Gene Therapy for Adult and Pediatric Patients with Severe Pyruvate Kinase Deficiency: Results from a Global Study of RP-L301

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Abstract # 4

American Society of Gene + Cell Therapy

Disclosures

- Honoria for Educational Lectures: Rocket Pharmaceuticals, Inc., Swedish Orphan Biovitrum AB (Sobi) , Novartis AG, Amgen Inc., Miltenyi Biotec, Sanofi SA
- Advisory Board Member: Rocket Pharmaceuticals, Inc., Swedish Orphan Biovitrum AB (Sobi) , Novartis AG, Amgen Inc.
- Research Funding: Rocket Pharmaceuticals, Inc.

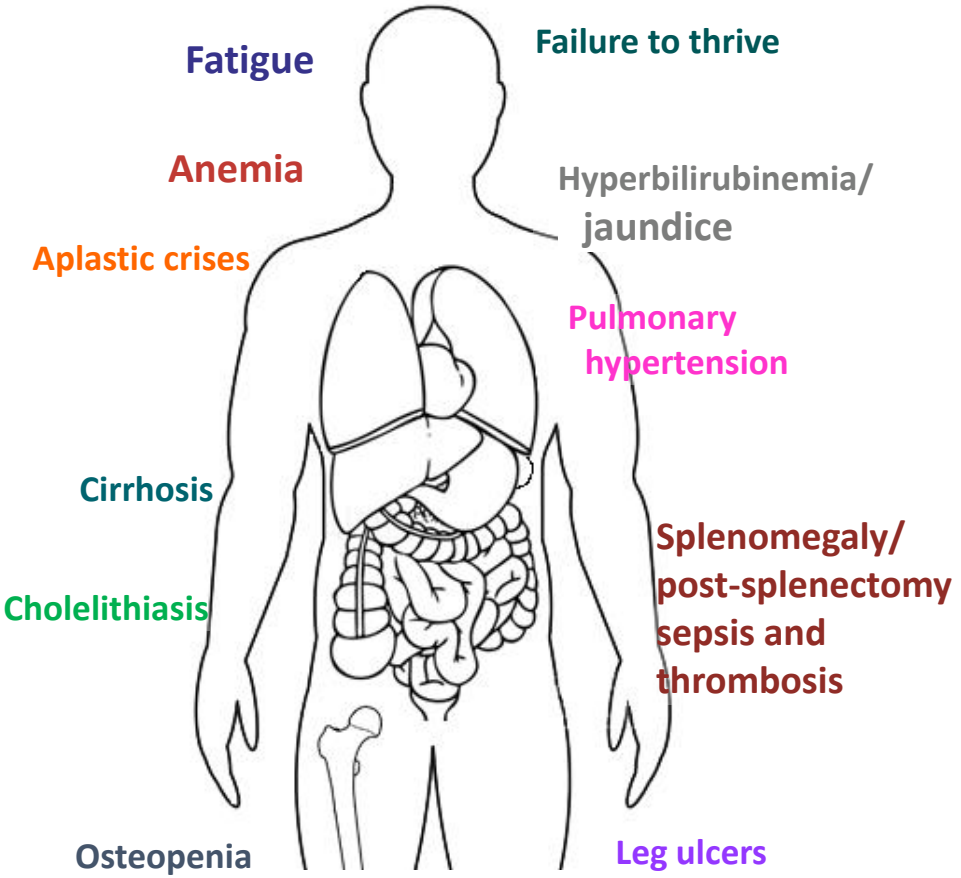
Pyruvate Kinase Deficiency (PKD)

- PKD is a rare inherited hemolytic anemia caused by ***PKLR*** gene mutations resulting in decreased red cell pyruvate kinase activity and impaired energy production (glycolysis)
- Clinical manifestations include anemia, reticulocytosis, hyperbilirubinemia/jaundice, 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 the United States
- Current therapies include enzyme activators or palliative measures limited to chronic blood transfusions, iron chelation therapy, and splenectomy
- Allogeneic HSCT has been performed in select cases and resulted in transfusion independence; this is not a standard-of-care due to significant toxicities and donor availability

