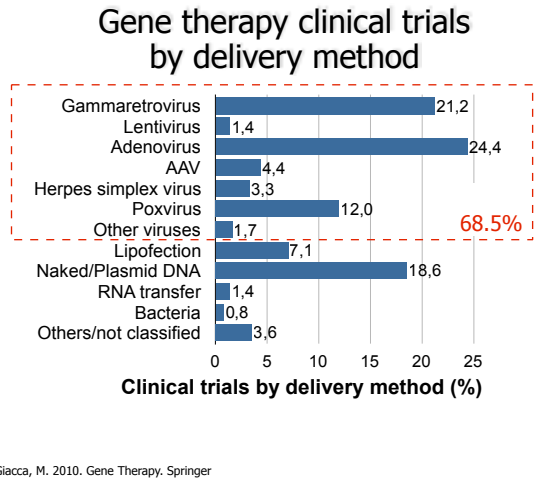
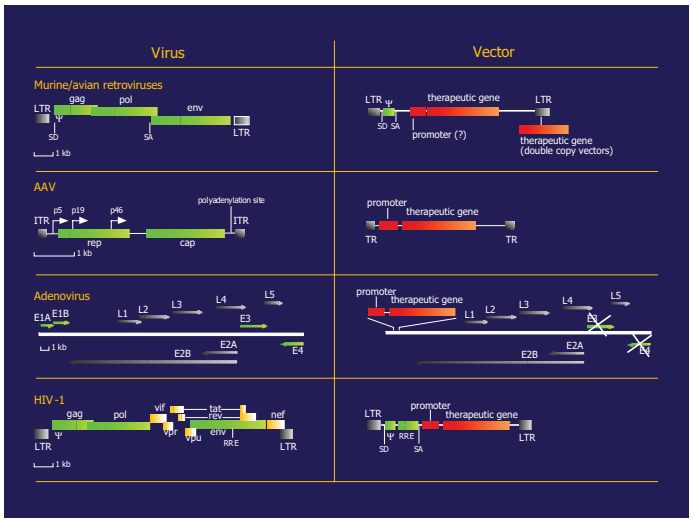
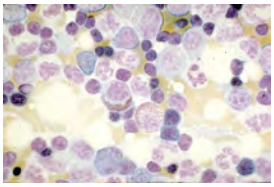
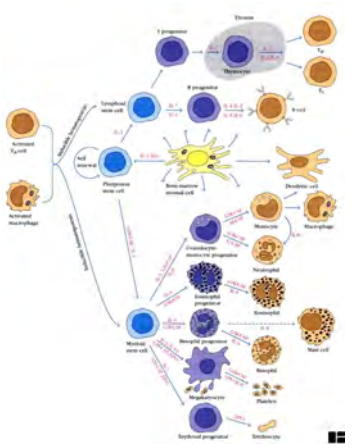


Giacca, M. 2010. Gene Therapy. Springer



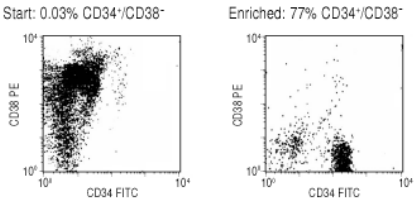
Hematopoiesis





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

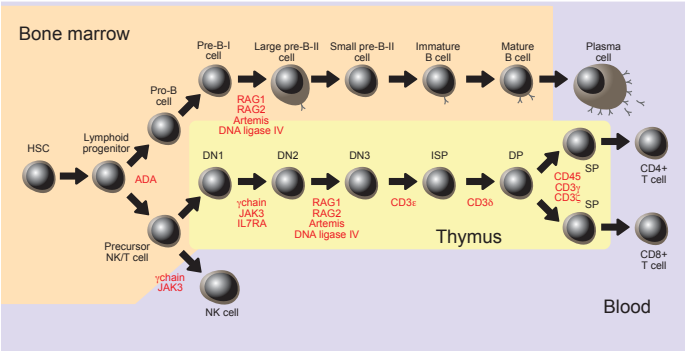


Principali difetti molecolare che portano allo sviluppo di SCID. AR: autosomica recessiva; X-L: legata al cromosoma X

