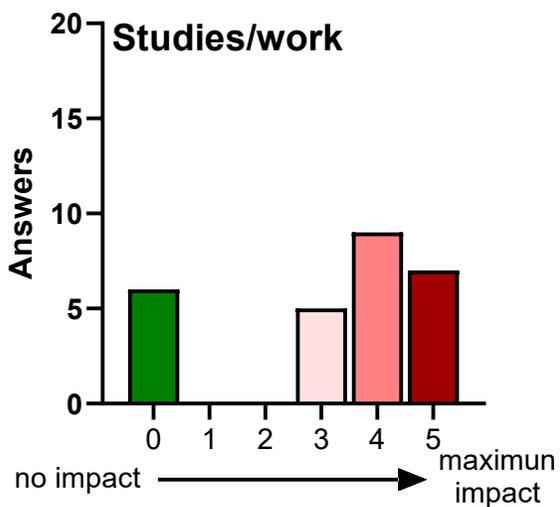
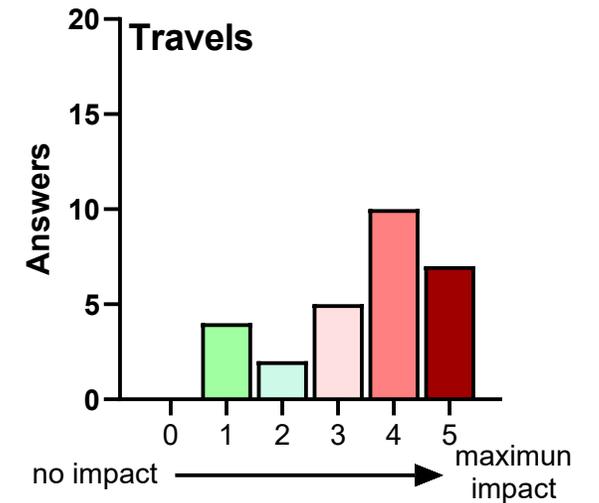
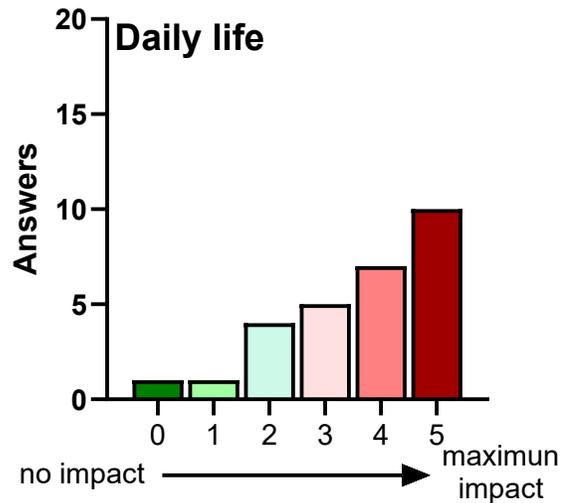
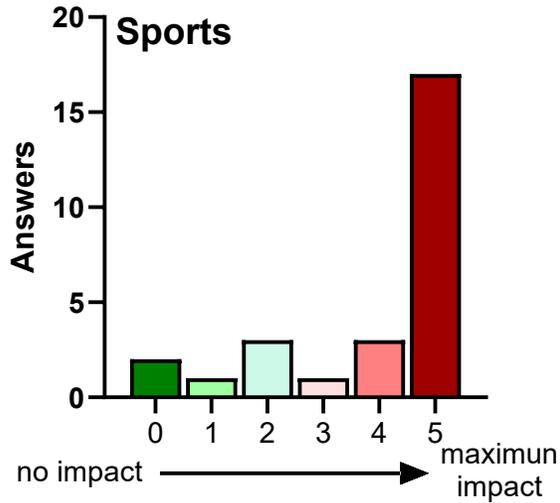
Español



Français



Italiano



Impact of the disease: What aspect of PKD or CDAll would you like to eliminate?



English



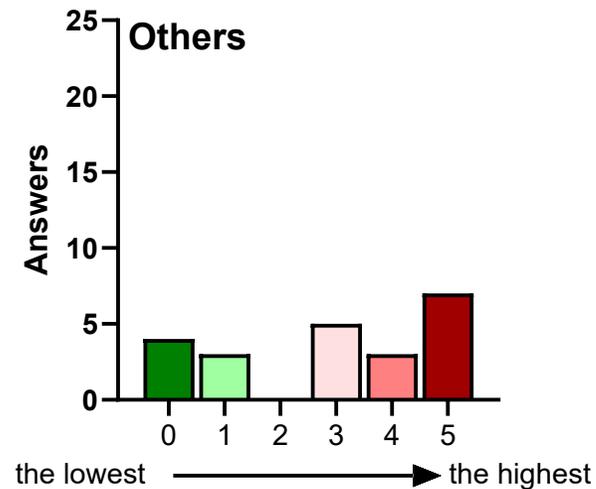
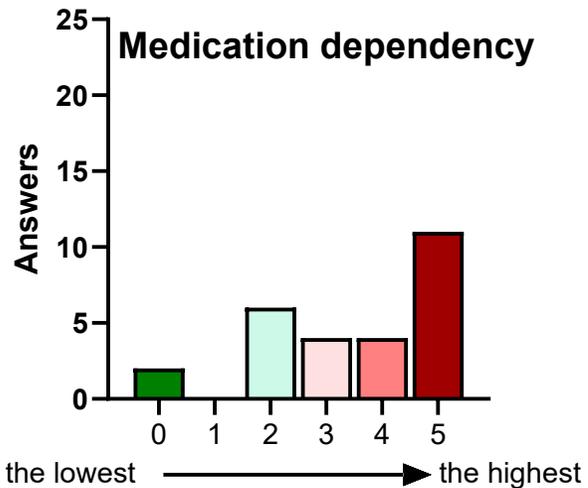
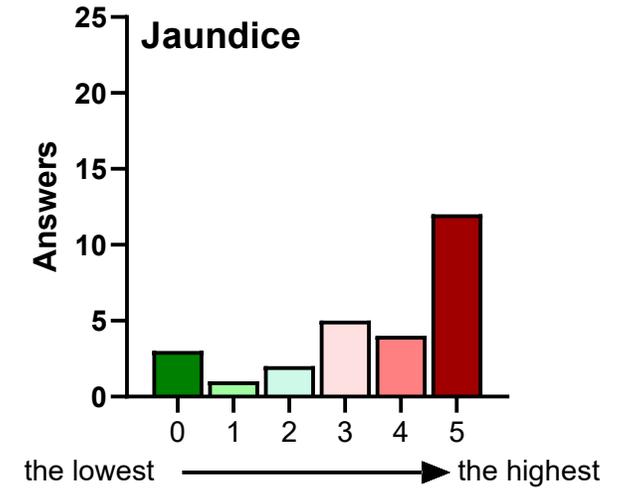
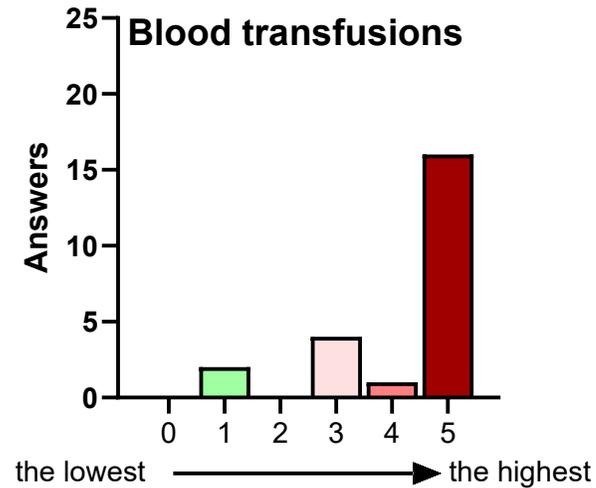
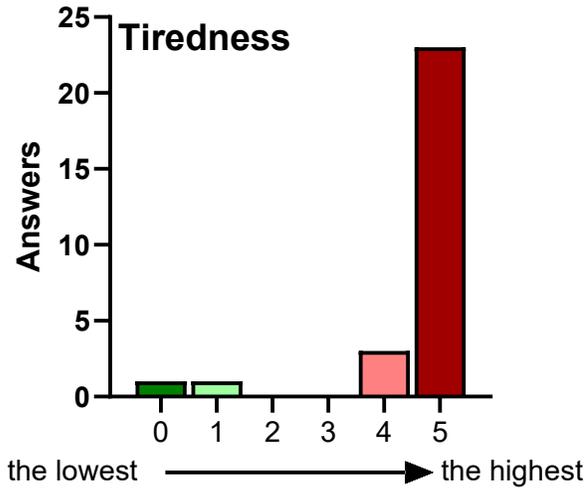
Español



Français



Italiano



Treatments: Which of the following should be the main characteristic of an ideal therapy

for PKD or CDA?



English



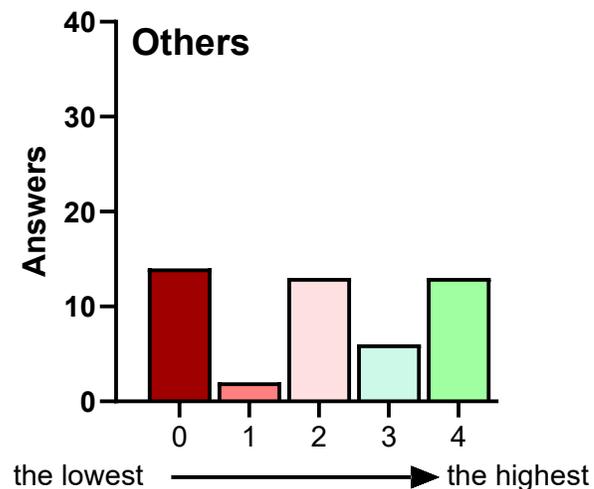
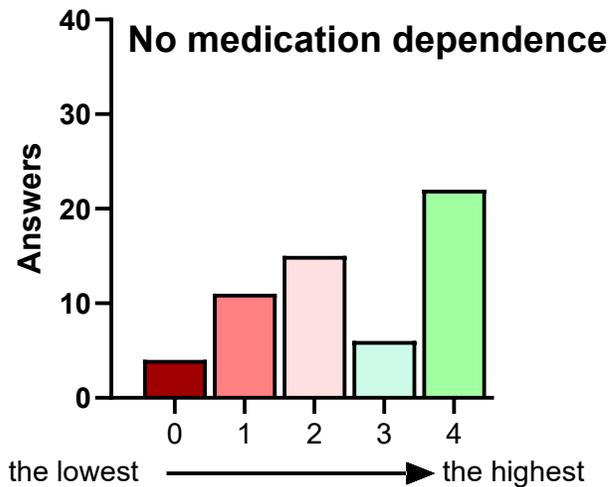
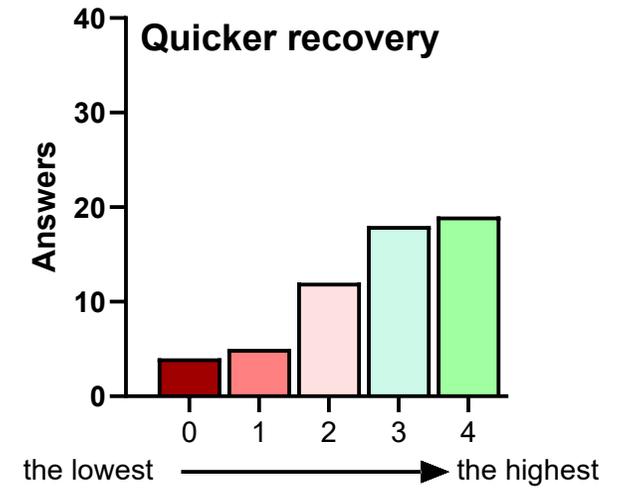
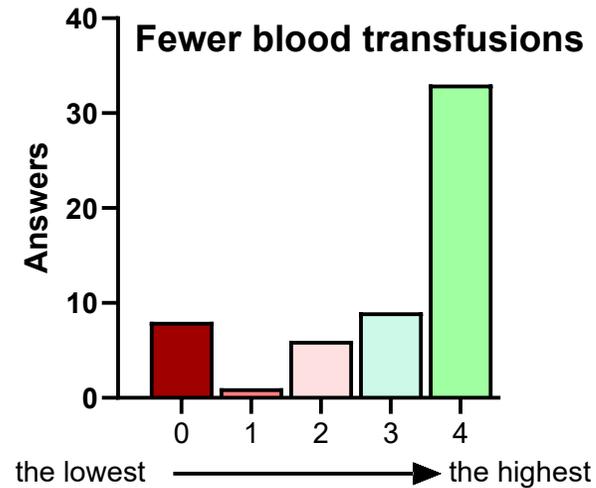
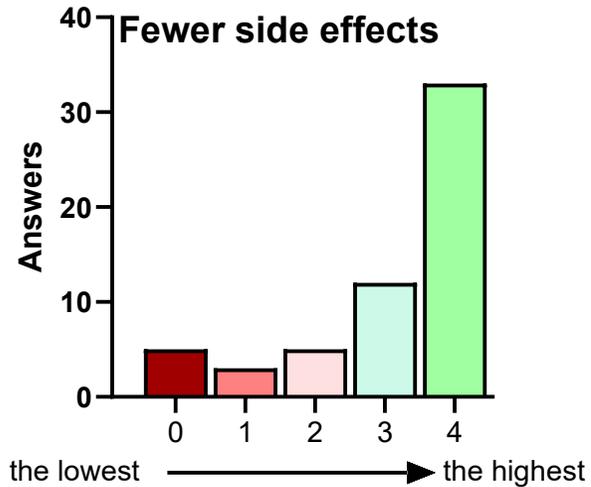
Español



Français



Italiano

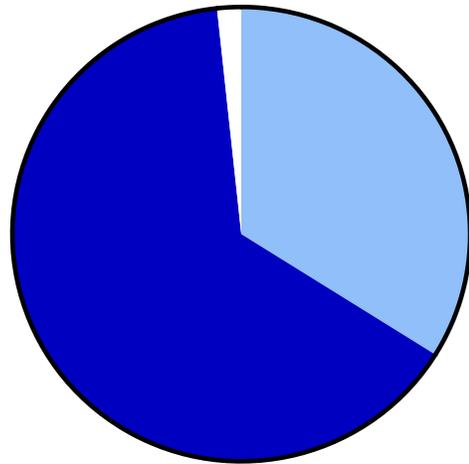




Gene therapy:

