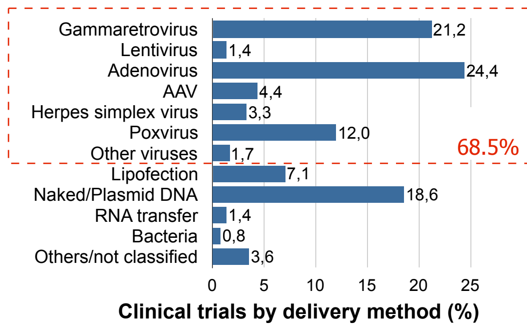
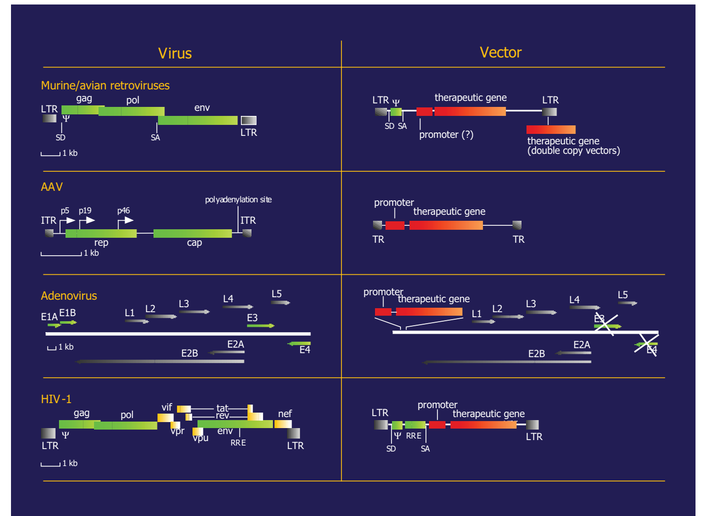
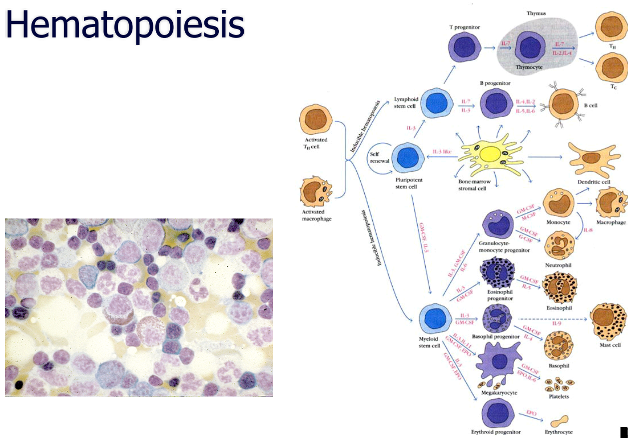
## Gene therapy clinical trials by delivery method




Giacca, M. 2010. Gene Therapy. Springer

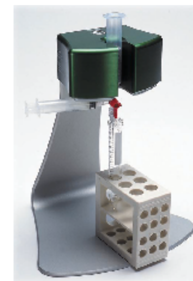


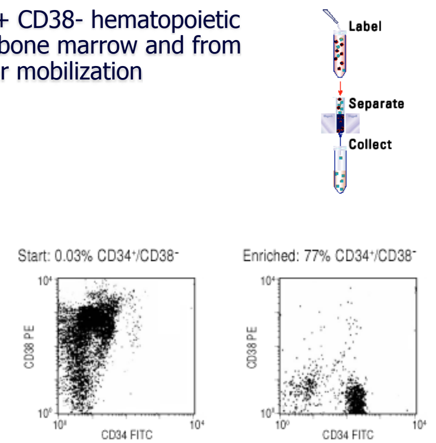

# Hematopoiesis



The whole hematopoietic system can be reconstituted by a single HSC

## Enrichment of CD34+ CD38- hematopoietic precursors from the bone marrow and from peripheral blood after mobilization



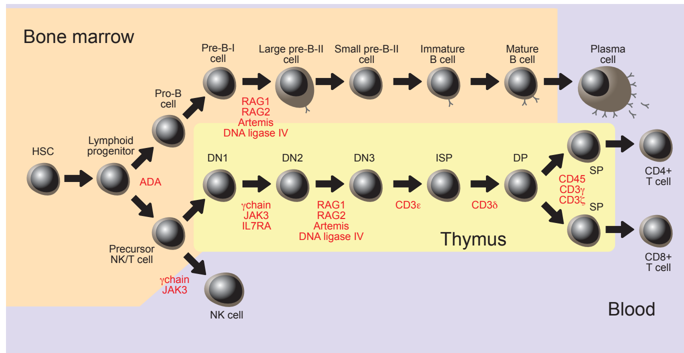



**Principali difetti molecolare che portano allo sviluppo di SCID.** AR: autosomica recessiva; X-L: legata al cromosoma X

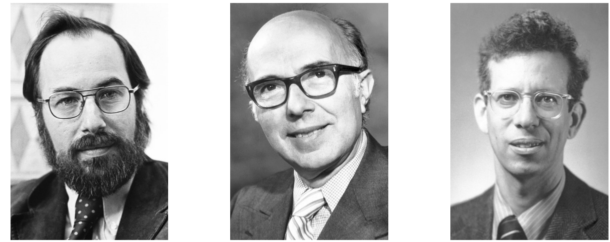
| Meccanismo | Gene mutato | Ereditarieta' | Cellule affette |
|---|---|---|---|
| Morte prematura delle cellule | ADA | AR | T, B, NK |
| Difetto nella sopravvivenza dovuto alla mancanza di segnali attivatori da parte di citochine | catena comune γ (cγ) | X-L | T, NK |
| | JAK-3 | AR | T, NK |
| | 1L7RA | AR | T |
| Difetto nel riarrangiamento V(D)J | RAG1 o RAG2 | AR | T, B |
| | Artemis | AR | T, B |
| Difetto nella segnalazione da parte del pre-TCR o del TCR | CD3 δ, ζ, ε | AR | T |
| | CD45 | AR | T |

- La convenzione classica della terapia genica non corregge ma aggiunge una copia sana del gene mutato
- Principali successi ad oggi ottenuti per le malattie AR
- Nuove prospettive con gene editing

## Defects leading to the development of severe combined immunodeficiency (SCID)

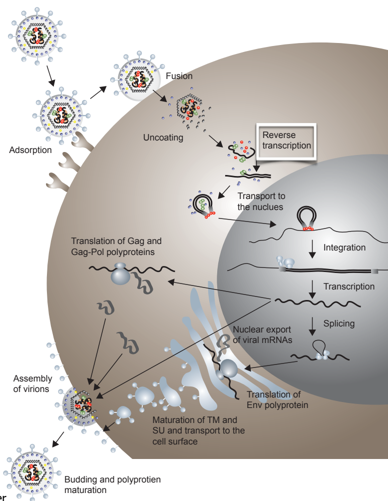



## The Nobel Prize in Physiology or Medicine 1975



The Nobel Prize in Physiology or Medicine 1975 was awarded jointly to David Baltimore, Renato Dulbecco and Howard Martin Temin "for their discoveries concerning the interaction between tumour viruses and the genetic material of the cell."

## Retrovirus life cycle

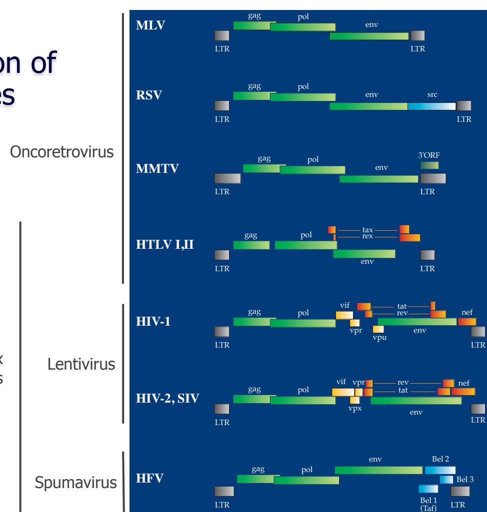



Giacca, M. 2010. Gene Therapy. Springer

## Taxonomy of the *Retroviridae* family

| Subfamily | Genus | Former classifications | Main species | Prototype viruses |
|---|---|---|---|---|
| *Orthoretrovirinae* | Alpharetrovirus | Avian type C retroviruses; Avian sarcoma/leukosis viruses (ASLV) | Avian leukosis virus | ALV |
| | | | Rous sarcoma virus | RSV |
| | Betaretrovirus | Mammalian type B retroviruses; Type D retroviruses | Mouse mammary tumor virus | MMTV |
| | | | Mason-Pfizer monkey virus | MPMV |
| | Gammaretrovirus | Mammalian type C retroviruses | Murine leukemia virus | Abelson-MLV, Friend-MLV, Moloney-MLV |
| | | | Feline leukemia virus | FeLV |
| | | | Gibbon ape leukemia virus | GaLV |
| | | | Harvey murine sarcoma virus | Ha-MSV |
| | | | Moloney murine sarcoma virus | Mo-MSV |
| | | | Simian sarcoma virus | SSV |
| | | | Reticuloendotheliosis virus | REV-A, REV-T |
| | Deltaretrovirus | BLV-HLTV group retroviruses | Bovine leukemia virus | BLV |
| | | | Primate T-lymphotropic viruses (human and simian) | HTLV-1, STLV-1, HTLV-2, STLV-2, STLV-3 |
| | Epsilonretrovirus | Fish retroviruses | Walleye dermal sarcoma virus | WDSV |
| | Lentivirus | | Bovine immunodeficiency virus | BIV |
| | | | Equine infectious anemia virus | EIAV |
| | | | Feline immunodeficiency virus | FIV-O, FIV-P |
| | | | Caprine arthritis encephalitis virus | CAEV |
| | | | Visna/Maedi virus | VISNA |
| | | | Human immunodeficiency virus 1 and 2 | HIV-1, HIV-2 |
| | | | Simian immunodeficiency virus | SIV-agm.155, SIV-cpz, SIV-mac |
| *Spumaretrovirinae* | Spumavirus | | Simian foamy virus | SFVmac (SFV-1 and SFV-2), SFVagm (SFV-3), SFVcpz and SFVcpz(hu) |
| | | | Bovine foamy virus | BFV |
| | | | Equine foamy virus | EFV |
| | | | Feline foamy virus | FFV |
| | | | Human foamy virus | HFV or HSRV |

