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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26th Annual Meeting of the American Society of Gene and Cell Therapy (ASGCT)

May 18, 2023

Abstract #218

Disclosures

- Advisory Board: Vertex Pharmaceuticals and Bluebird Bio

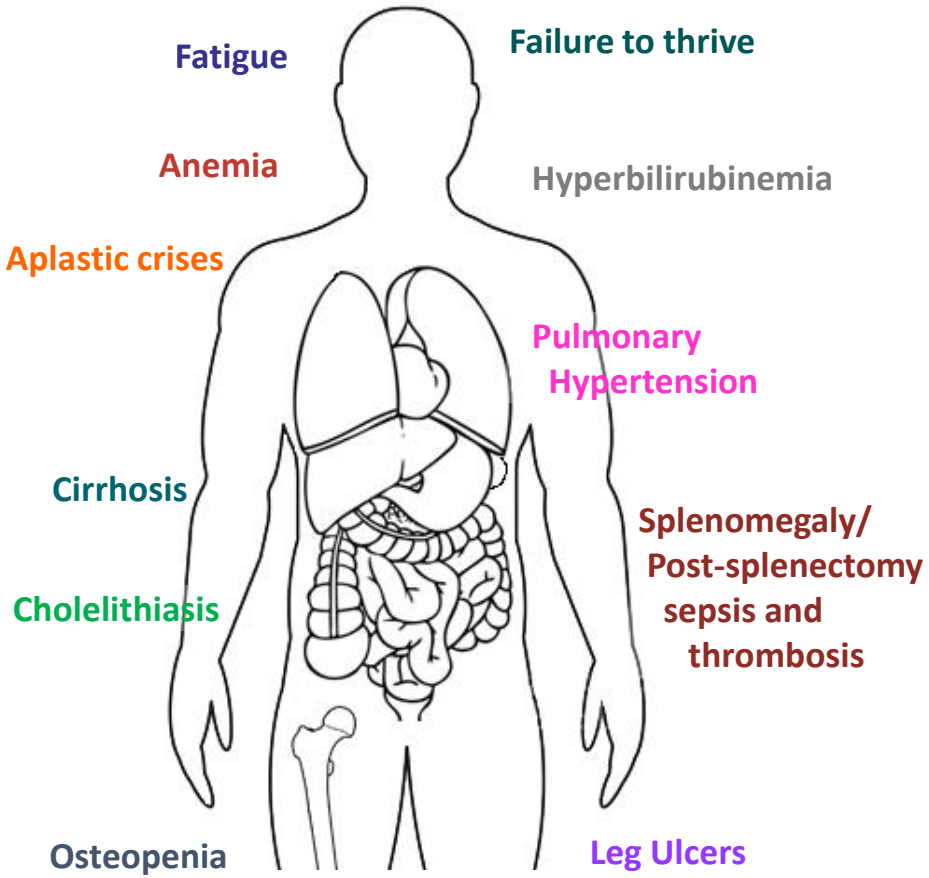
Pyruvate Kinase Deficiency (PKD)

- 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.

