Gene Therapy for Primary Immunodeficiency Diseases: Should we do it?

Lily E. Leiva, Ph.D.
Associate Professor and Immunology Lab Director
Department of Pediatrics, LSUHSC and Children’s Hospital
**Th0**

**APC**

**IL-3**

**Chemokines**

**Th1**

**IFN-γ**

**Recruitment**

**NK**

**PMN**

**B**

**CD40**

**CD40L**

**Alt C’ Pathway**

**Classic C’ Pathway**

**C3b**

**Bacteria**

**Treg**

**Activation**

**Extracell Pathogens**

**Virally infected cells**

**IL-12**

**IL-2**

**TGFβ**

**IL-10**

**IFN-γ**

**IFN-γ R**

**L.Leiva, 2012**
Primary Immunodeficiency Diseases (PID)

- Monogenic or complex genetic disorders that affect one or several components of the immune system and are present during the patient’s lifetime.

- PIDS can present clinically at any age and some improve due to compensatory mechanisms, others get worse with age.
Phenotype / Molecular / Genotype Diagnosis of Primary Immunodeficiencies

Gene abnormality

Receptor Enzyme Cytokine abnormalities

Neutrophils Complement Antibodies Lymphocytes

Infection Inflammation Autoimmunity Allergy

Protein-based and functional assays

>200
Defective microbicidal activity

Lymphocytes and platelets defects

Absence of T and NK cells

Absence of B cells

Lymphoproliferative disorder

Defective Ig switch

Defective leukocyte adhesion

X-Linked PIDs

- X-CGD (CYBB)
- WAS
- X-SCID (IL-2Rγ)
- XLA (btk)
- XLP (SH2D1A)
- HlgM (CD40L/gp39)
- LAD (β integrin)

X Linked PID:

- Defective microbicidal activity
- Lymphocytes and platelets defects
- Absence of T and NK cells
- Absence of B cells
- Lymphoproliferative disorder
- Defective Ig switch
- Defective leukocyte adhesion
Reticular dysgenesis

Stem cell

Myeloid progenitor

Lymphoid progenitor

SCID

Congenital agranulocytosis

Chronic granulomatous disease

Neutrophil

Monocyte

Pre-B cell

Pre-T cell

Leukocyte-adhesion deficiency

X-linked agammaglobulinemia

Common variable hypogammaglobulinemia

X-linked hyper-IgM syndrome

Selective immunoglobulin deficiency

Thymus

DiGeorge Syndrome

Bare-lymphocyte syndrome

Wiskott-Aldrich syndrome

Plasma cell

Memory B cell
Primary Immune Deficiencies: “Experiments of Nature”

Dr. Robert Good
1922 - 2003

Founder of Modern Immunology
In 1968 Performed the 1st successful human BM transplant
### Incidence of Some PID s

<table>
<thead>
<tr>
<th>Condition</th>
<th>Incidence</th>
</tr>
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<tbody>
<tr>
<td>Selective IgA</td>
<td>1:500</td>
</tr>
<tr>
<td>XLA</td>
<td>1:100,000</td>
</tr>
<tr>
<td>DiGeorge</td>
<td>1: 66,000</td>
</tr>
<tr>
<td>SCID</td>
<td>1: 66,000</td>
</tr>
<tr>
<td>CGD</td>
<td>1:250,000</td>
</tr>
<tr>
<td>Overall</td>
<td>1: 10,000</td>
</tr>
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</table>

- PID affect at least 10 million people worldwide.
- They are more common than childhood leukemia and lymphoma combined and have four times the incidence of cystic fibrosis.
- An estimated 70–90% of PID remain undiagnosed.
Classic Manifestations of PIDS

• Higher susceptibility to infections
• Infections with unusual microorganisms
• May also develop autoimmunity or autoinflammatory diseases
• May also develop malignancies
Importance of Identifying Molecular Defects in PIDs

• Pre-symptomatic diagnosis of PIDs:
  – based on family history
  – neonatal screening for PIDs

• Taylor management to gene defect specific treatments

• Identify candidates for gene therapy
Initial Laboratory Screening for PID

- CBC and differential
- Serum IgG, IgM, IgA, IgE levels
- Flow cytometry:
  - Total T cells (CD2, CD3)
  - T cell subsets (CD4, CD8)
  - B cells (CD19, CD20)
  - NK cells (CD16, CD56)
  - T Reg cells (CD4, CD25, CD127)
  - Activated T cells (CD3, HLA-DR)
- CH50
Severe Combined Immunodeficiencies

