Viral vectors in clinical gene therapies

lecture 6

24th November 2014

Prof. Józef Dulak, PhD, DSc
Department of Medical Biotechnology
Faculty of Biochemistry, Biophysics and Biotechnology
Room 3.025/3.07
Phone 664-63-75
Email: jozef.dulak@uj.edu.pl
In vivo and ex vivo gene therapy

Kaufman et al., EMBO Mol Med. 5: 1642-1661; 2013
Severe combined immunodeficiency diseases
First controlled trial of gene therapy - 1990

ADA deficiency – results in severe immunodeficiency syndrome
Gene therapy of ADA deficiency

Blease et al., 1990
First clinical trial of gene therapy - 1990

Retroviral vector containing correct ADA gene (cDNA) has been transduced into blood lymphocytes

This first clinical trial was not „pure” from the methodological point of view.

The patients have been treated concomitantly with enzyme injections – ADA-PEG.

Nevertheless, the marker transgene (neo) could be detected in the blood cells of the patients even more than 5 years after injection of modified cells.

Ashanti De Silva (patient)

Blease et al., 1990
Hematopoietic Stem Cells

10^6 new blood cells are produced every second
Hematopoiesis and main diseases in which the hematopoietic stem cells can be used for therapy

KB Kaufman et al., EMBO Mol Med 2013
X-linked severe combined immunodeficiency (X-SCID)

Lack of correct γc cytokine receptor gene

David Vetter

The „buble” boy
Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease

Science 2000: 28 April: 288: 669-672
Gene therapy is efficient in treatment of X-SCID

Stem cells without correct γc gene

Gene therapy

Retroviral vector with a correct γc gene
Gene therapy has been beneficial to most treated SCID-X1 patients!!!

- they can now cope with environment microorganisms and have a normal life in the absence of any specific therapy

- no evidence for $\gamma_c$ transgene silencing has been observed

France – 10 boys treated, in 9 immune system corrected
UK – 10 boys treated, in 10 immune system corrected
Potential risk of application of retroviral vectors

- **gag** - structural proteins
- **pol** - reverse transcriptase
- **env** - envelope proteins

- long-term expression & integration into cellular genome

random integration - risk of insertional mutagenesis

Retroviral vector

- ITR
- gag pol env
- ITR

Transgen

- ITR
- retrovirus
- ITR

- Department of Medical Biotechnology
Integration of γ-retroviral vector into the promoter of LMO2 gene

Serious side effects of SCID-X1 gene therapy

- Development of uncontrolled clonal T lymphoproliferative syndrome, similar to acute lymphoblastic leukemia (ALL) in 4 out of 10 treated children in Paris and 1 boy treated in London

- Due to the integration of a vector into an LMO2 gene either close to the promoter or in the first intron

Reasons: 1. LMO-2 locus is one of sites for retroviral integration

2. Cells with aberrant expression of LMO-2 could have been selected because they provide a clonal growth advantage
Side effects of MMLV-based retroviral vectors prompted investigations of the mechanisms of integration and search for the new, safer vectors
Recent clinical trials targeting X-SCID

Table 2
Summary of recent clinical trials targeting SCID-X1. T-ALL, T-cell acute lymphoblastic leukaemia bone marrow; LMO2, LIM domain only 2; SAE, serious adverse event.

<table>
<thead>
<tr>
<th>Trial centre</th>
<th>Vector</th>
<th>Conditioning</th>
<th>Patients</th>
<th>Outcome</th>
<th>SAE</th>
<th>Insertion site(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>France</td>
<td>γ-Retrovirus (amphotropic)</td>
<td>None</td>
<td>9</td>
<td>Significant clinical benefit to most</td>
<td>4 developed T-ALL; 1 died</td>
<td>LMO2</td>
<td>Hacein-Bey-Abina et al. (2002)</td>
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<td></td>
<td></td>
<td>CCND2</td>
<td>Hacein-Bey-Abina et al. (2003)</td>
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<td></td>
<td>BMI1</td>
<td>Hacein-Bey-Abina et al. (2008)</td>
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<td>Hacein-Bey-Abina et al. (2010)</td>
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<td>Thrasher et al. (2005)</td>
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<td>Gaspar et al. (2011a)</td>
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<td>Howe et al. (2008)</td>
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<td>Thrasher et al. (2005)</td>
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<td>Chinen et al. (2007)</td>
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<td>Personal communication</td>
</tr>
<tr>
<td>UK</td>
<td>γ-Retrovirus (GALV)</td>
<td>None</td>
<td>1</td>
<td>No clinical benefit (age related)</td>
<td>1 developed T-ALL</td>
<td>LMO2</td>
<td>Thrasher et al. (2005)</td>
</tr>
<tr>
<td>USA</td>
<td>γ-Retrovirus (GALV)</td>
<td>None</td>
<td>3</td>
<td>Limited clinical benefit (age-related)</td>
<td>None</td>
<td></td>
<td>Howe et al. (2008)</td>
</tr>
<tr>
<td>France, UK, USA</td>
<td>EF1α promoter</td>
<td>None</td>
<td>8</td>
<td>T-cell recovery observed (preliminary results)</td>
<td>None</td>
<td></td>
<td>Personal communication</td>
</tr>
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<td></td>
<td>Thrasher et al. (2005)</td>
</tr>
</tbody>
</table>
Gene therapy of HSC

- Peripheral/BM HSC harvest
- In-vitro Gene transduction
- Infusion of gene transduced HSC
- Conditioning regimen *

* - To be used if the gene transduced cells fails to engraft
# - High dose chemotherapy such as Busulfan
Allogeneic HSCT from matched sibling donors constitutes the treatment of choice for ADA-SCID (~88% success), but survival is significantly reduced following transplants from matched unrelated (66%) or haploidentical donors (43%). For transplanted patients who survived, long-term cellular and humoral immune recovery was achieved.