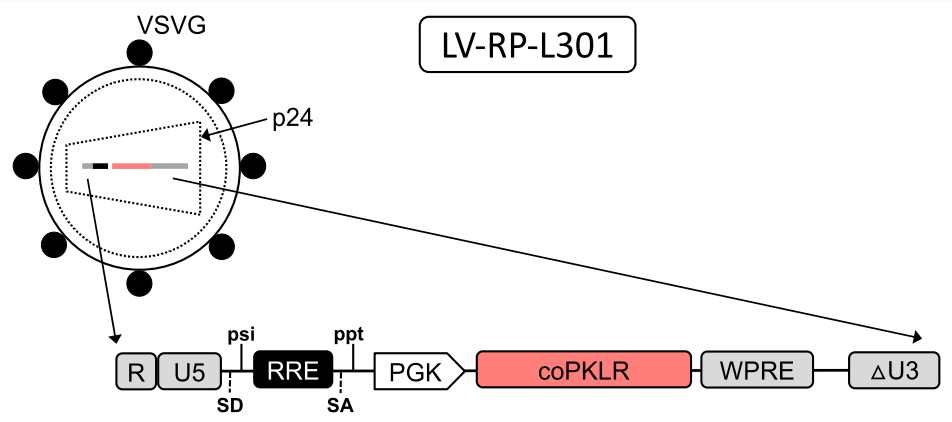
Complications of PKD



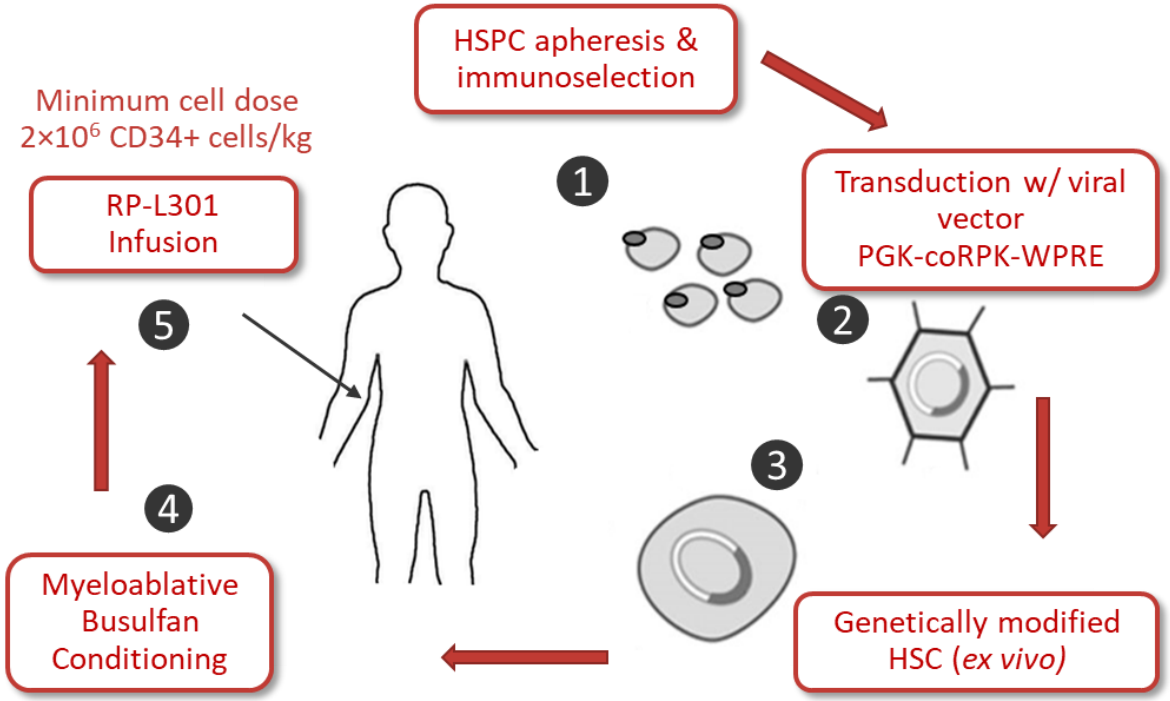
Al-Samkari H et al. N Engl J Med. 2022; 386:1432-1442.
Grace R et al. Blood. 2018; 131: 2183-2192.
Van Straaten S et al. Haematologica. 2018; 103(2): e82–e86
Zanella A et al. Br J Haematol. 2005;130(1):11-25.

Gene Therapy For PKD: RP-L301

Inserts a functional *PKLR* gene into autologous HSPCs with the intent of normalizing PK enzyme production, RBC function and lifespan



* Developed at CIEMAT by Dr. José Carlos Segovia



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)

TDM: therapeutic drug monitoring

Clinical Trial Design, Patient, and Drug Product Characteristics

Trial Design

- Non-Randomized Global Phase 1 Study

Key Eligibility Criteria

- 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

Primary Endpoint

Phase 1:

- Safety and toxicity of RP-L301

Secondary Endpoints

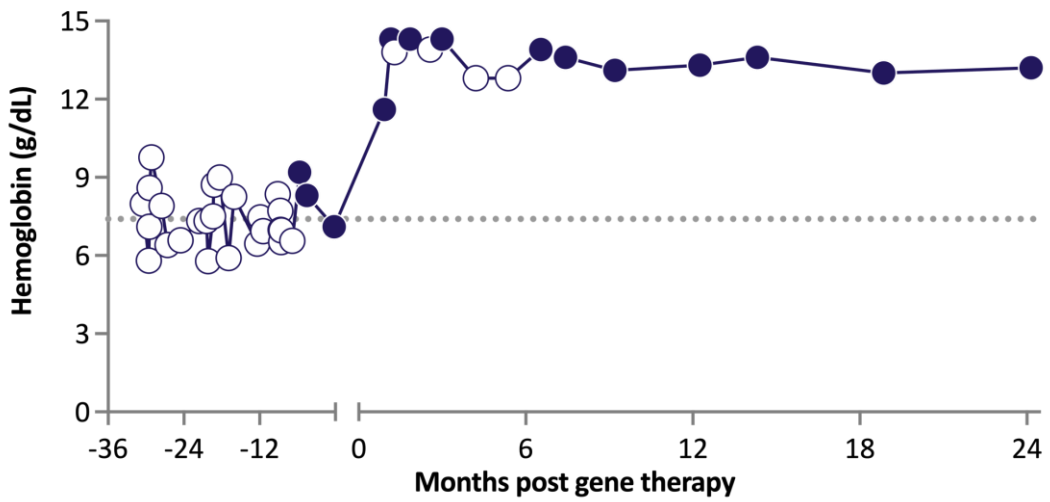
- Clinically significant reduction of anemia (Hb ↑)
- Transfusion independence at 12 months
- ≥50% reduction in transfusion requirements (when relevant) at 12 months
- Reduction of hemolysis
- Peripheral blood (PB) and bone marrow (BM) genetic correction as demonstrated by vector copy number (VCN)

