Lentiviral-Mediated Gene Therapy for Adults and Children with Severe Pyruvate Kinase Deficiency: Results from an Ongoing Global Phase 1 Study

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Disclosures

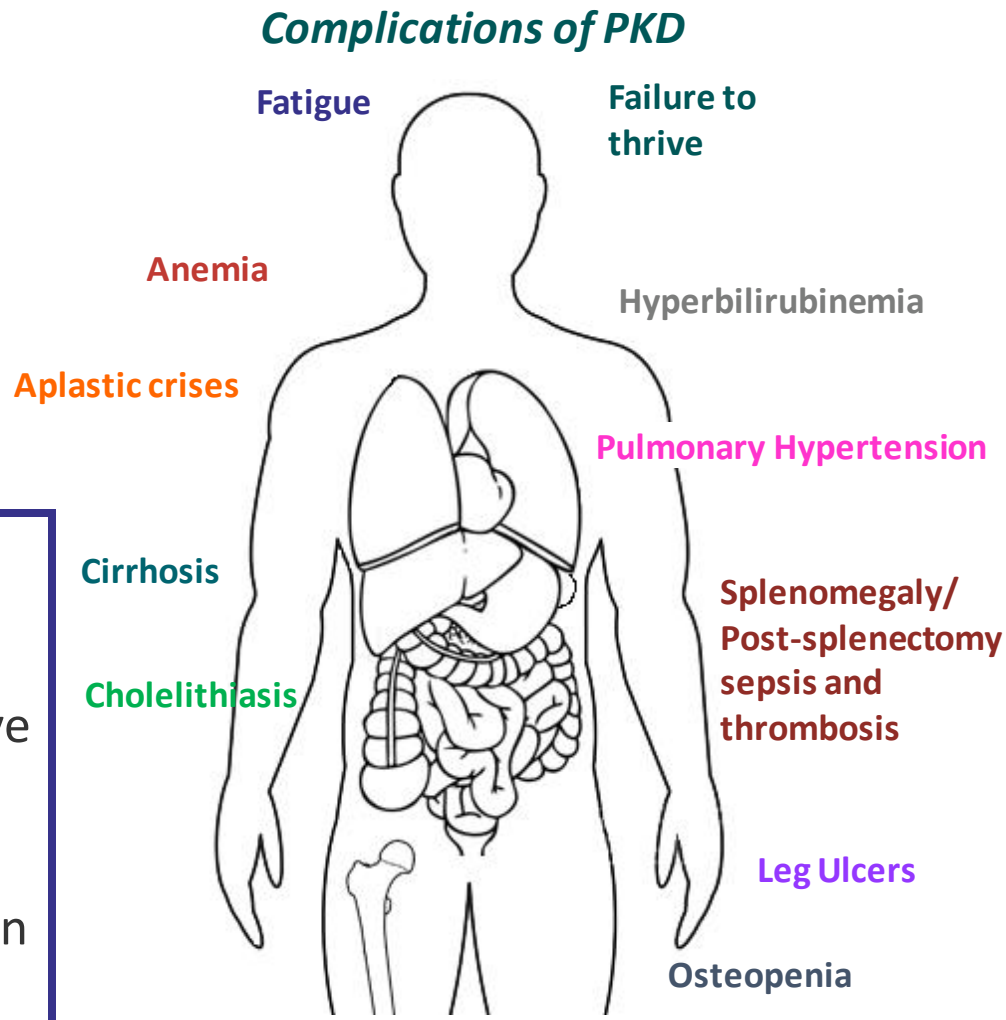
- Advisory Board: Vertex Pharmaceuticals and Bluebird Bio

Introduction

- Pyruvate kinase deficiency (PKD) is a rare inherited hemolytic anemia caused by mutations in the ***PKLR*** gene which result in decreased red cell pyruvate kinase activity and impaired erythrocyte energy production (glycolysis).
- Clinical manifestations include 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 Europe and North America.
- Currently available therapies include enzyme activators or palliative therapies limited to chronic blood transfusions, iron chelation therapy, and splenectomy.
- Allogeneic HSCT has been performed in select cases and resulted in transfusion independence; however, efficacy has been limited by significant toxicities and donor availability.



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Gene Therapy For PKD: Rationale

- Involves insertion of a functional *PKLR* gene into autologous hematopoietic stem and progenitor cells (HSPCs) with the intent of enabling lifelong red blood cell (RBC) pyruvate kinase enzyme production and normalized RBC function and lifespan
- Preclinical studies utilizing a clinically relevant PKD murine model have demonstrated safety and efficacy.
  - Limited evidence of lentiviral vector in non-hematopoietic organs, indicating very low risk of germline transmission
  - No evidence of replication competent lentivirus (RCL)
- Lentiviral gene therapy represents a potentially definitive treatment for PKD which addresses the underlying genetic defect and could ameliorate iron overload and end-organ damage.