Complications of PKD

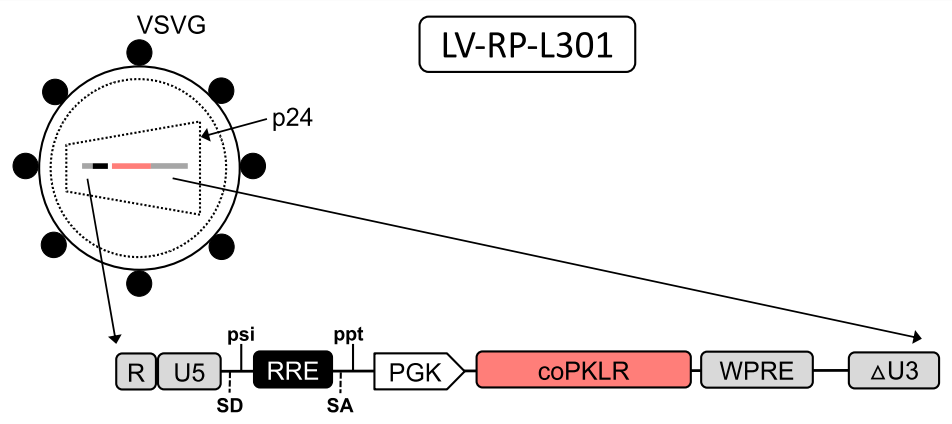


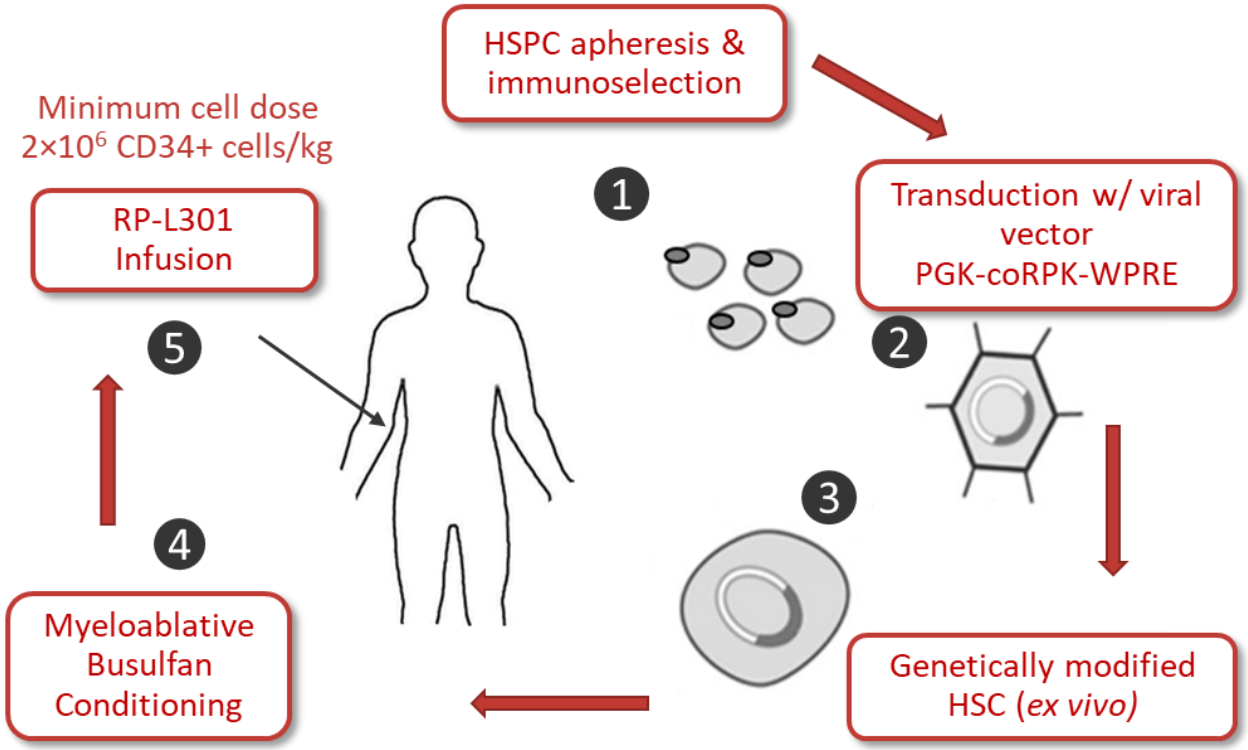
HSCT=hematopoietic stem cell transplant.

1. Al-Samkari H et al. *N Engl J Med.* 2022;386:1432-1442; 2. Grace R et al. *Blood.* 2018;131:2183-2192; 3. Van Straaten S et al. *Haematologica.* 2018;103(2):e82–e86; 4. 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





(Target AUC: 73,125 ng/mL*hour over 4 days; TDM)

*Developed at CIEMAT by Dr. José Carlos Segovia

AUC=area under curve; HSC=hematopoietic stem cell; HSPC=hematopoietic stem and progenitor cells; PK=pyruvate kinase; TDM=Therapeutic drug monitoring.

Clinical Trial Design

RP-L301-0119
NCT#04105166

Trial Design

- Single arm open-label

Key Eligibility Criteria

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

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

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)

Hb=hemoglobin; PKD=Pyruvate Kinase deficiency.

Pre-Treatment Patient and Drug Product Characteristics

		Adult Cohort		Pediatric Cohort	
		L301-006-1001	**L301-001-1002**	**L301-002-1005**	**L301-002-1004**
PATIENT CHARACTERISTICS	**Gender (Age at Initial Screening)**	Female (31.4 years)	Male (47.2 years)	Female (16.8 years)	Male (9.9 years)
	Age at RP-L301 Infusion	31.9 years	47.5 years	17.6 years	10.7 years
	PKD Mutation	c.721G>T; c.1529G>A	c.703GGG>AGG; c.1047A>AA; c.1744CGG>AGG	c.1116+2T>G; c.721G>T	c.204del; c.721G>T
	Average Hemoglobin* *over 2 years prior to enrollment*	7.3 g/dL (*Range:* 5.8–9.0 g/dL)	6.8 g/dL (*Range:* 6.7–6.8 g/dL)	7.3 g/dL (*Range:* 5.9–7.9 g/dL)	6.8 g/dL (*Range:* 5.2–7.6 g/dL)
	Total Bilirubin *at Screening Visit*	13.4 mg/dL	7.4 mg/dL	14.9 mg/dL	4.23 mg/dL
	Erythropoietin *at Screening Visit*	35.6 mIU/mL	57.2 mIU/mL	94.2 mIU/mL	45 mIU/mL
	RBC Transfusion History *over **2 years** prior to enrollment*	~14 transfusion episodes	~5 transfusion episodes	~3 transfusion episodes	~5 transfusion episodes
RP-L301 METRCIS	**CD34+ Cell Dose**	3.9×10^6 CD34+ cells/kg	2.4×10^6 CD34+ cells/kg	2.3×10^6 CD34+ cells/kg	6.5×10^6 CD34+ cells/kg
	VCN in Bulk Liquid Culture	2.73	2.08	1.98	4.59

