

# Changing the Treatment Paradigm for Pyruvate Kinase Deficiency with Lentiviral Mediated Gene Therapy: Interim Results from an Ongoing Global Phase 1 Study

**Ami J. Shah, MD**

**Center for Definitive and Curative Medicine**

**Department of Pediatrics, Division of Hematology, Oncology, Stem Cell Transplantation and Regenerative Medicine,  
Stanford University School of Medicine  
Lucile Packard Children's Hospital Stanford**

José Luis López Lorenzo, MD; Susana Navarro, PhD; Julián Sevilla, MD, PhD; Lucía Llanos, MD, PhD; Begoña Pérez de Camino Gaisse, MD; Sol Sanchez, MD; Bertil Glader, MD, PhD; May Chien, MD; Oscar Quintana-Bustamante, PhD; Miriam Zeini, PhD; Grace Choi, BS; Eileen Nicoletti, MD; Gayatri R. Rao, MD, JD; Maria Grazia Roncarolo, MD; Juan A. Bueren, PhD; Jonathan D. Schwartz, MD; José C. Segovia, PhD

American Society of Gene and Cell Therapy (ASGCT)

Monday, May 16, 2022

Abstract # 357



SEEKING GENE THERAPY CURES



**Stanford**  
Children's Health

Lucile Packard  
Children's Hospital  
**Stanford**

# Disclosures

---

- Advisory Board: Orchard Therapeutics

# 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 are palliative and limited to chronic blood transfusions, iron chelation therapy, and splenectomy.
- Side effects of these treatments include increased risk of disseminated infections and venous & arterial thromboembolic events.
- Therapies do not ameliorate PKD-related iron overload or end-organ damage.
- Allogeneic HSCT has been performed in select cases and resulted in transfusion independence; however, efficacy has been limited by significant toxicities and donor availability.

# 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 performed in Dr. Segovia's lab in a clinically relevant PKD murine model have demonstrated safety and efficacy.

## **PKD mice transplanted with gene corrected cells demonstrated phenotypic correction:**

- Significantly increased RBC count and half-life
- Decreased reticulocytosis, erythropoietin levels, and iron overload
- Normalized spleen and liver size (and structure)

## **Highly favorable safety profile:**

- No physical, behavioral, biochemical, hematologic, or morphologic abnormalities observed in transplanted mice
- Limited evidence of lentiviral vector in non-hematopoietic 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

- Clinically significant reduction of anemia (Hb ↑)
- Transfusion independence at 12 months
- $\geq 50\%$  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:

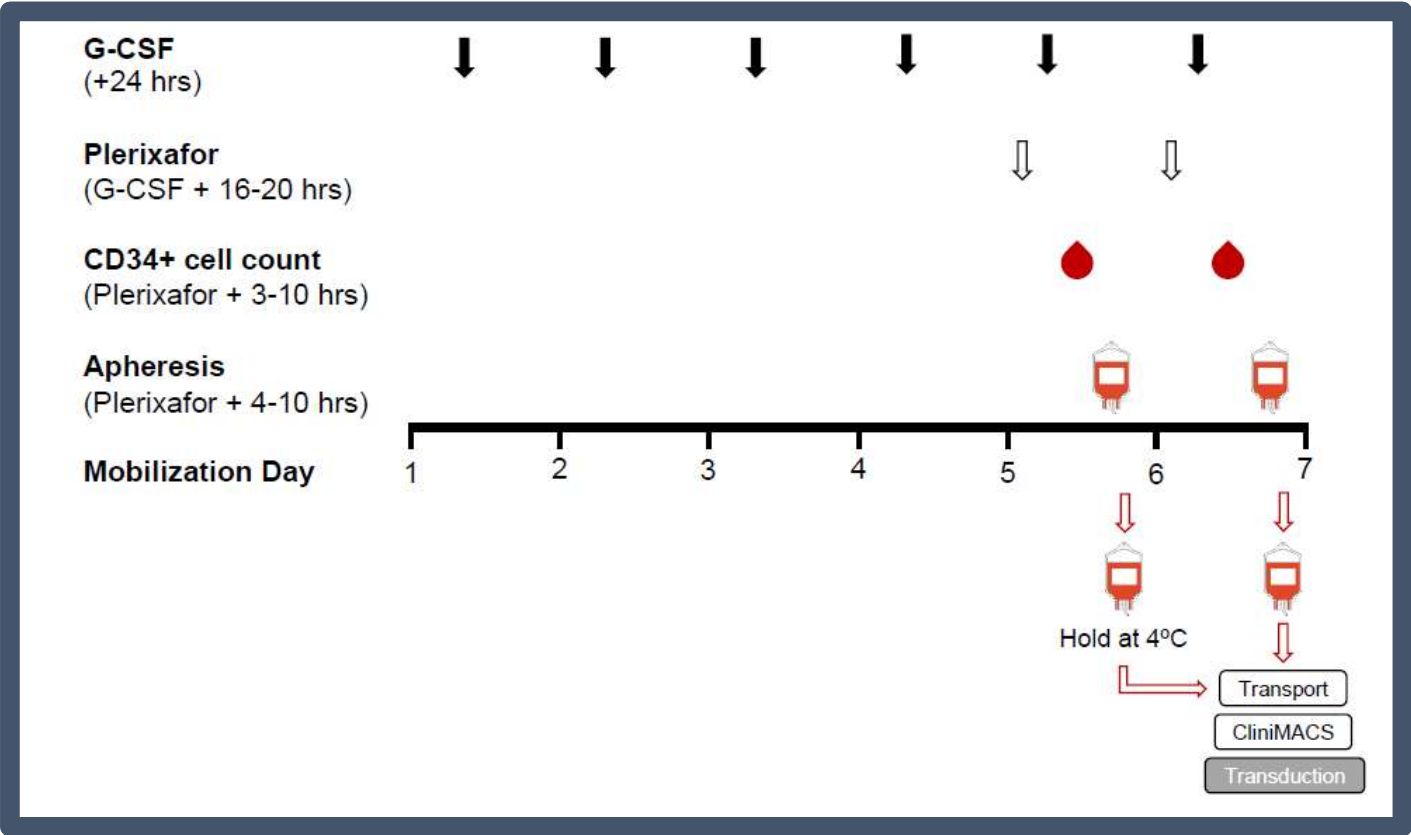
- PKD diagnosis with a confirmed *PKLR* mutation
- Age:
  - 1<sup>st</sup> cohort (N=2):  $\geq 18$  to 50 years
  - 2<sup>nd</sup> cohort (N=2):  $\geq 12$  to 17 years
  - 3<sup>rd</sup> cohort (N=2):  $\geq 8$  to 11 years
- Severe and/or transfusion-dependent anemia
- Splenectomized
- Adequate cardiac, 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