		Adult Cohort		Pediatric Cohort
		L301-006-1001	**L301-001-1002**	**L301-002-1005**
PATIENT CHARACTERISTICS	PKD Mutation	c.721G>T c.1529G>A	c.703GGG>AGG c.1047A>AA c.1744CGG>AGG	c.1116+2T>G c.721G>T
	Hemoglobin (g/dL)	7.4†	7.0†	5.9–10.2* (median: 7.9)
	Bilirubin (mg/dL)	13.4	7.4	14.9
	Erythropoietin (mIU/mL)	35.6	57.2	94.2
	Transfusion History (2y Prior to Enrollment)	~14 transfusion episodes	~5 transfusion episodes	~3 transfusion episodes
RP-L301 METRICS	CD34+ Cells/kg	3.9×10^6	2.4×10^6	2.3×10^6
	CFCs/kg	9.2×10^5	3.4×10^5	8.1×10^5
	Mean VCN: Liquid Culture	2.73	2.08	1.98

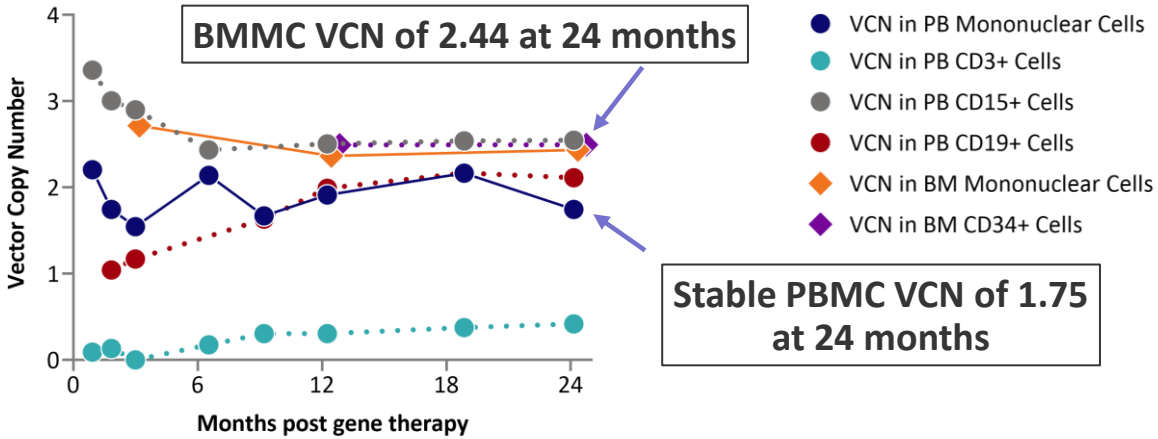
† Average hemoglobin calculated over 2 years prior to study enrollment

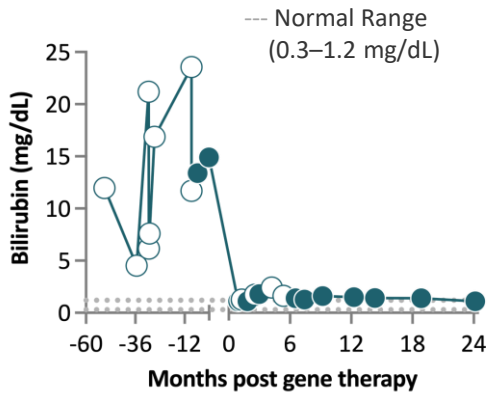
* Hemoglobin range over 2 years prior to study enrollment, *includes post-transfusion values*.

Patient L301-006-1001 Efficacy Results

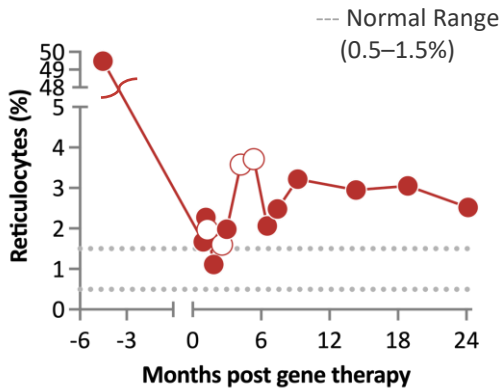


- Sustained **hemoglobin normalization from ~7.4 g/dL to 13.2 g/dL** 24 months post-RP-L301 infusion
- No red blood cell transfusions required following engraftment

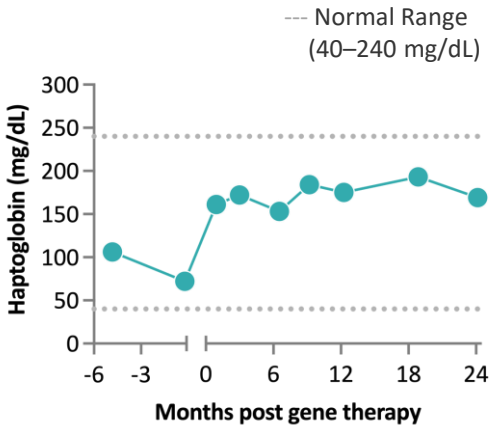




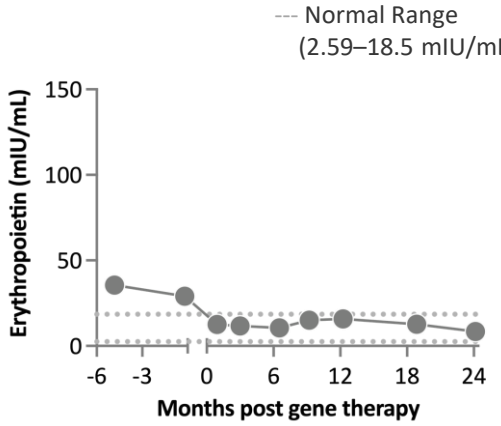
Significant bilirubin decrease from 13.4 mg/dL to 1.1 mg/dL