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

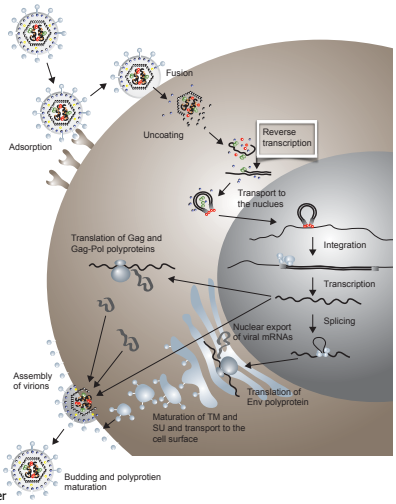
Defects leading to the development of severe combined immunodeficiency (SCID)



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

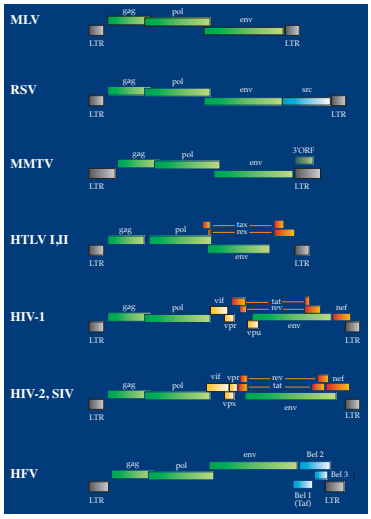


Giacca, M. 2010. Gene Therapy. Springer

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

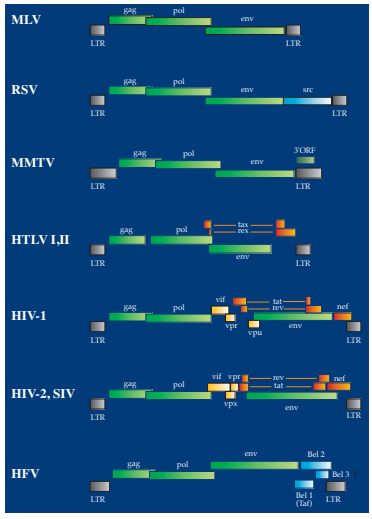


Common features of retroviruses

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

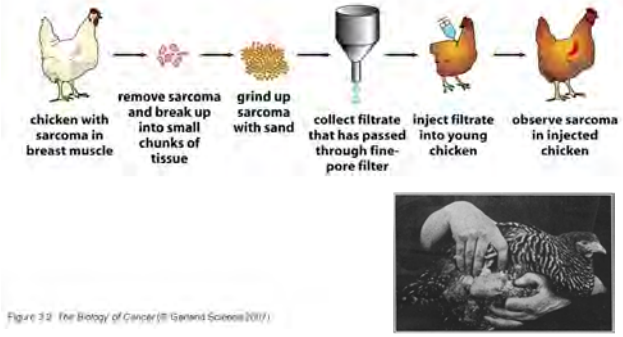
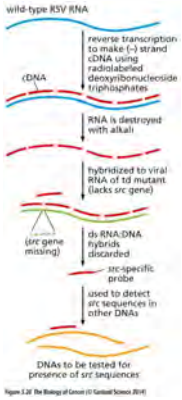
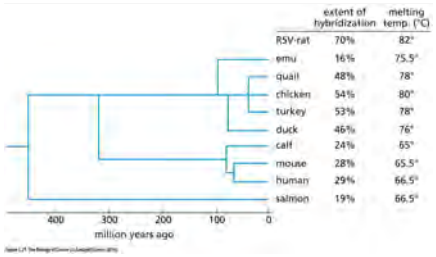


Figure 3.2 The Biology of Cancer (© Garland Science 2017)

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



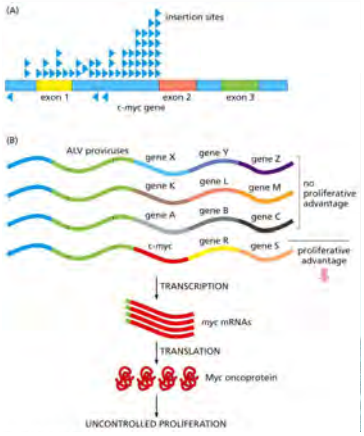


Examples of retroviruses carrying viral oncogenes (v-onc)

