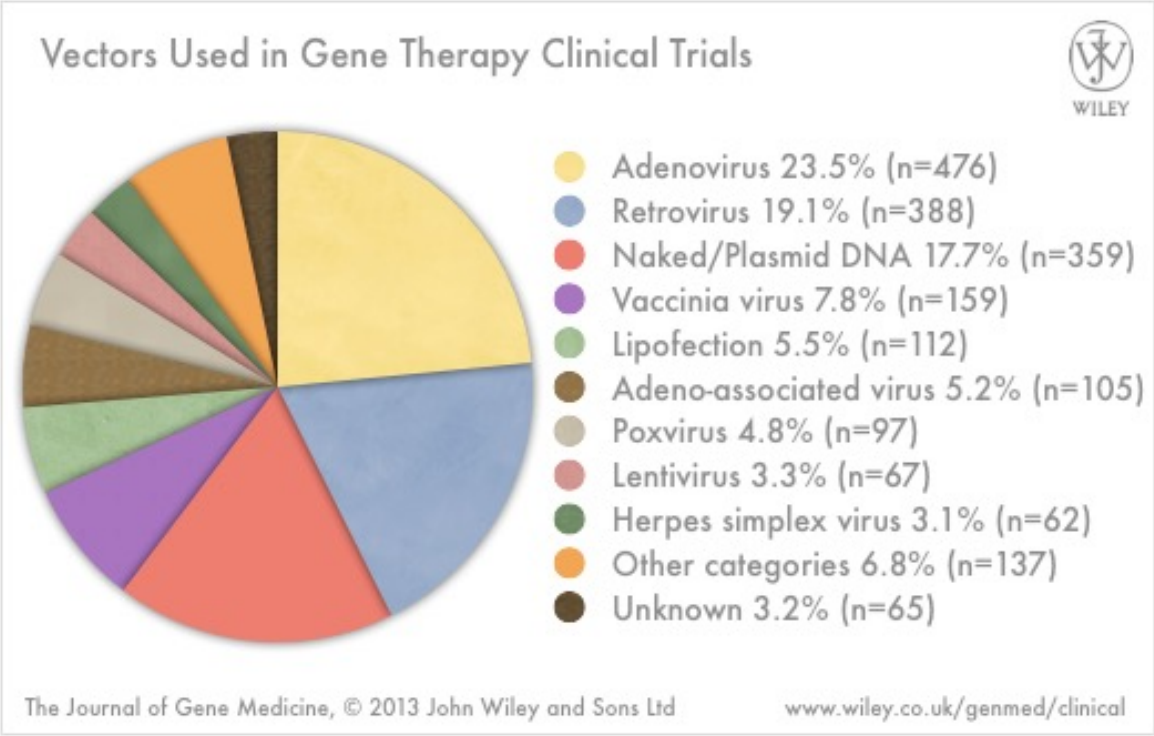
Most people say that it is the intellect which makes a great scientist. They are wrong: it is character.

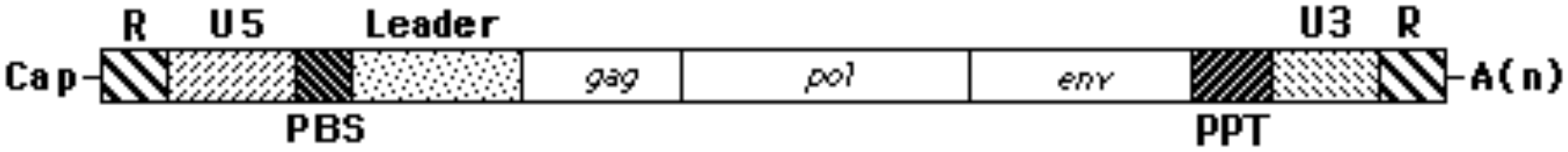
Albert Einstein

Which vectors for the genes



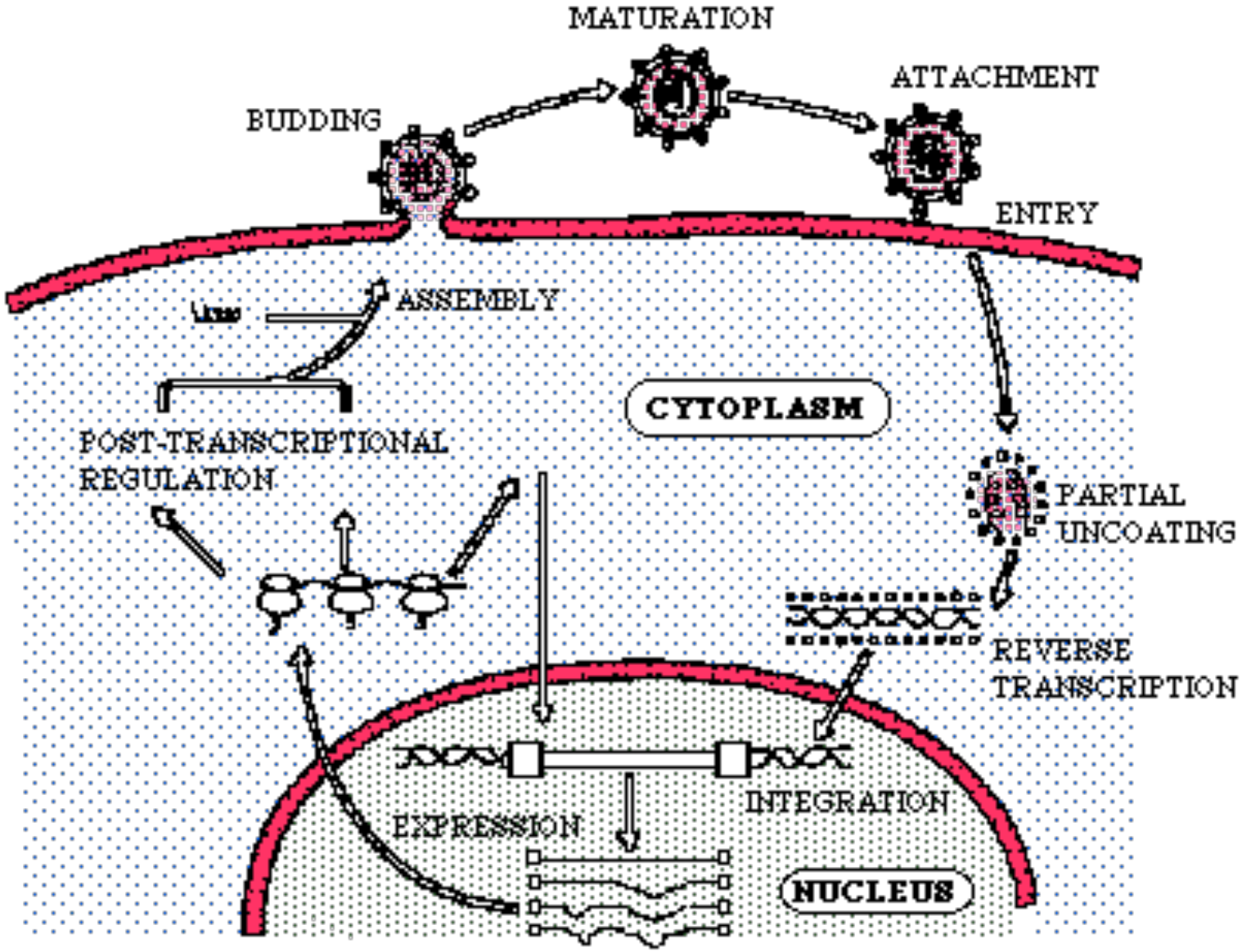
WHY retro: history of knowledge / integration

