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 FA NFANCA
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 malignancies and solid tumors. FA complementation group A (FANCA) accounts for 60-70% of FA. Estimated US-EU prevalence for FA ~4,000 patients. Allelic hematopoietic stem cell transplant (alloHCT) is frequently curative for 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 CSCR-enriched hematopoietic stem & progenitor cells (HSPCs) confers a preferable advantage and engraftment potential, and enables administration of gene therapy without conditioning – to minimizing toxicity in FA. Notably, the absence of conditioning, >12 months has been required for evidence of engraftment, phenotypic correction and stabilization/ Increased bone marrow cell counts as demonstrated in the FANCOLEN-I clinical trial (Rocket 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 FA-A CSCR-enriched hematopoietic stem and progenitor cells, transduced with the lentiviral vector (LV) carrying the FANCA gene (PGK-FANCA-WPRE) in 2 pediatric patients with FA. This current study incorporated modifications (“Process B”) to cell collection and manufacture, including commercial grade vector production, cell processing, and transduction enhancers. Based on evidence from the initial FANCOLEN-I HSCP collection study, eligibility criteria were established to focus enrollment on FA patients (predominantly younger) most likely to have sufficient bone marrow CDS4+ reserves to enable adequate harvest, transduction and subsequent engraftment to prevent BMF and obviate the need for alloHCT.

Study Schematic

CD34+ Mobilization protocol with G-CSF & plerixafor:

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

PB counts of at least 5x10^6 cells/mL were required to initiate apheresis.

If all parameters (Hb, ANC or Plt) were below lower limit of normal

CD34+ Mobilization

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

FA complementation group A (FANCA) accounts for 60-70% of FA. Fanconi anemia (FA) is a rare genetic disorder characterized by:

- Developmental abnormalities
- Progressive bone marrow failure (BMF)
- Risk of hematologic malignancies and solid tumors
- Developmental abnormalities
- Predisposition to solid tumors
- Predisposition to hematologic malignancies and solid tumors

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 ≥2.0x10^5 (μL from aspirate)
- If BM CD34+ ≥10-29/μL, then at least 2 of the following:
  - Hb ≥11g/dL
  - Platelets ≥100,000/μL

Exclusion criteria:

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

CD34+ Mobilization

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

Investigational Product

Treatment-emergent adverse events:

No adverse events were considered related to process B administration.

Adverse events considered related to mobilization/apheresis:

- Hypocalcemia, hypoproteinemia, hypobilirubinemia, hypomagnesemia, anemia, neutropenia, fatigue, urosepsis, bleeding

Adverse events considered related to mobilization:

- 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.

Objectives

Primary endpoint:

- To evaluate the safety of the infusion of investigational product RP-1102 autologous CDS4+ 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) CD34+ cell count (VCN;
- Progressive increase and nadir over time
- Phenotypic correction as evidenced by increased resistance of BM and PB cells to DNA-damaging agents mitomycin-C (MMC) and doxorubicin (DOX), respectively.

Preliminary Vector Copy Number

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

- Ph 1001: VCN ≤ 0.01
- Ph 1002: VCN ≤ 0.01

- For patients on initial FANCOLEN-I study who received optimal red cell (CFC) doses and VCNs (jets 2002 & 2006), PB VCNs at this early timepoint were in a similar range.
- In case of engraftment, increases in VCN are anticipated over 12 time frames.

Patient Characteristics

- Age (%) Gender 5 F 6 5 MCV (fl) 86.9 106.2
- WBC (x10^3) 4.0 6.000
- BM CD34+ (x10^6) 76 54
- Hb (g/dL) 11.9 8.9
- FA multi
- PAL (x10^6) 55,000 18,000

Conclusions

- This US Phase 1 trial confirms the HSCT 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 VCN: 9.36x10^6 to 1.0x10^7
  - CFC resistance (100nM MMC) in 30-50% range
  - CD34+ and CFC counts comparable to FANCOLEN-I pts who received optimal product and demonstrated engraftment, phenotype 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 ± 0.1
  - Increasing BM MM resistance at 6 mos.
  - Blood counts stable (potential trend increase) at 6 mos., in setting of multi-lineage decreases in 9.36 x 10^6 to 9.36x10^7 mos. prior to Apheresis

- Global Phase 2 study is underway: NCT# NCT04069533

- No resistance to this level of MMC was observed at pre-treatment baseline.

Preliminary Results: Blood Counts

- Blood count stability in both patients over 6 months following infusion, with trend increases (patient 1001 mos. 96; patient 1002 mos. 6-4).
- Blood count decreases in multiple lineages in both patients prior to infusion (patient 1001 over 36 mos. prior to 9 mos. pre-Apheresis).

- The CD34+ mobilization protocol was similar to original protocol with process modified (process B).

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