Navarro S *et al*. Mol. Ther. – Methods Clin. Dev. 2021; 22: 350-359.
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RP-L301-0119
NCT#04105166

Global Phase 1 PKD Gene Therapy Study

Clinical Sites:

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

Primary Endpoint

Safety and toxicity of RP-L301

Secondary Endpoints

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

Key Eligibility Criteria

Inclusion:

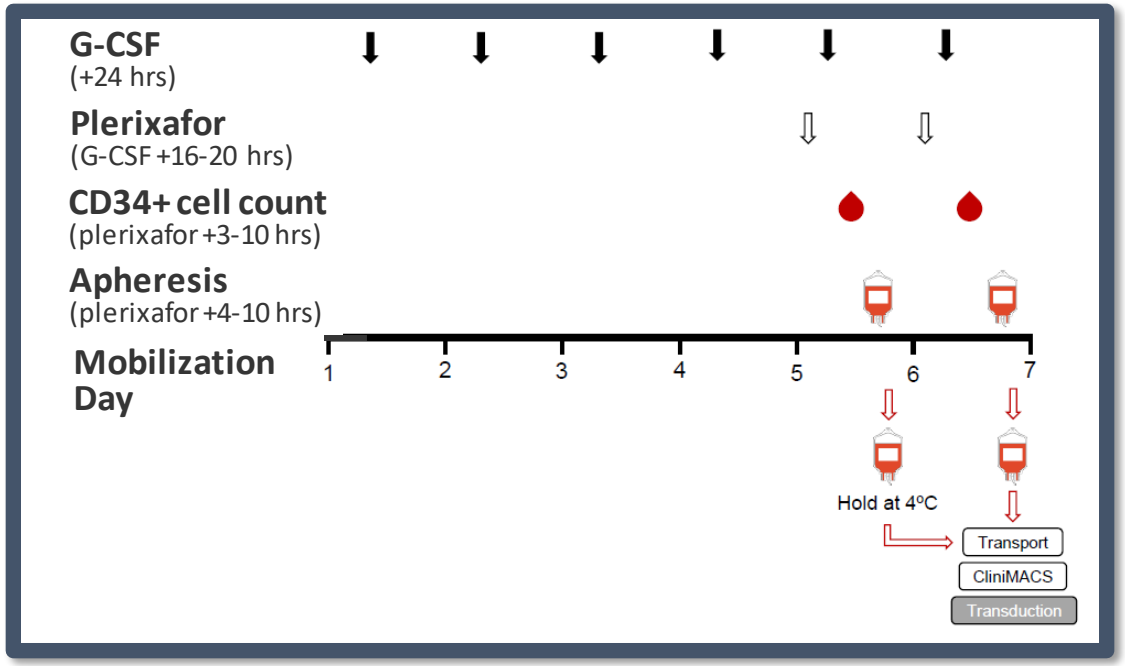
- PKD diagnosis with a confirmed *PKLR* mutation
- Age:
  - 1st cohort (N=2): ≥18 to 50 years
  - 2nd cohort (N=2-3): ≥8 to 17 years
- Severe and/or transfusion-dependent anemia
- Splenectomized
- Adequate cardiac, pulmonary, renal, and hepatic function

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

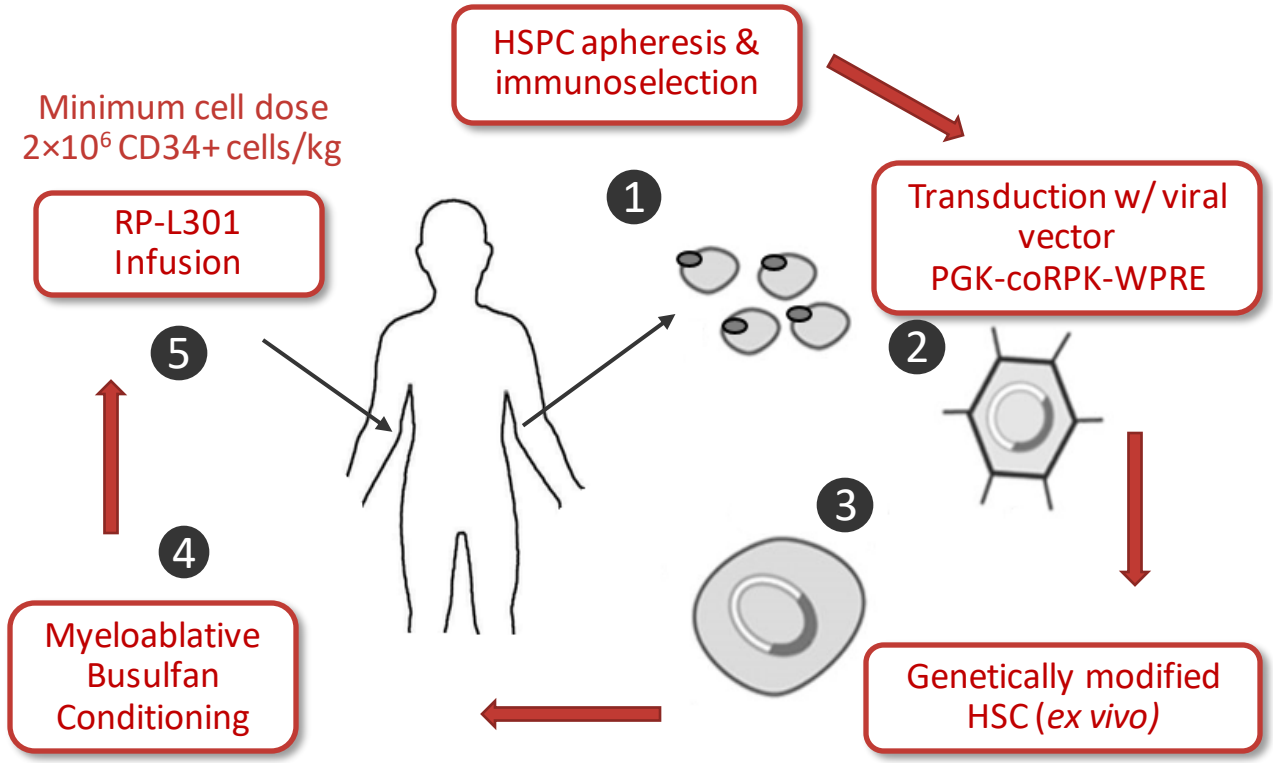
CD34+ cell mobilization protocol with G-CSF and plerixafor:



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/µL are required to initiate apheresis.

