Gene therapy

Alain Fischer

Hôpital Necker Enfants Malades, Inserm, Institut Imagine, Collège de France, Paris

3 March 1972, Volume 175, Number 4025

SCIENCE

Gene Therapy for Human Genetic Disease?

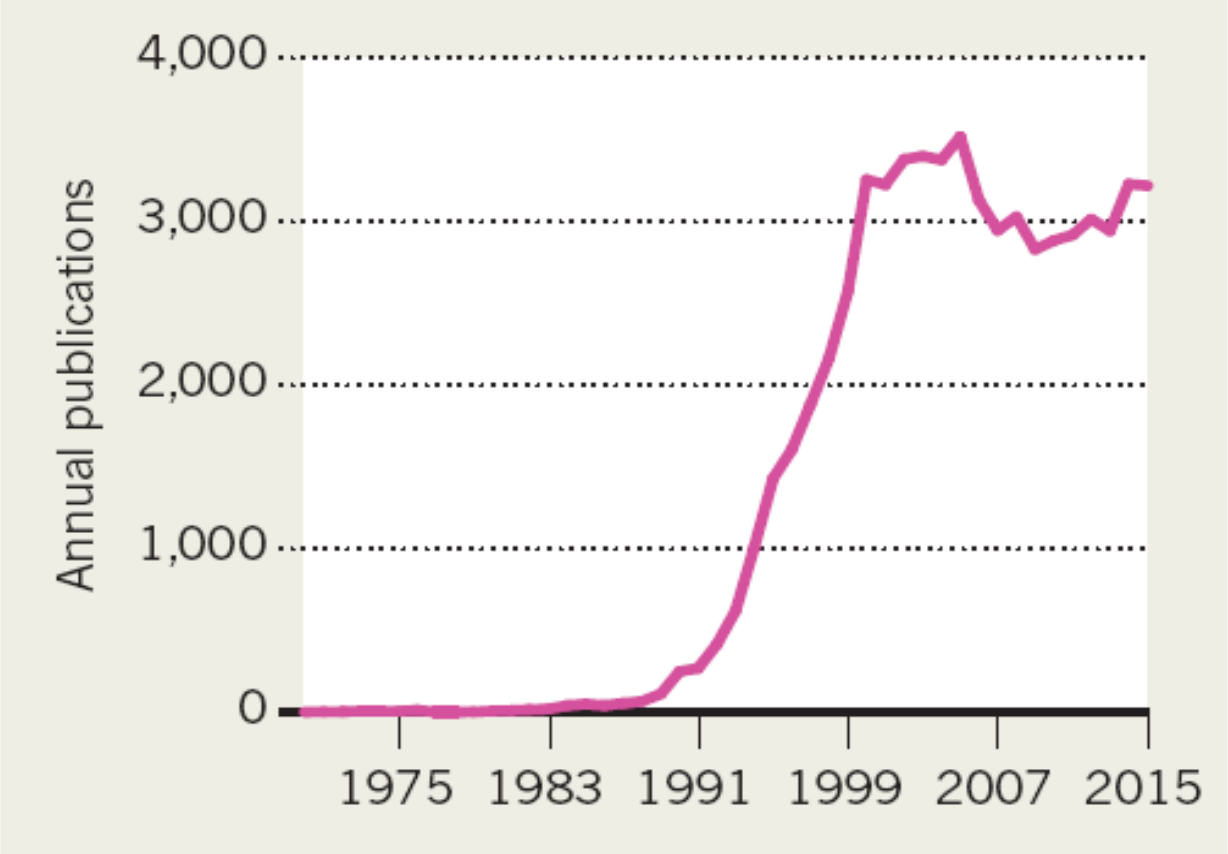
Proposals for genetic manipulation in humans raise difficult scientific and ethical problems.