T-cells -

B cells -

NK-cells -

Reticular dysgenesis

Rag 1 & 2 deficiency

γc-chain deficiency

NK-cells +

ADA deficiency

Rag 1 & 2 recombination deficiency

NK-cells -

JAK3 deficiency

NK-cells +

B cells +

NK-cells +

IL-7Rα deficiency

T cells + (clonal)

B-cells low

NK-cells +

Ommenn Syndrome
Severe Combined Immunodeficiency Disease (SCID) – Incidence 1:66,000
“The Boy in the Bubble”
David Vetter 1971-1984
Newborn Screening for SCID and T cell Lymphopenia by the TREC Assay
What Are TRECs?

TRECs: Occur During T Cell Receptor Chain Recombination

TRECs: T cell Receptor Excision Circles
Conditions Found by Screening for Low/Absent TREC

- Typical SCID, due to defects that include IL2RG (X-linked), ADA, IL7R, JAK3, RAG1, RAG2, DCLRE1C (Artemis), TCRD, TCRE, TCRZ, and CD45
- Leaky SCID or Omenn syndrome
- Variant SCID, with low T-cells but no defect in a known SCID gene
- Syndromes with variably affected cellular immunity that may be severe, including:
  - Complete or partial DiGeorge syndrome with low T-cells
  - CHARGE syndrome
  - Jacobsen syndrome
  - Trisomy 21
  - RAC2 dominant interfering mutation
  - DOCK8 deficient hyper-IgE syndrome
  - Cartilage hair hypoplasia

Immune Deficiency Foundation: www.primaryimmune.org
The National Newborn Screening in the USA
Implemented in the 1960s (PKU)

SOURCE:
The National Newborn Screening and Genetics Resource Center 10/11/06
(do not include mandated testing that is not yet in practice or pilot programs)
SCID Newborn Screening Campaign

• **On May 21, 2010** Kathleen Sebelius, Secretary of Health and Services announced the addition of SCID to the core panel of 29 genetic disorders as part of her recommendation to adopt the national Recommended Uniform Screening Panel. SCID is the first nominated condition to be added to the core panel of disorders.

• **Being used by 11 states with 7 additional States working on it**

www.SCID.net & the ID Foundation, 2013
NEW YORK, Nov. 14, 2013 /PRNewswire-USNewswire/
212 world renowned experts in Primary Immunodeficiency from 78 countries signed a "Berlin Declaration" calling for global implementation of newborn screening for Severe Combined Immunodeficiency (SCID), at the recently convened Berlin Summit organized by the Jeffrey Modell Foundation.
Deficiencies of Cell Mediated Immunity

Treatment

- IgG replacement before reconstitution
- Prevention and treatment of infections
- Supportive treatment

- Immunological reconstitution:
  - Stem Cell Transplantation
  - Gene Therapy
Immune Reconstitution with Hematopoietic Stem Cells (HSC)

• DONOR:
  – Related or Unrelated

• COMPATIBILITY:
  – Identical
  – Partially Identical - Haploidentical

• SOURCE OF CELLS:
  – Bone marrow
  – Cord blood
  – Peripheral blood
Factors which impact the outcome of Post Hematopoietic Stem Transplant

- Type of SCID
- Type of donor
- Infection at the time of transplant
  - Only 50% of SCID-X1 survive
- Conditioning - B cell reconstitution
### Various HCT regimens from various donor sources result in different survival rates for SCID

<table>
<thead>
<tr>
<th>Donor</th>
<th>n</th>
<th>years surveyed</th>
<th>conditioning</th>
<th>source</th>
<th>patient population</th>
<th>survival</th>
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</thead>
<tbody>
<tr>
<td>sibling</td>
<td>73</td>
<td>1990 on</td>
<td>no</td>
<td>SCETIDE (Europe)</td>
<td>all SCID</td>
<td>86% (10 y)</td>
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<tr>
<td>matched UD</td>
<td>78</td>
<td>1990 on</td>
<td>yes</td>
<td>SCETIDE (Europe)</td>
<td>all SCID</td>
<td>72% (10 y)</td>
</tr>
<tr>
<td>mismatched related</td>
<td>262</td>
<td>1990 on</td>
<td>yes/no</td>
<td>SCETIDE (Europe)</td>
<td>all SCID</td>
<td>59% (10 y)</td>
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<tr>
<td>sibling</td>
<td>15</td>
<td>1983-2004</td>
<td>no</td>
<td>Duke (US)</td>
<td>all SCID</td>
<td>100%</td>
</tr>
<tr>
<td>mismatched related</td>
<td>117</td>
<td>1983-2004</td>
<td>no</td>
<td>Duke (US)</td>
<td>all SCID</td>
<td>74%</td>
</tr>
</tbody>
</table>

(EBMT Meeting, 2008)
(Buckley, Annu Rev Immunol 2004)

Conclusions

In the European series, sibling matched HCT and closely matched unrelated donor HCT for B+ SCID results in 80% survival, mismatched related HCT in 60-70% survival.