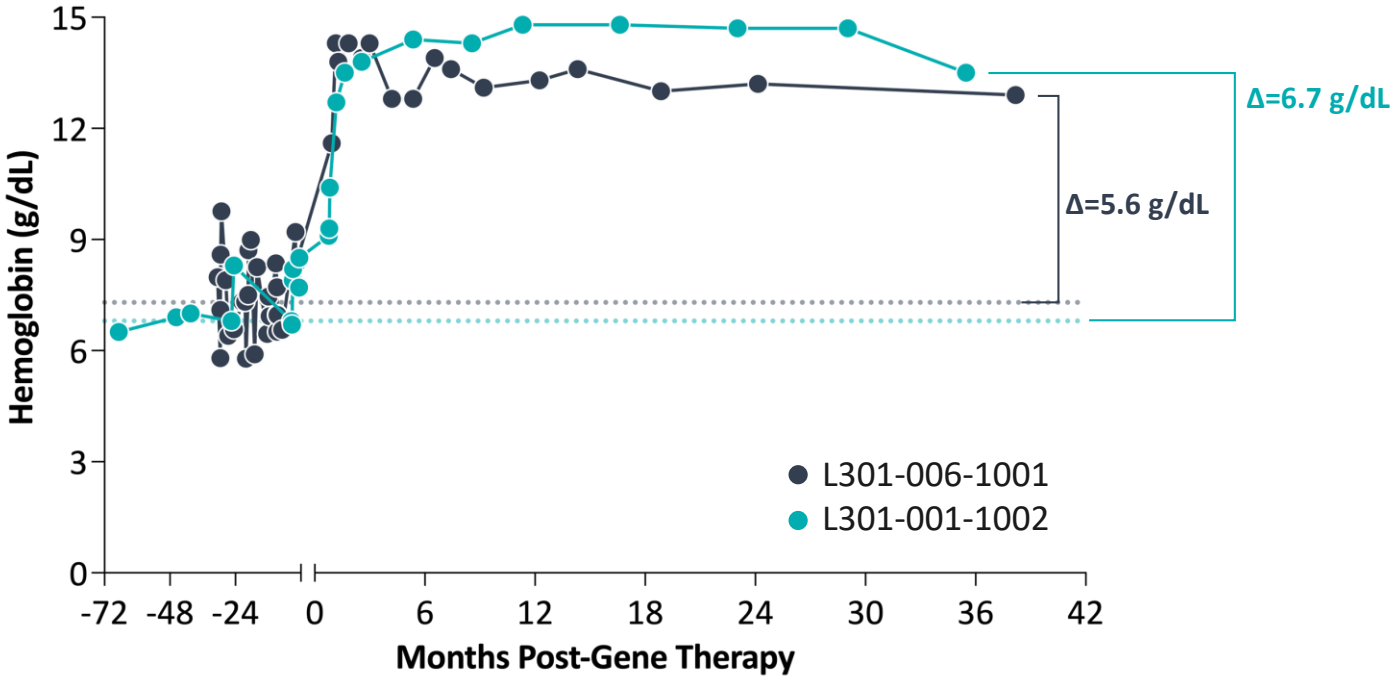
* Hemoglobin (Hb) range over 2 years prior to study enrollment; Hb values <61 days from prior transfusion were excluded.

PKD=Pyruvate Kinase Deficiency; RBC: Red blood cell; VCN: Vector copy number.

Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.

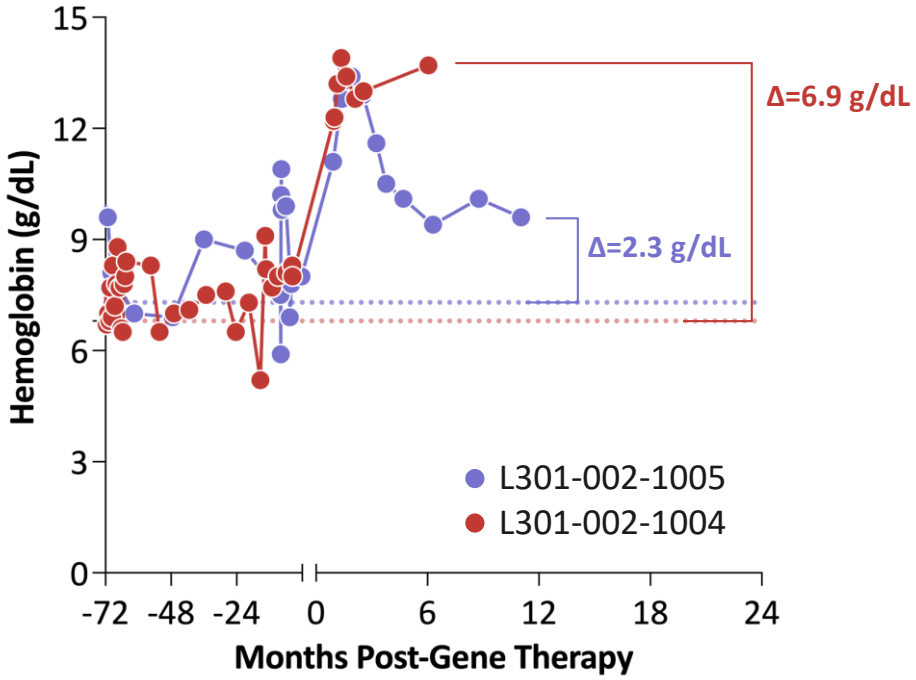
RP-L301 Confers Clinically Meaningful Hemoglobin Improvement

Adult Cohort



- RBC transfusion independence following neutrophil engraftment
- Sustained hemoglobin normalization up to 36 months

Pediatric Cohort



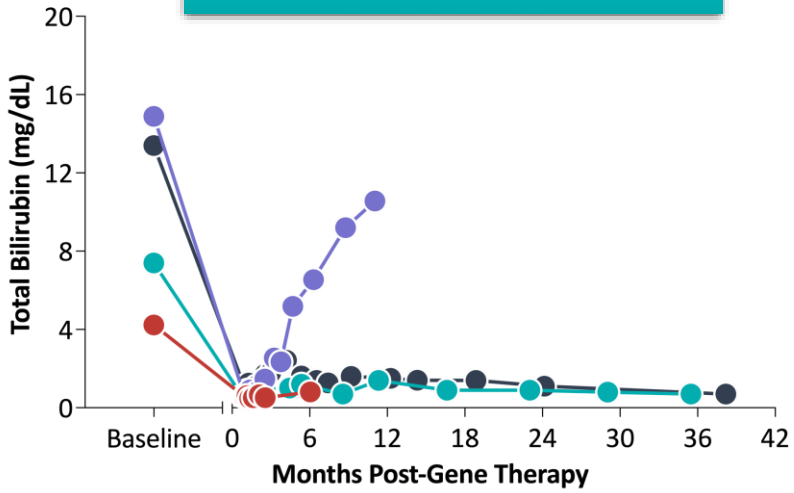
- RBC transfusion independence following neutrophil engraftment
- Hemoglobin normalization or improvement from pre-treatment baseline up to 12 months

RBC: Red blood cell.

