Ten years of gene therapy

2000-2010

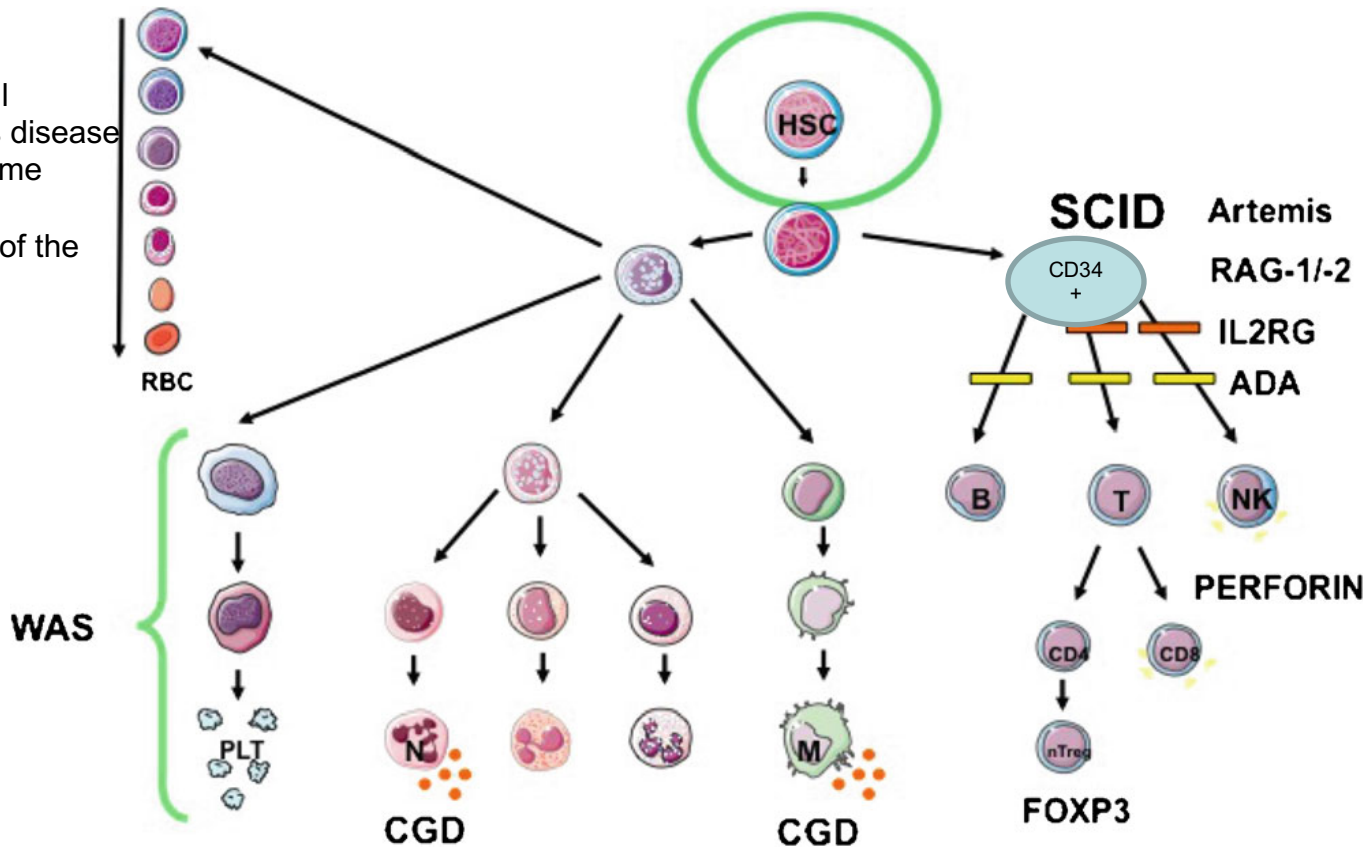
PID
primary immune deficiency

- Primary immunodeficiencies (PID) are rare genetic disorders of the innate and adaptive immune system.
- Among PID, severe combined immunodeficiencies (SCID) have poor prognosis.
- Over 120 different gene mutations have been identified which cause PID
- Transplant of hematopoietic stem cells (HSC) from an HLA-identical sibling donor is the treatment of choice for (SCID) and other types of PID with poor prognosis
- Gene therapy could represent a valid alternative to HSC transplantation when the genetic mutation of the patient has been identified.

PIDs-causing defects

HSC, hematopoietic stem cell
CGD, chronic granulomatous disease
WAS, Wiskott-Aldrich syndrome

IL2RG, encodes for γc chain of the IL2 receptor
ADA, encodes for Adenine DeAminase



Genetic defects in HSC progeny could cause block of development or functional failure at different levels of immune system. **Deficiencies affecting the common lymphoid progenitors** generate SCIDs (**Artemis, RAG-1/-2, IL2RG, ADA)** characterized by the **absence or the non-functional presence of B, T and NK populations.** If **FOXP3 gene is mutated, nTreg cells are lacking their suppressive phenotype**. In **CGD, neutrophils cannot produce oxidative burst to kill phagocytosed pathogens**. **WAS** protein deficiency impairs several immune cell functions, but **most importantly platelets**.

List of diseases, OMIM catalogue numbers and HUGO-approved gene symbols

– WAS (Wiskott-Aldrich syndrome), OMIM #301000, WAS gene
– CGD (X-linked Chronic Granulomatous Disease), OMIM #306400, CYBB gene
– ADA-SCID (Adenosine Deaminase deficiency), OMIM #102700, ADA gene
– Artemis deficiency, OMIM #602450, DCLRE1C gene
– RAG1/RAG2 (Recombination Activating Genes 1 and 2) deficiency, OMIM #601457, RAG1 and RAG2 genes
– SCIDX1 (X-linked Severe Combined Immunodeficiency or **γchain deficiency**), OMIM #300400, IL2RG gene
– XLP1 (X-linked lymphoproliferative disease type 1), OMIM #308240, SH2D1A gene
– XLP2 (X-linked lymphoproliferative disease type 2), OMIM #300635, XIAP gene
– IPEX (Immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome, OMIM #304790, FOXP3 gene

SCID, severe combined immunodeficiencies

11 different phenotupes and mutations in 10 genes

Rear diseases:1:50000-1:100000 nati vivi

Frequenza relativa rispetto a tutte le SCID

TABLE 1 Classification of severe combined immunodeficiencies[a]

Disease	Relative frequency[b] %		Affected cells	Gene product
Reticular dysgenesis	4	AR	T, B, NK, myeloid cells, platelets	Unknown
Alymphocytosis	15	AR	T, B	RAG-1, RAG-2
	15	AR	T, B	Artemis
X linked-SCID Absence of T lymphocytes and NK	40	X-L	T, NK	γc chain
	10	AR	T, NK	JAK-3
Absence of T lymphocytes	5	AR	T	IL-7 Rα chain
	$\leq$1	AR	T	CD45
	$\leq$1	AR	T	CD3δ
ADA deficiency	10–15	AR	T, B, NK	ADA

the γc chain is shared by several receptors including IL2, IL4,IL7,IL9 IL15

[a] Abbreviations: ADA, adenosine deaminase; AR, autosomal recessive; NK, natural killer cells; X-L, X-linked recessive.

[b] Frequency in our experience.

Toward SCID gene therapy

Gene therapy has several potential advantages over allogeneic transplant

- rapid access to treatment (allogenic stem cells)
- lack of graft-versus-host disease

Gene transfer approaches based on integrating vectors
possibly risk of insertional mutagenesis, which is dependent on the type of vector employed, the disease background, and the nature of the transgene