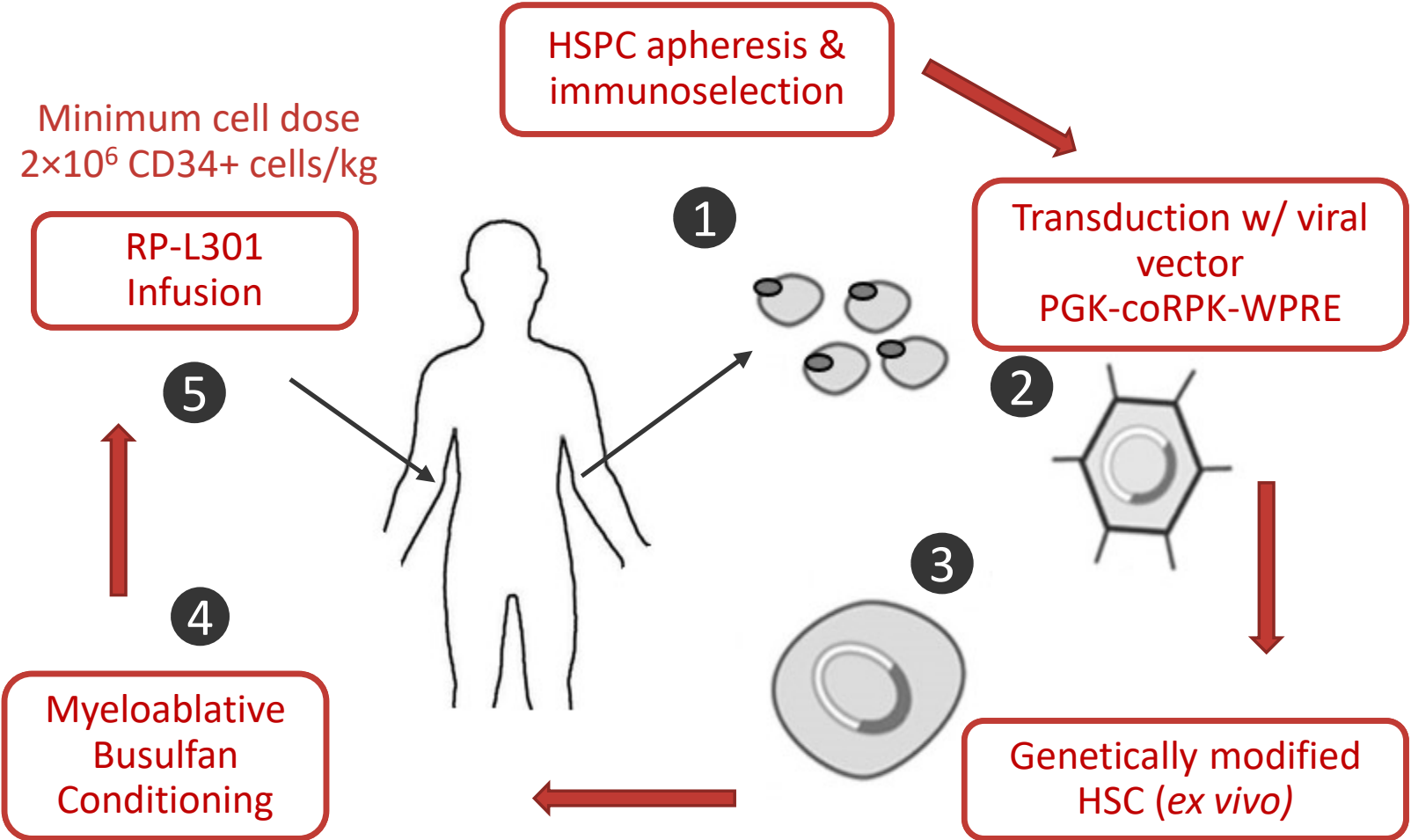
## CD34+ cell mobilization protocol with G-CSF and plerixafor:



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/ $\mu$ L are required to initiate apheresis.

## Schematic diagram of product manufacture and treatment:



Patients undergo HSPC 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; TDM)

# Patient L301-006-1001 Clinical Course

## Pre-treatment Characteristics

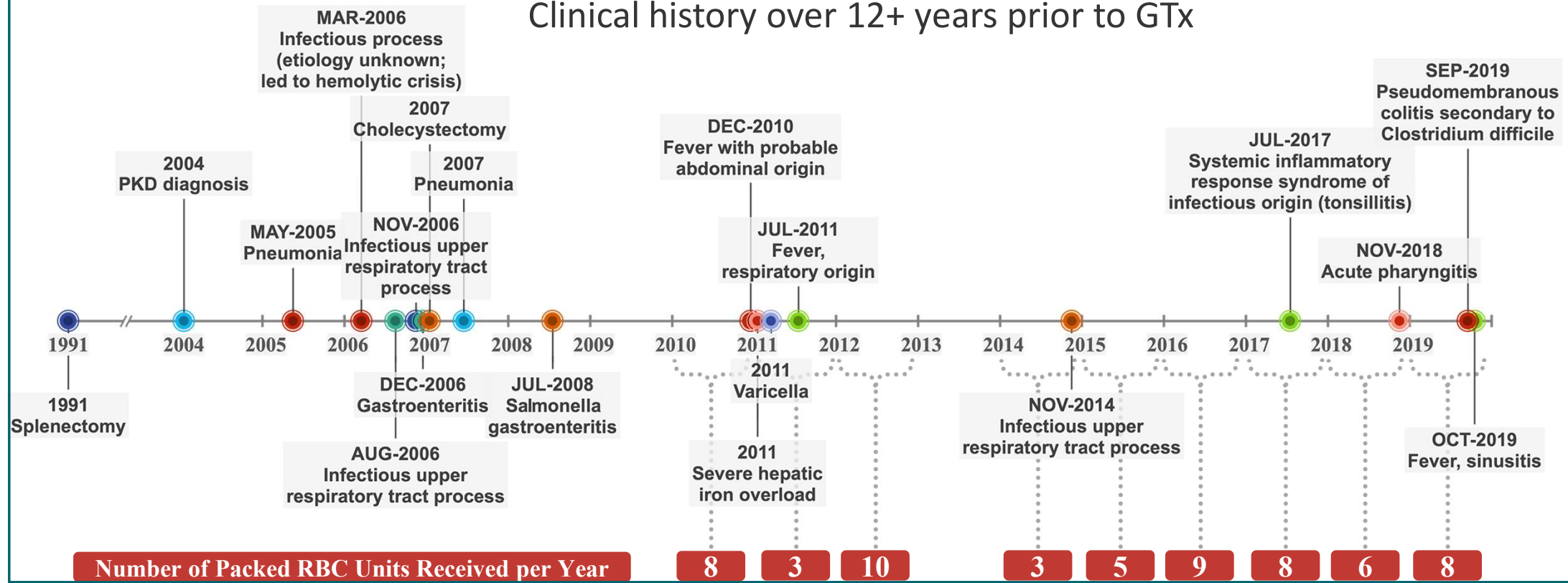
| Age (y) & Gender | PKD Mutation          | Hemoglobin (g/dL) | Bilirubin (mg/dL) | Erythropoietin (mIU/mL) | Transfusion Requirement for 2 Years Prior to Enrollment |
|------------------|-----------------------|-------------------|-------------------|-------------------------|---------------------------------------------------------|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