Only 50% of SCID-X1 patients who undergo haploidentical HCT when infected survive.

Unconditioned mismatched related HCT often does not reconstitute B cell function.

Brothers with Severe Combined Immunodeficiency (SCID-X1)
Children’s Hospital, New Orleans, 1999 and 2000
Clinical History

- 6-months-old boy
- *Pneumocystis jirovecii* pneumonia
- Persistent diarrhea
- Oral candidiasis
- Pseudomonas infection
- Failure to thrive
- Absence of thymus
- Lymphocyte phenotype: T–B+NK–
Treatment

- IVIG replacement therapy
- **1st TRANSPLANT**: T cell depleted haploidentical BM transplant from his father at age 7 months.
- Symptoms of GvHD appeared 2 weeks after first transplant.
- Transfusions: PBRC weekly, platelets every 2-3 d
- **2nd TRANSPLANT**: GCSF mobilized PBSC at age 19 months.
- Prednisone, GCSF, CsA, MMF.
Immune Reconstitution (A.R.)

- Months Post Stem Cell Transplant:
  - Lymphocytes/ul
  - Normal CD3+ cells

- Lymphs
- CD3
- CD19
- CD16/56
- CD4
- CD8

2nd
Immune Reconstitution (A.R.)

Lymphocytes/ul

- Lymphs
- CD3
- CD4
- CD8
- CD19
- CD16/56

Months Post Stem Cell Transplant

2nd
Gene Therapy
Primary Goal

• The transfer of exogenous genes to somatic cells of a patient in order to correct an inherited gene defect.
Basic Steps - Gene Therapy

• Diagnose a gene defect

• Identify and prepare the gene of interest

• Create a vector (a rocket and its load)

• Transduce the vector with the correct gene into patient’s stem cells cultured with a cocktail of cytokines
Gene Therapy

Viral Vector

Normal Gene

Abnormal Gene

Human Cell

Normal Protein
History of Gene Therapy

• 1990 First clinical trial for ADA deficiency at NIH
  mature lymphocytes

• 1992 Second ADA clinical trial in Italy
  mature lymphocytes and BM stem cells

• 1993 ADA clinical trial in 3 newborns
  cord blood stem cells

• 1996-1998 Four ADA in Europe and Japan

• 2000 First clinical trial for X-SCID ($\gamma_c$) Paris
SCID due to Adenosine Deaminase (ADA) Deficiency

**Clinical Signs**
- Growth failure
- Interstitial pneumonia
- Chronic diarrhea
- Persistent candidiasis

**Laboratory data**
- Severe lymphopenia
- Profound defects in T, B & NK cells
- Often increased liver enzymes

**Treatment**
- Polyethylene Glycol-Conjugated ADA (PEG-ADA)

First ADA-SCID patient receiving Gene Therapy in 1990
Brothers with Severe Combined Immunodeficiency (SCID-X1)  
Children’s Hospital, New Orleans, 2000
Hôpital Necker - Paris, France
Feb, 2000
Gene Therapy

BM

50 ml

Positive Selection of CD34+ Cells

5-10 x 10^6 CD34+ cells/Kg

Stem Cells Preactivated with SCF, FLT3L, IL-3 and MGDF

Moloney retrovirus + γc gene

3 Days

Scientists Report the First Success of Gene Therapy

Continued From Page A1

very long time." Gene therapy experts were exuberant. "It's a very exciting study," said Dr. R. Michael Blaun, who was a member of a medical team at the National Institutes of Health that tried the first gene therapy on a human patient nearly 18 years ago. Dr. Blaun, now the head of the human therapeutic divisions at Vail Gen in Newyork, Pa., added: "This would probably be the first example in any disease where gene therapy could be a fully successful treatment. You can't disconnect these patients from normal."