Sustained improvements in reticulocytes from 49.5% to 2.5%



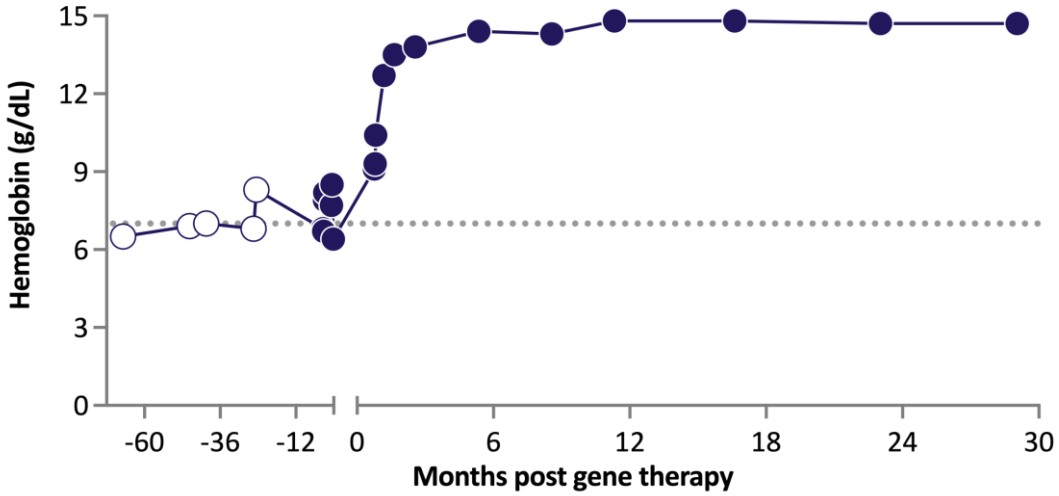
Haptoglobin remains within normal range



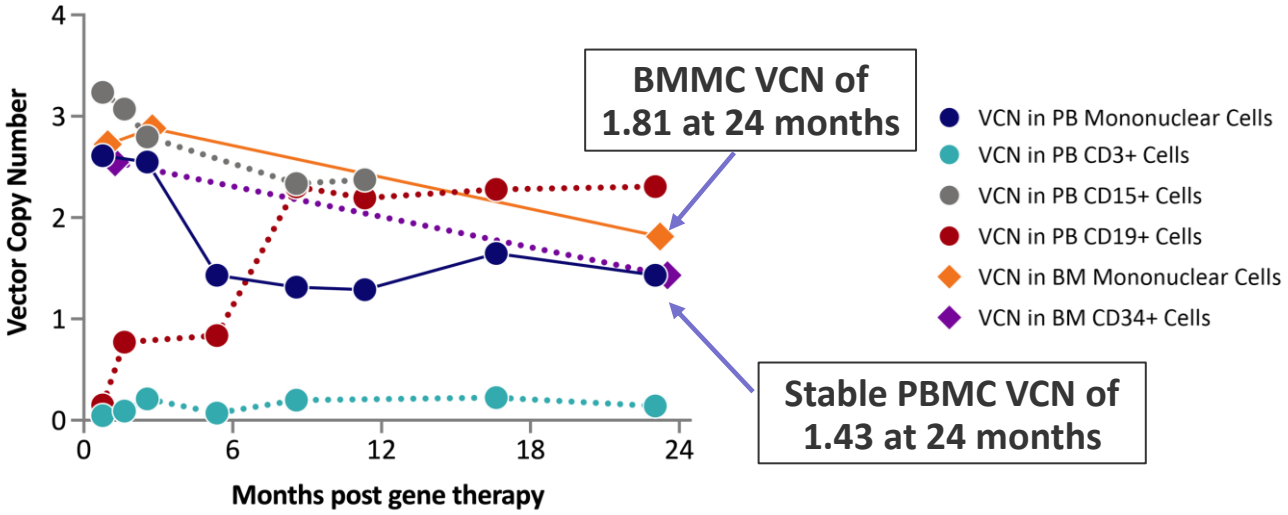
Continued erythropoietin normalization

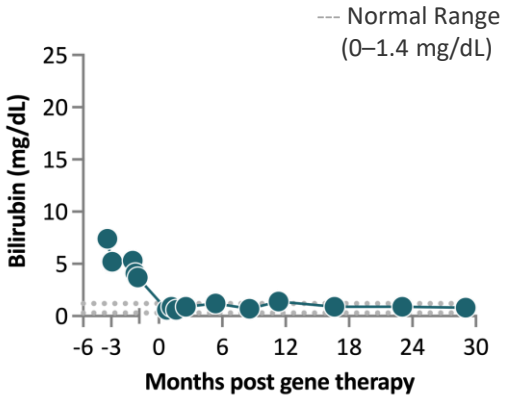
Data cut-off: May 3, 2023; Preliminary interim results are presented from the on-going clinical trial.