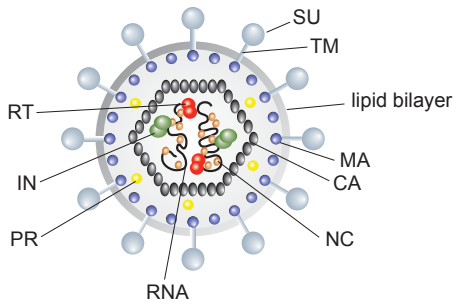
Parental /helper virus	Retrovirus	Acronym	v-onc
	Rous sarcoma virus	RSV	src
Avian leukosis virus (ALV)	Avian myeloblastosis virus	AMV	myb
	Avian erythroblastosis virus	AEV	erbA, B
	Avian myelocytomatosis virus 29	AMCV-29	myc
	Y73 sarcoma virus	Y73SV	yes
	Avian sarcoma virus 17	ASV-17	jun
Moloney-Murine 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

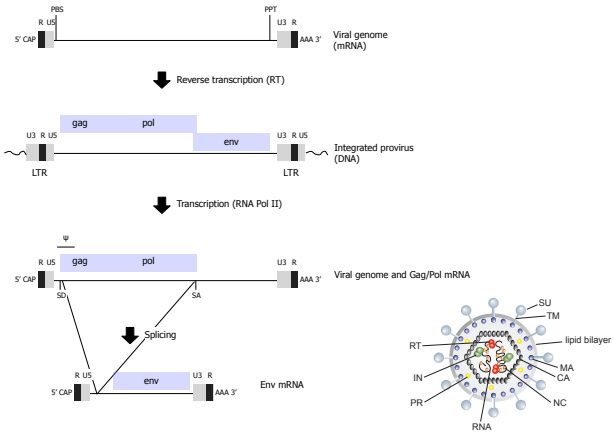
Insertional mutagenesis



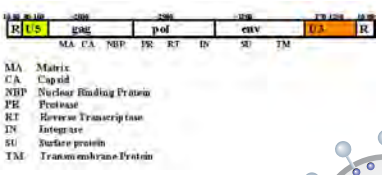
Retrovirus virion



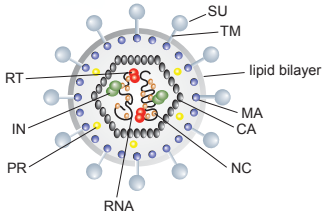
Genetic organization of generalized retrovirus



Genetic organization of generalized retrovirus

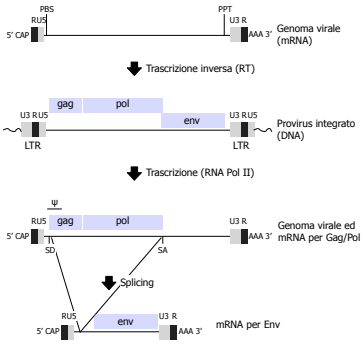


MA Matrix
CA Capsid
NBP Nuclear Binding Protein
PR Protease
RT Reverse Transcriptase
IN Integrase
SU Surface protein
TM Transmembrane Protein

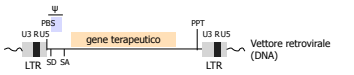


Elementi genetici dei Retrovirus

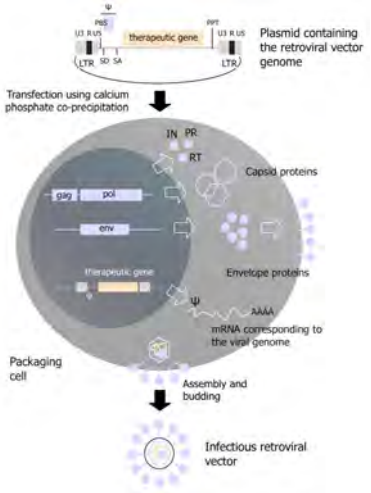
La trascrizione parte da LTR del virus integrato



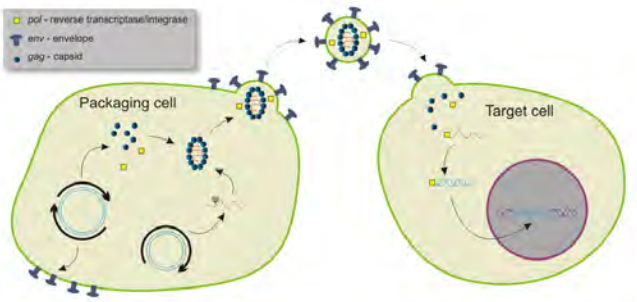
Nel vettore virale rimane:
- LTR che serve per la trascrizione,
- una regione di gag che serve per incapsidamento
- una porzione che produce un tRNA che funziona da primer per la trascrittasi inversa