(onco)Retrovirus (MuLV)

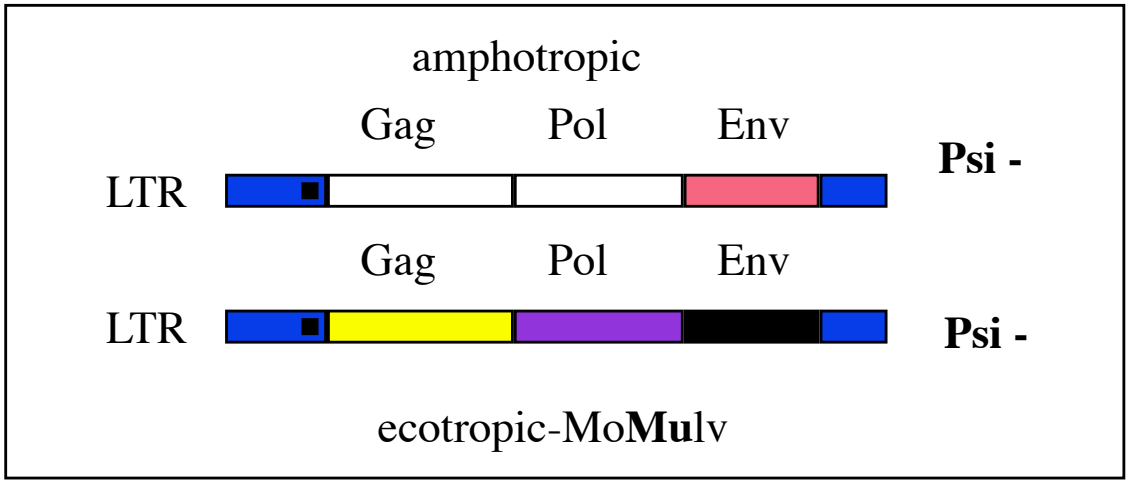


- Receptors: +aa transporter (ecotropic env), phosphate transporter (amphotropic env)
- Enveloped virus
- RNA genome (2 copies)
- dsDNA enters into the nucleus and integrates upon mitosis
- Enters the cell by fusion
- LTR: viral transcription, polyad, replication, integration
- 3 poly-proteins produced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

