Engraftment and Phenotypic Correction of Hematopoietic Stem Cells in Non-Conditioned Fanconi Anemia Patients Treated with Ex Vivo Gene Therapy

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Disclosure:
J.B. is a consultant, has licensed medicinal products and received funding from Rocket Pharma
Clinical Aspects of Fanconi Anemia

- Prevalence: $\approx 1$-5 per million
- 22 genes: $(\text{FANCA} > 60\%)$
- DNA repair pathway: ICL repair

Reduced numbers of HSCs in the BM of FA patients

Stem Cell Collection and Gene Transfer in Fanconi Anemia
Kelly et al. Molecular Therapy. 2007
Somatic Mosaicism: Natural Gene Therapy in FA HSCs

Phase I/II Gene Therapy Approach for FA-A Patients

- HSC numbers (PBC counts)
- Age

HSC collection

Transduction + Infusion of cHSCs
5. Infusion (No conditioning)

1. Mobilization of CD34+ cells: 2-3 x (G-CSF+Plerixafor)

2. ± Cryopreservation

3. CD34+ cell purification

4. Transduction (PGK-FANCA.Wpre* LV)

Approved Gene Therapy Trial for FA-A Patients
### Characteristics of the Medicinal Products

<table>
<thead>
<tr>
<th>PATIENT</th>
<th>No. Infused cells/kg</th>
<th>CD34+ cells/ kg</th>
<th>% CD34+</th>
<th>Mean VCN/cell (CFCs)</th>
<th>Transduced CD34+ cells/kg</th>
<th>Transduced CFCs/kg</th>
<th>MMC survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>02002 (Cryopr)</td>
<td>6.0x10^5</td>
<td>0.56x10^6</td>
<td>90.6%</td>
<td>0.45</td>
<td>2.5x10^5</td>
<td>1.7x10^4</td>
<td>30.3%</td>
</tr>
<tr>
<td>02004 (Cryopr)</td>
<td>1.2x10^6</td>
<td>0.72x10^6</td>
<td>57.0%</td>
<td>0.23</td>
<td>1.7x10^5</td>
<td>6.9x10^3</td>
<td>27.0%</td>
</tr>
</tbody>
</table>

PRE-STIMULATION: 8-10h  
TRANSUDATION: 10-12h  

PGK-FANCA.Wpre* LV
Kinetics of Gene-marked Cells in PB

02002 (Cryo)
2.5x10^6 cCD34+/Kg
1.7x10^5 cCFU/Kg

02004 (Cryo)
1.7x10^5 cCD34+/Kg
6.9x10^3 cCFU/Kg

02005 (Fresh)
2.3x10^5 cCD34+/Kg
2.8x10^3 cCFU/Kg

02006 (Fresh)
4.0x10^5 cCD34+/Kg
1.6x10^5 cCFU/Kg
Gene Marking in Purified PB and BM Cells from Patient 02002

Peripheral Blood

Bone Marrow

- CD14
- CD15
- CD3
- CD19

- Total BM
- CD34
- CD41
- CD42
- GlyA CD71

% Gene marked cells

Copies per genome

Months post Gene Therapy

Months post Gene Therapy
% corr PBCs: 0.03 0.27 0.14 N.D 2.1 6.5 9.2 12.1 16.5 22.1 34.4
Progressive Phenotypic correction of BM CFCs (MMC-Resistance)
Progressive Increase in the Chromosomal Stability of DEB-treated PB T cells

0 2 0 0 2

Healthy Donor
Mosaic FA
Non-reverted FA

% Aberrant-free cells
Months post Gene Therapy

0 2 0 0 4

Healthy Donor
Mosaic FA
Non-reverted FA

% Aberrant-free cells
Months post Gene Therapy

0 2 0 0 5

Healthy Donor
Mosaic FA
Non-reverted FA

% Aberrant-free cells
Months post Gene Therapy

0 2 0 0 6

Healthy Donor
Mosaic FA
Non-reverted FA

% Aberrant-free cells
Months post Gene Therapy
Peripheral Blood Cell Counts Prior to and After Gene Therapy

**02002 (Cryo)**
2.5x10^5 cCD34+/Kg
1.7x10^4 cCFU/Kg

**02004 (Cryo)**
1.7x10^5 cCD34+/Kg
6.9x10^3 cCFU/Kg

**02005 (Fresh)**
2.3x10^5 cCD34+/Kg
2.8x10^3 cCFU/Kg

**02006 (Fresh)**
4.0x10^5 cCD34+/Kg
1.6x10^5 cCFU/Kg
Kinetics of Corrected and Uncorrected PB Leukocytes prior to and after Gene Therapy

Uncorrected leukocytes/µL  Corrected leukocytes/µL

FA-02002

FA-02004

FA-02005

FA-02006

Leukocytes/µL

Leukocytes/µL

Leukocytes/µL

Leukocytes/µL

Months after Gene Therapy

Months after Gene Therapy

Months after Gene Therapy

Months after Gene Therapy

Uncorrected leukocytes/µL  Corrected leukocytes/µL
Conclusions

- No SAEs

- Collection of clinically relevant numbers of mPB CD34+ cells

- Infusion of corrected CD34+ cells in non-conditioned FA patients:
  - Engraftment and repopulation advantage of gene-corrected HSPCs
  - Poly/oligoclonal reconstitution and gene correction of multipotent HSCs
  - Phenotypic correction of BM progenitors and PB T cells
  - Amelioration of the BMF
Future Perspectives

- Infusion of higher numbers of corrected HSCs:
  - Collection / infusion of HSCs prior to BMF (Phase-II trial)
  - Improved transduction of HSCs (transduction enhancers)
**Preliminary result: Improved short-term engraftment after CD34+ cell transduction with Transduction Enhancers**

<table>
<thead>
<tr>
<th></th>
<th>Transduction Efficiency (Especif: 0.1-5 copies/cell)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No Transd. Enhancers</strong></td>
<td></td>
</tr>
<tr>
<td>Validation 1</td>
<td>0.39</td>
</tr>
<tr>
<td>Validation 2</td>
<td>0.33</td>
</tr>
<tr>
<td>Validation 3</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>With Transduction Enhancers</strong></td>
<td></td>
</tr>
<tr>
<td>Validation 1</td>
<td>2.08</td>
</tr>
<tr>
<td>Validation 2</td>
<td>2.56</td>
</tr>
<tr>
<td>Validation 3</td>
<td>3.13</td>
</tr>
</tbody>
</table>

**VCN in PB (copies per genome)**

- 0.5
- 2
- 0.5
- 2
- 0.5
- 1.5
- 0.5
- 2

**Months post Gene Therapy**

- 2002
- 2004
- 2005
- 2006
- 2003
Acknowledgments

Paula Río

Susana Navarro

Julián Sevilla
Eva Merino
Eva Gálvez

Cristina Díaz de Heredia
Raquel Hladun
Thanks!