<sup>†</sup> Average hemoglobin calculated over 2 years prior to study enrollment

## Product Metrics

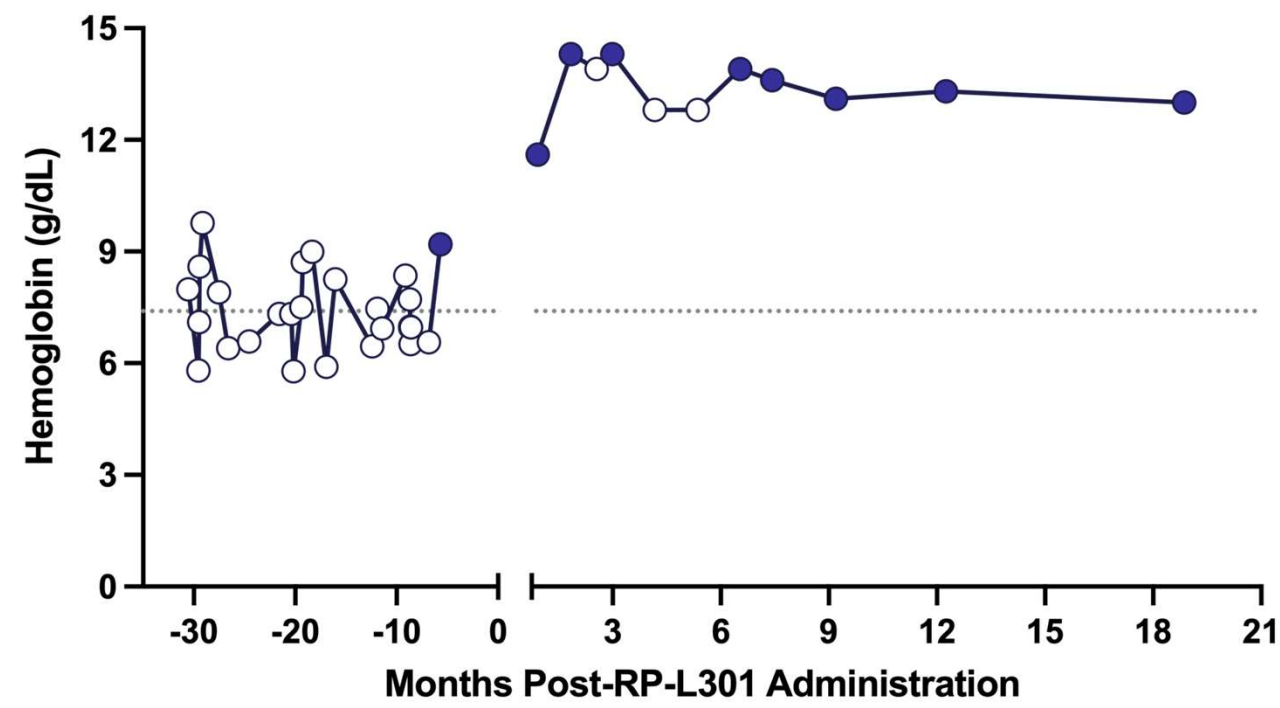
| Nucleated Cells/kg | CD34+ Cells/kg    | CFCs/kg           | Mean VCN: Liquid Culture |
|--------------------|-------------------|-------------------|--------------------------|
| $3.9 \times 10^6$  | $3.9 \times 10^6$ | $9.2 \times 10^5$ | 2.73                     |