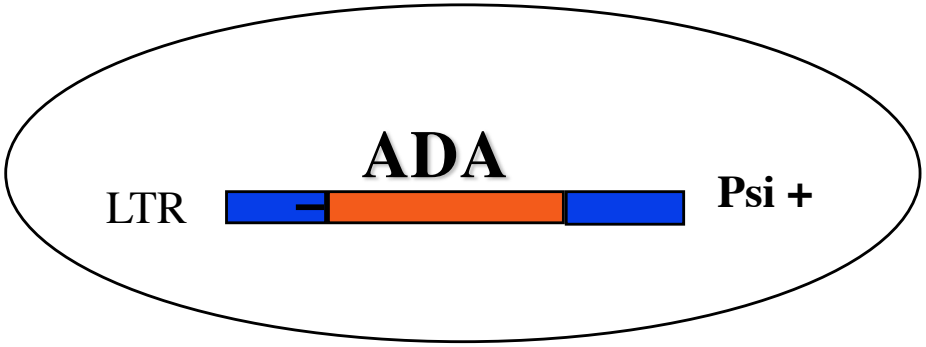
Retrovirus life cycle



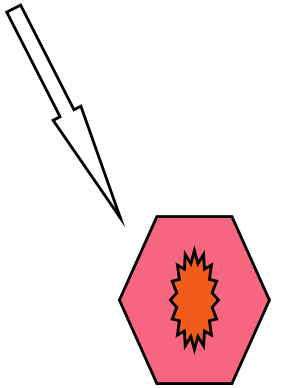
Retroviral vectors



encapsidation cell line

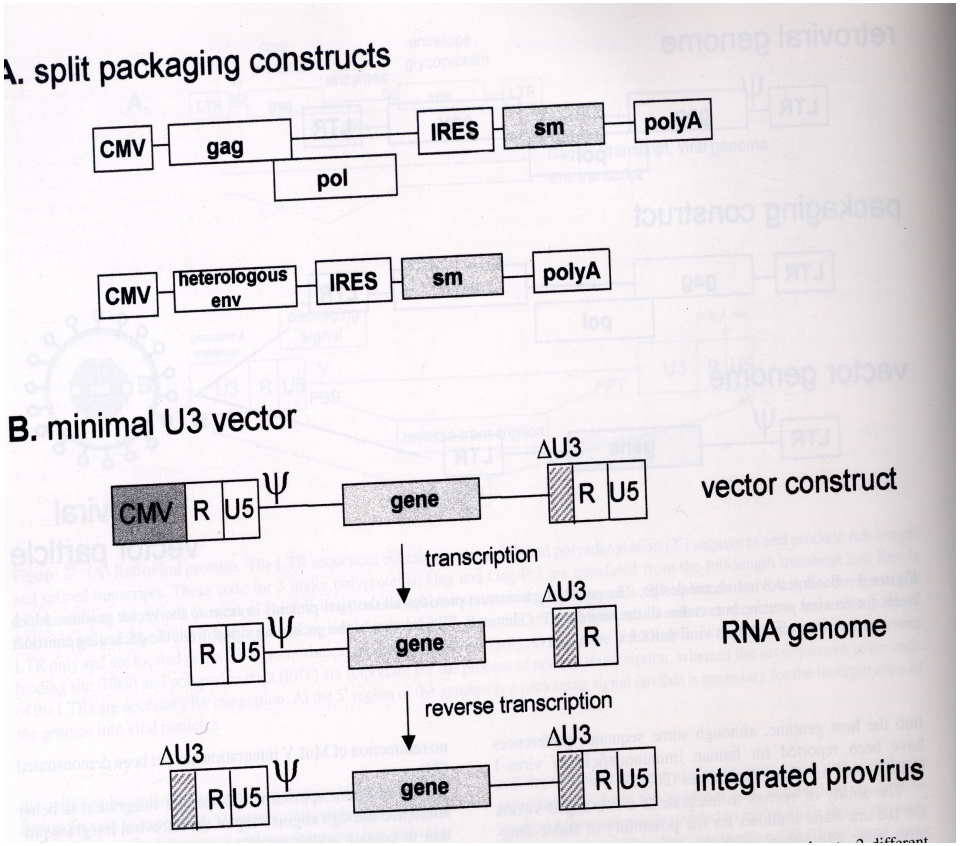


plasmid transfection



Retroviral vectors-ameliorations

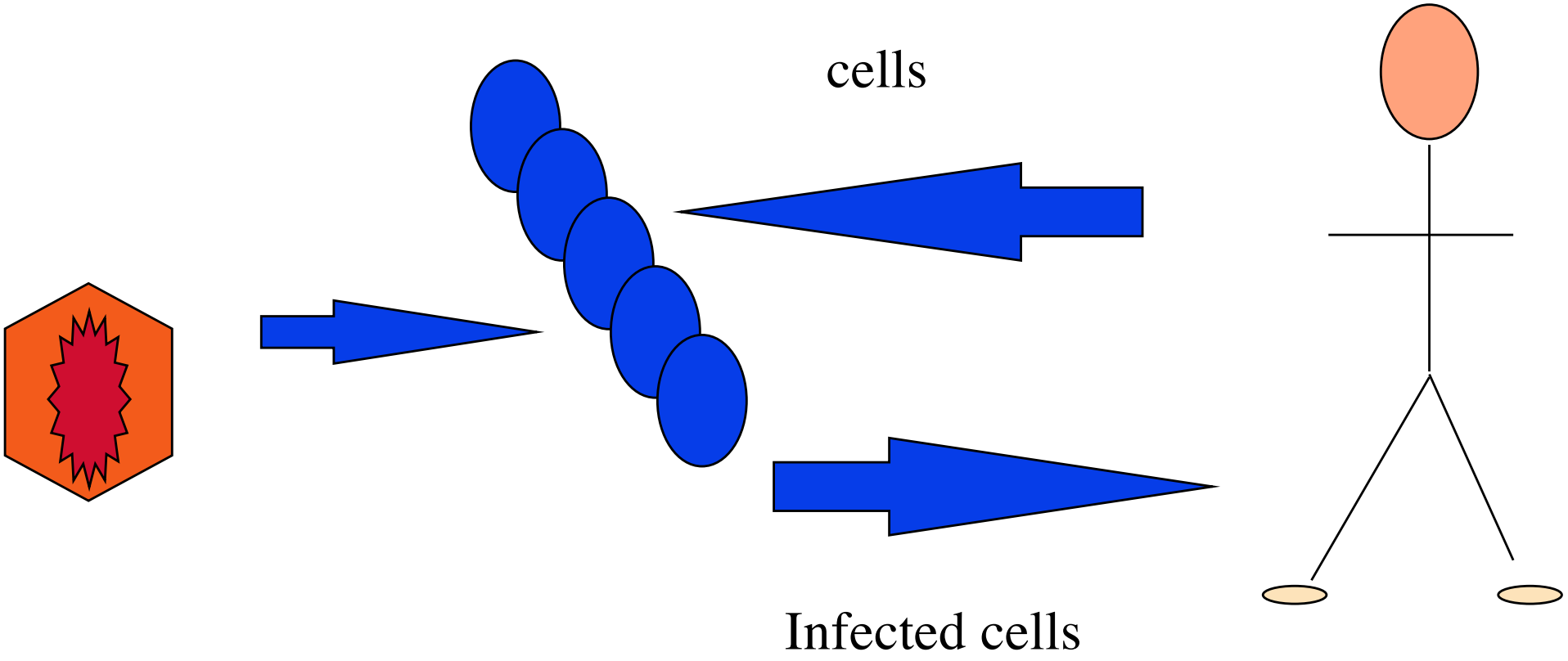
- heterologous envelope (VSV-G)
- Reduce overlap between packaging and vector (in gag and LTR)
- Use different promoters, no LTR
- Substitute the original packaging line NIH3T3 which contains endogenous MuLV like sequences