Milestones toward SCID gene therapy

HSC, human stem cells

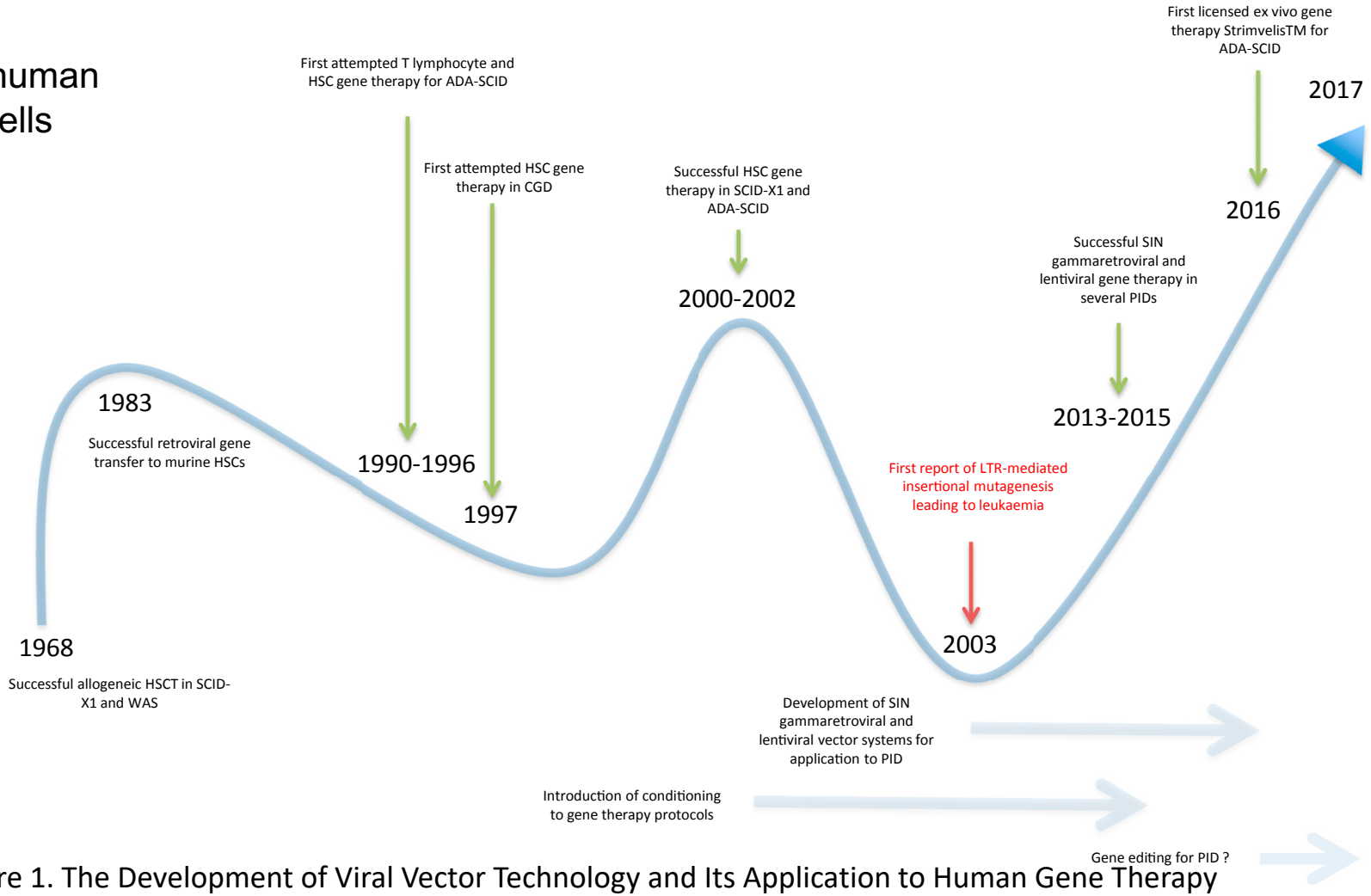


Figure 1. The Development of Viral Vector Technology and Its Application to Human Gene Therapy
The blue time line shows the various ups and downs of the field based on successes and setbacks.

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Gene therapy in SCIDs

Following the advent of the retroviral vector technology, clinical trials were successfully initiated for SCID-X1 (γc deficiency) in 1999 and then adenosine deaminase (ADA) deficiency.

To date, gene therapy results are available for:

- 35 patients with typical X-linked SCIDs
- five patients with atypical SCIDs
- 38 patients with ADA deficiency

(Aiuti et al., 2002, 2009; Candotti et al., 2012; Cavazzana-Calvo et al., 2000; Gaspar et al., 2011a, 2011b; Hacein-Bey-Abina et al., 2010)

ADA-SCID

The genetic defect

ADA- Adenine Deaminase dysfunction. ADA enzyme is responsible for the deamination of adenosine and deoxyadenosine in the purine salvage pathway. When metabolites accumulate, they become particularly toxic in the bone marrow, thymus and lymph nodes.

The clinic defects

ADA-deficiency is a SCID variant characterized by

- Impaired T, B, and NK cell development and functions
- Recurrent infections

non-immunological abnormalities have been described in several organs as result of the accumulation of purine toxic metabolites (hepatic, skeletal, neurologic, behavioral alterations and sensorineural deafness).

ADA-SCID (adenosine deaminase deficiency) affects 1:200,000 to 1:1,000,000 live births.

ADA therapies

Allogeneic transplant

Allogeneic transplant from mismatched related donors is affected by a higher morbidity and mortality as compared with other SCID variants

pegylated bovine ADA, PEG-ADA

ADA-SCID children who lack a compatible donor are often treated with enzyme replacement therapy (pegylated bovine ADA, PEG-ADA).
PEGADA results in clinical improvement and metabolic correction, but the immunological reconstitution is often incomplete and lifelong treatment is very expensive.

the patients

GENE THERAPY SUCCESSES AND FAILURES

SCID is often called "bubble boy disease". SCID became widely known during the 1970's and 80's, when the world learned of David Vetter, a boy with X-linked SCID, who lived for 12 years in a plastic, germ-free bubble. He died after a bone marrow transplant.

A recessive disorder of a mutation in the adenosine deaminase (ADA) gene causes SCID. Gene therapy successfully replaced this gene in several ADA patients

Culver, Anderson, and Blaese with gene therapy patients

Ashi DeSilva was born with ADA deficiency. In 1990 at the age of 4, she was the first successful gene therapy recipient

Negli anni 80 due ricercatori WF Anderson e RM Blaese unirono le loro competenze a quelle di due oncologi, S. Rosenberg e M Lotze ed insieme pensarono di

- Trattare una patologia ereditaria del sistema immunitario
- Con vettori virali

INITIAL TRIALS FOR ADA GENE THERAPY

Blaese RM, Culver KW, Miller AD, et al. T lymphocyte-directed gene therapy for ADA- SCID: initial trial results after 4 years. Science. 1995;270:475-480.

Bordignon C, Notarangelo LD, Nobili N, et al. Gene therapy in peripheral blood lymphocytes and bone marrow for ADA-immunodeficient patients. Science;1995;270:470-475.

T Lymphocyte–Directed Gene Therapy for ADA$^-$ SCID: Initial Trial Results After 4 Years

R. Michael Blaese,* Kenneth W. Culver, A. Dusty Miller, Charles S. Carter, Thomas Fleisher, Mario Clerici,† Gene Shearer, Lauren Chang, Yawen Chiang, Paul Tolstoshev, Jay J. Greenblatt, Steven A. Rosenberg, Harvey Klein, Melvin Berger, Craig A. Mullen,‡ W. Jay Ramsey, Linda Muul, Richard A. Morgan, W. French Anderson§

Because this disease is curable by allogeneic bone marrow transplantation given without pretransplantation cytoreductive conditioning, it was initially assumed that gene therapy should be directed at the bone marrow stem cell. However, initial attempts to use stem cell gene transfer in primates resulted in only low-level, transient gene expression, insufficient for clinical use. The observation that the only donor cells detected in some patients "cured" by allogeneic bone marrow transplantation was their T cells—the others remaining ADA-deficient (*5*)—raised the possibility that T cell–directed gene therapy also might be a useful treatment.

test reactivity] (*8–10*). Nearly all PEG-ADA–treated patients showed increased peripheral T cell counts, which provided a source of T cells for gene correction not available without enzyme therapy. Further-

Next, studies in mice, rabbits, and nonhuman primates using T cells modified with retroviral vectors showed normal cell survival and function after their reintroduction into recipient animals (*14*). Finally, Bordignon and colleagues (*15*) showed that ADA gene–corrected T cells acquired a survival advantage compared with uncorrected ADA-deficient cells when transplanted into immunodeficient, but ADA-

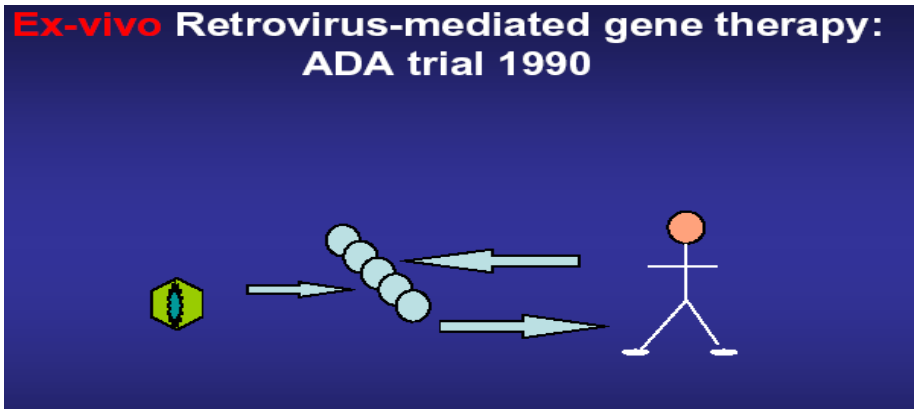
The adenosine deaminase complementary DNA (cDNA) (*11*) is 1.5 kb and fits within a retroviral vector. With the use of

Patients selection and enrollment

Patients with documented ADA SCID were eligible if they did not have a human lymphocyte antigen-matched sibling as a potential donor for marrow transplantation and if they had been treated with PEG-ADA for at least 9 months without full immune reconstitution

(*18*, *19*). Patient 1 presented with infection at 2 days of age and had recurrent infections and very poor growth until 26 months of age, [illegible] (30 U/kg/week)]. Treatment with PEG-ADA enzyme for approximately 2 years had resulted in significant, but incomplete, benefit. With [illegible] hemagglutinin titers (Table 1). At 4 years of age, she was enrolled in this trial.

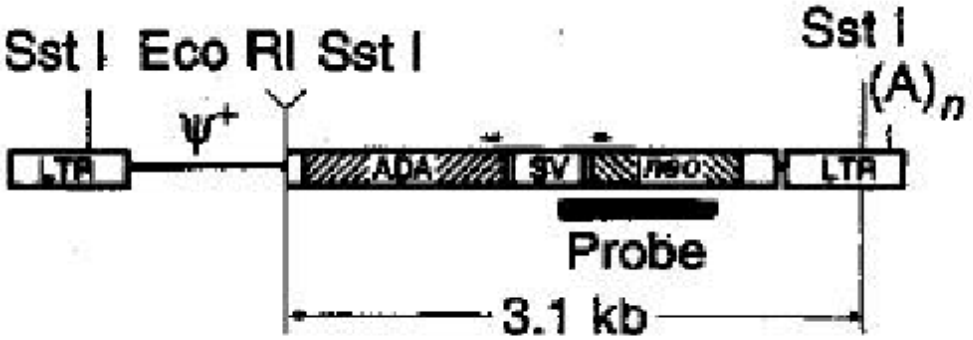
The course of disease in patient 2 (who was 9 years old when enrolled in the trial) was milder than that seen in classic SCID (*19*). She had her first serious infection at age 3, and septic arthritis at age 5; the diagnosis was finally established at age 6 when significant lymphopenia with ADA deficiency was confirmed. This patient had an excellent initial improvement in peripheral T cell numbers after the start of PEG-ADA therapy (30 U/kg/week) at age 5, but lymphopenia recurred in the third and fourth years of enzyme treatment (Fig. 1B).