Theodore Friedmann and Richard Roblin

Schematic Model of Genetic Disease

Some aspects of a hypothetical human genetic disease in which an enzyme is defective are shown in Fig. 1. The consequences of a gene mutation which renders enzyme E_3 defective could be (i) failure to synthesize required compounds D and F; (ii) accumulation of abnormally high concentrations of compound C and its further metabolites by other biochemical pathways; (iii) failure to regulate properly the activity of enzyme E_1, because of loss of the normal feedback inhibitor, compound F; and (iv) failure of a regulatory step in a linked pathway because

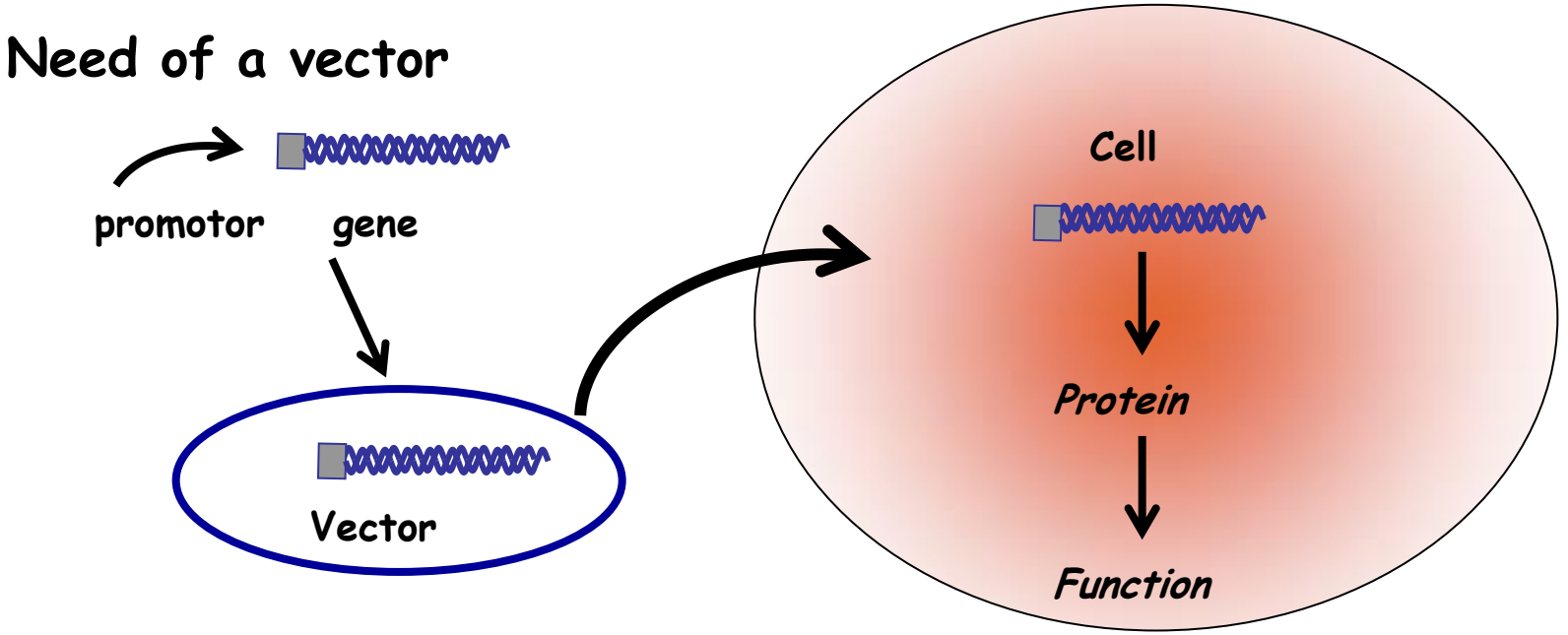
Research papers in gene therapy



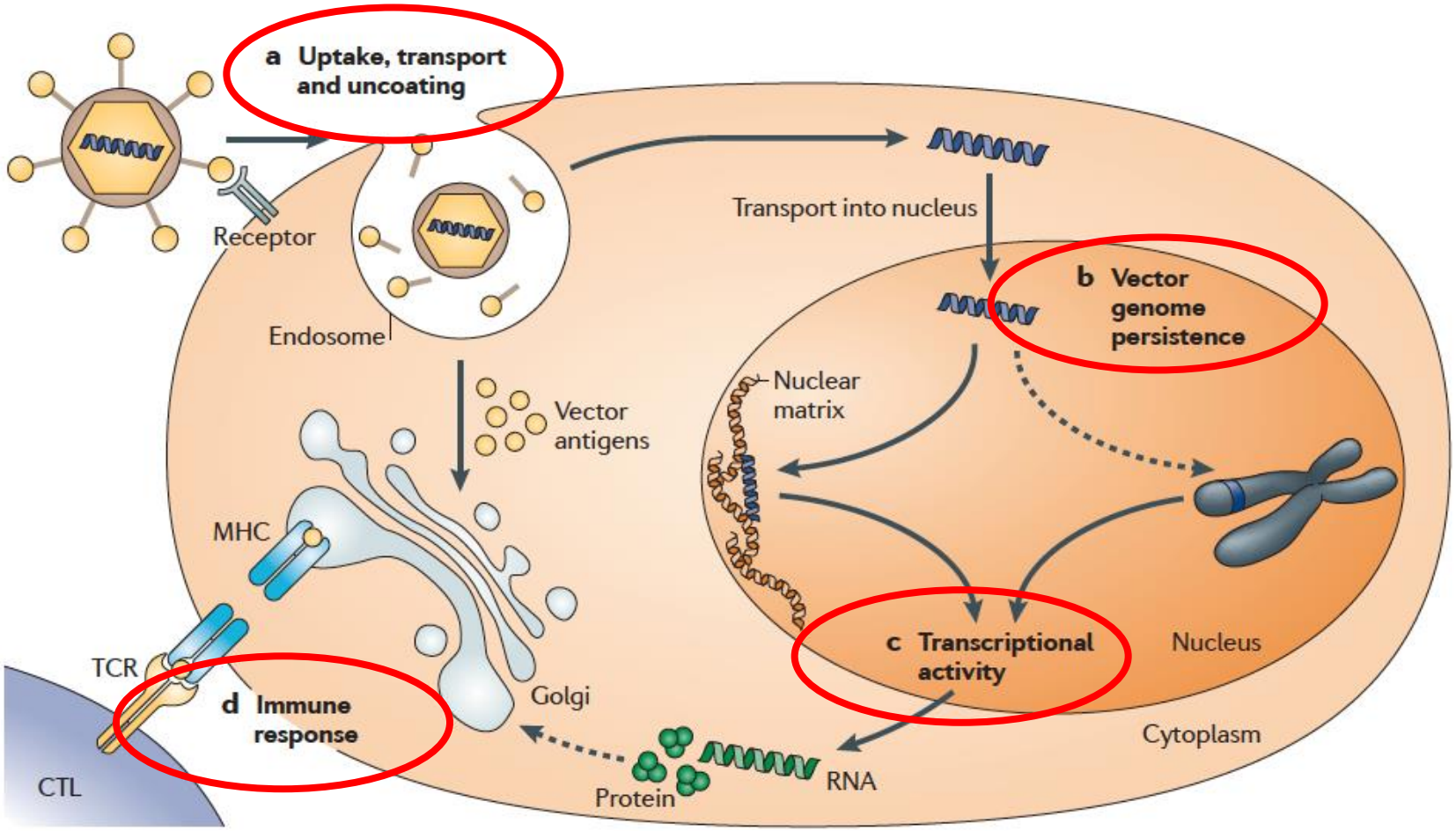
Nature, 2016

Strategies for gene therapy

- To add a functional copy of a mutated gene
- To inhibit the expression of a (mutated) gene
- To fix a mutation
- To add a "new gene" to provide a new function



