Changing the Natural History of Fanconi Anemia Complementation Group-A with Gene Therapy: Early Results of U.S. Phase I Study of Lentiviral-mediated *Ex-vivo FANCA* Gene Insertion in Human Stem and Progenitor Cells

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Introduction

Fanconi anemia (FA) is a rare genetic disorder characterized by:

- Defective cellular deoxyribonucleic acid (DNA) repair
- Developmental abnormalities
- Progressive bone marrow failure (BMF)
  - in ~80% patients
  - frequently during the 1st decade of life
- Predisposition to hematologic malignancies and solid tumors.

FA complementation group A (FANCA) accounts for 60-70% of FA. Estimated US+EU prevalence for FA ~4,000 patients.

Allogeneic hematopoietic stem cell transplant (alloHSCT) is frequently curative of FA-associated BMF. However its utilization & efficacy are limited by:

- donor availability
- graft-versus-host disease (GVHD)
- acute & long-term toxicities including increased solid tumor risk (particularly in patients with GVHD).

Ex-vivo insertion of a functional *FANCA* gene into autologous FA-A CD34+ enriched hematopoietic stem & progenitor cells (HSPCs) confers a proliferative advantage and engraftment potential, and enables administration of gene therapy without conditioning – vital to minimizing toxicity in FA. Notably, in the absence of conditioning, ≥ 12 months has been required for evidence of engraftment, phenotypic correction and stabilization/increases of blood counts as demonstrated in the FANCOLEN-I Phase 1/2 clinical trial (Río P *et al*. Nat Med 2019; 25:1396-1401).

We conducted a Phase 1 US clinical trial at Stanford University to evaluate the feasibility and safety of autologous CD34+ cells transduced with the lentiviral vector (LV) carrying the *FANCA* gene (PGK-FANCA-WPRE) in 2 pediatric patients with FA-A.

This current study incorporated modifications ("Process B") to cell collection and manufacture, including commercial grade vector & cell processing, and transduction enhancers. Based on evidence from the initial FANCOSTEM HSPC collection study, eligibility criteria were established to focus enrollment on pts (predominantly younger) most likely to have sufficient bone marrow CD34+ reserves to enable adequate harvest, transduction and subsequent engraftment to prevent BMF and obviate the need for alloHSCT.

Objectives

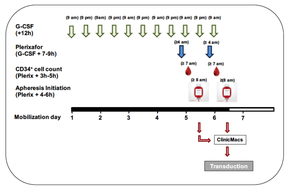
Primary endpoint:
To evaluate the safety of the infusion of investigational product RP-L102: autologous CD34+ enriched cells transduced with LV carrying the *FANCA* gene in FA-A patients.

Secondary endpoints:

- Clinical response: prevention of BMF
- Engraftment as determined by peripheral blood (PB) and bone marrow (BM) vector copy number (VCN);
  - progressive increases are anticipated over time
- Phenotypic correction as evidenced by increased resistance of BM and PB cells to DNA-damaging agents mitomycin-C (MMC) and diepoxybutane (DEB), respectively.

Study Schemata

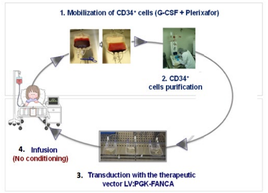
CD34+ mobilization protocol with G-CSF & plerixafor:



G-CSF & plerixafor were administered over an approximate 6-day sequence with apheresis conducted on 2 consecutive days.

PB counts of at least 5 CD34+ cells/µL were required to initiate apheresis.

Schematic diagram of product manufacture & treatment:



Patients underwent CD34+ mobilization, followed by CD34+ immunoselection, transduction and subsequent infusion without conditioning.

At least 2x10^5 CD34+ cells/kg were required as starting material following apheresis.

Lentiviral Vector & Transduction

Schematic diagram of PGK-FANCA-WPRE:

PGK-FANCA-WPRE is a self-inactivating LV carrying the therapeutic *FANCA* gene.

Under hypoxic conditions (5% O_2), CD34+ enriched cells from FA patients are pre-stimulated (8-12h) & transduced (10-16h) in the presence of transduction enhancers prior to final formulation & infusion as a fresh product with no cryopreservation.

Key Eligibility Criteria

Inclusion criteria:

- FA complementation group A
- Age 1-12 years
- At least 1 parameter (Hb, ANC or Plt) below lower limit of normal
- BM CD34+ concentration ≥ 30/µL (from aspirate)
- If BM CD34+ of 10-29/µL, then at least 2 of the following:
  - Hb ≥ 11g/dL
  - PMN ≥ 900/µL
  - Platelets ≥ 60,000/µL

Exclusion criteria:

- Available & eligible HLA-identical sibling donor
- Lansky PS ≤ 60%
- MDS or leukemia (including associated cytogenetic abnormalities)
- Mosaicism with stable/improved blood counts

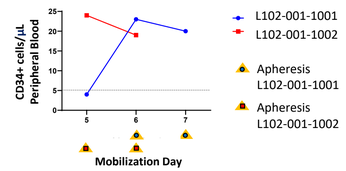
Patient Characteristics

	1001	1002
Age (y) Gender	5 F	6 F
WBC (/µL)	4,000	4,600
PMN (/µL)	1,280	1,340
Hb (g/dL)	11.9	8.9
Plt (/µL)	55,000	38,000