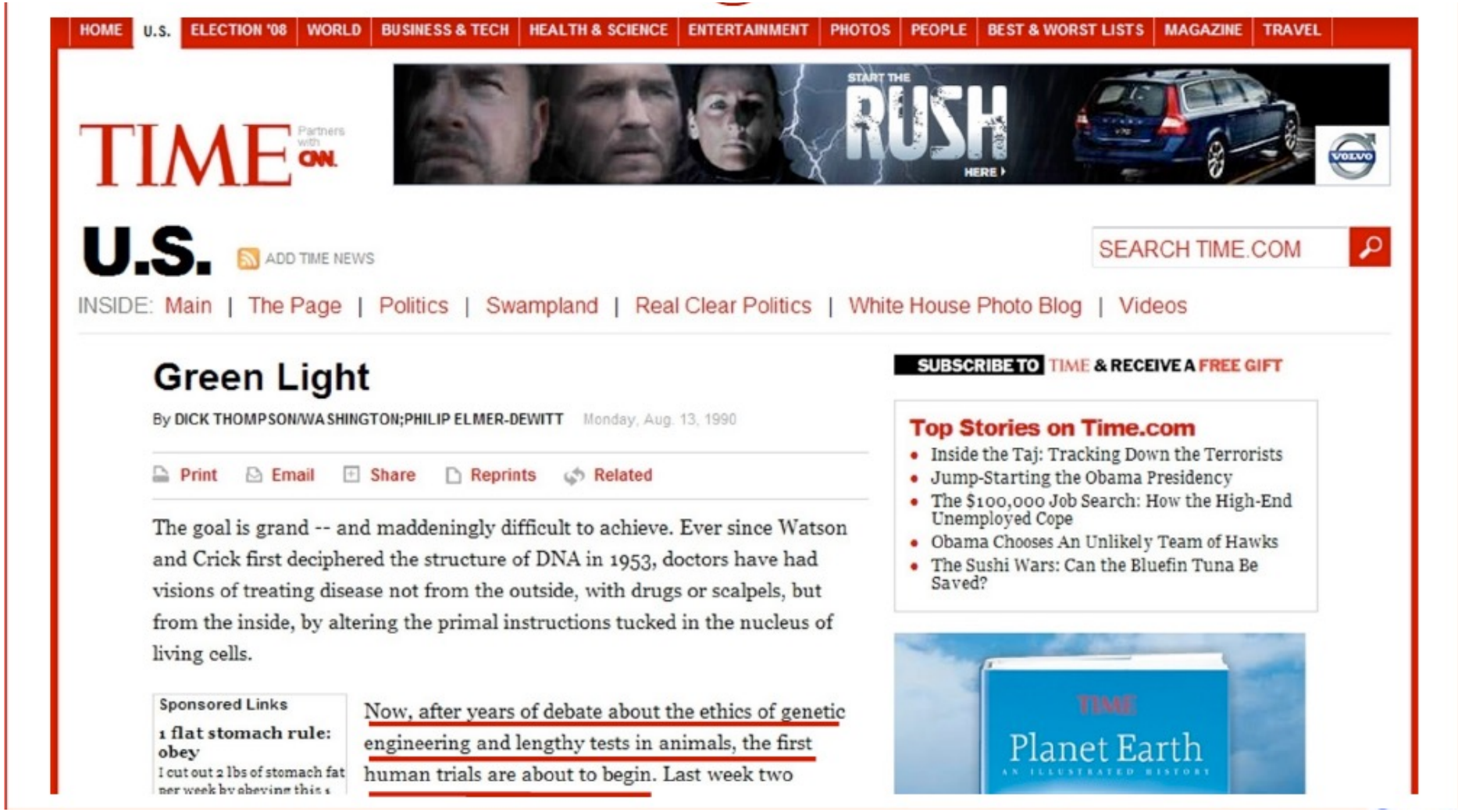
Pros and Cons viral vectors

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



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Green Light

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

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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

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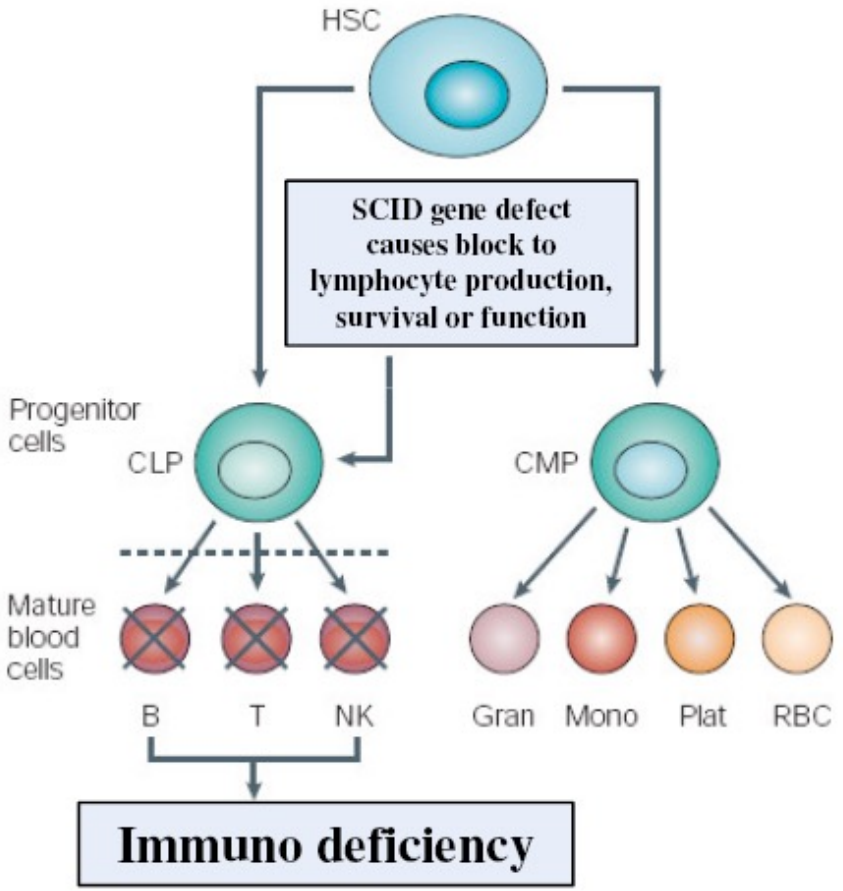
What is SCID