Schematic diagram of product manufacture and treatment:



Patients undergo HSPC mobilization and collection. Immunoselection of CD34+ cells and lentiviral-mediated *PKLR* gene transduction are conducted *ex vivo*. RP-L301 is infused following myeloablative busulfan conditioning.

(Target area under the curve: 73,125 ng/mL*hour over 4 days; TDM)

Patient L301-006-1001's Clinical Course Prior to RP-L301 Administration

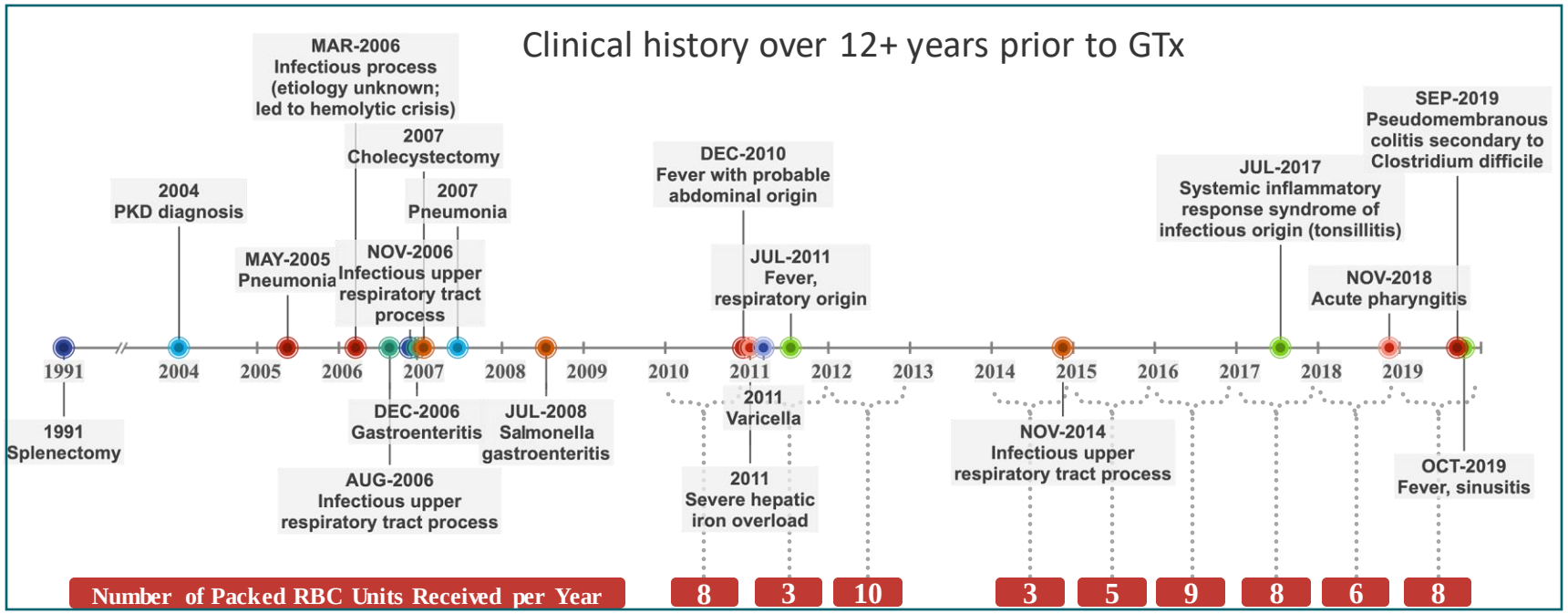
Pre-treatment Characteristics

Age (y) & Gender	PKD Mutation	Hemoglobin (g/dL)	Bilirubin (mg/dL)	Erythropoietin (mIU/mL)	Transfusion Requirement for 2 Years Prior to Enrollment
31 F	c.721G>T c.1529G>A	7.4†	13.4	35.6	~14 transfusion episodes

† Average hemoglobin calculated over 2 years prior to study enrollment

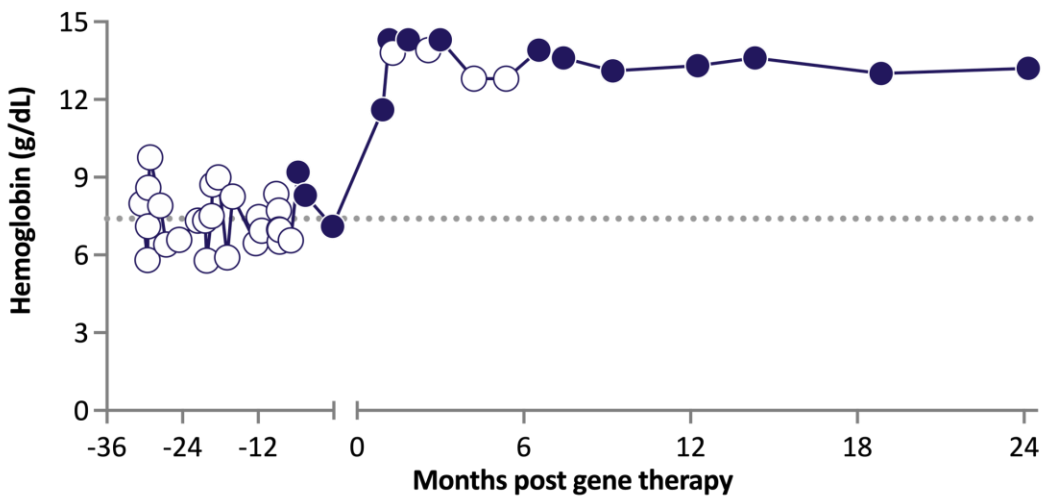
Product Metrics

Nucleated Cells/kg	CD34+ Cells/kg	CFCs/kg	Mean VCN: Liquid Culture
3.9×10^6	3.9×10^6	9.2×10^5	2.73

