Bringing curative gene therapies to patients with rare, undertreated diseases

Gene Therapy for Fanconi Anemia: A Primer
What is Fanconi Anemia (FA)?

• Inherited DNA-repair disorder that results in bone marrow failure (BMF), malformations and a cancer predisposition
  - When cells divide in the body, DNA can get damaged. Normally, cells can repair damaged DNA; in FA, cells cannot repair DNA as efficiently

  [Diagram showing DNA damage, repair, and cell division for Healthy Cell and FA Cell]

  When this occurs in bone marrow (BM) cells for example, it leads to BMF or leukemia
What is FA?

• Results from mutations in Fanconi Anemia (FANC) genes
• FANC genes encode for a group of proteins essential for DNA repair
• At least 23 FANC genes (known as complementation groups) have been described to-date
• Mutations in many of these FANC genes may result in FA
  - Fanconi A (FANCA): 60-70% of FA cases
  - Fanconi C (FANCC): 10-15% of FA cases
  - Fanconi G (FANCG): 10% of FA cases
*Each of the other subtypes account for 3% or fewer of cases
What are Common Manifestations of FA?

Many different parts of the body may be affected.

- A) Short stature
- B) Microcephaly
- C) Abnormal thumbs
- D) Café-au-lait spots

How is FA Inherited?

- **Autosomal recessive pattern of inheritance in 98% of cases**
  - Both parents must pass along 1 copy of the mutated gene
  - Each child has a 25% chance of inheriting a defective gene from both parents
  - Likelihood of a male or female having FA is equal

- **Other patterns of inheritance (rare):**
  - FANCB: X-linked
  - RAD51: Autosomal dominant
How is FA Diagnosed?

1. **Chromosome Fragility Test with DEB (or MMC)**
   - DNA is packaged on chromosomes
   - DEB and MMC are DNA damaging agents
     - DEB = Diepoxybutane
     - MMC = mitomycin-C
   - This test assesses the number of chromosomal breaks in a patient’s white blood cells (WBCs). WBCs are removed from the patient and mixed with DNA damaging agents in a laboratory.

2. Genetic testing for mutations in a FANC gene
Chromosome Fragility Test with DEB (or MMC)

1. Blood is collected from patient
2. White blood cells (WBCs) are stimulated to divide
3. DEB or MMC (DNA damaging agent) is added
4. Chromosomal breaks are assessed

No/minimal breaks → NORMAL
More than minimal breaks → FA

Because normal cells can repair damage to DNA caused by DEB or MMC, no/minimal breaks are seen in the chromosomes.

Because FA cells lack ability to repair DNA, exposure to DEB or MMC results in chromosomal breaks.

International Fanconi Anemia Registry: The Rockefeller University
Current Standard of Care for FA

• Allogeneic hematopoietic stem cell transplantation (HSCT) is the standard of care for correcting the hematologic aspects of FA after symptoms present, including BMF or leukemia
  - Allogeneic means the cells have been donated by an individual other than the patient

• HSCT is also known as a bone marrow transplant (BMT)

1 Source: https://www.fanconi.org/explore/treatment
What is an Allogeneic HSCT?

Allogeneic HSCT is a medical procedure that:

1. Destroys existing blood stem cells (also known as hematopoietic stem cells or HSCs) in a patient’s BM through chemotherapy with or without radiation (also known as conditioning); and

2. Replaces the BM of the patient with blood stem cells (HSCs) from a matched or partially matched, related or unrelated, donor’s blood stem cells

An allogeneic HSCT requires:

1) a **donor** who is able and willing to give stem cells and
2) a patient to **undergo conditioning**
Hematopoietic Stem Cells (HSCs), i.e., Blood Stem Cells

1 in every 10,000-15,000 BM cells is thought to be a stem cell

HSCs give rise to WBCs, RBCs, platelets

Hematopoietic Stem Cells

Common Myeloid Progenitor Cell

Common Lymphoid Progenitor Cell

WBCs fight infections

White Blood Cells (WBCs)

Monocyte
Eosinophil
Neutrophil
Basophil

Red Blood Cells

Platelets

RBCs carry oxygen
Platelets help blood clot

T- Lymphocyte
B- Lymphocyte
Natural Killer Cell

White Blood Cells (WBCs)

The average body produces 200bn RBCs, 10bn WBCs and 400bn platelets per day
Allogeneic HSCT Considerations

Conditioning, which is necessary for an allogeneic HSCT, can cause significant side effects.

