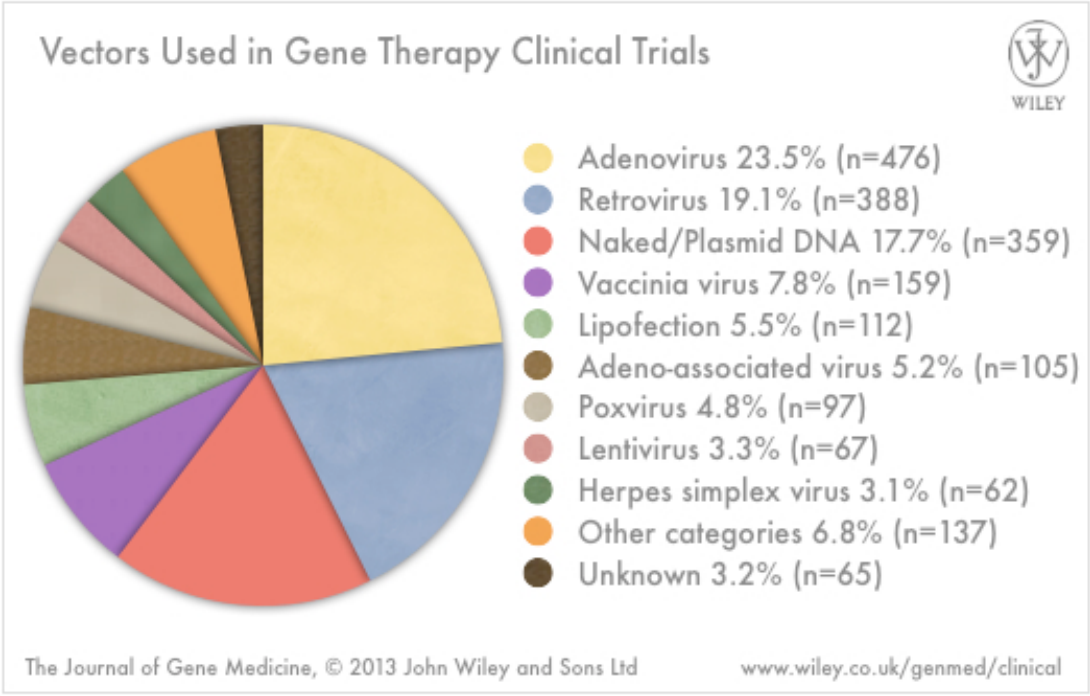
Most people say that it is the intellect which makes a great scientist. They are wrong: it is character.

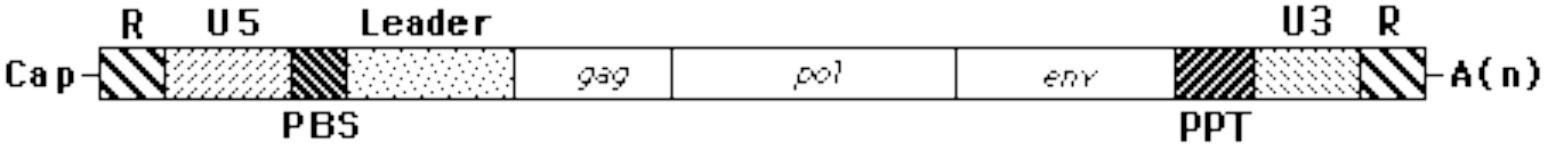
Albert Einstein

Which vectors for the genes



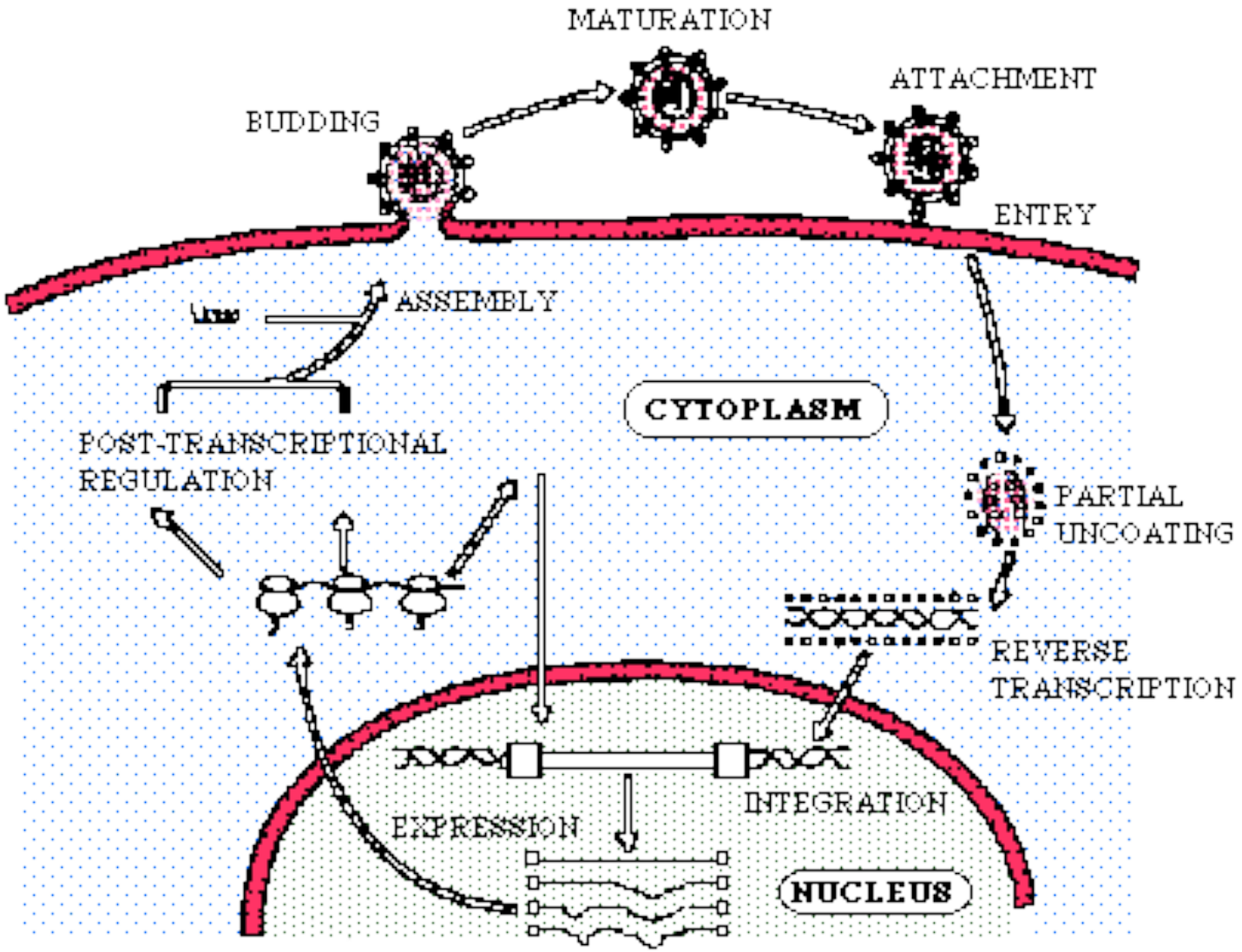
WHY retro: history of knowledge / integration

(onco)Retrovirus (MuLV)