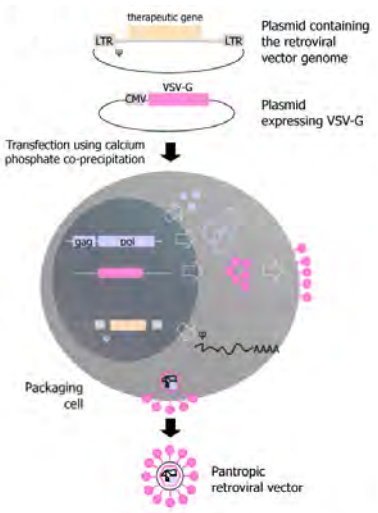
Packaging of gammaretroviral vectors



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



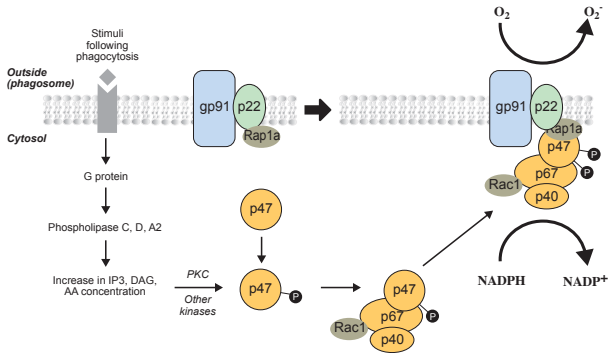
Pseudotyping of gammaretroviral vectors



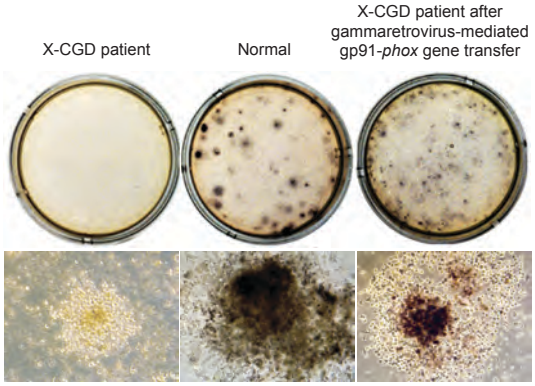
Monogenic hereditary disorders for which gene therapy clinical trials were conducted by gene transfer into HSCs

Disease group	Disease	Defective gene
Severe combined immunodeficiency syndromes (SCID)	SCID-X1	Gamma common (γc) chain of interleukin receptors
	ADA-SCID	Adenosine deaminase
		JAK-3
	PNP-SCID	Purine-nucleoside phosphorylase (PNP)
Lysosomal storage disorders	Hurler's disease (MPS I)	α-L-iduronidase
	Hunter's disease (MPS II)	Iduronate-2-sulfatase
	Gaucher's disease	Glucocerebrosidase (β-glucosidase)
	Fabry's disease	α-galactosidase A
	Sly syndrome (MPS VII)	β-glucuronidase
Defects of phagocytes	Chronic granulomatous disease (CGD)	gp91^{phox}, p47^{phox}
	Leukocyte adhesion disorder	CD18 (β2-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 gp91^{phox} cDNA into CD34$^+$ hematopoietic stem cells

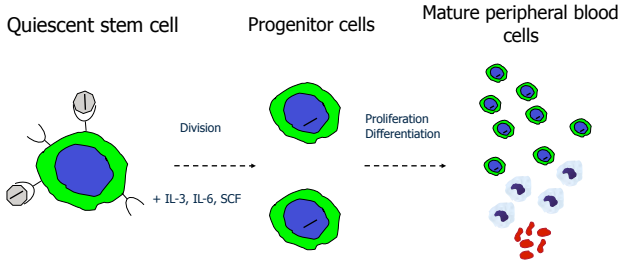


Zentilin, L, et al. 1996. Exp. Cell. Res. 225, 257.