## Genetic organization of retroviruses

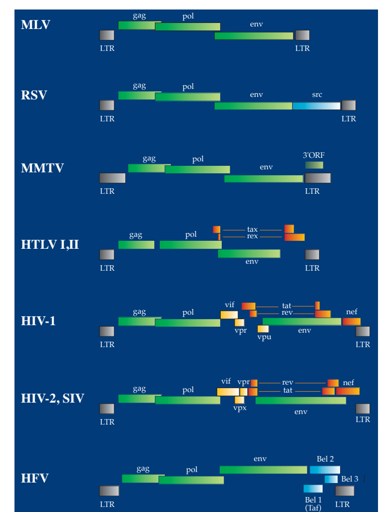



## Common features of retroviruses

They all contain LTRs (400-700 nt), which form in the integrated pro-virus

Viral particles contain mRNA

They all contain gag, pol and env genes



## RNA Tumor Viruses - The Rous Sarcoma Virus Story

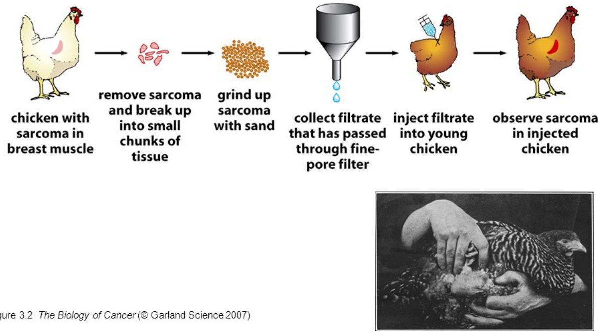
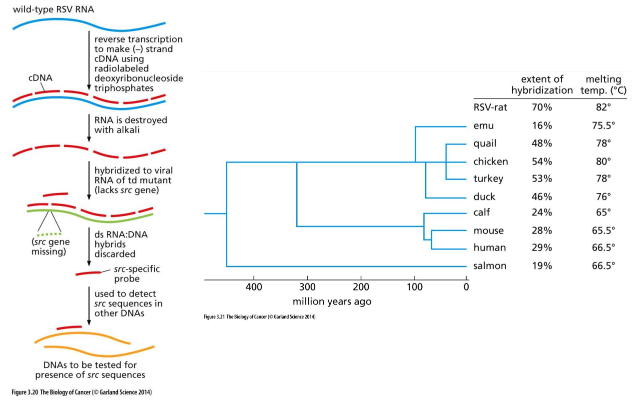



Figure 3.2 *The Biology of Cancer* (© Garland Science 2007)

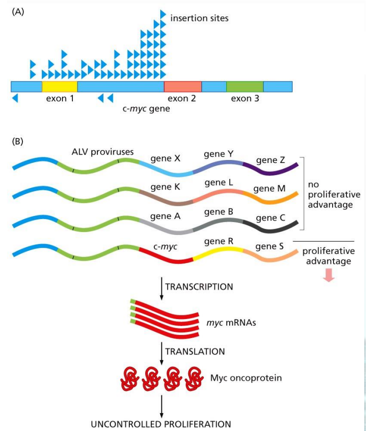
## The discovery of proto-oncogenes: a version of the src gene carried by RSV is also present in uninfected cells



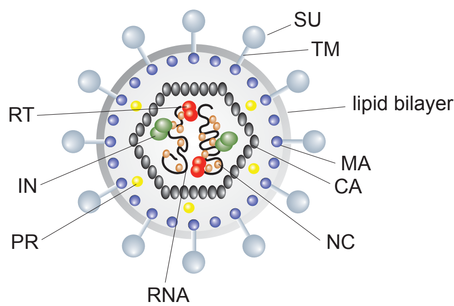

## Examples of retroviruses carrying viral oncogenes (v-onc)

| Parental /helper virus | Retrovirus | Acronym | v-onc |
|---|---|---|---|
| | Rous sarcoma virus | RSV | src |
| Avian leukosis virus (ALV) | Avian myeloblastosis virus | AMV | myb |
| | Avian erythroblastosis virus | AEV | erbA, B |
| | Avian myelocytomatosis virus 29 | AMCV-29 | myc |
| | Y73 sarcoma virus | Y73SV | yes |
| | Avian sarcoma virus 17 | ASV-17 | jun |
| Moloney-Murine leukemia virus (Mo-MLV) | Abelson murine leukemia virus | Ab-MLV | abl |
| | Harvey murine sarcoma virus | Ha-MSV | ras |
| | Moloney murine sarcoma virus | Mo-MSV | mos |
| | Finkel-Biskis-Jinkins murine sarcoma virus | FBJ-MSV | fos |
| Feline leukemia virus (FeLV) | Snyder-Theilen feline sarcoma virus | ST-FeSV | fes |
| | Gardner-Arnstein feline sarcoma virus | GA-FeSV | |
| | Susan McDonough feline sarcoma virus | SM-FeSV | fms |
| | Hardy-Zuckerman 4 feline sarcoma virus | HZ4-FeSV | kit |
| Simian sarcoma virus (SSV) | Woolly monkey sarcoma virus | WMSV | sis |

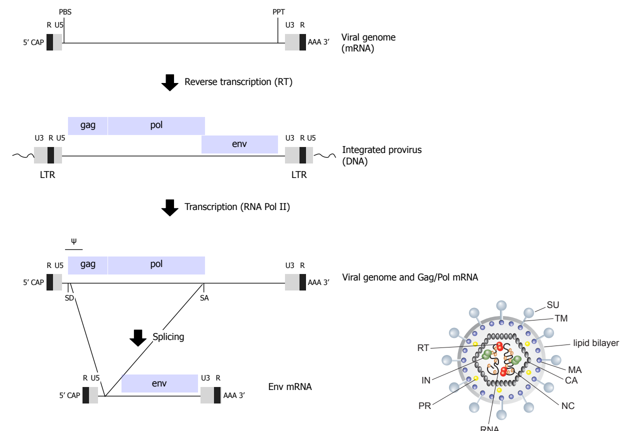
**Slowly transforming retroviruses activate protooncogenes by inserting their genomes adjacent to these cellular genes**



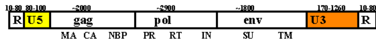

## Retrovirus virion



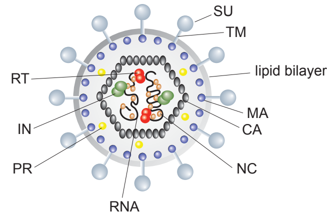

## Genetic organization of generalized retrovirus



## Genetic organization of generalized retrovirus

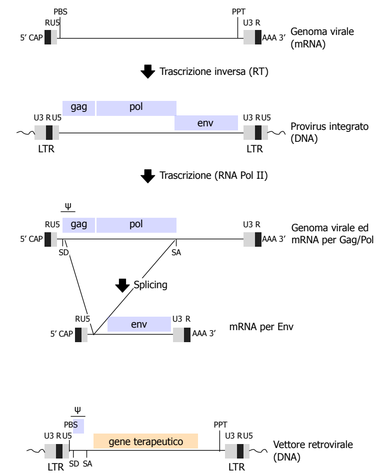



MA Matrix
CA Capsid
NBP Nuclear Binding Protein
PR Protease
RT Reverse Transcriptase
IN Integrase
SU Surface protein
TM Transmembrane Protein



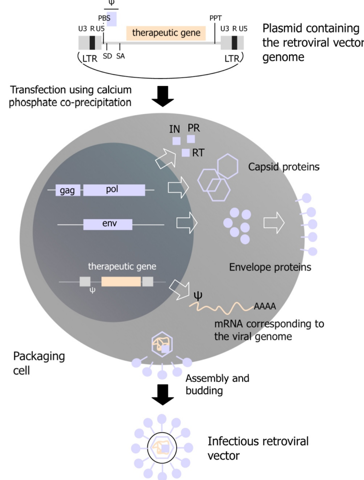

## Elementi genetici dei Retrovirus

La trascrizione parte da LTR del virus integrato

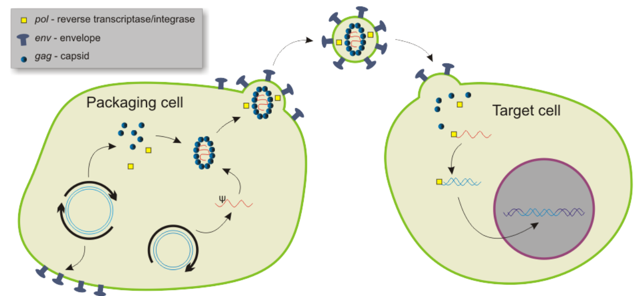



Nel vettore virale rimane:
– LTR che serve per la trascrizione,
– una regione di gag che serve per incapsidamento
– una porzione che produce un tRNA che funziona da primer per la trascrittasi inversa