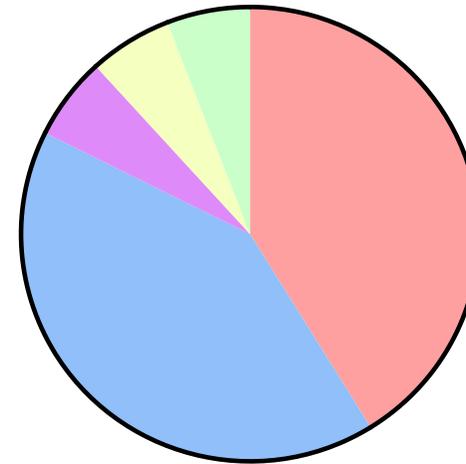


Total=59



- 33.90% Yes
- 64.41% No
- No answer

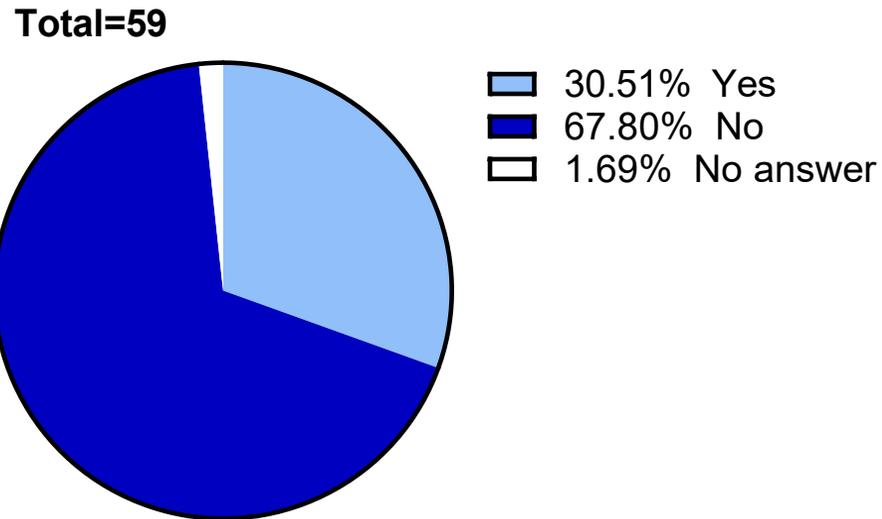
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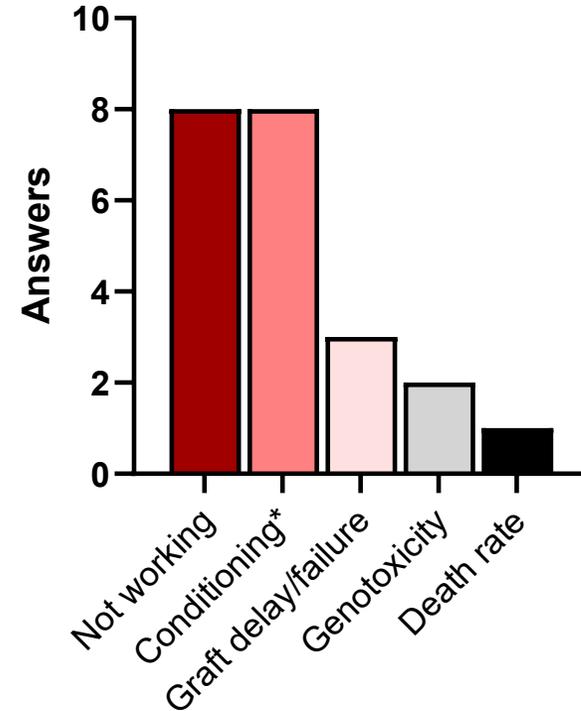
- 7 Thal-SCD
- 7 PKD
- 1 Fanconi anemia
- 1 Hemophilia
- 1 CPS1 deficiency



Gene therapy: Do you know of the **side effects of gene therapy** for inherited blood diseases?



The percentages of people who know about diseases treated with gene therapy, the steps of gene therapy, and its side effects are similar.



**Conditioning associated side effects: risk of infection, organ damage, infertility, cancer risk*

Gene therapy for PKD or CDA: What are your opinions on a possible gene therapy-based treatment for PKD or CDA?



English



Español



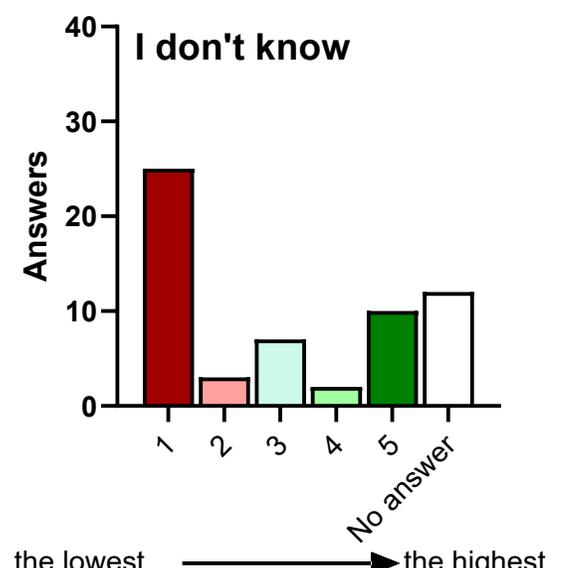
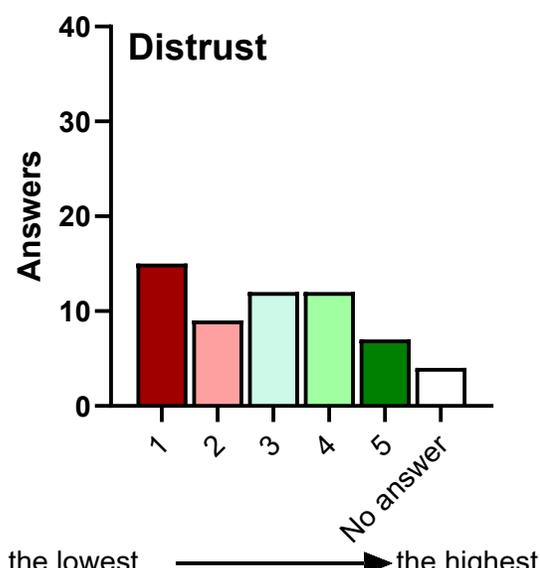
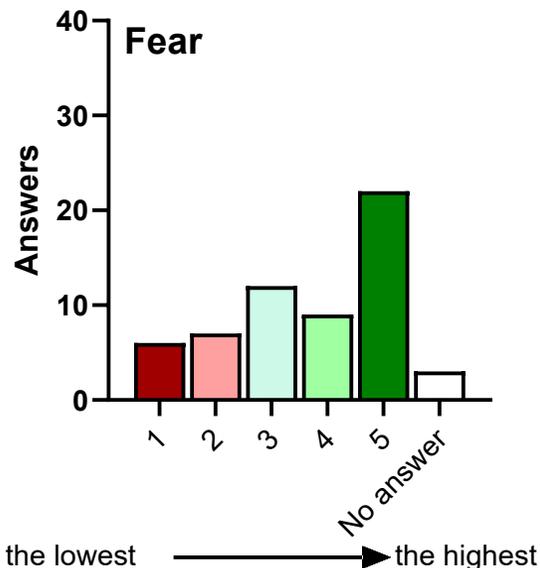
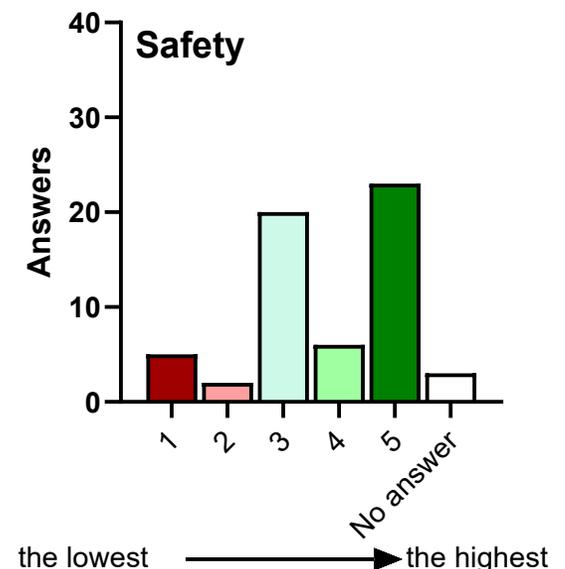
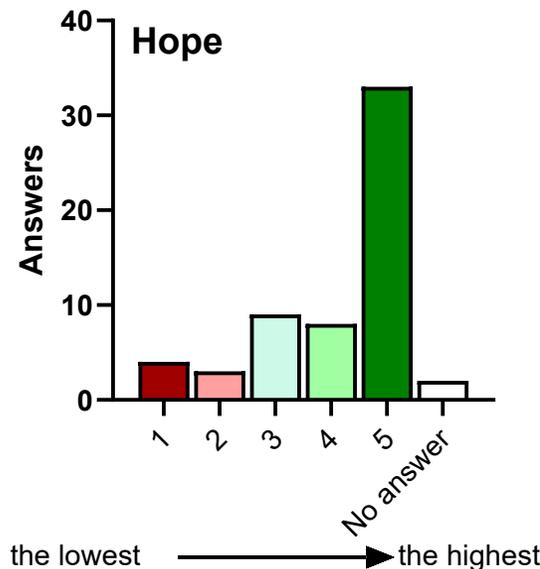
Français



Italiano



treatment for PKD or CDA?



Gene therapy for PKD or CDA: Do you think gene therapy might be a potential treatment for PKD or CDA?



 English



 Español



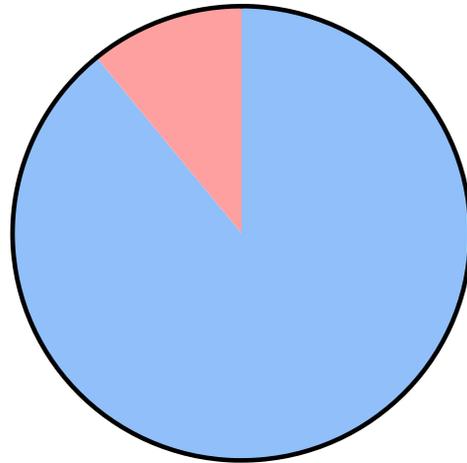
 Français



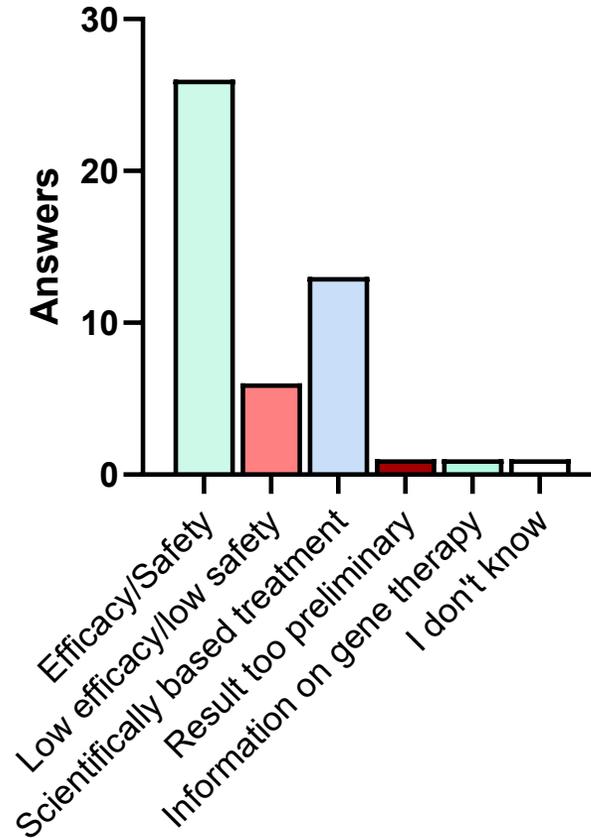
 Italiano



Total=55



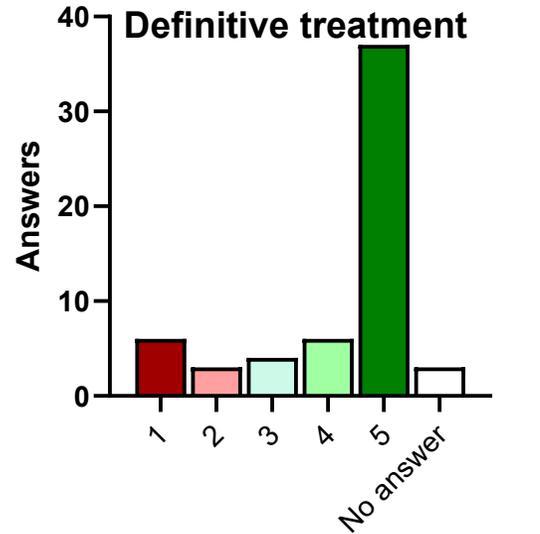
89.09% Yes
10.91% No



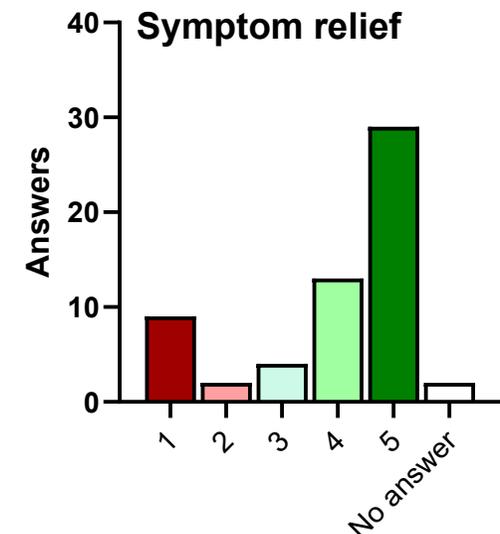
Gene therapy for PKD or CDA: What are your expectations for a potential gene therapy for



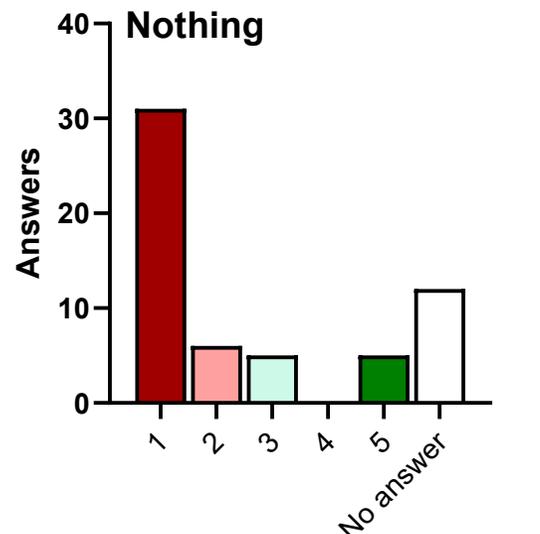
PKD or CDA?