Gene therapy protocol

- T cells were obtained from their blood by apheresis,
- induced to proliferate in Culture,
- transduced with the ADA retroviral vector LASN,
- culture-cxpanded, and
- then rcinfused into the patient after 9 to 12 days (17).
- No selection procedure was used to enrich for gcne-transduced cell

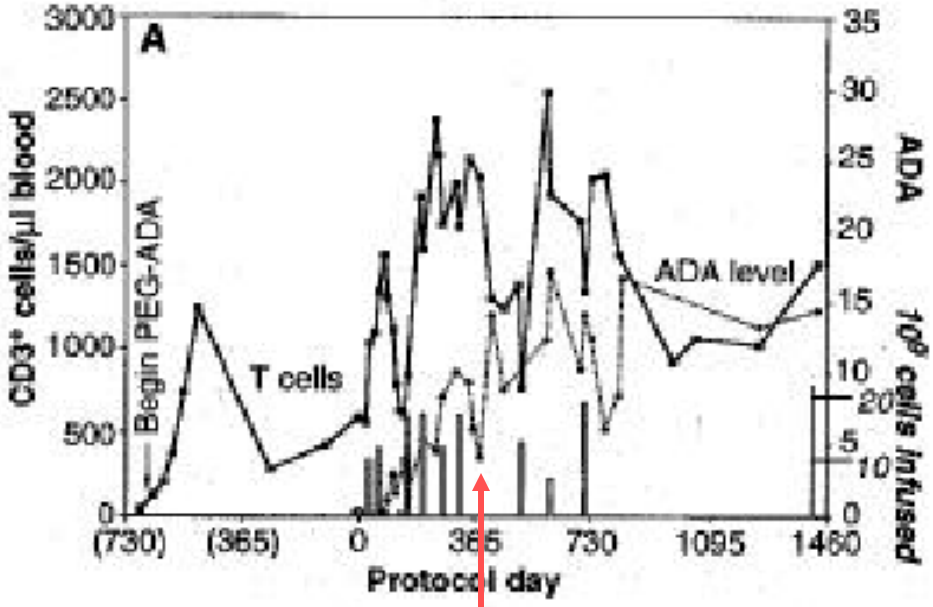
ADA vector

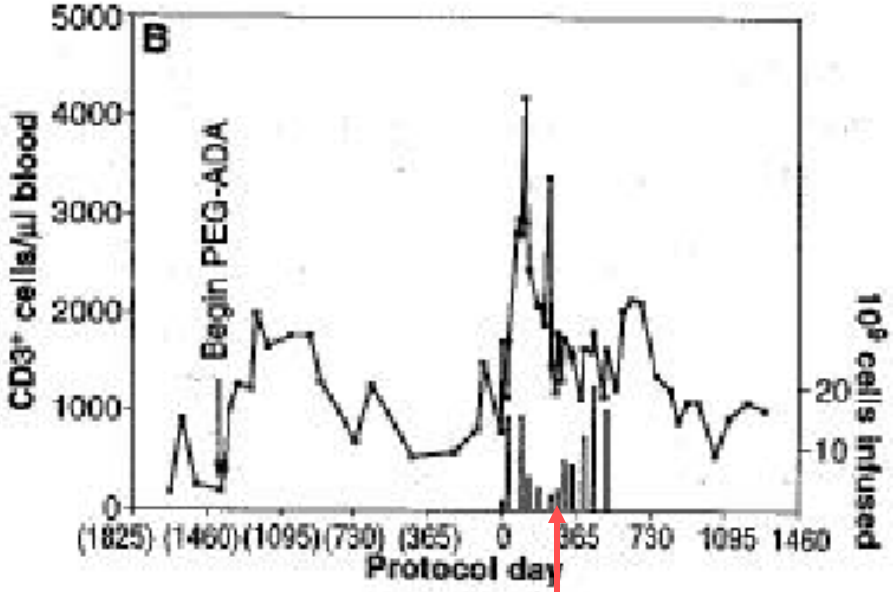


- Il cDNA di ADA (1,5 kb) clonato in un vettore retrovirale
- Linfociti T trasfettati esprimevano valori normali di ADA
- Linfociti T trasfettati in topi, conigli e primati mostravano una sopravvivenza normale
- Se inoculati in un background ada^-, essi avevano un vantaggio selettivo

Patient 1 (4 years)

Patient 2 (9 years)





Aumento di T cells
Aumento dei livelli di ADA

Aumento lievi di T cells
Aumento lievo dei livelli di ADA

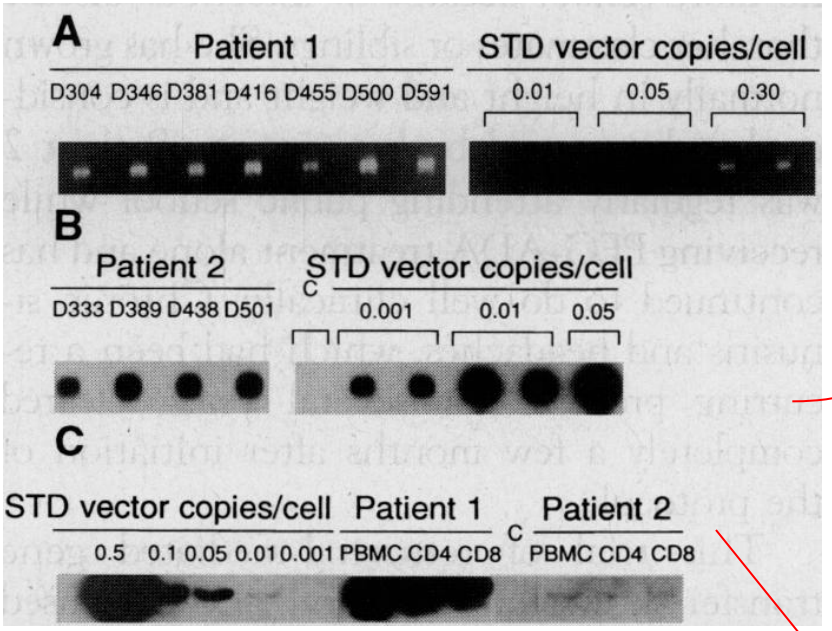


Fig. 2. PCR evaluation of the frequency of LASN vector–positive cells in the blood of patients 1 and 2 at various protocol days. (**A**) Cells from patient 1 for protocol days (D) 304 to 591 (see Fig. 1A). PCR analysis was performed as described (*26*) in an ethidium-stained gel. (**B**) Cells from patient 2 for protocol days (D) 333 to 501 (see Fig. 1B). PCR products were probed with ^{32}P-labeled *neo* gene as described (*26*). (**C**) Purified CD4$^+$ and CD8$^+$ cell subpopulations from patient 1 (D1480) and patient 2 (D1198) prepared by separation of peripheral blood mononuclear cells (PBMCs) by fluorescence-activated cell sorting (FACS). The purity of the separated T cell subpopulations from which DNA was extracted exceeded 98%, as confirmed by FACS analysis. Direct PCR with [^{32}P]deoxycytosine triphosphate was performed as described (*27*). Standards (STD) were prepared from DNA obtained from cell mixtures of a known proportion of LASN-transduced cells containing a single vector insert mixed with vector-negative cells. C, vector-negative control cells.

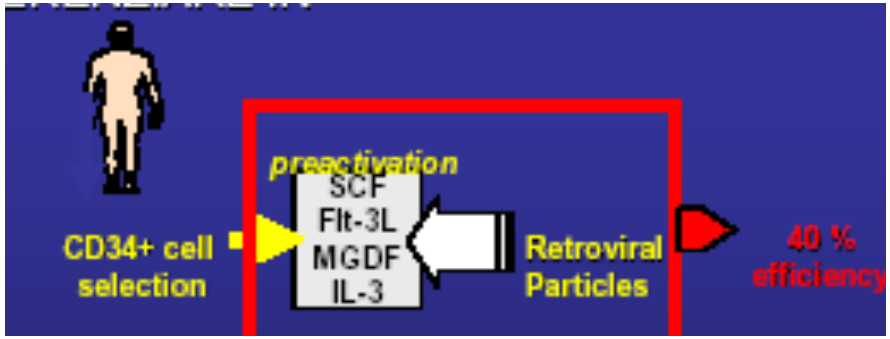
conclusion

In 1990, a clinical trial was started using retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency (ADA^- SCID). The number of blood T cells normalized as did many cellular and humoral immune responses. Gene treatment ended after 2 years, but integrated vector and ADA gene expression in T cells persisted. Although many components remain to be perfected, it is concluded here that gene therapy can be a safe and effective addition to treatment for some patients with this severe immunodeficiency disease.

Gene therapy outcome

T lymphocytes transduction with ADA was not sufficient to achieve significant clinical benefit.

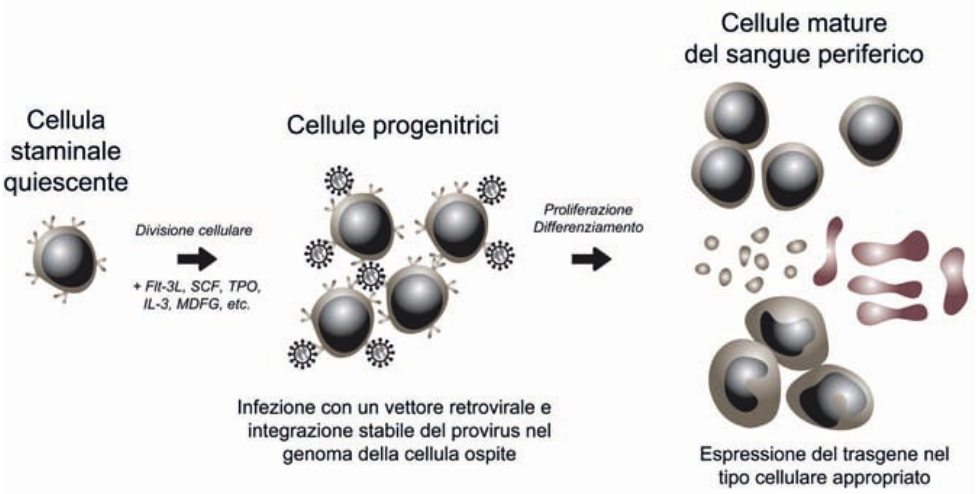
gene-corrected HSC
(hematopoietic stem cells)



HSC gene therapy

HSC, hematopoietic stem cells

HSC CD34+/CD38- is a small non-differentiated cells



2 Gene therapy protocol[1,9]

Cell harvesting: Under general anaesthesia, bone marrow was harvested from the patient into a closed collection bag. Cells carrying the CD34 antigen (haemopoietic progenitor cells) were selected using the CliniMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany).