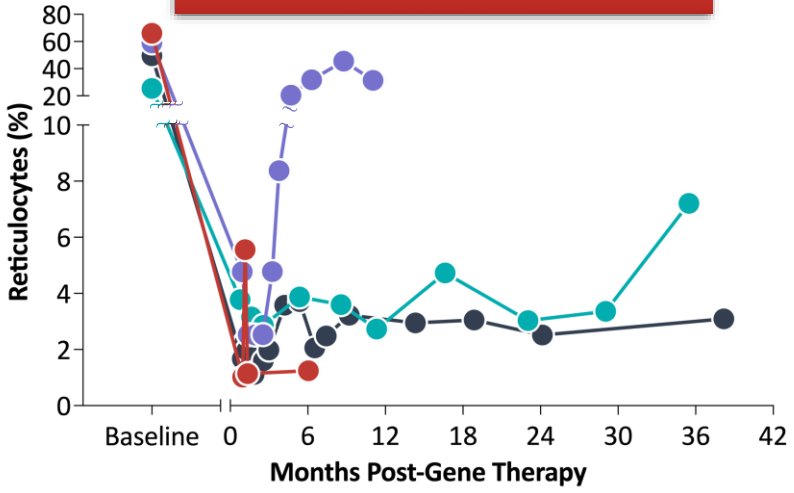
Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.

Concurrent Improvement in Biochemical Markers

TOTAL BILIRUBIN



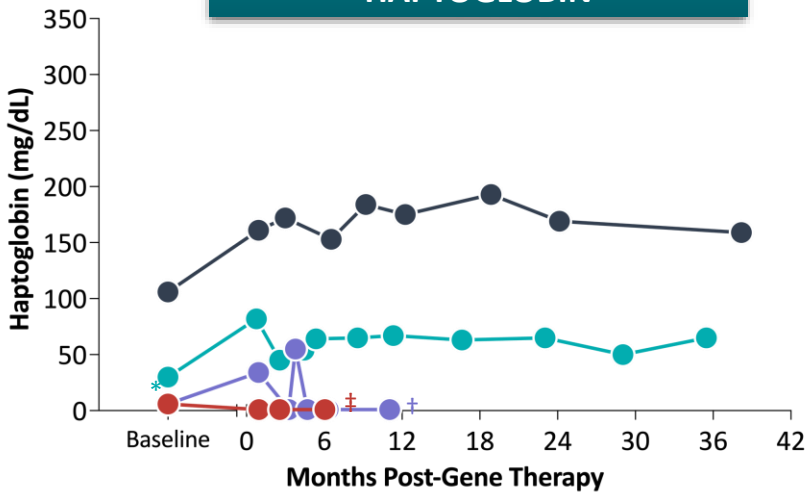
RETICULOCYTES



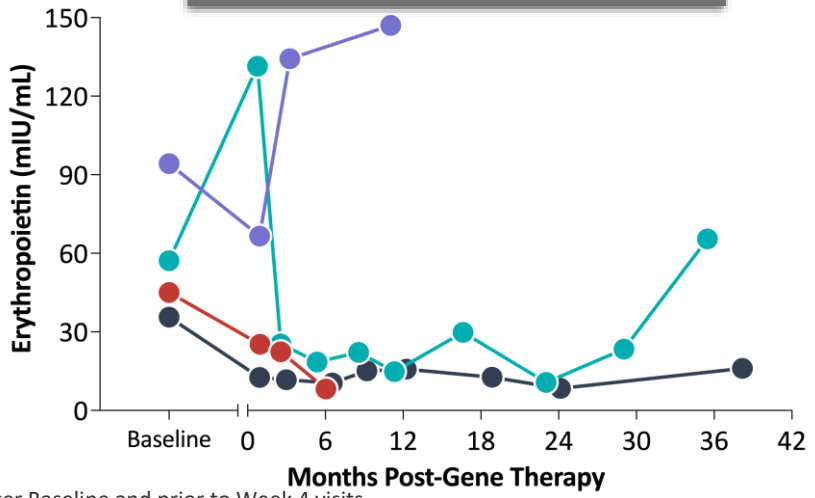
Subject ID

- L301-006-1001
- L301-001-1002
- L301-002-1005
- L301-002-1004

HAPTOGLOBIN



ERYTHROPOIETIN



Baseline hemolysis markers were obtained at the initial screening visit for each patient. Graphs exclude results after Baseline and prior to Week 4 visits.
mo=months; w=week.

* L301-001-1002 haptoglobin <30 mg/dL at baseline

† L301-002-1005 haptoglobin <1 mg/dL at 3mo, ~4.7mo, 6mo, and 12mo.

‡ L301-002-1004 haptoglobin <1 mg/dL at 4w, 3mo, and 6mo.

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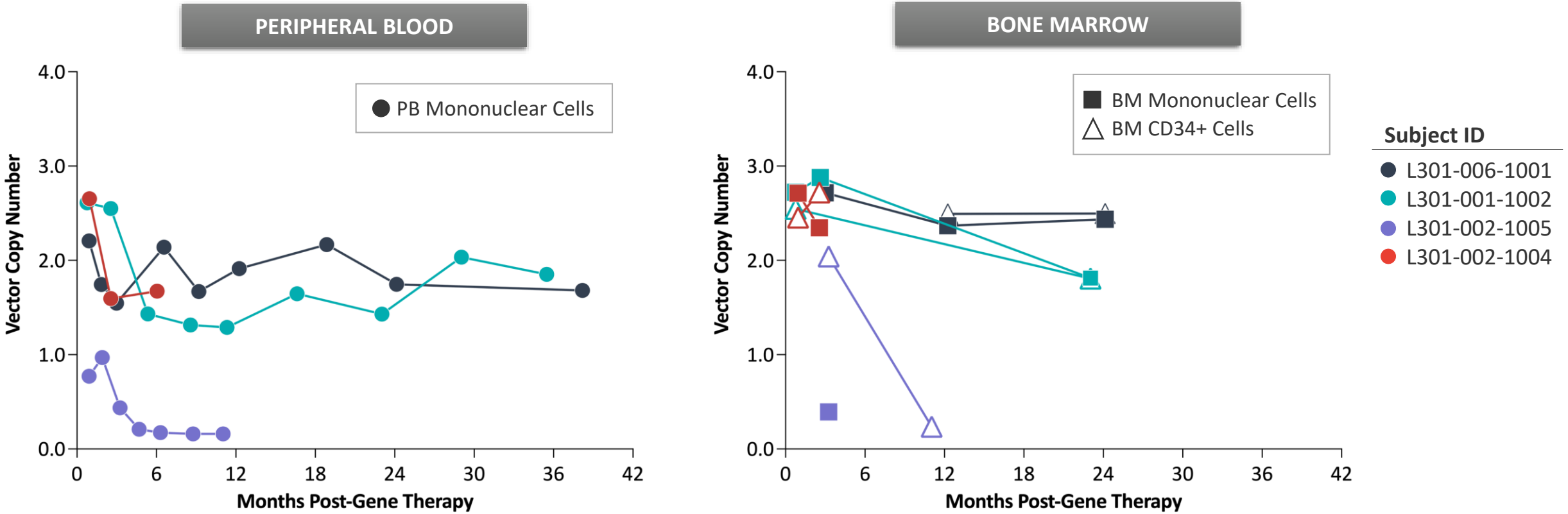
Genetic Correction is Associated with Hemoglobin Increases