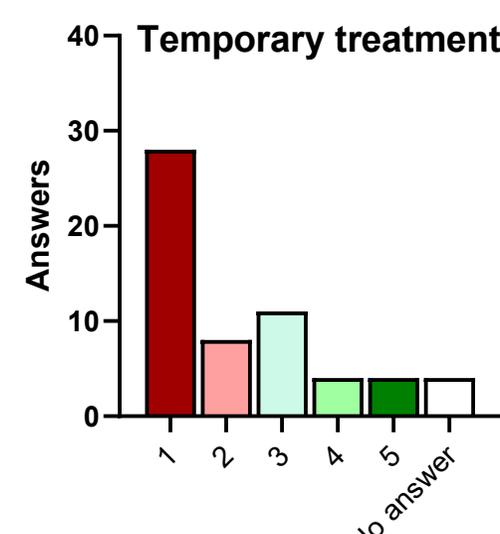
the lowest → the highest



the lowest → the highest



the lowest → the highest



the lowest → the highest

Gene therapy for PKD or CDA: Would you undergo gene therapy to correct PKD or CDA if available?



 English



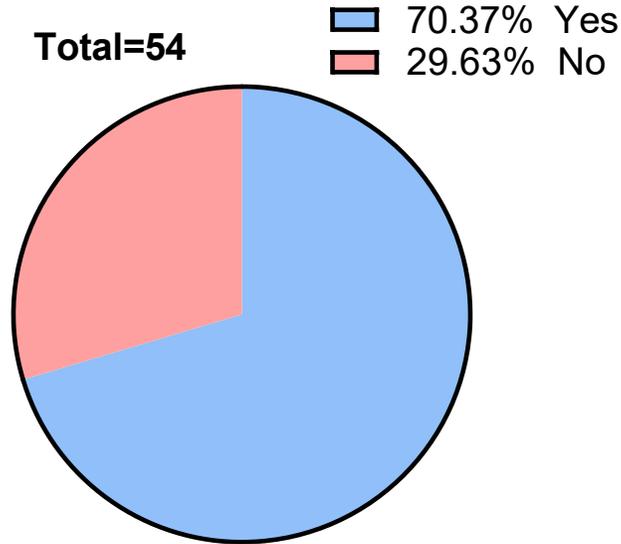
 Español



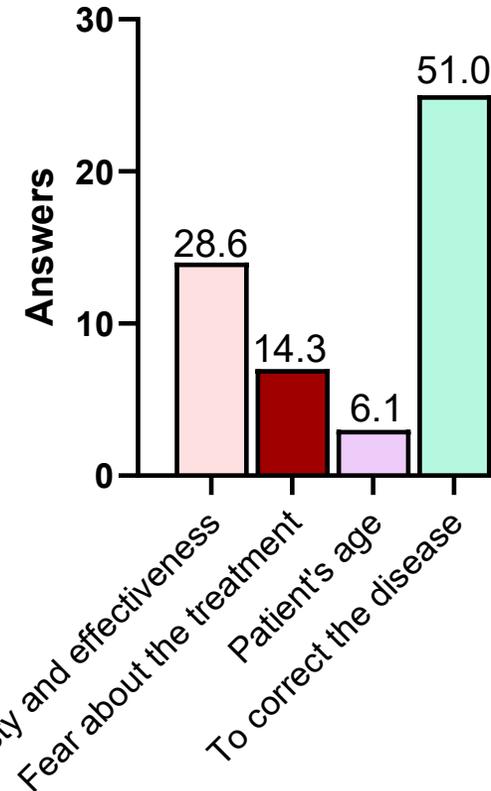
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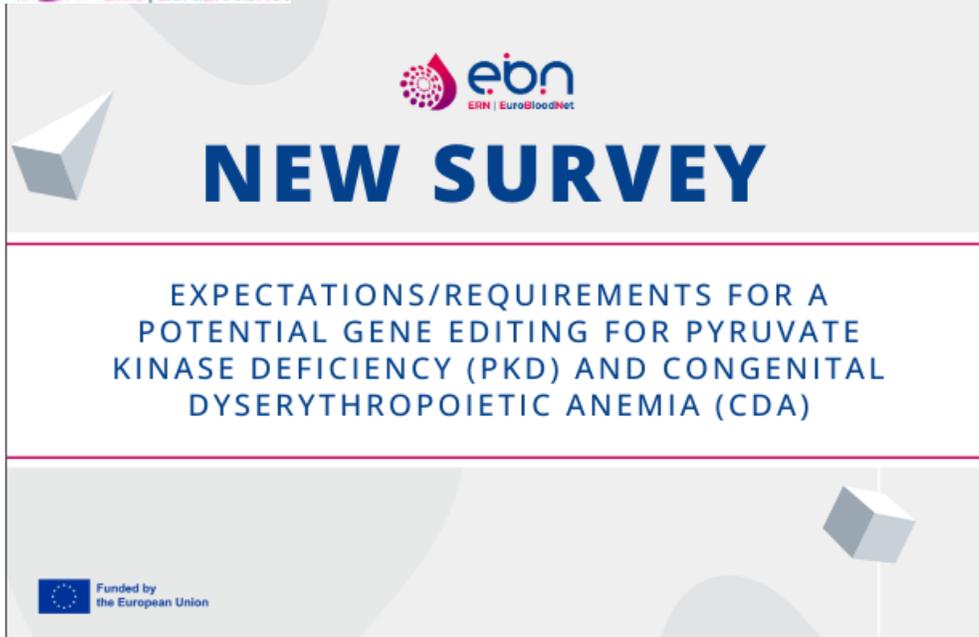
Although more people would undergo gene therapy, the percentage is slightly lower than the percentage of people who view gene therapy as a potential treatment.



**Percentages are indicated*



NEWSLETTER – June 2025



NEW SURVEY

EXPECTATIONS/REQUIREMENTS FOR A
POTENTIAL GENE EDITING FOR PYRUVATE
KINASE DEFICIENCY (PKD) AND CONGENITAL
DYSERYTHROPOIETIC ANEMIA (CDA)

Funded by
the European Union

t.ly/nbYe2



Español



Français



English



Italiano



Please participate in the survey and share it with others.

Your input is important!

Thank you!

Open until: December 31, 2025.



Patients Webinar

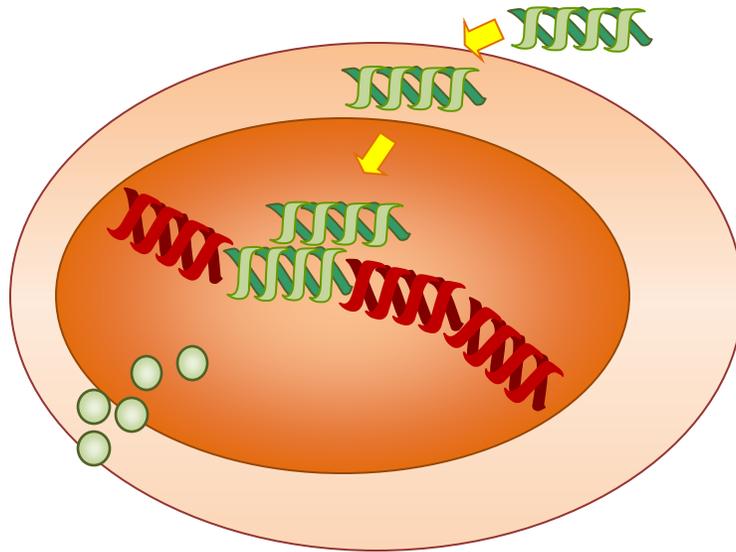
Gene therapy for Pyruvate Kinase Deficiency and Congenital Dyserythropoietic Anemia Type II

02

Gene Therapy for Pyruvate Kinase Deficiency:

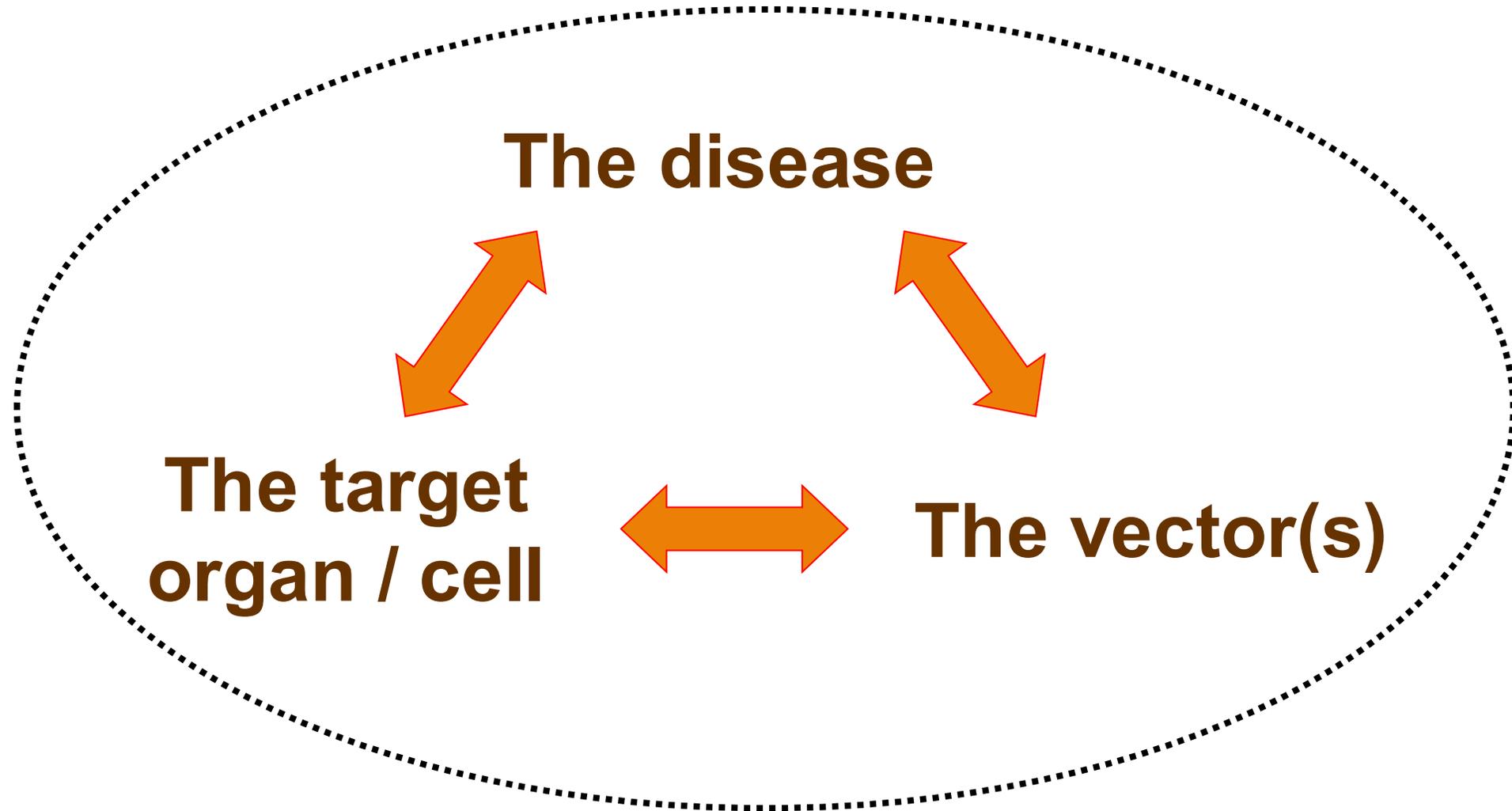
Speaker José Carlos Segovia Sanz
CIEMAT/CIBERER/IIS-FJD

Gene Therapy



To introduce nucleic acid sequences in the cell to allow the expression of the healthy protein that the patient lacks because of mutations in the inherited gene

Gene Therapy



Unmet Need: Pyruvate Kinase Deficiency (PKD)



Disease

- Monogenic rare genetic disorder characterized by **premature destruction of red blood cells** due to energetic deficit
- >300 mutations identified in **PKLR gene**, condition inherited in an autosomal recessive pattern.