Clinical history over 12+ years prior to GTx

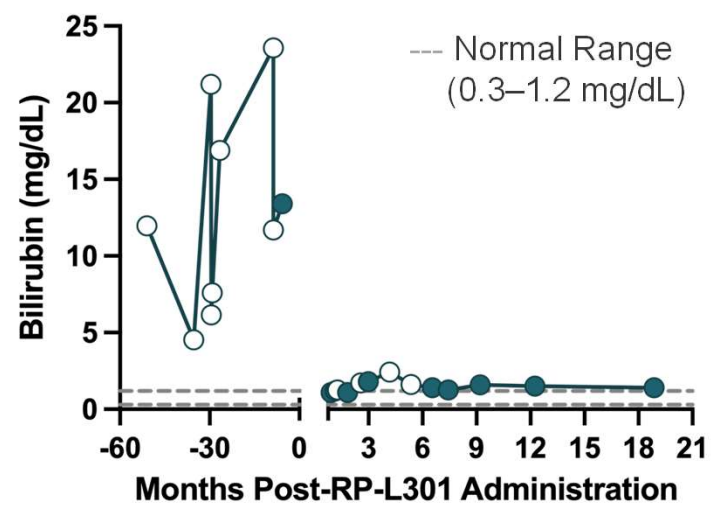
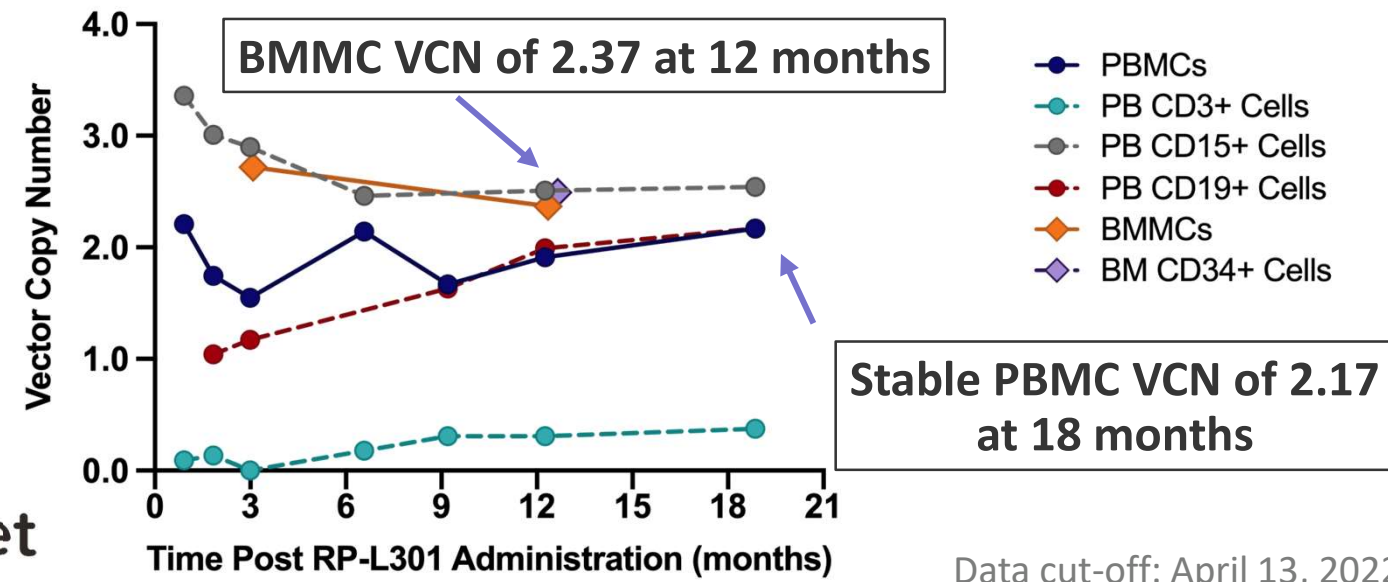


- Patient had history of multiple infections precipitating hemolytic crises & requiring multiple transfusions and hospitalizations.
- Patient had hepatic iron overload secondary to transfusions and underlying PKD.

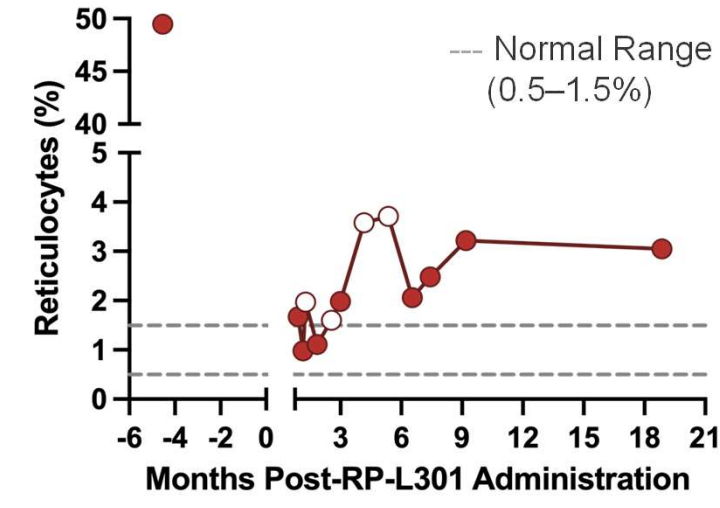
# Preliminary Efficacy Results: L301-006-1001



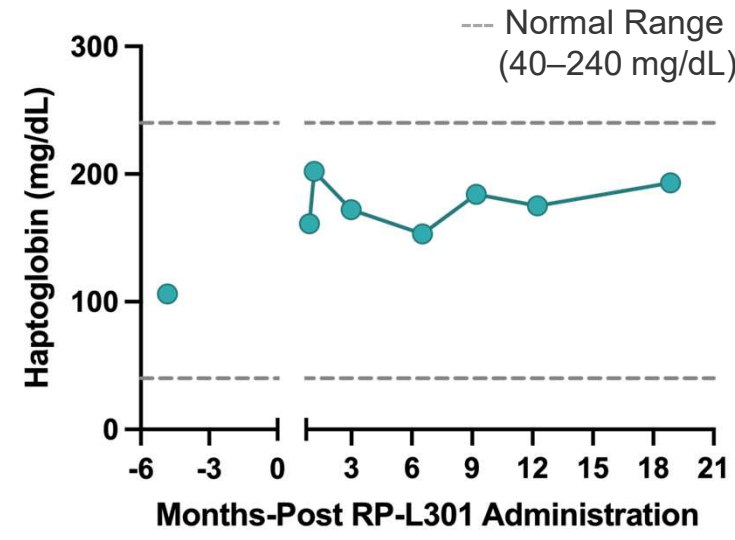
- Sustained **hemoglobin improvement from ~7.4 g/dL to 13.0 g/dL** over 18 months post-RP-L301 infusion
- No red blood cell transfusion requirements following engraftment



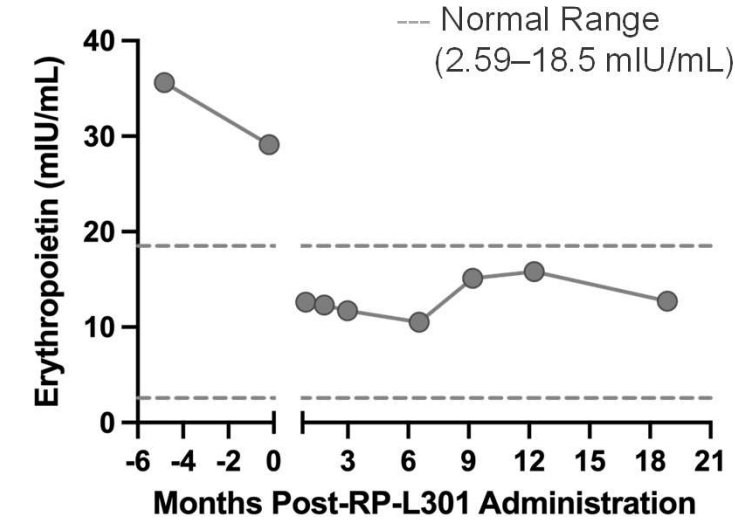
*Significant bilirubin decrease from 13.4 mg/dL to 1.4 mg/dL*



