

Lentiviral Mediated Gene Therapy for Pyruvate Kinase Deficiency: A Global Phase 1 Study for Adult and Pediatric Patients

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- There are no relationships to disclose.

Introduction

- Pyruvate kinase disease (PKD) is a rare inherited hemolytic anemia caused by a mutation in the ***PKLR*** gene which results in decreased red cell pyruvate kinase (RPK) activity, impaired erythrocyte metabolism, and diminished red blood cell lifespan.
- PKD is characterized by anemia, reticulocytosis, hyperbilirubinemia, splenomegaly, and iron overload and may be life threatening in severely affected individuals.

PKD represents a significant unmet medical need

- There are up to 8,000 cases in EU and US.
- Currently available therapies are palliative and limited to chronic blood transfusions, iron chelation therapy, and splenectomy.
- Side effects of these treatments include increased infection susceptibility and thromboembolic risk.
- Therapies do not ameliorate PKD-related iron overload or end-organ damage.
- Allo-HSCT has been performed in select cases and resulted in transfusion independence; however, efficacy has been limited by toxicities and availability of a suitable donor.

Preclinical Studies Demonstrate Safety and Efficacy of Lentiviral-mediated Gene Therapy

- *In vivo* experiments were conducted using Lin⁻ BM cells from male PKD mice transduced with the therapeutic vector PGK-coRPK-WPRE and then transplanted into lethally irradiated female PKD mice.

PKD Murine Model:

- **Hemolytic anemia**
- **Splenomegaly**
- **Histologic signs of hepatic extra-medullary erythropoiesis**



Recombinant congenic AcB55 carrying a loss-of function mutation (269 T>A affecting exon 2 of *PKLR* gene)

Min-Oo, et al. *Nat Gene*. 2003; 35(4): 357-362.

Min-Oo, et al. *Genes Immun*. 2004; 5(3): 168-175.

PKD mice transplanted with gene-corrected cells demonstrated phenotypic correction:

- Significant increase in RBC count and half-life
- Decreased erythropoietin levels
- Normalized spleen and liver size & structure, with no evidence of erythroid clusters or iron deposits
- Improvement in red cell pyruvate kinase enzymatic pathway as assessed by metabolomic assays

Highly Favorable Safety Profile:

- No physical, behavioral biochemical, hematologic, or morphologic abnormalities observed in transplanted mice
- Limited evidence of PGK-coRPK-WPRE in nonhematopoietic organs, indicating very low risk of germline transmission
- No evidence of replication competent lentivirus (RCL)

Global Phase 1 PKD Gene Therapy Study

Clinical Sites:

Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain
Stanford University, Palo Alto, California, United States
Hospital Infantil Universitario Niño Jesús, Madrid, Spain

Primary Endpoint

Evaluation of the safety and toxicity of RP-L301

Secondary Endpoints

- Peripheral blood (PB) and bone marrow (BM) genetic correction as demonstrated by vector copy number (VCN)
- Transfusion independence (when relevant) at 12 months
- Achievement of 50% reduction in transfusion requirements (when relevant) at 12 months
- Clinically significant reduction of anemia
- Reduction of hemolysis

Key Eligibility Criteria

Inclusion:

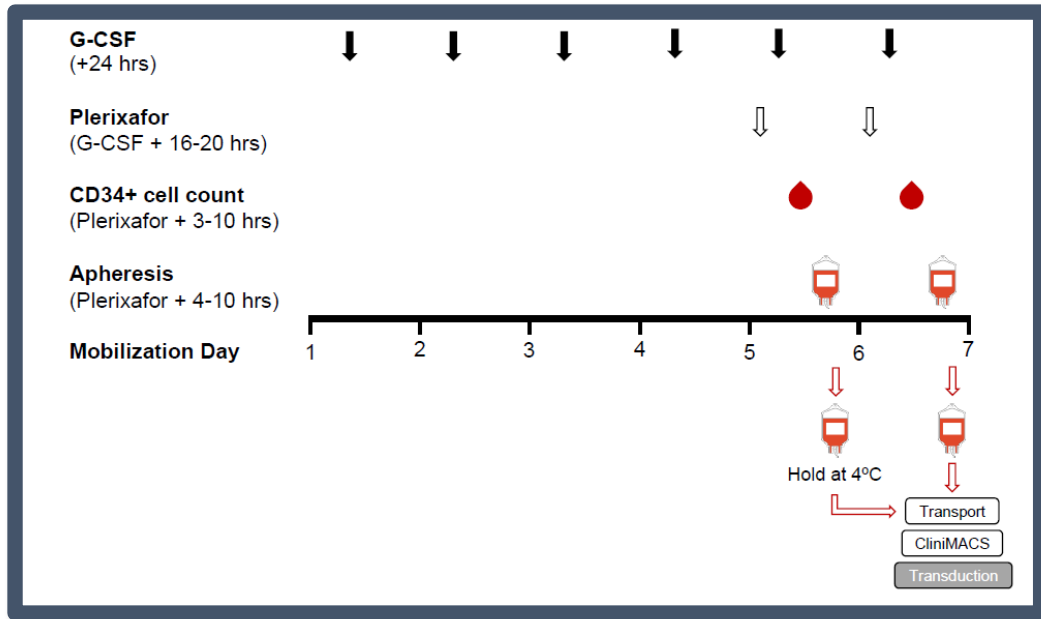
- PKD diagnosis with a confirmed *PKLR* mutation
- Age:
 - 1st cohort (N=2): ≥18 to 50 years
 - 2nd cohort (N=2): ≥12 to 17 years
 - 3rd cohort (N=2): ≥ 8 to 11 years
- Severe and/or transfusion-dependent anemia
- Adequate cardiac, pulmonary, renal, and hepatic function

Exclusion:

- Presence of another known cause of hemolysis
- Venous or arterial thromboembolic event in prior 12 months
- Severe iron overload
- Other significant medical condition

Study Design

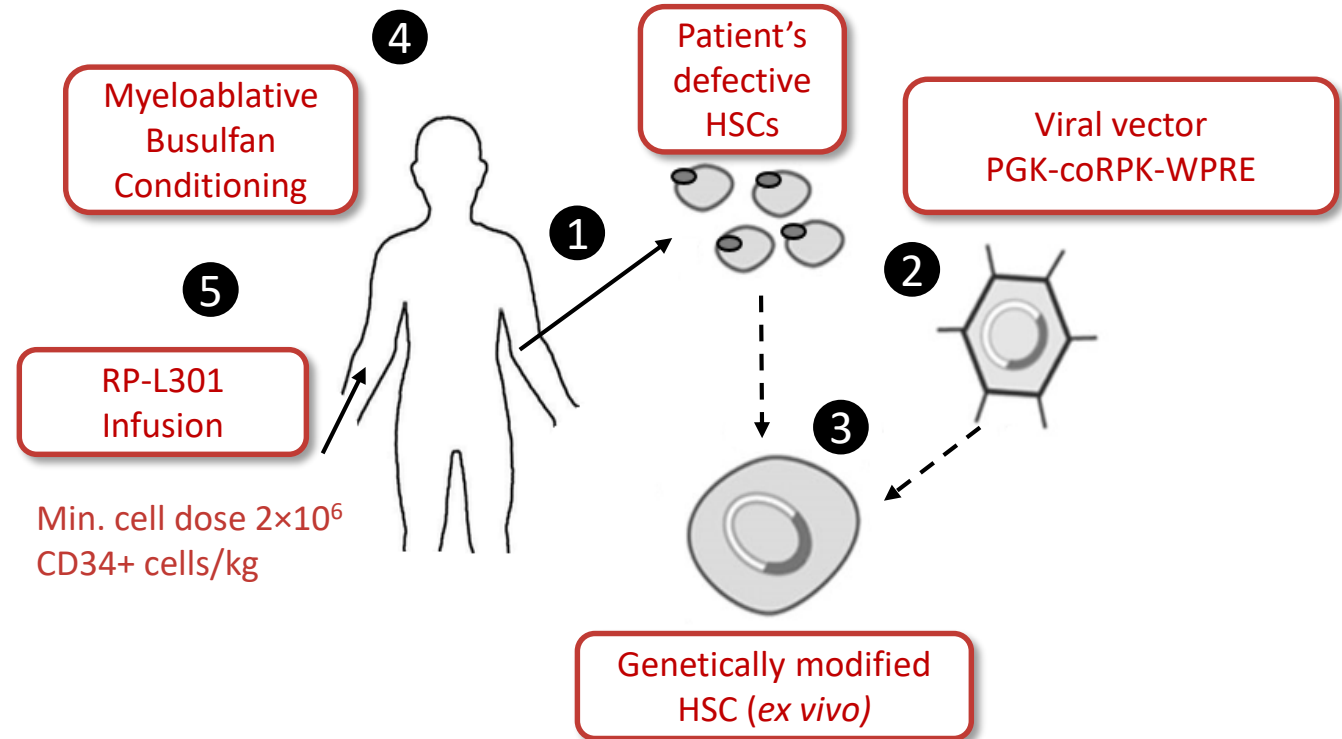
CD34+ cell mobilization protocol with G-CSF and plerixafor:



G-CSF and plerixafor are administered over a 6-day sequence with apheresis conducted on 2 consecutive days.

PB counts of at least 10 CD34+ cells/ μ L are required to initiate apheresis.

Schematic diagram of product manufacture and treatment:



Patients undergo HSC mobilization and collection, followed by CD34+ immunoselection and transduction. RP-L301 is infused subsequent to myeloablative busulfan conditioning. (Target area under the curve: 73,125 ng/mL*hour over 4 days)

Subject Characteristics and Product Metrics

Subject Characteristics

Subject	Age (y) and Gender	PKD Mutation	Hemoglobin (g/dL)	Bilirubin (mg/dL)	Erythropoietin (mIU/mL)	Transfusion Requirement for 2 Years Prior to Enrollment
L301-006-1001	31 F	c.721G>T c.1529G>A	7.4 [†]	13.4 mg/dL	35.6 mIU/mL	~14 transfusion episodes
L301-001-1002*	47 M	c.703GGG>AGG c.1047A>AA c.1744CGG>AGG	7.0 [‡]	7.4 mg/dL	57.2 mIU/mL	~5 transfusion episodes

Product Metrics

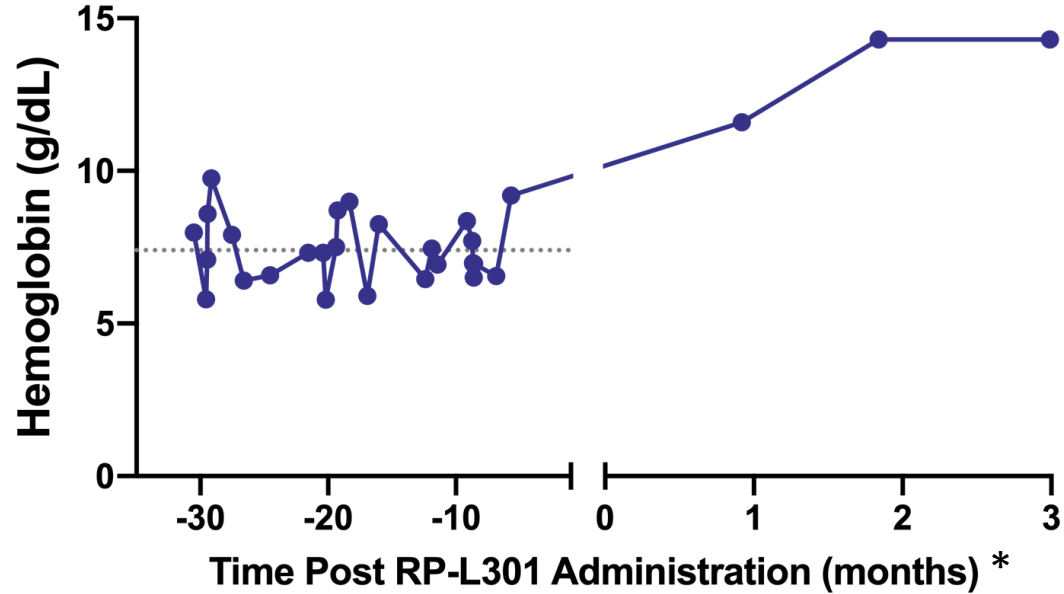
Subject	Nucleated Cells/kg	CD34+ Cells/kg	CFCs/kg	Mean VCN: Liquid Culture
L301-006-1001	3.93 x 10 ⁶	3.9 x 10 ⁶	9.16 x 10 ⁵	2.73
L301-001-1002*	2.42 x 10 ⁶	2.4 x 10 ⁶	3.38 x 10 ⁵	2.08