Allogeneic HSCT corrects the hematologic aspects of FA (e.g., BMF and leukemia); patients must still be regularly examined for signs of cancer (e.g., head and neck cancer).

Allogeneic HSCT may be complicated by:
- Graft versus host disease (GvHD), which is when donor cells attack patient cells.
- A further increased risk of solid tumors (e.g., head and neck cancer, and others).
Somatic Mosaicism in FA – “Natural” Gene Therapy

• In some FA patients a rare, spontaneous second mutation can occur in an FA HSC which corrects/reverses the original FA mutation

• Corrected HSCs, with the reversion mutation, have a growth advantage over FA cells
How is Gene Therapy a Potential Treatment for FA

• Gene therapy is a technique which involves the insertion of a functional gene into a patient’s cells in the hopes that the correct gene compensates for the abnormal gene
  - Utilizes a virus (which is disabled so it cannot reproduce or cause disease) to deliver the “normal” gene
    • The mutated or uncorrected gene is not removed
  - The virus carrying the “normal” gene is known as a vector
  - Viral vector can be delivered either:
    • Directly into the body (*in-vivo*); or
    • Into cells that are removed from the patient and transplanted back into the patient after the cells have been genetically modified (*ex-vivo*)
Delivery of Therapeutic ("Normal") Gene

"Normal" gene

Viral vector carrying the "normal" gene

Viral vector enters patient cell and delivers "normal" gene

"Normal" gene incorporated in patient’s DNA
In a Nutshell: How Does Rocket’s Gene Therapy Work?

Rocket’s gene therapy is an *ex vivo* lentiviral vector (LVV) therapy intended to treat patients with *FANCA* mutations. Here’s how:

1. Blood stem cells (HSCs) are pushed from the BM into the blood with special medicines and then collected from patients via a procedure called apheresis.

2. HSCs are genetically modified in a lab by introducing a functional copy of the *FANCA* gene using a viral vector:
   - The type of viral vector (VV) used is a lentiviral vector.

3. Genetically modified HSCs are returned back to the patient with no conditioning.
Why Is Conditioning NOT Currently Used in FA Gene Therapy?

• What is conditioning (recap)?
  - Use of chemotherapy with or without radiation to destroy existing HSCs in a patient’s BM

• Why is conditioning used in allogeneic HSCT?
  - To remove FA patient’s HSCs in order to make room in the BM for healthy donor cells and prevent rejection of donor cells

• Why is conditioning not currently used in gene therapy for FA?
  - Because when an FA HSC is genetically modified to include a “normal” copy of the *FANCA* gene, that HSC has a selective survival and growth advantage over the unmodified FA HSC because unlike the FA HSC, the genetically-modified HSC can repair damaged DNA, proliferate, and potentially generate healthy blood and BM cells
  - Gene therapy in FA is expected to mimic naturally occurring somatic mosaicism in FA, and is not expected to increase the risk of subsequent solid organ cancers (Note: it is not known whether gene therapy will decrease the risk of leukemia)
Selective Advantage of Gene-Modified HSCs: Why Conditioning is NOT Currently Used in Gene Therapy

Unlike the FA HSCs, genetically-modified HSCs can repair damaged DNA and proliferate normally.
Gene Therapy As A Preventive Measure

- Potential for correction of blood and bone marrow defect without conditioning, at juncture when HSCs are available
- GTx implemented as preventative measure to avert bone marrow failure; BMT is indicated for patients in whom more severe marrow failure has occurred
STEP 1: Collect HSCs
~ 1 week – Patient is hospitalized

A
1. Apheresis catheter is inserted by a surgeon while a patient is under anesthesia
2. Patient is given medication (G-CSF and Plerixafor) as injections under the skin to make HSCs from the BM enter the blood (this is called mobilization)

B
1. If enough HSCs mobilize to the blood, then blood is drawn from the apheresis catheter to the apheresis machine
2. HSCs are separated from other blood cells in apheresis machine
3. HSCs are collected
4. Remaining blood is returned to patient

Patient is hooked up to the apheresis machine for several hours
HSCs collected from the patient by apheresis are modified (transduced) in a lab to introduce a "normal" copy of the FANCA gene using a LVV.

Gene-modified HSCs are infused into the vein via the catheter for ~30 minutes. HSCs find their way to the BM and then produce new blood cells containing the corrected gene and DNA-repair proteins.