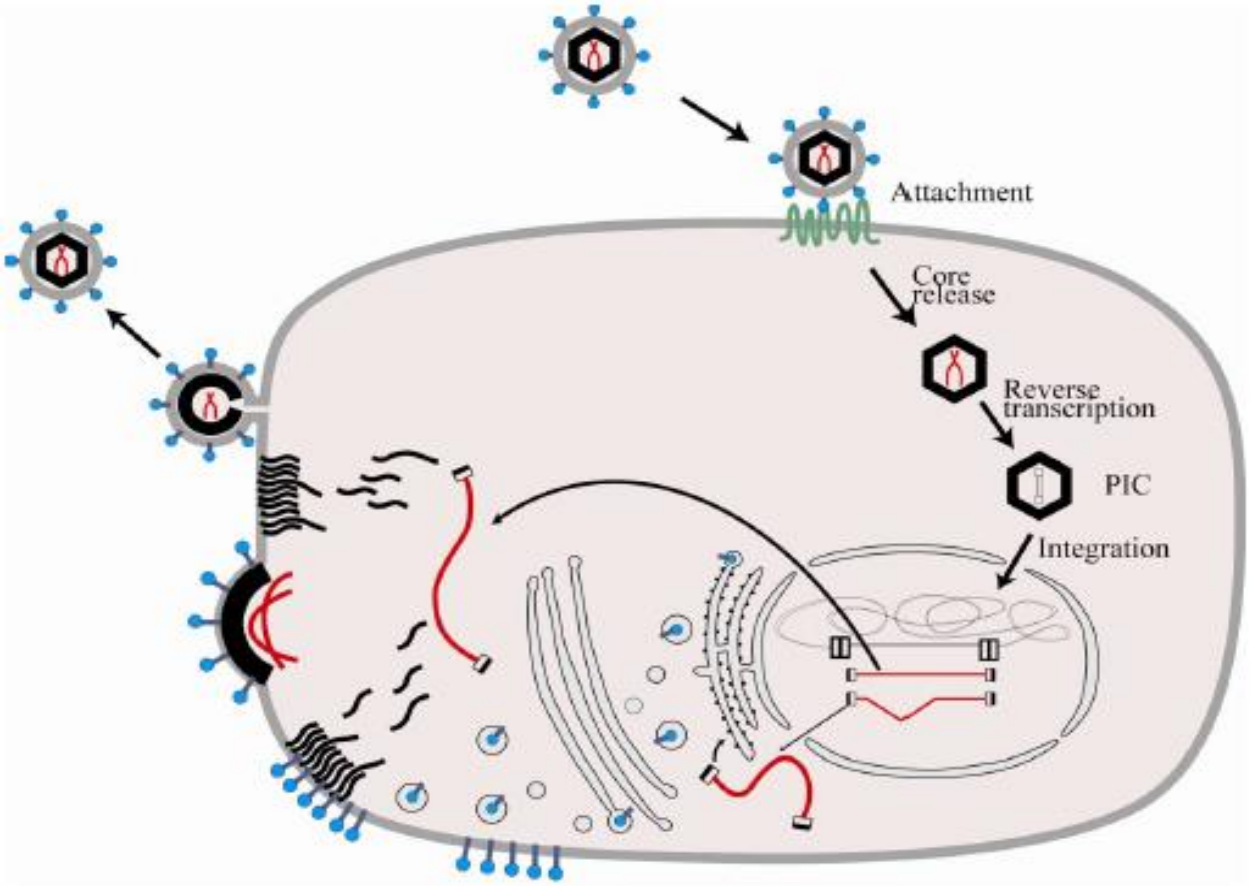
4 hurdles



M.A. Kay, Nature Reviews Genetics 2011

2 challenges: safety and long term efficacy