*Sustained reduction in reticulocytes*



*Haptoglobin remains within normal range*



*Continued erythropoietin normalization*



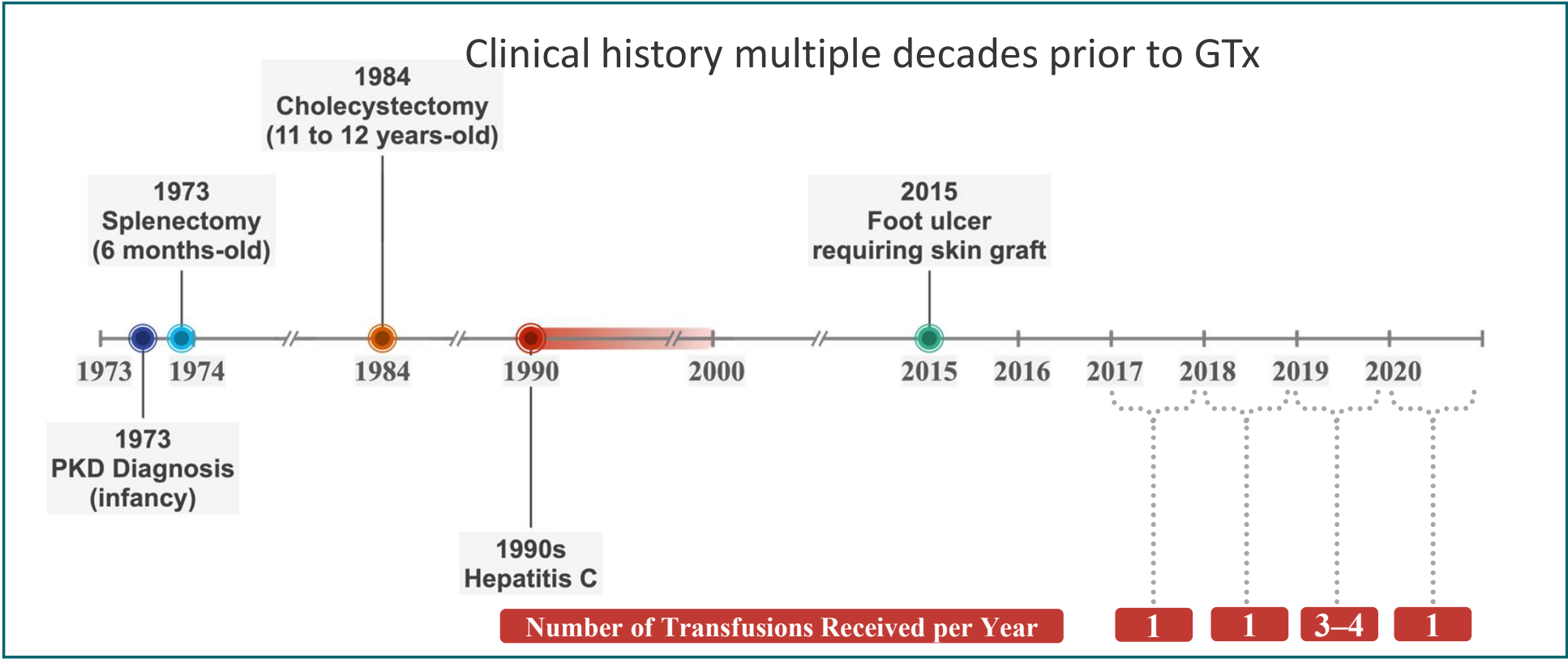
Data cut-off: April 13, 2022; Preliminary interim results are presented from the ongoing clinical study.

# Patient L301-001-1002 Clinical Course

## 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<br>c.1047A>AA<br>c.1744CGG>AGG | 7.0 <sup>‡</sup>  | 7.4               | 57.2                    | ~5 transfusion episodes                                 |

<sup>‡</sup> Average hemoglobin calculated over 2 years prior to study enrollment

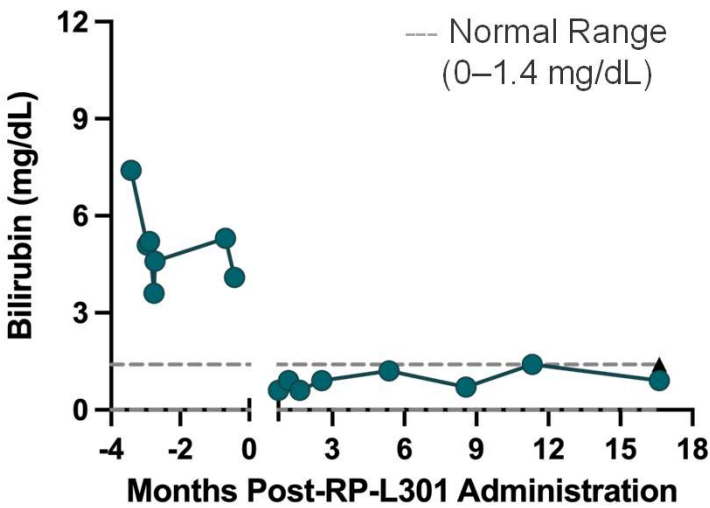
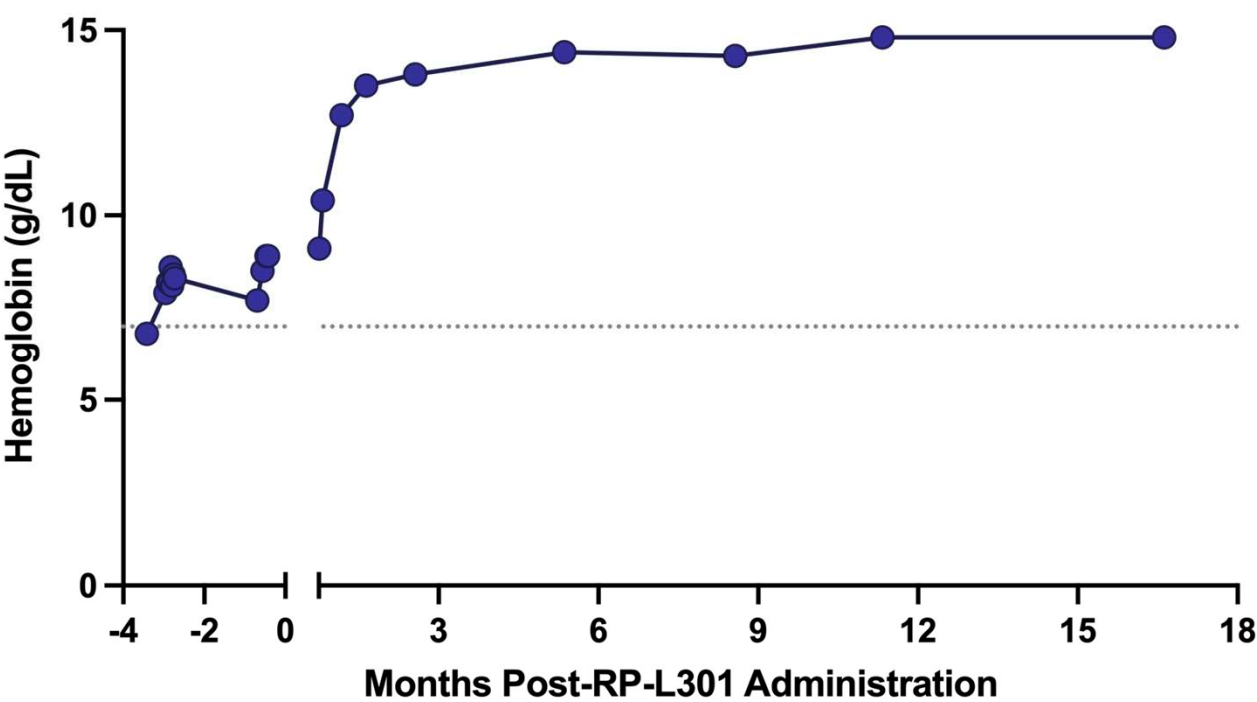