The success comes on the heels of a tumultuous decade in which, according to the National Institutes of Health, more than 390 gene therapy studies were initiated, involving more than 4,900 patients and more than a dozen medical conditions. While those doing the research always expressed confidence in its promise, critics said many of the companies formed to capitalize on the technology exaggerated preliminary data in the hope of raising capital.

And, at times, some scientists promised more than the technology then was ready to deliver, leaving the field a target for accusations that patients were being endangered by reckless experiments that were doomed to fail.

"We've all been so burned by saying, 'Ah, this looks like it worked, that looks like it worked,'" said Dr. W. French Anderson of the Keck School of Medicine at the University of Southern California and a member of the team that attempted the first gene therapy in 1990. "Now when it finally looks like something is working, I don't want to be in the position of saying the same words. We've all been criticized for hyping too much."

Anderson said his team's treatment by Dr. Fischer had a rare disorder known as severe combined immunodeficiency-XI, or SCID-XI, which almost exclusively affects boys, occurring once in every 75,000 live births. It is caused by mutations that destroy the function of a gene that is needed to make T cells, a class of white blood cells.

Even better, if functioning genes actually get into the marrow cells of patients with SCID-XI, those genetically corrected cells will proliferate and displace cells with the defective gene. That is because, as the body tries to grow a complete immune system, it sends waves of chemical signals to the bone marrow to stimulate it into producing T cells.

T cells with the defective gene start to grow and then die. Any cells with a functioning gene will be fueled by the body's hormones and will grow rapidly to populate the bone marrow.

Dr. Fischer said he began working on gene therapy for SCID-XI in 1985.

The first successful gene therapy has been reported by Dr. Alain Fischer and Dr. Maria Cavanata-Cavalcioni and colleagues in Paris.

Therapy is said to allow two born with a debilitating disease to live normally.

Treatment could work in this particular disease, and the success with the first babies is the result.

Scientists, of course, will assure all that SCID-XI and other rare genetic diseases of immune system cells would be perfect for gene therapy. In fact, the very first human

A CLOSER LOOK

A Gene Therapy Breakthrough

French researchers have developed a method of gene therapy to treat a genetic disorder known as severe combined immunodeficiency-XI, a condition that forces patients to live within a sterile bubble to avoid threats to their nonfunctioning immune system.

The Process

Bone marrow is extracted from the patient. A set of blood stem cells is separated from the marrow.

The cells are mixed with a virus containing the corrective gene. The virus helps carry the gene into the cells.

The cells containing the corrective gene are implanted back into the patient.

The genetically corrected cells proliferate and displace cells with the defective gene.

Gene Therapy: A New Hope for Patients with SCID

Scientists express cautious optimism about the future of gene therapy.
IMMUNOLOGICAL RECONSTITUTION AFTER GENE THERAPY IN X-LINKED SCID

Hacein-Bey et al., 2002
Immunoscope TCRvβ
RD (P5)

<table>
<thead>
<tr>
<th>Time</th>
<th>BV1</th>
<th>BV2</th>
<th>BV23</th>
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<tr>
<td>13 months</td>
<td>0.4%</td>
<td>11.8%</td>
<td>1.5%</td>
</tr>
<tr>
<td>31 months</td>
<td>0.5%</td>
<td>12.9%</td>
<td>0.5%</td>
</tr>
<tr>
<td>34 months</td>
<td>15%</td>
<td>64%</td>
<td>17%</td>
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<tr>
<td>Leukemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>80 months</td>
<td>0.5%</td>
<td>9.5%</td>
<td>1.5%</td>
</tr>
<tr>
<td>112 months</td>
<td>0.4%</td>
<td>10.5%</td>
<td>1.1%</td>
</tr>
</tbody>
</table>
LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1

S. Hacein-Bey-Abina,1,2* C. Von Kalle,6,7,8 M. Schmidt,6,7 M. P. McCormack,9 N. Wulffraat,10 P. Leboulch,11 A. Lim,12 C. S. Osborne,13 R. Pawliuk,11 E. Morillon,2 R. Sorensen,19 A. Forster,9 P. Fraser,13 J. I. Cohen,15 G. de Saint Basile,1 I. Alexander,16 U. Wintergerst,17 T. Frebourg,18 A. Aurias,19 D. Stoppa-Lyonnet,20 S. Romana,3 I. Radford-Weiss,3 F. Gross,2 F. Valensi,4 E. Delabesse,4 E. Macintyre,4 F. Sigaux,20 J. Soulier,21 L. E. Leiva,14 M. Wissler,6,7 C. Prinz,6,7 T. H. Rabbitts,9 F. Le Deist,1 A. Fischer,1,5†† M. Cavazzana-Calvo1,2†