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

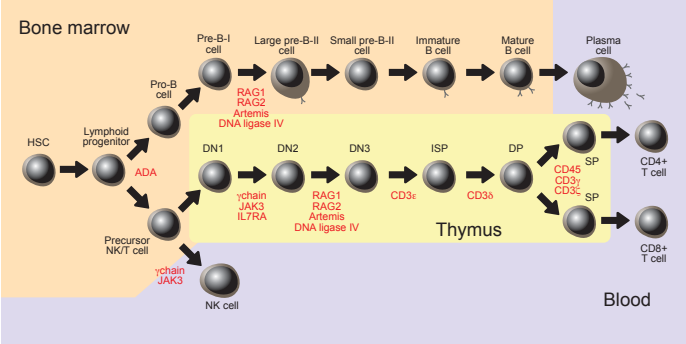


binding of retrovirus to specific receptors of the cell surface

reverse transcription and integration into the host cell genome

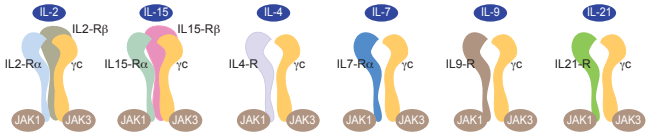
expression of the transgene in the appropriate cell type

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



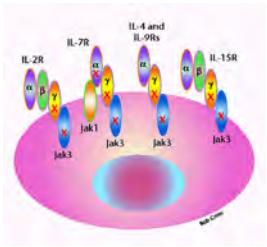
SCID-X1, the bubble boy disease

Molecular structure of the interleukin receptors



γ_c gene therapy trial

A. Fisher, Paris 2000



Eligibility

SCID-X1 (proven γ_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 γ_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".

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

Anno 143° — Numero 264

GAZZETTA UFFICIALE

DELLA REPUBBLICA ITALIANA

PARTE PRIMA — Roma - Lunedì, 11 novembre 2002

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DECRETO DEL PRESIDENTE DEL CONSIGLIO DEI MINISTRI 4 novembre 2002

DECRETI, DELIBERE E ORDINANZE MINISTERIALI

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Ministero della salute

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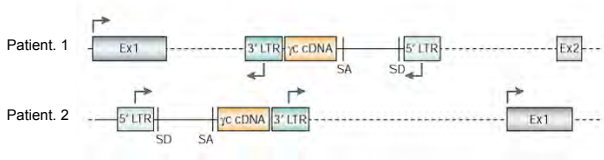
DECRETO 31 ottobre 2002

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

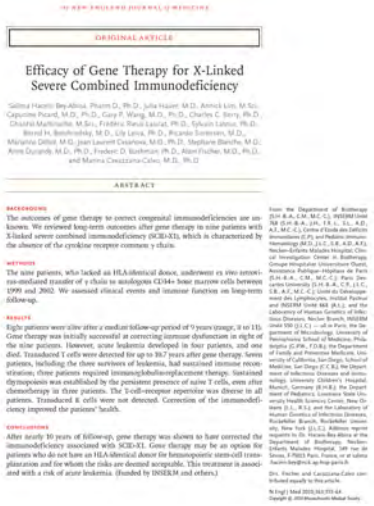
Gene Therapy Insertional Mutagenesis Insights

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 γ*c* loci provided genetic evidence for cooperativity between LMO2 and γc

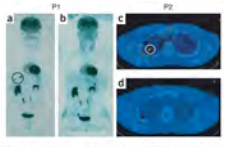
In most gene therapy trials, the transplanted gene is unlikely to be oncogenic and occurrences of insertional mutagenesis will be low, as has been seen in trials conducted during the past several years



ORIGINAL ARTICLE

Efficacy of Gene Therapy for X-Linked Severe Combined Immunodeficiency

ABSTRACT



nature medicine

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

Marion G Ott[1,10], Manfred Schmidt[2–8,10], Kerstin Schwarzwaelder[1–3,10], Stefan Stein[6,10], Ulrich Siler[7,10], Ulrike Koehl[9], Hanno Glimm[2,3], Klaus Kühlcke[9], Andrea Schilz[9], Hana Kunkel[9], Sonja Naundorf[9], Andrea Brinkmann[3], 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[7,10] & Manuel Grez[6,10]

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

JANUARY 29, 2009

Gene Therapy for Immunodeficiency Due to Adenosine Deaminase Deficiency

ABSTRACT

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

Variable	Patients with Normal Value
	no./total no.
Cell count	
CD4+ T cells	5/9
CD8+ T cells	4/9
Natural killer cells	3/9
B cells	4/9
In vitro proliferative responses	
PHA mitogen	8/9
Anti-CD3 mitogen	9/9
Candida albicans	7/9
Alloantigens	8/9
TT	5/5
Serum immunoglobulins	
IgG	3/9
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 (Ellis et al.,[18] Comans-Bitter et al.,[19] 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)

