FINDING THE MINIMUM VECTOR COPIES PER CELL NEEDED TO REACH PHENOTYPIC CORRECTION IN A MOUSE MODEL OF ERYTHROCYTE PYRUVATE KINASE DEFICIENCY USING A CLINICALLY APPLICABLE LENTIVIRAL VECTOR

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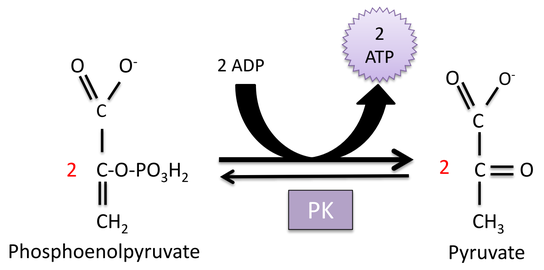
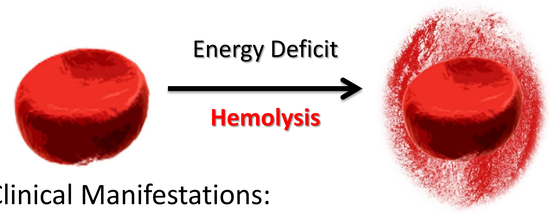
INTRODUCTION

PYRUVATE KINASE DEFICIENCY AND THERAPEUTIC LENTIVIRAL VECTOR

Pyruvate Kinase (PK),catalyzes the last step of Glycolysis



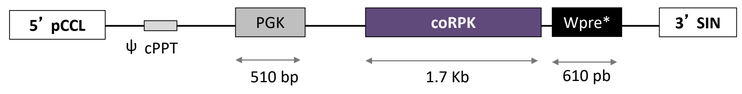
Loss in PK activity impairs the cell metabolism causing **Pyruvate Kinase Deficiency (PKD)**, an autosomal recessive disease caused by mutations in the *PKLR* gene (Liver and Red Blood Cell isoforms.)



Clinical Manifestations:

- Chronic non-spherocytic haemolytic anemia (CNSHA.)
- Splenomegaly.
- Reticulocytosis.
- Fatal in some severely affected patients during early childhood.

We have designed a successful preclinical protocol in a PKD mouse model based on autologous transplantation of hematopoietic stem cell (HSC) genetically corrected by a **Therapeutic Lentiviral Vector** (PGK-coPKLR.Wpre*-LV).



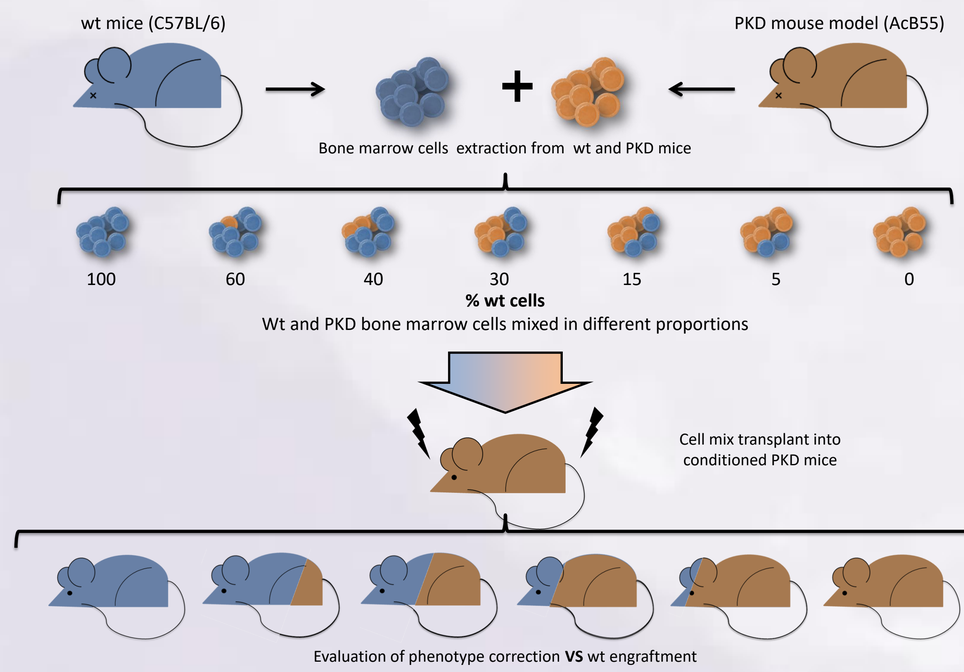
This vector have already being designated as **Orphan Drug** by the European Medicines Agency (EMA) and Food and Drug Administration (FDA).