Among 10 patients with ADA deficiency, restoration of immune functions has been achieved in 9.

No serious side effects (leukemias) have been observed.
Hematopoietic Stem Cell Gene Therapy for Adenosine Deaminase–Deficient Severe Combined Immunodeficiency Leads to Long-Term Immunological Recovery and Metabolic Correction

H. Bobby Gaspar,†,‡ Samantha Cooray,†,‡ Kimberly C. Gilmour,†,‡ Kathryn L. Parsley,†,‡ Fang Zhang,† Stuart Adams,§ Emma Bjorkegren,† Jinhua Bayford,†,‡ Lucinda Brown,†,‡ E. Graham Davies,†,‡ Paul Veys,§ Lynette Fairbanks,∥ Victoria Bordon,¶ Theoni Petropoulou,|| Christine Kinnon,† Adrian J. Thrasher†,‡

(Published 24 August 2011; revised 16 January 2013)

Genetic defects in the purine salvage enzyme adenosine deaminase (ADA) lead to severe combined immunodeficiency (SCID) with profound depletion of T, B, and natural killer cell lineages. Human leukocyte antigen–matched allogeneic hematopoietic stem cell transplantation (HSCT) offers a successful treatment option. However, individuals who lack a matched donor must receive mismatched transplants, which are associated with considerable morbidity and mortality. Enzyme replacement therapy (ERT) for ADA-SCID is available, but the associated suboptimal correction of immunological defects leaves patients susceptible to infection. Here, six children were treated with autologous CD34-positive hematopoietic bone marrow stem and progenitor cells transduced with a conventional gammaretroviral vector encoding the human ADA gene. All patients stopped ERT and received mild chemotherapy before infusion of gene-modified cells. All patients survived, with a median follow-up of 43 months (range, 24 to 84 months). Four of the six patients recovered immune function as a result of engraftment of gene-corrected cells. In two patients, treatment failed because of disease-specific and technical reasons: Both restarted ERT and remain well. Of the four reconstituted patients, three remained off enzyme replacement. Moreover, three of these four patients discontinued immunoglobulin replacement, and all showed effective metabolic detoxification. All patients remained free of infection, and two cleared problematic persistent cytomegalovirus infection. There were no adverse leukemic side effects. Thus, gene therapy for ADA-SCID is safe, with effective immunological and metabolic correction, and may offer a viable alternative to conventional unrelated donor HSCT.
Treatment options for ADA deficiency

1. Matched-donor bone marrow transplantation

2. Enzyme replacement therapy with pegylated bovine ADA

3. Autologous HSC therapy

- Since 2000, over 40 patients in Italy, the UK and the USA have been treated with retroviral vectors encoding ADA and low-intensity conditioning;
- All patients are alive, and in the majority of them, PEG-ADA is no longer required;
- An increase in lymphocyte number, improvement of immune response and metabolic detoxification were achieved;
- No leukemic or oncogenic events were revealed, even if integration into known oncogenes has been reported.

In UK and USA – phase I/II clinical trials with lentiviral vector have been recently approved
Gene therapy of primary T cell immunodeficiencies

Alain Fischer a,b,c,*, Salima Hacein-Bey-Abina a,b,d,e, Marina Cavazzana-Calvo a,b,d,e

a INSERM U768, Paris, France
b Paris Cité, Université Paris Descartes, Imagine Institute, Paris, France
c Immunology and Pediatric Hematology Department, Assistance Publique-Hôpitaux de Paris, Paris, France
d Biotherapy Department, Necker Children's Hospital, Assistance Publique-Hôpitaux de Paris, Paris, France
e Biotherapy Clinical Investigation Center, Groupe Hospitalier Universitaire Ouest, Assistance Publique-Hôpitaux de Paris, INSERM, Paris, France
Review

Gene therapy for PIDs: Progress, pitfalls and prospects

Sayandip Mukherjee\textsuperscript{a}, Adrian J. Thrasher\textsuperscript{a,b,*}

\textsuperscript{a} Centre for Immunodeficiency, Molecular Immunology Unit, University College London Institute of Child Health, 30 Guilford Street, London WC1N1EH, UK
\textsuperscript{b} Great Ormond Street Hospital for Children, National Health Service Trust, London WC1N1EH, UK
Hematopoiesis and main diseases in which the hematopoietic stem cells can be used for therapy
Chronic granulomatous disease (CGD)

- A rare inherited immunodeficiency characterized by recurrent, often life-threatening bacterial and fungal infections due to a functional defect in the microbial-killing activity of phagocytic neutrophils.
- A result of mutations in genes encoding a multicomponent enzyme complex, the NADPH oxidase, that catalyses the respiratory burst.
- The majority of patients (70%) have an X-linked form of the disease which is associated with mutations in a membrane-bound component gp91phox.
- HLA-matched allogeneic HSC transplantation can be curative, but for patients without suitable donors, genetic modification of autologous hematopoietic stem cells is an attractive alternative.
Chronic granulomatous disease