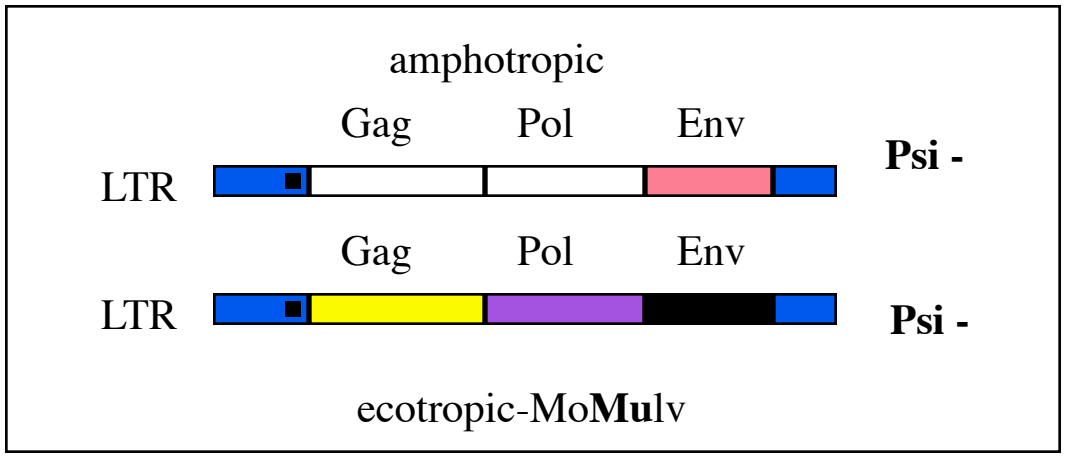


- Receptors: +aa transporter (ecotropic env), phosphate transporter (amphotropic env)
- Enveloped virus
- RNA genome (2 copies)
- dsDNA enters into the nucleus and integrates upon mitosis
- Enters the cell by fusion
- LTR: viral transcription, polyad, replication, integration
- 3 poly-proteins produced by alternate splicing, further processed

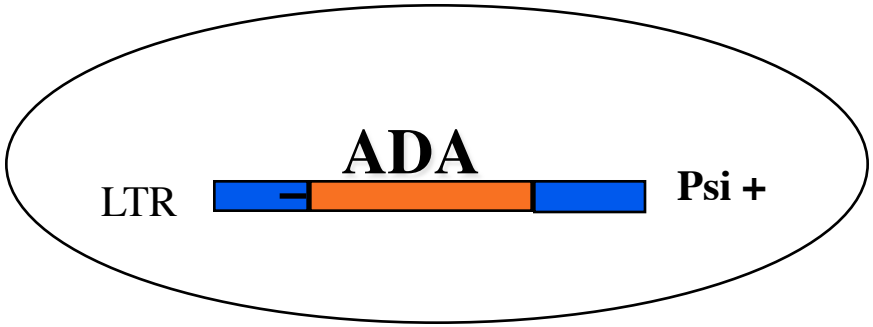
Retrovirus life cycle



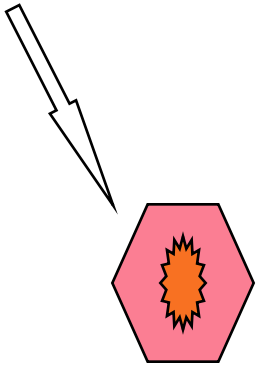
Retroviral vectors



encapsidation cell line

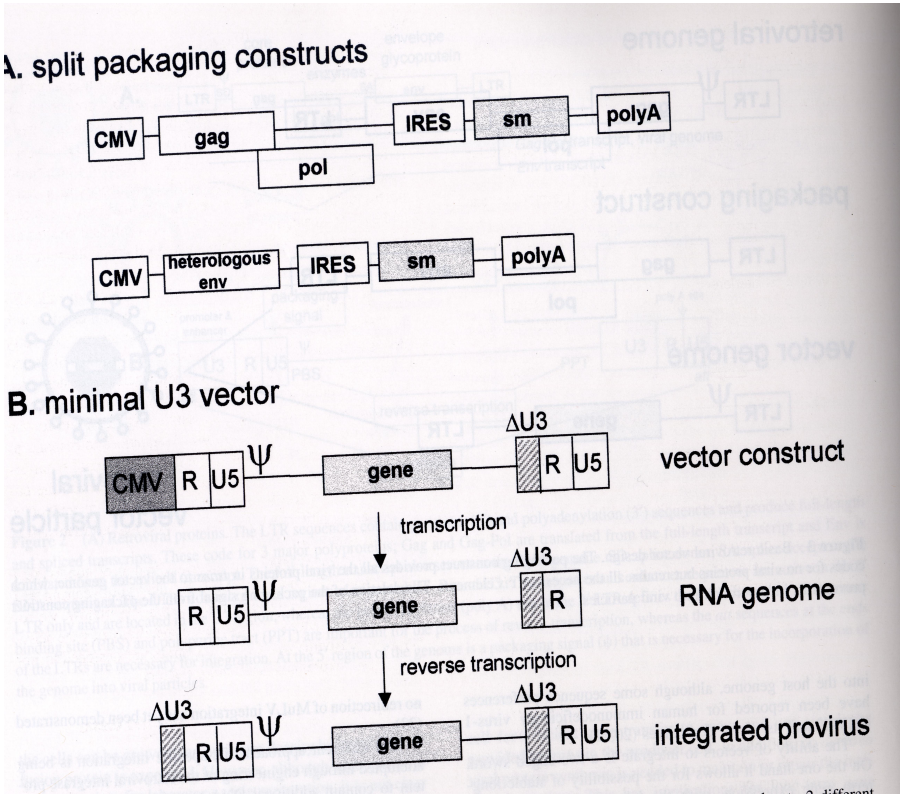


plasmid transfection



Retroviral vectors-ameliorations

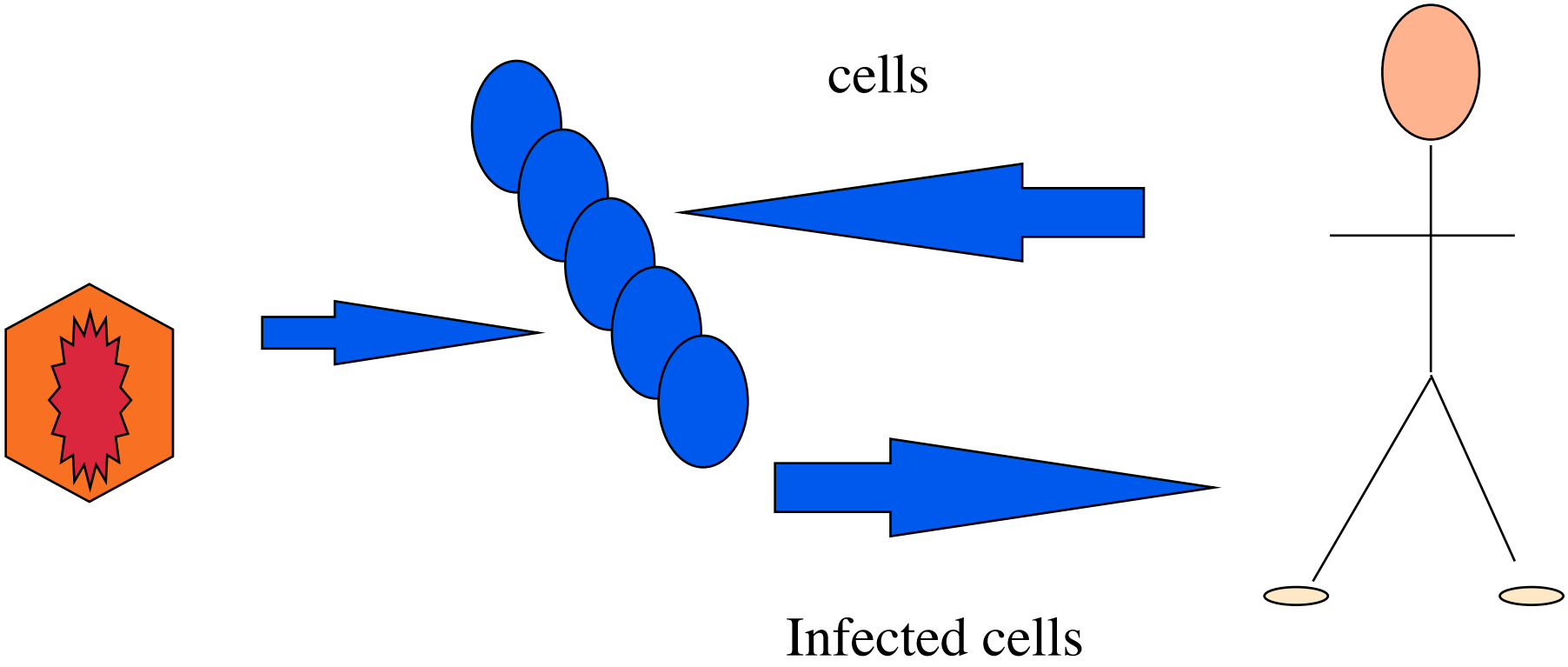
- heterologous envelope (VSV-G)
- Reduce overlap between packaging and vector (in gag and LTR)
- Use different promoters, no LTR
- Substitute the original packaging line NIH3T3 which contains endogenous MuLV like sequences