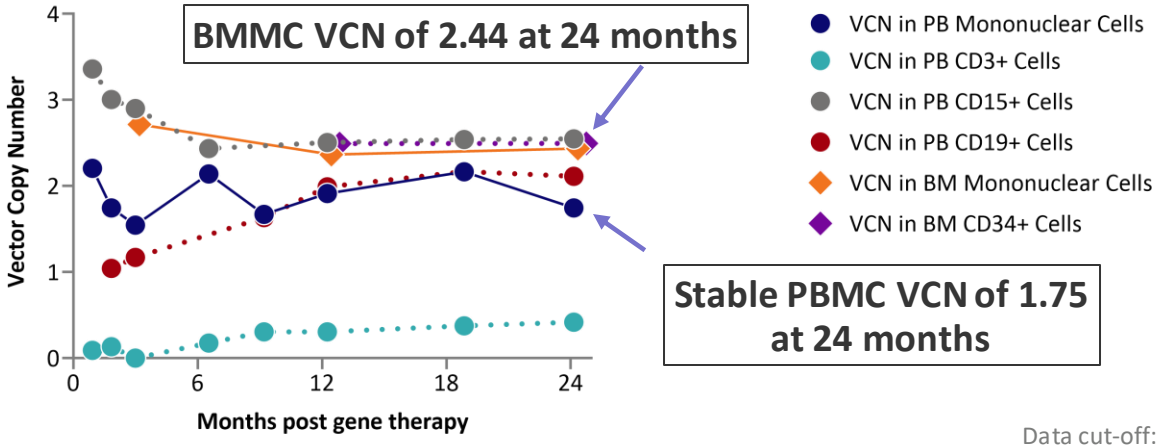


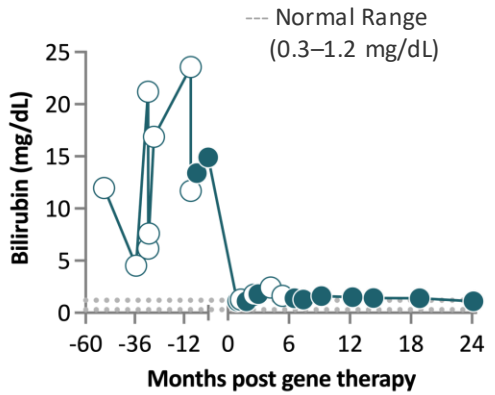
- Patient had a history of multiple infections precipitating hemolytic crises and requiring multiple transfusions and hospitalizations.
- Patient had hepatic iron overload secondary to transfusions and underlying PKD.

Patient L301-006-1001 Preliminary Efficacy Results

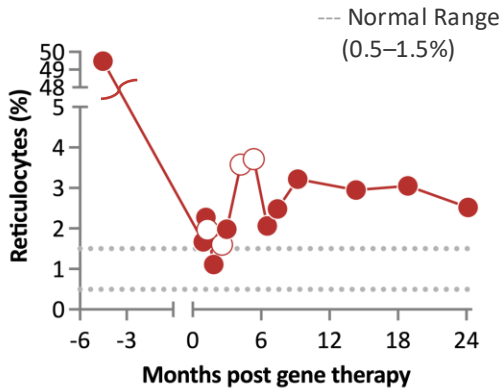


- Sustained **hemoglobin normalization from ~7.4 g/dL to 13.2 g/dL** 24 months post-RP-L301 infusion
- No red blood cell transfusions required following engraftment

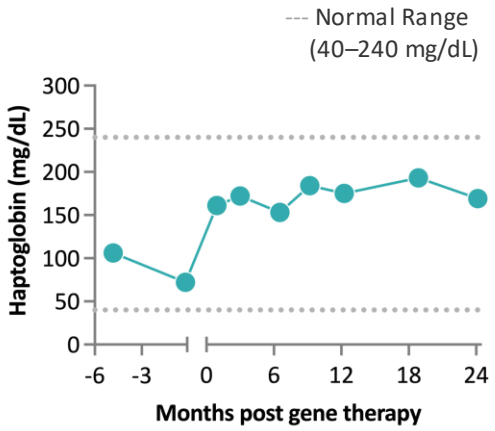




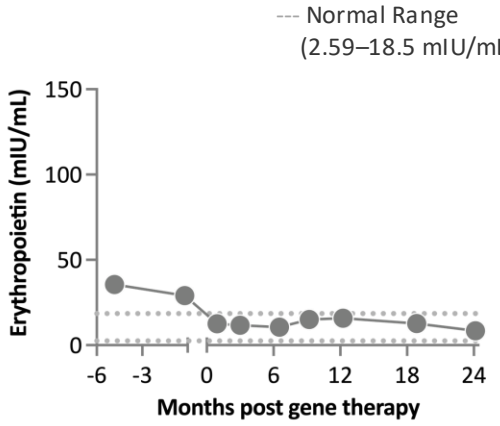
Significant bilirubin decrease from 13.4 mg/dL to 1.1 mg/dL