We have previously shown correction of X-linked severe combined immunodeficiency [SCID-X1, also known as γ chain (γc) deficiency] in 9 out of 10 patients by retrovirus-mediated γc gene transfer into autologous CD34 bone marrow cells. However, almost 3 years after gene therapy, uncontrolled exponential clonal...
Third cancer case halts gene therapy

WASHINGTON — The Food and Drug Administration has suspended several U.S. gene-therapy experiments after learning that a third child who underwent treatment in France has developed cancer as a result, a development that has...
Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1

Status of Gene Therapy Clinical Trials for PIDS

- ADA-SCID – 42 pts
- SCID-X1 – 32 pts
- Chronic Granulomatous Disease (CGD) – 24 pts
- Wiskott-Aldrich Syndrome (WAS) – 15 pts

- Transatlantic Gene Therapy Consortium
Gene Therapy Vectors

- γ-retrovirus
- Lentivirus
- Self-inactivating (SIN) vectors
  - SIN γ-retrovirus
  - SIN Lentivirus
- Improved safety standards for vectors
### Summary of the Clinical Experience of HSC-Gene Therapy for ADA-SCID

<table>
<thead>
<tr>
<th>Center</th>
<th>No. pts treated</th>
<th>Conditioning</th>
<th>Outcome</th>
<th>Serious Adverse Event</th>
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</thead>
<tbody>
<tr>
<td>Italy</td>
<td>18</td>
<td>Busulfan</td>
<td>15/18 off ERT</td>
<td>None</td>
</tr>
<tr>
<td>UK</td>
<td>8</td>
<td>Melphalan or Busulfan</td>
<td>4/8 off ERT</td>
<td>None</td>
</tr>
<tr>
<td>USA</td>
<td>14</td>
<td>Busulfan</td>
<td>10/14 off ERT</td>
<td>None</td>
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<tr>
<td>UK, USA</td>
<td>2</td>
<td>Busulfan</td>
<td>Follow-up less than a year</td>
<td>None</td>
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*Mukherjee S, Thrasher AJ, Gene 525, 2013*
## Summary of the Clinical Experience of HSC-Gene Therapy for SCID-X1

<table>
<thead>
<tr>
<th>Center</th>
<th>No. pts treated</th>
<th>Conditioning</th>
<th>Outcome</th>
<th>Serious adverse events</th>
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</thead>
<tbody>
<tr>
<td>France</td>
<td>9</td>
<td>None</td>
<td>Significant clinical benefit</td>
<td>T cell ALL (4 pts)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>None</td>
<td>No clinical benefit</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>10</td>
<td>None</td>
<td>Significant clinical benefit</td>
<td>T cell ALL (1 pt)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>None</td>
<td>No clinical benefit</td>
<td></td>
</tr>
<tr>
<td>USA, France</td>
<td>3</td>
<td>None</td>
<td>Limited clinical benefit</td>
<td>No</td>
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<tr>
<td></td>
<td>8</td>
<td>None</td>
<td>T cell recovery</td>
<td></td>
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</tbody>
</table>

*Mukherjee S, Thrasher AJ, Gene 525, 2013*
Chronic Granulomatous Disease (CGD)
Recent liver abscess and two previous pleural effusion drainages in a 12-year-old boy with XL-CGD

S. aureus isolated in all occasions
Treatment of CGD

• When first discovered, CGD was usually fatal in early childhood.

• Current Treatment Options Include:
  – Aggressive Antibiotic Therapy
  – Antifungal prophylaxis with Itraconazole or Voriconazole
  – Subcutaneous Interferon IFN-γ after the first 6-12 months of life.
  – Stem cell transplantation
  – Gene therapy
## Summary of the Clinical Experience of HSC-Gene Therapy for CGD

Mukherjee S, Thrasher AJ, Gene 525, 2013

<table>
<thead>
<tr>
<th>Center</th>
<th>No. pts treated</th>
<th>Conditioning</th>
<th>Outcome</th>
<th>Serious adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>USA</td>
<td>5 5 3</td>
<td>None None Busulfan</td>
<td>No clinical benefit No clinical benefit Transient clinical benefit</td>
<td>None None None</td>
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<tr>
<td>Germany</td>
<td>2</td>
<td>Busulfan</td>
<td>Long term clinical benefit</td>
<td>Both developed MDS w/monosomy 1 died of sepsis</td>
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<tr>
<td>Switzerland</td>
<td>2</td>
<td></td>
<td>Transient clinical benefit</td>
<td>1 pt - MDS</td>
</tr>
<tr>
<td>UK</td>
<td>1</td>
<td>Melphalan</td>
<td>Transient clinical benefit</td>
<td>None</td>
</tr>
<tr>
<td>Korea</td>
<td>2</td>
<td>None</td>
<td>Transient clinical benefit</td>
<td>None</td>
</tr>
</tbody>
</table>
What does it take to set up a Gene Therapy Lab from scratch?
Personnel