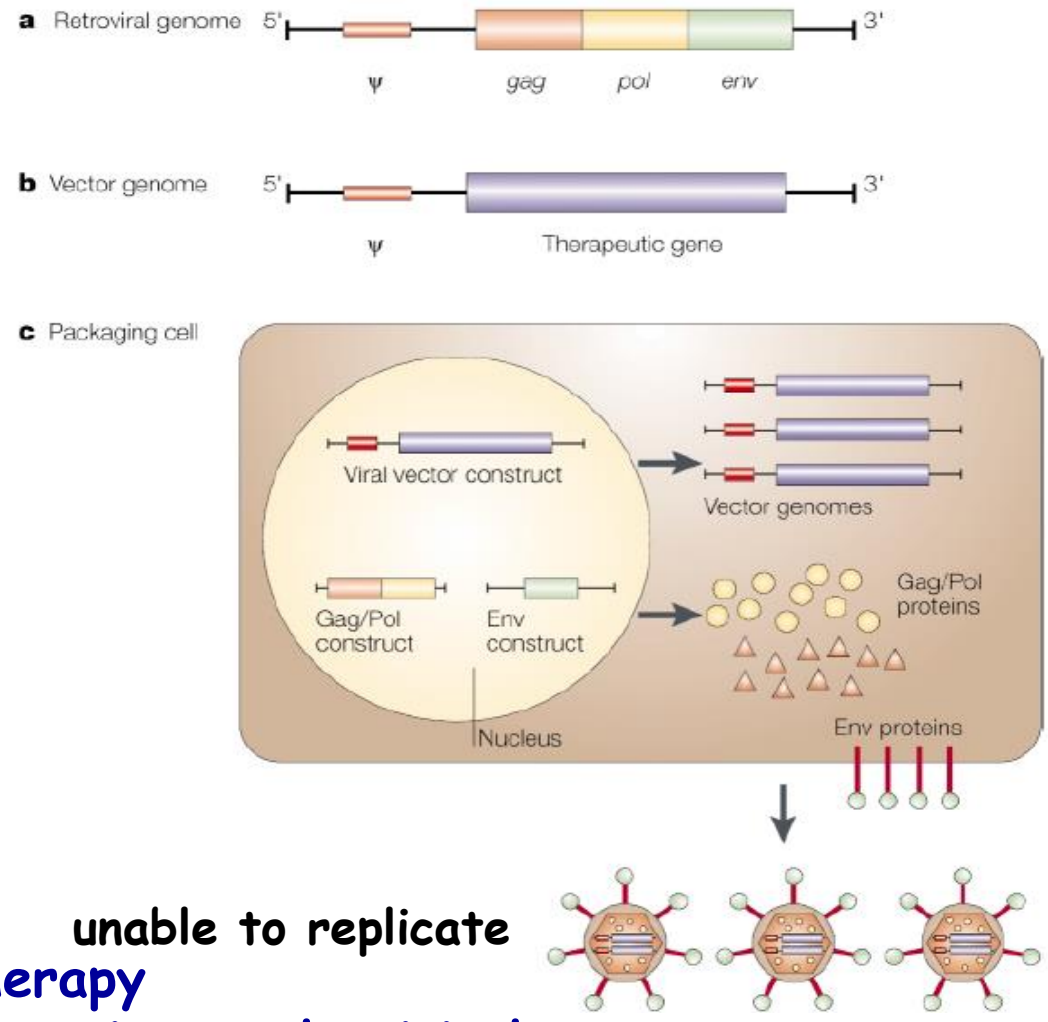
Retrovirus



integration into the genome, replication and transcription
exactly what do one needs for gene therapy in stem cells