BM=bone marrow; PB=Peripheral blood; VCN=vector copy number.

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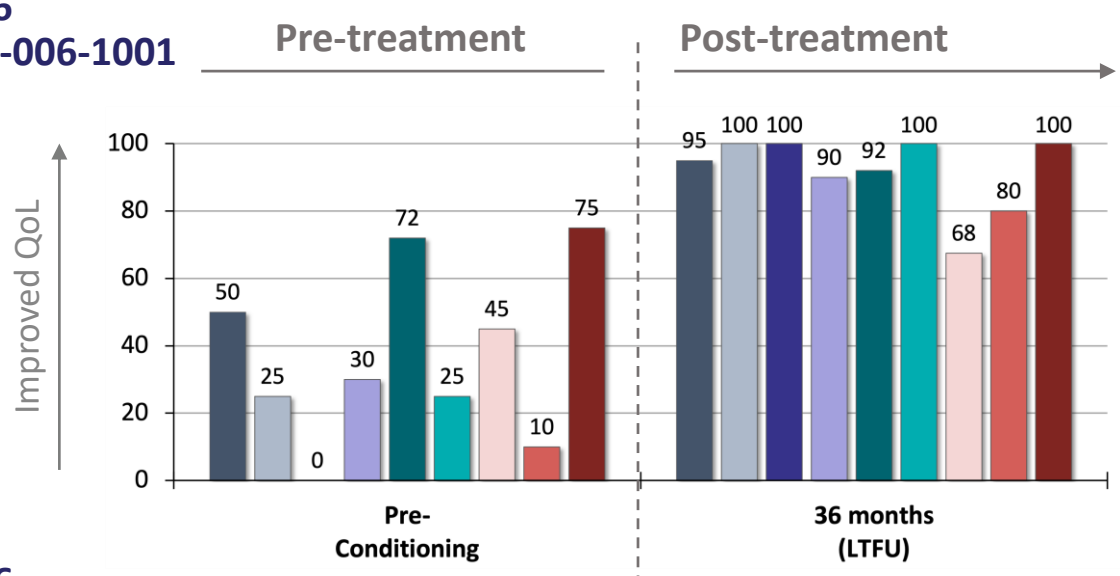
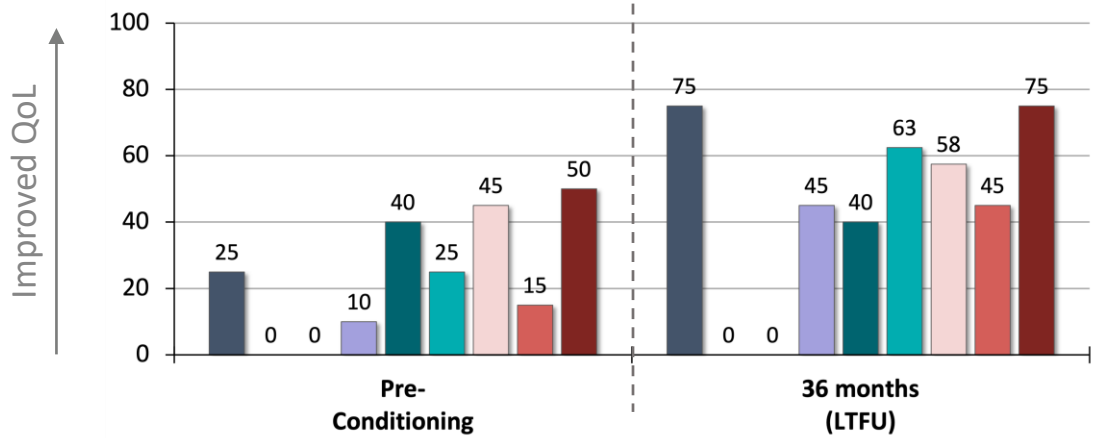
Improved Quality of Life by SF-36 and FACT-Anemia (not depicted)
up to 36 months following RP-L301 (adult cohort)*

SF-36
L301-006-1001



SF-36
L301-001-1002



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 reported for:

- **physical functioning (■)**
- **energy/fatigue (■)**
- **general health (■)**

Similar findings with the *FACT-Anemia QoL questionnaire*:
Improved QoL is demonstrated by increase in FACT-Anemia total and component scores from baseline.

*Previously presented data at screening and at 3 months, 6 months, 9 months, 12 months, 18 months, 24 months and 30 months post-gene therapy is not shown.
LTFU=long term follow-up.

Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.