Epidemiology

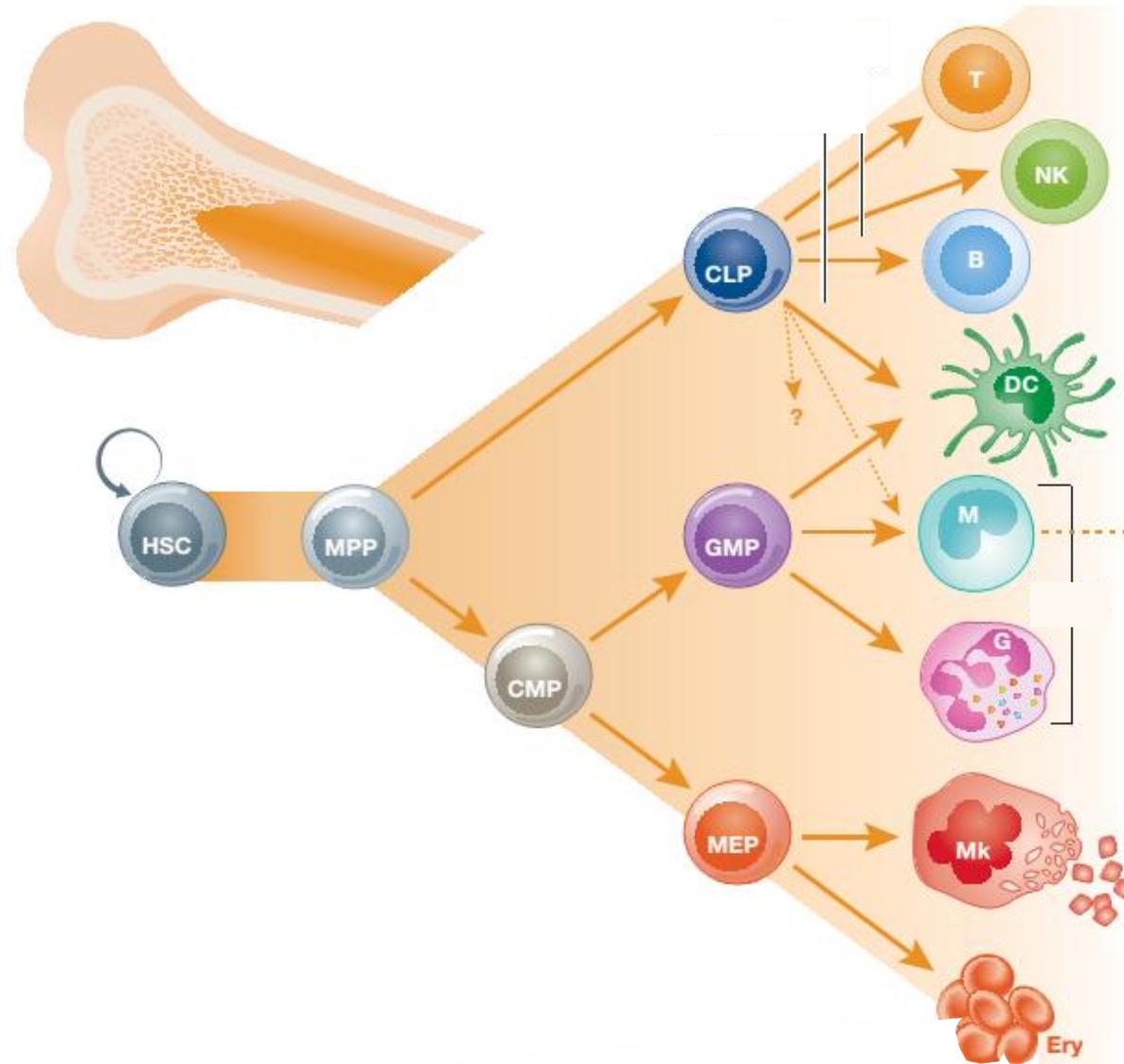
- PREVALENCE: **1/ 2 x 10⁴** in Caucasian population.
 - Published 51 per million in non-Hispanic caucasians. Conservative estimates conclude a number from **3,000 to 8,000 in the US+EU combined.**
- INCIDENCE: Unknown, underestimated



Current treatment options

- PALLIATIVE: Chronic blood transfusions, splenectomy—side effects include iron overload and extensive end-organ damage
- CURATIVE (not in the routine practice): **Bone marrow transplant** (important side effects); **Gene therapy**

Gene Therapy in the Hematopoietic System



Why Hematopoietic Stem Cells are the target?



Erythrocytes / Red Blood Cells

- Short half-life: 90 days
- Need to be replenish

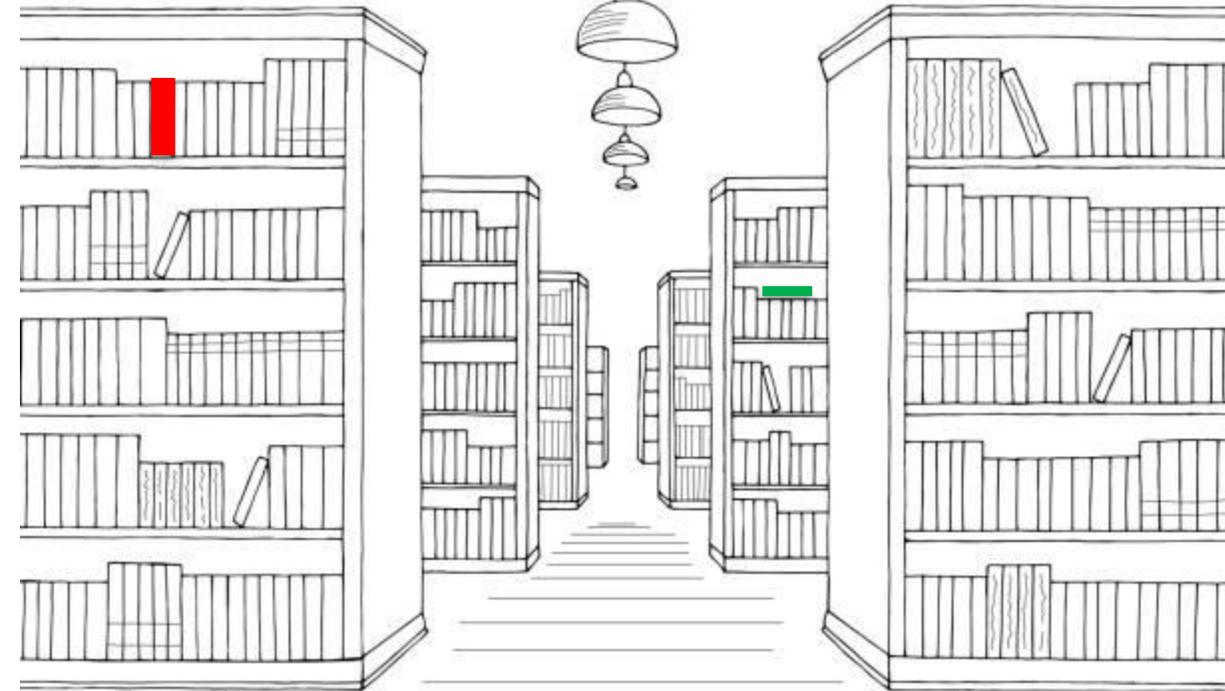


Hematopoietic Stem Cell (HSC)

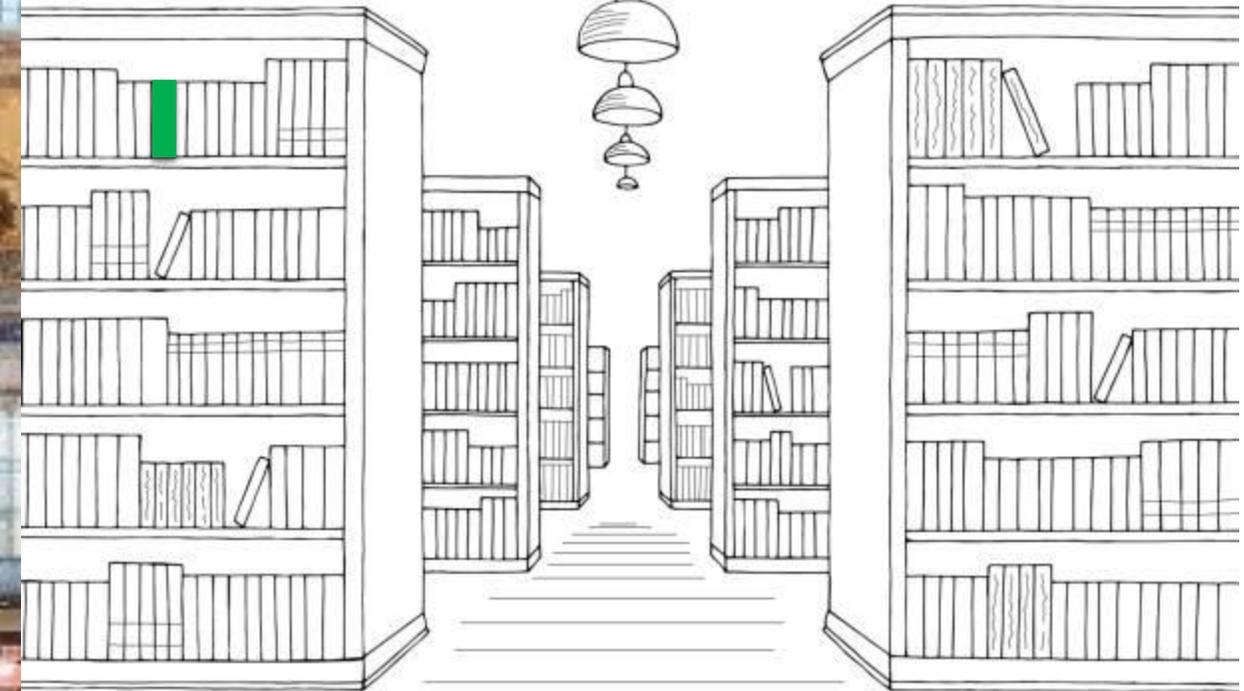
- Lives all life long
- Generates new HSC
- Its job is to produce all blood cells all life long

Gene Addition vs Gene Editing

Lentiviral vector Gene Addition



CRISPR Gene Editing

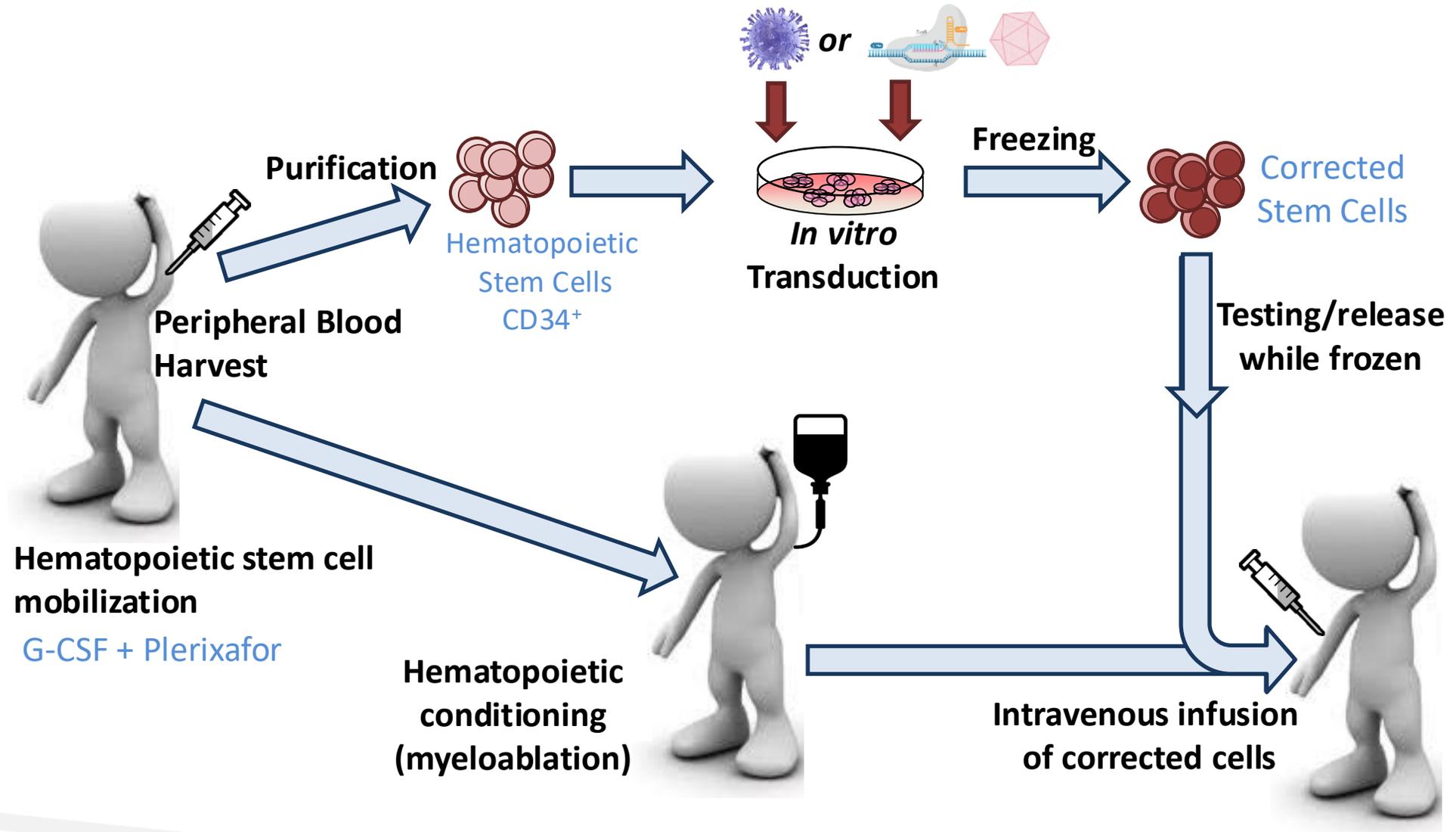


- Very efficient
- Already approved



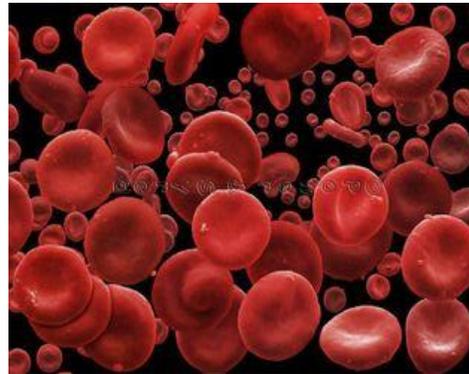
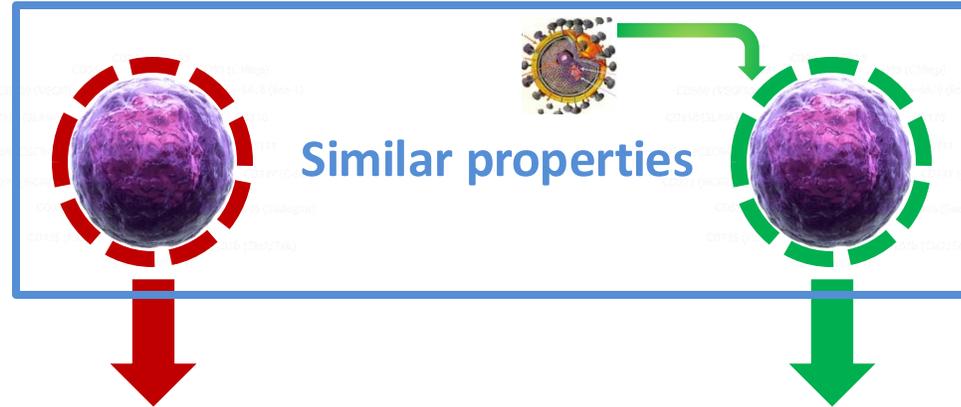
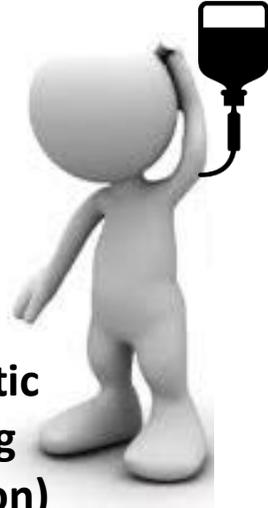
- Less efficient
- Require improvements

Overview of the Clinical Protocol



Why do we need conditioning (myeloablation)?