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Gene Therapy Treatment Process

STEP 4: Follow-Up

- 2-3 days post-infusion – Patient monitored in the hospital for infusion reactions; catheter is removed and patient is discharged from hospital
- Every few months post-infusion for 3 years – Outpatient follow-up at clinical center

The patient will be monitored to determine if the therapy is working and if it has any negative effects. This includes monitoring the patient’s blood and BM
- To monitor blood: Blood is drawn from a needle injected into the patient’s vein
- To monitor BM: BM is drawn from a needle inserted into the hip bone while the patient is under anesthesia (i.e., similar to annual BM testing FA patients typically undergo)

Note: Patients will be followed long-term (additional 12 years) primarily by their local physician to monitor for safety
Gene Therapy Treatment Timeline

- **Gene Therapy Treatment**: ~2 week total hospitalization at clinical site for HSC mobilization, HSC collection, HSC transduction and post-infusion monitoring
- **Follow-Up**: ~13 follow-up outpatient visits at clinical site over 3 years post-infusion (1, 2, 4, 6, 9, 12, 15, 18, 21, 24, 28, 32 and 36-months)
- **Long-Term Safety Follow-Up**: Annual outpatient check-ups for 12 years primarily by local physician
What Is Expected to Happen When Gene Therapy is Given to FA Patients?

1. The FA HSC is genetically modified with the "normal" FANCA gene, infused into the patient, and makes its way to the BM.

2. The genetically-modified FA HSCs then give rise to genetically corrected WBCs, RBCs, and platelets in the BM over a period of months.

3. The genetically corrected WBCs, RBCs, and platelets move from the BM into the bloodstream.

Blood cells in BM = BM cells
Blood cells in bloodstream = Peripheral Blood (PB) cells
What Should One Hope to See After Receiving Gene Therapy?

1. Genetic correction of BM cells – i.e., engraftment
   - The goal of gene therapy is for the gene-modified HSCs to produce healthy red cells, white cells and platelets in the BM and blood
   - How is engraftment of gene modified cells measured?
     • By assessing vector copy number (VCN)
     • VCN represents the average number of vector (corrected gene) copies per cell following genetic modification with LVV
     • Increases in the number of gene corrected cells is slow; engraftment is typically observed at the 1-year mark

Slow, progressive increases in the % of gene-corrected BM cells are expected to continue to over time
What Should One Hope to See After Receiving Gene Therapy?

2. Functional correction of BM cells

- Since gene-modified BM cells have a “normal” copy of the FANCA gene, these BM cells should be able to repair damage to DNA and survive in the presence of DNA damaging agents.

- How is functional correction of BM cells measured?
  - By assessing resistance of BM cells when exposed to DNA damaging agents, like MMC.

Increases in the % of MMC resistant BM cells are expected over time.
What Should One Hope to See After Receiving Gene Therapy?

3. Functional correction of peripheral blood (PB) cells

- After gene-modified HSCs are infused into the patient, it is expected that gene-modified BM cells will be produced and will then enter the bloodstream, this will include circulating WBCs.

- PB cells may therefore be expected to have a functional copy of the FANCA gene and will potentially be able to repair damage to DNA.

- How is functional correction of PB cells measured?
  - By assessing resistance of PB cells to DNA damaging agents, like DEB.

Increases in the proportion of DEB-resistant PB cells are expected over time.
Understanding FA Cells, Healthy Cells and Gene Therapy Cells

- **FA Blood Cell**
  - MMC DEB
  - Experiences further damage
  - Eventual cell death

- **Healthy Cell**
  - MMC DEB
  - Repairs itself
  - Production of more healthy cells

- **Post Gene Therapy**
  - MMC DEB
  - "Normal" copy of the gene allows for DNA repair
  - Production of cells with ability to repair DNA
4. Hematologic Correction

- Over time, many FA patients experience BMF and their blood counts (hemoglobin (Hb), WBCs, platelets) decrease

- Since gene-modified HSCs are infused into the patient, patients are expected to produce gene-modified blood cells in the BM, which then enter the bloodstream

Blood counts (Hb, WBCs, platelets) are expected to stabilize and/or improve gradually over time
## Summary of What FA Patients Should Hope to See After Receiving Gene Therapy