Safety Profile of RP-L301 Appears Highly Favorable

- **Hematopoietic stem and progenitor cell mobilization and collection appear safe and feasible in patients with severe PKD**

- **Neutrophil engraftment occurred by day +15 post RP-L301 administration**

  - Infusion was well tolerated in all treated patients (N=4)
  - Patients discharged from hospital within 1 month following RP-L301 infusion

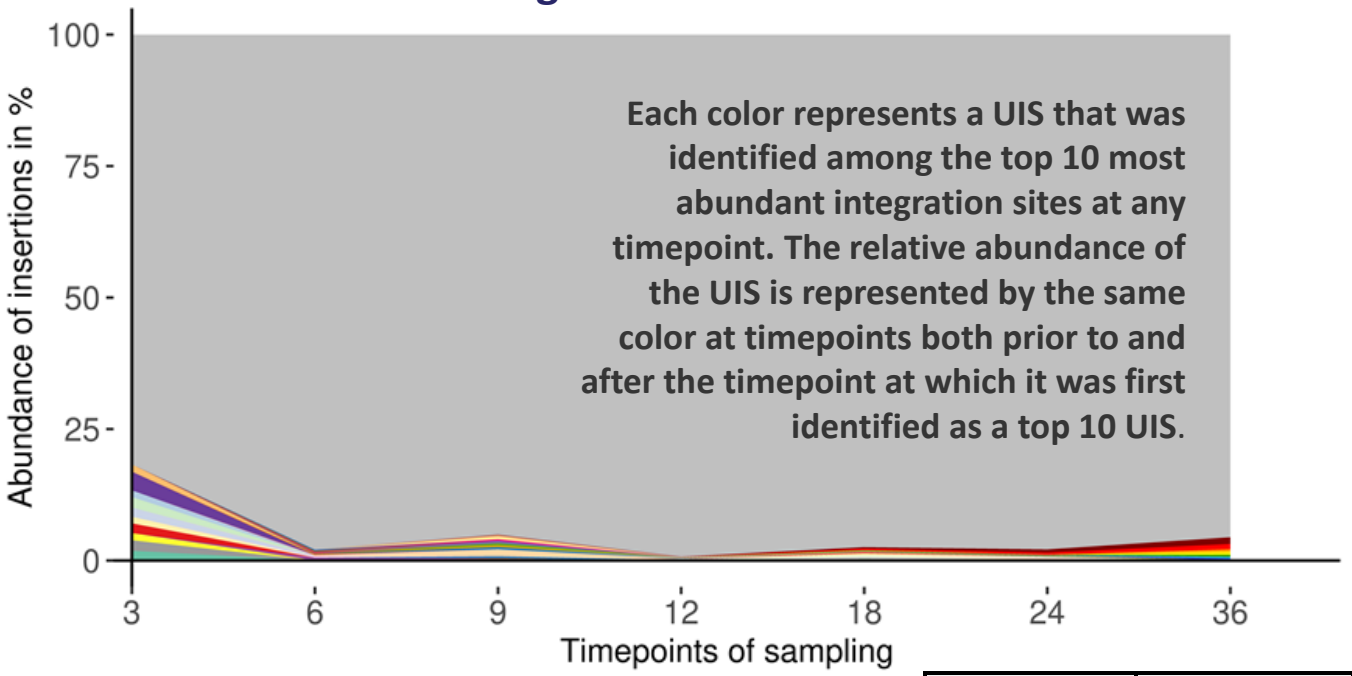
- **No RP-L301-related serious adverse events (SAEs) up to 36 months post- infusion**

PKD=Pyruvate Kinase Deficiency.

Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.

Highly Polyclonal Insertion Patterns Evident from ISA (PB) over 36 months post-RP-L301 (Patient 1001)

L301-006-1001: Integration Site Abundance Over Time



Top 10 integrations accounted for <5% of all identified UISs through 36 months

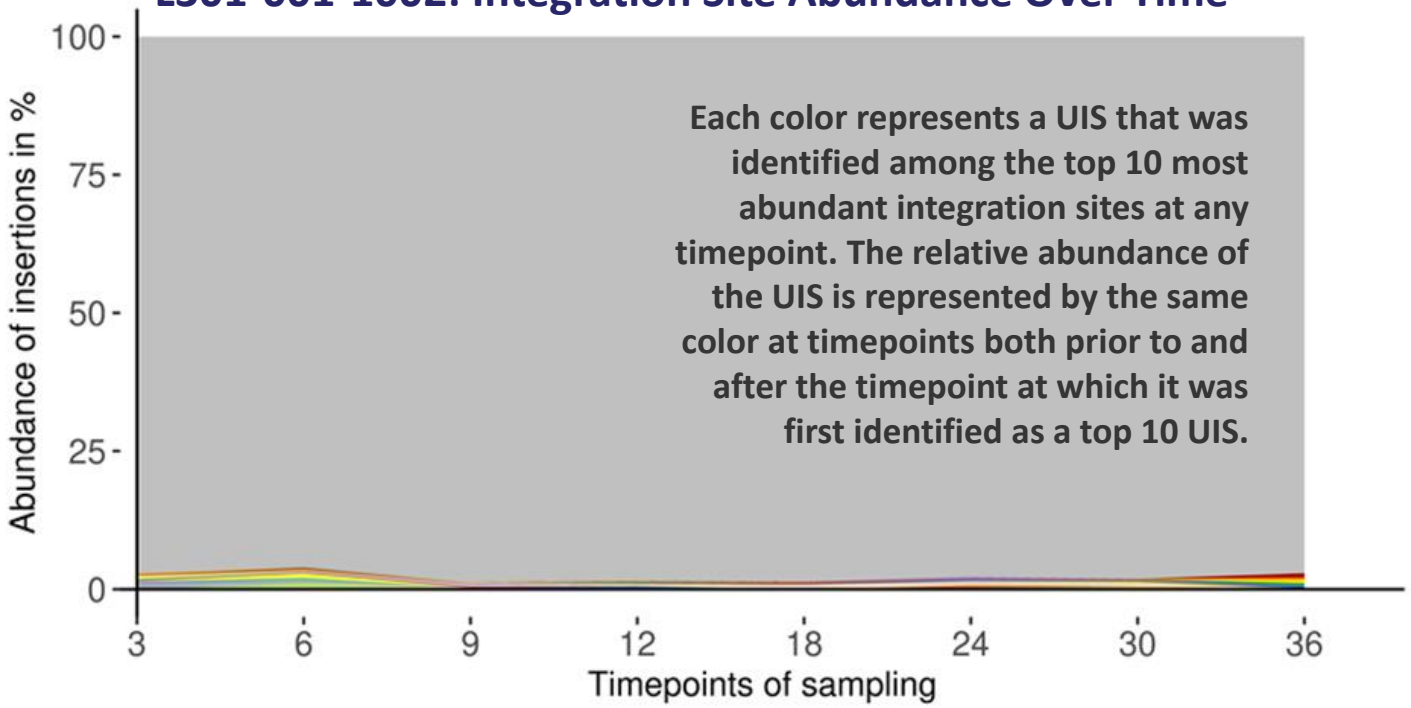
Month 3		Month 6		Month 9		Month 12		Month 18		Month 24		Month 36	
% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene
3.55	HLA-DOA	0.54	TNRC6C*	1.28	MIR8074	0.12	HINT1	0.38	RBM4B*	0.62	NPLOC4	1.27	NPLOC4
1.98	MARCHF8*	0.22	BAZ1A*	0.43	ABHD16A*	0.09	EPS15~*	0.33	POU2F1*	0.39	BRD4~*	1.00	BRD4~*
1.98	ZNF543	0.16	ACOX1*	0.43	CARMIL1*	0.08	MTMR3~*	0.31	NPLOC4	0.18	MSH5*	0.35	SUPT3H*
1.87	ZNF546	0.16	C21orf91-OT1	0.43	CEP192*	0.07	NFAT5*	0.31	SHMT1*	0.18	NFAT5*	0.32	BAX~*
1.84	RPTOR*	0.16	CD79A~*	0.43	EPB41*	0.06	EDC3*	0.27	BRD4~*	0.14	ATF7*	0.27	OSBPL8*
1.76	NUMA1~*	0.16	COBLL1*	0.43	SH3RF1*	0.06	TP53BP1*	0.18	EZH2~*	0.14	NDEL1*	0.27	PDIK1L
1.37	FOXK2*	0.16	GUF1*	0.43	WNK1*	0.05	CNN2*	0.18	FAM78A*	0.14	PRPSAP1*	0.24	DERA*
1.37	UBR2*	0.16	LINC02220*	0.32	ACOX1*	0.05	LAIR1	0.18	FCHSD2*	0.13	LAIR1	0.24	STAT5B*
1.28	LINC02569	0.16	MFSD14B*	0.32	ADAM6	0.05	NPLOC4	0.16	HMCES*	0.09	BIN2*	0.21	INTS13
1.26	PPP6R3*	0.16	PACSIN2~*	0.32	CLCN5*	0.04	ABCA3~*	0.16	MKLN1*	0.09	ZNF251*	0.21	NFATC3*