Sustained improvements in reticulocytes from 49.5% to 2.5%



Haptoglobin remains within normal range



Continued erythropoietin normalization

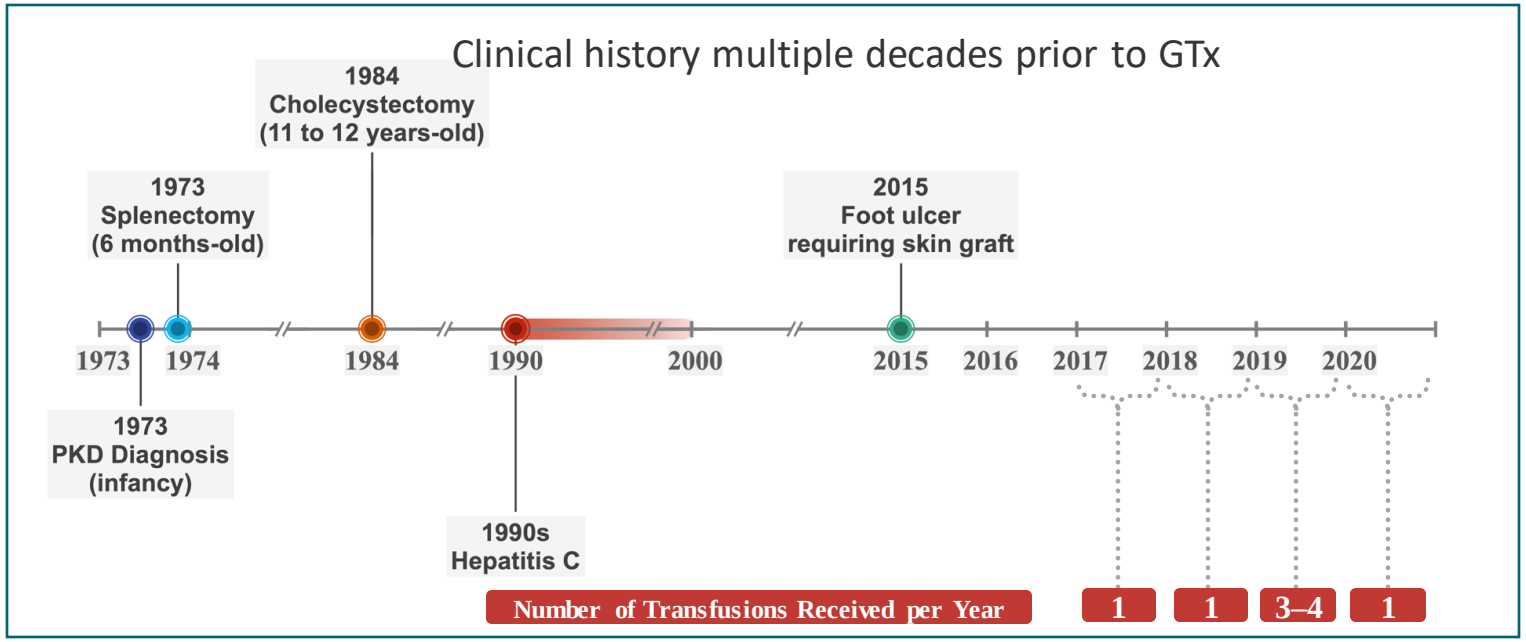
Data cut-off: October 26, 2022; Preliminary interim results are presented from the ongoing clinical study.

Patient L301-001-1002's Clinical Course Prior to RP-L301 Administration

Pre-treatment Characteristics

Age (y) & Gender	PKD Mutation	Hemoglobin (g/dL)	Bilirubin (mg/dL)	Erythropoietin (mIU/mL)	Transfusion Requirement for 2 Years Prior to Enrollment
47 M	c.703GGG>AGG c.1047A>AA c.1744CGG>AGG	7.0‡	7.4	57.2	~5 transfusion episodes

‡ Average hemoglobin calculated over 2 years prior to study enrollment



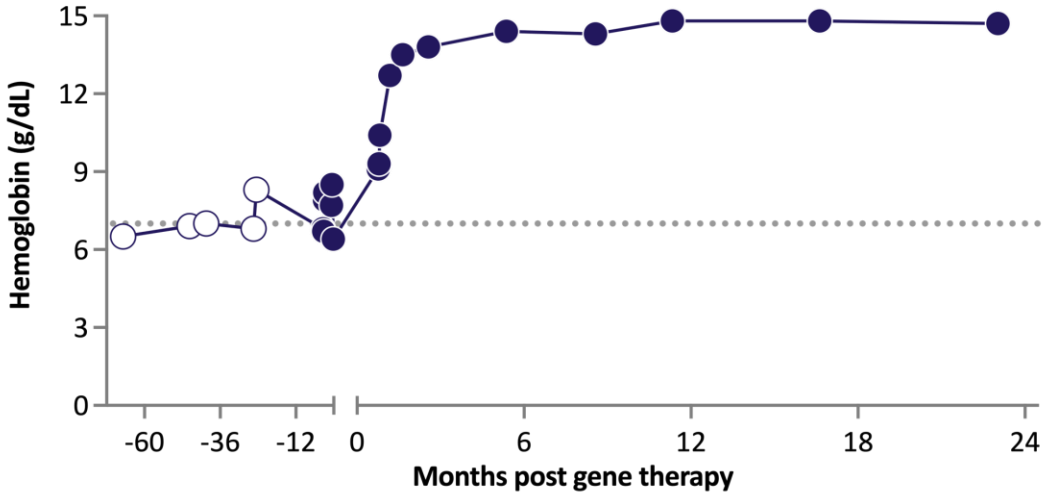
Product Metrics

Nucleated Cells/kg	CD34+ Cells/kg	CFCs/kg	Mean VCN: Liquid Culture
2.4×10^6	2.4×10^6	3.4×10^5	2.08