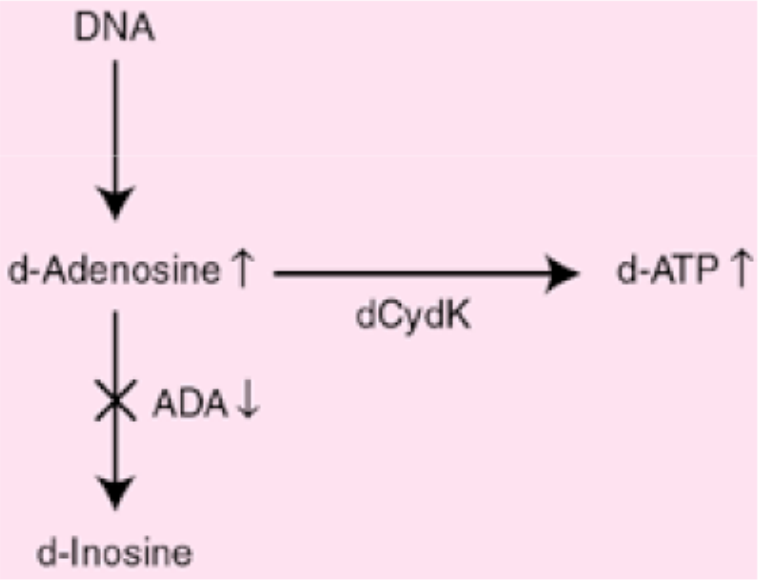
The Buble boy: David Phillip Vetter
(September 21, 1971– February 22, 1984)
Texas (USA)



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

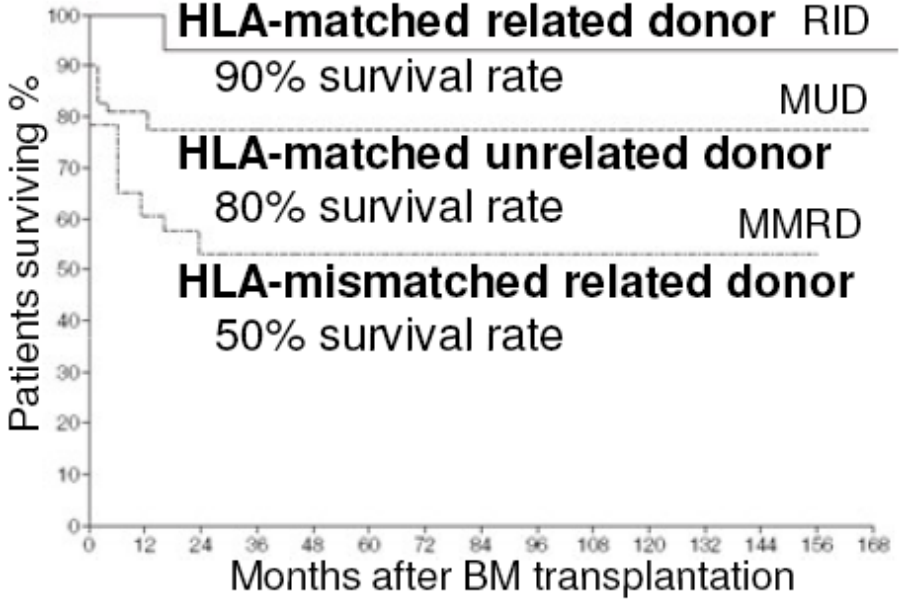
- **recurrent infections**
- **failure to thrive.**
- **multi-system pathologic changes**

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

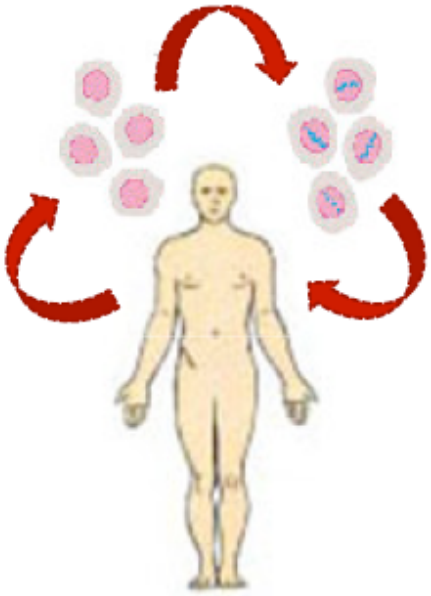
occurrence of neutralizing antibodies or autoimmunity.

Gene therapy advantages

Autologous cells

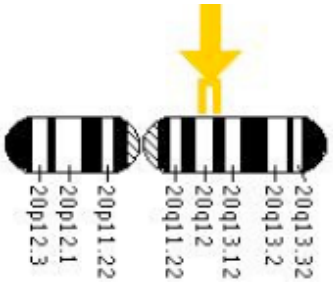
-no HvGG/GvHD

-Available for all patients

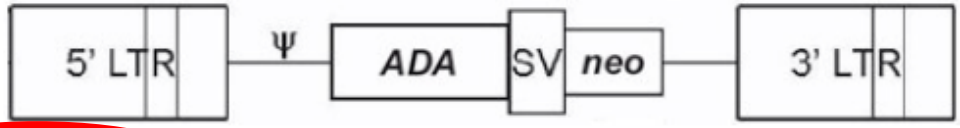


Radical correction of genetic defect of disease

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.