Hematopoietic conditioning (myeloablation)



Defective erythrocytes



Healthy erythrocytes

- To assure engraftment of corrected cells

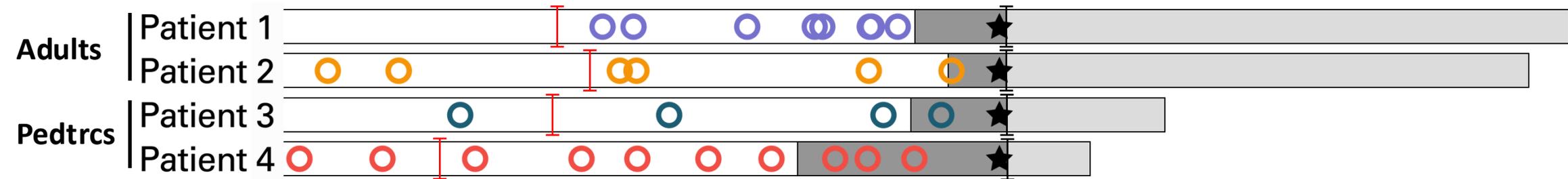
- I think the worst thing is that it doesn't work
- I know if you're younger you can recover quicker, if you're older you might have a more difficult time recovering from the phase where they administer the stuff



Clinical studies

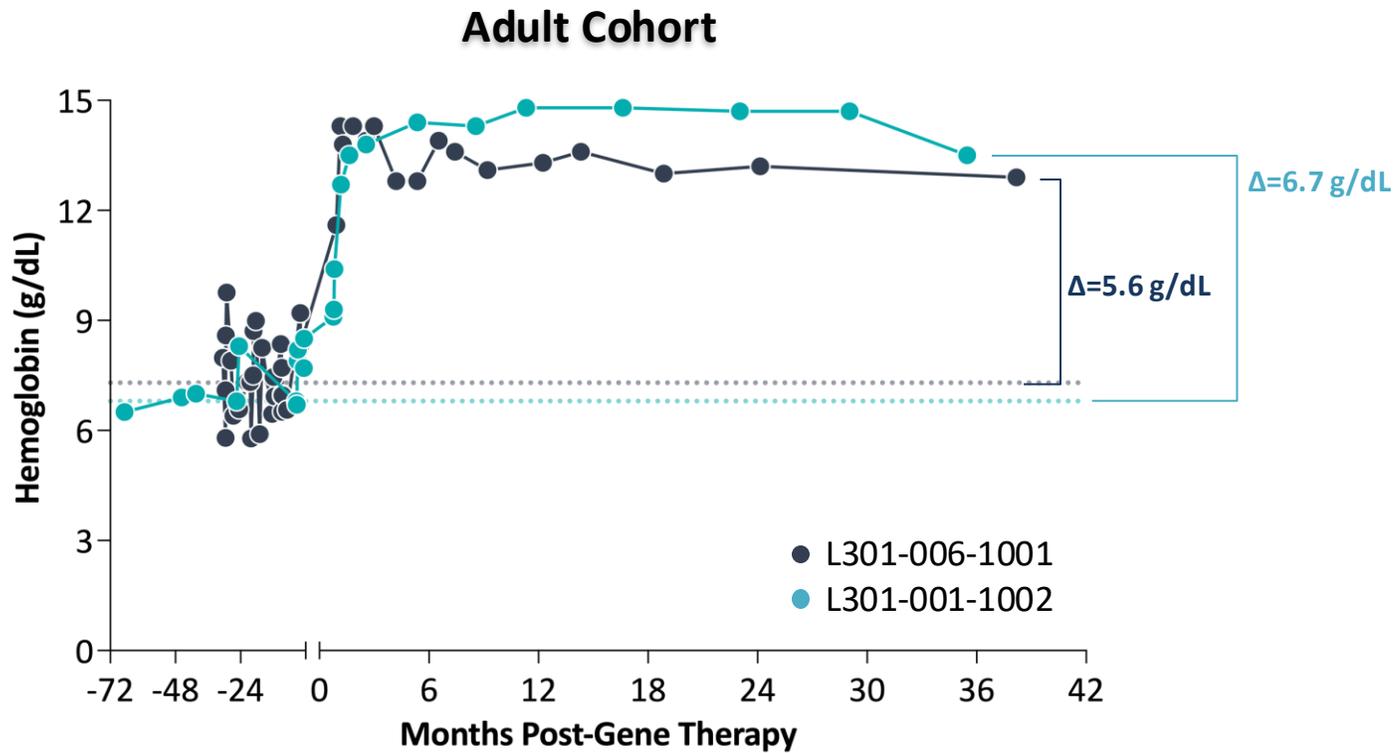
Gene Therapy Clinical Trial: RP-L301-0119

Red Blood Cell transfusion requirements stopped after engraftment of RP-L301 Drug Product

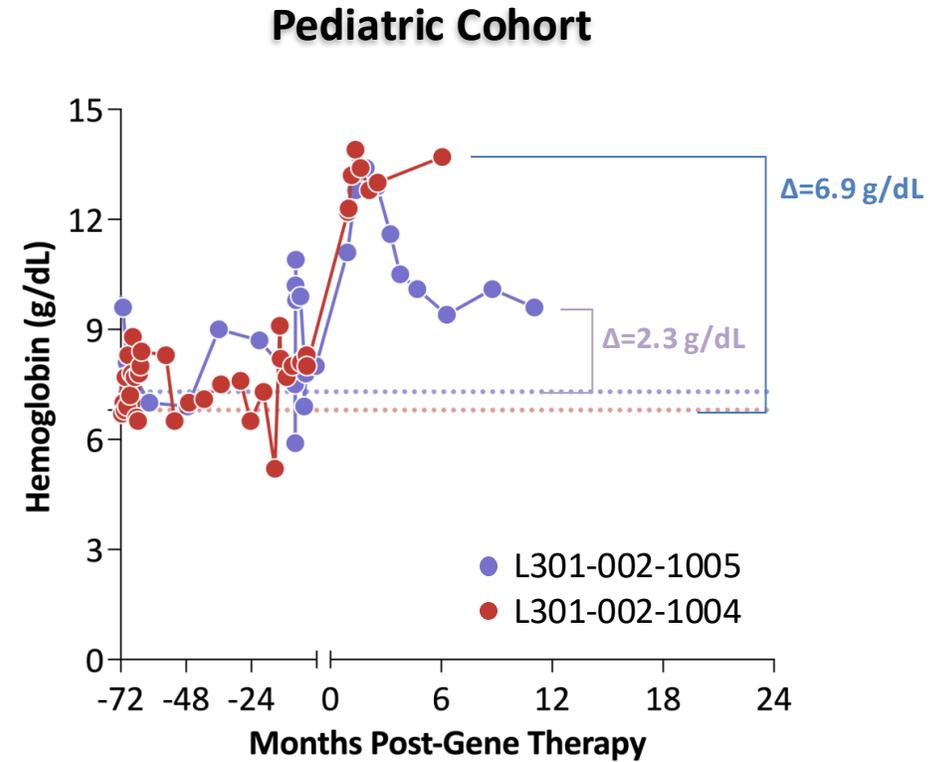


- RBC Transfusions
- I 2 years Prior to Enrollment
- ★ RP-L301 Infusion
- I Engraftment
- Screening to Engraftment Period
- Post-Engraftment Follow-up Period

RP-L301 Confers Clinically Meaningful Hemoglobin Improvement



Sustained hemoglobin normalization up to 5 years



Hemoglobin normalization or improvement from pre-treatment baseline up to 18 months

Questions / Concerns

- Possibility of your body rejecting the new bone marrow.
- Side effects...most caused by the busulfan (conditioning):
 - Risk of infection, liver injury, lose hair
 - Risk of future cancer unknown
 - Temporary low immunity, infections,
 - Fatigue from the chemotherapy before the procedure, but most of them are short-term and improve with medical follow-up
 - Acute life-threatening infection

Questions / Concerns

- Sterility
- Possibly affect future fertility

Principal Clinical Investigator of the adult cohort

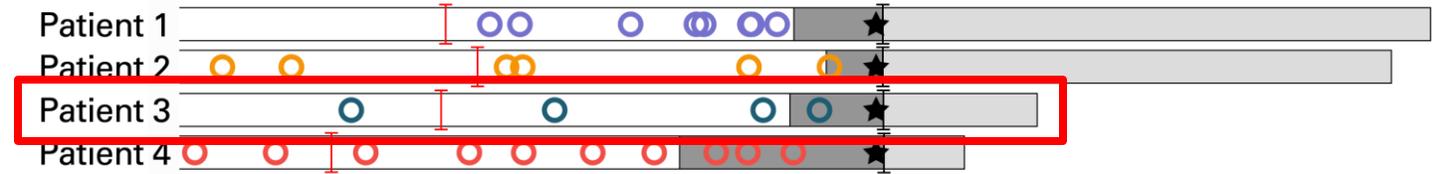
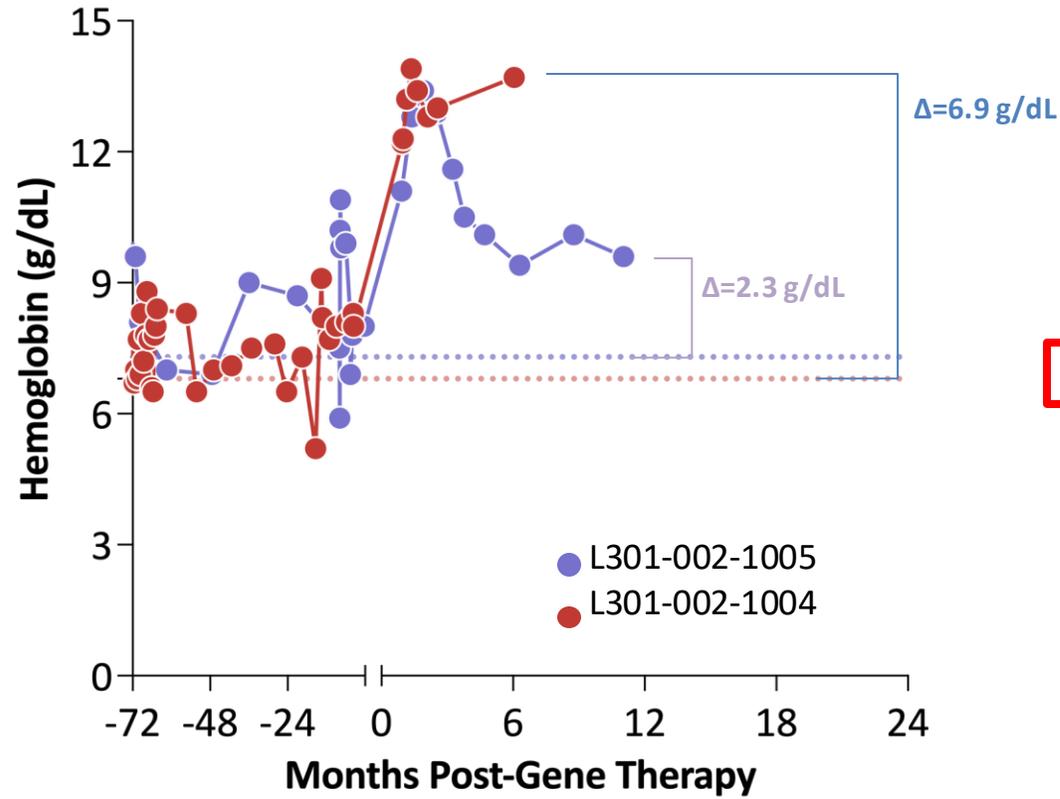


Dr. Jose-Luis López-Lorenzo

- Possibility of not complete cure after gene therapy

Blood transfusion independent – better Hb levels

Pediatric Cohort



- RBC Transfusions
- I 2 years Prior to Enrollment
- ★ RP-L301 Infusion
- I Engraftment
- Screening to Engraftment Period
- Post-Engraftment Follow-up Period

Questions / Concerns

- There have been too many deaths in gene therapy trials
- Changes to other genes which can have unknown effects in the long term

Some numbers about LVV gene therapy

With Lentiviral Vectors	in Hematopoietic Rare Diseases
1350	~300

Disease	Patients Treated	Deaths Reported	Deaths Attributed to Gene Therapy/Lentivirus
ADA-SCID	62	0	0
Wiskott-Aldrich	30	1	No direct link
Fanconi Anemia	15	0	0
Sickle Cell Disease	50	2	Unclear/linked primarily to conditioning
Beta-Thalassemia	22	0	0
Adrenoleucodystrophy (CALD)	67	2	Deaths attributed to disease progression or transplantation complications / to vector?
Pyruvate Kinase Deficiency	4	0	0
Leukocyte Adhesion Deficiency type I	9	0	0
	259	5	2?