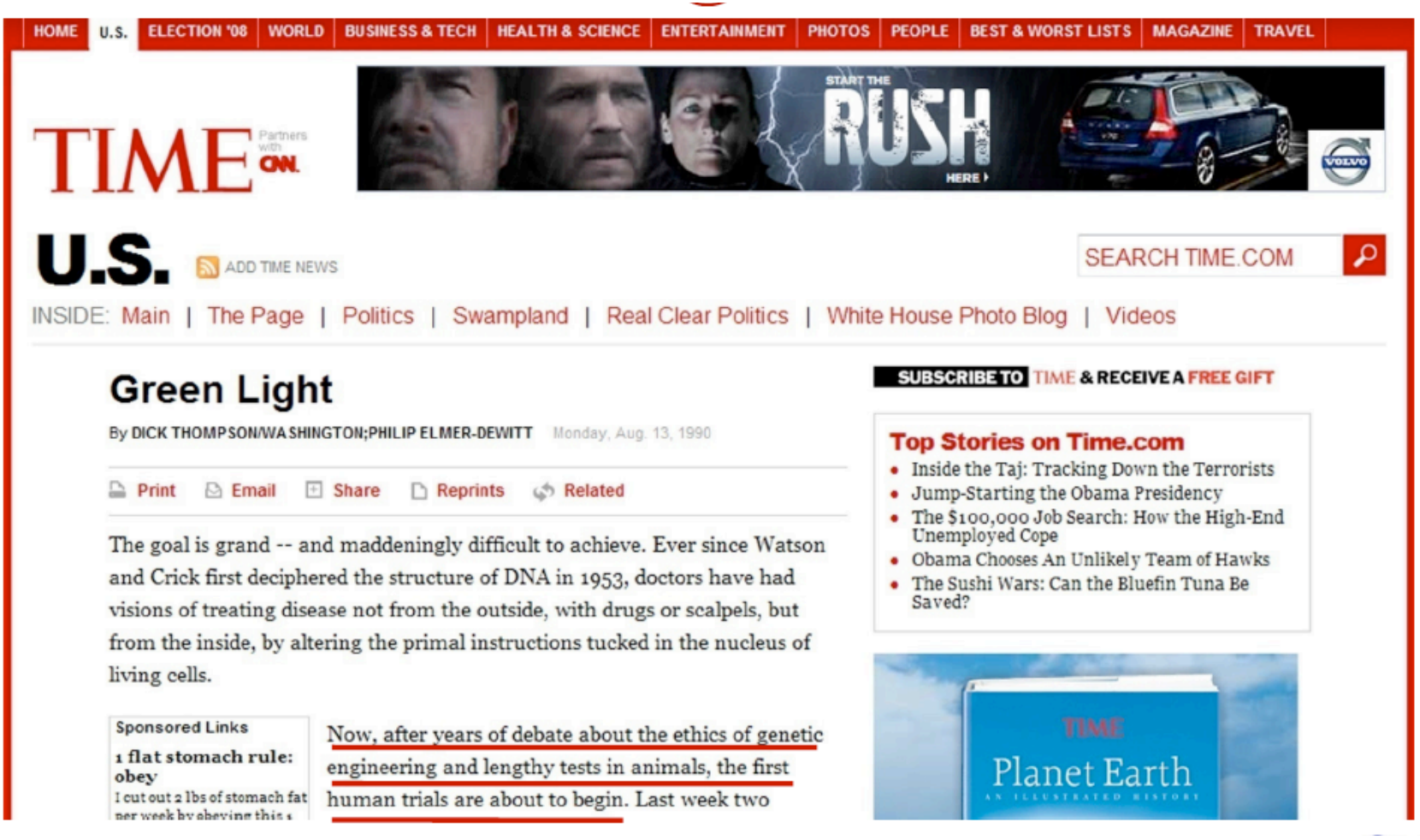
Pros and Cons viral vectos

Vector	Genetic material	Packaging capacity	Tropism	Inflammatory potential	Vector genome forms	Main limitations	Main advantages
Enveloped							
Retrovirus	RNA	8 kb	Dividing cells only	Low	Integrated	Only transduces dividing cells; integration might induce oncogenesis in some applications	Persistent gene transfer in dividing cells
Lentivirus	RNA	8 kb	Broad	Low	Integrated	Integration might induce oncogenesis in some applications	Persistent gene transfer in most tissues
HSV-1	dsDNA	40 kb* 150 kb‡	Strong for neurons	High	Episomal	Inflammatory; transient transgene expression in cells other than neurons	Large packaging capacity; strong tropism for neurons
Non-enveloped							
AAV	ssDNA	<5 kb	Broad, with the possible exception of haematopoietic cells	Low	Episomal (>90%) Integrated (<10%)	Small packaging capacity	Non-inflammatory; non-pathogenic
Adenovirus	dsDNA	8 kb* 30 kb§	Broad	High	Episomal	Capsid mediates a potent inflammatory response	Extremely efficient transduction of most tissues

Ex vivo gene therapy



1990 first gene therapy trial approved



HOME | U.S. | ELECTION '08 | WORLD | BUSINESS & TECH | HEALTH & SCIENCE | ENTERTAINMENT | PHOTOS | PEOPLE | BEST & WORST LISTS | MAGAZINE | TRAVEL

TIME Partners with CNN

U.S. ADD TIME NEWS

SEARCH TIME.COM

INSIDE: Main | The Page | Politics | Swampland | Real Clear Politics | White House Photo Blog | Videos

Green Light

By DICK THOMPSON/WASHINGTON;PHILIP ELMER-DEWITT Monday, Aug. 13, 1990

Print Email Share Reprints Related

The goal is grand -- and maddeningly difficult to achieve. Ever since Watson and Crick first deciphered the structure of DNA in 1953, doctors have had visions of treating disease not from the outside, with drugs or scalpels, but from the inside, by altering the primal instructions tucked in the nucleus of living cells.

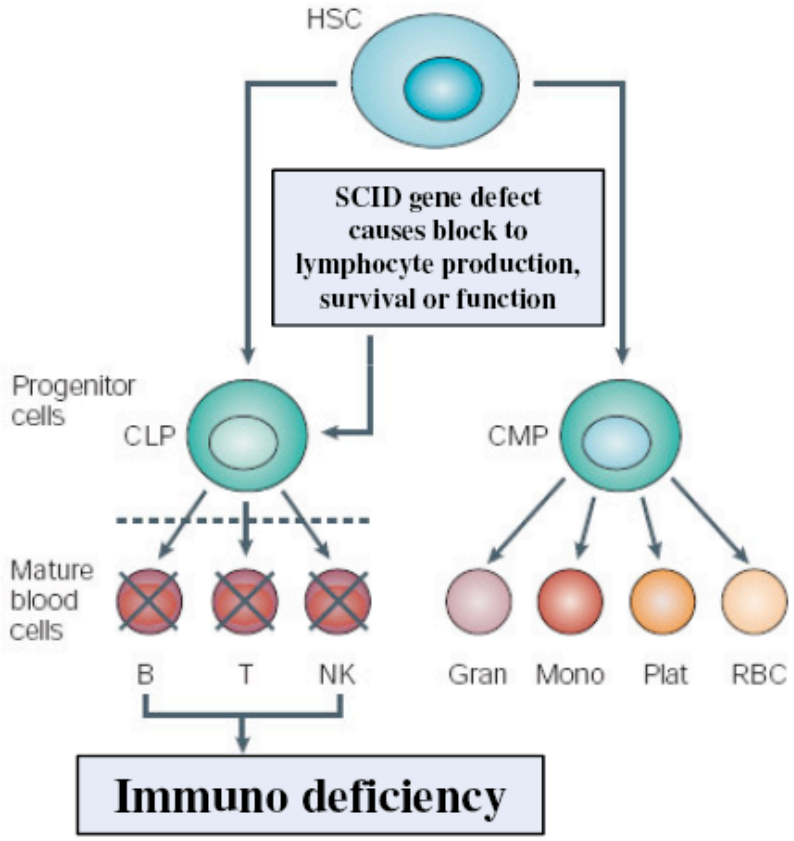
Now, after years of debate about the ethics of genetic engineering and lengthy tests in animals, the first human trials are about to begin. Last week two

Top Stories on Time.com

- Inside the Taj: Tracking Down the Terrorists
- Jump-Starting the Obama Presidency
- The $100,000 Job Search: How the High-End Unemployed Cope
- Obama Chooses An Unlikely Team of Hawks
- The Sushi Wars: Can the Bluefin Tuna Be Saved?