↓

Pre-activation of cells with cytokines: CD34+ cells were cultured in gas-permeable bags (Baxter, Deerfield, Ill) in a purpose-built clean-room. Cells were stimulated to grow at a concentration of 5×10^5 cells/mL in X-vivo 10 medium (BioWhittaker, Walkersville, Md) with 4% fetal bovine serum (CSL, Australia); 60 ng/mL IL-3 (Stem Cell Technologies, Canada); 300 ng/mL stem-cell factor (Amgen, Thousand Oaks, Calif); 300 ng/mL Flt-3 ligand (R&D Systems, Minneapolis, Minn); and 100 ng/mL human recombinant thrombopoietin (R&D Systems).

↓

Genetic modification by retroviral vector: After 24 hours of preactivation, cells were harvested and transferred to fresh bags coated with RetroNectin (52 μg/mL, TaKaRa, Japan) (a recombinant peptide of human fibronectin that enhances retrovirus-mediated gene transfer) containing retroviral vector supernatant manufactured and supplied at a functional titre of 8.2×10^5 transducing units (TU)/mL by Genopoietic (Lyon, France). Protamine sulfate (2 μg/mL, Rhone-Poulenc Rorer Pharmaceuticals, Collegeville, PA) and the above cytokines were also added. The cells underwent gene transfer over 3 days during which the retroviral vector and cytokine mix were replaced every 24 hours.

↓

Quality control testing: After each transfer cycle, cultures were evaluated for sterility, viability, persistence of CD34+ expression and acquisition of γc expression.

Results from the first approach using HSC-corrected cells

In patients receiving gene-corrected HSC, vector-transduced cells remained below the therapeutic threshold.

Immune cells were not proven to be sustained by gene-corrected cells, since all patients continued to receive enzyme replacement therapy

Withdrawal of PEG-ADA

Withdrawal of PEG-ADA favored the selective accumulation of gene-corrected T cells, leading to improved immune response
however
transduced T cells appeared not sufficient to allow adequate systemic detoxification.

Mol Ther. 2006 Oct;14(4):505-13. Epub 2006 Aug 14.

Successful reconstitution of immunity in ADA-SCID by stem cell gene therapy following cessation of PEG-ADA and use of mild preconditioning.

Gaspar HB[1], Bjorkegren E, Parsley K, Gilmour KC, King D, Sinclair J, Zhang F, Giannakopoulos A, Adams S, Fairbanks LD, Gaspar J, Henderson L, Xu-Bayford JH, Davies EG, Veys PA, Kinnon C, Thrasher AJ.

Abstract

Gene therapy is a promising treatment option for monogenic diseases, but success has been seen in only a handful of studies thus far. We now document successful reconstitution of immune function in a child with the adenosine deaminase (ADA)-deficient form of severe combined immunodeficiency (SCID) following hematopoietic stem cell (HSC) gene therapy. An ADA-SCID child who showed a poor response to PEG-ADA enzyme replacement was enrolled into the clinical study. Following cessation of enzyme replacement therapy, autologous CD34(+) HSCs were transduced with an ADA-expressing gammaretroviral vector. Gene-modified cells were reinfused following one dose of preconditioning chemotherapy. Two years after the procedure, immunological and biochemical correction has been maintained with progressive increase in lymphocyte numbers, reinitiation of thymopoiesis, and systemic detoxification of ADA metabolites. Sustained vector marking with detection of polyclonal vector integration sites in multiple cell lineages and detection of ADA activity in red blood cells suggests transduction of early hematopoietic progenitors. No serious side effects were seen either as a result of the conditioning procedure or due to retroviral insertion. Gene therapy is an effective treatment option for the treatment of ADA-SCID.

Pazienti: pt 1 (3 years old)

- One month before GT, PEG-ADA was dismessed
- day -2: treatment with Melphalane (140 mg/kg)
- Day 0, isolation of CD34+ cells, transduction with GIADA (retroviral vector with ADA gene); efficiency of transduction, 25-30%
- Reinfusion of 1,4 x 10^6 cells/kg

Toward the best protocol

A major improvement was obtained after the introduction of a reduced dose of intravenous busulfan (4 mg/kg) prior cell reinfusion to make space in the bone marrow for gene corrected HSC10 and allow multilineage reconstitution and ADA expression.

busulfan is a non-myeloablative drug

myeloid or **myelogenous cells** are blood cells that arise from a progenitor cell for granulocytes, monocytes, erythrocytes, or platelets

Lymphoid progenitor cells that give rise to B and T cells

Correction of ADA-SCID by Stem Cell Gene Therapy Combined with Nonmyeloablative Conditioning

Alessandro Aiuti, *et al.*

Science **296**, 2410 (2002);

Pazienti: pt 1 (7 mesi) e pt 2 (2 anni e 6 mezzo)

Treatment (busulfonano 4 mg/kg)

Cells CD34+, transduced with GIADA (viral vector with ADA cDNA)

pt1, 8,6 x 10^6 cells/kg (25% trasdotte); pt2, 0,9x 10^6 cells/kg (21% trasdotte)

Quantitative PCR analysis for vector-containing cells

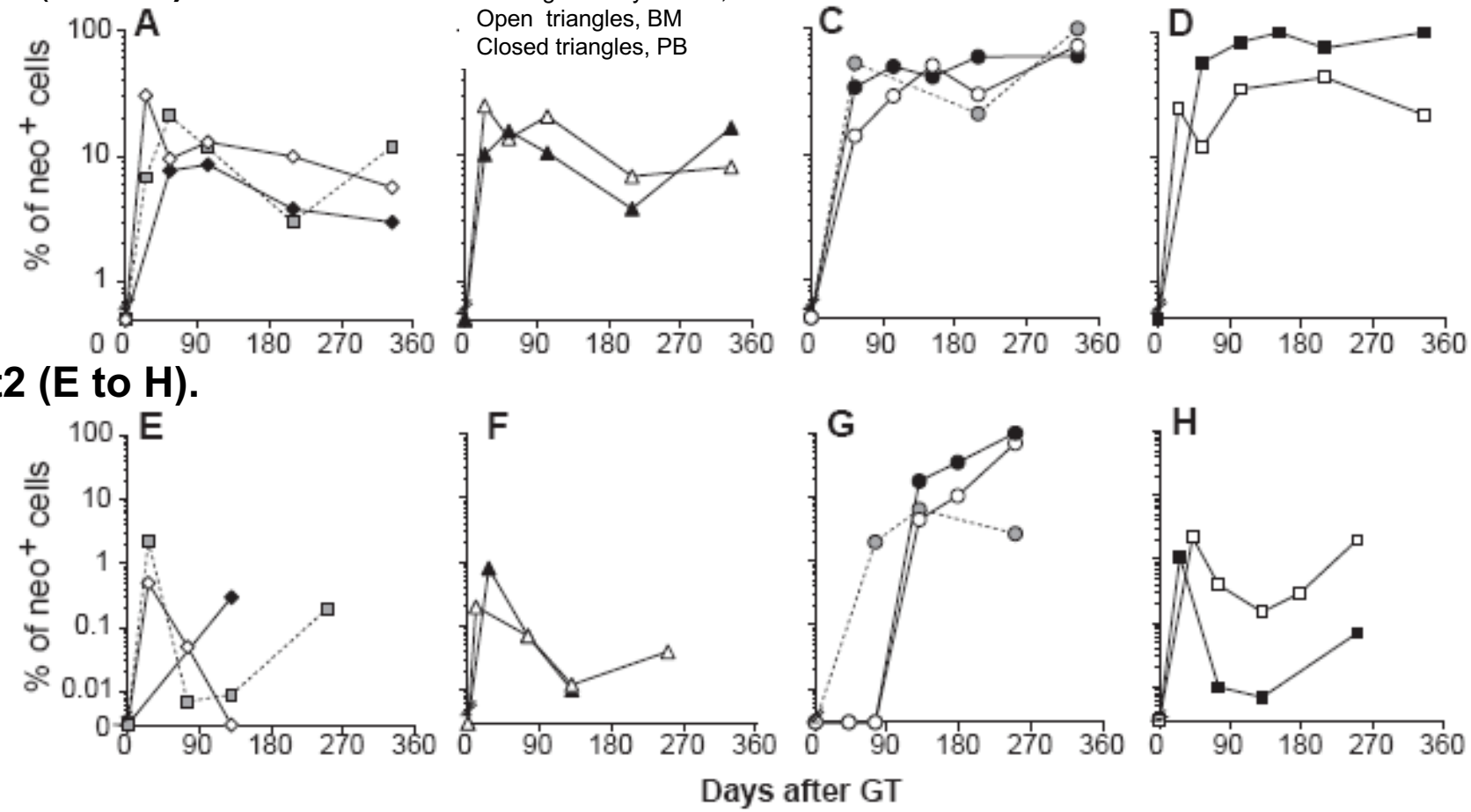
CD34 progenitor cells (gray squares),
CD61 megakaryocytic cells (solid diamonds),
glycophorin A erythroid cells (open diamonds

from the PB
CD3 T cells (open circles),
CD56/CD16 NK cells (gray circles)
T-cell lines (solidmcircles)