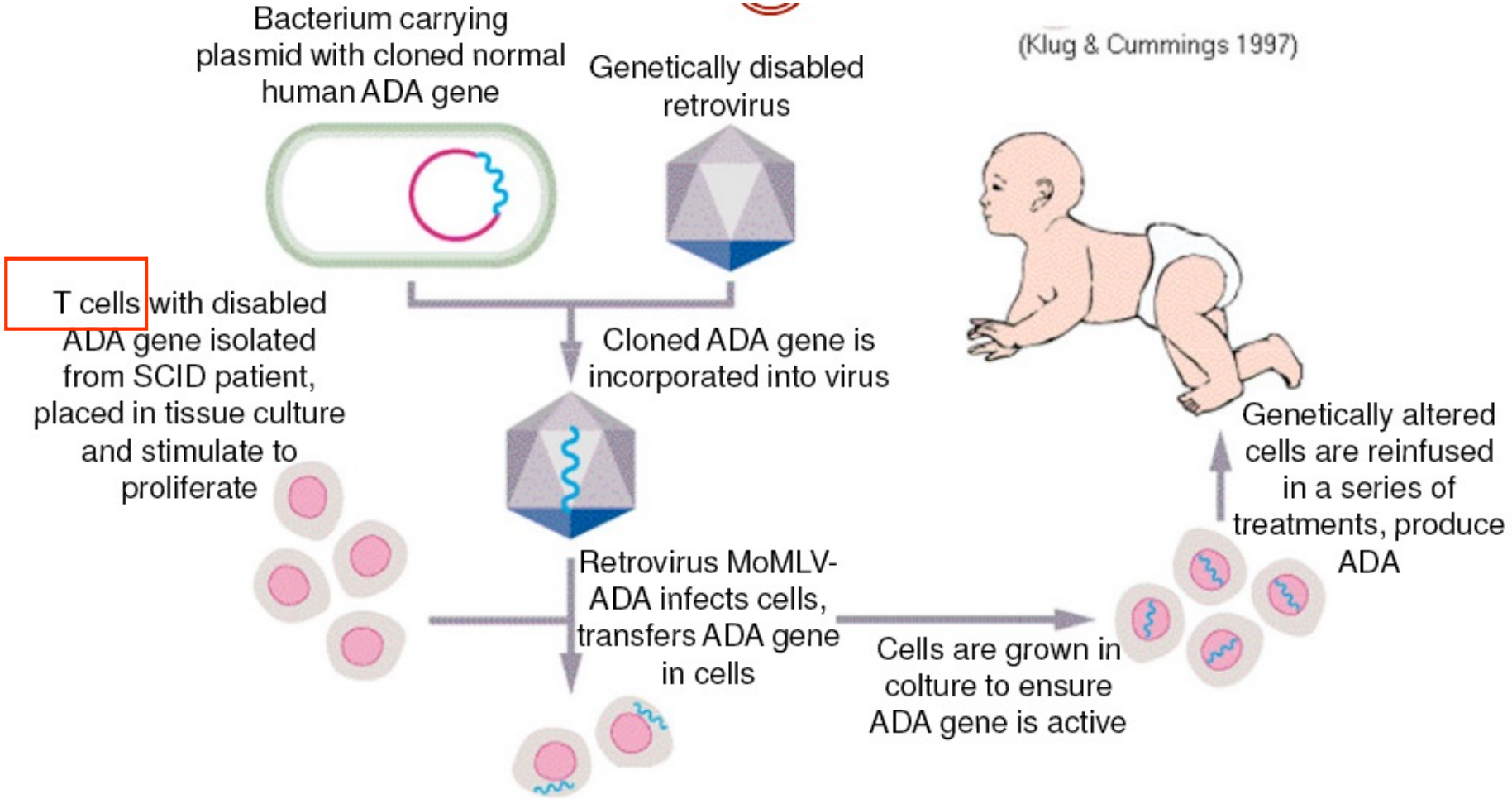
-Wild type or gene corrected cells were shown to carry a strong selective survival advantage over deficient cells in hematopoietic cell transplantation and preclinical gene therapy model

NIH trial

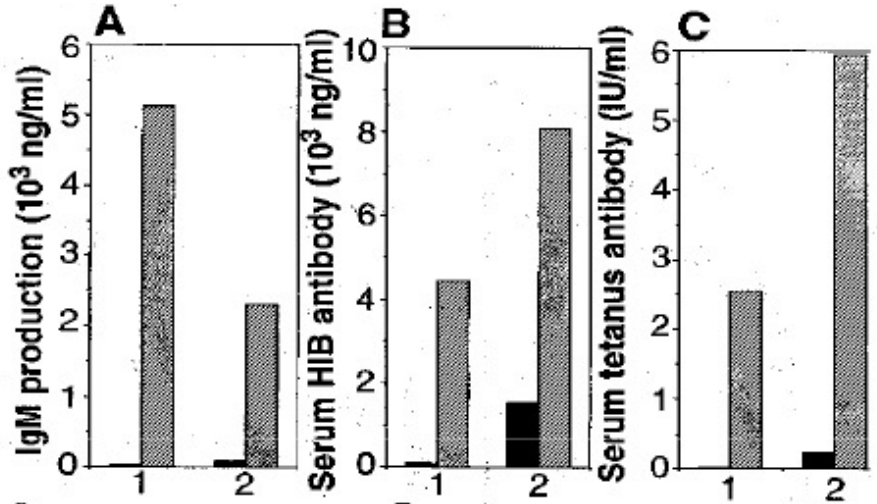
Culver, Anderson, and Blaese with gene therapy patients (Ashanthi De Silva and Cynthia Cutshall).
Courtesy of Dr. Kenneth Culver, Novarti Pharmaceuticals Corp.

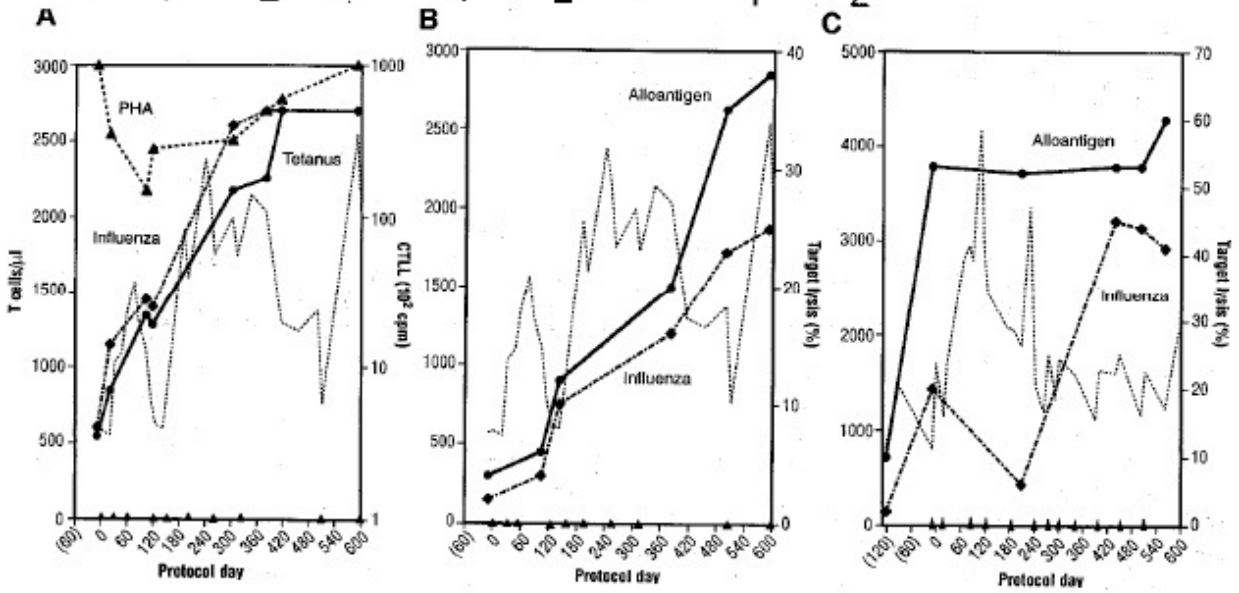
W. French Anderson (NIH); in the late summer of 1990, the FDA was sufficiently convinced by the preliminary laboratory data to approve the first human gene therapy trials using the MoMLV-based delivery vector

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]	2	Transduction after stimulation with antiCD3 monoclonal antibody and IL2
Onodera et al[4]	1	
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.

