**Título:** HEMATOPOIETIC STEM CELL GENE EDITING FOR THE TREATMENT OF PYRUVATE KINASE DEFICIENCY

**Nombre:** Fañanás Baquero, Sara

**Universidad:** Universidad Autónoma de Madrid

**Departamento:** Bioquímica

**Fecha de lectura:** 18/09/2020

**Mención a doctor europeo:** concedido

**Programa de doctorado:** Programa de Doctorado en Biociencias Moleculares por la Universidad Autónoma de Madrid

**Dirección:**

> **Director:** JOSE CARLOS SEGOVIA SANZ

> **Codirector:** OSCAR QUINTANA BUSTAMANTE

**Tribunal:**

> **presidente:** LLUIS MONTOLIU JOSE

> **secretario:** MANUEL RAMIREZ ORELLANA

> **vocal:** JAIME JUAN CARVAJAL GARCIA-VALDECASAS

> **vocal:** Giuliana Ferrari

> **vocal:** FRANCISCO MARTIN MOLINA

**Descriptores:**

> INGENIERIA GENETICA

> BIOLOGIA MOLECULAR

> VIROLOGIA

**El fichero de tesis** ya ha sido incorporado al sistema

> http://hdl.handle.net/10486/693686

**Localización:** REPOSITORIO DE TESIS – BIBLIOTECA UAM

**Resumen:** Pyruvate Kinase Deficiency (PKD) is an autosomal recessive disorder caused by mutations in the PKLR gene, and constitutes the main cause of chronic non-spherocytic hemolytic anemia. PKLR gene encodes the erythroid pyruvate kinase protein (RPK) implicated in the last step of the anaerobic glycolysis in red blood cells. The defective enzyme fails to produce normal ATP levels, and erythrocytes from PKD patients suffer hemolysis. The only curative treatment applied so far, is the hematopoietic stem and progenitor cells (HSPC) transplant of a compatible donor, with the associated limitations this technique involves. In this work, we conducted precise gene editing in the endogenous locus. We intended to follow two different approaches based on the homology directed repair pathway (HDR). On one side, we attempted to specifically correct a PKD

patient’s mutation (occurring in exon 3 of PKLR gene) by electroporation of a specific ribonucleoprotein (RNP) and a single-stranded
oligonucleotide with the corrective sequence in a lymphoblastic cell line derived from the patient. We demonstrated the feasibility of the process although the efficacy needs to be improved to achieve clinical importance. On the other side, we developed a wider strategy, potentially applicable to more patients with a variety of mutations, based in the knockin of a therapeutic donor in the PKLR locus. We tested its introduction in the second intron of the gene, mediated by TALEN nucleases. The donor carried a codon optimized sequence of RPK cDNA and a puromycin selection gene. Although we observed high levels of gene editing in the puromycin resistant cells, the editing efficacy in the engrafted cells was low. To optimize the protocol, we incorporated the use of adeno-associated vectors to mediate the delivery of the therapeutic donor. We were able to efficiently edit HSPCs by using a selection-free system. The ex vivo gene edited cells efficiently reconstituted the human hematopoiesis in primary and secondary recipients, and cells that have undergone HDR were found within the mature and primitive cell subsets. Finally, we mediated gene editing in HSPCs from 3 different PKD patients and edited cells were differentiated in vitro towards the
erythroid lineage. At the end of the process, only the cells edited with the therapeutic donor were able to produce normal levels of ATP, demonstrating the restoration of the glycolysis functionality in edited cells and situating this gene editing strategy as a potential therapeutic alternative for the treatment of PKD.