Gene Therapy for X-CGD

1. Myelosuppression
2. Reinfusion of genetically modified cells

G-CSF Mobilisation
Leukapheresis

Quality Control
Retroviral mediated Gene Transfer

Isolation of CD34+ Cells

Gene Transfer Vector
Blood Stem Cells (CD34+ cells)

gp91phox
Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1

Marion G Ott¹,¹⁶, Manfred Schmidt²⁻⁴,¹⁶, Kerstin Schwarzwaelder³⁻⁵,¹⁶, Stefan Stein⁶,¹⁶, Ulrich Siler⁷,¹⁶, Ulrike Koehl⁸, Hanno Glimm²,³, Klaus Kühlcke⁹, Andrea Schilz⁹, Hana Kunkel⁶, Sonja Naundorf⁹, Andrea Brinkmann⁸, Annette Deichmann³,⁴, Marlene Fischer²,³,⁵, Claudia Ball³⁻⁵, Ingo Pilz³,⁵, Cynthia Dunbar¹⁰, Yang Du¹¹, Nancy A Jenkins¹¹, Neal G Copeland¹¹, Ursula Lüthi¹², Moustapha Hassan¹³, Adrian J Thrasher¹⁴, Dieter Hoelzer¹, Christof von Kalle²⁻⁴,¹⁵,¹⁶, Reinhard Seger⁷,¹⁶ & Manuel Grez⁶,¹⁶

Gene transfer into hematopoietic stem cells has been used successfully for correcting lymphoid but not myeloid immunodeficiencies. Here we report on two adults who received gene therapy after nonmyeloablative bone marrow conditioning for the treatment of X-linked chronic granulomatous disease (X-CGD), a primary immunodeficiency caused by a defect in the oxidative antimicrobial activity of phagocytes resulting from mutations in gp91phox. We detected substantial gene transfer in both individuals' neutrophils that lead to a large number of functionally corrected phagocytes and notable clinical improvement. Large-scale retroviral integration site–distribution analysis showed activating insertions in MDS1-EVI1, PRDM16 or SETBP1 that had influenced regulation of long-term hematopoiesis by expanding gene-corrected myelopoiesis three- to four-fold in both individuals. Although insertional influences have probably reinforced the therapeutic efficacy in this trial, our results suggest that gene therapy in combination with bone marrow conditioning can be successfully used to treat inherited diseases affecting the myeloid compartment such as CGD.
Correction of neutrophil bacteriocidal function by overexpression of gp91phox subunit of NADPH oxidase

Figure 1 Gene-corrected neutrophils in the blood of an individual with CGD after HSPC gene therapy. Whereas CGD neutrophils lack NADPH activity (green cytosol), the progeny of HSPCs engrafted after gene therapy have reconstituted enzyme activity (blue cytosol) resulting from the presence of a functional gene replaced by a retroviral vector. Gene-corrected cells differ for the site of vector insertion (nucleus in different color), reflecting the origin from distinct progenitors. Early after gene therapy, many progenitors contribute to the neutrophil pool but become exhausted with time. At later times, gene-corrected cells from a unique progenitor progressively expand until accounting for the majority of circulating cells. In these cells, the vector integrated near a growth-promoting gene which is activated by insertional mutagenesis and confers a growth advantage. Total neutrophil number does not increase with time, suggesting that the expanding cells are subjected to normal control. Because the clinical benefit is dependent on the number of circulating gene-corrected cells (gray line), the expansion enables efficacious and sustained correction of the disease.

Side effects as a result of gene therapy for SCIDs

Table 1 Patients developing insertional mutagenesis/uncontrolled clonal expansions

<table>
<thead>
<tr>
<th>Study</th>
<th>Vector</th>
<th>Complication</th>
<th>Insertion sites</th>
<th>Additional chromosomal abnormalities</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCID-X1 (P4, Paris)</td>
<td>MFG-Ampho</td>
<td>T-ALL, mature T cell, T-ALL</td>
<td>LMO2</td>
<td>CDKN2A del</td>
<td>Died</td>
</tr>
<tr>
<td>SCID-X1 (P5, Paris)</td>
<td>MFG-Ampho</td>
<td>Late cortical T cell, T-ALL</td>
<td>LMO2</td>
<td>Notch mutation</td>
<td>CR</td>
</tr>
<tr>
<td>SCID-X1 (P7, Paris)</td>
<td>MFG-Ampho</td>
<td>T-ALL, late cortical T cell, T-ALL</td>
<td>CCND2</td>
<td>CDKN2A del</td>
<td>CR</td>
</tr>
<tr>
<td>SCID-X1 (P10, Paris)</td>
<td>MFG-Ampho</td>
<td>T-ALL, late cortical T cell, T-ALL</td>
<td>LMO2</td>
<td>Notch mutation</td>
<td>CR</td>
</tr>
<tr>
<td>SCID-X1 (P8, London)</td>
<td>MFG-GALV</td>
<td>T-ALL</td>
<td>BMI1</td>
<td>CDKN2A deletion</td>
<td>CR</td>
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<td></td>
<td></td>
<td></td>
<td>LMO2</td>
<td>Notch 1 mutation</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CDKN2A deletion</td>
<td></td>
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<tr>
<td>CGD (Frankfurt)</td>
<td>SFFV-GALV</td>
<td>Clonal myeloid expansions,</td>
<td>MDS1-EVI1</td>
<td>Monosomy-7</td>
<td>Died</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myelodysplasia</td>
<td>PRDM16</td>
<td>Died sepsis</td>
<td></td>
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<td></td>
<td>SETBP1</td>
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<td></td>
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<td>MDS1-EVI1</td>
<td>Monosomy-7</td>
<td>Transgene silencing</td>
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<td></td>
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<td>PRDM16</td>
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</tbody>
</table>