Expanding Gene Therapy to other diseases

- There are more inherited erythroid rare diseases:
 - Pyruvate Kinase Deficiency (PKD)
 - Sickle Cell Diseases (SCD)
 - Beta-Thalassemia (B-Thal)
- Congenital Dyserythropoietic Anemia type II (CDAII)
- Glucose Phosphate Isomerase Deficiency (GPI)

Expanding Gene Therapy to other diseases

- There are more inherited erythroid rare diseases:
 - Pyruvate Kinase Deficiency (PKD)
 - Sickle Cell Diseases (SCD)
 - Beta-Thalassemia (B-Thal)
- **Congenital Dyserythropoietic Anemia type II (CDAII)**
 - Mercedes Dessy, PhD
- Glucose Phosphate Isomerase Deficiency (GPI)

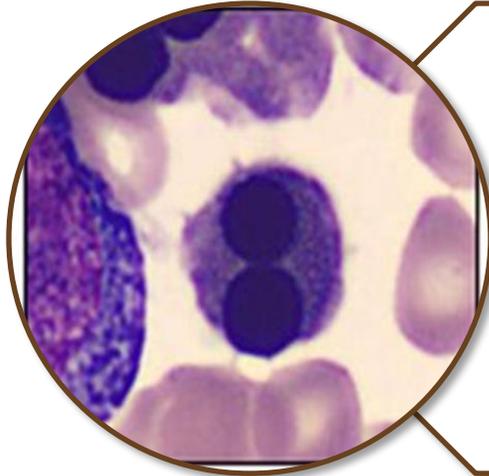
02

Gene Therapy for Congenital Dyserythropoietic Anemia type II

Speaker Mercedes Dessy Rodríguez
CIEMAT/CIBERER/IIS-FJD



Congenital Dyserythropoietic Anemia Type II: What is CDAII?



Disease

- Rare inherited disorder caused by **mutations in the *SEC23B* gene**
- Very low incidence: **0.71 cases/million**
- **Ineffective erythropoiesis**
- Accumulation of **bi/multinucleated erythroblasts** in bone marrow



Current treatment options

- PALLIATIVE: Chronic blood transfusions, iron chelation, splenectomy
- CURATIVE (not in the routine practice): **Bone marrow transplant** (important side effects)

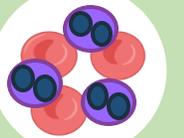


Aim: New therapeutic approaches for CDAII

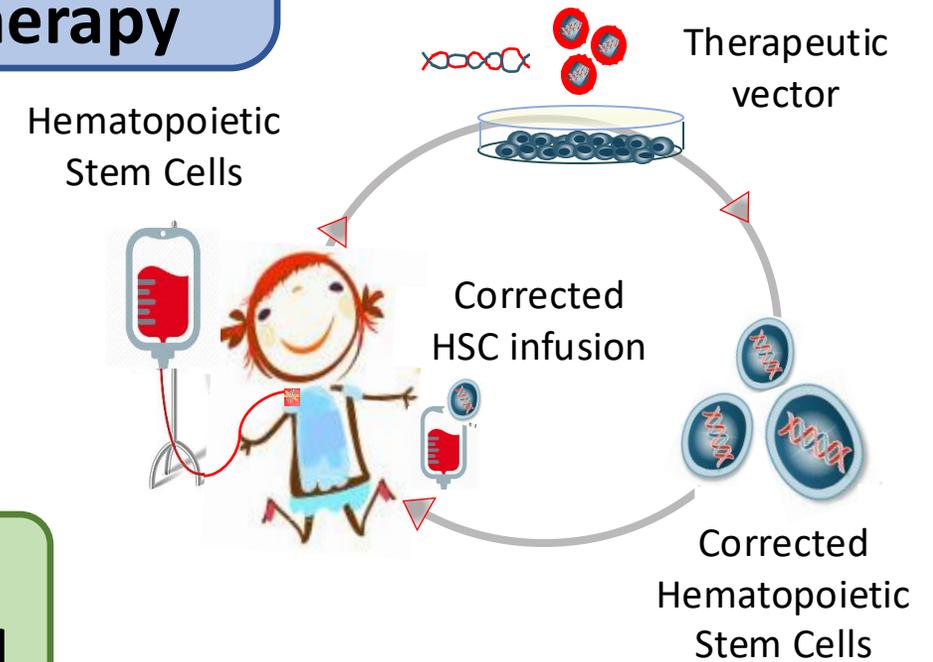
**New
therapeutic
approaches
for CDAII**



**Lentiviral-mediated
hematopoietic stem
cell gene therapy**

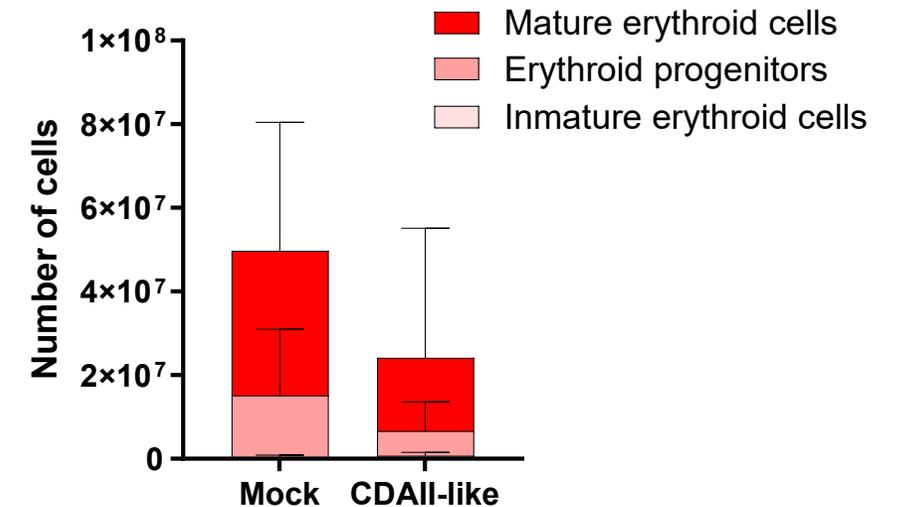
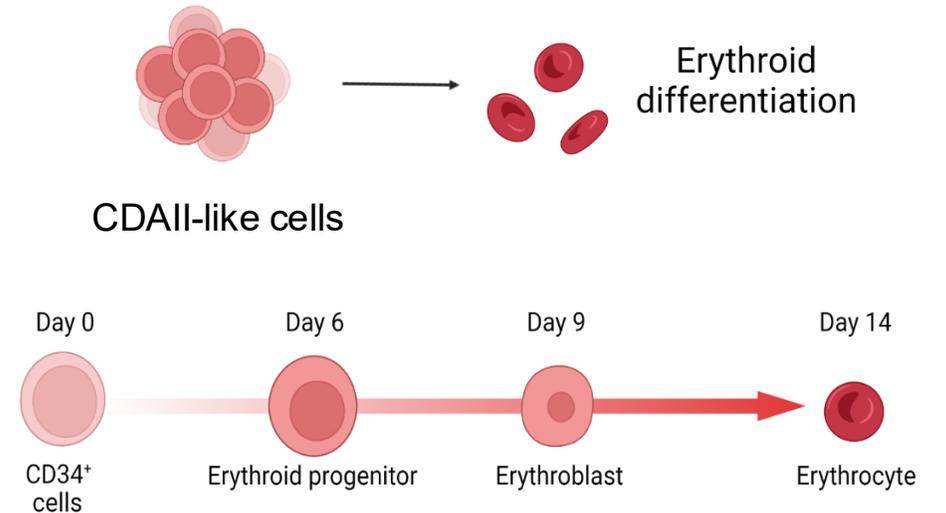
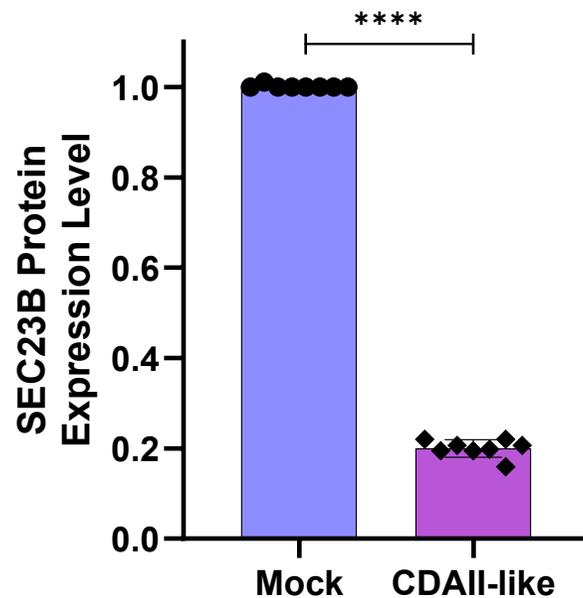
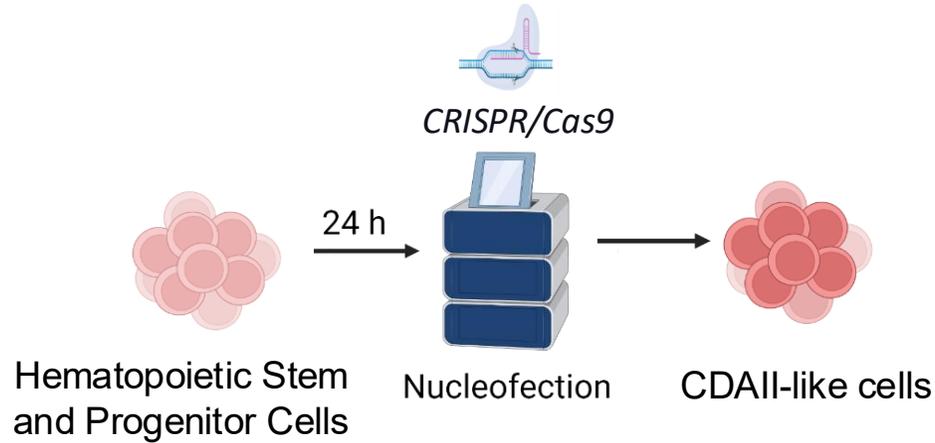


**Human cell
model of CDAII**





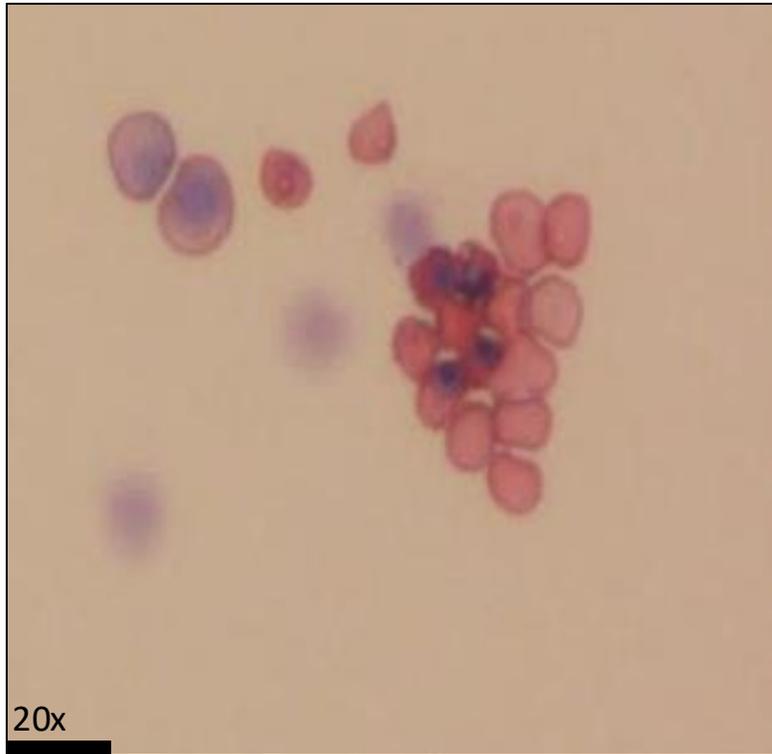
Cellular model reproduces CDAll patients' characteristics *in vitro*



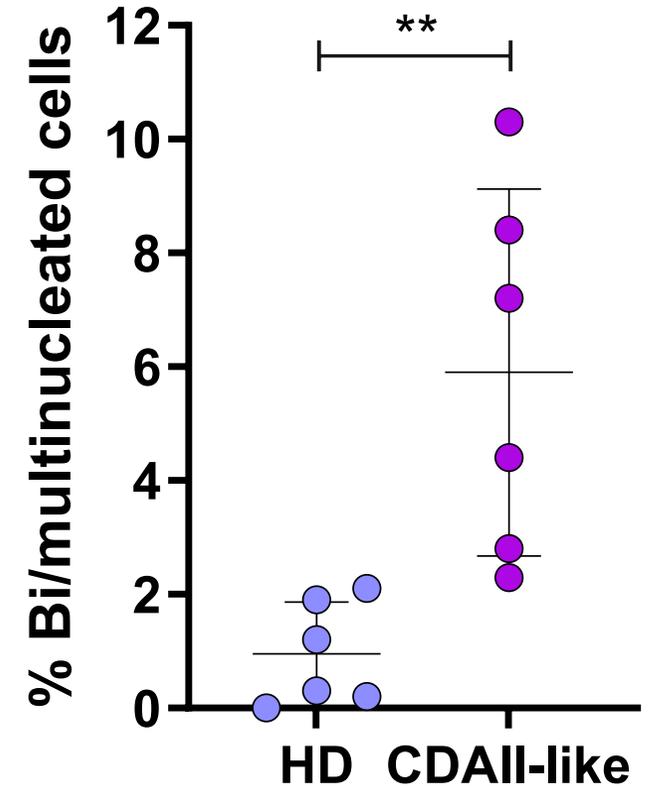
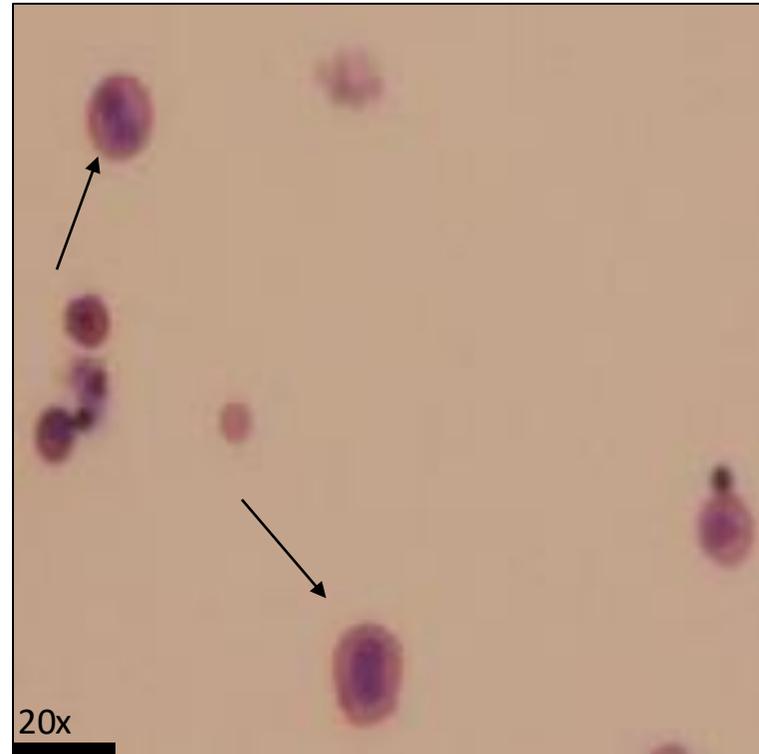


