Establishing multicenter international gene therapy trials for blood genetic diseases

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Inherited diseases of the blood and immune system

Challenges

• Low prevalence
• High number of different rare diseases
• High phenotypic heterogeneity

Opportunities for gene therapy in this focused area

• Novel therapies, technologies and common knowledge applicable to several of such rare diseases
• International networks to translate research results into the marketing of orphan drugs
• Improved benefit to patient and society
Basic principle of hematopoietic gene therapy

Gene correction

Self-renewal
### Gene Therapy for PIDS: Proof of concept and limitations

#### Table 1: Referenced hematopoietic gene therapy studies conducted for primary immune deficiencies in the past 10 years

<table>
<thead>
<tr>
<th>Disease</th>
<th>Type of vector</th>
<th>Centers</th>
<th>No. of patients</th>
<th>Efficacy</th>
<th>Toxicity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA-SCID</td>
<td>Gamma retroviral vector LTR-driven transgene expression</td>
<td>Italy, UK, US, Japan</td>
<td>36</td>
<td>Yes for most patients receiving conditioning prior to gene therapy</td>
<td>No</td>
<td>[16,17]</td>
</tr>
<tr>
<td>SCID-X1</td>
<td>Gamma retroviral vector LTR-driven transgene expression</td>
<td>France, UK, US</td>
<td>22</td>
<td>Yes (patients &lt;10 year old)</td>
<td>Yes, insertional mutagenesis in 5 patients</td>
<td>[18–21]</td>
</tr>
<tr>
<td>CGD</td>
<td>Gamma retroviral vector, LTR-driven transgene expression</td>
<td>Germany, UK, Switzerland, US, South Korea</td>
<td>17</td>
<td>Initial benefit only, 1 patient died of sepsis</td>
<td>Yes, insertional mutagenesis in 3 patients</td>
<td>[22–26]</td>
</tr>
<tr>
<td>Wiskott–Aldrich syndrome</td>
<td>Gamma retroviral vector, LTR-driven transgene expression</td>
<td>Germany</td>
<td>10 included, 2 reported</td>
<td>Yes</td>
<td>Yes, insertional mutagenesis in 1 patient</td>
<td>[27**]</td>
</tr>
</tbody>
</table>

LTR, long-terminal repeat.
Efficient, stable and safe gene transfer in hematopoietic stem cells

Not pathogenic and non-replicative

Different genomic insertion pattern and lower genotoxicity compared to gammaretroviral vectors

Pharmaceutical qualities: Processes for purification, concentration, formulation and cryopreservation

Technological advancement with rHIV-1-derived LV

Adapted from Modlich et al. Mol Ther 2009
Manufacture of clinical-grade lentiviral vectors

Large-scale production (50L)
  Transient transfection of HEK 293 T cells
  Cell Factory Trays (CF10)

Down-stream processing: membrane-based and chromatography steps
  200 fold volumic concentration (250mL)
  3 log protein and DNA reduction

Formulated rec. viral particle (XVivo 20)

Cryopreserved

Final product is sterile, biologically-active, stable

Merten et al., Human Gene Therapy, 2010
The Wiskott-Aldrich Syndrome

Very rare (1/250 000) X-linked PID

Multiple symptoms
- Hemorrhages, microthrombocytopenia
- Immunodeficiency
- Eczema
- Auto-immunity
- Lymphoreticular malignancies

Lack of WASp, a cytoskeletal regulator in leukocytes

Conventional treatment of WAS is allogeneic HSCT.