<table>
<thead>
<tr>
<th>#</th>
<th>Description</th>
<th>Measured By</th>
<th>Expect to See</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gene correction of BM cells (engraftment)</td>
<td>VCN</td>
<td>Progressive increase in % of gene-corrected BM cells</td>
</tr>
<tr>
<td>2</td>
<td>Functional correction of BM cells</td>
<td>Resistance to MMC</td>
<td>Progressive increased survival of BM cells in presence of MMC</td>
</tr>
<tr>
<td>3</td>
<td>Functional correction of PB cells</td>
<td>Resistance to DEB</td>
<td>Progressive decrease in aberrant PB cells in presence of DEB</td>
</tr>
<tr>
<td>4</td>
<td>Hematologic correction</td>
<td>Blood counts: Hemoglobin, WBC, Platelets</td>
<td>Stabilization or improvement of blood counts</td>
</tr>
</tbody>
</table>

If/when these changes are observed will vary from patient to patient
May take several months and in some cases, longer, to see some of these changes
What If Gene Therapy Does Not Work?

**IMPORTANT!**

- Gene therapy is **not** expected to prevent a patient from receiving a successful allogeneic HSCT in the future if this is needed
  - (This has **not** been studied extensively but is believed to be the case based on available information and results)

- It is not known whether participation in the current study will prevent participation in future gene therapy studies
  - Future studies will be designed to allow patients who have had prior unsuccessful gene therapy to participate, but this determination will also require agreement from health authorities (FDA, EMA, etc.) regarding the safety of gene therapy in the setting of prior unsuccessful therapy
Potential Risks Associated with the Gene Therapy Itself

- **Risk of blood cancer (myelodysplasia or leukemia)**
  - May occur depending on where the gene is inserted in the chromosome of the patient’s HSC
  - Risk is minimized with use of lentivirus (LV) as viral vector, which is why this vector was chosen for the gene therapy
    - No patients in clinical studies using LV (>150 patients worldwide) have developed LV-related blood cancers

- **Replication Competent Lentivirus (RCL)**
  - LV used has a small piece taken from HIV but that piece lacks the parts needed for HIV to grow or reproduce so HIV infection is considered extremely unlikely
  - No patients in clinical studies using LV have developed infection

- **Bacterial infection resulting from contamination during manufacturing of gene therapy**

- **Allergic/immune reaction to the gene therapy**

Although these risks are believed to be very low, the study team will closely monitor all patients
Other Potential Risks

• Risks associated with the following study procedures, while very low, include:

1. BM biopsy: Bruising/pain/bleeding/infection at collection site
2. Insertion of apheresis catheter: Bleeding/bruising/infection
3. Drugs used to produce and mobilize HSCs from BM into the blood. These drugs include:
   • G-CSF – Nausea, vomiting, diarrhea, fatigue, headache, bone pain, hair loss
   • Plerixafor – Nausea, vomiting, diarrhea, injection site reaction, fatigue, joint pain, headache, dizziness, increased WBCs, decreased platelets
4. Red blood cell and platelet transfusions are likely to be required during apheresis
   • The number of transfusions during apheresis is likely to be limited (1-2)

• There may be risks that are currently unknown

The study team will closely monitor all patients
11 patients have been treated with the current gene therapy (RP-L102)

9 patients treated in Madrid, Spain at Hospital Infantil Universitario Niño Jesús

2 patients treated in the USA at the Lucile Packard Children’s Hospital (The Center for Definitive and Curative Medicine at Stanford University)

NOTE: 3 additional patients were treated on a prior study with a similar gene therapy utilizing an LV at Fred Hutchinson Cancer Center (Seattle, WA, USA); RP-L101. This therapy is not currently under development but was considered safe and was not associated with serious long-term side-effects
Preliminary Data Shows: Evidence of Engraftment

MAIN TAKEAWAY: There is evidence that the proportion of gene-modified cells in the blood are increasing over time, i.e., there is engraftment

• 30 months after receiving gene therapy, approximately 50% of the cells in the blood of Patient FA-02002 are likely to be gene-modified cells with VCN >0.50

• 18 months after receiving gene therapy, approximately >10% of the cells in the blood of Patient FA-02006 are likely to be gene-modified cells with VCN of 0.13

• Progressive increases in gene-modified cells and VCN also noted in Patients FA-02004 and FA-02005

Source: Rio et al., Nature Medicine, Sep. 9, 2019

cCFU = Corrected Colony Forming Units; pVCN: Product VCN *Minimally Acceptable Dose
Recap

- MMC is a DNA damaging agent that is toxic to (uncorrected) FA BM cells
- If FA BM cells are corrected, they can repair DNA damage and are therefore resistant to MMC
- So functional correction of BM cells can be measured by resistance to MMC

Data shows that in two of the initially treated patients (FA-02002 and FA-02004) the % of BM progenitor cells that survive in the presence of MMC (and so are resistant to MMC) is steadily increasing over time.