## Product Metrics

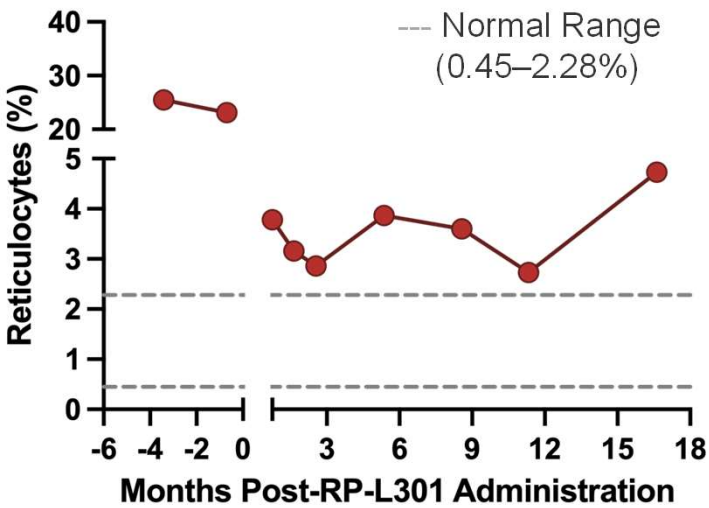
| Nucleated Cells/kg | CD34+ Cells/kg    | CFCs/kg           | Mean VCN: Liquid Culture |
|--------------------|-------------------|-------------------|--------------------------|
| $2.4 \times 10^6$  | $2.4 \times 10^6$ | $3.4 \times 10^5$ | 2.08                     |

- 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

# Preliminary Efficacy Results: L301-001-1002

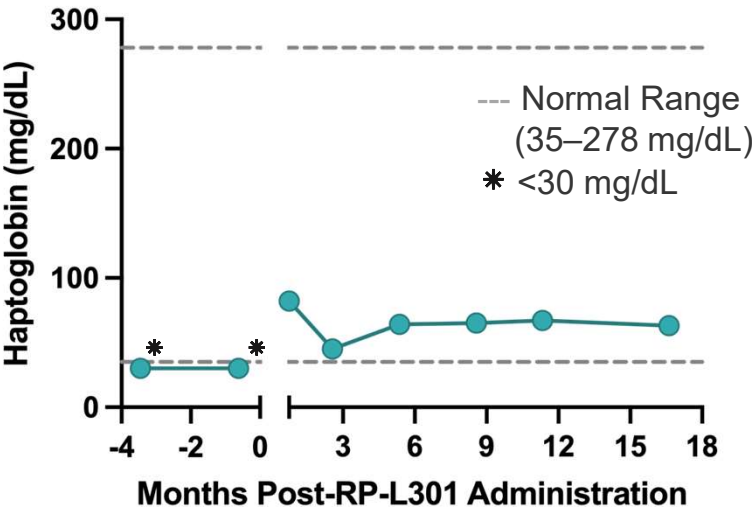
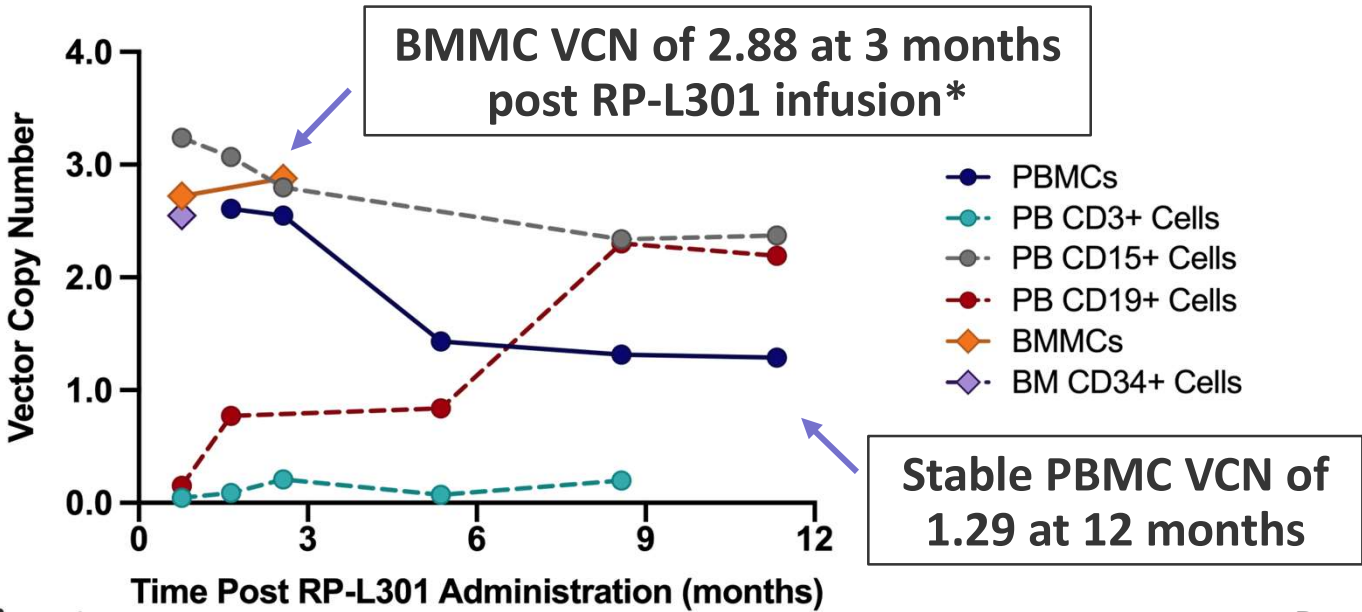