What is SCID

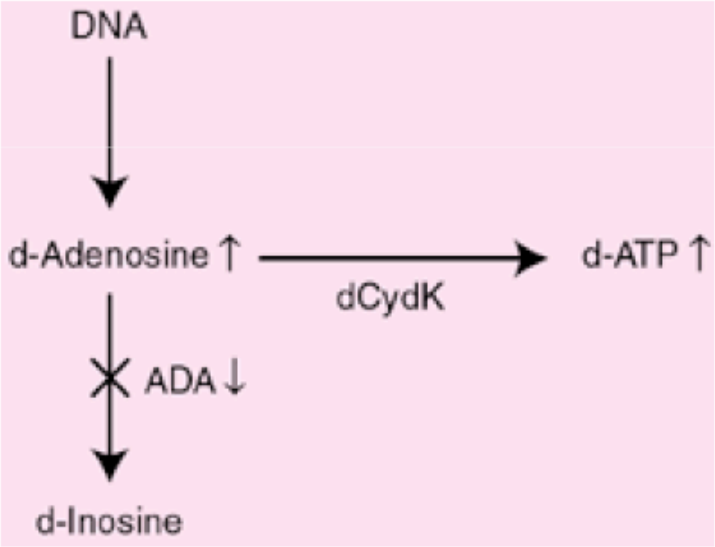
The Buble boy: David Phillip Vetter
(September 21, 1971– February 22, 1984)
Texas (USA)



ADA SCID, 15-20% of all SCIDs

Genotype

Mutations in ADA gene mapped to chromosome 20q12-q13.11



ADA deficiency => accumulation of purine metabolites

Phenotype

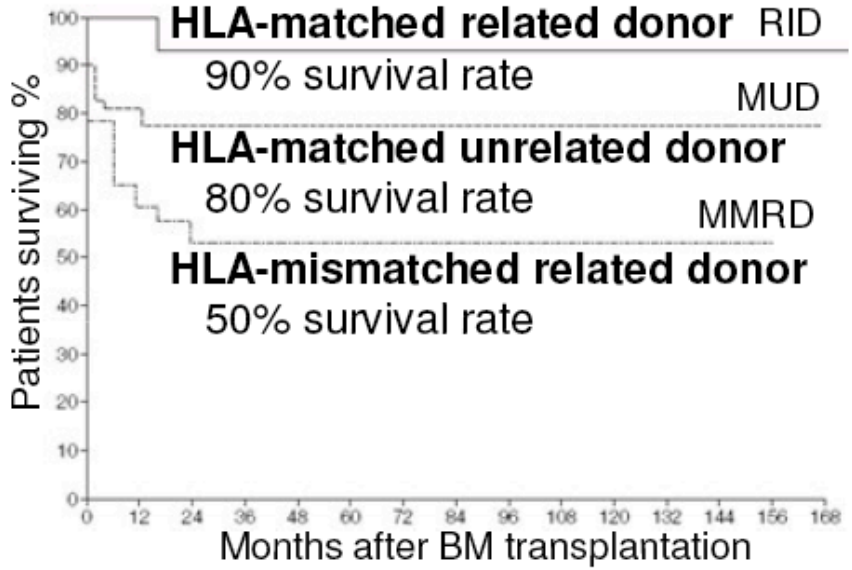
- **recurrent infections**
- **failure to thrive.**
- **multi-system pathologic changes**

Conventional treatment

- **Life in germ-free environment**
- **HSCT**
- **PEG-ADA**

Conventional treatment of ADA

HSC transplant



Complications	No. of Patients/Total (%)		
	RID BMT	MUD BMT	MMRD BMT
Survival	12/13 (92.3)	33/41 (80.5)	21/40 (52.5)
Fatal interstitial pneumonitis	0/13	1/41 (2.4)	11/40 (27.5)
Graft failure	0/13	3/41 (7.3)	12/40 (30.0)
Acute graft-vs-host disease	4/13 (30.7)	30/41 (73.1)	18/40 (45.0)
Abnormal T-cell receptor diversity	3/8 (37.5)	1/19 (5.3)	7/18 (38.9)

PEG-ADA

Corrects the metabolic alterations of the disease

BUT variable degree of immune recovery

high costs 200 000 euro /year

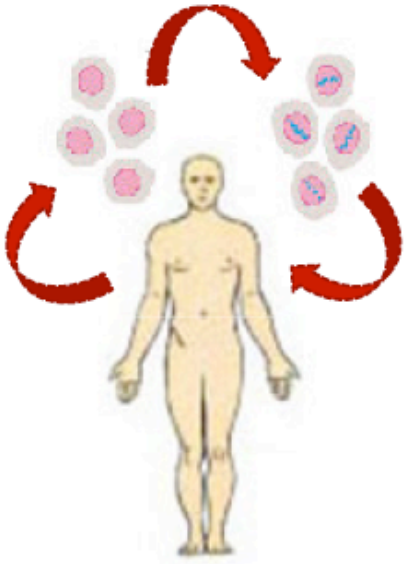
occurrence of neutralizing antibodies or autoimmunity.

Gene therapy advantages

Autologous cells

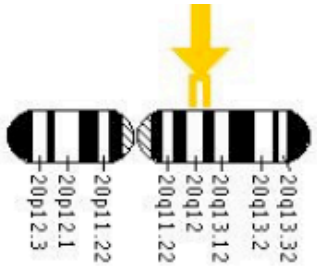
-no HvGG/GvHD

-Available for all patients



Radical correction of genetic defect of disease

Rationale

- Monogenic disease. 

-ADA gene is a housekeeping gene, expressed in all tissues, which can be inserted into gene transfer vectors under constitutive promoters such as the one present in standard gamma-retroviral vectors.

-Because as low as 10% of ADA activity can allow normal immune functions in healthy individuals, it was hypothesised that even relatively low amount of correction and/or of engrafted HSC would have resulted in successful therapy.