Abbreviations: ALL, acute lymphocytic leukaemia; CDKN2A, cyclin-dependent kinase inhibitor 2A; CGD, chronic granulomatous disease; CR, complete remission; Evi1, ecotropic viral integration site 1; GALV, Gibbon Ape leukaemia virus; HSCT, haematopoietic stem cell transplantation; LMO2, LIM domain only 2; MDS, myelodysplasia syndrome 1; MUD-HSCT, matched unrelated donor haematopoietic stem cell transplantation; PRDM16, PR domain containing 16; SCID, severe combined immunodeficiency; SETBP1, SET binding protein 1; SFFV, Spleen focus forming virus; TCR, T-cell receptor.

Final outcome – treatment of disease was effective, but transient

Quasim et al., Gene Therapy 2009
Table 3
Summary of recent clinical trials targeting CGD. SAE, serious adverse event.

<table>
<thead>
<tr>
<th>Trial centre</th>
<th>Vector</th>
<th>Conditioning</th>
<th>Patients</th>
<th>Outcome</th>
<th>SAE</th>
<th>Insertion site(s)</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>US</td>
<td>γ-Retroviral</td>
<td>None</td>
<td>5</td>
<td>No clinical benefit</td>
<td>None</td>
<td>–</td>
<td>Malech et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>(amphotropic)</td>
<td>None, Busulfan (10 mg/kg)</td>
<td>5</td>
<td>No clinical benefit</td>
<td>None</td>
<td>–</td>
<td>Malech (2000)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>Transient clinical benefit</td>
<td>None</td>
<td>–</td>
<td>Goebel and Dinauer (2003)</td>
</tr>
<tr>
<td>Germany</td>
<td>γ-Retroviral (SFFV LTR)</td>
<td>Busulfan (8.8 mg/kg)</td>
<td>2</td>
<td>Long term clinical benefit</td>
<td>Both developed MDS with monosomy 7; 1 died from sepsis</td>
<td>MDS EVII</td>
<td>Kang et al. (2010)</td>
</tr>
<tr>
<td>Switzerland</td>
<td></td>
<td></td>
<td>2</td>
<td>Transient clinical benefit</td>
<td>1 patient developed MDS</td>
<td>None</td>
<td>Ott et al. (2006)</td>
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<td>Bianchi et al. (2009)</td>
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<td>Bianchi et al. (2011)</td>
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<tr>
<td>UK</td>
<td>γ-Retroviral (MLV LTR)</td>
<td>Melphalan (140 mg/m²)</td>
<td>1</td>
<td>Transient clinical benefit</td>
<td>None</td>
<td>–</td>
<td>Personal communication</td>
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<td></td>
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<td></td>
<td>Thrasher AJ</td>
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<tr>
<td>Korea</td>
<td>γ-Retroviral</td>
<td>Busulfan (6.4 mg/kg) + Fludarabine (120 mg/m²)</td>
<td>3</td>
<td>Transient clinical benefit</td>
<td>None</td>
<td>–</td>
<td>Kang et al. (2011)</td>
</tr>
<tr>
<td>Switzerland, Germany, France, UK</td>
<td>SIN lentivector, myeloid promoter</td>
<td>Busulfan (12-16 mg/kg)</td>
<td>2</td>
<td>Trial open</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Retrovirus gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils.

Laboratory of Host Defenses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA. ekang@niaid.nih.gov

Abstract

Chronic granulomatous disease (CGD) is associated with significant morbidity and mortality from infection. The first CGD gene therapy trial resulted in only short-term marking of 0.01% to 0.1% of neutrophils. A recent study, using busulfan conditioning and an SFFV retrovirus vector, achieved more than 20% marking in 2 patients with X-linked CGD. However, oxidase correction per marked neutrophil was less than normal and not sustained. Despite this, patients clearly benefited in that severe infections resolved. As such, we initiated a gene therapy trial for X-CGD to treat severe infections unresponsive to conventional therapy. We treated 3 adult patients using busulfan conditioning and an MFGS retroviral vector encoding gp91(phox), achieving early marking of 26%, 5%, and 4% of neutrophils, respectively, with sustained long-term marking of 1.1% and 0.03% of neutrophils in 2 of the patients. Gene-marked neutrophils have sustained full correction of oxidase activity for 34 and 11 months, respectively, with full or partial resolution of infection in those 2 patients. Gene marking is polyclonal with no clonal dominance. We conclude that busulfan conditioning together with an MFGS vector is capable of achieving long-term correction of neutrophil oxidase function sufficient to provide benefit in management of severe infection. This study was registered at www.clinicaltrials.gov as #NCT00394316.
Wiskott-Aldrich syndrome (WAS)