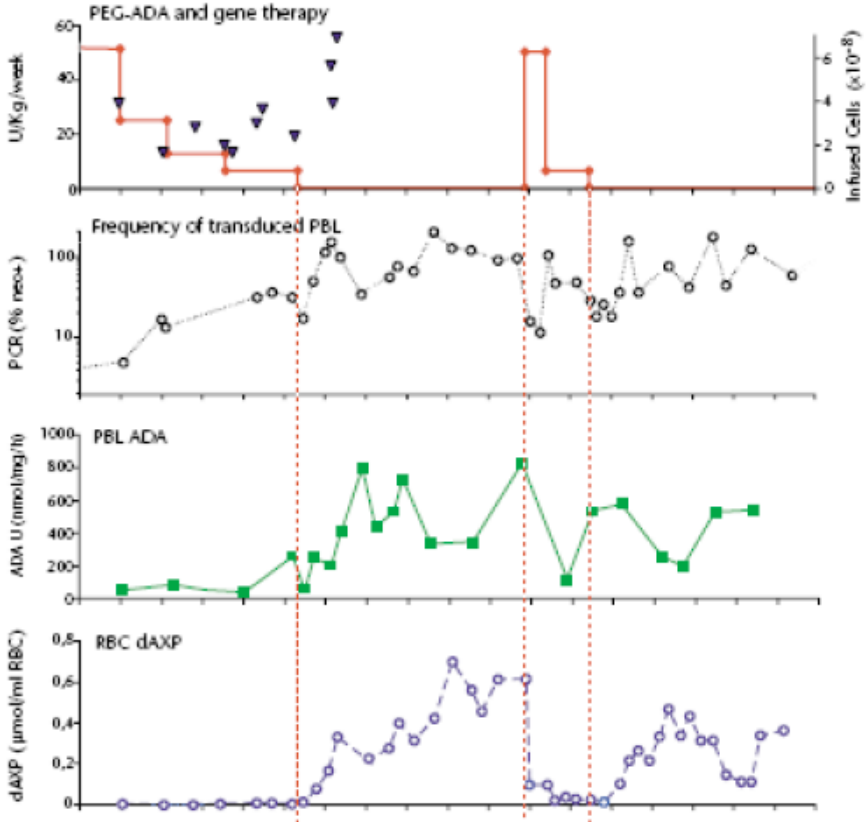
Better growth factors/matrices/serum-free media developed that are capable of stimulating early HSC to divide, become transduced and retain pluripotency.

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)

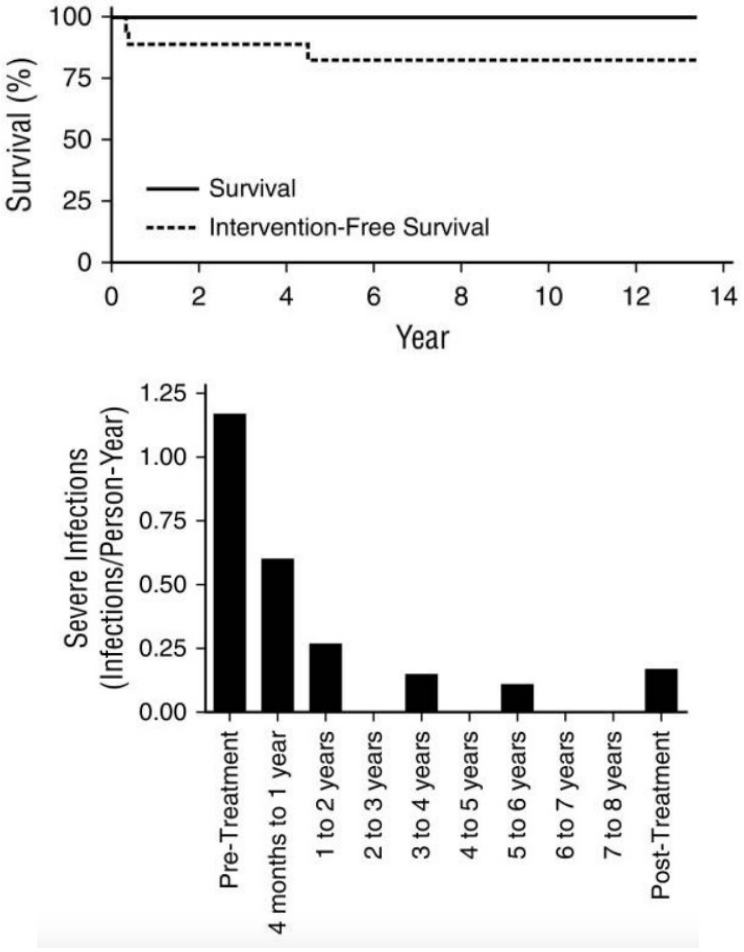
[1]**Haematopoietic stem cells gene therapy for ADA-SCID**.
Aiuti et al. 2008.Blood Cells Mol Dis 40:248

