Gene Therapy for Chronic Granulomatous Disease:

From Bench to Bedside

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The acute response of the phagocytic system
X-linked Chronic Granulomatous Disease (X-CGD)

- inability of phagocytes to kill ingested microbes
  several defects in the NADPH oxidase complex
  70% X-linked: dysfunctional gp91phox protein

- congenital immunodeficiency disorder
  (incidence: 1: 250,000)

- clinical manifestation:
  severe recurrent bacterial & fungal infections
  decreased life expectancy

- curative treatment options:
  allogeneic HSC transplantation / gene therapy
Gene Replacement Therapy for X-CGD

- G-CSF mobilization
- Leukapheresis
- Isolation of CD34+ cells
- Myelosuppression
- Quality control

Gene transfer

Blood Stem Cells (CD34+)
Transfer Vector (SF71gp91phox)

Ott et al., 2006
Gene Therapy for X-CGD: Patient Characteristics

Mutations:

- **P1** exon 9: 1041A>C T343P
- **P2** exon 10: 5 bp deletion stop 501

Clinical History:

**P1**
- 26 years
- Liver abscess
- *Pseudomonas* Septicemia
- Candida Oesophagitis
- Granulomas Ureter/Bladder
- **Prior to GT:**
  - 2 liver abscesses (*S. aureus*)

**P2**
- 25 years
- Liver abscess
- Parotis abscess
- Hidradenitis
- Lung aspergillosis
- **Prior to GT:**
  - Lung cavity with infected wall

Cross & Segal, 2004, modified
Gene Therapy for X-CGD: Clinical Benefit

P1

a  

b  

P2

c  

d  

Ott et al., 2006
Clonal Expansion and Silencing of Transgene Expression

Ott et al., 2006
Integration Site Analysis by LAM-PCR

Schmidt et al., 2001
Schmidt et al., 2002
Ailles et al., 2002
Woods et al., 2003
United States Patent No. 06514706 B1
Retroviral Insertion Site Analysis Revealed a Reduction in the Number of Clones Contributing to final Hematopoiesis in P1

Ott et al., 2006
Common Integration Sites Involved in Clonal Proliferation

$MDS1 \ (\sim 514.6 \text{ kbp})$

$EVI1 \ (\sim 61.5 \text{ kbp})$

$PRDM16 \ (\sim 369.4 \text{ kbp})$

Ott et al., 2006
Detection of Monosomy 7 in P1 and P2

EVI1 is highly expressed in Cells from P1 and P2

Increased Genomic Instability in Cells from P1 and P2

EVI1 but not gp91^{phox} causes Centrosomal Aberrations in Human Diploid Fibroblasts

Stein et al., 2010
Clonal Expansion and Silencing of Transgene Expression

Ott et al., 2006
CpG Methylation Analysis of the Clinical Vector SF71gp91\textsuperscript{phox}

Proivirus

![Diagram of Provirus with SFFV, gp91\textsuperscript{phox}, U3, R, U5, SD, SA, ATG]

- 6 CpGs
- 17 CpGs

Bisulfite Treatment

- Unmethylated DNA: N C G N C G N
- Methylated DNA: N C G N C G N

Cloning in TOPO and Colony Sequencing

- BS Treatment
- PCR

Schultze-Straßer, 2010
Methylation-dependent Inactivation of Gp91^{phox} Expression

Stein et al., 2010
Gene Therapy for X-CGD: Clinical Course

Progressive $MDS1$–$EVI1$ clonal dominance
- Loss of NADPH oxidase activity
- CpG vector promoter methylation
- Cytopenias, myelodysplasia
- $-$Chr 7, DNA breaks

Dunbar & Larochelle, 2010; Schwäble et al., 2011; modified
Gene Therapy for X-CGD: Conclusions

• Gene therapy can provide a significant clinical benefit for CGD patients

but:

• Therapeutic effect was compromised by vector silencing
• Insertional Oncogenesis led to MDS with monosomy 7

• P1 died 27 months after gene therapy
• P2 underwent allogeneic HSCT
## Current status of CGD gene therapy clinical trials

<table>
<thead>
<tr>
<th>Center</th>
<th>Patients treated</th>
<th>Total Conditioning</th>
<th>Vector type used$^a$</th>
<th>% Transduction efficiency$^a$</th>
<th>Total dose of infused CD34+ cells per kg</th>
<th>Significant Engraftment &gt; 3 months</th>
<th>Initial Clinical Benefit</th>
<th>Genotoxicity</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frankfurt</td>
<td>2</td>
<td>Liposomal busulfan 8.0 mg/kg</td>
<td>SF71gp91phox (SFFV-LTR)</td>
<td>P1: 45.0% P2: 39.5%</td>
<td>P1: $11.3 \times 10^6$ P2: $9.0 \times 10^6$</td>
<td>15% gene marking in CD15$^+$ cells</td>
<td>Yes</td>
<td>Both patients developed clonal myeloproliferation and MDS with monosomy 7</td>
<td>Stein et al. Ott et al.</td>
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<tr>
<td>Zürich</td>
<td>2</td>
<td>Liposomal busulfan 7.0-8.8 mg/kg</td>
<td>SF71gp91phox (SFFV-LTR)</td>
<td>P1: 25.3% P2: 32.7%</td>
<td>P1: $19.0 \times 10^6$ P2: $8.0 \times 10^6$</td>
<td>20% gene marking in CD15$^+$ cells</td>
<td>Yes</td>
<td>Development of clonal myeloproliferation</td>
<td>Bianchi et al. and R. Seger, personal communication</td>
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<tr>
<td>London</td>
<td>1</td>
<td>Melphalan 140 mg/m$^2$</td>
<td>MFGS-gp91phox (MLV-LTR)</td>
<td>5-20%</td>
<td>0.2 – $10^6$</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>A. Thrasher personal communication</td>
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<tr>
<td>NIH</td>
<td>3</td>
<td>Busulfex 10 mg/kg</td>
<td>MFGS-gp91phox (MLV-LTR)</td>
<td>25% - 73%</td>
<td>18.9 – $71.0 \times 10^6$</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Kang et al.</td>
</tr>
<tr>
<td>Seoul</td>
<td>2</td>
<td>Busulfex 6.4 mg/kg + Fludarabine 120 mg/m$^2$</td>
<td>MT-gp91phox (MLV-LTR)</td>
<td>P1: 10.5% P2: 28.5%</td>
<td>P1: $5.4 \times 10^6$ P2: $5.8 \times 10^6$</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Kim et al.</td>
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$^a$ Transduction efficiencies were measured either by the analysis of gp91phox cell surface expression using a FITC-labeled monoclonal murine anti-human antibody (7D5) and flow cytometry (Frankfurt, Zürich, London, Seoul), or by analysis of intracellular gp91phox expression using the 7D5 antibody (NIH).

$^a$ All vectors were pseudotyped with the GALV envelope and produced on PG13 packaging cells, except for MFGS-gp91phox vector which was produced in 293-producer cells using an amphotropic envelope.
# X-CGD GT-Working Party

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