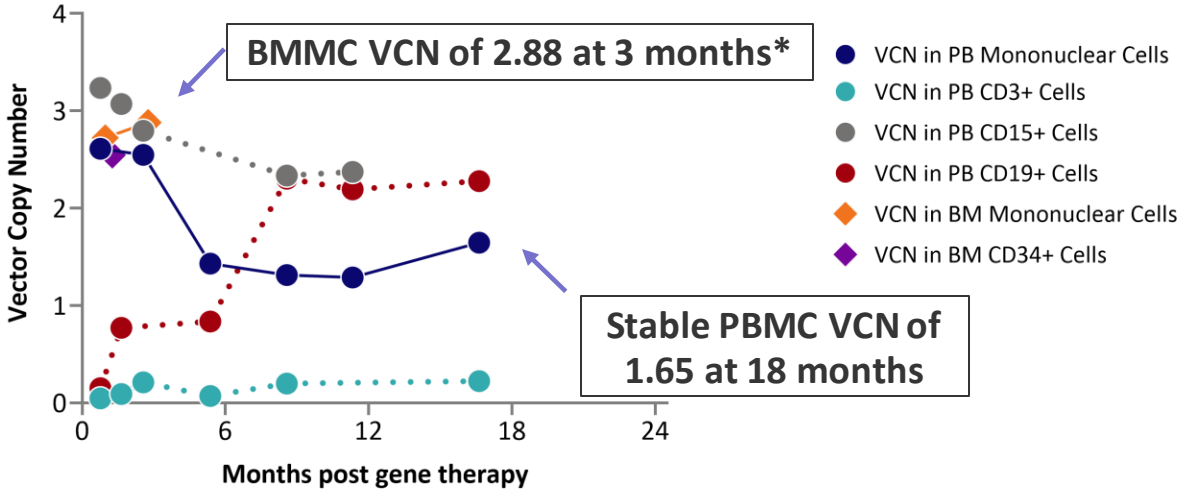
- Patient had severe anemia, but declined frequent red blood cell transfusions.
- Additional complications included liver iron overload and foot ulcer requiring skin graft.
- Patient had participated in mitapivat (AG-348) clinical trial without significant Hb increase

Patient L301-001-1002 Preliminary Efficacy Results

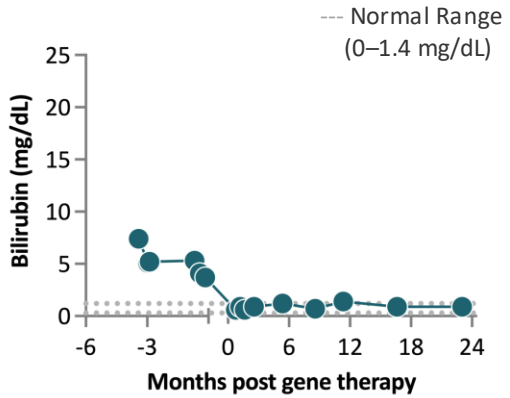
● Assessment Performed at Clinical Site
○ Assessment Performed at Local Laboratory



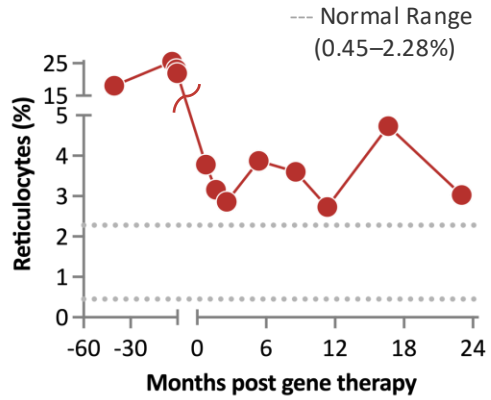
- Sustained **hemoglobin normalization from ~7.0 to 14.7 g/dL** 24 months post-RP-L301 infusion
- No red blood cell transfusions required following engraftment



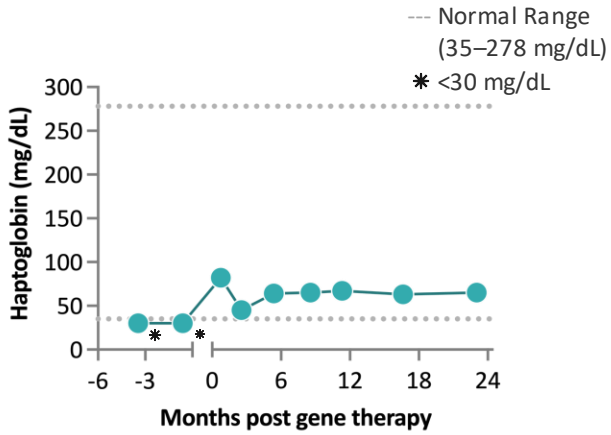
*BMMC VCN unable to be evaluated at 12 months due to technical difficulties with assay.