- an X-linked, complex primary immunodeficiency disorder caused by mutations in the WAS gene;
- characterized by recurrent infections, thrombocytopenia, eczema, autoimmunity, and an increased risk of lymphoma;
- The WAS protein (WASP) is a key regulator of actin polymerization in HSCs;
- The complex biology of this disease results from dysfunction in different leukocyte subsets, including defective T and B cell function, disturbed formation of the natural killer (NK) cell immunological synapse, and impaired migratory responses of all leukocyte subsets;
- Severe WAS leads to early death because of infections, hemorrhage, or malignancy;
- For these patients, the standard curative therapy consists of allogeneic hematopoietic stem cell transplantation (HSCT).
- Although effective, allogeneic HSCT is associated with considerable morbidity and mortality, in particular if no human leukocyte antigen (HLA)-matched HSC donor is available.
CD34+ HSC were transduced with WASP-expressing retroviral vectors that were created with backbone vector CMMP and pseudotyped with gibbon ape leukemia virus (GALV) envelope protein; the cells were then reinfused 4 days later.

Before reinfusion of CD34+ cells, busulfan was administered at a dose of 4 mg per kilogram of body weight per day on days 3 and 2 before the procedure.

\[ \gamma \text{-retroviral gene therapy} \]
Wiskott-Aldrich syndrome gene therapy

- HSC gene therapy trial using a $\gamma$-retroviral vector, 9 of 10 patients showed sustained engraftment and correction of WAS protein (WASP) expression in lymphoid and myeloid cells and platelets.
- GT resulted in partial or complete resolution of immunodeficiency, autoimmunity, and bleeding diathesis.
- Analysis of retroviral insertion sites revealed >140,000 unambiguous integration sites and a polyclonal pattern of hematopoiesis in all patients early after GT.
- Seven patients developed acute leukemia [one acute myeloid leukemia (AML), four T cell acute lymphoblastic leukemia (T-ALL), and two primary T-ALL with secondary AML associated with a dominant clone with vector integration at the LMO2 (six T-ALL), MDS1 (two AML), or MN1 (one AML) locus]. Disease occurred between 16 months and 5 years after GT.
- Cytogenetic analysis revealed additional genetic alterations such as chromosomal translocations.
- This study shows that hematopoietic stem cell GT for WAS is feasible and effective, but the use of $\gamma$-retroviral vectors is associated with a substantial risk of leukemogenesis.

Table 1. Clinical characteristics and treatment modalities of the study patients. AIHA, autoimmune hemolytic anemia; BCG, Bacille Calmette-Guérin.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at GT (years)</th>
<th>WAS mutation</th>
<th>Clinical status before GT</th>
<th>Infused CD34+ cells/kg (×10^6)</th>
<th>Vector copies per cell</th>
<th>Follow-up month</th>
<th>Clinical status after GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>IVS6+1 G&gt;T</td>
<td>AIHA, hemorrhagia, eczema, disseminated BCG infection, mollusca contagiosa, panniculitis</td>
<td>18.6</td>
<td>2.4</td>
<td>81</td>
<td>Sequela of pneumococcal meningitis, T-ALL (day 1813), AML during maintenance therapy, allogeneic HSCT</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>Arg86His</td>
<td>AIHA, hemorrhagia, colitis, severe eczema, vasculitis, bacterial sepsis</td>
<td>13.5</td>
<td>2.7</td>
<td>80</td>
<td>Well</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Glu133Lys</td>
<td>AIHA, hemorrhagia, colitis, severe eczema, cellulitis, gastrointestinal infections</td>
<td>2.9</td>
<td>3.0</td>
<td>15</td>
<td>Well, status after haplo-identical HSCT</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Arg34X</td>
<td>Hemorrhagia, eczema, gluteal abscess</td>
<td>24.9</td>
<td>3.2</td>
<td>53</td>
<td>Well</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>Glu31Lys</td>
<td>Hemorrhagia, colitis, eczema, otitis, bacterial arthitis, pulmonary infections</td>
<td>17.9</td>
<td>5.2</td>
<td>44</td>
<td>T-ALL (day 1073); relapse shortly after allogeneic HSCT and death</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>IVS8+1_4 delGAGT</td>
<td>Hemorrhagia, eczema</td>
<td>20.6</td>
<td>3.0</td>
<td>25</td>
<td>T-ALL (day 488), now well and in remission after allogeneic HSCT</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>IVS3+1 G&gt;T</td>
<td>AIHA, hemorrhagia, colitis, eczema, growth retardation</td>
<td>13.5</td>
<td>2.7</td>
<td>46</td>
<td>Weak colitis responsive to meselazine; T-ALL (day 1105), in remission after allogeneic HSCT</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>Ala134Thr</td>
<td>Hemorrhagia, colitis, eczema, pneumonia</td>
<td>20.9</td>
<td>1.7</td>
<td>44</td>
<td>T-ALL (day 792), AML during maintenance therapy, haploidentical HSCT, pulmonary insufficiency and hemorrhage, death</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>Val303fsX4</td>
<td>Hemorrhagia colitis, eczema, vasculitis, pneumonia, lymphadenitis, arthrits</td>
<td>21.1</td>
<td>2.3</td>
<td>43</td>
<td>AML (day 1163), in remission after allogeneic HSCT</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>His30del</td>
<td>Hemorrhagia, colitis, eczema, vasculitis, cellulitis, osteomyelitis, otitis, sepsis</td>
<td>9.7</td>
<td>3.5</td>
<td>43</td>
<td>Recurring small skin infections and weaker vasculitis compared to status before GT until 2012; T-ALL (day 1364), currently in remission and undergoing allogeneic HSCT</td>
</tr>
</tbody>
</table>
Wiskott-Aldrich syndrome **lentiviral** gene therapy