POC for autologous HSCT-GT
LV for enhanced efficacy and safety in WAS

- Self-inactivating lentiviral vector derived from rHIV1 and pseudotyped with VSVg
- Encodes the human WAS cDNA driven by its endogenous 1.6 kb promoter recapitulating a physiological expression pattern
Reproducible manufacture of the WAS LV

<table>
<thead>
<tr>
<th>Vector:</th>
<th>w1.6_hWASP_WPRE (VSVg) lentiviral vector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production site:</td>
<td>Genethon ETGC</td>
</tr>
<tr>
<td>First lot release:</td>
<td>2008</td>
</tr>
<tr>
<td>Output:</td>
<td>6 lots manufactured and released conform to date +1</td>
</tr>
<tr>
<td>Titers:</td>
<td>$0.9 - 2.4 \times 10^9$ IG/ml  (spec $&gt; 2 \times 10^8$)</td>
</tr>
<tr>
<td></td>
<td>$1.5 - 3.4 \times 10^4$ ngP24/ml  (spec $&gt; 1.6 \times 10^3$)</td>
</tr>
<tr>
<td>Infectivity:</td>
<td>$3.1 - 9.2 \times 10^4$ IG/ng P24 (spec $&gt; 2.4 \times 10^4$)</td>
</tr>
<tr>
<td>Total batch size:</td>
<td>$2.5 - 6 \times 10^{11}$ IG  (aim $&gt; 10^{11}$IG)</td>
</tr>
<tr>
<td>QC:</td>
<td>Identity, integrity, purity, biopotency, sterility, bioburden, RCL</td>
</tr>
<tr>
<td></td>
<td>EP tests EP monograph and US P tests</td>
</tr>
<tr>
<td>Stability:</td>
<td>48 months</td>
</tr>
</tbody>
</table>

Oct 2012
International Gene Therapy Studies for Wiskott-Aldrich Syndrome (WAS)

Pr Adrian Thrasher, Pr H. Bobby Gaspar, **LONDON**
EUDRACT Number: **2007-004308-11**
OPEN 2010
Treated 2 patients

Pr Marina Cavazzana-Calvo
Pr Alain Fischer
Pr Salima hacein-Bey Abina, **PARIS**
EUDRACT Number: **2009-011152-22**
OPEN 2010
Treated 4 patients

Dr; Sung-Yun Pai
Pr Luigi Notarangelo
Pr David Williams, **BOSTON**
IND **14728**
NHLBI funded
OPEN 2012, patients enrolled
Three centers testing the same vector in very similar clinical protocols

Great Ormond Street Hospital, London: 5 patients
Necker Hospital, Paris: 5 patients
Children’s Hospital, Boston: 5 patients

Pilot and feasibility studies, open labeled, non-randomized

Single infusion of autologous CD34+ bone marrow cells transduced with the lentiviral vector (w1.6_hWASP_WPRE (VSVg))

Primary objectives: Safety and sustained engraftment of WASP+ transduced cells, reconstitution of immunity and correction of thrombopenia

Secondary objectives: To improve health of patient (immunity and bleeding).
• **Inclusion criteria:**
  Males of all ages with Severe WAS (clinical score 3-5).
  Confirmed molecular diagnosis.
  Lack of geno-identical donor or 10-9/10 HLA-matched unrelated BM or CB donor

• **Exclusion criteria:**
  Patients with geno-identical donor or MUD
  Contra-indication to procedure
  HIV seropositivity
Haplo Id donor or Leukapheresis * or following G-CSF + Plerixafor (day -8 to day -3)
Preparation of the ATMP

Magnetic cell sorted autologous CD34+ cells

Prestimulation of CD34+ cells with 4 cytokines
Flt3-ligand; c-kit ligand; TPO; IL-3

Serum-free medium

2 hits of vector at $1 \times 10^8$ IG/mL

< 4 days culture - Fresh cells

QC pre-infusion and post-infusion

Quality assurance across centers
Same reagent-providers and lots (cytokines, retronectin, XVivo, Vector)
Transduction protocols validated in each center
VCN qPCR calibration centralized by GNT
Centralized safety evaluation in EU (GNS) and IS (Upenn/Bushman)
Preliminary Results on the WAS trials (Fr – UK)

- Treated 6 patients
- Feasibility: all products conform, all patients engrafted
- No SAE linked to vector
- Stable transduction in vivo
- Multilineage WASp expression
- Diverses IS
- Selective advantage (T)

- Clinical status
  Death of Fr02: viral infections, not linked to vector
  Improvement seen in some patients: eczema, vasculitis, platelets counts, T cell counts
X-linked chronic granulomatous disease