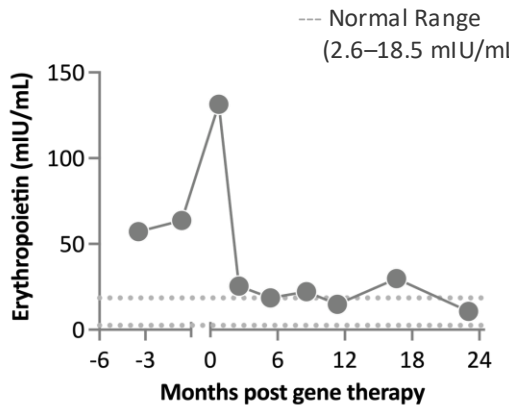
Normalized bilirubin from 7.4 mg/dL to 0.9 mg/dL



Significant improvement in reticulocytes from 25.5% to 3.0%



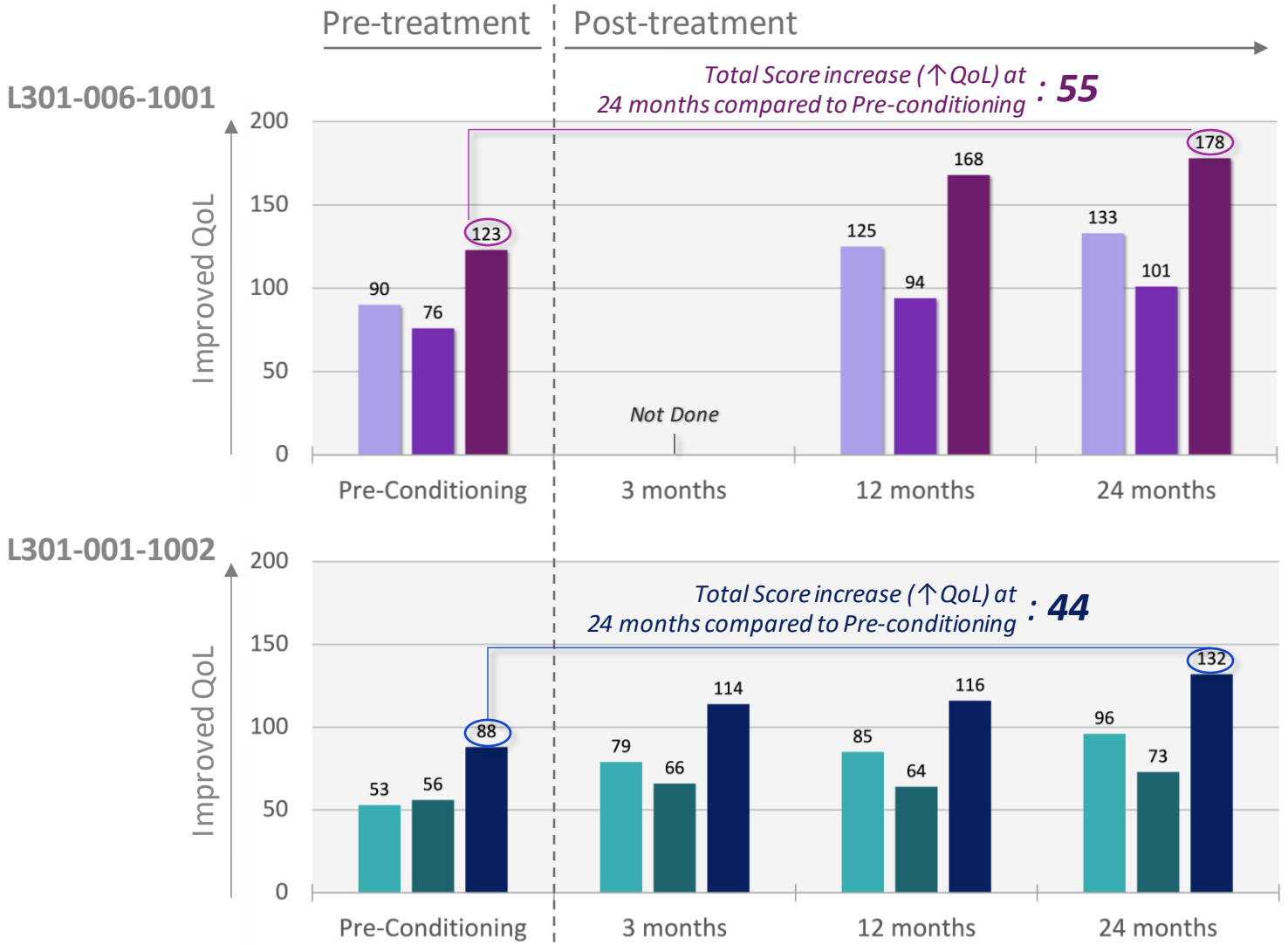
Sustained haptoglobin normalization



Significant decrease in erythropoietin levels

Data cut-off: October 26, 2022; Preliminary interim results are presented from the ongoing clinical study.

Preliminary Results Indicate Improved Quality of Life by FACT-Anemia
over 24 months subsequent to RP-L301 therapy



Trial Outcome Index (TOI) Score *(Score range: 0–136)*

General (G) Score *(Score range: 0–108)*

Total Score *(Score range: 0–188)*

Total Score is derived from subscales evaluating:

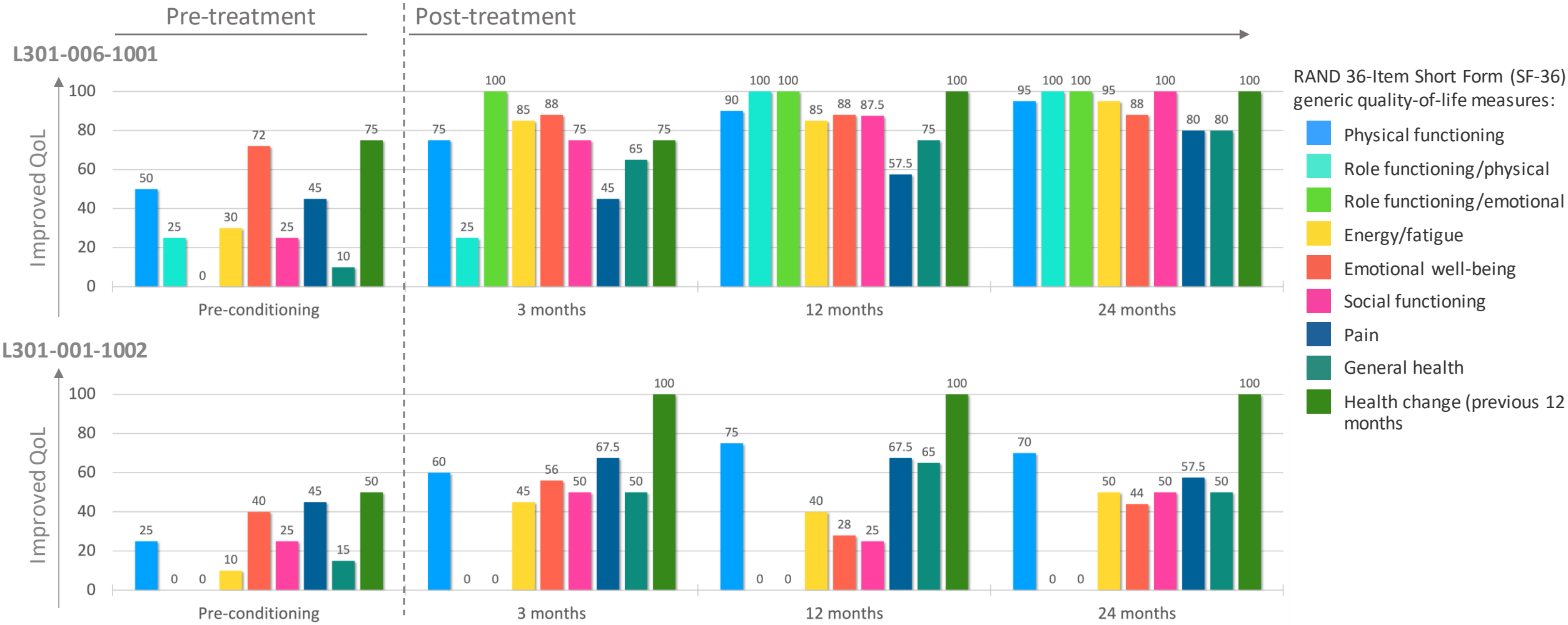
- Physical well-being
- Social/family well-being
- Emotional well-being
- Functional well-being
- Anemia

Both patients have anecdotally reported improved quality of life (QoL) following RP-L301 administration; Improved QoL has also been demonstrated by increase in FACT-An Total and component scores from baseline.

Previously presented data at Screening and at 6 months, 9 months, and 18 months post-gene therapy is not shown.
Data cut-off: October 26, 2022; Preliminary interim results are presented from the ongoing clinical study.

Preliminary Results Indicate Improved Quality of Life by SF-36

over 24 months subsequent to RP-L301 therapy



Substantial improvements have been reported for physical functioning, energy/fatigue, and general health components.

Previously presented data at Screening and at 6 months, 9 months, and 18 months post-gene therapy is not shown.
Data cut-off: October 26, 2022; Preliminary interim results are presented from the ongoing clinical study.

Safety Profile of RP-L301 appears highly favorable

- **Hematopoietic stem and progenitor cell mobilization and collection appears safe and feasible in the initial adult patients with severe PKD**

- **Hematopoietic reconstitution occurred within 2 weeks post RP-L301 administration**
  - Infusion well tolerated in (N=2)
  - Patients discharged from hospital within 1 month following RP-L301 infusion

- **No RP-L301-related serious adverse events (SAEs) through 24 months post- infusion in adult patients**
  - Transient transaminase elevation seen in both subjects post conditioning and infusion with no clinical stigmata of liver injury; fully resolved

- **Insertion site analyses in PB and BM for both adult patients through 12 months post-RP-L301 demonstrated highly polyclonal patterns and there has been no evidence of insertional mutagenesis**
  - Additional ISA testing for both patients is ongoing

Conclusions

- **Clinical efficacy and safety data indicate that RP-L301 is a potential treatment for patients with severe PKD, including those who did not derive benefit from available therapies (i.e. mitapivat)**
- **Investigational product RP-L301 was successfully manufactured**
  - Liquid culture VCNs >2.0; CD34+ cell doses 2.4–3.9×10^6/kg
- **Robust and sustained efficacy in both patients at 24-months post-RP-L301 infusion demonstrated by:**
  - ***Normalized hemoglobin*** **(from baseline levels in 7.0 – 7.5 g/dL range)**
  - Improved hemolysis parameters
  - ***Transfusion independence***
- **Both patients reported improved (and quantified) quality of life following treatment**

Adult and pediatric enrollment is complete. Phase 2 trial initiation is anticipated in 2023. Please contact PKDclinicaltrial@rocketpharma.com for more information.

Acknowledgements

Ciemat

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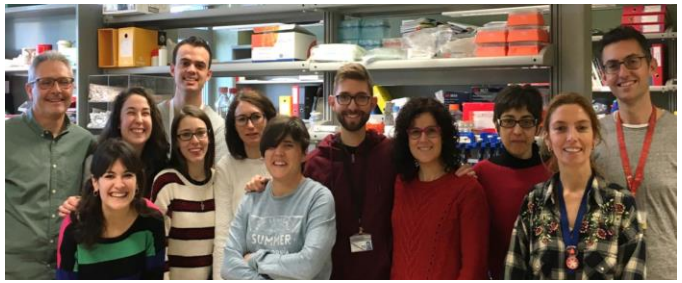

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