This demonstrates functional correction of BM cells because it shows that more BM cells are able to repair damaged DNA

1Source: Rio et al., *Nature Medicine*, Sep. 9, 2019
3 Preliminary Data Shows: Functional Correction of PB Cells by Increased Resistance to DEB

MAIN TAKEAWAY: The % of aberrant PB cells in the presence of DEB is decreasing over time, showing that an increasing number of PB cells are able to repair damaged DNA

Recap
- DEB, like MMC, is also a DNA damaging agent that is toxic to (uncorrected) FA peripheral blood (PB) cells
- So functional correction of PB cells can be measured by a reduction in % of aberrant cells when exposed to DEB

Data shows that in at least two of the initially treated patients (FA-02002 and FA-02006), the % of aberrant cells in the presence of DEB decreases over time

This demonstrates functional correction of PB cells because it shows that more PB cells are able to repair damaged DNA

1Source: Rio et al., Nature Medicine, Sep. 9, 2019
Preliminary Data Shows: Hematologic Correction

**MAIN TAKEAWAY:** Blood counts, including platelets, WBCs (neutrophils) and Hb appear to be stabilizing over time, after substantial decreases prior to gene therapy.

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1Source: Rio et al., *Nature Medicine*, Sep. 9, 2019

BM = BM; cCD34+ = Corrected CD34+ cells; cCFU = Corrected Colony Forming Units
Current Trials (as of Oct. 2019)

- European Phase 1 Trial (Sponsor HNJ): Enrollment Complete (n=9)
- U.S. Phase 1 Trial (Sponsor Rocket Pharma): Enrollment Complete (n=2)
- Global Phase 2 Trial (Sponsor Rocket Pharma): Currently Enrolling (n=10)
  - Sites in Europe (FANCOLEN-II): Hospital Infantil Universitario Niño Jesús (Madrid) and additional centers to potentially participate
  - Sites in U.S.: Lucile Packard Children’s Hospital (The Center for Definitive and Curative Medicine at Stanford University, California) and additional centers to potentially participate
    - University of Minnesota to conduct centralized evaluation of BM MMC-resistance and engage in advisory activities
  - Enrollment Eligibility
    - FA as diagnosed by chromosomal fragility assay
    - Patients of the complementation group FA-A
    - Age: Pediatrics (≥ 1-year); minimum weight of 8kg
    - At least 30 CD34+ cells/μL in one BM aspiration within 3 months prior to CD34+ cell collection OR if C34+ cells/μL in BM is 10-29, PB parameters should meet 2 of the 3 following criteria: —Hemoglobin: ≥11g/dL, —Neutrophils: ≥900 cells/μL, —Platelets: ≥60,000 cells/μL

Phase 1: Evaluates safety of the gene therapy (& preliminary efficacy)
Phase 2: Evaluates safety & efficacy of the gene therapy
Q. What are genes?
Genes are inherited from our parents and make us unique. They are responsible for things like eye color and the color and texture of our hair. Genes are made of strands of a molecule called DNA and are housed in chromosomes. There are about 25,000 genes in most cells of the human body. Each gene contains a “blueprint” for the manufacture of a protein; proteins perform numerous functions. The Fanconi Anemia proteins are responsible for repairing damage to genetic material (DNA) in cells throughout the body. A mutation or change in a normal gene can lead to a disorder, like Fanconi Anemia (FA).

Q. What is gene therapy?
For many diseases, gene therapy is considered experimental, which means that it has not been proven to be helpful or a standard treatment for that disease. However, it does offer the potential to substantially improve or possibly cure diseases that are caused by mutations in a single gene. In the gene therapy used in this trial, the correct gene is put into the patient’s blood stem cells (blood forming cells) with the intent of enabling them to work correctly.

Q. What is a Hematopoietic stem cells (HSCs)?
HSCs are a type of immature cell that give rise to red and white blood cells and platelets.