CD19 **B cells** from the BM (open boxes)
the PB (solid boxes

Pt1 (**A to D)**

CD15 granulocytic cells,
Open triangles, BM
Closed triangles, PB



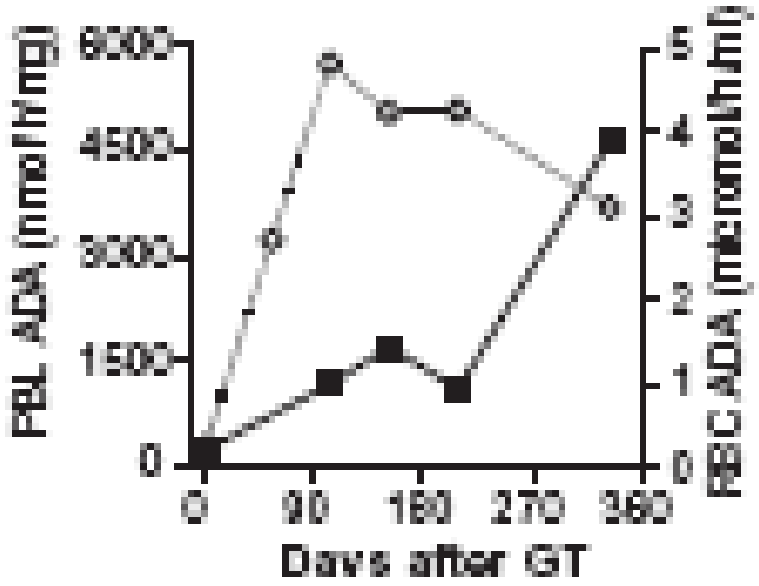
Pt2 (E to H).

BM, bone marrow
PB, periferal blood

Caratterizzazione biochimica di ADA in pt1 e pt2

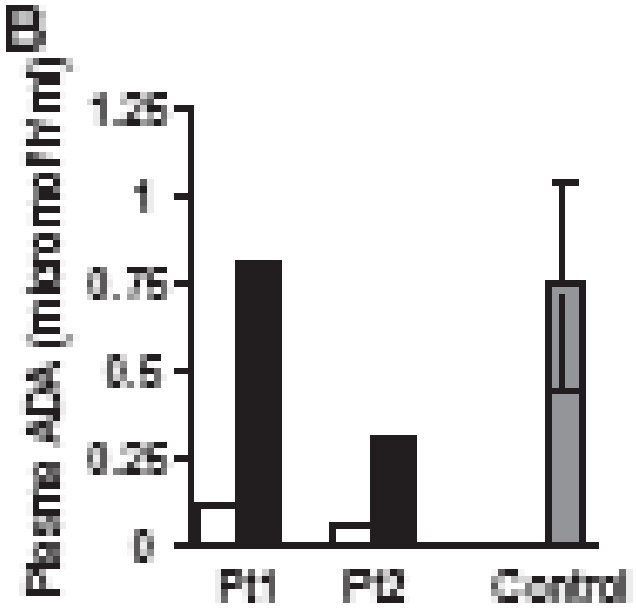
Normal ADA activity in PBL 1350+/- 650 nmol/hr/mg, in RBC 12 +/-2 μmol/h/ml values

A Intracellular ADA activity in Pt1



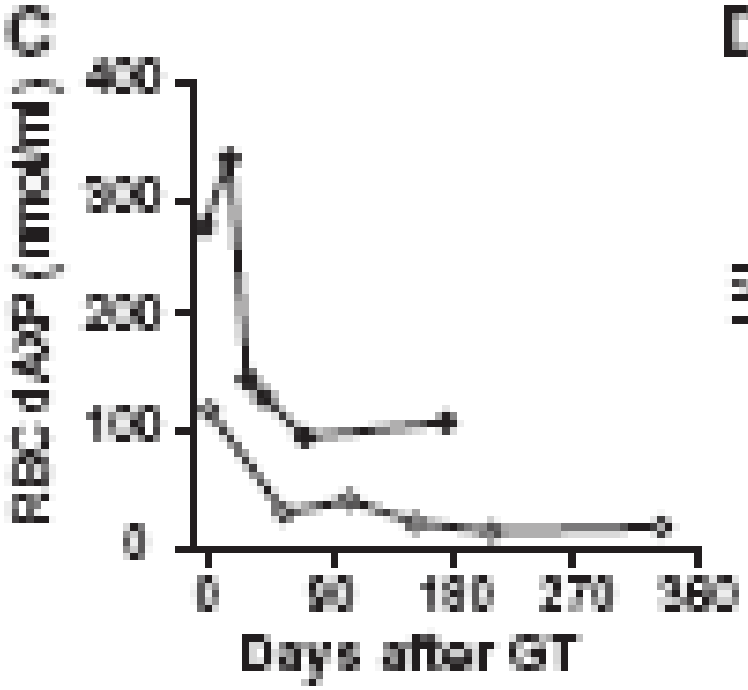
ADA activity in PBL (peripheral blood lymphocytes ■) and RBC (red blood cells ○)

B

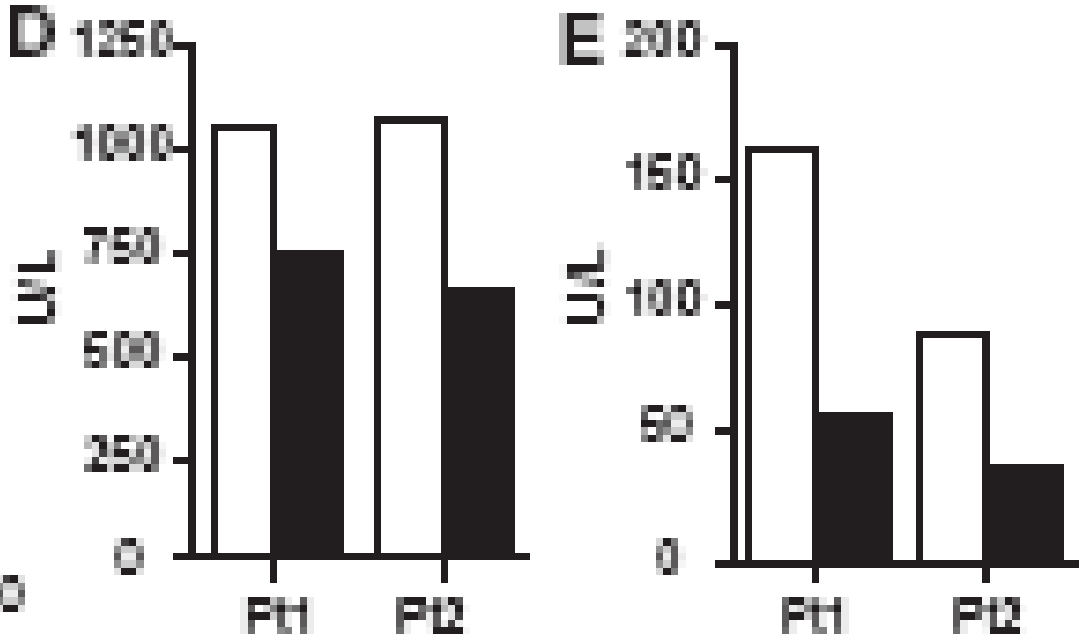


ADA activity in the plasma, before (white) after (black) treatment

ADA and purine metabolites



C Concentration of dAXP purine metabilites in RBC in Pt1 ○ and Pt2 ●



D Serum level of LDH and
E aspartate aminotransferase
prior (white) and after (black)
the ttreatment

LDH and aspartate aminotransferase are markers of cytoxicity.

conclusion

Hematopoietic stem cell (HSC) gene therapy for adenosine deaminase (ADA)–deficient severe combined immunodeficiency (SCID) has shown limited clinical efficacy because of the small proportion of engrafted genetically corrected HSCs. We describe an improved protocol for gene transfer into HSCs associated with nonmyeloablative conditioning. This protocol was used in two patients for whom enzyme replacement therapy was not available, which allowed the effect of gene therapy alone to be evaluated. Sustained engraftment of engineered HSCs with differentiation into multiple lineages resulted in increased lymphocyte counts, improved immune functions (including antigen-specific responses), and lower toxic metabolites. Both patients are currently at home and clinically well, with normal growth and development. These results indicate the safety and efficacy of HSC gene therapy combined with nonmyeloablative conditioning for the treatment of SCID.

Since the year 2000, at TIGET15 patients without HLA-identical sibling donors have been treated with transduced autologous bone marrow CD34+ cells according to this experimental protocol

Table 2. Summary of the clinical experience of hematopoietic stem cell (HSC)-gene therapy for adenosine deaminase-deficient severe combined immunodeficiencies (ADA-SCID) in the last decade.