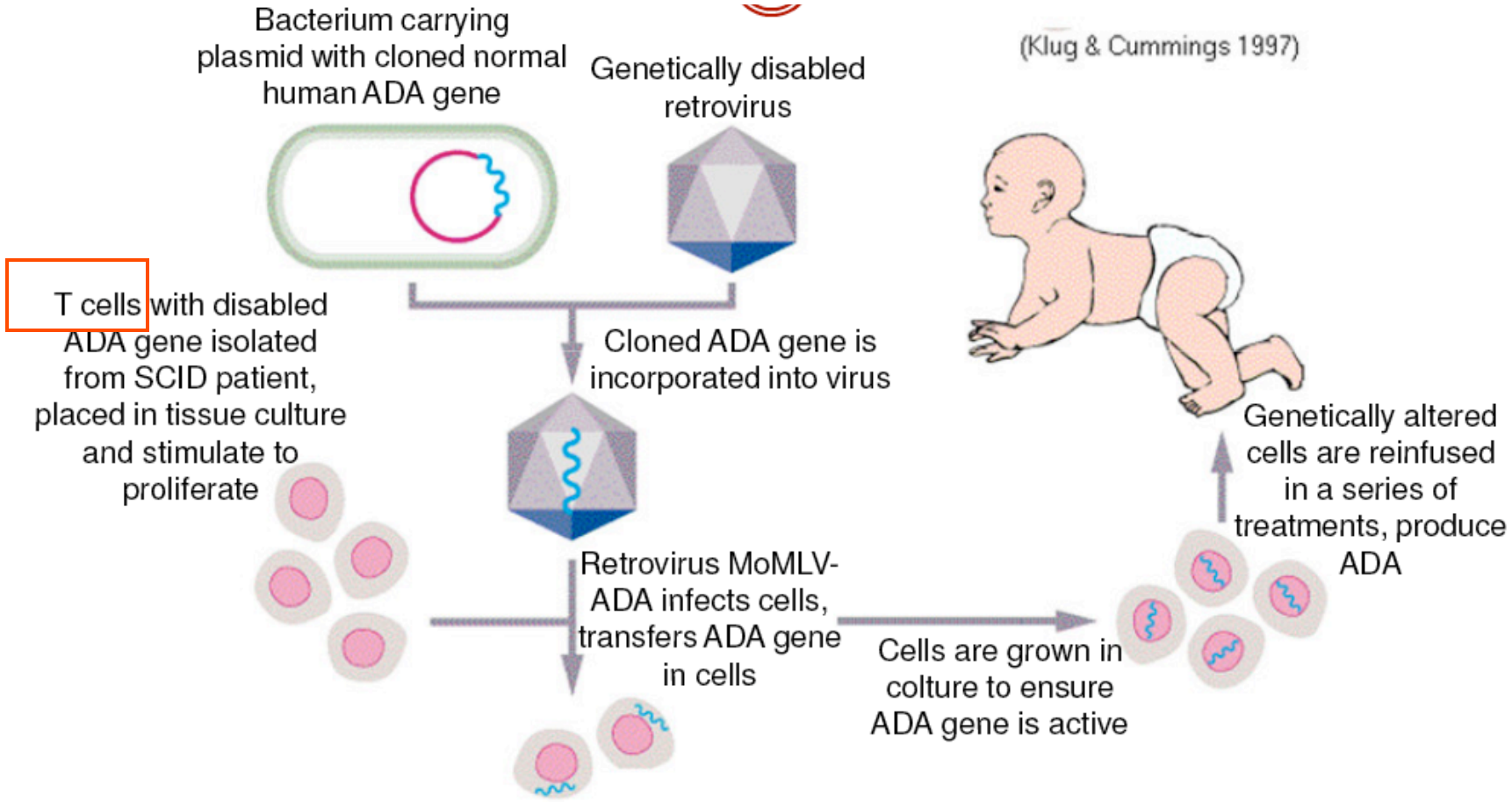
-Wild type or gene corrected cells were shown to carry a strong selective survival advantage over deficient cells in hematopoietic cell transplantation and preclinical gene therapy model

NIH trial

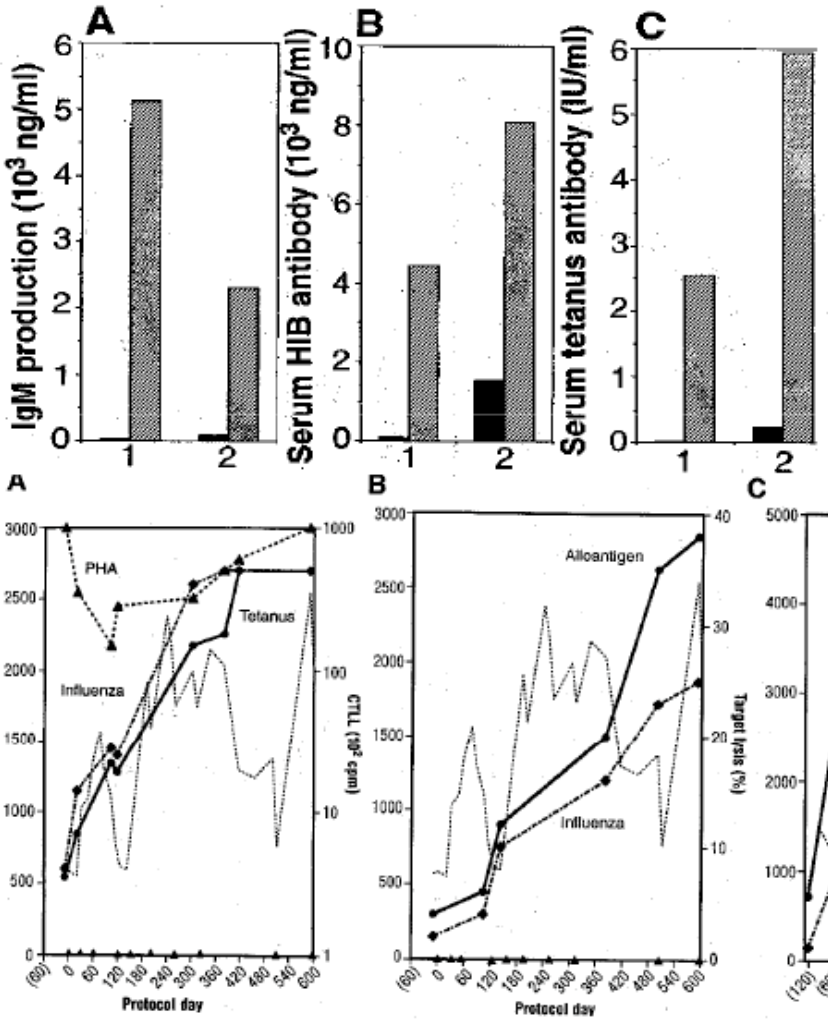
Culver, Anderson, and Blaese with gene therapy patients (Ashanthi De Silva and Cynthia Cutshall).
Courtesy of Dr. Kenneth Culver, Novarti Pharmaceuticals Corp.

W. French Anderson (NIH); in the late summer of 1990, the FDA was sufficiently convinced by the preliminary laboratory data to approve the first human gene therapy trials using the MoMLV-based delivery vector

Protocol


Bacterium carrying plasmid with cloned normal human ADA gene
Genetically disabled retrovirus
(Klug & Cummings 1997)
T cells with disabled ADA gene isolated from SCID patient, placed in tissue culture and stimulate to proliferate
Cloned ADA gene is incorporated into virus
Retrovirus MoMLV-ADA infects cells, transfers ADA gene in cells
Cells are grown in colture to ensure ADA gene is active
Genetically altered cells are reinfused in a series of treatments, produce ADA

Results



T-cell count increasing
Improvement of cellular immune function
Improvement of humoral immune function

Results, trial with PBLs

PBL gene therapy trials		
Investigators	**Patients**	**Gene transfer protocol**
Blaese et al. [1,2] Onodera et al[4]	2 1	Transduction after stimulation with antiCD3 monoclonal antibody and IL2
Bordignon et al[3]	6	Transduction after stimulation with PHA + IK2

[1]**T-Lymphocyte-Directed Gene Therapy for ADA-SCID: Initial Trial Results After 4 Yeasrs.**
Blaese RM et al. Science 1995

[2]**Persistence and expression of the adenosine deaminase gene for 12 years and immune reaction to gene transfer components: long-term results of the first clinical gene therapy trial.**
Mull et al. GeneTherapy 2003.

[3]**Gene therapy in peripheral blood lymphocytes and bone marrow for ADA immunodeficiency patients.**
Bordignon C et al. 1995. Science 270:470-5

[4]**Succesful peripheral T-Lymphocyte-directed gene transfer for a patient with severe combined immunedeficiency caused by adenosine deaminase deficiency.**
Onodera M et al. 1998. Blood 91:30-36.

Results, trial with HSCs

HSC gene therapy trials		
Investigators	**Patients**	**Gene transfer protocol**
Bordignon et al. [1]	2	Infection of mononuclear cell with viral supernatant, no cytokines added
Kohn et al[2]	3	Infection of UCB $CD34^+$ cell with viral supernatant, in presence of cytokines (IL3, IL6, CSF)
Hoogerbrugge et al[3]	3	Co-culture of BM $CD34^+$ cells on irradiated producer with IL3

[1]**Gene therapy in peripheral blood lymphocytes and bone marrow for ADA immunodeficiency patients.**
Bordignon C et al. 1995. Science 270:470-5
[2]**Engraftment of gene-modified umbilical cord blood cells in neonates with adenosine deaminase deficiency.**
Kohn DB et al. 1995. Nat Med 1:1017.
[3]**Bone marrow gene transfer in three patients with adenosine deaminase deficiency.**
Hoogerbrugge PM et al. 1996. Gene Ther. 3:179.

HSCs, progresses

Better vectors made to high titers.