Q. What is an Lentiviral vector (LVV)?
LVV is a type of vector used in gene therapy that incorporates foreign DNA into both dividing and non-dividing cells to deliver a gene copy to the cells. A vector (also known as a carrier) is genetically modified to deliver the “normal” gene. Select viruses can serve to deliver the gene by infecting the cell and introducing the DNA into the chromosome.

Q. What is somatic mosaicism?
A term that refers to two genetically distinct populations of cells within an individual.
**Frequently Asked Questions**

**Q. What is the purpose of this study?**
The purpose of this study is to determine whether gene therapy in FA is safe and effective in preventing bone marrow failure in patients with Fanconi Anemia, complementation Group A (FA-A). Gene therapy will involve placement of an intact (non-mutated) copy of the FANCA gene (Fanconi Anemia causing gene) inside blood stem cells. To do this, blood stem cells are collected from a patient and genetically modified in a specialized manufacturing laboratory by introducing a “normal” copy of the FANCA gene using a lentiviral vector (this process is called “transduction”). The corrected stem cells are then given back to the patient without using any medication, such as chemotherapy, to remove existing bone marrow cells.

**Q. How is FA currently treated?**
Current treatment medications such as androgens may help to raise blood counts to safe levels. These treatments do not work for everyone, are associated with side effects, and the benefits are sometimes only for a short time. The only available therapy that can possibly cure the blood portion of the disease is bone marrow transplantation (BMT). This procedure involves medications such as chemotherapy that kill a patient’s existing blood forming and immune cells followed by an infusion of blood stem cells from a donor to replace the patient’s bone marrow. This is usually done when the patient’s own bone marrow is not working any more or if there is evidence of blood cancers, such as leukemia, because there are many associated risks and side effects.

**Q. How may this investigational gene therapy help my child?**
We hope that this investigational gene therapy will prevent bone marrow failure in your child but we don’t know for sure if it will. However, we can share that as of July 2019, this therapy has been given to 11 children, with over 1 year follow-up available for 4 of them. Results from these 4 patients have been promising and show that the gene-corrected stem cells are growing in the bone marrow and making new red blood cells, white blood cells, and platelets, commonly referred to as engraftment.
Frequently Asked Questions

Q. Who is eligible to participate in the gene therapy clinical trial?
FANCA patients who have not developed severe bone marrow failure or dysplasia, do not have an HLA-identical sibling donor, and are not on other experimental therapies. The trial is expected to enroll 12 patients globally.

Q. Where will the gene therapy clinical trial be conducted?
Clinical centers which will be enrolling patients in this Phase 2 trial including:
• Hospital del Niño Jesús in Madrid, Spain and Great Ormand Street Hospital in London, England
• Lucile Packard Children’s Hospital (The Center for Definitive and Curative Medicine at Stanford University), California

Q. How is the mobilization and apheresis process performed?
Normally, stem cells live in the bone marrow and there are not many in the bloodstream. To increase the availability of stem cells in the blood, medications called granulocyte colony-stimulating factor (GCSF) and plerixafor will be given by a daily subcutaneous injection, which is an injection that goes just under the skin, for 5-6 days to make more of the stem cells in your child’s bone marrow move (temporarily) into the bloodstream. Blood tests will be done each day to check and see if there are enough stem cells in the bloodstream to collect. Under anesthesia, a special intravenous tube called a central catheter will be placed in a large vein in the chest or groin area (note: for larger adult-sized patients, access might be through veins in the arms). When a patient is ready to undergo collection of stem cells, the catheter will be hooked up to an apheresis machine which collects stem cells from the blood and returns the other cells and blood back to your child. The process takes several hours and you can stay with your child during the procedure. Your child will be carefully monitored. It is likely that 2 of these procedures will be necessary to collect enough stem cells; these procedures will be performed on consecutive days. It is likely that the intravenous leukapheresis catheter (apheresis catheter) will be required for 2-3 days, after which time it will be removed. As part of the apheresis procedure, transfusion of platelets and possibly red blood cells will be required (likely on the second day of apheresis). It is likely that any blood product transfusions required will be given only around the time of the apheresis procedure (and that they will not be required subsequently).
Frequently Asked Questions

Q. Will this study require conditioning?
The gene-corrected stem cells are believed to have a survival advantage over diseased stem cells. Once infused, these corrected stem cells have the potential to repopulate bone marrow and blood and to prevent bone marrow failure. The ability of the gene-corrected stem cells to engraft without any prior conditioning has been demonstrated in the patients treated to-date.