Patient L301-001-1002 Efficacy Results

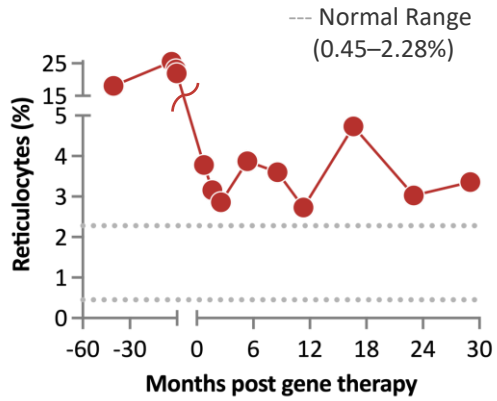


- Sustained **hemoglobin normalization from ~7.0 to 14.7 g/dL** at 30 months post-RP-L301 infusion
- No red blood cell transfusions required following engraftment

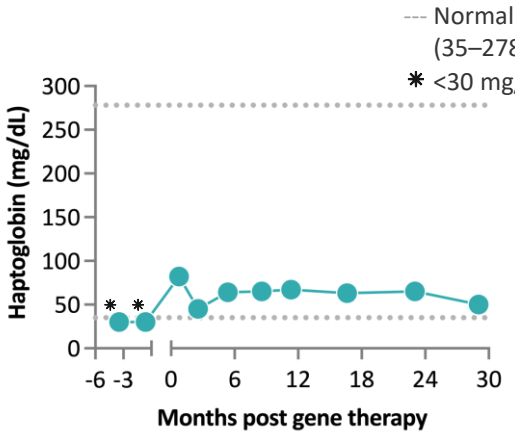




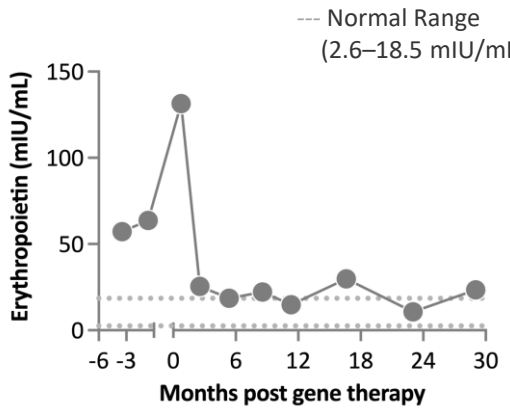
Normalized bilirubin from 7.4 mg/dL to 0.8 mg/dL