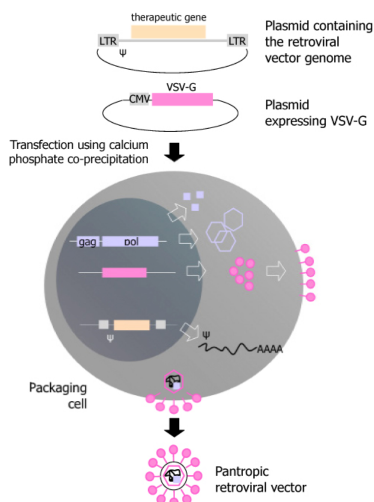
## Packaging of gammaretroviral vectors




## Retroviral vector integration results in transgene transcription (no additional particles produced)



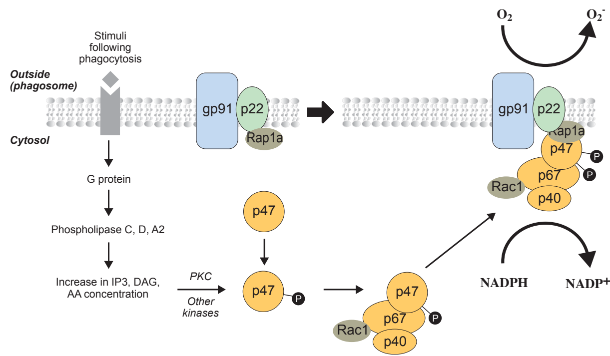

## Pseudotyping of gammaretroviral vectors



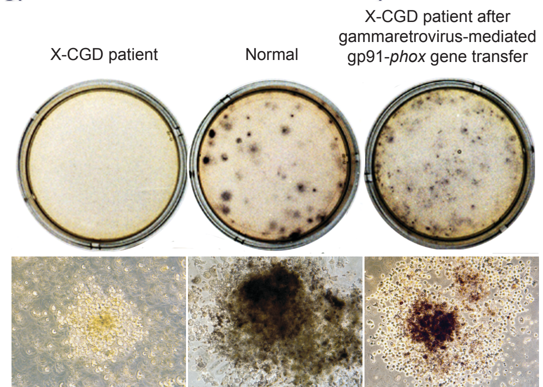

## Monogenic hereditary disorders for which gene therapy clinical trials were conducted by gene transfer into HSCs

| Disease group | Disease | Defective gene |
|---|---|---|
| Severe combined immunodeficiency syndromes (SCID) | SCID-X1 | Gamma common ($\gamma$c) chain of interleukin receptors |
| | ADA-SCID | Adenosine deaminase |
| | | JAK-3 |
| | PNP-SCID | Purine-nucleoside phosphorylase (PNP) |
| Lysosomal storage disorders | Hurler's disease (MPS I) | $\alpha$-L-iduronidase |
| | Hunter's disease (MPS II) | Iduronate-2-sulfatase |
| | Gaucher's disease | Glucocerebrosidase ($\beta$-glucosidase) |
| | Fabry's disease | $\alpha$-galactosidase A |
| | Sly syndrome (MPS VII) | $\beta$-glucuronidase |
| Defects of phagocytes | Chronic granulomatous disease (CGD) | $gp91^{phox}$, $p47^{phox}$ |
| | Leukocyte adhesion disorder | CD18 ($\beta$2-integrin) |
| Other diseases | Fanconi anemia, group C | FANCC |

## Activation of phagocyte NAPDH oxidase




## Functional correction of NAPDH activity in myeloid colonies from an X-CGD patient after gene transfer of the gp91phox cDNA into CD34+ hematopoietic stem cells

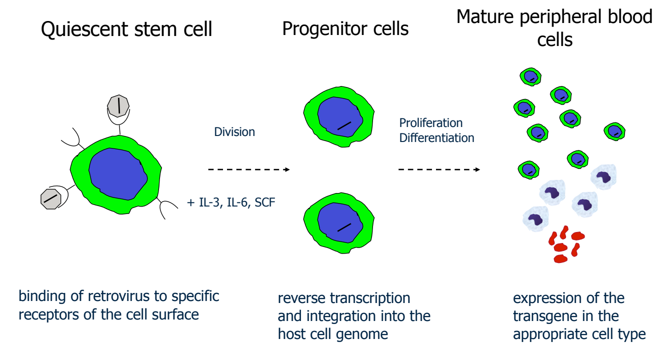



Zentilin, L, et al. 1996. Exp. Cell. Res. 225, 257.

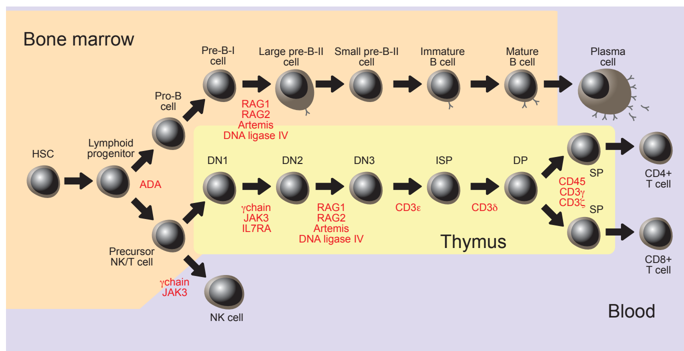
## Gene therapy of hematopoietic stem cells: Conclusions from clinical trials

- Virus-positive cells are detectable in peripheral blood after several years from treatment
- Only a very small fraction (0.01-0.1%) of reconstituting HSCs are transduced with the currently available protocols

## Gene therapy of hematopoietic stem cells

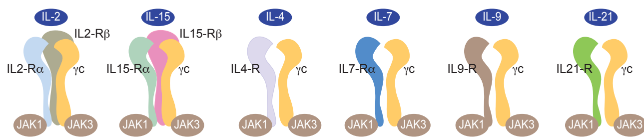



## Defects leading to the development of severe combined immunodeficiency (SCID)



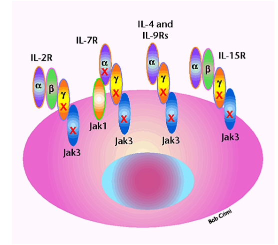

## SCID-X1, the bubble boy disease

## Molecular structure of the interleukin receptors




## $\gamma_c$ gene therapy trial

A. Fisher, Paris 2000




**Eligibility**

SCID-X1 (proven $\gamma_c$ gene mutation)
Lack of an HLA identical donor

**Protocol**

Bone marrow harvesting (30-100 ml)
CD34+ cell separation (immunomagnetic micro beads)
One day pre-activation with SCF, FLT 3L, IL-3 and MGDF
Three rounds of infection with the MFG $\gamma_c$ vector-containing supernatants in CH-296 fibronectin fragment-coated bags
I.V. infusion

## Gene Therapy of Human Severe Combined Immunodeficiency (SCID)–X1 Disease


Marina Cavazzana-Calvo,*[1,2,3] Salima Hacein-Bey,*[1,2,3] Geneviève de Saint Basile,[1] Fabian Gross,[2] Eric Yvon,[3] Patrick Nusbaum,[2] Françoise Selz,[1] Christophe Hue,[1,2] Stéphanie Certain,[1] Jean-Laurent Casanova,[1,4] Philippe Bousso,[5] Françoise Le Deist,[1] Alain Fischer[1,2,4]†



Severe combined immunodeficiency–X1 (SCID-X1) is an X-linked inherited disorder characterized by an early block in T and natural killer (NK) lymphocyte differentiation. This block is caused by mutations of the gene encoding the γc cytokine receptor subunit of interleukin-2, -4, -7, -9, and -15 receptors, which participates in the delivery of growth, survival, and differentiation signals to early lymphoid progenitors. After preclinical studies, a gene therapy trial for SCID-X1 was initiated, based on the use of complementary DNA containing a defective γc Moloney retrovirus–derived vector and ex vivo infection of CD34+ cells. After a 10-month follow-up period, γc transgene–expressing T and NK cells were detected in two patients. T, B, and NK cell counts and function, including antigen-specific responses, were comparable to those of age-matched controls. Thus, gene therapy was able to provide full correction of disease phenotype and, hence, clinical benefit.



Science. 2000. Vol. 288, pp. 669-672


## SCID-X1 has been a suitable and attractive setting for the clinical translation of targeted gene correction strategies and adoptive transfer of gene-corrected cells, as cells bearing a functional gamma chain show a positive selective advantage in vivo in the affected patients

## Leukemia case triggers tighter gene-therapy controls

Trials of gene therapy for SCID were halted in the United States and France following the report that a three-year-hold patient treated by Alain Fischer in Paris had developed leukemia after being treated with a retroviral vector (ex *vivo* transduction of bone marrow stem cells).

In October 2002 an advisory committee to the FDA ruled that gene therapy trials of that kind should now continue. However, there must be increased monitoring for adverse events (abnormal activity of certain cells, integration sites), and patients must receive modified informed consent forms to explain the chances of this side effect occurring. "One adverse event, as serious as it is, in the context of the whole field us not enough to put all programs on hold".

PA Photos
A baby cured of SCID by gene therapy

Using a PCR-based technique, it was discovered that the retroviral vector had inserted into more than 40 sites in the genome of different repopulating cells. In the T-cell clone that grew abnormally, it had inserted in the *LMO-2* oncogene, causing increased expression of the gene. Increased activity of the T-cell clone carrying the *LMO-2* integration was detected in blood samples taken from the boy as early as 13 months after treatment, well before he showed any clinical symptoms. However, this event was probably not sufficient for leukemia, but a second event was required for cancer to ensue.
Another question is the possibility that the boy had a genetic predisposition to leukemia, as there have been two childhood cancers in the family.