Q. What are the side effects from the gene therapy?
In any research study where a new therapy is being tested, there is a chance the patient may get sick from the new treatment. With a gene therapy, there is a small chance that the patient may get an infection or an allergic reaction. Older gene therapies that used a different type of vector called a gamma-retroviral vector, to deliver the correct gene, caused leukemia in some of the patients who received these therapies. The current FA trial uses a lentiviral vector (LVV). It is believed that lentiviral vectors are not likely to cause cancer and more than 200 patients have received gene therapy with these vectors for various disorders over recent years without any treatment-related leukemias identified. We will monitor patients carefully to evaluate whether any of these side effects develop. You should also know that some of the tests or other medicines we give your child during this process may make him/her feel sick or have other side effects. This therapy has been given to 11 pediatric patients with long-term follow-up available for 4 of them. To-date, the safety profile has been highly favorable.

Q. Has this study been approved?
This study has been approved by relevant health authorities, including the US Food and Drug Administration (FDA), and the Spanish Health Authority (AEMPS), as well as the individual Institutional Review Boards (IRB) at the clinical centers where the study will be conducted. Health authorities, like the FDA, are responsible for ensuring that the study is scientifically sound and appropriate to conduct with children. The IRB is responsible for ensuring the safety of all patients who agree to participate.
Frequently Asked Questions

Q. Can my child receive other therapies while enrolled in the clinical trial?
Since the therapy in the trial is still experimental, it is important to make sure that the all the effects of the treatment are fully understood. Thus, no other treatments that may affect the bone marrow function are allowed if your child continues to participate in the trial until the 3-year study period is over. Should you or your [child’s] doctor decide to pursue another therapy, withdrawal from the trial is permitted at any time with no penalty.

Q. How does this study differ from other studies in FA using oral medicines to prevent cell stress or cancer?
Studies looking at medications that may prevent cancer or reduce the stress on blood cells are using medications that are approved for other conditions, and there is evidence from laboratory studies that these drugs can reduce cell stress or reduce chemical processes that lead to cancer formation. The specific effects in FA are not known, and it not known if these therapies will prevent cell damage or for how long. These medications are believed to be generally safe. Gene therapy is intended to reverse the root cause of the blood portion of FA, by providing blood stem cells with an intact (non-mutated) FANCA gene. Similarly, it is not proven whether gene therapy will prevent bone marrow failure or other long-term blood complications in FA; it is believed that if there is a positive effect on blood cells, this is likely to increase over time, and that the benefit could be durable since healthy blood stem cells can generate blood over the course of someone’s entire life.

Gene therapy will involve more direct inconvenience in the short-term relative to an oral medication, since it will involve hospitalization for approximately one week, and will involve stem cell collections which require a large intravenous catheter, two apheresis procedures and possible platelet and red blood cell transfusions. These procedures will not be ongoing, and once a gene therapy patient has received the infusion of gene-corrected cells, they will likely leave the hospital over the following 2-3 days and will not require ongoing medications. Gene therapy patients will be asked to participate in ongoing follow-up visits to enable assessment of blood, bone marrow, and other health-related parameters.
Frequently Asked Questions

Q. The gene therapy is intended to prevent bone marrow failure, but will there be a risk that the remaining, non-corrected blood stem cells transform to leukemia over time?

It is hoped that gene therapy will enable a sufficient number of blood stem cells to grow in the bone marrow and blood so that a patient will not experience subsequent bone marrow failure. It is likely that some non-corrected HSCs will persist over time. It is hoped that the presence of the gene-corrected blood and bone marrow cells will reduce the stress on these non-corrected cells, so that they may be less likely to transform into leukemia, but the existence or extent of this reduction in stress and leukemia is not known. We will try to study this as part of the gene therapy trial. (It has been shown that FA mosaic patients who have normal blood counts are relatively unlikely to develop leukemia or other blood cancers. However, MDS and leukemia have been reported in some FA patients with mosaicism).

Q. Is gene therapy going to be available for patients who have other FA subtypes, such as FANCC or FANCG? What about the very rare subtypes?

The gene therapy program started in FANCA (complementation group A) because the majority of FA patients are part of this subtype. It is planned that once a reasonable degree of improvement is seen in the FANCA study, programs will be developed for the other common complementation groups (FANCC and FANCG). If these appear successful (safe and corrective of the blood component of FA) then we will work with the FDA and other health authorities to understand if there is a way to develop more customized programs for the very rare subtypes.