Cellular model reproduces CDAll patients' characteristics *in vitro*

Mock

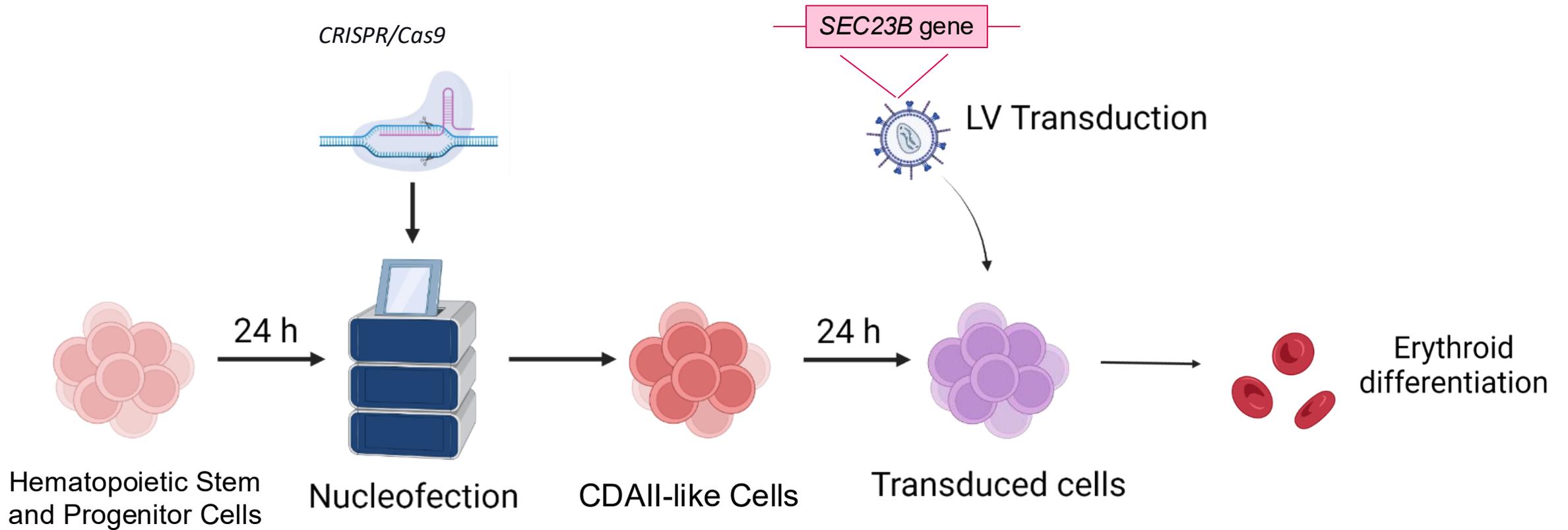


CDAll-like



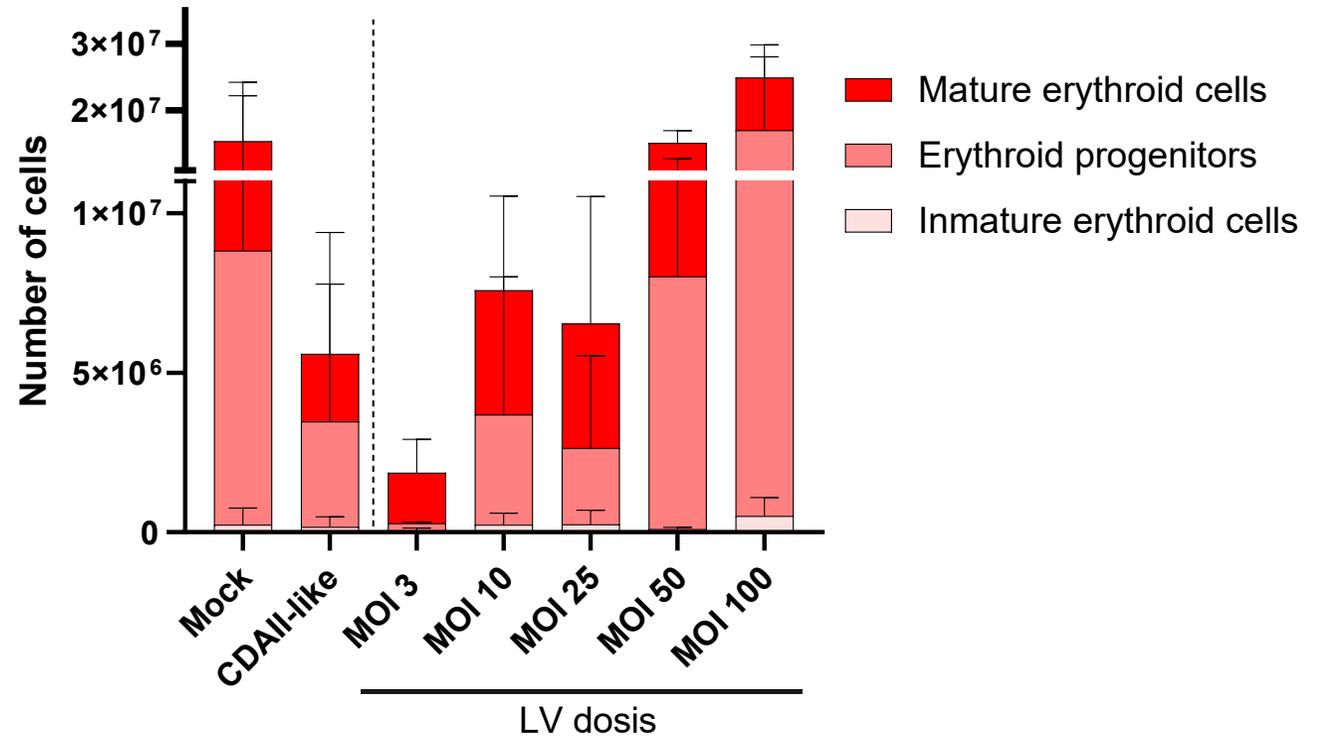
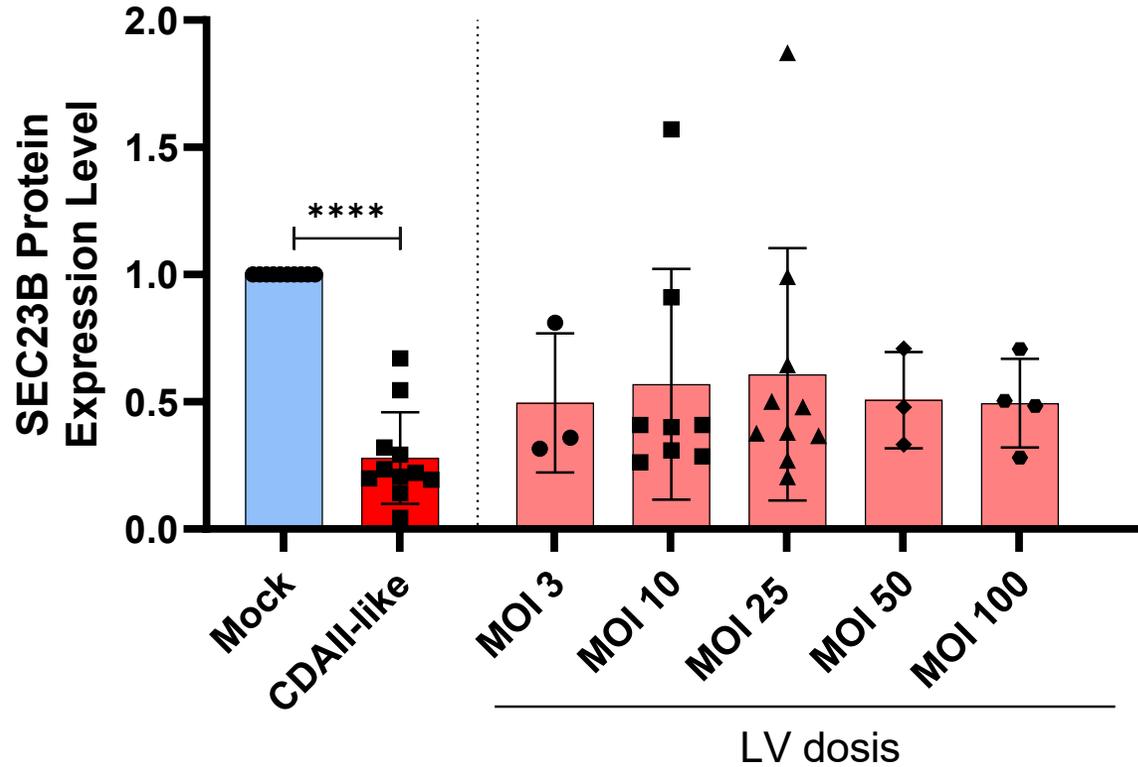
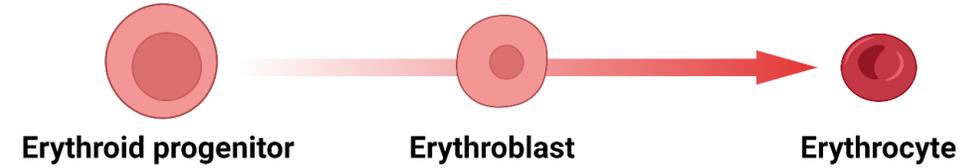