- Rare primary immunodeficiency: (1:70,000)
- Inability of phagocytes to kill ingested microbes
- Defects in the NADPH oxidase complex
- X-linked: defect in gp91phox
- Severe recurrent bacterial and fungal infections
- Inflammation, granulomas
- Life expectancy: 30 – 40 years with poor quality of life

• X-CGD Treatment
  – Antibiotic, anti-fungal prophylaxis
  – IFN-gamma
  – Granulocyte infusions for severe infections

• Only one curative option
  – Stem Cell Transplantation (SCT) (only HLA-id siblings or unrelated donors). The underlying proinflammatory disease favors GvHD and transplant rejection and requires immunoablation/suppression.

• Hematopoietic gene therapy
  – Has been attempted but lacks long-term benefit and LTR-RV showed some toxicity (insertional mutagenesis, transgene silencing)
A SIN rHIV-derived lentiviral vector has been developed to express the CYBB gene (gp91phox) preferentially in myeloid cells.

Shows stable and regulated expression in monocytes and granulocytes
Multi-center Phase I/II trial of gene therapy for X-CGD

- **High titer clinical-grade G1XCGD vector** manufactured at Genethon

- **Multicenter trial sponsored by Genethon**
  - London Institute of Child Health - UCL
  - Paris Necker Hospital Paris
  - Frankfurt Georg Speyer Haus, FU
  - Zurich Zurich University Hospital

- **Patient number** Approximately 5 patients per site

- **Objectives and challenges to meet:**
  - Stability of engraftment
  - Efficacy of gene transfer and improved health
  - Safety
Orphan drug development for X-CGD

- Standardization, coordination, quality

- PIP submitted to EMA

- Orphan drug designation obtained in Feb 2012

- Clinical trial authorization by MHRA in London (2013)

- International network of partners supported by FP7 Net4CGD (www.net4CGD.eu)
FP7 HEALTH 2012-INNOVATION: Orphan drugs

The site www.net4CGD.eu is under construction

Net4CGD, Gene Therapy for X-linked Chronic Granulomatous Disease (CGD), is a large-scale integrating project in Health Research of the European Commission 7th framework programme (FP7). The Net4CGD project is focused on the clinical development of a new orphan drug of gene therapy that can rapidly become a new treatment option for patients with the X-linked form of CGD. The Net4CGD consortium consists of 11 partners institutions and is coordinated by Genethon, France.

Public Presentation of the Net4CGD consortium
April 30, 2013 - 14h30

Plenary talk by Pr. Reinhard Seger University of Zurich, Switzerland.
"From bone marrow transplantation to gene therapy: Specific challenges and therapeutical progress in chronic granulomatous disease (CGD)"

Address of the venue: Genocentre, 1 rue de l’Internationale, 91002 Evry, France (www.genocentre.fr).
Contact: Candice Quinon
International Gene Therapy trials for two rare PIDs

Reiteration leads to acceleration of projects

International aspect

Additional complexity for product development and manufacture trial approval and conduct

However, strong advantage

Number of patients
Expertise
Validation of product
Delivery to patient

Future challenges

Extend
Ameliorate process
Reduce costs
GENETHON – Evry – France

*Immunology and Gene Therapy*

*Inserm U951*

A Galy, S. Charrier, A Viornery, K. Seye, G. Cedrone, J. Perea

HIV-vector

O. Merten, M. Gasmi, F. Barnay-Toutain, E. Galene, Than Hoa-Le

Regulatory, Clinical Affairs, QA

G. Honnet, D. Caizergues, M. Keen, M. Souquieres, H. Haddad, A. Kobrine, P. Guérin, F. Mavilio

GENOSAFE – Evry – France

M. Audit, A. Botrel, M. Dessainte

MHH (Hannover Med. School)

C. Baum, U. Modlich, S. Knoss

TIGET, Milan (PC work)

L. Naldini, MG Roncarolo, A. Aiuti

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A.M. McNichol, S. Swift, M. Blundell, G. Bouma

N. Himmoudi

NECKER HOSPITAL, Paris

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UNIVERSITY OF PENNSYLVANIA

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Georg Speyer Haus

M. Grez