December 2002

## Second Child in French Trial Is Found to Have Leukemia

**Gene Therapy Studies Under Review**

| Lead investigator | Institution | Disease | Status |
|---|---|---|---|
| A. Fischer | Necker Hospital, Paris | X-SCID | on hold |
| H. Malech and J. Puck | NIH | X-SCID | on hold |
| B. Sorrentino and R. Buckley | St. Jude Children's, Memphis; Duke | JAK-3 deficiency | on hold |
| A. Thrasher | Inst. of Child Health, London | X-SCID | on hold |
| K. Weinberg and D. Kohn | NIH and Children's Hospital, LA | X-SCID | on hold |
| F. Condotti and D. Kohn | NIH and Children's Hospital, LA | ADA-SCID | on hold |
| C. Bordignon | San Raffaele Institute, Milan | ADA-SCID | on hold |

SERIE GENERALE

Spediz. abb. post. 45% - art. 2, comma 20/b
Legge 23-12-1996, n. 662 - Filiale di Roma

Anno 143° — Numero 264

# GAZZETTA UFFICIALE
## DELLA REPUBBLICA ITALIANA

PARTE PRIMA — Roma - Lunedì, 11 novembre 2002 — SI PUBBLICA TUTTI I GIORNI NON FESTIVI

DIREZIONE E REDAZIONE PRESSO IL MINISTERO DELLA GIUSTIZIA - UFFICIO PUBBLICAZIONE LEGGI E DECRETI - VIA ARENULA 70 - 00100 ROMA
AMMINISTRAZIONE PRESSO L'ISTITUTO POLIGRAFICO E ZECCA DELLO STATO - LIBRERIA DELLO STATO - PIAZZA G. VERDI 10 - 00100 ROMA - CENTRALINO 06 85081

La **Gazzetta Ufficiale,** *oltre alla* **Serie generale,** *pubblica quattro Serie speciali, ciascuna contraddistinta con autonoma numerazione:*
- **1ª Serie speciale:** *Corte costituzionale* (pubblicata il mercoledì)
- **2ª Serie speciale:** *Comunità europee* (pubblicata il lunedì e il giovedì)
- **3ª Serie speciale:** *Regioni* (pubblicata il sabato)
- **4ª Serie speciale:** *Concorsi ed esami* (pubblicata il martedì e il venerdì)

SOMMARIO


DECRETI PRESIDENZIALI

DECRETO DEL PRESIDENTE DEL CONSIGLIO DEI MINISTRI 11 ottobre 2002.
**Adozione di un emblema rappresentativo da parte del Dipartimento della protezione civile della Presidenza del Consiglio dei Ministri** . . . Pag. 4

DECRETO DEL PRESIDENTE DEL CONSIGLIO DEI MINISTRI 4 novembre 2002.
**Modifica del decreto relativo alla Commissione nazionale per la previsione e la prevenzione dei grandi rischi** . . . Pag. 7

DECRETI, DELIBERE E ORDINANZE MINISTERIALI

Ministero dell'economia e delle finanze

DECRETO 23 ottobre 2002.
**Indicazione del prezzo medio ponderato dei buoni ordinari del Tesoro a novantadue e trecentosessantacinque giorni relativi all'emissione del 15 ottobre 2002** . . . Pag. 7

DECRETO 28 ottobre 2002.
**Riapertura delle operazioni di sottoscrizione dei buoni del Tesoro poliennali 3,50%, con godimento 15 settembre 2002 e scadenza 15 settembre 2005, settima e ottava tranche** . . . Pag. 8

DECRETO 7 novembre 2002.
**Emissione di buoni ordinari del Tesoro a novantuno giorni.** . . . Pag. 9

DECRETO 7 novembre 2002.
**Emissione di buoni ordinari del Tesoro a trecentosessantaquattro giorni** . . . Pag. 10

Ministero della salute

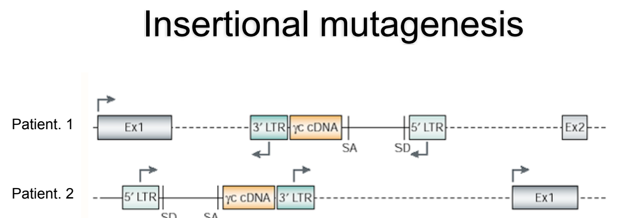
ORDINANZA 10 ottobre 2002.
**Sospensione su tutto il territorio nazionale delle sperimentazioni cliniche con prodotti per terapia genica, che prevedono l'impiego di vettori retrovirali** . . . Pag. 10

Ministero delle attività produttive

DECRETO 22 ottobre 2002.
**Proroga della gestione commissariale della società cooperativa agricola «Valcivica», in Vetralla** . . . Pag. 11

DECRETO 31 ottobre 2002.
**Rettifica all'allegato n. 2 al decreto ministeriale 8 ottobre 2002, recante approvazione delle proposte formulate dalle regioni e dalle province autonome di Trento e Bolzano ai sensi del decreto ministeriale 3 luglio 2000, concernente il testo unico delle direttive per la concessione e l'erogazione delle agevolazioni alle attività produttive nelle aree depresse di cui alla legge n. 488/1992, riferite alle domande presentate per il bando del 2002 del settore industria** . . . Pag. 11


# Insertional mutagenesis




- LMO2 encodes a LIM domain protein that binds to transcription factors SCL/TAL1, GATA1, GATA2
- Expressed by haematopoietic progenitors and cells of myeloid lineage, but not in post-thymic T cells
- LMO2 is activated in childhood ALL and in other spontaneous human T cell leukaemias
- LMO2 is leukaemogenic when overexpressed in transgenic mice

# Good news for gene therapy

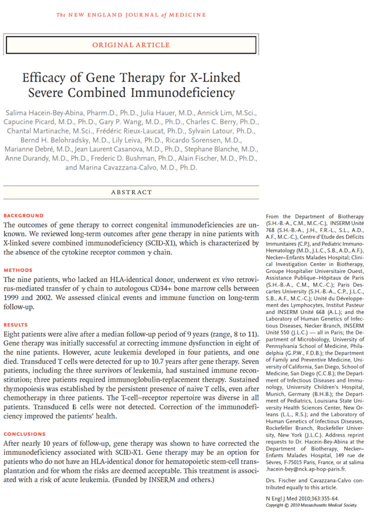
## Gene Therapy Insertional Mutagenesis Insights

**Utpal P. Davé, Nancy A. Jenkins, Neal G. Copeland***

SCIENCE VOL 303 16 JANUARY 2004

**The finding that a retrovirally induced mouse leukaemia contains integrations at both *Lmo2* and γc loci provided genetic evidence for cooperativity between LMO2 and γc**

In most gene therapy trials, the transplanted gene is unlikely to be oncogenic and occurrences of insertional mutagenesis will be low, as has been seen in trials conducted during the past several years



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

## Efficacy of Gene Therapy for X-Linked Severe Combined Immunodeficiency

Salima Hacein-Bey-Abina, Pharm.D., Ph.D., Julia Hauer, M.D., Annick Lim, M.Sci., Capucine Picard, M.D., Ph.D., Gary P. Wang, M.D., Ph.D., Charles C. Berry, Ph.D., Chantal Martinache, M.Sci., Frédéric Rieux-Laucat, Ph.D., Sylvain Latour, Ph.D., Bernd H. Belohradsky, M.D., Lily Leiva, Ph.D., Ricardo Sorensen, M.D., Marianne Debré, M.D., Jean Laurent Casanova, M.D., Ph.D., Stephane Blanche, M.D., Anne Durandy, M.D., Ph.D., Frederic D. Bushman, Ph.D., Alain Fischer, M.D., Ph.D., and Marina Cavazzana-Calvo, M.D., Ph.D.

ABSTRACT

**BACKGROUND**
The outcomes of gene therapy to correct congenital immunodeficiencies are unknown. We reviewed long-term outcomes after gene therapy in nine patients with X-linked severe combined immunodeficiency (SCID-X1), which is characterized by the absence of the cytokine receptor common γ chain.

**METHODS**
The nine patients, who lacked an HLA-identical donor, underwent ex vivo retrovirus-mediated transfer of γ chain to autologous CD34+ bone marrow cells between 1999 and 2002. We assessed clinical events and immune function on long-term follow-up.

**RESULTS**
Eight patients were alive after a median follow-up period of 9 years (range, 8 to 11). Gene therapy was initially successful at correcting immune dysfunction in eight of the nine patients. However, acute leukemia developed in four patients, and one died. Transduced T cells were detected for up to 10.7 years after gene therapy. Seven patients, including the three survivors of leukemia, had sustained immune reconstitution; three patients required immunoglobulin-replacement therapy. Sustained thymopoiesis was established by the persistent presence of naive T cells, even after chemotherapy in three patients. The T-cell–receptor repertoire was diverse in all patients. Transduced B cells were not detected. Correction of the immunodeficiency improved the patients' health.