I. Verma, Science 23 August 2013

A. Aiuti et al., , Science 23 August 2013
### Wiskott-Aldrich syndrome **lentiviral** gene therapy

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infectious manifestations</strong></td>
<td>Recurrent ENT</td>
<td>Pneumonias, colitis, arthritis(cellulitis, URTI, UTI</td>
</tr>
<tr>
<td><strong>Pathogens</strong></td>
<td>VZV, CMV, HSV, EBV</td>
<td>CMV, HHV-6, candida</td>
</tr>
<tr>
<td><strong>Thrombocytopenia manifestations</strong></td>
<td>Skin petechiae</td>
<td>Skin petechiae, GI bleeding</td>
</tr>
<tr>
<td><strong>Eczema</strong></td>
<td>Moderate-severe</td>
<td>Moderate-severe</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>Developmental disorder, allergy</td>
<td>Failure to thrive, elevated inflammatory indexes/vasculitis, hepatosplenomegaly</td>
</tr>
<tr>
<td><strong>WAS mutation</strong></td>
<td>Exon 10: C&gt;T 995 (R321X)</td>
<td>IVS10del11nt</td>
</tr>
<tr>
<td><strong>WASP expression</strong></td>
<td>&lt;5%</td>
<td>&lt;5%</td>
</tr>
<tr>
<td><strong>Zhu score</strong></td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>Age at treatment (years)</strong></td>
<td>5.9</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Infused CD34+ cells (×10^6/kg)</strong></td>
<td>3.66 (BM) + 5.25 (MPB)</td>
<td>14.1</td>
</tr>
<tr>
<td><strong>Vector copies/genome</strong></td>
<td>1.9 (BM) – 1.4 (MPB)</td>
<td>2.4</td>
</tr>
<tr>
<td><strong>Transduction efficiency (CFC)</strong></td>
<td>92% (BM) – 88% (MPB)</td>
<td>97%</td>
</tr>
<tr>
<td><strong>Follow-up (months)</strong></td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td><strong>Current clinical conditions</strong></td>
<td>A&amp;W, no eczema, no major bleeding or petechiae, off IVIG</td>
<td>A&amp;W, no eczema, no major bleeding or petechiae</td>
</tr>
</tbody>
</table>

A. Aiuti et al., *Science* 23 August 2013
In contrast to γ-retroviral gene therapy, LV-based GT did not induce in vivo selection of clones carrying integrations near oncogenes;

- No clonal expansions in the patients for up to 20-32 months after gene therapy

**Lentiviral vector gene therapy offers safety advantages over γ-retroviral, but a longer follow-up time is needed for validation**
Concerns on SIN-lentiviral vectors

- May upregulate the expression of genes flanking the integration site when carrying strong enhancer/promoter sequences in internal positions;

- Induce aberrant splicing and/or premature termination of endogenous transcripts of the targeted gene;

- Cause loss-of-function or gain-of-function mutations;

- Thus, large number of transduced Cells may increase the risk of insertional mutagenesis or tumor onset;

- Certain HIV-1 integrations might contribute to the expansion and persistence of transduced cells.

AV Sauer et al., Curr Opinion Allergy Clin Immunol, December 2014
Past, present and future of gene therapy in primary immunodeficiencies

AV Sauer et al., Curr Opinion Allergy Clin Immunol, December 2014
Clinical trials of leukodystrophies - lentiviral vectors

Leukodystrophies (LDs) - a group of inherited diseases in which molecular abnormalities of glial cells are responsible for exclusive or predominant defects in myelin formation and/or maintenance within the central and, sometimes, the peripheral nervous system
Adrenoleukodystrophy (ALD)

This X-linked recessive disease, with an estimated frequency of 1/20,000 men, presents in a variety of phenotypes [24].

In the most severe late infantile or juvenile cerebral form, which has a mean age of onset of about 7 years and constitutes 40 to 50% of the cases, neurological symptoms predominate.

Initial behavioral and school problems are followed by gait disturbances, visual and hearing impairment, varying alterations of cognitive functions with progressive dementia and a devastating downhill course toward an apparent vegetative state in 3 to 5 years.

People with ALD accumulate high levels of saturated, very long chain fatty acids in their brain and adrenal cortex because the fatty acids are not broken down by an enzyme in the normal manner.