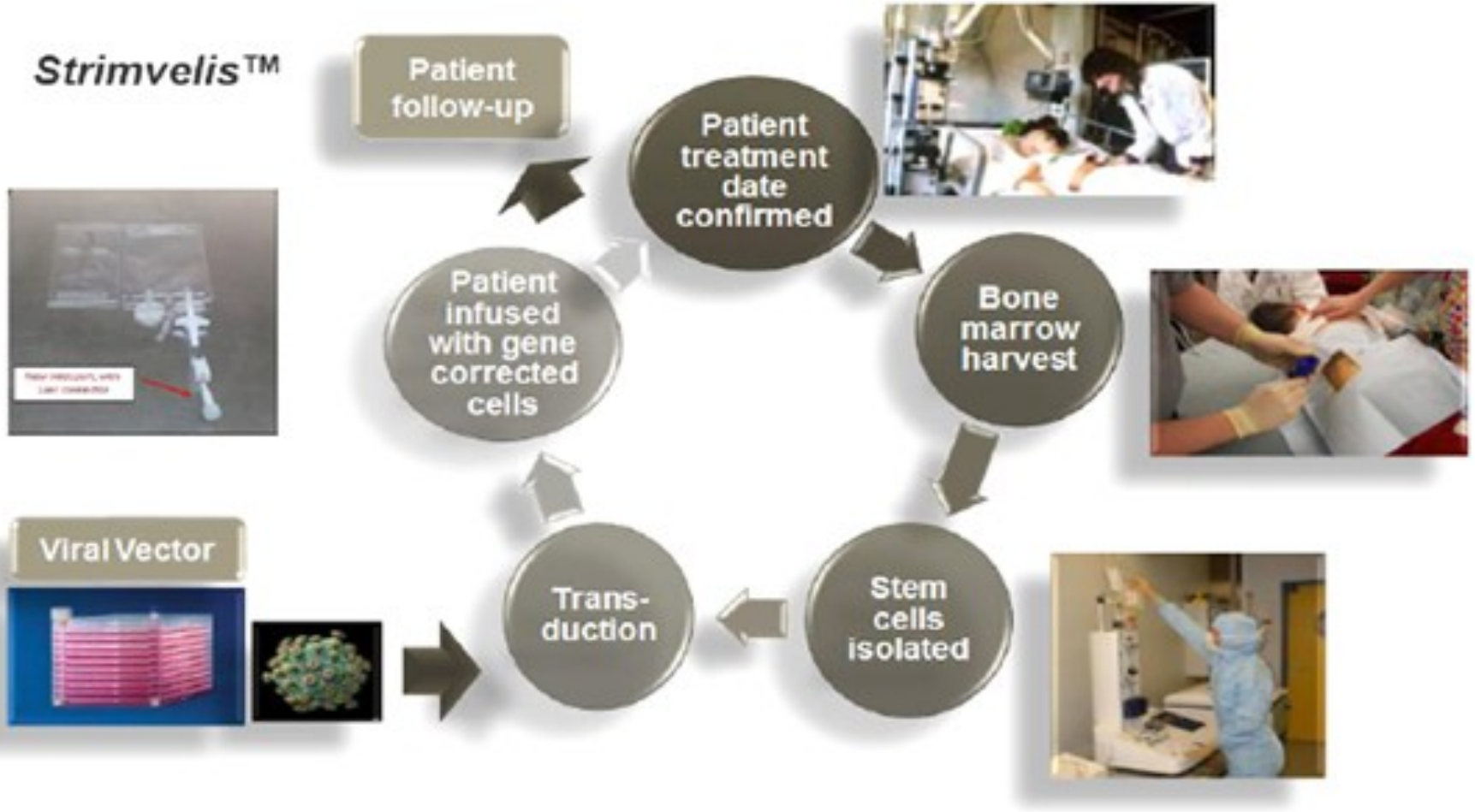
Study	No. pts treated	Conditioning	Longest follow-up	Efficacy	Toxicity
HSR-TIGET[11,14]	15	Busulfan (4 mg/kg)	8 y	Yes	No
GOSH[11,19]	5	Melphalan (140 mg/m^2)	5.5 y	Yes	No
CHLA/NIH[17]	4	No	8 y	No	No
CHLA/NIH[11,17,18]	6	Busulfan (75-90 mg/m^2)	2 y	Yes	Pancytopenia due to pre-existing cytogenetic abnormality (1 pt)

GOSH indicates Great Ormond Street Hospital; CHLA, Children's Hospital Los Angeles.

GOSH, London
HSR, TIGET, Milan
CHLA, Los Angeles

Strimvelis™ approval in Europe

- 18 ADA-SCID patients successfully treated in 2000 -2011
- Follow up for 8 years
- 100% survival



Gene Therapy for "Bubble Boy" Disease

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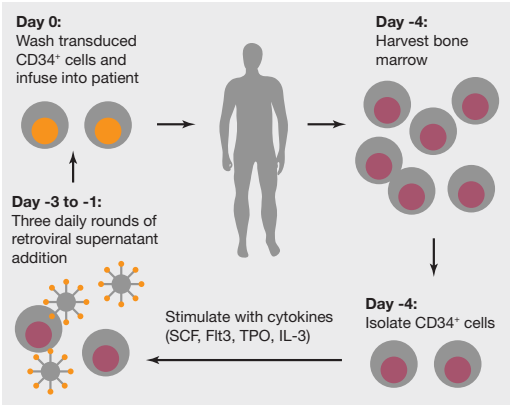
http://dx.doi.org/10.1016/j.cell.2016.06.049

Cell



Adenosine deaminase (ADA) deficiency results in the accumulation of toxic metabolites that destroy the immune system, causing severe combined immunodeficiency (ADA-SCID), often referred to as the "bubble boy" disease. Strimvelis is a European Medicines Agency approved gene therapy for ADA-SCID patients without a suitable bone marrow donor.

NAME
Strimvelis (GSK2696273); international nonproprietary name: autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector that encodes for the human ADA cDNA sequence

APPROVED FOR
Treatment of severe combined immunodeficiency due to adenosine deaminase deficiency (ADA-SCID) in patients who cannot be treated by a bone-marrow transplant because they do not have a suitable, matched, related donor

TYPE
Gene therapy: autologous CD34+ enriched cell fraction that contains CD34+ cells transduced with retroviral vector (LXSN vector) that encodes for the human ADA cDNA sequence

CELLULAR TARGETS
CD34+ hematopoietic cells

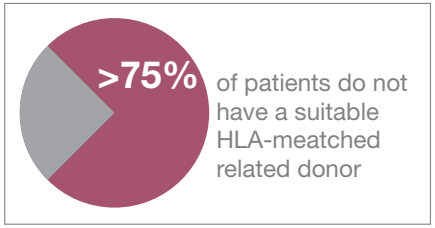
EFFECTS ON TARGETS
Replaces defective adenosine deaminase in immune cells

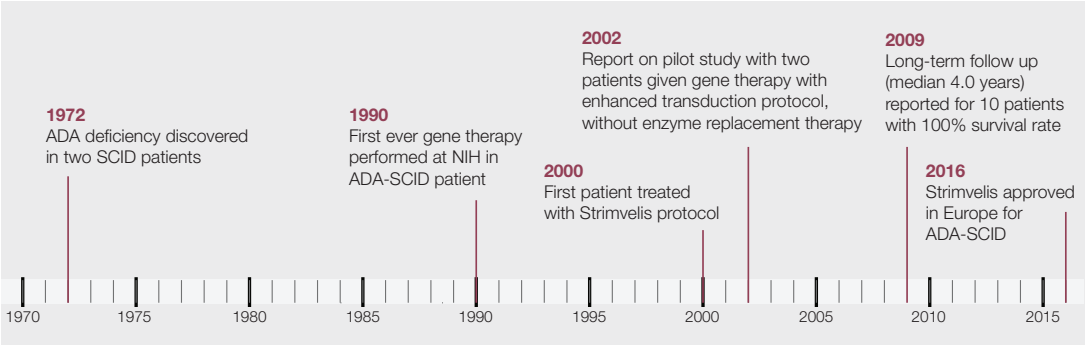
DEVELOPED BY
GlaxoSmithKline, MolMed, San Raffaele Telethon Institute for Gene Therapy

Disease frequency

Treatments to date

HLA-matched related donors





References for further reading are available with this article online: www.cell.com/cell/fulltext/S0092-8674(16)30857-1

Gene therapy in ADA deficiency: a summary

Adenosine deaminase deficiency has now been treated with modern gene therapy techniques, following the inclusion of **38** patients in three trials (performed in Italy, the UK and the USA) (Aiuti et al., 2002, 2009; Cavazzana-Calvo et al., 2012; Ferrua et al., 2010).

An important difference, with other GT protocols, was related to the use of a mild conditioning regimen (4 mg/kg busulfan for most patients), in order to improve transduced stem cell engraftment. This choice was motivated by the fact that ADA deficiency is a metabolic disease in which increasing the number of transduced cells within the different cell lineages could be advantageous.

Efficacy (judged in terms of T cell development and the absence of clinical indications for supplementing patients with pegylated ADA) has been seen **in 28 of the 30 patients**, whereas the 10 others are alive and on enzyme replacement therapy.
The median follow-up is 3.5 years (range: 1 to 11.5 years).

Significant transduced B, NK lymphocyte and myeloid cell counts have been detected as a consequence of the mild myeloablation and the transduced stem cells' good engraftment. These results are very encouraging and suggest that gene therapy is a coherent therapeutic option for patients with ADA deficiency.

Gene therapy for X-SCID

X-linked SCID

Severe immunodeficiency X-linked, characterized by deficiency in T and NK cells

Most frequent form of SCID (46%)
More frequent in male than in female
The gene was identified as the coding of the gamma chain of IL-2 receptor
The gene localizes in the X chromosome.