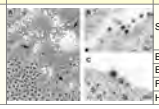
1970 Reverse Transcriptase (Temin and Mizutani; Baltimore)

1976 Probe for src oncogene hybridizes with cellular DNA (Stehelin)

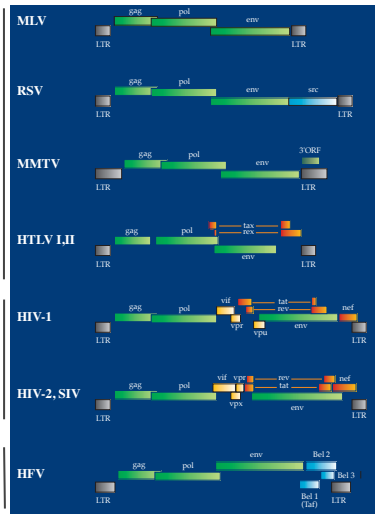
1980/82: First human retrovirus (HTLV-I)

1983: HIV-1

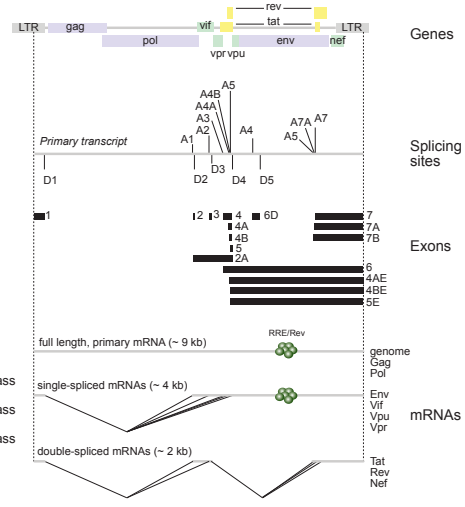
Modern taxonomy of the *Retroviridae* family

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

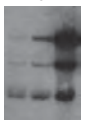


HIV-1 genomic organization and transcripts



Time after HIV-1 infected cell stimulation (h):

1 8 24

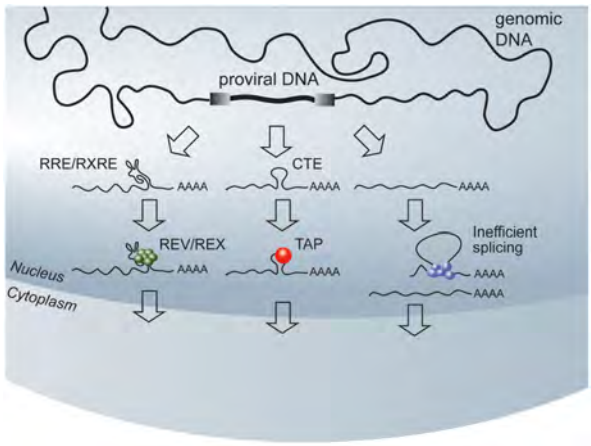


9 kb mRNA class

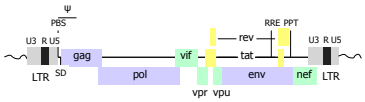
4 kb mRNA class

2 kb mRNA class

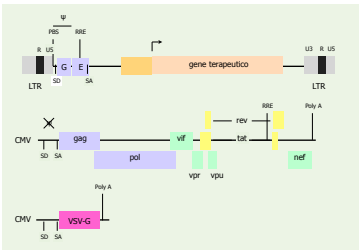
Export of unspliced viral mRNAs outside the nucleus



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

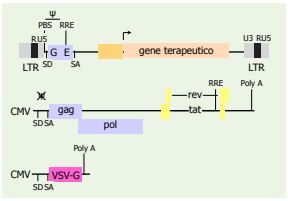
3. VSV-G (env di HIV lega CD4, espresso essenzialmente in linfociti e macrofagi)

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

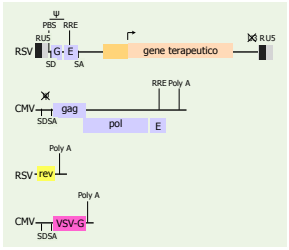
Vettori lentivirali

Vettori lentivirali di seconda generazione



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

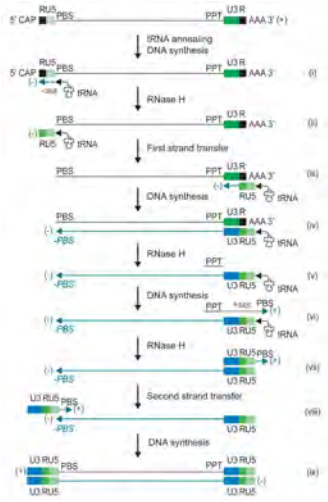
Vettori lentivirali di terza generazione