The biochemical pathogenesis that leads to the massive demyelination

The gene responsible for X-linked ALD has been cloned and shown to be an ABC transporter protein. To date, the substrate transported by the ALD protein and the relationship between its transport function and VLCFA-CoA synthase activation are unknown.
Treatment of adrenoleukodystrophy

X-linked adrenoleukodystrophy – accumulation of large amounts of very long chain saturated fatty acids – leads to demyelination and early death

1. Bone marrow transplantation
2. Lorenzo’s oil

4:1 mixture of glyceryl trioleate and glyceryl trierucate (so called: Lorenz’s oil) in combination with a diet low in VLCSFA (very long chain saturated fatty acids), have been used with limited success, especially before disease symptoms appear

1984 r. Michaela & Augusto Odone (in movie played by Susan Sarandon & Nick Nolte)
Hematopoiesis and main diseases in which the hematopoietic stem cells can be used for therapy
Gene therapy of adrenoleukodystrophy

L. Naldini, Science 2009
Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy

Nathalie Cartier, 1,2* Salima Hacein-Bey-Abina, 3,4,5* Cynthia C. Bartholomae, 6 Gabor Veres, 7 Manfred Schmidt, 6 Ina Kutschera, 6 Michel Vidaud, 1 Ulrich Abel, 6 Liliane Dal-Cortivo, 3,5 Laure Caccavelli, 3,5 Nizar Ma Hannaoui, 8 Véronique Kiermer, 9 Denice Mittelstaedt, 10 Céline Bellesme, 2 Najiba Lahlou, 11 François Lefrère, 3 Stéphane Blanche, 8 Muriel Audit, 12 Emmanuel Payen, 13,14 Philippe Leboulch, 13,14,15 Bruno l’Homme, 13 Pierre Bougnères, 2 Christof Von Kalle, 6 Alain Fischer, 4,8 Marina Cavazzana-Calvo, 3,4,5* Patrick Aubourg 1,2*†

X-linked adrenoleukodystrophy (ALD) is a severe brain demyelinating disease in boys that is caused by a deficiency in ALD protein, an adenosine triphosphate–binding cassette transporter encoded by the ABCD1 gene. ALD progression can be halted by allogeneic hematopoietic cell transplantation (HCT). We initiated a gene therapy trial in two ALD patients for whom there were no matched donors. Autologous CD34+ cells were removed from the patients, genetically corrected ex vivo with a lentiviral vector encoding wild-type ABCD1, and then re-infused into the patients after they had received myeloablative treatment. Over a span of 24 to 30 months of follow-up, we detected polyclonal reconstitution, with 9 to 14% of granulocytes, monocytes, and T and B lymphocytes expressing the ALD protein. These results strongly suggest that hematopoietic stem cells were transduced in the patients. Beginning 14 to 16 months after infusion of the genetically corrected cells, progressive cerebral demyelination in the two patients stopped, a clinical outcome comparable to that achieved by allogeneic HCT. Thus, lentiviral-mediated gene therapy of hematopoietic stem cells can provide clinical benefits in ALD.
Gene therapy of adrenoleukodystrophy

Brain MRI

N. Cartier et al., Science, 6th November 2009
MLD – neurodegenerative lysosomal storage disease
- caused by arylsulfatase A deficiency; enzyme substrate sulfatide accumulates & causes widespread demyelination and neurodegeneration;
- primarily affects children and invariably leads to premature death

I. Verma, Science 23 August 2013

A. Aiuti et al., Science 23 August 2013
Gene therapy prevents progression of metachromatic leukodystrophy

HSC gene therapy can prevent progression of metachromatic leukodystrophy. Magnetic resonance (MR) images of the brain of a patient (MLD01) before and after gene therapy. The brain of this patient appeared largely normal 2 years after treatment. In contrast, the brain of an untreated, age-matched late infantile MLD patient (UT LI MLD) showed severe demyelination associated with diffuse atrophy. (Top) Axial T2 weighted fast spin-echo MR images. (Bottom) Fluid-attenuated inversion recovery (FLAIR) MR images.

A. Biffi et al., Science 23 August 2013;
1. Autosomal inherited blood disorder

2. Patients have defects in either alpha or beta globin chain gene (unlike sickle-cell disease) – abnormal red blood cells are produced

3. Therapy for thalassaemia primarily involves chelation or removal of excessive iron from the blood
   - patients with severe thalassaemia require blood transfusion
   - bone marrow transplant (BMT) from compatible donor (sibling’s)
   - BMT from haploidentical mother to child
Treatment of beta-thalassemia

Figure 1 | Gene-therapy procedure. a, Cavazzana-Calvo et al.² collected haematopoietic stem cells (HSCs) from the bone marrow of a patient with β-thalassaemia and maintained them in culture. b, The authors then introduced lentiviral-vector particles containing a functional β-globin gene into the cells and allowed them to expand further in culture. c, To eradicate the patient’s remaining HSCs and make room for the genetically modified cells, the patient underwent chemotherapy. d, The genetically modified HSCs were then transplanted into the patient.
Transfusion independence and \textit{HMGA2} activation after gene therapy of human \(\beta\)-thalassaemia