Significant improvement in reticulocytes from 25.5% to 3.4%



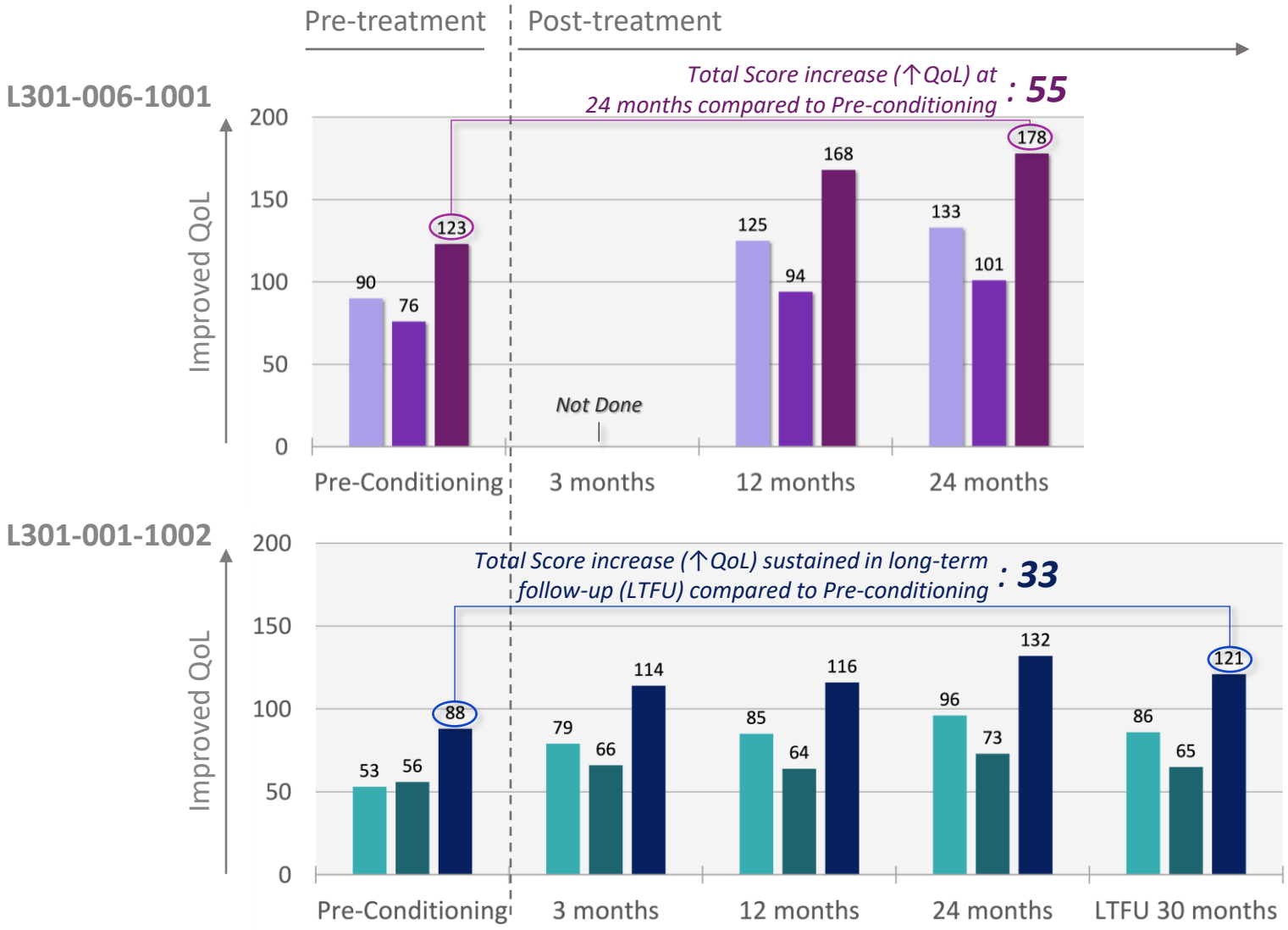
Sustained haptoglobin normalization



Significant decrease in erythropoietin levels

Data cut-off: May 3, 2023; Preliminary interim results are presented from the on-going clinical trial.

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)*

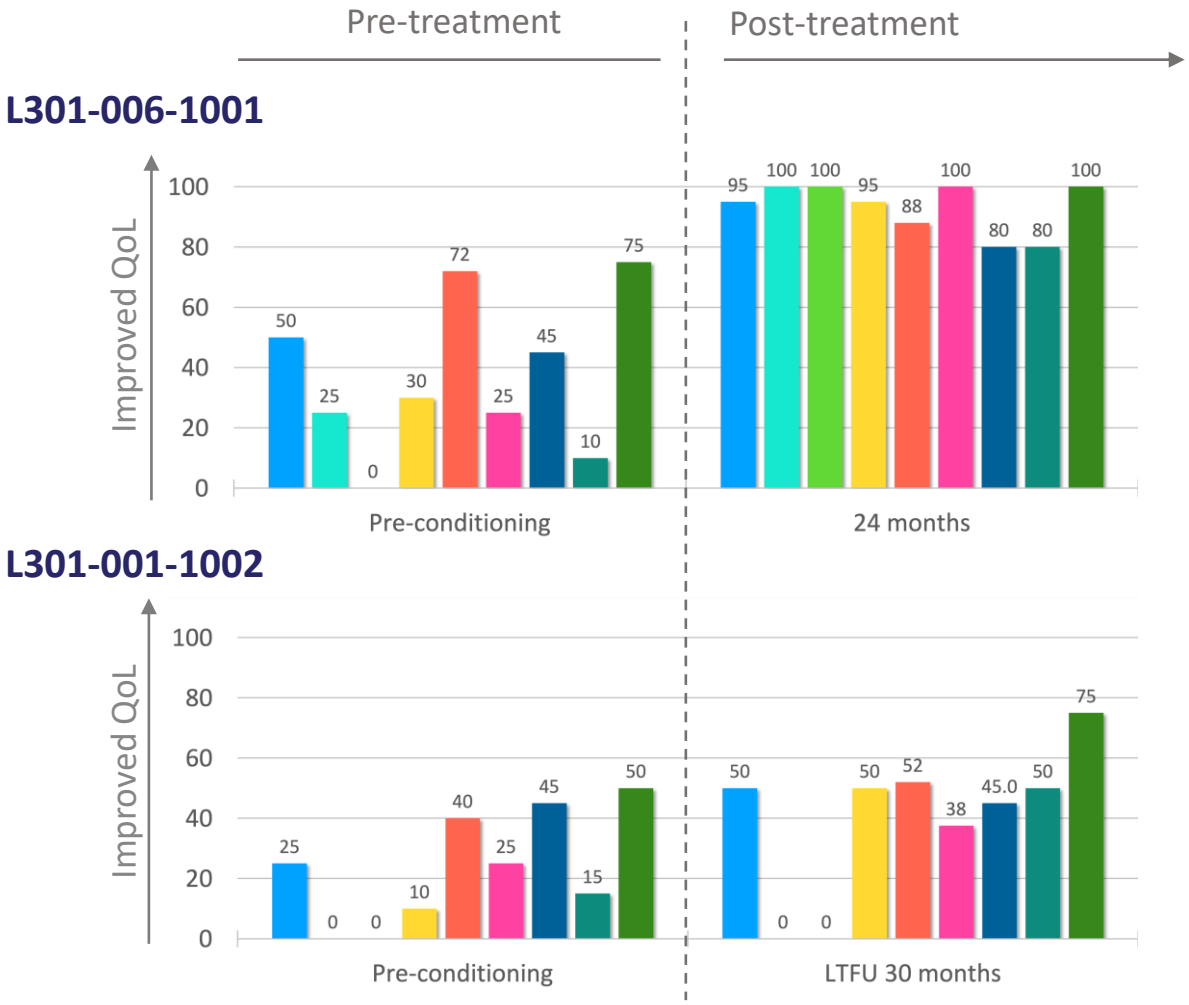
Total Score is derived from subscales evaluating:

- 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: April 26, 2023

Results Indicate Improved Quality of Life by SF-36
over 24 months subsequent to RP-L301 therapy



RAND 36-Item Short Form (SF-36) generic quality-of-life measures:

- Physical functioning
- Role functioning/physical
- Role functioning/emotional
- Energy/fatigue
- Emotional well-being
- Social functioning
- Pain
- General health
- Health change (previous 12 months

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: April 26, 2023

Safety Profile of RP-L301 in Adult Cohort appears highly favorable

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

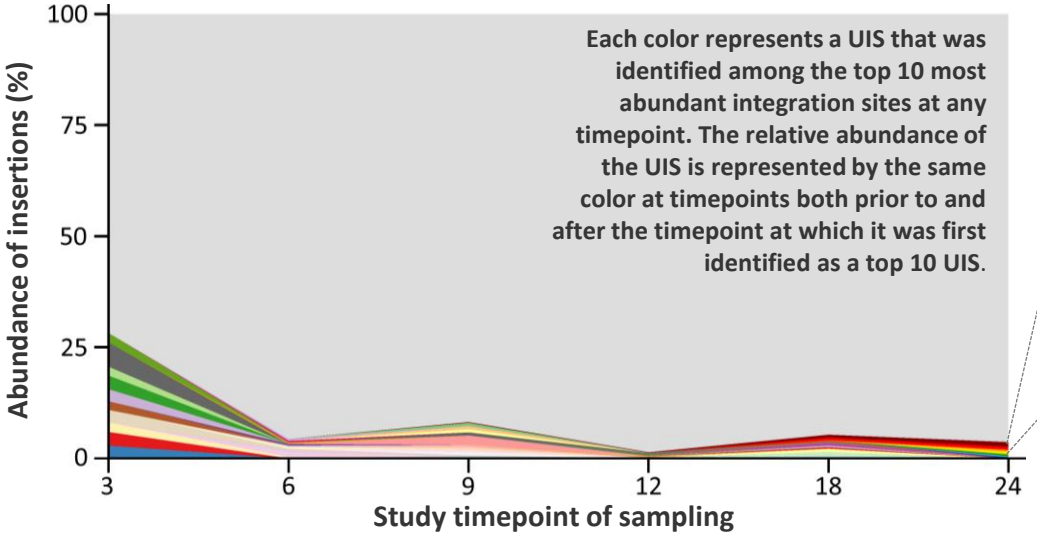
- **Hematopoietic reconstitution occurred within 2 weeks post RP-L301 administration**
  - Infusion well tolerated in (N=2)
  - Patients discharged from hospital within 1 month following RP-L301 infusion

- **No RP-L301-related serious adverse events (SAEs) up to 30 months post- infusion**
  - Transient transaminase elevation seen in both adult 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 24 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

Highly Polyclonal Insertion Patterns Evident from ISA (PB) over 24 months post-RP-L301

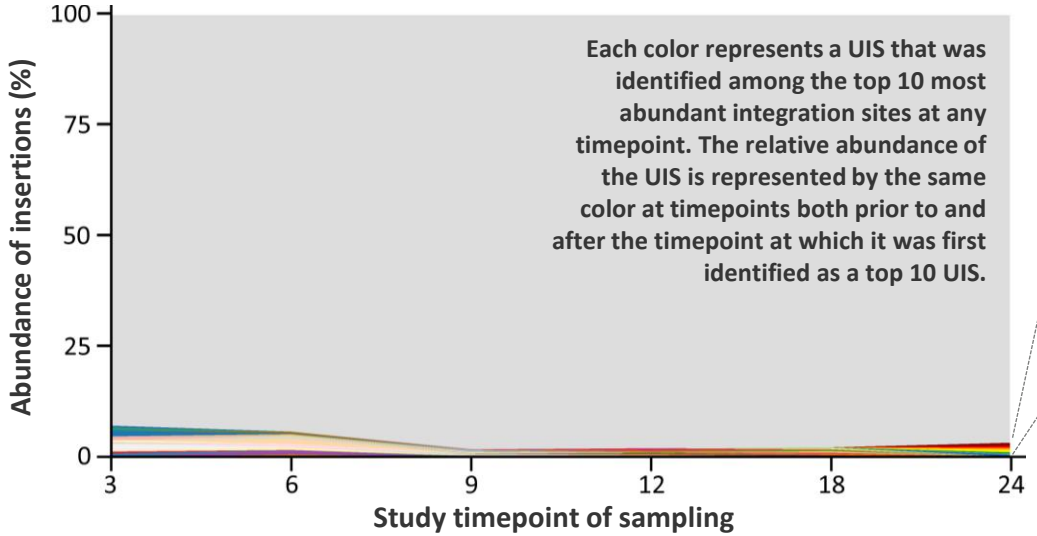
L301-006-1001: Integration Site Abundance Over Time