A vector (also known as a carrier) is genetically modified to deliver the “normal” gene. Select viruses can serve as to deliver the gene by infecting the cell and introducing the DNA into the chromosome.

- Delivery of the vector can be done directly into the body (in-vivo) or cells can be modified outside the patient’s body and the correct version is transplanted back into the patient (ex-vivo).
Frequently Asked Questions

Q. Why do DEB and MMC sensitivity/resistance matter?
Increased resistance of stem cells and blood cells to DNA-damaging agents such as DEB and MMC means that these cells now have the ability to repair DNA damage, leading to improved survival. With this improved survival, blood count stabilization and potentially improvement in blood counts is anticipated.

Q. How long will patients be followed up?
Patients will be followed for 3-years. Patients will have the ability to enroll in long-term follow-up for an additional 12-years to ensure safety.

Q. Why does the # of gene corrected cells matter?
It is believed that the greater the number of gene corrected HSCs are infused, the higher the likelihood of correction in the blood and bone marrow.

Q. Is there still a risk for myelodysplastic syndrome (MDS)?
It is unknown whether or not the therapy will lower the risk of MDS in patients who receive gene therapy. It is hoped that the risk of MDS will be decreased as the gene-corrected cells will now have the ability to repair DNA damage which predisposes patients to developing DNA mutations resulting in blood cancers.

Q. How young do patients need to be to be eligible?
Ages 1 and above. We are targeting patients who have not developed bone marrow failure. Younger patients are believed to be more likely to have a greater stem cell reserve in bone marrow; this study will enable us to better understand this.

Q. Eligibility criteria for Phase 1; whether that will change for Phase 2
There are no major changes to eligibility from Phase 1 to Phase 2 although patients above the age of 12 will be eligible for the phase 2 study.
Q. Why are there only results presented from the four patients in the materials and not 11?
The FANCOSTEM (stem cell mobilization and collection study) enrolled 10 patients, however two of the patients did not have sufficient stem cell quantities to enable mobilization of stem cells to the blood, and did not undergo apheresis or proceed into the second (treatment) study. The remaining eight patients were eventually treated on the FANCOLEN-I (treatment) study, as was an additional patient from France whose cells were mobilized and collected in France (the patient was treated in Spain) — accounting for nine patients treated to date in Europe. An additional two patients received treatment earlier this year on an initial US study (at Stanford), making for a total of 11 patients.

The presentations to-date have focused on the initial four patients because these were the patients with the most extensive follow-up, and in the absence of conditioning, assessments of potential efficacy during the initial 6-months following therapy are rather limited. As the other patients are followed for longer periods of times their progress will also be disclosed.

Q. What is the rationale for having frozen stem cells for two patients and fresh stem cells for the other two patient?
Drs. Sevilla, Bueren and their team recognized that in order to collect an adequate number of stem cells, collection was to be performed as early as possible, before blood counts had diminished beyond a certain extent, and this collection study required that blood counts be above pre-specified thresholds. Because this was a first in human study, the health authorities did not want the initial investigational treatment to occur in patients whose blood counts were relatively good, and required that to qualify for the therapy-study, counts had to have decreased to moderate/severe levels such that an intervention (like a transplant) would be warranted. (This is different from the studies currently underway, where the intent is to collect and treat as early as possible). As a result of this complex but necessary arrangement, some patients had stem cells collected and then had to wait until their blood counts decreased during subsequent years to qualify for treatment. These patients’ cells were frozen. There was however some overlap in the criteria between the two studies, and some patients met criteria for both FANCOSTEM and FANCOLEN-I at the same time; these patients received the transduced stem cells within several days of collection, as will be the case with the patients in the studies underway.
Glossary of Abbreviations

- Bone marrow (BM)
- Bone marrow failure (BMF)
- Bone marrow transplant (BMT)
- Diepoxybutane (DEB)
- Fanconi Anemia (FA)
- Fanconi Anemia genes (FANC)
- Graft versus host disease (GvHD)
- Hematopoietic stem cells (HSCs)
- Hematopoietic stem cell transplantation (HSCT)
- Hemoglobin (Hb)
- Lentiviral vector (LVV)
- Lentivirus (LV)
- Mitomycin-C (MMC)
- Peripheral blood (PB)
- Red blood cells (RBCs)
- Replication competent lentivirus (RCL)
- Vector copy number (VCN)
- Viral vector (VV)
- White blood cells (WBCs)