Marina Cavazzana-Calvo\textsuperscript{1,2a}, Emmanuel Payen\textsuperscript{3,4,5a}, Olivier Negre\textsuperscript{3,4,5a}, Gary Wang\textsuperscript{2}, Kathleen Hehir\textsuperscript{8}, Floriane Fusi\textsuperscript{4,5}, Julian Down\textsuperscript{8}, Maria Denaro\textsuperscript{8}, Troy Brady\textsuperscript{7}, Karen Westerman\textsuperscript{8,9}, Resy Cavalcreso\textsuperscript{9}, Beatrix Gillet-Legrand\textsuperscript{4}, Laure Caccavelli\textsuperscript{1,2}, Riccardo Sgara\textsuperscript{10}, Leila Maouche-Chrétien\textsuperscript{5,9}, Françoise Bernaudin\textsuperscript{11}, Robert Girot\textsuperscript{12}, Ronald Dorazio\textsuperscript{8}, Geert-Jan Mulder\textsuperscript{6}, Axel Polack\textsuperscript{8}, Arthur Bank\textsuperscript{13}, Jean Soulier\textsuperscript{2}, Jérôme Larghero\textsuperscript{2}, Noël Kabbara\textsuperscript{2}, Bruno Dallé\textsuperscript{6}, Bernard Gourmelon\textsuperscript{9}, Gérard Socie\textsuperscript{9}, Stany Chrétien\textsuperscript{5,9}, Nathalie Cartier\textsuperscript{14}, Marie Ligozat\textsuperscript{5,9}, Eric Galacteros\textsuperscript{10}, Yves Beuzard\textsuperscript{13,15}, Eliane d’Elbeuf\textsuperscript{2}, and Jean-Paul Leboulch\textsuperscript{5,9,16}.

The \(\beta\)-haemoglobinopathies are the most prevalent inherited disorders worldwide. Gene therapy of \(\beta\)-thalassaemia is particularly challenging given the requirement for massive haemoglobin production in a lineage-specific manner and the lack of selective advantage for corrected haematopoietic stem cells. Compound \(\beta^E/\beta^0\)-thalassaemia is the most common form of severe thalassaemia in southeast Asian countries and their diaspora\textsuperscript{1,2}. The \(\beta^E\)-globin allele bears a point mutation that causes alternative splicing. The abnormally spliced form is non-coding, whereas the correctly spliced messenger RNA expresses a mutated \(\beta^E\)-globin with partial instability\textsuperscript{1,2}. When this is compounded with a non-functional \(\beta^0\)-allele, a profound decrease in \(\beta\)-globin synthesis results, and approximately half of \(\beta^E/\beta^0\)-thalassaemia patients are transfusion-dependent\textsuperscript{1,2}. The only available curative therapy is allogeneic haematopoietic stem cell transplantation, although most patients do not have a human-leukocyte-antigen-matched, geno-identical donor, and those who do still risk rejection or graft-versus-host disease. Here we show that, 33 months after lentiviral \(\beta\)-globin gene transfer, an adult patient with severe \(\beta^E/\beta^0\)-thalassaemia dependent on monthly transfusions since early childhood has become transfusion independent for the past 21 months. Blood haemoglobin is maintained between 9 and 10 g dl\textsuperscript{−1}, of which one-third contains vector-encoded \(\beta\)-globin. Most of the therapeutic benefit results from a dominant, myeloid-biased cell clone, in which the integrated vector causes transcriptional activation of \textit{HMGA2} in erythroid cells with further increased expression of a truncated \textit{HMGA2} mRNA insensitive to degradation by let-7 microRNAs. The clonal dominance that accompanies therapeutic efficacy may be coincidental and stochastic or result from a hitherto benign cell expansion caused by dysregulation of the \textit{HMGA2} gene in stem/progenitor cells.

In 2007, Cavazzana-Calvo and colleagues\textsuperscript{2} treated an 18-year-old male patient who had HbE/\(\beta\)-thalassaemia — a form of the disorder in which haemoglobin production is severely compromised. They treated the patient’s HSCs with an HIV-derived lentiviral vector containing a functional \(\beta\)-globin gene (Fig. 1). In a bold move, the investigators gave the patient a high dose of chemotherapy before administering his genetically modified HSCs. Their aim was to eliminate most, if not all, of the diseased HSCs in the patient’s body. This severe degree of pre-transplant ‘conditioning’ seems to have been crucial for the success of the treatment. Had the conditioning been less intense, the genetically corrected HSCs might have become diluted by residual host HSCs, possibly compromising the outcome.

Blood cells: the levels of genetically modified cells rose from less than 2% in the first few months to 11% at 33 months post-transplant. Concomitantly, levels of the normal \(\beta\)-globin protein increased, with 10–20% of reconstituted HSCs containing the transferred globin gene; this resulted in the improved production and quality of red blood cells. Remarkably, a year after the treatment, the patient no longer needed blood transfusions. Although, three years on, he remains mildly anaemic and shows signs of compensatory expansion of red-blood-cell precursors in his bone marrow, absence of the need for blood transfusions means that this case can be viewed as a clinical success.
HSC gene therapy timeline

Figure 3. HSC gene therapy timeline. History of gene therapy and the milestones that contributed to the implementation of gene therapy for monogenic disorders using haematopoietic cells (adapted from Appelbaum, 2007; Wirth et al, 2013). Milestones in HSCT are highlighted in light blue whereas major contributions in the field of gene transfer are coloured violet. Although no haematological disorder can be treated with Glybera (dark blue), its market approval is a milestone for the entire field of gene therapy. BMT, bone marrow transplantation; disease abbreviations as in the text.

KB Kaufman et al., EMBO Mol Med 2013
Gene therapy on the move

Kerstin B. Kaufmann¹, Hildegard Büning², Anne Galy³, Axel Schambach⁴,⁵, Manuel Grez¹*

Keywords: clinical trials; iPS; monogenic disorders; stem cell therapy; viral vectors

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The other successful application of gene therapy for monogenic diseases

Next week...