AIMS

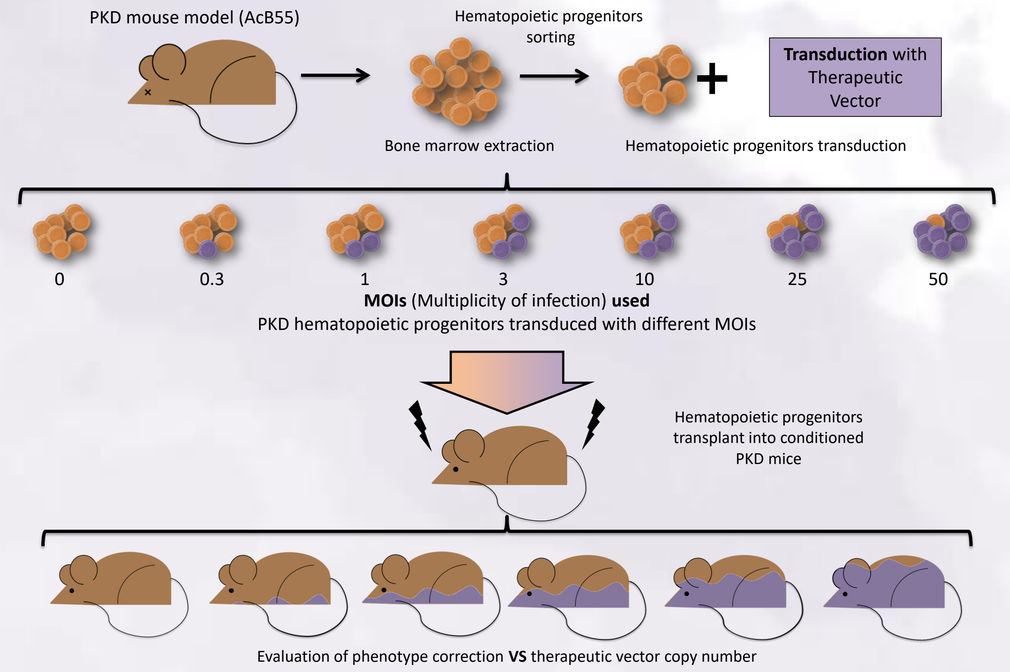
Define the minimal proportion of corrected cells required to achieve a therapeutic effect in PKD patients