**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 the Department of Biotherapy (S.H.-B.-A., C.M., M.C.-C.), INSERM Unité 768 (S.H.-B.-A., J.H., F.R.-L., S.L., A.D., A.F., M.C.-C.), Centre d'Etude des Déficits Immunitaires (C.P.), and Pediatric Immuno-Hematology (M.D., J.L.C., S.B., A.D., A.F.), Necker–Enfants Malades Hospital; Clinical Investigation Center in Biotherapy, Groupe Hospitalier Universitaire Ouest, Assistance Publique–Hôpitaux de Paris (S.H.-B.-A., C.M., M.C.-C.); Paris Descartes University (S.H.-B.-A., C.P., J.L.C., S.B., A.F., M.C.-C.); Unité du Développement des Lymphocytes, Institut Pasteur and INSERM Unité 668 (A.L.); and the Laboratory of Human Genetics of Infectious Diseases, Necker Branch, INSERM Unité 550 (J.L.C.) — all in Paris; the Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia (G.P.W., F.D.B.); the Department of Family and Preventive Medicine, University of California, San Diego, School of Medicine, San Diego (C.C.B.); the Department of Infectious Diseases and Immunology, University Children's Hospital, Munich, Germany (B.H.B.); the Department of Pediatrics, Louisiana State University Health Sciences Center, New Orleans (L.L., R.S.); and the Laboratory of Human Genetics of Infectious Diseases, Rockefeller Branch, Rockefeller University, New York (J.L.C.). Address reprint requests to Dr. Hacein-Bey-Abina at the Department of Biotherapy, Necker–Enfants Malades Hospital, 149 rue de Sèvres, F-75015 Paris, France, or at salima.hacein-bey@nck.ap-hop-paris.fr.

Drs. Fischer and Cavazzana-Calvo contributed equally to this article.

N Engl J Med 2010;363:355-64.
Copyright © 2010 Massachusetts Medical Society.

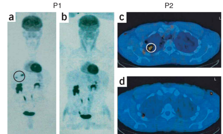



**Figure 6** Fused PET scans of P1 (**a**,**b**) and fused PET-CT scans of P2 (**c**,**d**) before (**a**,**c**) and 50 (**b**) or 53 d (**d**) after gene therapy. Circle in **a** denotes two active abscesses due to *Staphylococcus aureus* infection in the liver of P1, and the circle in **c** shows $^{18}$F-FDG uptake in the wall of a lung cavity of P2 due to *A. fumigatus* infection.

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

Marion G Ott[1,16], Manfred Schmidt[2–4,16], Kerstin Schwarzwaelder[3–5,16], Stefan Stein[6,16], Ulrich Siler[7,16], Ulrike Koehl[8], Hanno Glimm[2,3], Klaus Kühlcke[9], Andrea Schilz[9], Hana Kunkel[6], Sonja Naundorf[9], Andrea Brinkmann[8], Annette Deichmann[3,4], Marlene Fischer[2,3,5], Claudia Ball[3–5], Ingo Pilz[3,5], Cynthia Dunbar[10], Yang Du[11], Nancy A Jenkins[11], Neal G Copeland[11], Ursula Lüthi[12], Moustapha Hassan[13], Adrian J Thrasher[14], Dieter Hoelzer[1], Christof von Kalle[2–4,15,16], Reinhard Seger[7,16] & Manuel Grez[6,16]

**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 gp91$^{phox}$. 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.**

NATURE MEDICINE VOLUME 12 | NUMBER 4 | APRIL 2006

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

**Alessandro Aiuti,[1] Shimon Slavin,[2] Memet Aker,[2] Francesca Ficara,[1] Sara Deola,[1] Alessandra Mortellaro,[1] Shoshana Morecki,[2] Grazia Andolfi,[1] Antonella Tabucchi,[3] Filippo Carlucci,[3] Enrico Marinello,[3] Federica Cattaneo,[1] Sergio Vai,[1] Paolo Servida,[4] Roberto Miniero,[5] Maria Grazia Roncarolo,[1,6] * Claudio Bordignon[1,6]*†**

Science, 2002

The first report of immune restoration in 2 patients with ADA-deficient SCID

These subjects were not treated with PEG-ADA enzyme-replacement therapy, thought to reduce selective advantage of the genetically corrected cells

These subjects received BM cytoreduction with a moderate dosage of busulphan, which could promote engraftment of the genetically modified cells

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 JANUARY 29, 2009 VOL. 360 NO. 5

# Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

Alessandro Aiuti, M.D., Ph.D., Federica Cattaneo, M.D., Stefania Galimberti, Ph.D., Ulrike Benninghoff, M.D., Barbara Cassani, Ph.D., Luciano Callegaro, R.N., Samantha Scaramuzza, Ph.D., Grazia Andolfi, Massimiliano Mirolo, B.Sc., Immacolata Brigida, B.Sc., Antonella Tabucchi, Ph.D., Filippo Carlucci, Ph.D., Martha Eibl, M.D., Memet Aker, M.D., Shimon Slavin, M.D., Hamoud Al-Mousa, M.D., Abdulaziz Al Ghonaium, M.D., Alina Ferster, M.D., Andrea Duppenthaler, M.D., Luigi Notarangelo, M.D., Uwe Wintergerst, M.D., Rebecca H. Buckley, M.D., Marco Bregni, M.D., Sarah Marktel, M.D., Maria Grazia Valsecchi, Ph.D., Paolo Rossi, M.D., Fabio Ciceri, M.D., Roberto Miniero, M.D., Claudio Bordignon, M.D., and Maria-Grazia Roncarolo, M.D.

## ABSTRACT

**BACKGROUND**

We investigated the long-term outcome of gene therapy for severe combined immunodeficiency (SCID) due to the lack of adenosine deaminase (ADA), a fatal disorder of purine metabolism and immunodeficiency.

**METHODS**

We infused autologous CD34+ bone marrow cells transduced with a retroviral vector containing the *ADA* gene into 10 children with SCID due to ADA deficiency who lacked an HLA-identical sibling donor, after nonmyeloablative conditioning with busulfan. Enzyme-replacement therapy was not given after infusion of the cells.

**RESULTS**

All patients are alive after a median follow-up of 4.0 years (range, 1.8 to 8.0). Transduced hematopoietic stem cells have stably engrafted and differentiated into myeloid cells containing ADA (mean range at 1 year in bone marrow lineages, 3.5 to 8.9%) and lymphoid cells (mean range in peripheral blood, 52.4 to 88.0%). Eight patients do not require enzyme-replacement therapy, their blood cells continue to express ADA, and they have no signs of defective detoxification of purine metabolites. Nine patients had immune reconstitution with increases in T-cell counts (median count at 3 years, $1.07 \times 10^9$ per liter) and normalization of T-cell function. In the five patients in whom intravenous immune globulin replacement was discontinued, antigen-specific antibody responses were elicited after exposure to vaccines or viral antigens. Effective protection against infections and improvement in physical development made a normal lifestyle possible. Serious adverse events included prolonged neutropenia (in two patients), hypertension (in one), central-venous-catheter–related infections (in two), Epstein–Barr virus reactivation (in one), and autoimmune hepatitis (in one).

**CONCLUSIONS**

Gene therapy, combined with reduced-intensity conditioning, is a safe and effective treatment for SCID in patients with ADA deficiency. (ClinicalTrials.gov numbers, NCT00598481 and NCT00599781.)

From the San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET) (A.A., F.C., U.B., B.C., L.C., S. Scaramuzza, G.A., M.M., I.B., S.M., M.-G.R.), University of Milan-Bicocca (S.G., M.G.V.), Ospedale San Giuseppe (M.B.), San Raffaele Scientific Institute (F.C.), Università Vita–Salute San Raffaele (C.B., M.-G.R.), and MolMed (C.B.) — all in Milan; Tor Vergata University (A.A., P.R.) and Children's Hospital Bambino Gesù (P.R.) — both in Rome; University of Siena, Siena (A.T., F.C.); and University of Turin, Turin (R.M.) — all in Italy; Immunologische Tagesklinik, Vienna, Austria (M.E.); Hadassah University Hospital, Jerusalem, Israel (M.A., S. Slavin); King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia (H.A.-M., A.A.G.); Hôpital Universitaire des Enfants Reine Fabiola–Université Libre de Bruxelles, Brussels (A.F.); University Children's Hospital, Bern, Switzerland (A.D.); Children's Hospital, Harvard Medical School, Boston (L.N.); Universitäts-Kinderklinik München, Munich, Germany (U.W.); and Duke University Medical Center, Durham, NC (R.H.B.). Address reprint requests to Dr. Roncarolo at HSR-TIGET, Via Olgettina 58, 20132 Milan, Italy, or at m.roncarolo@hsr.it.

N Engl J Med 2009;360:447-58.

Copyright © 2009 Massachusetts Medical Society.

**Table 3. Long-Term Immune Reconstitution after Gene Therapy.***

| Variable | Patients with Normal Value (no./total no.) |
|---|---|
| Cell count | |
| CD3+ T cells | 5/9 |
| CD4+ T cells | 4/9 |
| Natural killer cells | 3/9 |
| B cells | 4/9 |
| In vitro proliferative responses | |
| PHA mitogen | 9/9 |
| Anti-CD3 mitogen | 9/9 |
| *Candida albicans* | 7/9 |
| Alloantigens | 8/9 |
| TT | 5/5 |
| Serum immunoglobulins | |
| IgG | 5/9 |
| IgM | 7/9 |
| IgA | 5/9 |
| Antibodies to specific antigens | |
| Vaccine including TT, DT, BPT, and Hib | 5/5 |
| Pneumococcus (IgM) | 4/5 |
| MMR vaccine or other viral antigens† | 5/5 |

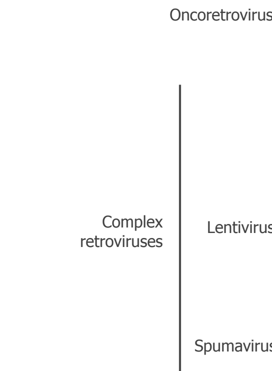
* Results are from the most recent time point at which the patient was not receiving polyethylene glycol–modified bovine adenosine deaminase (PEG-ADA). Data for Patient 8 are not included here; this patient was evaluated for safety only because PEG-ADA was reintroduced 0.4 year after gene therapy. Some results are listed only for the five patients whose serum IgG levels were within the normal range after discontinuation of intravenous immune globulin supplementation. The normal values of cell counts, serum immunoglobulin levels, and in vitro proliferative responses are those reported for age-matched subjects (Eibl et al.,[19] Comans-Bitter et al.,[24] and laboratory controls). BPT denotes *Bordetella pertussis* toxin; DT diphtheria toxin; Hib *Haemophilus influenzae* type b; MMR measles, mumps, and rubella; PHA phytohemagglutinin; and TT tetanus toxoid.

