Phase I/II Gene therapy trial of Fanconi anemia patients with a new Orphan Drug consisting of a lentiviral vector carrying the FANCA gene: A Coordinated International Action

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1. Technical aspects of *EUROFANCOLEN*

- Main characteristics of FA
- Antecedents of FA gene therapy
- Aim of the Project
- Technical Description of the project

2. Impacto esperado del Proyecto en el tratamiento de pacientes con AF

3. Dimensión internacional del Proyecto
A 20-year Perspective on the International Fanconi Anemia Registry (IFAR)

• Recessive disease associated to mutations in a family of 15 FANC genes
• Prevalence: ≈ 1-5 per million
• Mean Survival: 25 years
## Distribution of FA Patients by Complementation Groups

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D1</th>
<th>D2</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>I</th>
<th>J</th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>Group</th>
<th>Reference</th>
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<tbody>
<tr>
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<td>0.8</td>
<td>9.5</td>
<td>3.3</td>
<td>3.3</td>
<td>2.5</td>
<td>2.1</td>
<td>8.7</td>
<td>1.6</td>
<td>1.7</td>
<td>0.4</td>
<td>R</td>
<td>R</td>
<td>EUFAR</td>
<td>Levitus et al. Blood 103: 2498, 2004</td>
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<td>15</td>
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<td>3.0</td>
<td>1.0</td>
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<td>1.6</td>
<td>0.1</td>
<td>R</td>
<td>R</td>
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<td>Taniguchi and Andrea. Blood: 107:4223, 2006</td>
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<tr>
<td>81.4</td>
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<td>R</td>
<td>R</td>
<td>SNFA</td>
<td>Casado et al. J. Med Genet. 44:241, 2007</td>
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<td>R</td>
<td>R</td>
<td>GEFA</td>
<td>Rosenberg et al. Haematologica 93:511, 2008</td>
</tr>
</tbody>
</table>

R: Rare
Preferential treatment of FA patients:

- Allogeneic Transplantation from HLA identical siblings

Main limitations of BMT in FA:

- Reduced proportion of patients with a related HLA identical donor
- Higher morbidity and mortality in transplants from alternative donors
- Higher risks of SCCs in transplanted FA patients

Alternative:

- Infusion of gene-corrected autologous HSCs: GENE THERAPY
Fanconi anemia

ADA; X1-SCID; WAS

B-Thal
WAS
CGD; MLD; ALD

erythrocyte
eosinophil
basophil
monocyte
macrophage
platelets
neutrophil

megakaryocyte

B-cell
T-cell
NK-cell

HSC
HPC
CMP (CFU-S)
CFU-GEMM
CFU-GM

CLP
Conclusions from Previous Clinical Trials in FA Patients

  - Transient detection of FANCC transduced cells in PB and BM cells.
  - Transient improvement in BM cellularity

- Stem Cell Collection and Gene Transfer in Fanconi Anemia. Kelly et al. Mol Ther. 2007
  - The target CD34+ cell dose of 2x10^6/kg future weight was not obtained in any patient.
  - Only transient improvements in hemoglobin and platelet counts were observed.
  - Gene correction was transient, likely owing to the low dose of gene-corrected cells infused.

Main limitations of previous FA gene therapy approaches:

- Very limited numbers of CD34+ cells.
- Use of gamma-retroviral vectors associated with prolonged ex vivo transduction periods of the HSCs.
AIM of EUROFANCOLEN

To develop a clinically efficient gene therapy trial in FA patients (Initially FA-A)

- Using more efficient procedures of HSC collection.
- Using more efficient and safer gene transfer vectors (A new LV developed by EUROFANCOLEN members; The Orphan Drug).
- Reducing the ex vivo manipulation of FA HSCs.
- If necessary, using optimized procedures of patient’s conditioning.
WP1: To determine the genetic and hematopoietic characteristics of FA patients

- Fanconi anemia diagnosis.
- Diagnosis of the pathogenic mutations.
- Early diagnosis of myelodysplastic syndromes or leukemia.
- Diagnosis of mosaic patients with revertant mutations accounting for spontaneous hematological recovery.
- Subtyping of Fanconi anemia patients.
- Prediction of the hematopoietic reserve of the patients.
WP2: To assess the safety and efficacy of an improved mobilization and HSC collection method based on a new mobilization regimen for FA patients with plerixafor and filgrastim.