Month 3		Month 6		Month 9		Month 12		Month 18		Month 24	
%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene
5.49	HLA-DOA	1.16	TNRC6C*	2.14	MIR8074	0.23	HINT1	0.83	RBM4B*	1.08	NPLOC4
3.07	MARCHF8*	0.47	BAZ1A*	0.71	ABHD16A*	0.17	EPS15~*	0.71	POU2F1*	0.68	BRD4~*
3.07	ZNF543	0.35	COBLL1*	0.71	CARMIL1*	0.15	MTMR3~*	0.67	NPLOC4	0.31	MSH5*
2.89	ZNF546	0.35	GUF1*	0.71	CEP192*	0.13	NFAT5*	0.67	SHMT1*	0.31	NFAT5*
2.85	RPTOR*	0.35	PLEKHB2*	0.71	EPB41*	0.11	EDC3*	0.59	BRD4~*	0.25	ATF7*
2.72	NUMA1~*	0.35	RGS12*	0.71	SH3RF1*	0.11	TP53BP1*	0.39	EZH2~*	0.25	NDEL1*
2.12	FOXK2*	0.35	RNPEPL1*	0.71	WNK1*	0.10	CNN2*	0.39	FAM78A*	0.25	PRPSAP1*
2.12	UBR2*	0.35	SMYD3*	0.53	PUM1*	0.10	LAIR1	0.39	FCHSD2*	0.23	LAIR1
1.99	LINC02569	0.35	SNX27*	0.53	RLF~*	0.10	NPLOC4	0.35	HMCES*	0.17	BIN2*
1.94	PPP6R3*	0.35	WDR41*	0.53	SDHB~*	0.08	RABGAP1L*	0.35	MKLN1*	0.17	ZNF251*

* Integration within a transcription unit
~ Integration within 50 kb of proto-oncogene

L301-001-1002: Integration Site Abundance Over Time



Month 3		Month 6		Month 9		Month 12		Month 18		Month 24	
%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene	%Contribution	Closest Gene
1.37	GPATCH8*	1.23	NSRP1*	0.32	FOXK2*	0.43	MCUB*	0.30	LAIR1	0.70	IL7*
1.37	RFPL1S	1.07	ZNF609*	0.19	LIMS4,LIMS3*	0.23	ATP6V0A2*	0.23	ARHGAP27*	0.45	SETX*
0.88	MYO9B*	0.75	PMVK	0.19	MARK2*	0.23	C12orf4*	0.23	ATP8A2*	0.30	CTDSPL*
0.78	VPS16*	0.64	TNRC6C*	0.16	TTC9C	0.18	HERC4*	0.23	HCG25*	0.30	PAK1~*
0.69	C11orf80*	0.48	ZNF81*	0.16	ZNF609*	0.15	CD4*	0.19	CLIC4*	0.30	VPS13D*
0.39	ATAD5*	0.32	CSNK1G1*	0.13	ACTR3*	0.15	COPS7A*	0.19	HLA-DMB	0.25	NCOA6~*
0.39	PLEC*	0.32	NCAPD2~*	0.13	APPL1*	0.15	SBF2*	0.19	IP6K1*	0.20	ARL6*
0.39	PLXND1*	0.27	FOXJ3*	0.13	C2CD3*	0.15	TNRC6C*	0.19	MAP3K5*	0.20	FOXJ3*
0.39	TRAF7~*	0.27	KCTD2*	0.13	RIPOR2	0.13	BABAM2*	0.19	NAA15*	0.20	IP6K1*
0.39	VMP1*	0.27	NOTCH4~*	0.13	SIL1*	0.13	RNF115*	0.19	ROCK2*	0.20	UBA7~

* Integration within a transcription unit
~ Integration within 50 kb of proto-oncogene

Top 10 integrations accounted for <5% of all identified UIS at 24 months for both patients

Data cut-off: April 26, 2023

Pediatric Cohort Update: Patient L301-002-1005

- **RP-L301 infusion tolerated well**
  - Engraftment achieved at day +15
  - Hospital discharge <1 month post-infusion

- **Hemoglobin normalized at 6 weeks post-RP-L301 infusion**
  - Hemoglobin 13.4 g/dL at 8 week visit (from median baseline of 7.9 g/dL)
  - No red blood cell transfusion requirements following engraftment

Data cut-off: May 3, 2023; Preliminary interim results are presented from the on-going clinical trial.

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)
- Robust and sustained efficacy in both adult patients up to 30 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 adult patients reported improved (and quantified) quality of life following treatment
- Early pediatric data suggests similar clinical efficacy as seen in adult cohort long-term efficacy data

Adult and pediatric enrollment is complete. Phase 2 trial initiation is anticipated in Q4 2023. Please contact PKDclinicaltrial@rocketpharma.com for more information.

Acknowledgements

José-Carlos Segovia, PhD

Susana Navarro, PhD

José Luis Lopez Lorenzo, MD
Lucía Llanos, MD, PhD
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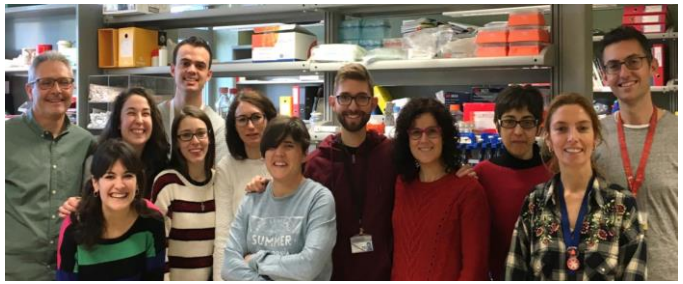

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