A.W. Nienhuis, Blood 2008

Construction of retroviral vectors



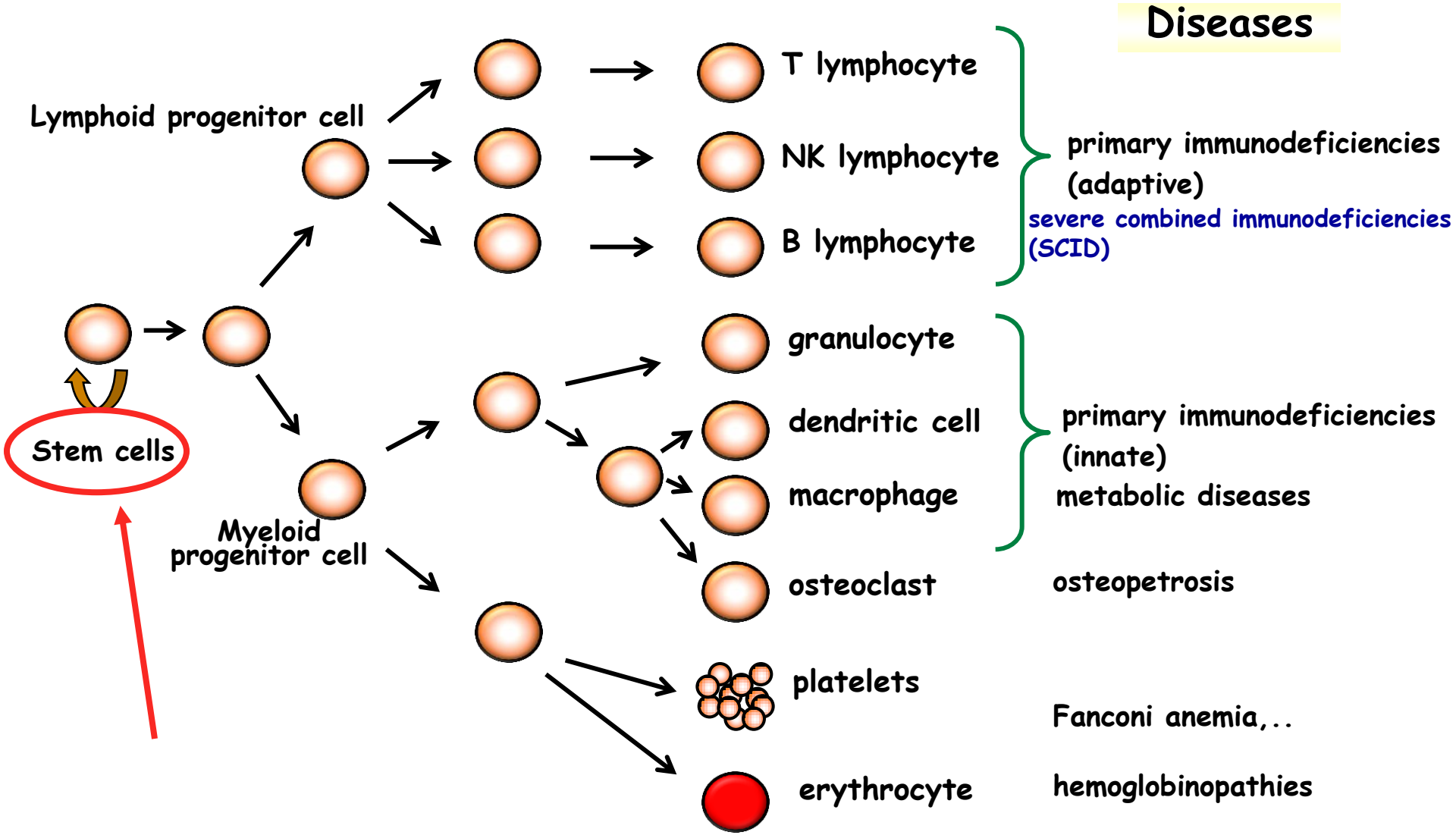
unable to replicate

Ex vivo gene therapy

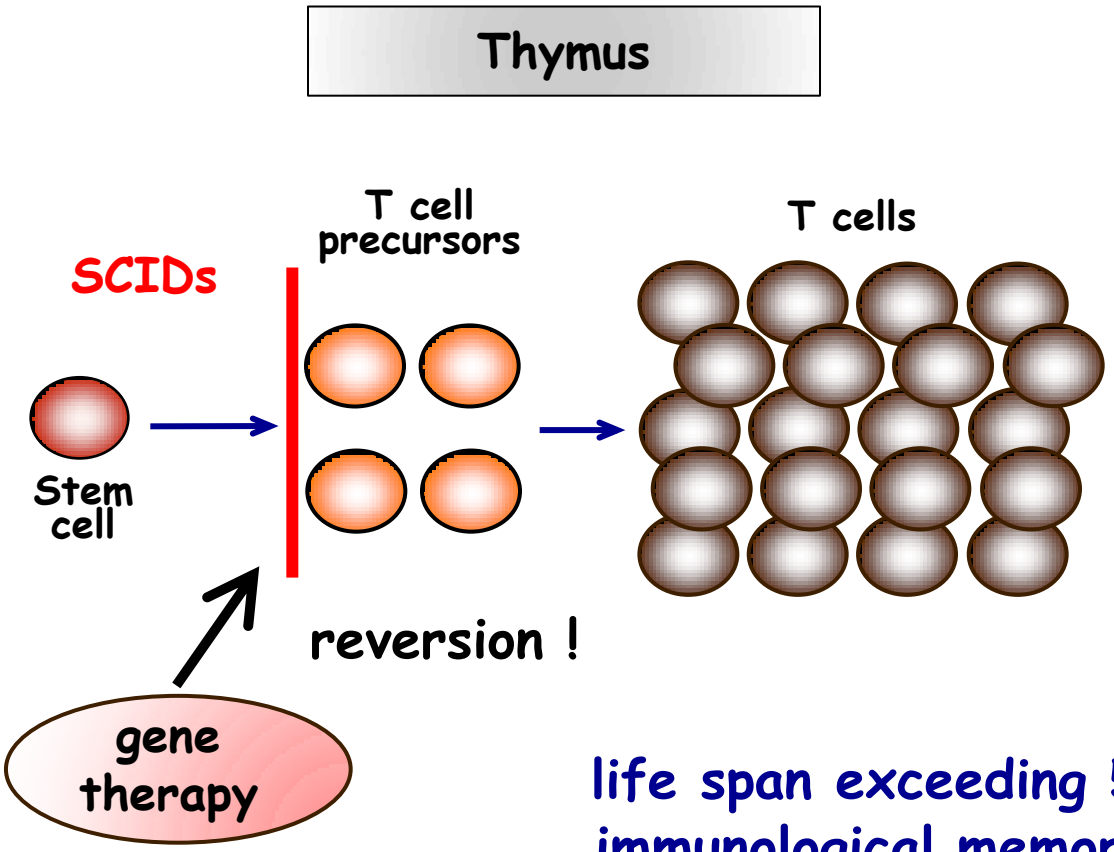
From gamma retrovirus to lentiviral vectors