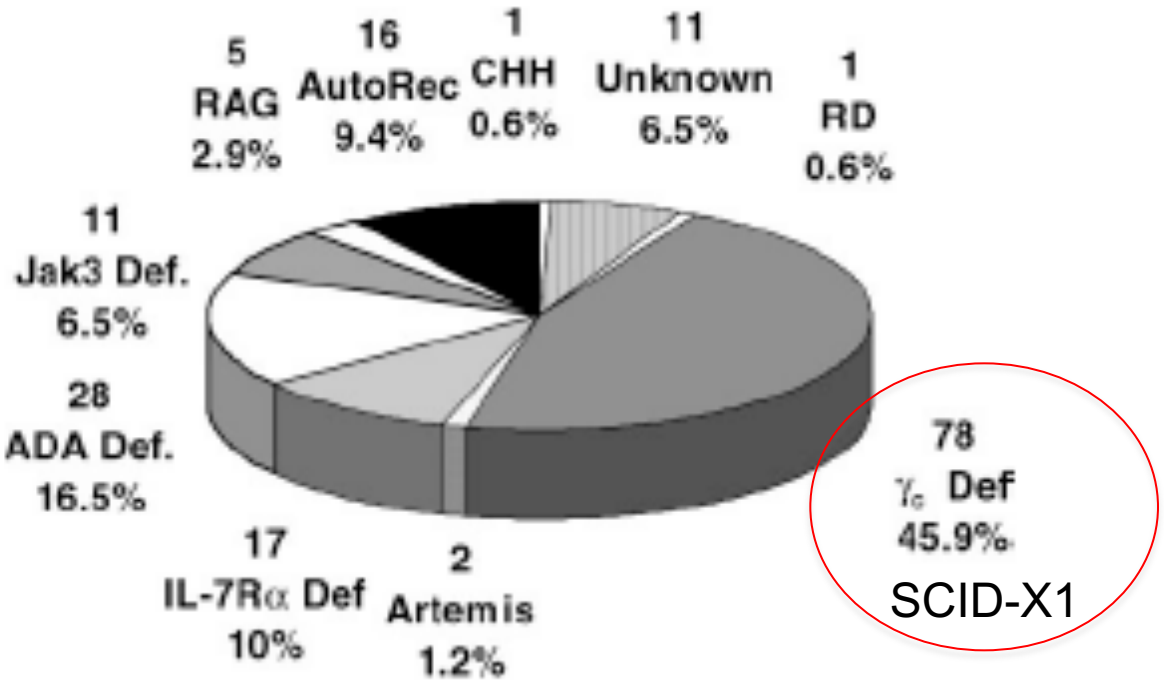
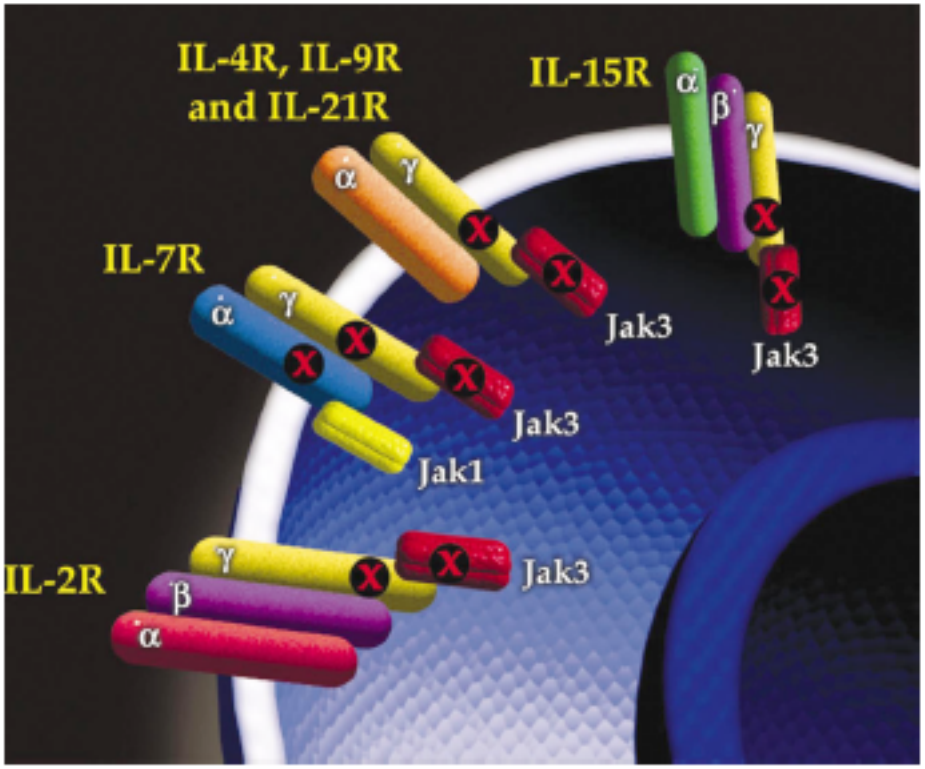


Figure 4 Relative frequencies of the different genetic types of SCID among 170 patients seen consecutively by the author over 3.5 decades.

Gene therapy for X-SCID

The genetic defect

SCID-X1 is caused by γc deficiency. IL2RG encodes for the **common** cytokine receptor **gamma** chain (**γc**), which is part of the receptors for IL-2 and five other cytokines (IL-4, IL-7, IL-9, IL-15, and IL-21).



The clinic defects

SCID-X1 patients lack T and NK cells, while B cells are present but functionally impaired

Multiple defects are caused by the absence of IL2RG

Patient phenotype ($T^-B^+NK^-$)

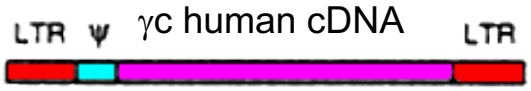
Absence of T (T-) – alteration of IL-7 signalling; block in the development of thymocytes at the double negative stage before the production rearrangement of the TCR genes. This cytokine also contributes with IL-15 to the survival of memory CD8 + T lymphocytes.

Absence NK (NK-) - IL-15 is a critical factor for the development and survival of NK cells and the absence of these cells in the SCIDs is the result of their developmental block in the absence of a correct action of IL-15.

Presence of B (B+) – development of B cells is independent from IL-7. However, the function of B cells (defective activation, lack of switches) is compromised not only by the absence of T cells but also by the absence of IL-4 and IL-21.

First gene therapy for SCID-X1

The first clinical gene therapy protocol was conducted at Hôpital Necker (Paris) and was based on ex vivo γc gene transfer using retroviral vectors into autologous CD34+ bone marrow cells



Retroviral vector and virus-producing cell line. Human γc chain cDNA extending from the initiation codon ATG (nucleotides numbered 1 to 1114) was generated by reverse transcription polymerase chain reaction (RT-PCR) from mRNA of control B-cell lines. Forward primer 5′-GCAAGCGACATGTTGAAGCC-3′, and reverse primer: 5′-GAGGATCCGGGTTCAGGTTTCAG-3′ contain AFL III and *Bam*HI site, respectively for γc chain insertion in the retroviral vector. Correct γc sequence was assessed by direct sequencing of the entire PCR amplified fragment. Human γc chain was then inserted at the *Nco* I, *Bam*HI site of the MFG (B2)/Mo-LTR vector[18] MFG (B2), which uses the Moloney murine leukemia virus (MO-MLV) LTRs for transcription of the viral genome and contains the B2 mutation corresponding to a single G to A transition at position +160 of the MO-MLV sequence.[19] This vector, named MFG (B2)-γc, does not contain a selection marker.

Hacein-Bein et al, Blood 87(8): 3108, 1996

2 Gene therapy protocol[1,9]

Cell harvesting: Under general anaesthesia, bone marrow was harvested from the patient into a closed collection bag. Cells carrying the CD34 antigen (haemopoietic progenitor cells) were selected using the CliniMACS system (Miltenyi Biotec, Bergisch Gladbach, Germany).

Pre-activation of cells with cytokines: CD34+ cells were cultured in gas-permeable bags (Baxter, Deerfield, Ill) in a purpose-built clean-room. Cells were stimulated to grow at a concentration of 5 × 10^5 cells/mL in X-vivo 10 medium (BioWhittaker, Walkersville, Md) with 4% fetal bovine serum (CSL, Australia); 60 ng/mL IL-3 (Stem Cell Technologies, Canada); 300 ng/mL stem-cell factor (Amgen, Thousand Oaks, Calif); 300 ng/mL Flt-3 ligand (R&D Systems, Minneapolis, Minn); and 100 ng/mL human recombinant thrombopoietin (R&D Systems).

Genetic modification by retroviral vector: After 24 hours of preactivation, cells were harvested and transferred to fresh bags coated with RetroNectin (52 µg/mL, TaKaRa, Japan) (a recombinant peptide of human fibronectin that enhances retrovirus-mediated gene transfer) containing retroviral vector supernatant manufactured and supplied at a functional titre of 8.2 × 10^5 transducing units (TU)/mL by Genopoietic (Lyon, France). Protamine sulfate (2 µg/mL, Rhone-Poulenc Rorer Pharmaceuticals, Collegeville, PA) and the above cytokines were also added. The cells underwent gene transfer over 3 days during which the retroviral vector and

Quality control testing: After each transfer cycle, cultures were evaluated for sterility, viabilit persistence of CD34+ expression and acquisition of γc expression.

Cell harvesting and washing: Cells were then harvested, washed and resuspended in 4% human albumin solution (CSL).

Reinfusion: Cells were re-infused into the patient, without preconditioning, via a central line.