* Infusion planned

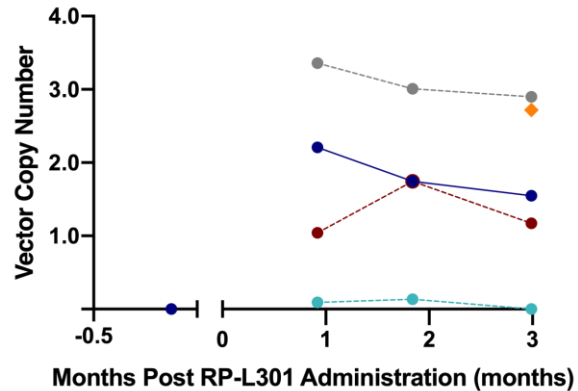
[†] Average hemoglobin calculated over 2 years prior to study enrollment

[‡] Average hemoglobin calculated over 2 years prior to study enrollment; subject has declined red blood cell transfusions

Preliminary Efficacy Results: L301-006-1001

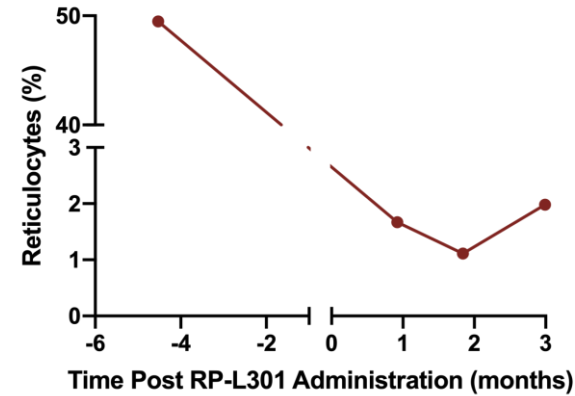


- Significant hemoglobin improvement ~7.4 g/dL to 14.3 g/dL
- No transfusion requirements following engraftment