*Integration within a transcription unit'; ~Integration within 50 kb of proto-oncogene.
ISA=insertion site analysis; PB=peripheral blood; UIS(s)=unique integration site(s).

Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.

Highly Polyclonal Insertion Patterns Evident from ISA (PB) over 36 months post-RP-L301 (Patient 1002)

L301-001-1002: Integration Site Abundance Over Time



Top 10 integrations accounted for <5% of all identified UISs through 36 months

Month 3		Month 6		Month 9		Month 12		Month 18		Month 24		Month 30		Month 36	
% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene	% Contr.	Closest Gene
0.54	GPATCH8*	0.86	NSRP1*	0.25	FOXK2*	0.33	MCUB*	0.18	LAIR1	0.49	IL7*	0.37	SF3A3*	0.46	CEP20
0.54	RFPL1S	0.74	ZNF609*	0.15	LIMS4,LIMS3*	0.18	ATP6V0A2*	0.14	ARHGAP27*	0.31	SETX*	0.26	AQR*	0.37	IL12RB1
0.35	MYO9B*	0.52	PMVK	0.15	MARK2*	0.18	C12orf4*	0.14	ATP8A2*	0.21	CTDSPL*	0.19	KHDRBS2	0.32	ZBTB3
0.31	VPS16*	0.45	TNRC6C*	0.12	TTC9C	0.14	HERC4*	0.14	HCG25*	0.21	PAK1~*	0.17	DOCK10*	0.28	BAX~*
0.27	C11orf80*	0.33	ZNF81*	0.12	ZNF609*	0.12	CD4*	0.11	BRF1*	0.21	VPS13D*	0.12	CHAMP1*	0.23	BCL7A~*
0.15	ATAD5*	0.22	CSNK1G1*	0.10	ACTR3*	0.12	COPS7A*	0.11	CLIC4*	0.17	NCOA6~*	0.12	FBXW2*	0.23	TNFSF12*
0.15	PLEC*	0.22	NCAPD2~*	0.10	APPL1*	0.12	SBF2*	0.11	HLA-DMB	0.14	ANXA1	0.12	HCG27	0.23	YLPM1*
0.15	PLXND1*	0.19	C19orf38*	0.10	C2CD3*	0.12	TNRC6C*	0.11	IFT81*	0.14	ARL6*	0.12	INO80D*	0.19	ABCA3~*
0.15	TRAF7~*	0.19	EPS15L1*	0.10	CLDN14	0.10	AGGF1*	0.11	IP6K1*	0.14	CARMIL1*	0.11	QRICH1*	0.19	AKT2~*
0.15	VMP1*	0.19	FOXJ3*	0.10	DICER1~*	0.10	ANTXR2*	0.11	KMT5B*	0.14	FOXJ3*	0.11	UBA7~	0.19	BRCC3*

*Integration within a transcription unit'; ~Integration within 50 kb of proto-oncogene.
ISA=insertion site analysis; PB=peripheral blood; UIS(s)=unique integration site(s).

Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.

Safety Profile of RP-L301 Appears Highly Favorable

- **Hematopoietic stem and progenitor cell mobilization and collection appear safe and feasible in patients with severe PKD**
- **Neutrophil engraftment occurred by day +15 post RP-L301 administration**
  - Infusion was well tolerated in all treated patients (N=4)
  - Patients discharged from hospital within 1 month following RP-L301 infusion
- **No RP-L301-related serious adverse events (SAEs) up to 36 months post- infusion**

- **Insertion site analyses in PB and BM for both adult patients through 36 months post-RP-L301 demonstrated highly polyclonal patterns -- no clonal dominance or insertional mutagenesis**
  - Pediatric patients more recently treated; ISA testing ongoing

BM=bone marrow; ISA=insertion site analysis; PB=peripheral blood; PKD=Pyruvate Kinase Deficiency.

Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.

Conclusions

- RP-L301 is a potential treatment for patients with PKD
  - Anemia resolution in patients who did not derive benefit from available therapies (mitapivat, splenectomy)
- Sustained and clinically meaningful hemoglobin improvement in all patients (up to 36 months following therapy)
  - Hemoglobin normalization in 3 of 4 patients (range: 12.9 g/dL to 13.7 g/dL)
  - No red blood cell transfusion requirements following neutrophil engraftment
  - Decreased hemolysis
  - Quality of life improvement
- Favorable RP-L301 safety profile
  - No product related SAEs
  - Busulfan conditioning well tolerated; neutrophil engraftment by day +15 in all patients and hospital discharge within 1 month following therapy

Preparation for Phase 2 trial is underway

Please contact PKDclinicaltrial@rocketpharma.com for more information.

PKD=Pyruvate Kinase Deficiency; SAEs=serious adverse events.

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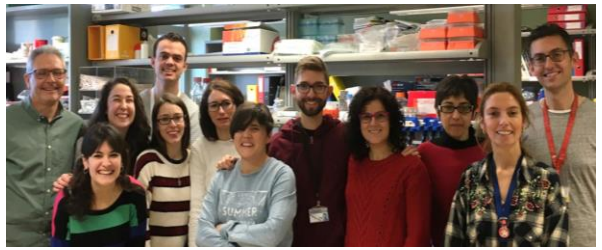
Acknowledgements

José-Carlos Segovia, PhD

Susana Navarro, PhD



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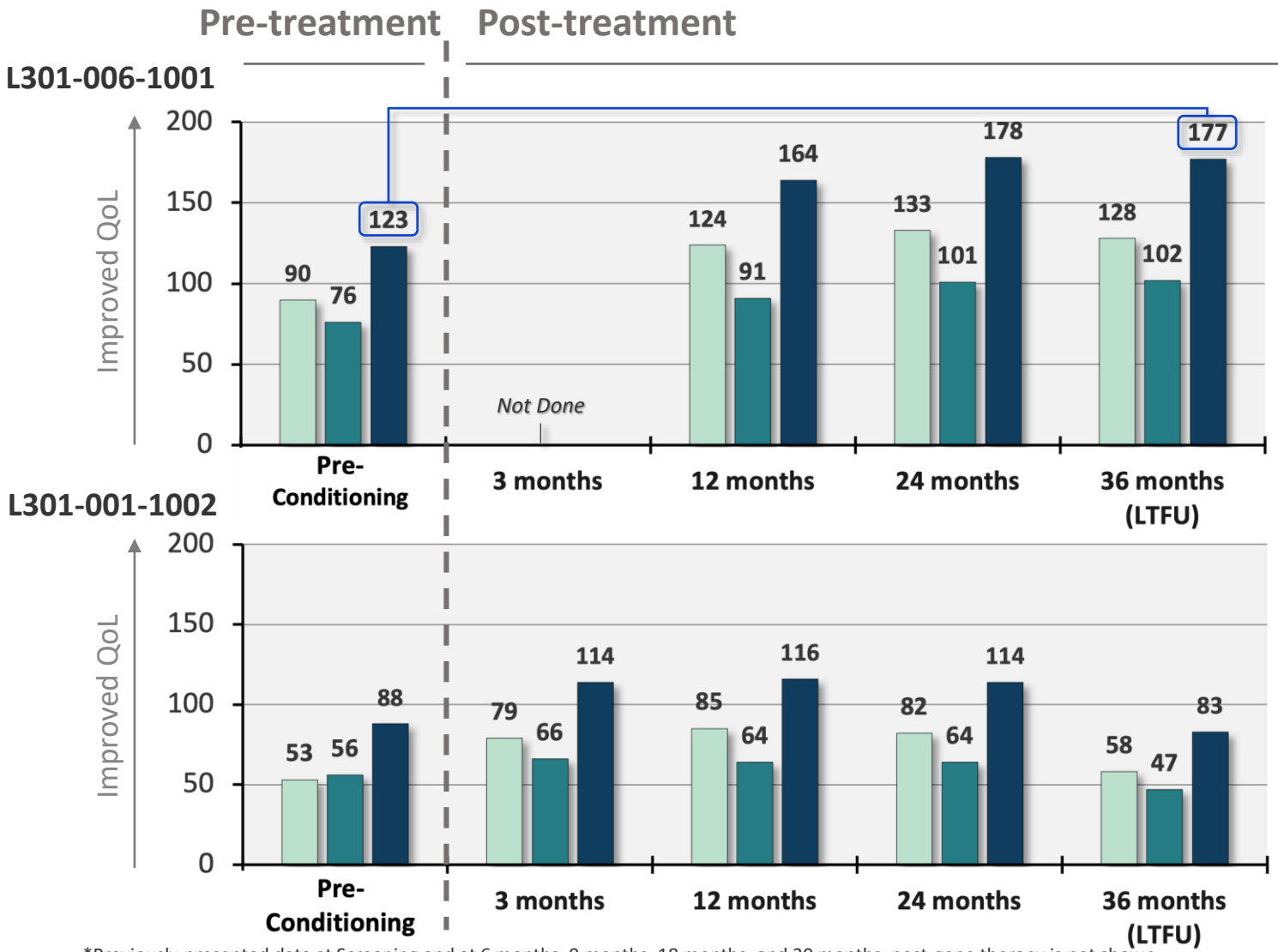
Improved Quality of Life (QoL) by FACT-Anemia
up to 36 months following RP-L301 (adult cohort)*



*Pt 1001: Total Score increase (↑QoL) at 36 months compared to Pre-conditioning : **54***

- **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 QoL following RP-L301 administration; Improved QoL is demonstrated by increase in FACT-Anemia total and component scores from baseline.

*Previously presented data at Screening and at 6 months, 9 months, 18 months, and 30 months post-gene therapy is not shown.
FACT=Functional assessment of cancer therapy; LTFU=long term follow-up; Pt=patient.

Data cut-off: February 5, 2024; Preliminary interim results are presented from the on-going clinical trial.