† Viral antigens consisted of antibodies against varicella, Epstein–Barr virus, or cytomegalovirus.

# Retroviruses: historical introduction

- 1908 Chicken leukosis is caused by a virus (Ellerman and Bang)
- 1911 Cell-free transmission of sarcoma in chickens (Rous)
- 1936 Mammary carcinoma in mice caused by a filterable agent (Bittner)
- 1958 Development of the focus assay for RSV (Temin and Rubin)
- 1964 Provirus hypothesis (generation of viral DNA copy and integration in cellular genome) (Temin)
- 1970 Reverse Transcriptase (Temin and Mizutani; Baltimore)
- 1976 Probe for src oncogene hybridizes with cellular DNA (Stehelin)
- 1980/82: First human retrovirus (HTLV-I)
- 1983: HIV-1

# Modern taxonomy of the *Retroviridae* family

| Subfamily | Genus | Former classifications | Main species | Prototype viruses |
|---|---|---|---|---|
| *Orthoretrovirinae* | Alpharetrovirus | Avian type C retroviruses; Avian sarcoma/leukosis viruses (ASLV) | Avian leukosis virus | ALV |
| | | | Rous sarcoma virus | RSV |
| | Betaretrovirus | Mammalian type B retroviruses; Type D retroviruses | Mouse mammary tumor virus | MMTV |
| | | | Mason-Pfizer monkey virus | MPMV |
| | Gammaretrovirus | Mammalian type C retroviruses | Murine leukemia virus | Abelson-MLV, Friend-MLV, Moloney-MLV |
| | | | Feline leukemia virus | FeLV |
| | | | Gibbon ape leukemia virus | GaLV |
| | | | Harvey murine sarcoma virus | Ha-MSV |
| | | | Moloney murine sarcoma virus | Mo-MSV |
| | | | Simian sarcoma virus | SSV |
| | | | Reticuloendotheliosis virus | REV-A, REV-T |
| | Deltaretrovirus | BLV-HLTV group retroviruses | Bovine leukemia virus | BLV |
| | | | Primate T-lymphotropic viruses (human and simian) | HTLV-1, STLV-1, HTLV-2, STLV-2, STLV-3 |
| | Epsilonretrovirus | Fish retroviruses | Walleye dermal sarcoma virus | WDSV |
| | Lentivirus | | Bovine immunodeficiency virus | BIV |
| | | | Equine infectious anemia virus | EIAV |
| | | | Feline immunodeficiency virus | FIV-O, FIV-P |
| | | | Caprine arthritis encephalitis virus | CAEV |
| | | | Visna/Maedi virus | VISNA |
| | | | Human immunodeficiency virus 1 and 2 | HIV-1, HIV-2 |
| | | | Simian immunodeficiency virus | SIV-agm.155, SIV-cpz, SIV-mac |
| *Spumaretrovirinae* | Spumavirus |  | Simian foamy virus | SFVmac (SFV-1 and SFV-2), SFVagm (SFV-3), SFVcpz and SFVcpz(hu) |
| | | | Bovine foamy virus | BFV |
| | | | Equine foamy virus | EFV |
| | | | Feline foamy virus | FFV |
| | | | Human foamy virus | HFV or HSRV |



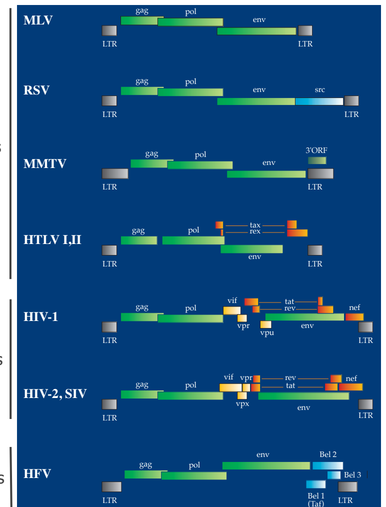
# Genetic organization of retroviruses

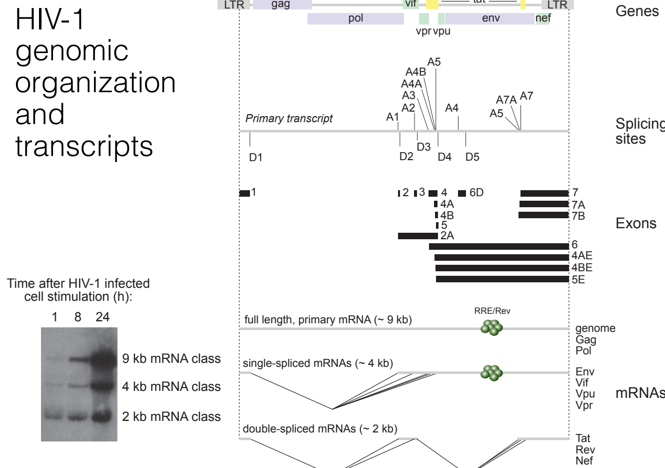
Oncoretrovirus

Complex retroviruses

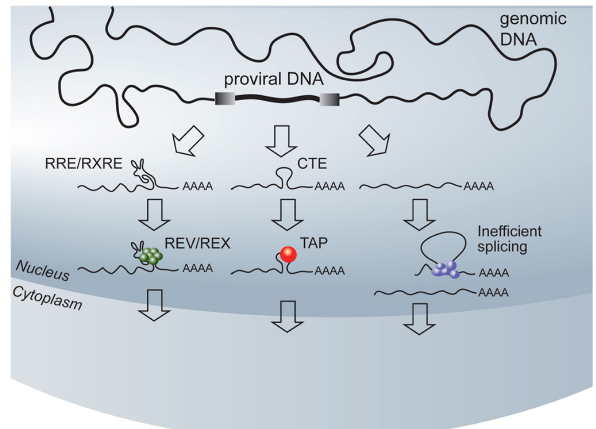
Lentivirus

Spumavirus

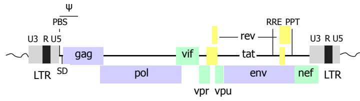


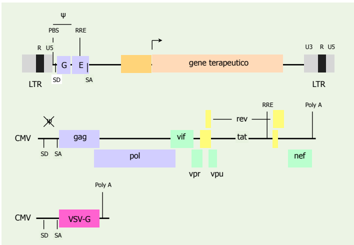

# Export of unspliced viral mRNAs outside the nucleus



# Vettori lentivirali




Vettori Lentivirali di prima generazione




3 plasmidi

1. segnali regolatori, sito di legame per REV (RRE), promotore e gene terapeutico

2. gag, pol e 6 geni accessori

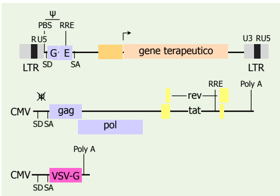
3. VSV-G (env di HIV lega CD4, espresso essenzialmente in linfociti e macrofagi)

# Safety Concerns Specific to Lentiviral vectors

- Recombination during manufacture may generate a replication-competent lentivirus (RCL)
  - HIV a known human pathogen
  - vesicular stomatitis virus (VSV-G) envelope broadens tropism
- Recombination with wild type virus in HIV+ subjects
- Lentiviral vector mobilization by wild type virus

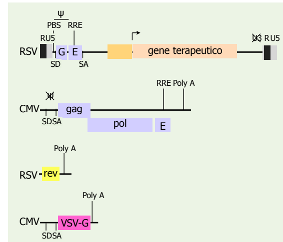
# Vettori lentivirali

Vettori lentivirali di seconda generazione




assenza dei fattori di virulenza vif, vpr, vpu, nef

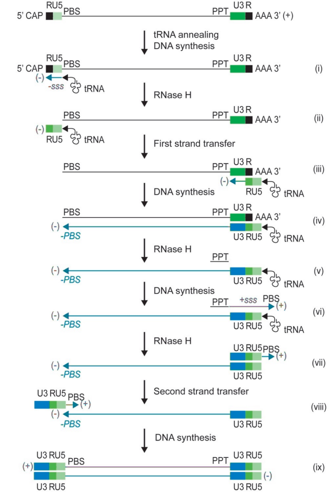
Vettori lentivirali di terza generazione



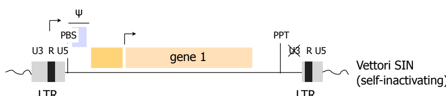

rev in un plasmide separato: ridotta probabilità di ricombinazione

tat non necessaria se trascrizione attivata da CMV

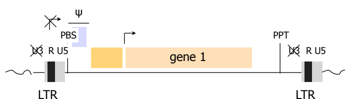
# Reverse transcription




# Variazioni nella costruzione dei vettori gammaretrovirali



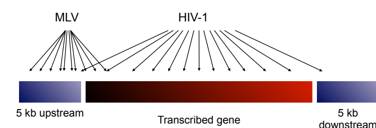

Trascrizione inversa




la delezione in U3 viene mantenuta quando il genoma viene retrotrascritto, il che distrugge l'attività di promotore/enhancer del LTR