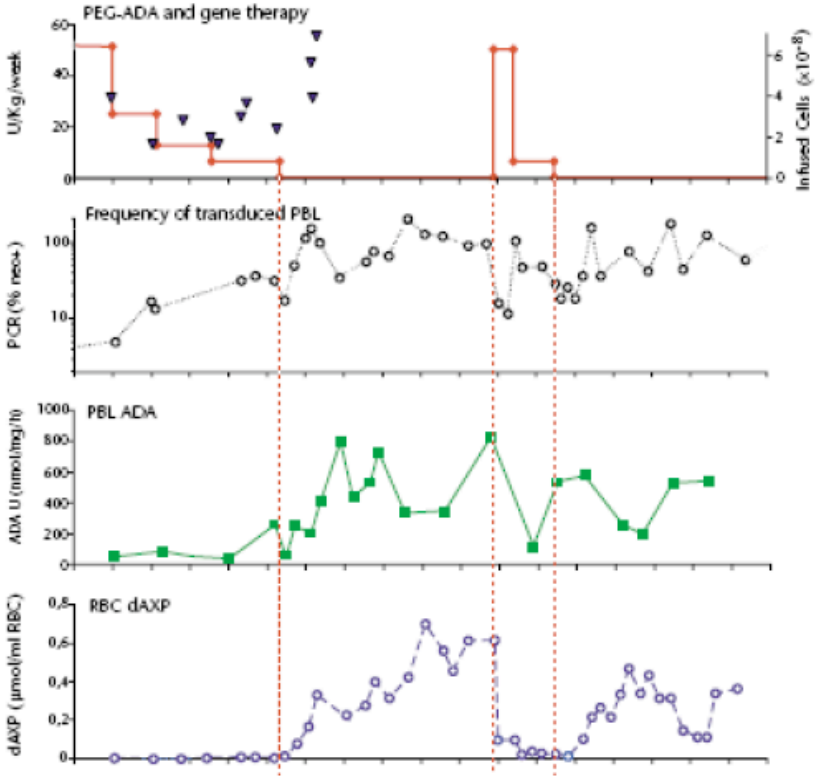
Better growth factors/matrices/serum-free media developed that are capable of stimulating early HSC to divide, become transduced and retain pluripotency.

In large animal models of gene transfer/HSCT, the levels of gene-marking increase 10-100X using these methods

➡ **2° generation of clinical trials for SCID initiated in late 1990's**

PEG-ADA discontinuation (PBLs)

Immune reconstitution in ADA-SCID after PBL gene therapy and discontinuation of enzyme replacement. Aiuti et al. 2002. Nat Med 8:423-5



DISCONTINUATION OF PEG-ADA

Selective growth advantage og gene-transduced T-Lymphocytes

Intracellular PBL ADA activity raised

Red blood cells dAXP increased

Conclusions early ADA trials (1990-1998)

- safety of viral gene transfer
- Persistence
- PEG ADA impairs effective gene/cell therapy

Gene therapy and non-myeloablative conditioning

Two children in this study never got PEG-ADA.

Radical approach: **non-myeloablative conditioning** make more room for transgenic T-cells by suppressing host BM.

Results:
improved immune functions
(including antigen-specific responses),
lower toxic metabolites.
Both patients are currently at home and clinically well, with normal growth and development.

Aiuti A et al., 2002 (Science)

Gene therapy and non-myeloablative conditioning

HSC gene therapy trials		
Investigators	**Patients**	**Gene transfer protocol**
Aiuti et al. (Milan)[1] Kohn et al. (USA)[2] Gasper et al. (London)[3]	12 4 4	Infection of BM CD34+ cells with viral supernatant in presence of retronectin and cytokines (SCF, TPO, FLT3ligand, IL3)

[1]**Haematopoietic stem cells gene therapy for ADA-SCID**.
Aiuti et al. 2008.Blood Cells Mol Dis 40:248

[2]**Corrective gene transfer into bone marrow CD34+ cells for adenosine deaminase (ADA) deficiency: results in four patients after one year follow up.**
Candotti F , Khon BD et al. 2003. Mol Ther 7:S448.

[3]**Successful reconstitution of immunity in ADA-SCID by stem cells gene therapy following cessation of PEG-ADA and use of mild preconditioning.**
Gasper HB et al. 2006. Mol Ther 14:505.

On the news

guardian.co.uk

Search: guardian.co.uk Search

News | Sport | Comment | Culture | Business | Money | Life & style | Travel | Environment | Blogs | Video | Jobs | A-Z

News › Science › Genetics

French claim gene therapy breakthrough

Gene patenting: special report

Clue: A major city

Tim Radford
guardian.co.uk, Friday April 28 2000 01.21 BST
Article history

French scientists claim today to have succes
therapy" to two infants condemned to a life se

The babies, aged 8 and 11 months, were bor
called human severe combined immunodefici
children are forced to live in a sterile "bubble"
immune systems and would be unable to resi
waiting for a bone marrow transplant.

BBC | NEWS | SPORT | WEATHER | WORLD SERVICE | A-Z INDEX | SEARCH | Go

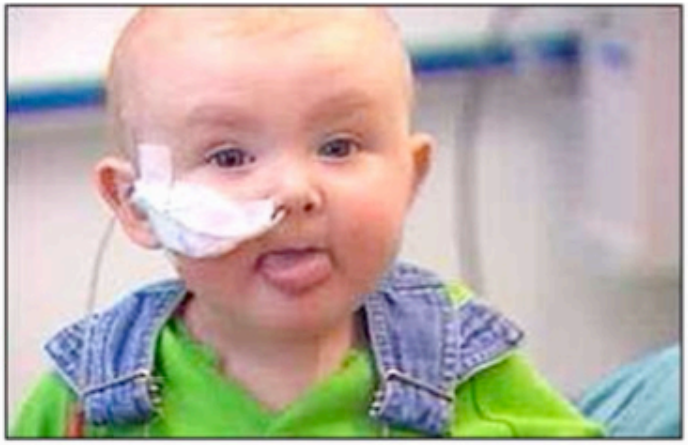
BBC NEWS WORLD EDITION

You are in: Science/Nature

News Front Page
Africa
Americas
Asia-Pacific
Europe
Middle East
South Asia
UK
Business
Entertainment
Science/Nature
Technology
Health

Thursday, 27 April, 2000, 18:14 GMT 19:14 UK

Gene therapy frees 'bubble babies'



Scid children must live in sterile rooms

WATCH/LISTEN REAL MEDIA
ON THIS STORY
Dr Alain Fischer
Bone marrow transplants are not wholly successful
The BBC's David Concar
"Until now the only way out was a bone marrow transplant"

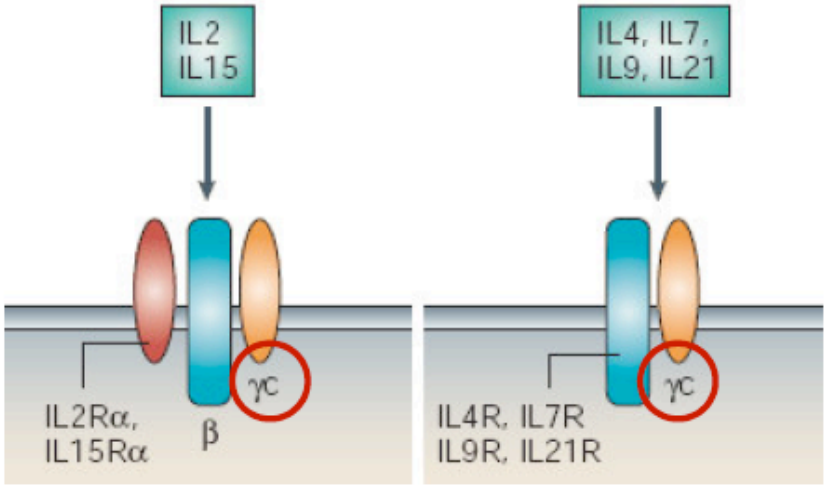
See also:

- 02 Mar 00 | Science/Nature
  Surprise gene therapy success
- 20 Feb 00 | Health
  Gene therapy 'advance' for Aids
- 11 Feb 00 | Health
  First steps to heart disease 'jab'