Principal Investigators
Adrian Thrasher
Bobby Gaspar
Waseem Qasim

Production Team
Nourredine Himoudi
Karen Buckland
Hong Zhan
Catherine Irving

Patient Assays
Christine Rivat
Kimberly Gilmour

Regulation
Anne-Marie McNicol
Sue Swift

Pharmacy QA/QP
Olufemi Rabiu
Annette Hogg
Farzin Fazaneh
Peter Brown
Quality Assurance and GMP (Good Manufacturing Practice)

Sue Swift PhD FSB
QA Officer for Gene Therapy
Premises

Good air quality (low particles, high flow)
Maintain sterility and reduce cross-contamination
Frequent cleaning and monitoring

Grade A – isolators
Grade B - incubators in aseptic room
Grade C - room including centrifuge, freezer
Double changing rooms
Equipment – regular monitoring, testing, calibration and validation

Show that the equipment does the job properly
Validation

USR – user requirement specification
IQ – installation qualification
OQ – operational qualification
PQ – performance qualification
PV – performance validation

Validation Policy – overall concept
Validation Master Plan – activities and schedule
Validation Protocol – method for each activity
Validation Report – data, deviations and conclusion
Validation Schedule
Production and raw materials

Each material used in manufacture has to be validated, prepared to a high standard of quality to ensure correct activity and avoid cross-contamination.

- **Cytokines**
- **Human serum/albumin**
- **Cell culture media**
- **Viral vectors**
- **Retronectin**
- **Cells**
DAY 1
Bone marrow or leukapheresis harvest from patient
Cells selected using CliniMacs procedure
Cells cultured overnight

Day 2
Lentiviral vector added to CD34+ cells
First round of transduction

Day 3
Transduced cells prepared for infusion
Cells infused into patient following:
\- Gram-ve result
\- CD34 cell count
\- Viability result

Day 4
Repeat for 2\textsuperscript{nd} round of transduction (20-22 hours)

Day 5
Repeat for 3\textsuperscript{rd} round of transduction (6 hours)
Transduced cells prepared for infusion
Cells infused into patient following:
\- Gram -ve result
\- CD34 cell count
\- Viability result

Day 6-7
Bead removal

Day 8
CliniMacs selection of CD34+ (transduced) cells

Day 9
Gram -ve result
CD34 cell count
Viability result
Cryopreservation
Quality Control – sterility, gene marking, cell number, identification

Quality Control – tests on raw materials and finished product

BacAlert (infection)
qPCR result (successful gene transfer)
Flow cytometry (count, viability, identification)
## Conclusions

### Stem Cell Transplant vs Gene Therapy

<table>
<thead>
<tr>
<th>Stem Cell Transplant</th>
<th>Gene Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Repairs the immune system</td>
<td>• Repairs the immune system</td>
</tr>
<tr>
<td>• Requires a compatible donor</td>
<td>• Autologous cells</td>
</tr>
<tr>
<td>• Risk of GvHD</td>
<td>• No GvHD</td>
</tr>
<tr>
<td>• Imm. reconstitution takes longer (4-6 months)</td>
<td>• Imm. reconstitution is faster (2-4 months)</td>
</tr>
<tr>
<td>• Long term immune reconstitution</td>
<td>• Long term immune reconstitution</td>
</tr>
<tr>
<td></td>
<td>• Pitfalls: risk of insertional mutagenesis</td>
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</tbody>
</table>
Gene Therapy
Should we do it?
Aknowledgements

Principal Investigators
Adrian Thrasher
Bobby Gaspar
Waseem Qasim

Production Team
Nourreddine Himoudi
Karen Buckland
Hong Zhan
Catherine Irving

Patient Assays
Christine Rivat
Kimberly Gilmour

Regulation
Anne-Marie McNicol
Sue Swift

Pharmacy QA/QP
Olufemi Rabiu
Annette Hogg
Farzin Fazaneh
Peter Brown

Ricardo U. Sorensen, MD
Thank you