Normalized bilirubin from 7.4 mg/dL to 0.9 mg/dL

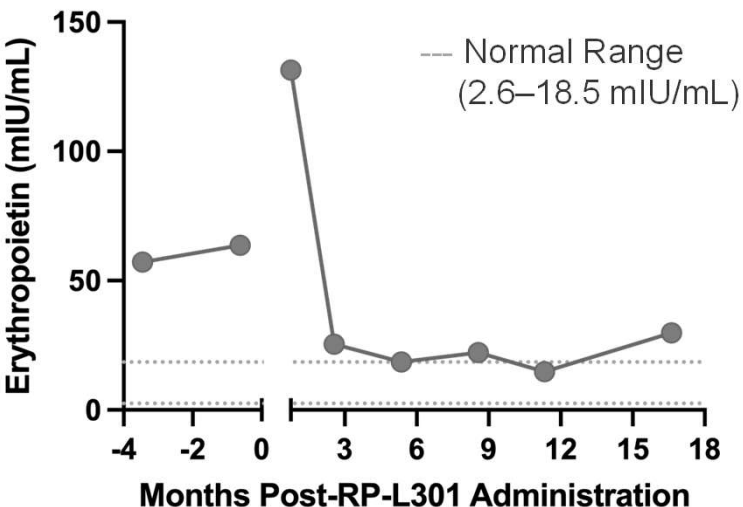


Significant improvement in reticulocytes from 25.5% to 4.73%

- Hemoglobin normalized to 14.8 g/dL at ~18 months post-rx
- No red blood cell transfusion requirements following engraftment



Sustained haptoglobin normalization



Significant decrease in erythropoietin levels

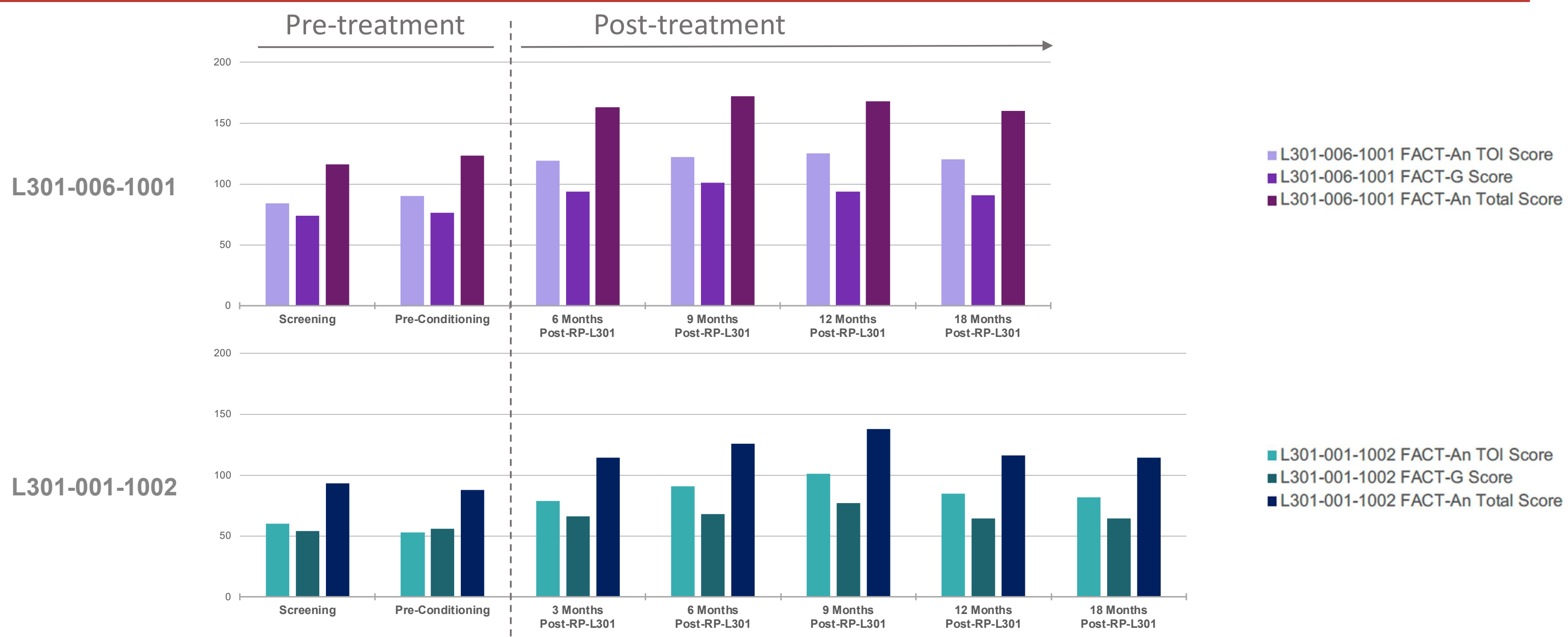


\*BMMC VCN unable to be evaluated at 12 months due to technical difficulties with assay.

Data cut-off: April 13, 2022; Preliminary interim results are presented from the ongoing clinical study.