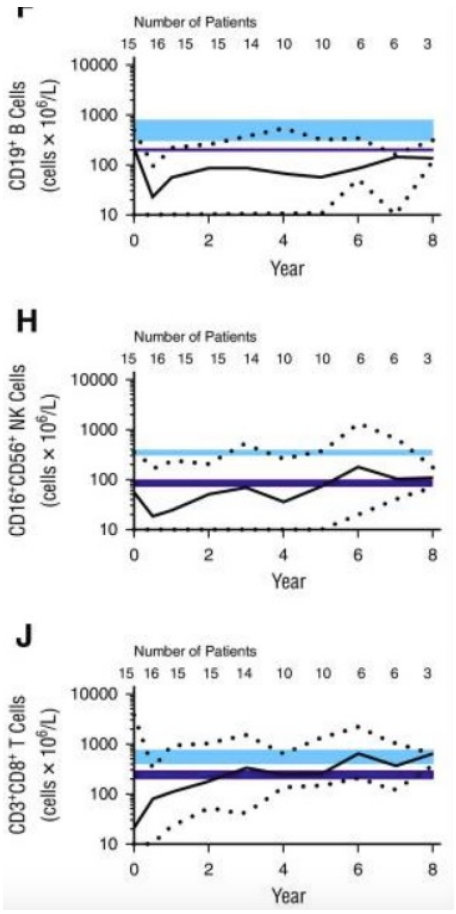
[2]**Corrective gene transfer into bone marrow CD34+ cells for adenosine deaminase (ADA) deficiency: results in four patients after one year follow up.**
Candotti F , Khon BD et al. 2003. Mol Ther 7:S448.

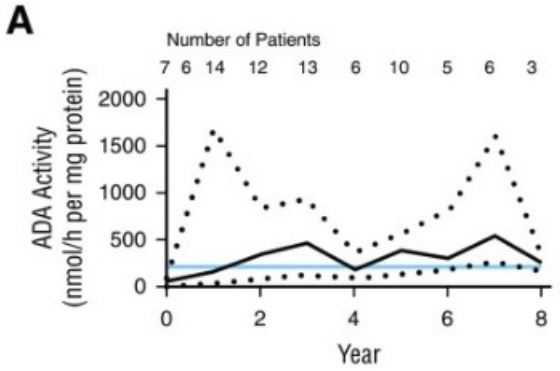
[3]**Successful reconstitution of immunity in ADA-SCID by stem cells gene therapy following cessation of PEG-ADA and use of mild preconditioning.**
Gasper HB et al. 2006. Mol Ther 14:505.

Gene therapy and non-myeloablative conditioning

Long Term Results



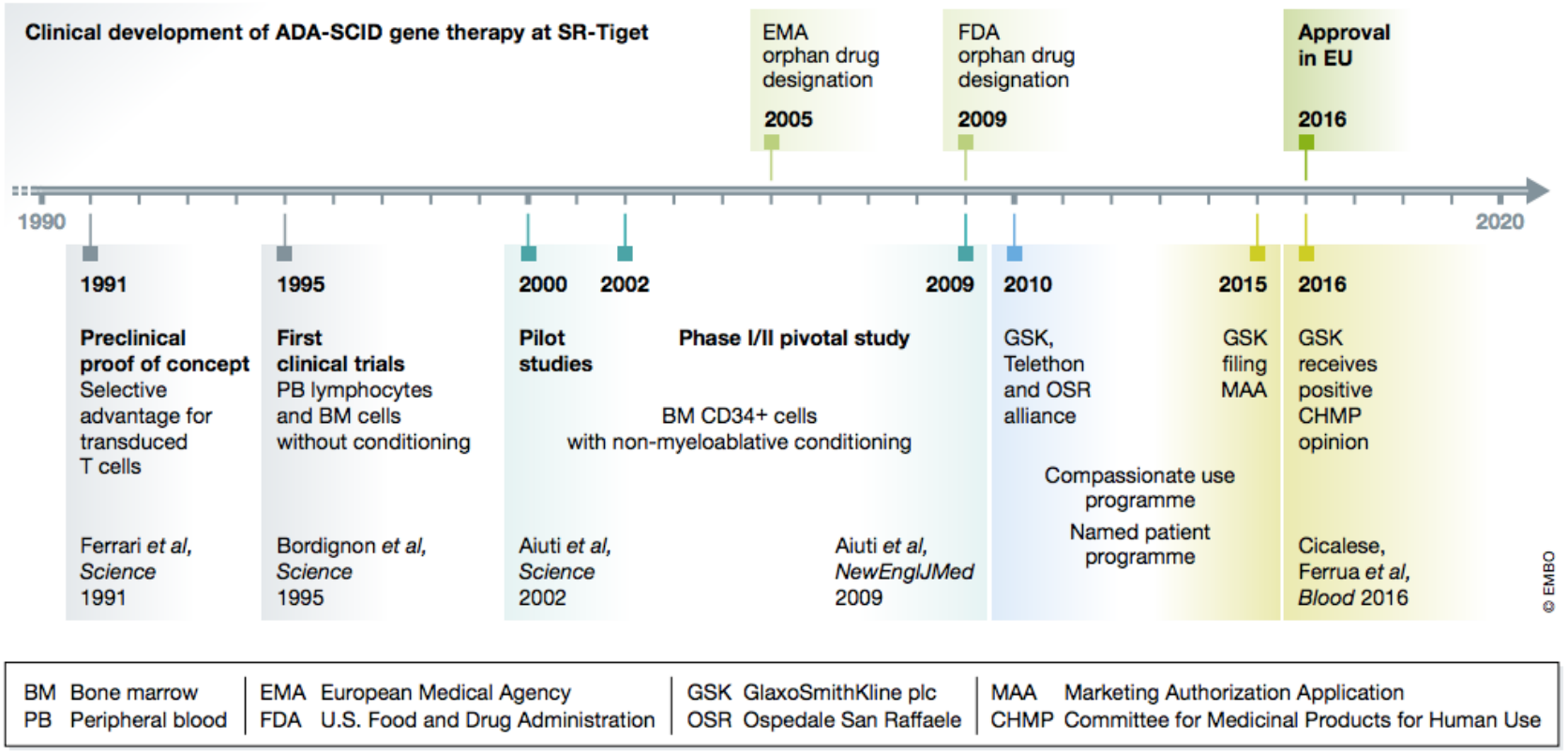




No events indicative of myelodysplasia or leukemic transformation were reported in any patient at any time.

Update on the safety and efficacy of retroviral gene therapy for immunodeficiency due to adenosine deaminase deficiency. Cicalese et al., Blood, 2016

Gene therapy and non-myeloablative conditioning



Aiuti et al, Embo Molecular Medicine, 2017

QUESTIONS?