Purezza cell CD34+: 79%

Tot N= 5x10^6 cells

Retrovirus codificante per human γc 8,2x10^5 TU

eMJA The Medical Journal of Australia

Research

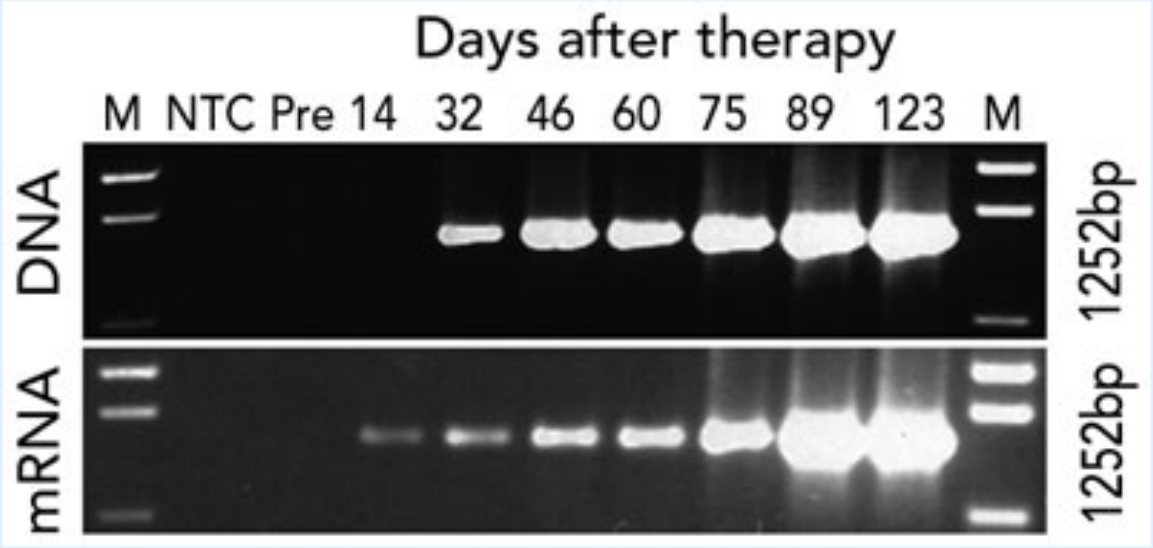
Treatment of an infant with X-linked severe combined immunodeficiency (SCID-X1) by gene therapy in Australia

Samantha L Ginn, Julie A Curtin, Belinda Kramer, Christine M Smyth, Melanie Wong, Alyson Kakakios, Geoffrey B McCowage, Debbie Watson, Stephen I Alexander, Margot Latham, Sharon C Cunningham, Maolin Zheng, Linda Hobson, Peter B Rowe, Alain Fischer, Marina Cavazzana-Calvo, Salima Hacein-Bey-Abina and Ian E Alexander

MJA 2005; 182 (9): 458-463

Paziente:
Child, 9 month affetto da SCID-X1

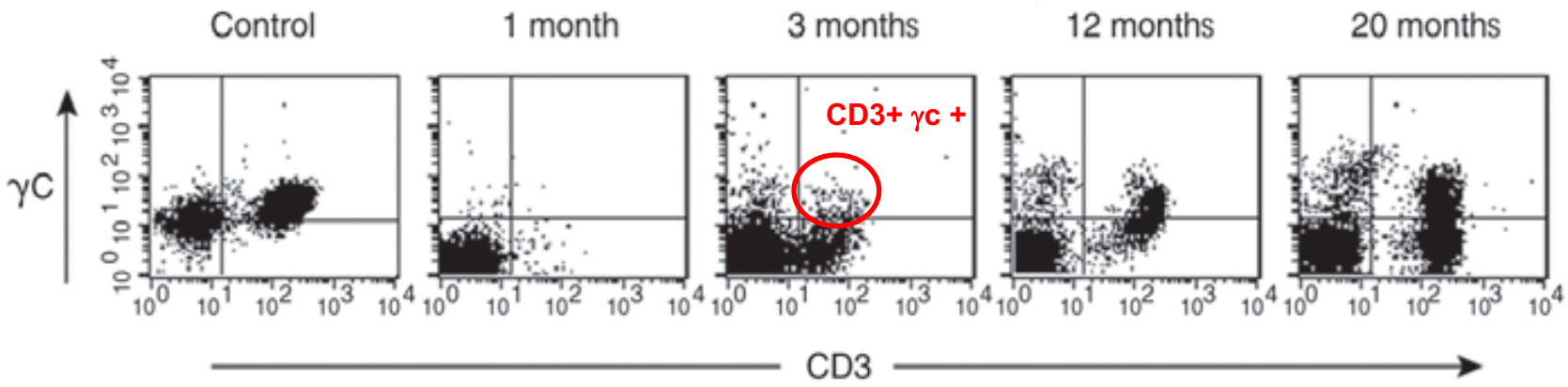
3 Detection of gene-corrected cells in peripheral blood after gene therapy



A. Detection by polymerase chain reaction (PCR) of cells containing integrated vector DNA (upper panel) and cells expressing vector-encoded mRNA (lower panel). The PCR primers used amplify a 1252 base-pair product encompassing the γc gene.

M = molecular weight marker; NTC = no template control; Pre = pre-treatment sample.

Characterisation of T cells in peripheral blood after gene therapy



Detection of cell surface γc protein expression on T cells in peripheral blood by fluorescence activated cell sorting. The first panel shows blood from a normal control patient with T (CD3+) cells expressing γ c in the upper right quadrant. Such cells were absent from our patient's blood 1 month after gene therapy, but appeared progressively from 3 months.

Results

- T cells observed after 75 days from the treatment
- Two weeks after treatment general improvement of the patient's condition was observed
- T cells level did not reach physiological level, nor complete reconstitution of the immune system was observed
- The patient underwentbone marrow transplantation

A low dose of CD34 + cells with the correct gene was used

Other trials with the same vector

1999-2002

Patients:	10 bambini, 0-5 year
Dose	1-3 x 10^6 CD34+/kg

Overall, 17 out of the 20 SCID-X1 patients enrolled in both clinical trials benefited from gene therapy.
In nearly all patients T-cell counts reached normal levels and the cells were functionally competent, as demonstrated by normal responses to mitogens and specific antigens.

Table 3. Summary of the clinical experience of hematopoietic stem cell (HSC)-gene therapy for SCID-X1.

Study	No. pts treated	Longest follow-up	Efficacy	Toxicity
Hôpital Necker[22,25]	10	10 y	Yes	T-cell leukemia (4 pts)
GOSH [23,24]	10	7 y	Yes	T-cell leukemia (1 pt)
GOSH, Necker,[27] NIH[26]	5 (age 10-20)	3 y for pts with engraftment	No, improvement in 1 pt	No

GOSH indicates Great Ormond Street Hospital.

treatment of SCID-X1 patients at later stage of disease was not efficacious

Five patients were **treated later in life (at between 10 and 20 years of age)** because of either an atypical SCID-X1 caused by hypomorphic mutation or poorly reconstituted T cell immunity years after HSCT.
Despite technically efficient gene transfer, the results have been disappointing — with **little or no improvement in T cell immunity.**

Defective residual thymic function at a later age in SCID patients very probably accounts for these failures and raises the question of how long the thymus remains potentially functional in a patient lacking effective thymopoiesis.

SCID-X1 mooving forward

A new clinical trial has been reinitiated for which a **SIN retroviral vector** with a satisfactory in vitro safety data (Modlich et al., 2009) containing the c gene has been designed. This international (running in the United Kingdom, United States and France) trial has been initiated two years ago and should provide within the next couple of years the expected informations on its combined safety/efficacy profile.

Adverse events

activation of the proto-oncogene LMO2 caused by the retroviral LTR

LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy for SCID-X1

S. Hacein-Bey-Abina, *et al.*

Science **302**, 415 (2003);
DOI: 10.1126/science.1088547

Four patients in the French trial and one in the English trial developed clonal T-cell proliferation that became evident 2 to 6 years after treatment.

This leukemia-like disease was the result of vector-mediated up-regulation of host cellular oncogenes by the MLV LTR.

Chemotherapy allowed sustained remission in 4 cases, and these patients ontinued to benefit from gene therapy, but 1 patient died due to refractory leukemia

CONCLUSIONS

After nearly 10 years of follow-up, gene therapy was shown to have corrected the immunodeficiency associated with SCID-X1. Gene therapy may be an option for patients who do not have an HLA-identical donor for hematopoietic stem-cell transplantation and for whom the risks are deemed acceptable. This treatment is associated with a risk of acute leukemia. (Funded by INSERM and others.)

From Hacein-Bey-Abina 2010

Published and Ongoing Clinical Trials for Primary Immune Deficiencies

Disease	*Mutated gene/ protein*	*Target cell/Vector*	*Conditioning*	*Year reported reported/reference*
ADA-SCID	ADA (adenosine deaminase)	PBL/RV	N	1995(21)
		PBL,HSC/RV	N	1995(22)
		CB/RV	N	1995 (23)
		HSC/RV	N	1996 (24)
		HSC/RV	Y (Bu RIC)	2002 (25,27,28,30)
		HSC/RV	Y (Melph Bu RIC)	2006 (26,32)
		HSC/RV	N/Y (Bu RIC)	2012(29,31)
		HSC/LV	Y (Bu RIC)	ongoing
SCID-X1	IL2RG (gc)	HSC/RV	N	2000(42,44,48,51)
		HSC/RV	N	2000 (43,45,49,50)
		HSC/RV	N	2007 (65)
		HSC/SIN-RV	N	2014 (62)
		HSC/LV	Y (Bu RIC)	2016 (66) ongoing
CGD	NCF-1/p47phox	HSC/RV	N	1997(76)
	CYBB (gp91phox)	HSC/RV	Y (Bu RIC)	2006(77)
		HSC/RV	Y (Bu RIC)	2010(78)
		HSC/RV	Y (Bu/Flu RIC)	2012 (79)
		HSC/LV	Y (Bu FIC)	ongoing (81)
WAS	WASP (WASp)	HSC/LV	Y (Bu/Flu RIC)	2013(88,89) ongoing
		HSC/LV	Y (Bu/Flu FIC)	2015(88,90) ongoing
		HSC/LV	Y (Bu/Flu FIC)	ongoing (88,US)

PBL, peripheral blood lymphocytes; CB, cord blood; HSC, haematopoietic stem cells
Bu, Busulphan; Flu, fludarabine, Melph, Melphalan; RIC, reduced intensity conditioning; FIC, full myeloablative conditioning

From: Molecular Therapy Vol. 25 No 5 May 2017

Da consultare

Fisher et al 2013 “Gene therapy for primary T cell immunodeficiencies. Gene 525: 170-173

Twenty-Five Years of Gene Therapy for ADA-SCID: From *Bubble Babies* to an Approved Drug

Francesca Ferrua[1,2] and Alessandro Aiuti[1,2,*]

[1]*San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), Pediatric Immunohematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milan, Italy;* [2]*Vita-Salute San Raffaele University, Milan, Italy.*

Aiuti A., Roncarolo M.G., Naldini L. (2017) Gene therapy for ADA-SCID, the first marketing approval of an *ex vivo* gene therapy in Europe: paving the road for the next generation of advanced therapy medicinal products. *EMBO Molecular Medicine* 9