**AIM:** To collect $4 \times 10^6$ CD34+ cells / kg of weight projected to 5 years.

- **HSC mobilization:** Filgrastim (10-12 μg / kg every 12 hours) for up to 7 days and plerixafor (240 μg / kg) up to 4 days, 6 to 11 hours before starting apheresis

<table>
<thead>
<tr>
<th>Days:</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>Filgrastim</td>
<td>↓</td>
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<td>↓</td>
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<td>Mozobil</td>
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<tr>
<td>Apheresis</td>
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</tr>
</tbody>
</table>
WP2: To assess the safety and efficacy of an improved mobilization and HSC collection method based on a new mobilization regimen for FA patients with plerixafor and filgrastim.

**Main Inclusion Criteria:**

- Patients with a confirmed diagnosis of FA.
- Normal PBC counts to moderate aplasia: Hemoglobin $\geq 8.0$ g/dL, or Neutrophils $\geq 750$/mm$^3$, or Platelets $\geq 30,000$/mm$^3$.

**Main Exclusion Criteria:**

- Evidence of myelodysplastic syndrome or leukemia, or cytogenetic abnormalities predictive these syndromes in BM aspirates obtained $<3$ months prior to HSC collection.
- Evidence of somatic mosaicism associated with hematological improvement.
WP3: To validate the safety and efficacy of the therapeutic clinical-grade lentiviral vector

Antecedents:

Orphan Medicinal Product Designation: EU 3/10/822

Lentiviral vector containing the Fanconi anemia A (FANCA) gene
WP3: To validate the safety and efficacy of the therapeutic clinical-grade lentiviral vector

AIMs:

- To produce the therapeutic vector under GMP conditions.

- To validate the GMP manufacturing process of the medicinal product: Genetically modified FA-A CD34+ cells.

- To validate the safety of the medicinal product.
WP4: To assess the safety and efficacy of the infusion of CD34+ cells in FA patients, after transduction with the therapeutic lentiviral vector

**AIM**

To demonstrate the safety and obtain the first evidences of clinical efficacy associated to the infusion of the medicinal product: Genetically modified autologous CD34+ cells.

**Inclusion Criteria**

- Patients complementation group: FA-A
- Moderate to severe aplasia

**Exclusion Criteria**

- Patients with a HLA-identical related donor
- Nº of cryopreserved or fresh CD34+ cells: <10^5 CD34+/kg weight
- Evidence of CD34+ cells transformation
- Evidence of somatic mosaicism in HSCs associated with hematological improvement

Approved 12/4/2013
**Combined FA Clinical Trial**  
*(FancoSTEM+FancoLEN)*

1. Plerixafor/Filgrastim mobilization of CD34+ cells  
(As early as possible once the FA diagnosis is confirmed)

2. Cryopreservation

3. Genetic correction with LV:PGK.FANCA.Wpre*

4. Infusion of transduced CD34+ cells  
First without conditioning

* To optimize the recovery of stem cells  
* To prevent the collection of leukemic stem cells.

CD34+ purification

FA patient
3.- Management Committees and Advisory Boards

1.- PARTNERS

P1 (Co-ordinator): J. Bueren. CIEMAT/CIBERER (Madrid. Sp)

P2: M. Cavazzana-Calvo (AP-HP)
P3: A. Thrasher (UCL)
P4: J. Sevilla. (SERMAS)
P5: C. Díaz-Heredia (ICS-HUVH)
P6: A. Galy (Genethon)
P7: J. Soulier. (UPD)
P8: J. Surrallés (UAB)
P9: J. Kenklies (GATC)
P10: M. Schmidt (NCT)
P11: L del Pozo (IDETRA)

2.- Sub-Contractors

Sc 1: C.R.O.
Sc 2: Safety Analyses
Sc 3: DNA Sequencing
Sc 4: Clinical Q.C.

4. Associated Institutions and stakeholders

FARF
CIBERER (Orph.Drug Promoter)
IFAGTWG
Fanconi anemia Hope (UK)
Spanish Association for FA (Sp)
Scientific and Ethic Advisory Committee

- Prof. Eliane Gluckman:

- Prof Jakub Tolar: Director of the University of Minnesota’s Stem Cell Institute.

- Prof. Michael Fucs (Institute for Science and Ethics. Bonn. Ger)
International Dimension of the Project

The FARF and EUROFANCOLEN have promoted the creation of the “International Fanconi Anemia Gene Therapy Working Group” with USA and EU members.

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Gene Therapy for Fanconi Anemia: One Step Closer to the Clinic

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