Lentiviral gene therapy for CDAll





Lentiviral gene therapy rescues CDAll phenotype in our in vitro cellular model



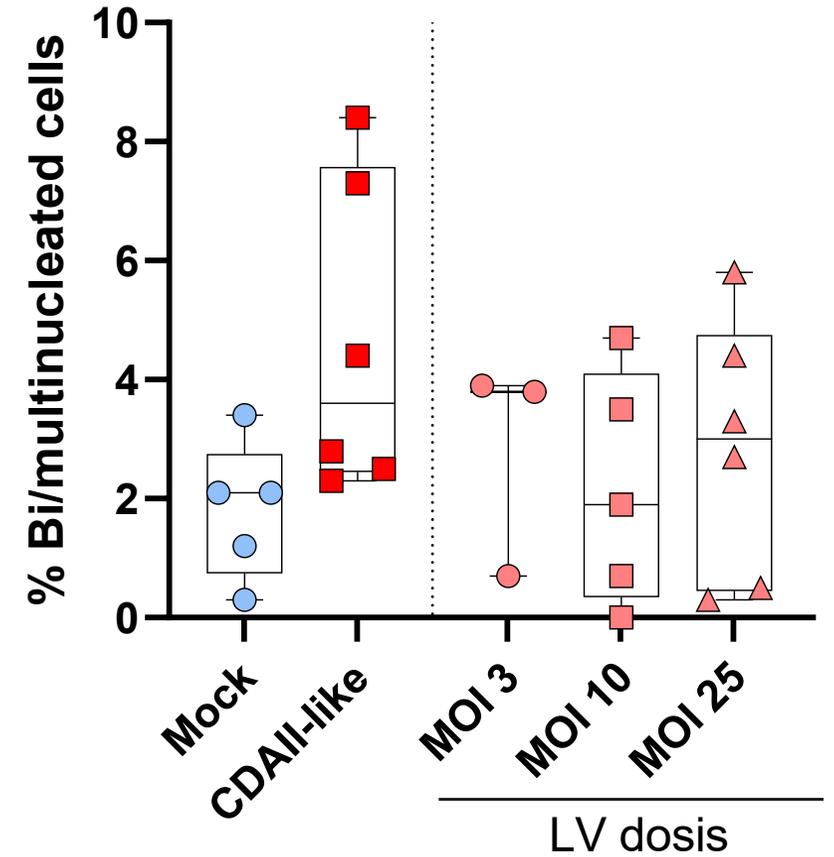


Lentiviral gene therapy rescues CDAll phenotype in our in vitro cellular model

CDAll-like

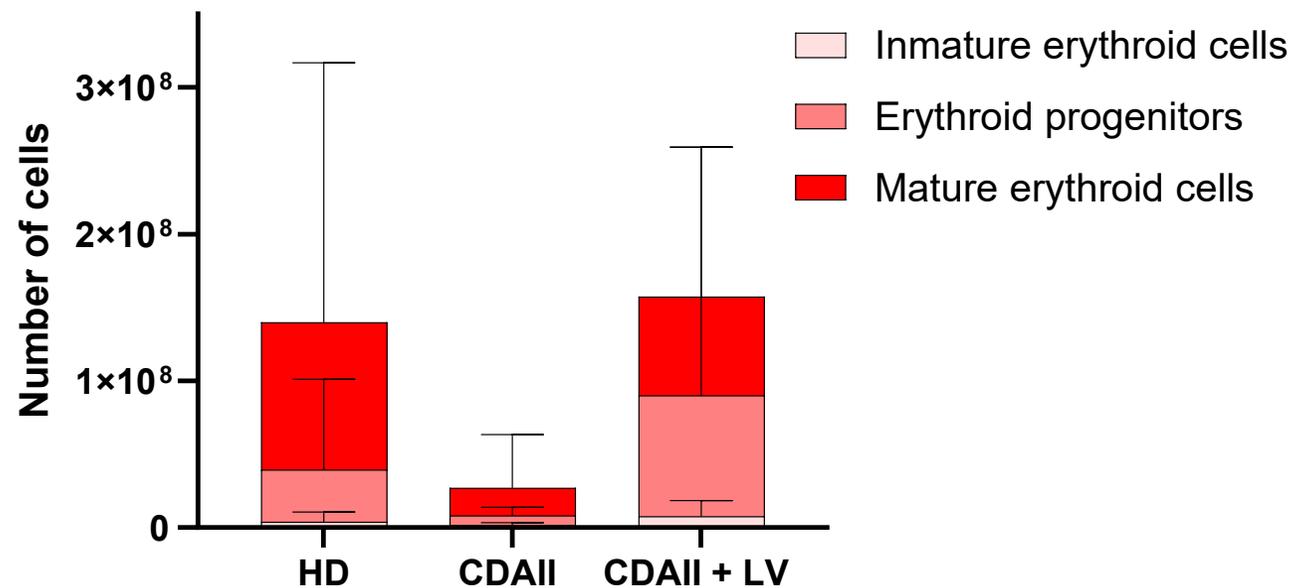
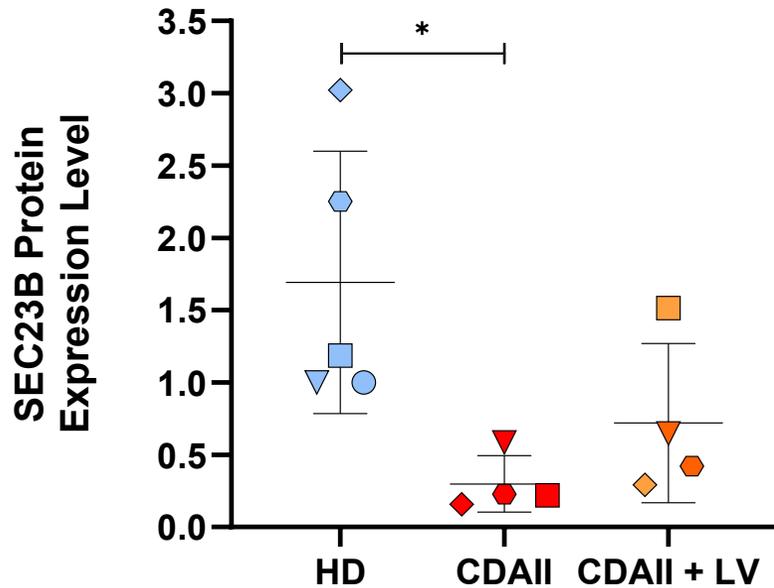
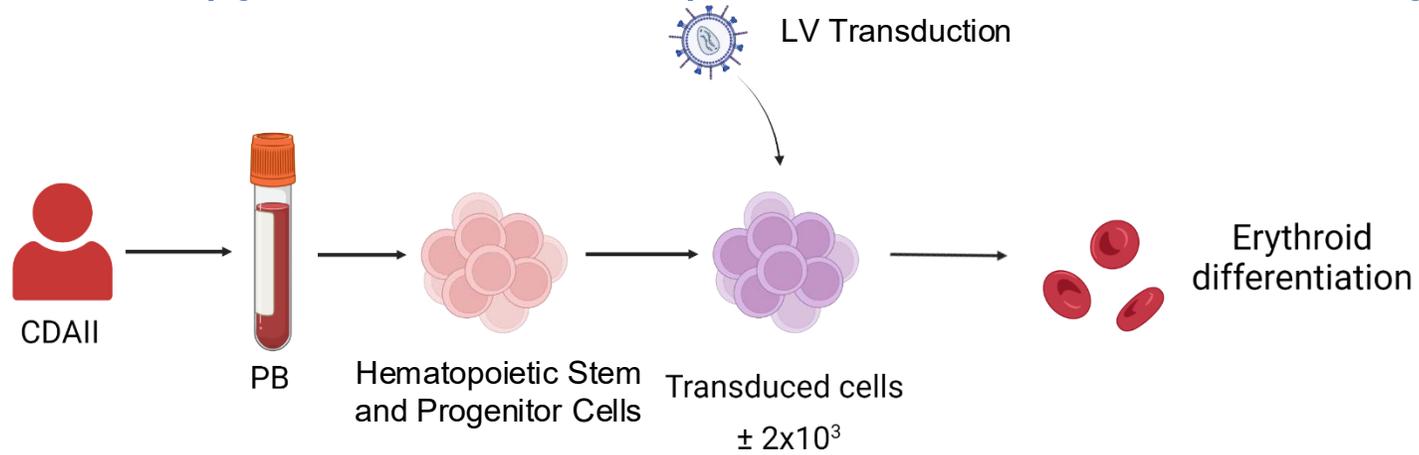


SEC23B LV





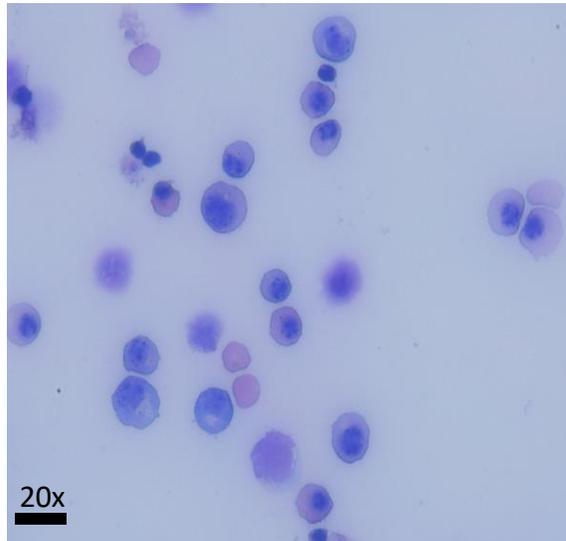
Lentiviral gene therapy seems to compensate SEC23B deficiency in CD411 patients



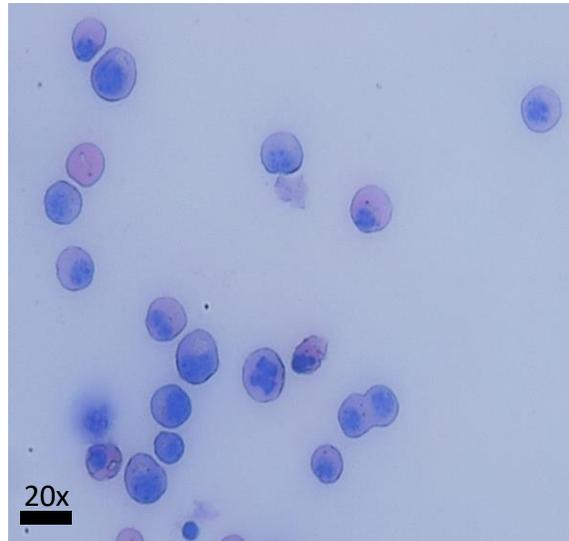


Lentiviral gene therapy seems to compensate SEC23B deficiency in CDAII patients

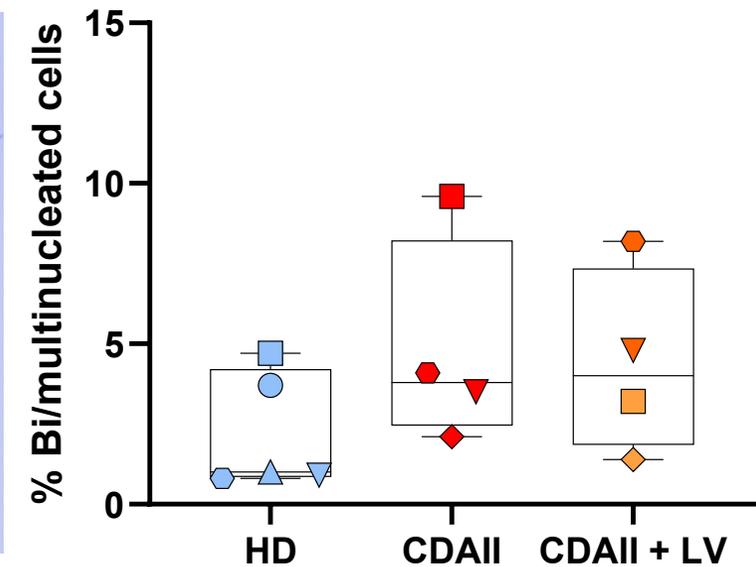
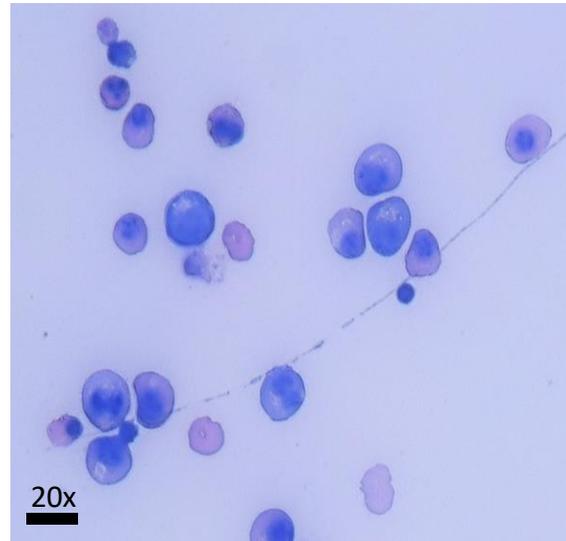
HD



CDAII

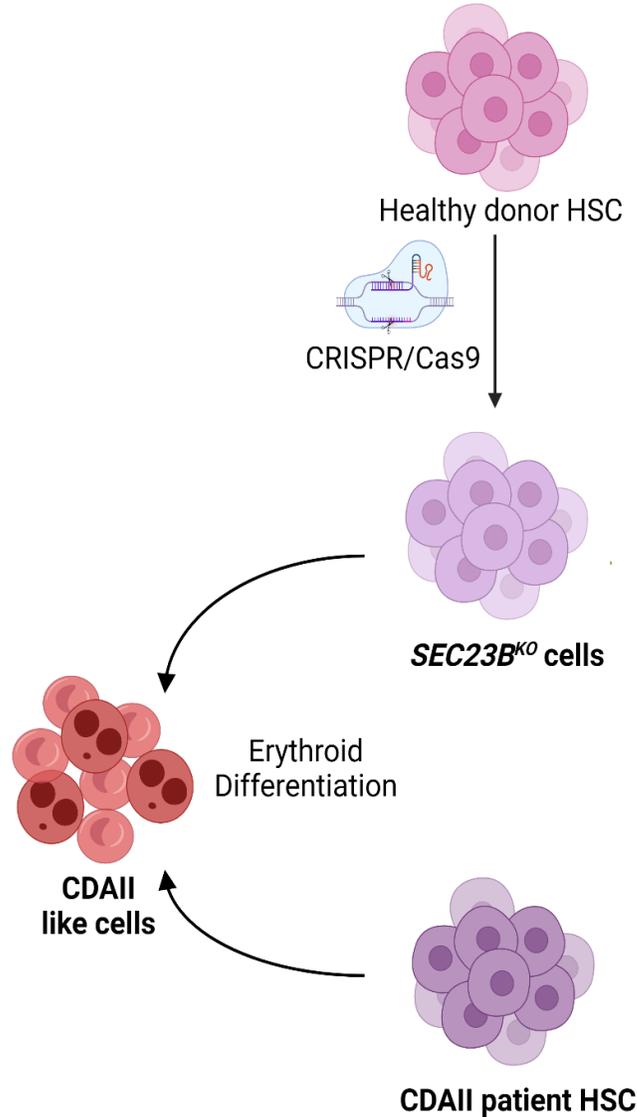


CDAII + LV





To sum up...



Erythroid cells derived from *CD41-like* HSPCs show CD41-like phenotype

Acknowledgements



TecnoCel



Purchasing dept. staff
Management dept. staff
Animal facility staff



Desarrollo De Aplicaciones
Y Sistemas Informáticos
Almudena Bailador Ferreras



Patients and healthy donors

Patients Webinar

Gene therapy for Pyruvate Kinase Deficiency and Congenital Dyserythropoietic Anemia Type II



Hospital Universitario
Fundación Jiménez Díaz
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Gavin Mc Donough
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FUNDACIÓN
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Giovanna Zanella
Marie Fertin
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Lucía Guerrero Fernández (H Rio Carrión)
Miriam Vara Pampliega (HU Cruces)
Leonor Senent (HU de la Fe)
Amanda Pérez (H Clinic)
Anna Gaya (H Clinic)



Ami Shah, MD
Maria Grazia Roncarolo, MD
Bert Glader, MD, PhD
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Daniel P. Dever
Joab Camarena
Annalisa Lattanzi
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Matthew Porteus



Rolf Turk
Mollie S. Schubert
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Jose Bonafont
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S2017/BMD-3692



PID2023-152564OB-I00
Making accessible gene editing for
inherited anemias (GENERA)



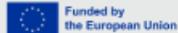


NEWSLETTER – June 2025



NEW SURVEY

EXPECTATIONS/REQUIREMENTS FOR A
POTENTIAL GENE EDITING FOR PYRUVATE
KINASE DEFICIENCY (PKD) AND CONGENITAL
DYSERYTHROPOIETIC ANEMIA (CDA)



t.ly/nbYe2



Español



Français



English



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Open until: December 31, 2025.

THANK YOU



Patients



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Eurobloodnet - European Reference Network on Rare Hematological Diseases



ERN-EuroBloodNet's EDUcational Youtube channel



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Additional slides

Why do we need Hematopoietic Stem Cell Mobilization?



Peripheral Blood
Harvest

Hematopoietic stem cell
mobilization

G-CSF + Plerixafor

- To facilitate their collection:
 - Hematopoietic stem cells are located in the Bone Marrow
 - These drugs induce their exit to the peripheral blood
 - While they are circulating, they can be collected easily
 - Correction of the hematopoietic stem cells will assure correction all life long

Some numbers about LVV gene therapy

Total treated	With Lentiviral Vectors	In rare hematopoietic rare diseases
21.000	1350	~300

Disease	Patients Treated	Deaths Reported	Deaths Attributed to Gene Therapy/Lentivirus
ADA-SCID	62	0	0
Wiskott-Aldrich	30	1	No direct link
Fanconi Anemia	15	0	0
Sickle Cell Disease	50	2	Unclear/linked primarily to conditioning
Beta-Thalassemia	22	0	0
Adrenoleucodystrophy (CALD)	67	2	Deaths attributed to disease progression or transplantation complications, not vector
Pyruvate Kinase Deficiency	4	0	0
Leukocyte Adhesion Deficiency type I	9	0	0
	259	5	

Trial Design

- Non-Randomized Global Phase 2 Study

Key Eligibility Criteria

- PKD diagnosis with a confirmed *PKLR* mutation
- Age: ≥ 8 to 50 years (n:12)
- Severe and/or transfusion-dependent anemia
- Splenectomized (To be discussed with Regulatory Agencies)