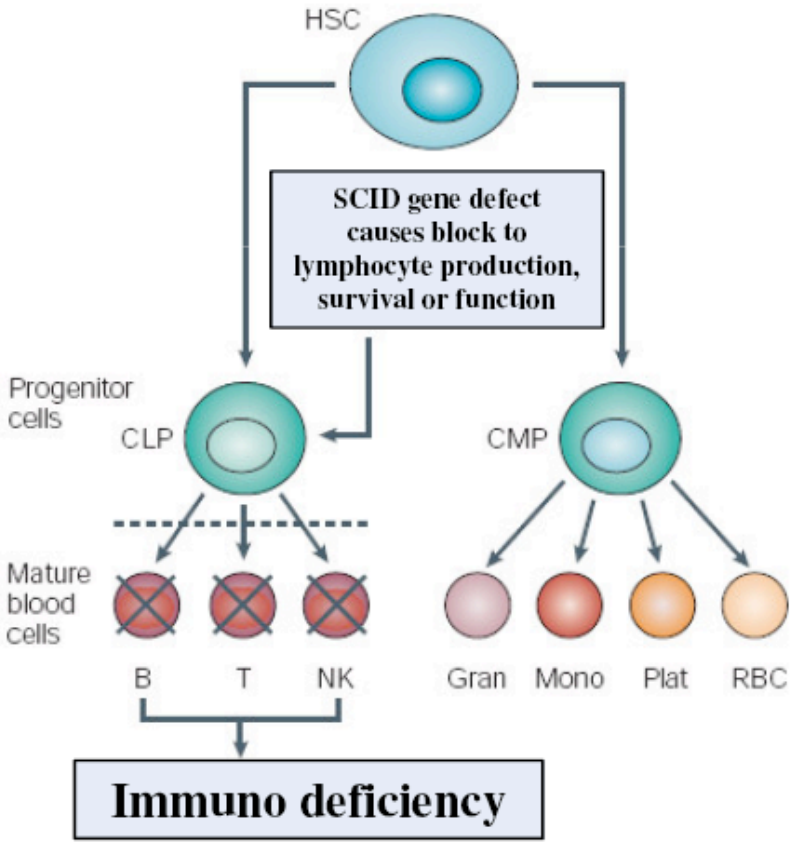
SCIDX1 (50% SCID cases)

Genotype

Mutations in gamma c gene mapped to chromosome Xq13



Phenotype



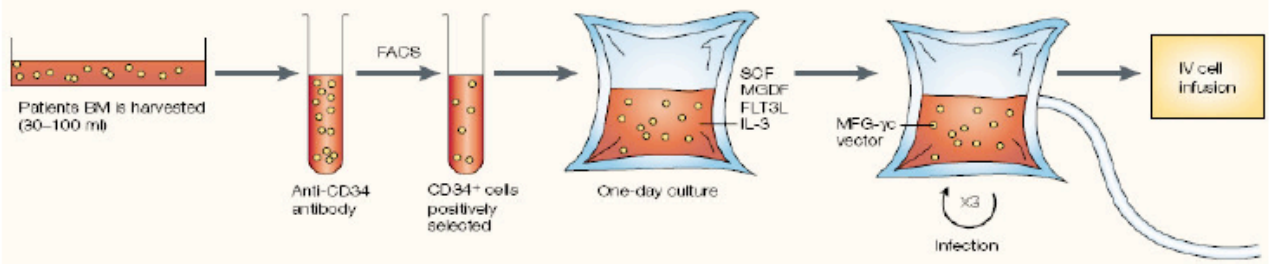
Ex-vivo Retrovirus-med. gene therapy: SCIDX1 trial 1998, A. Fisher France

- Recessive disease
- X linked
- Defect in the γc gene, receptor for cytokines => block in T and NK differentiation
- Ex-vivo gene therapy on CD34+cells: MuLV- gc 20x106 cells/Kg

Ex-vivo Retrovirus-med. gene therapy: SCIDX1I trial 1998, A. Fisher France

Enrolled 10 children under the age of 1 year between March 1999 and May 2002.

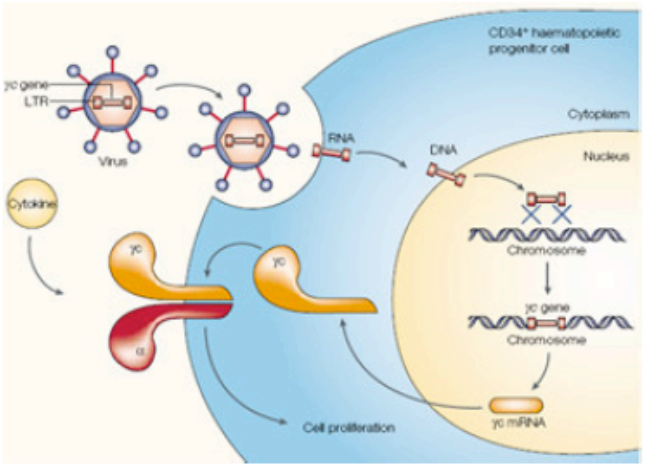
PROTOCOL



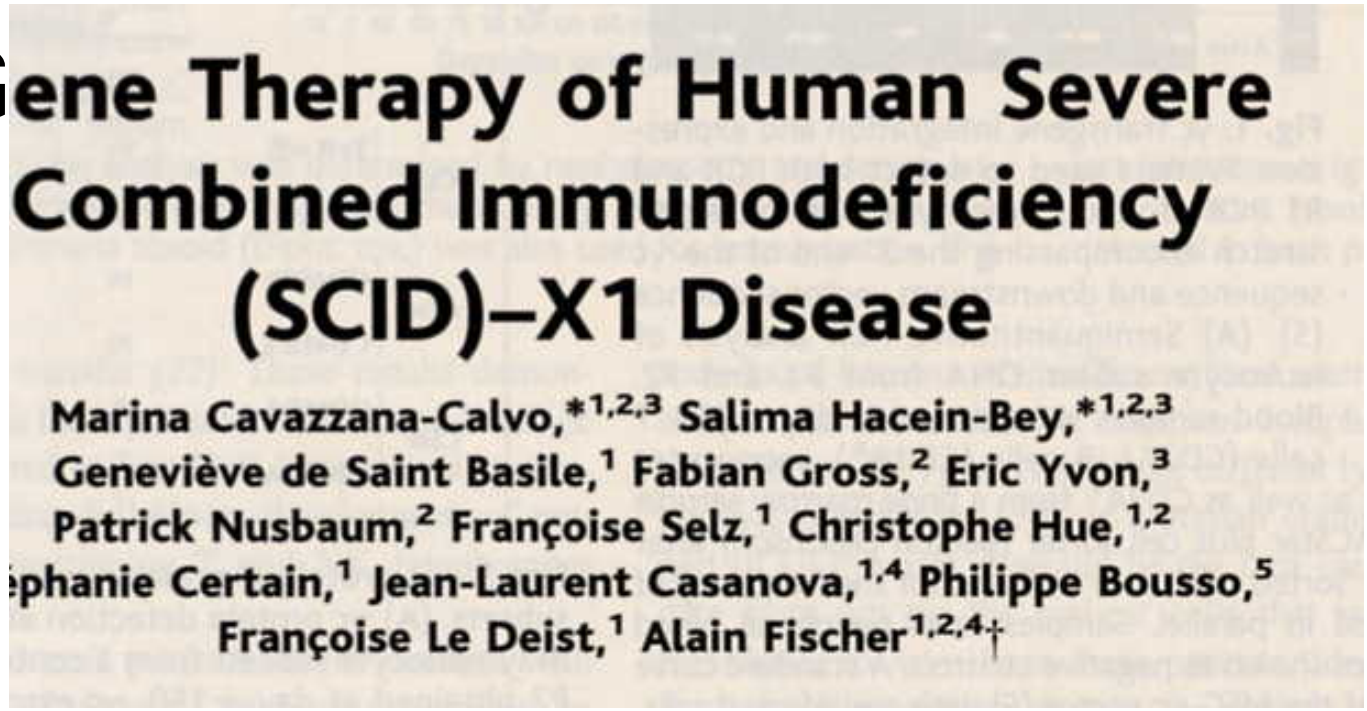
Ex vivo transduction of CD34+ bone-marrow cells harvested from the iliac crest.

VECTOR:

γc cDNA under the control of the viral LTR, the defective MLV was produced using an amphotropic packaging cell line.



SCIDX1 trial



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]†

Science. 2000 Apr 28;288(5466):669-72.