# Retroviruses integrate near transcriptionally active regions of DNA

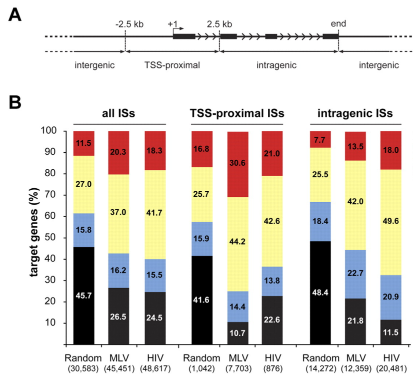
- Acceptor sites for retroviral integrations map near DNase I hypersensitive sites in chromatin (S. Vijaya et al. J. Virol. 1986)
- Retrovirus integration and chromatin structure: Moloney murine leukemia proviral integration sites map near DNase I-hypersensitive sites (H. Rohdewohld et al. J. Virol. 1987)
- Chromosome structure and human immunodeficiency type 1 cDNA integration: centromeric alphoid repeats are a disfavored target (S. Carteu et al. J. Virol. 1998)
- HIV-1 integration in the human genome favors active genes and local hotspots (A.R.W. Schroder et al. Cell 2002)



## Integration is not random

MLV: transcriptional start site

HIV: transcriptional units




# Lentiviral Hematopoietic Stem Cell Gene Therapy Benefits Metachromatic Leukodystrophy

Alessandro Biffi,[1,2,3]*† Eugenio Montini,[1]* Laura Lorioli,[1,2,3,4] Martina Cesani,[1] Francesca Fumagalli,[2,4,5] Tiziana Plati,[1] Cristina Baldoli,[6] Sabata Martino,[7] Andrea Calabria,[1] Sabrina Canale,[2] Fabrizio Benedicenti,[1] Giuliana Vallanti,[8] Luca Biasco,[1] Simone Leo,[9] Nabil Kabbara,[10] Gianluigi Zanetti,[9] William B. Rizzo,[11] Nalini A. L. Mehta,[12] Maria Pia Cicalese,[2,3] Miriam Casiraghi,[2] Jaap J. Boelens,[13] Ubaldo Del Carro,[5] David J. Dow,[12] Manfred Schmidt,[14] Andrea Assanelli,[3,15] Victor Neduva,[12] Clelia Di Serio,[8] Elia Stupka,[16] Jason Gardner,[17] Christof von Kalle,[14] Claudio Bordignon,[4,8] Fabio Ciceri,[3,15] Attilio Rovelli,[18] Maria Grazia Roncarolo,[1,2,3,19] Alessandro Aiuti,[1,2,3] Maria Sessa,[2,5] Luigi Naldini[1,4]†

Metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. Patients with MLD exhibit progressive motor and cognitive impairment and die within a few years of symptom onset. We used a lentiviral vector to transfer a functional *ARSA* gene into hematopoietic stem cells (HSCs) from three presymptomatic patients who showed genetic, biochemical, and neurophysiological evidence of late infantile MLD. After reinfusion of the gene-corrected HSCs, the patients showed extensive and stable *ARSA* gene replacement, which led to high enzyme expression throughout hematopoietic lineages and in cerebrospinal fluid. Analyses of vector integrations revealed no evidence of aberrant clonal behavior. The disease did not manifest or progress in the three patients 7 to 21 months beyond the predicted age of symptom onset. These findings indicate that extensive genetic engineering of human hematopoiesis can be achieved with lentiviral vectors and that this approach may offer therapeutic benefit for MLD patients.

# Lentiviral Hematopoietic Stem Cell Gene Therapy in Patients with Wiskott-Aldrich Syndrome

Alessandro Aiuti,[1,2,3,4]* Luca Biasco,[1]† Samantha Scaramuzza,[1]† Francesca Ferrua,[2,3,5] Maria Pia Cicalese,[2,3] Cristina Baricordi,[1] Francesca Dionisio,[1] Andrea Calabria,[1] Stefania Giannelli,[1] Maria Carmina Castiello,[1,5] Marita Bosticardo,[1] Costanza Evangelio,[2,3] Andrea Assanelli,[3,6] Miriam Casiraghi,[2] Sara Di Nunzio,[2] Luciano Callegaro,[2] Claudia Benati,[7] Paolo Rizzardi,[7] Danilo Pellin,[8] Clelia Di Serio,[8] Manfred Schmidt,[9] Christof Von Kalle,[9] Jason Gardner,[10] Nalini Mehta,[11] Victor Neduva,[11] David J. Dow,[11] Anne Galy,[12] Roberto Miniero,[13] Andrea Finocchi,[4] Ayse Metin,[14] Pinaki P. Banerjee,[15] Jordan S. Orange,[15] Stefania Galimberti,[16] Maria Grazia Valsecchi,[16] Alessandra Biffi,[1,2,3] Eugenio Montini,[1] Anna Villa,[1,17] Fabio Ciceri,[3,6] Maria Grazia Roncarolo,[1,2,3,5]‡ Luigi Naldini[1,5]‡

Wiskott-Aldrich syndrome (WAS) is an inherited immunodeficiency caused by mutations in the gene encoding WASP, a protein regulating the cytoskeleton. Hematopoietic stem/progenitor cell (HSPC) transplants can be curative, but, when matched donors are unavailable, infusion of autologous HSPCs modified ex vivo by gene therapy is an alternative approach. We used a lentiviral vector encoding functional WASP to genetically correct HSPCs from three WAS patients and reinfused the cells after a reduced-intensity conditioning regimen. All three patients showed stable engraftment of WASP-expressing cells and improvements in platelet counts, immune functions, and clinical scores. Vector integration analyses revealed highly polyclonal and multilineage haematopoiesis resulting from the gene-corrected HSPCs. Lentiviral gene therapy did not induce selection of integrations near oncogenes, and no aberrant clonal expansion was observed after 20 to 32 months. Although extended clinical observation is required to establish long-term safety, lentiviral gene therapy represents a promising treatment for WAS.

www.sciencemag.org SCIENCE VOL 341 23 AUGUST 2013

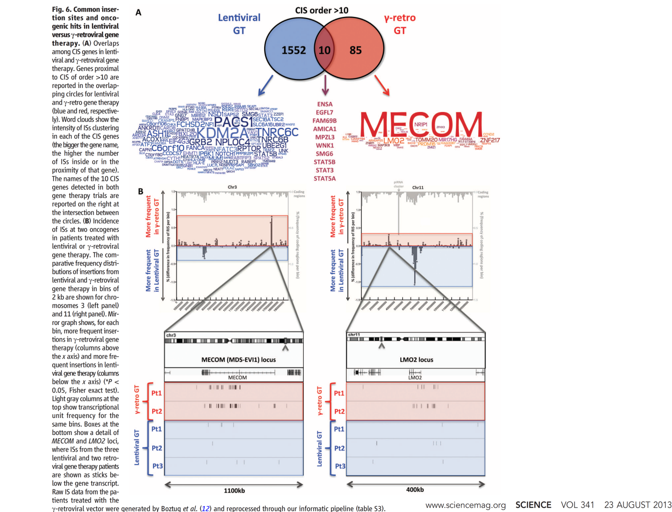



**Fig. 6. Common insertion sites and oncogenic hits in lentiviral versus γ-retroviral gene therapy.** (**A**) Overlaps among CIS genes in lentiviral and γ-retroviral gene therapy. Genes proximal to CIS of order >10 are reported in the overlapping circles for lentiviral and γ-retro gene therapy (blue and red, respectively). Word clouds show the intensity of ISs clustering in each of the CIS genes (the bigger the gene name, the higher the number of ISs inside or in the proximity of that gene). The names of the 10 CIS genes detected in both gene therapy trials are reported on the right at the intersection between the circles. (**B**) Incidence of ISs at two oncogenes in patients treated with lentiviral or γ-retroviral gene therapy. The comparative frequency distributions of ISs from lentiviral and γ-retroviral gene therapy in bins of 2 kb are shown for chromosomes 3 (left panel) and 11 (right panel). Mirror graph shows, for each bin, more frequent insertions in γ-retroviral gene therapy (columns above the *x* axis) and more frequent insertions in lentiviral gene therapy (columns below the *x* axis) (*$P < 0.05$, Fisher exact test). Light gray columns at the top show transcriptional unit frequency for the same bins. Boxes at the bottom show a detail of *MECOM* and *LMO2* loci, where ISs from the three lentiviral and two retroviral gene therapy patients are shown as sticks below the gene transcript. Raw IS data from the patients treated with the γ-retroviral vector were generated by Boztug *et al.* (12) and reprocessed through our informatic pipeline (table S3).

www.sciencemag.org SCIENCE VOL 341 23 AUGUST 2013

MEDICINE

# Gene Therapy That Works

Gene therapy trials show a beneficial effect in children suffering from a neurodegenerative disorder or an immunodeficiency disease.

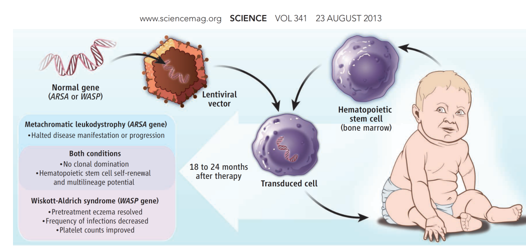
Inder M. Verma

The concept of gene therapy is disarmingly simple: Introduce a healthy gene in a patient and its product should alleviate the defect caused by a faulty gene or slow the progression of disease (*1*). Why then, over the past three decades, have there been so few clinical successes in treating patients with this approach? A major obstacle has been the delivery of genes to the appropriate cell, tissue, and organ. How does one introduce a gene into the brain with trillions of cells, or the liver with billions of cells, or the rare hematopoietic adult stem cell that has the potential to populate all lineages of lymphoid and myeloid cells? Much effort has been devoted to finding ways to efficiently deliver a therapeutic gene to the desired cell type, resulting in sustained production of the gene product, ideally through the entire life of the recipient, without unwanted side effects like genotoxicity or unsettling the immune balance (*2*). On pages 864 and 865 in this issue, Biffi *et al*. (*3*) and Aiuti *et al*. (*4*) report encouraging results using lentivirus-mediated gene therapy to treat children with rare genetic defects.