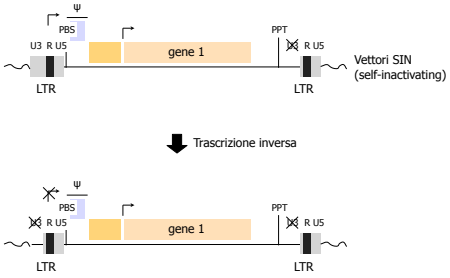
rev in un plasmide separato: ridotta probabilità di ricombinazione

tat non necessaria se trascrizione attivata da CMV

Reverse transcription



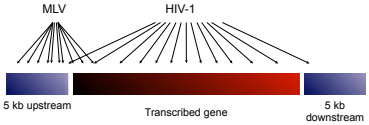
Variazioni nella costruzione dei vettori gammaretrovirali



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

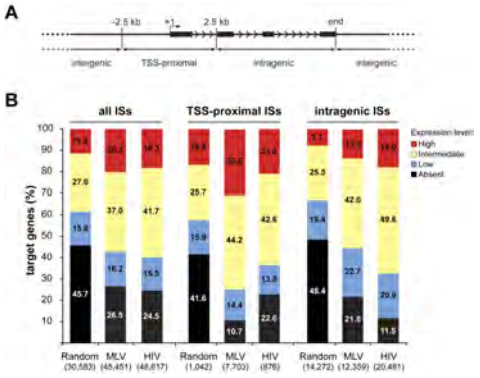
- Acceptor sites for retroviral integrations map near DNase I hypersensitive sites in chromatin (S. Vijaya et al. J. Virol. 1986)
- Retrovirus integration and chromatin structure: Moloney murine leukemia proviral integration sites map near DNase I-hypersensitive sites (H. Rohdewohld et al. J. Virol. 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

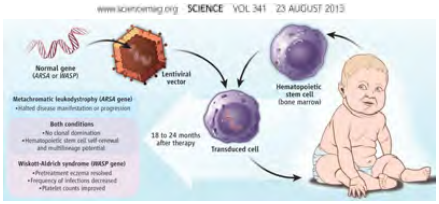
Alessandra Biffi,[1,2,3,*†] Eugenio Montini,[1,*] Laura Lorioli,[1,2,3,4] Martina Cesani,[1] Francesca Fumagalli,[2,4,5] Tiziana Plati,[1] Cristina Baldoli,[6] Sabata Martino,[7] Andrea Calabria,[1] Sabrina Canale,[2] Fabrizio Benedicenti,[1] Giuliana Vallanti,[8] Luca Biasco,[1] Simone Leo,[9] Nabil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

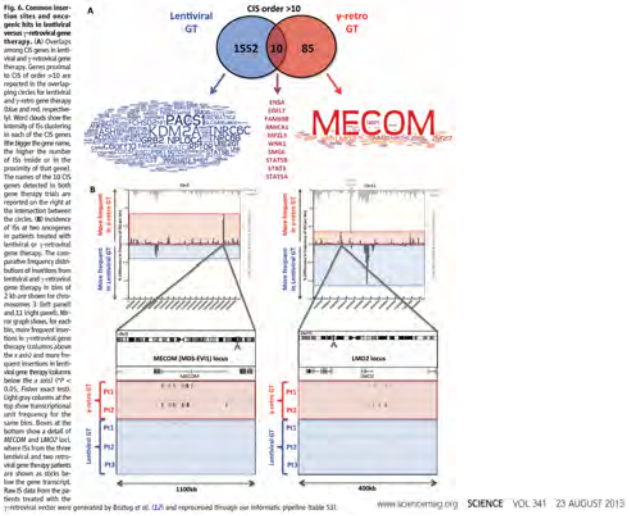
Metachromatic leukodystrophy (MLD) is an inherited lysosomal storage disease caused by arylsulfatase A (ARSA) deficiency. Patients with MLD exhibit progressive motor and cognitive impairment and die within a few years of symptom onset. We used a lentiviral vector to transfer a functional ARSA gene into 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 hematopoietic 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