EXPERIMENTAL DESIGN

1st Approach: wt and PKD total bone marrow mixes

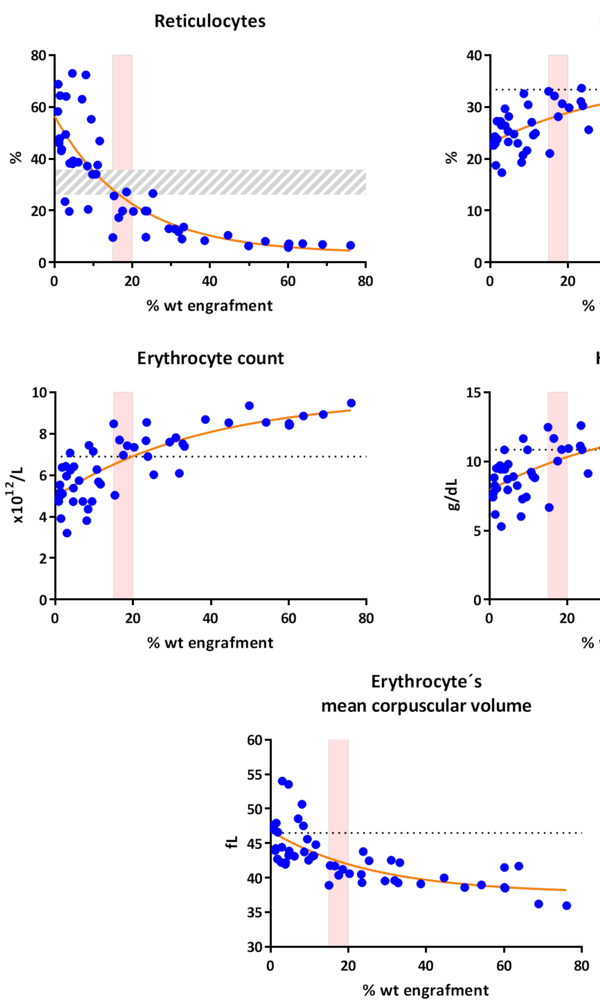


2nd Approach: Transduction of PKD hematopoietic progenitors



RESULTS

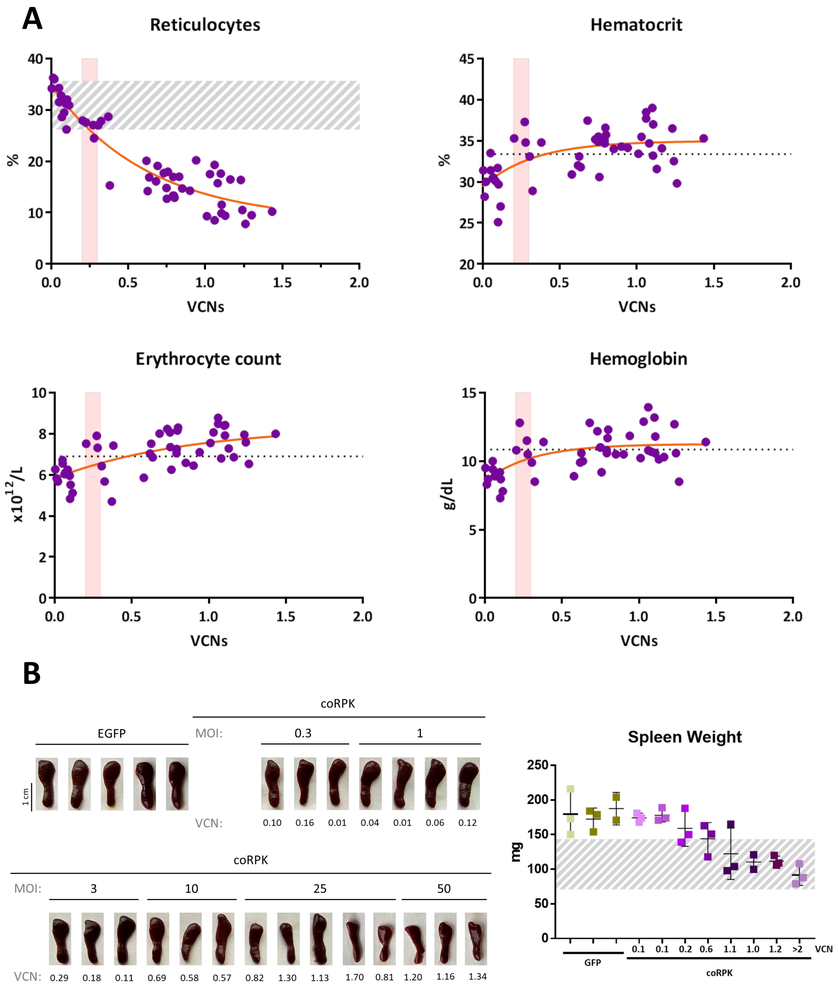
Phenotype rescue depending on the wt/PKD cell proportion transplanted



Erythroid lineage features of transplanted mice with respect to the wt/PKD proportion. Orange line, non-lineal correlation. Dashed interval in reticulocytes graph represents standard deviation of control non-transplanted PKD mice. Dotted horizontal lines are the thresholds set based on ROC curves performed in control non-transplanted PKD mice.

Pink columns correspond to 15 to 20% of wt cells among all engrafted cells, interval which fits with the minimum therapeutic percentage. Mice transplanted with cell mixes containing less than 15-20% wt cells started to show hematological features that fit with deficient mice.

Phenotype rescue depending on therapeutic vector MOI used



(A) Erythroid lineage features of transplanted mice with different MOI of therapeutic vector. Orange line, non-lineal correlation. Dashed interval in reticulocytes graph (standard deviation non-transplanted PKD mice). Dotted horizontal lines are the thresholds set based on ROC curves performed in non-transplanted PKD and non deficient mice.

Pink columns correspond to 0.2 and 0.3 VCNs (vector copy number), interval which fits with the minimum therapeutic VCN**.** Mice that showed VCN lower 0.3 in peripheral blood were not capable to overcome the deficient phenotype.

(B) Spleen size (left) and weight (right) at endpoint. Both parameters are correlated with the Vector Copy Number at end point

CONCLUSIONS

- PKD correction was found when total bone marrow contained at least 20% of non-deficient cells
- PKD correction by the therapeutic vector was achieved when at least 0.3 copies of the vector were detected among the total peripheral blood cell populations
- Spleen Size and weight confirmed results obtained in peripheral blood and corroborated the therapeutic properties of the developed clinical vector

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