	1001	1002
MCV (fL)	86.9	106.2
BM34+ (/µL)	78	34
FA mutn	c.2606A>C, p.(Gln869Pro) c.2813A>G, p.(Asp944Gly) c.3703C>G, p.(Gln1235Glu)	c.(?_-1)(522+1_523-1)del encompassing exons 1-5 c.(?_-1)(283+1_284-1)del encompassing exons 1-3

CD34+ Mobilization

Peripheral Blood CD34+ counts on Days 5-7 of mobilization:



Investigational Product

Subject	Nucleated Cells/kg	CD34+ Cells/kg^	CFCs/kg^	Mean VCN: Liquid Culture	Mean VCN: CFCs	CFC Survival MMC 10nM (%)
1001	7.8 x 10^6	2.0 x 10^5	5.2 x 10^4	2.08	1.10	33
1002	2.4 x 10^6	3.7 x 10^5	5.0 x 10^4	2.21	0.93*	47

* Mean CFC VCN was assessed from a cryopreserved drug product sample.
^ Per NC200 automated count (results in ~50% lower assessment vs. manual used in FANCOLEN-I). CFCs: colony forming cells; MMC: mitomycin-C

Adverse Events

Treatment-emergent Adverse Events:

Event	Adverse Events Grade				
	any	1	2	3	4
Pyrexia	1	1	--	--	--
Croup (infectious)	1	--	--	1	--
Dyspnea	1	--	--	1	--

No adverse events were considered related to RP-L102 administration.

Drug product and patients' blood post-infusion (2m & 6m) negative for replication competent lentivirus.

Adverse events considered related to mobilization/apheresis:

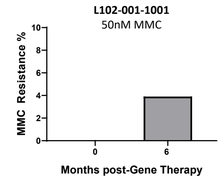
- Hypocalcemia, hypoproteinemia, hypoalbuminemia, hypokalemia, nausea, fatigue, catheter site pain & bleeding and hypotension; each occurred in n=1 patient and were Grade 1.
- Both patients experienced transient anemia (Hb 6.9 & 7.1 g/dL respectively) and thrombocytopenia (plt 18 & 9K/µL respectively) and received a portion of the RBC priming unit as part of apheresis procedure; 1 patient received platelet transfusion post apheresis.

Preliminary Vector Copy Number

Preliminary qPCR results at 4 months post infusion (PBMC):

- Pt 1001: VCN ~0.01
- Pt 1002: VCN ~0.01
  - For patients on initial FANCOLEN-I trial who received optimal cell/CFC doses and VCNs (pts 2002 & 2006), PB VCNs at this early timepoint were in a similar range.
  - In absence of conditioning, increases in VCN are anticipated over ≥ 12m timeframe.

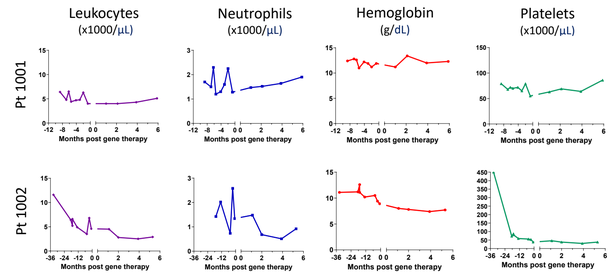
Preliminary Bone Marrow MMC-resistance



Resistance to 50nM MMC was demonstrated in 4% of bone marrow progenitors (CFCs) from patient 1001 at 6 months post-infusion. No resistance to this level of MMC was observed at pre-treatment baseline.

Bone marrow specimen for patient 1002 insufficient to enable MMC-assay.
(Bone marrow evaluations at 12 & 18 months planned).

Preliminary Results: Blood Counts



- Blood count stability in both patients over 6m following infusion, with trend increases (patient 1001 mos. 0⇢6; patient 1002 mos. 4⇢6)
- Blood count decreases in multiple lineages in both patients prior to infusion (patient 1001 over 36 mos. pre-Rx; patient 1002 over 9 mos. pre-Rx).

Conclusions

- This US Phase 1 trial confirms the HSPC collection, transduction and viability demonstrated in the FANCOLEN-I clinical study and establishes the safety and feasibility of commercial Process B vector/cell manufacturing in FA.
- Investigational product metrics show consistency with parameters comparable or favorable relative to earlier processes:
  - Liquid culture VCNs >2.0 and CFC VCNs ~1.0
  - CFC resistance (10nM MMC) in 30-50% range
  - CD34+ and CFC counts comparable to FANCOLEN-I pts who received optimal product and demonstrated engraftment, phenotypic correction and hematologic stability/improvement over 24-36 mos.
- At 6 mos., both patients are clinically stable with early indicators of engraftment in the absence of conditioning:
  - Preliminary gene marking (VCN) in PB at 4 mos.
  - Increasing BM MMC-resistance at 6 mos.
  - Blood counts stable (potential trend increase) at 6 mos., in setting of multi-lineage decreases in 9-36 mos. prior to gene therapy.
- Global Phase 2 study is underway: NCT# NCT04069533
  - Initial patient received infusion in Madrid (Nov 2019)
  - Registration-enabling study with primary endpoint of BM MMC-resistance at 1-3 years post-infusion.

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