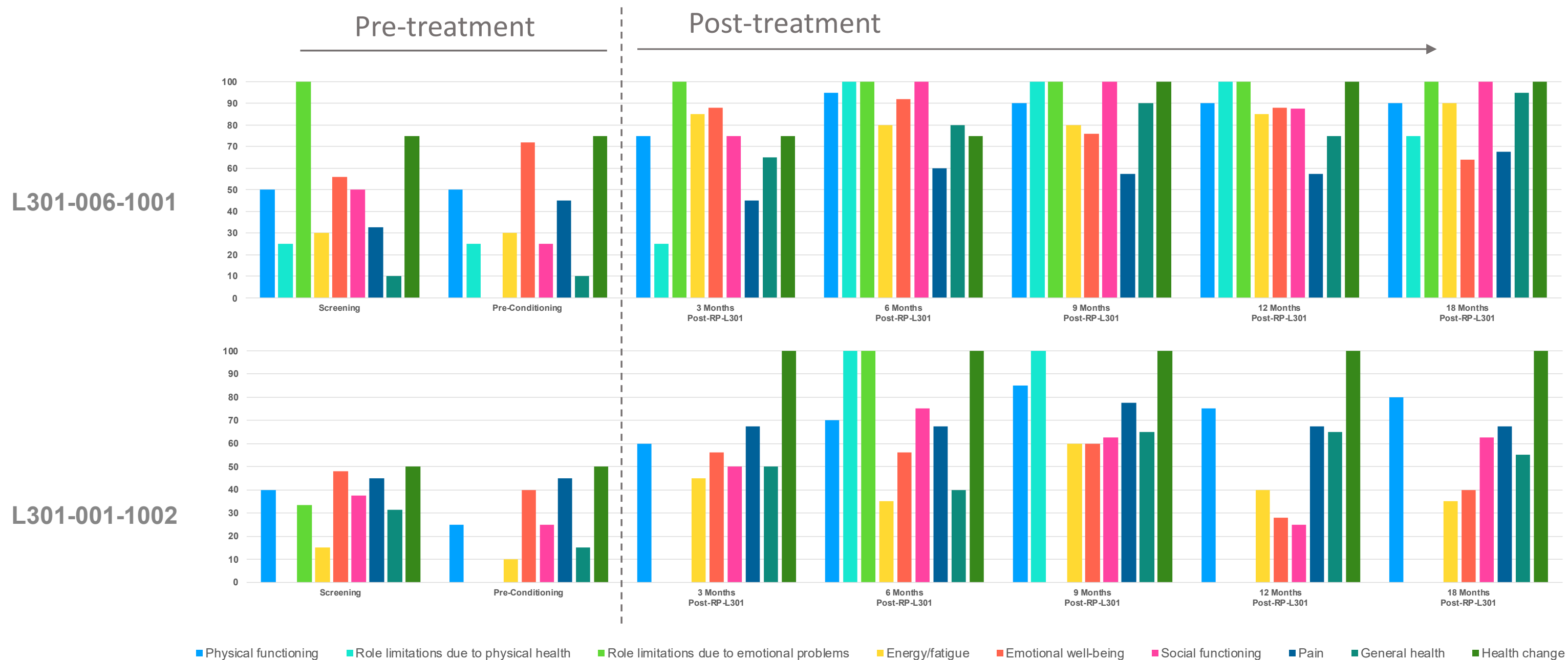


# Preliminary Results Indicate Improved Quality of Life (FACT-An) during 18 months subsequent to RP-L301 therapy



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.

# Preliminary Results Indicate Improved Quality of Life (SF-36) during 18 months subsequent to RP-L301 therapy



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

# Safety Profile of RP-L301 appears highly favorable

---

- Hematopoietic stem and progenitor cell 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 IP-related serious adverse events (SAEs) at 18 months post- infusion in adult patients
  - Transient transaminase elevation seen in both subjects post conditioning and infusion with no clinical stigmata of liver injury; now resolved
- Insertion site analyses in PB and BM for both adult patients at 12 months post-RP-L301 demonstrated highly polyclonal patterns and no demonstrable insertions in proximity to potential oncogenic loci
  - Additional ISA testing for both patients is ongoing

# Conclusions

---

- **Investigational product RP-L301 was successfully manufactured**
  - Liquid culture VCNs >2.0; CD34+ cell doses  $2.4\text{--}3.9 \times 10^6/\text{kg}$
- **Robust and sustained efficacy in both patients at 18-months post-RP-L301 infusion demonstrated by:**
  - *Normalized hemoglobin*
  - Improved hemolysis parameters
  - *No red blood cell transfusion requirements* following engraftment (transfusion independence)
- **Both patients reported improved quality of life following treatment**

*Pediatric cohort is currently enrolling patients.*

*Please contact [PKDclinicaltrial@rocketpharma.com](mailto:PKDclinicaltrial@rocketpharma.com) for more information.*

# Acknowledgements



José-Carlos Segovia, PhD



Susana Navarro, PhD



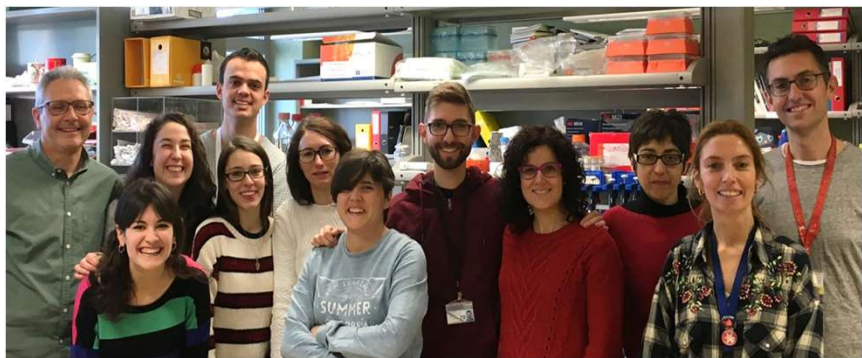
José Luis Lopez Lorenzo, MD  
Lucía Llanos, MD, PhD  
Begoña Pérez Camino de Gaisse, MD  
Sol Sanchez, MD  
Javier Cornago, MD



Maria Grazia Roncarolo, MD



Bert Glader, MD, PhD  
May Chien, MD  
Elisabeth Merkel, RN



CIEMAT Team



Oscar Quintana-Bustamante, PhD



Juan Bueren, PhD



Julián Sevilla, MD, PhD



Eileen Nicoletti, MD  
Gayatri R. Rao, MD, JD  
Grace Choi, BS  
Miriam Zeini, PhD  
Jonathan Schwartz, MD  
Kinnari Patel, PharmD, MBA  
Gaurav Shah, MD