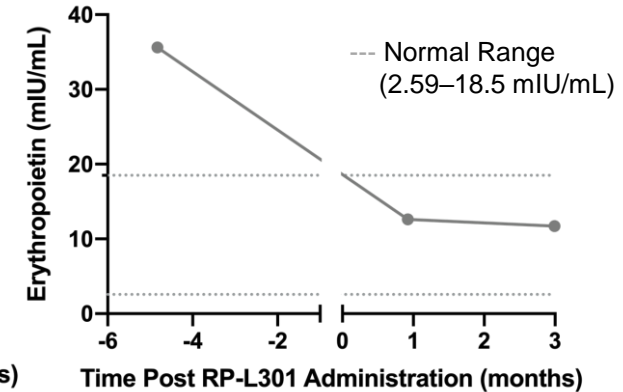


VCN in PBMCs 1.55 and VCN in BMMCs 2.72 at 3 months post RP-L301

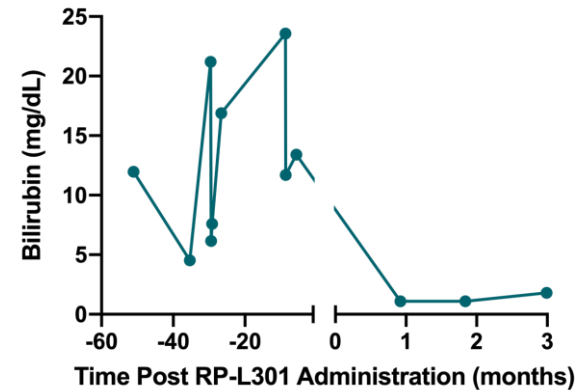
At 1–3 months post RP-L301 *



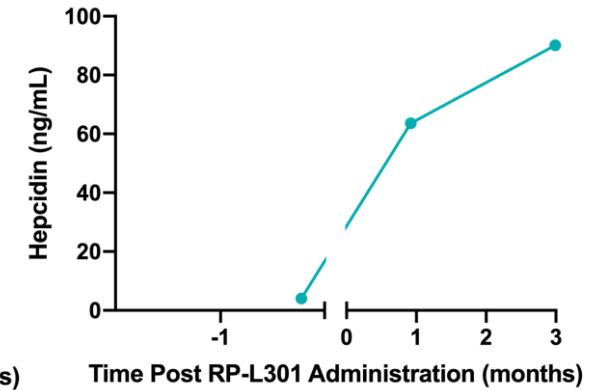
Reticulocytes decreased



Erythropoietin normalized



Bilirubin decreased from 13.4 mg/dL to 1.8 mg/dL



Hepcidin increased from <4.0 ng/mL to 90.1 ng/mL

* Lab Values during mobilization/apheresis & post-conditioning period were not included

Preliminary Safety Results

Treatment-emergent Adverse Events (Grade 3 or higher) (N=1 subject)

Event System Organ Class (NCI CTCAE v. 5.0)	Adverse Events Grade		
	Any	3	4
Blood and lymphatic system disorders			
Neutropenia	1	1	–
Gastrointestinal disorders			
Stomatitis	1	1	–
Investigations			
AST increased	1	1	–
ALT increased	1	1	–
Metabolism and nutrition			
Hypertriglyceridemia	1	–	1

- ▶ *No RP-L301 related adverse events*
- ▶ *Subject L301-006-1001 achieved neutrophil engraftment on day +13*

Adverse events considered related to mobilization/apheresis (N=2 subjects): Grade 2 SAE (chest pain, dyspnea, and nausea) during apheresis collection. These events were considered related to hyperleukocytosis and the mobilizing agents. They resolved with supportive care and without sequelae. Other events included Grade 2 bone pain and Grade 3 leukocytosis.

Conclusions

- Hematopoietic stem and progenitor cell collection appears safe and feasible in the initial adult subjects with severe PKD
- Investigational product RP-L301 was successfully manufactured
 - Liquid culture VCN >2.0
- Safety profile of RP-L301 appears favorable
 - Infusion well tolerated in (N=1); no IP-related SAEs or AEs
 - Hematopoietic reconstitution in less than 2 weeks
- Preliminary efficacy evident during initial 3 months after RP-L301
 - Subject L301-006-1001 with peripheral blood VCN of 2.21 at 1-month, normalized hemoglobin and hemolysis markers (baseline Hb ~7.4; Hb 14.3 g/dL at 3 months post RP-L301)
- Second adult patient treatment is planned in the coming weeks

Additional investigation in adult, adolescent & pediatric patients with severe PKD is forthcoming

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Ciemat



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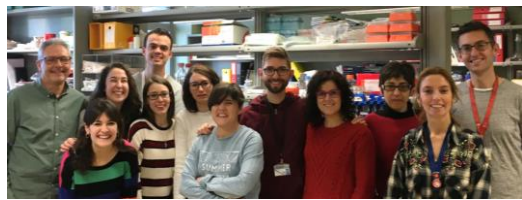
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