Alessandra Aiuti,[1,2,3,*†] Luca Biasco,[1†] Samantha Scaramuzza,[1†] Francesca Ferrua,[1,2,3] Maria Pia Cicalese,[2,3] Cristina Baricordi,[2] Francesca Dionisio,[3] Andrea Calabria,[1] Stefania Giannelli,[1] Maria Carmina Castiello,[1,2] Marita Bosticardo,[1] Costanza Evangelio,[1,2] Andrea Assanelli,[3,4] Miriam Casiraghi,[2] Sara Di Nunzio,[2] Luciano Callegaro,[2] Claudia Benati,[2] Paola Rizzardi,[2] Danilo Pellin,[4] Clelia Di Serio,[4] Manfred Schmidt,[5] Christof Von Kalle,[5] Jason Gardner,[6] Nalini Mehta,[11] Victor Neduva,[11] David J. Dow,[11] Anne Galy,[12] Roberto Miniero,[13] Andrea Finocchi,[14] Ayse Metin,[15] Pinaki P. Banerjee,[15] Jordan S. Orange,[16] Stefania Gallimberti,[18] Maria Grazia Valsecchi,[18] Alessandra Biffi,[1,2,3] Eugenio Montini,[1] Anna Villa,[1,17] Fabio Ciceri,[3,4] Maria Grazia Roncarolo,[1,2,3,3‡] Luigi Naldini[1,2‡]

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.





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-

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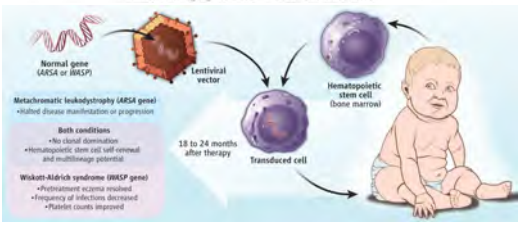

Leukemia (2018) 32:1529–1541
https://doi.org/10.1038/s41375-018-0106-0

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

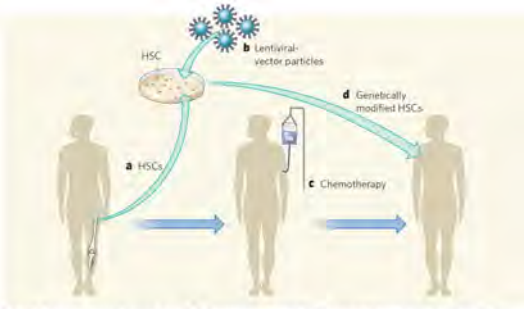

Figure 1 | Gene-therapy procedure. **a**, Cavazzana-Calvo *et al.*[2] collected haematopoietic stem cells (HSCs) from the bone marrow of a patient with β-thalassaemia and maintained them in culture. **b**, The authors then introduced lentiviral-vector particles containing a functional β-globin gene into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

LETTERS

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

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 β^E/β^0-thalassaemia is the most common form of severe thalassaemia in southeast Asian countries and their diasporas[1,2]. The β^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 β^E-globin with partial instability[1,2]. When this is compounded with a non-functional β^0 allele, a profound decrease in β-globin synthesis results, and approximately half of β^E/β^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 β^E/β^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.

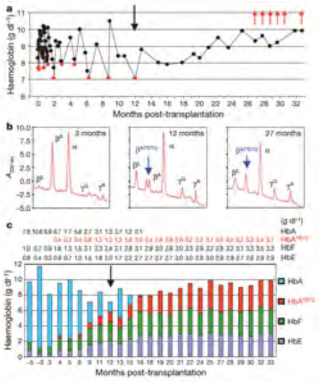

Figure 1 | Conversion to transfusion independence. **a**, Total Hb concentrations in whole blood. Red dots, transfusion time points; black vertical arrow, the last time the patient was transfused; red arrows, phlebotomies (200 ml each) to remove excess iron. **b**, HPLC blood globin chain profiles. Note that β^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

- Can carry large transgenes (up to 8 Kb)
- Efficient gene transfer
- Infects dividing and non-dividing cells
- No immunogenic proteins generated
- Stable integration into the host genome and stable expression of the transgene

- Potential for generation of RCL
- 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
- In vivo inactivation by the complement system
- Do not work in all tissues (muscle, heart, vessels)
- No packaging cells for scaling up

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

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

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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.

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