A. PCR:
detection of γc DNA

B: RT PCR
Detection of γc RNA

protein expression

Lymphocyte subsets

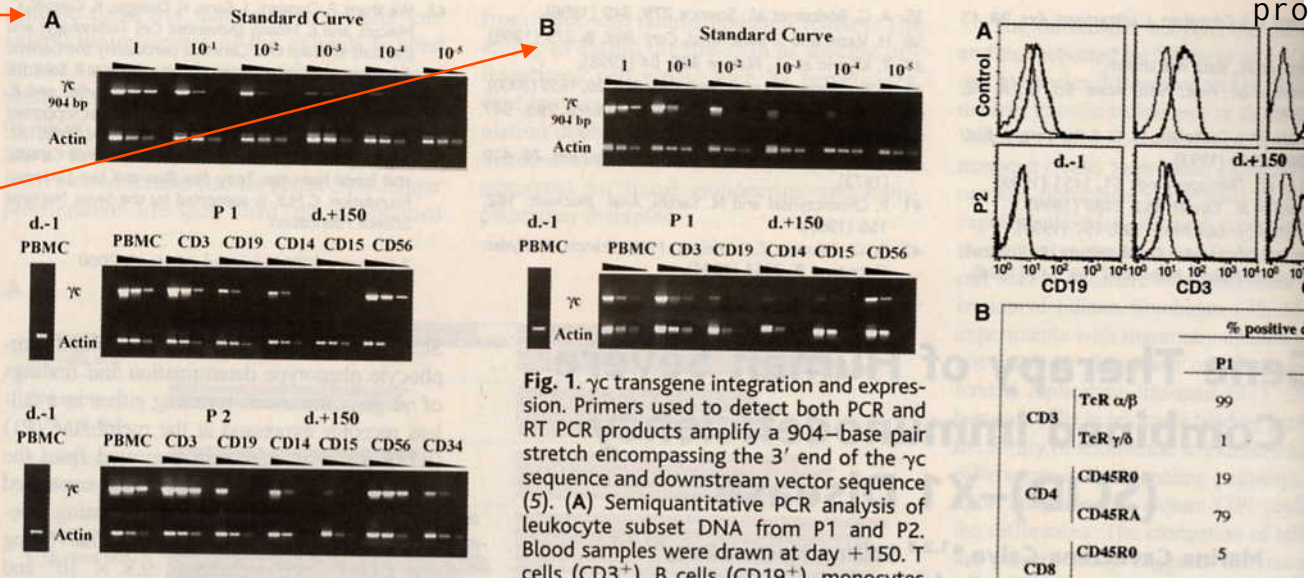


Fig. 1. γc transgene integration and expression. Primers used to detect both PCR and RT PCR products amplify a 904–base pair stretch encompassing the 3′ end of the γc sequence and downstream vector sequence (*5*). (**A**) Semiquantitative PCR analysis of leukocyte subset DNA from P1 and P2. Blood samples were drawn at day +150. T cells ($CD3^+$), B cells ($CD19^+$), monocytes ($CD14^+$), granulocytes ($CD15^+$), and NK cells ($CD56^+$) as well as $CD34^+$ from a bone marrow sample obtained at day +150 from P2 were isolated by a FACStar plus cell sorter (Becton Dickinson) after staining with appropriate mAbs (*19*). Purity was >99%. Sorted cells were analyzed for the frequency of vector-containing cells (*17*). Actin DNA was amplified in parallel. Samples from peripheral blood mononuclear cells (PBMC) obtained before treatment are shown as negative controls. A standard curve was constructed by diluting cells containing one copy of the MFG γc vector (*5*) with noninfected cells. All specimens were tested at three dilutions: 1:1, 1:20, and 1:200. (**B**) Semiquantitative RT-PCR analysis of leukocyte-subset RNA from P1. The same blood sample as in (A) was used. Actin cDNA was amplified in parallel as a control of RNA content. The standard curve was constructed as in (A) (*17*). No signal was detected in the absence of reverse transcriptase (not shown). Each specimen was diluted to 1:1, 1:500, and 1:5000.

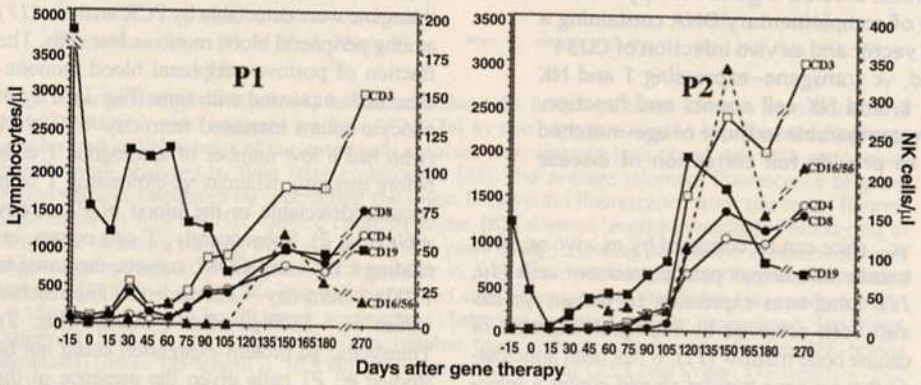


Fig. 2. Longitudinal study of lymphocyte subsets from patient 1 (P1) and patient 2 (P2). Absolute counts of T cells ($CD3^+$, $CD8^+$, and $CD4^+$), B cells ($CD19^+$), and [illegible] ($CD16^+56^+$) are shown as a function of time. Day 0 is the date of treatment. The scale for NK cells is on the right-hand side of each panel.

B

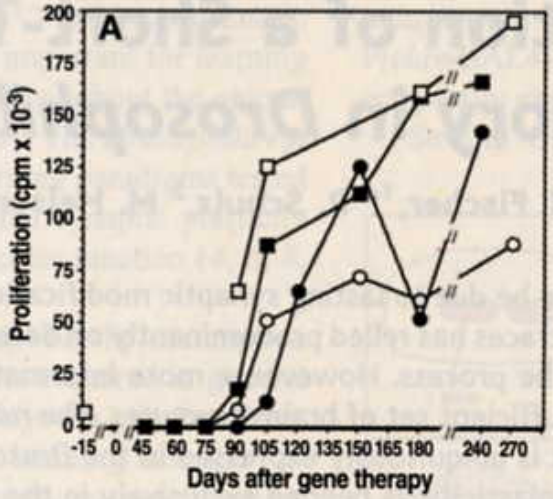
		% positive (P1)
CD3	TcR α/β	99
	TcR γ/δ	1
CD4	CD45R0	19
	CD45RA	79
CD8	CD45R0	5
	CD45RA	94

Fig. 3. γc protein expression and ly
subsets. (**A**) γc protein detection at t
of lymphocyte subsets from a control
P2 obtained at day +150. γc expres
cells from P2 after treatment was un
(not shown). The *y* axis depicts the re
number, and the *x* axis shows the log
arbitrary immunofluorescence units.
are isotype controls; thick lines, stain
anti-γc. Similar results were observed
samples obtained at days 275 (P1)
(P2). (**B**) The percentage of CD45
$CD45RA^+$ among CD4 and CD8 T cell
and P2 obtained at day +275 and 24
tively, as well as the percentage
expressing either an αβ TCR or a γδ

P1. As determined by semi-quantita
and reverse transcriptase–PCR analys
observed that in both cases, a low fra
cells carry and express the γc transgen
It is therefore unknown whether ant
sponses are provided by untransduc
few transduced B cells. Residual persi
1%) of administered intravenous immu
[illegible] (last given 5 months before [illegible]
antibody response) could, in part, als
ute. The γc-expressing NK cells were

Science. 2000 Apr 28;288(5466):669-72.

Functional characteristics of transduced cells



B

	IgG	IgA	IgM	Antibodies to : Tet. tox. (iu/ml)	Dipht. Tox. (iu/ml)	Polio virus I	II	III
		mg/dl				Titer x 10^{-1}		
P1 (d. + 270)	323	41	202	.53	.86	320	160	160
P2 (d. + 240)	309	0	46	.93	.63	640	640	160
Control (age matched)	420-850	16-80	40-90	>.20	>.20	>80		

Science. 2000 Apr 28;288(5466):669-72.