For scientists in the field of gene therapy, good news, tinged with occasional setbacks, has been trickling in over the past decade, starting with the successful clinical trials of children with X-linked severe combined immunodeficiency disease (SCID) (*5*). Currently, more than 1700 clinical trials are under way worldwide, drawing on a wide array of gene therapy approaches for both acquired and inherited diseases (*6*). The approach involves genetically engineering a virus so that it infects a target cell to deliver a gene, but does not cause disease. Retroviruses (such as lentiviruses) integrate their genetic material, including the new gene, in to the host cell genome. Such transduced host cells are transplanted back into the patient and proliferate with the correct gene, producing healthy cells (see the figure). Biffi *et al*. and Aiuti *et al*. provide new hope to children with metachromatic leu-

Laboratory of Genetics, The Salk Institute, La Jolla, CA 92037, USA. E-mail: verma@salk.edu

www.sciencemag.org SCIENCE VOL 341 23 AUGUST 2013




Leukemia (2018) 32:1529–1541
https://doi.org/10.1038/s41375-018-0106-0

REVIEW ARTICLE

Acute lymphoblastic leukemia

## Clinical use of lentiviral vectors

Michael C. Milone[1,2] · Una O'Doherty[1]

**Table 1** Ongoing clinical trials using lentiviral vectors to modify hematopoietic stem cells

| Condition | Phase | NCT number |
| --- | --- | --- |
| Transfusion-dependent β-thalassemia | 1/2 | NCT02453477 |
| | 3 | NCT02906202 |
| Cerebral adrenoleukodystrophy | 2/3 | NCT01896102 |
| Sickle cell disease | 1 | NCT02140554 |
| | 1 | NCT02193191 |
| Metachromatic leukodystrophy and adrenoleukodystrophy | 1/2 | NCT02559830 |
| Wiskott-Aldrich syndrome | 1/2 | NCT01347346 |
| | 1/2 | NCT01347242 |
| | 1/2 | NCT02333760 |
| X-SCID | 1/2 | NCT01306019 |
| | 1/2 | NCT01512888 |
| ADA-SCID | 1/2 | NCT02999984 |
| | 1/2 | NCT01380990 |
| Fanconi anemia | 2 | NCT02931071 |
| X-linked chronic granulomatous disease | 1/2 | NCT02234934 |

*ADA* adenosine deaminase, *SCID* severe combined immunodeficiency

GENE THERAPY

# Targeting β-thalassaemia

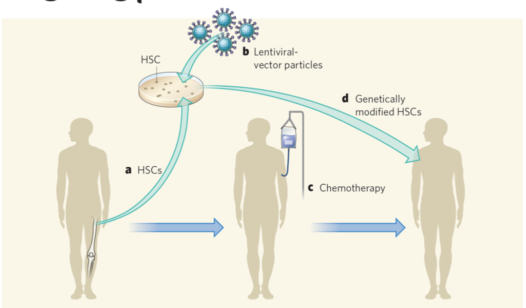



**Figure 1 | Gene-therapy procedure. a,** Cavazzana-Calvo *et al.*[2] collected haematopoietic stem cells (HSCs) from the bone marrow of a patient with β-thalassaemia and maintained them in culture. **b,** The authors then introduced lentiviral-vector particles containing a functional β-globin gene into 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.

NATURE | Vol 467 | 16 September 2010

nature Vol 467 | 16 September 2010 | doi:10.1038/nature09328

LETTERS

## Transfusion independence and *HMGA2* activation after gene therapy of human β-thalassaemia

Marina Cavazzana-Calvo[1,2]*, Emmanuel Payen[3,4,5]*, Olivier Negre[3,4,5,6], Gary Wang[7], Kathleen Hehir[8], Floriane Fusil[3,4,5], Julian Down[8], Maria Denaro[8], Troy Brady[7], Karen Westerman[8,9], Resy Cavallesco[9], Beatrix Gillet-Legrand[6], Laure Caccavelli[1,2], Riccardo Sgarra[10], Leila Maouche-Chrétien[3,4], Françoise Bernaudin[11], Robert Girot[12], Ronald Dorazio[8], Geert-Jan Mulder[8], Axel Polack[8], Arthur Bank[13], Jean Soulier[5], Jérôme Larghero[5], Nabil Kabbara[5], Bruno Dalle[5], Bernard Gourmel[5], Gérard Socie[5], Stany Chrétien[3,4,9], Nathalie Cartier[14], Patrick Aubourg[14], Alain Fischer[1,2], Kenneth Cornetta[15], Frédéric Galacteros[16], Yves Beuzard[3,4,5], Eliane Gluckman[5], Frederick Bushman[7], Salima Hacein-Bey-Abina[1,2]* & Philippe Leboulch[3,4,9]*

**The β-haemoglobinopathies are the most prevalent inherited disorders worldwide. Gene therapy of β-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 diasporas[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[1,2]. When this is compounded with a non-functional $\beta^0$ allele, a profound decrease in β-globin synthesis results, and approximately half of $\beta^E/\beta^0$-thalassaemia patients are transfusion-dependent[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 β-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$^{-1}$, of which one-third contains vector-encoded β-globin. Most of the therapeutic benefit results from a dominant, myeloid-biased cell clone, in which the integrated vector causes transcriptional activation of *HMGA2* in erythroid cells with further increased expression of a truncated *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 *HMGA2* gene in stem/progenitor cells.**

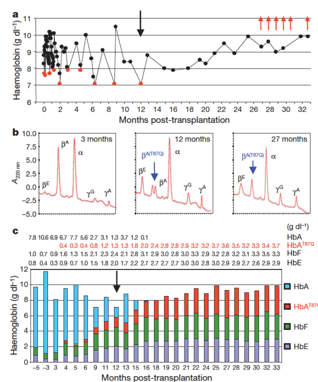



**Figure 1 | Conversion to transfusion independence.** **a**, Total Hb concentrations in whole blood. Red dots, transfusion time points; black vertical arrow, the last time the patient was transfused; red arrows, phlebotomies (200 ml each) to remove excess iron. **b**, HPLC blood globin chain profiles. Note that $\beta^A$ only derives from blood transfusions. **c**, Contribution of each Hb species, quantified by HPLC, to total blood Hb concentrations (in g dl$^{-1}$). Actual numbers for each Hb species are indicated above the chart.

## Lentiviral Vector Generations Summary Table

| | First Generation | Second Generation | Third Generation |
|---|---|---|---|
| Plasmids | 3 | 3 | 4 |
| Deletion in 3' LTR - SIN | No | No | Yes |
| Packaging plasmids with HIV genes | 1 | 1 | 2 |
| Accessory genes: vif, vpr, vpu, nef | All absent | All absent | All absent |
| tat and rev genes | On a single packaging plasmid | On a single packaging plasmid | tat is absent; rev on a separate plasmid |
| gag and pol genes | Same plasmid | Same plasmid | Same plasmid |
| Recombination events needed to generate Replication Competent Lentiviruses (RCL)* | 2 recombinations | 3 recombinations | 4 recombinations between plasmids without homology & must pick a promoter to complement SIN deletion |

## Pro and cons of lentiviral vectors

| Pros | Cons |
|---|---|
| Can carry large transgenes (up to 8 Kb) | Potential for generation of RCL |
| Efficient gene transfer | Potential for insertional mutagenesis: Even replication-incompetent lentiviruses with human tropism are able to infect human cells and integrate their genome into the host cells→risk in case of accidental exposure |
| Infects dividing and non-dividing cells | In vivo inactivation by the complement system |
| No immunogenic proteins generated | Do not work in all tissues (muscle, heart, vessels) |
| Stable integration into the host genome and stable expression of the transgene | No packaging cells for scaling up |

## Real applications for ex vivo gene therapy (HSC, epithelia)

Articles

## Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: interim results of a non-randomised, open-label, phase 1/2 clinical study

*Francesca Ferrua*, Maria Pia Cicalese*, Stefania Galimberti, Stefania Giannelli, Francesca Dionisio, Federica Barzaghi, Maddalena Migliavacca, Maria Ester Bernardo, Valeria Calbi, Andrea Angelo Assanelli, Marcella Facchini, Claudia Fossati, Elena Albertazzi, Samantha Scaramuzza, Immacolata Brigida, Serena Scala, Luca Basso-Ricci, Roberta Pajno, Miriam Casiraghi, Daniele Canarutto, Federica Andrea Salerio, Michael H Albert, Antonella Bartoli, Hermann M Wolf, Rossana Fiori, Paolo Silvani, Salvatore Gattillo, Anna Villa, Luca Biasco, Christopher Dott, Emily J Culme-Seymour, Koenraad van Rossem, Gillian Atkinson, Maria Grazia Valsecchi, Maria Grazia Roncarolo, Fabio Ciceri, Luigi Naldini, Alessandro Aiuti*

**Interpretation** Data from this study show that gene therapy provides a valuable treatment option for patients with severe Wiskott-Aldrich syndrome, particularly for those who do not have a suitable HSPC donor available.

**Funding** Italian Telethon Foundation, GlaxoSmithKline, and Orchard Therapeutics.

www.thelancet.com/haematology **Vol 6 May 2019**