N. Somia & I. Verma, Nature Reviews Genetics 2000

Gene therapy ⇨ hematopoietic stem cells



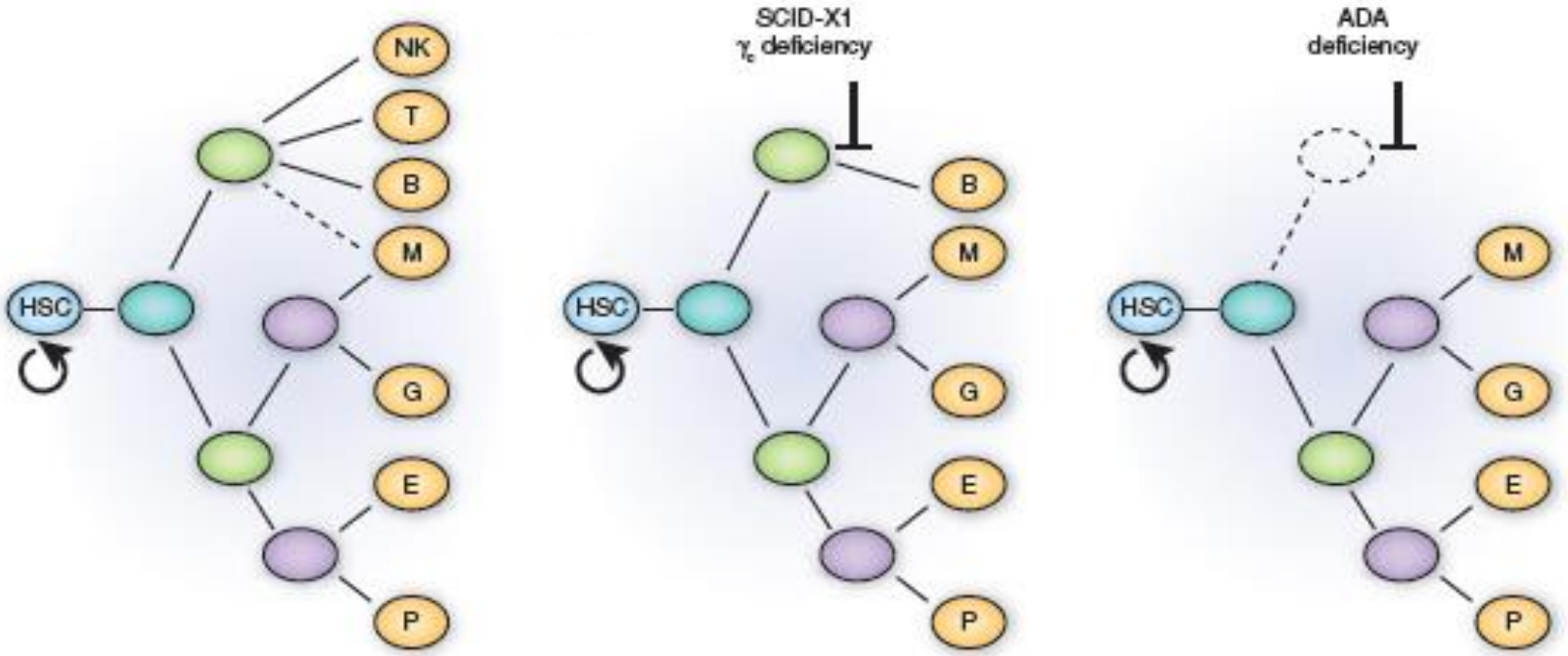
Why SCIDs were the optimal condition to probe gene therapy



life span exceeding 50 years
immunological memory

Gene therapy for SCID

SCID X1 and ADA deficiency



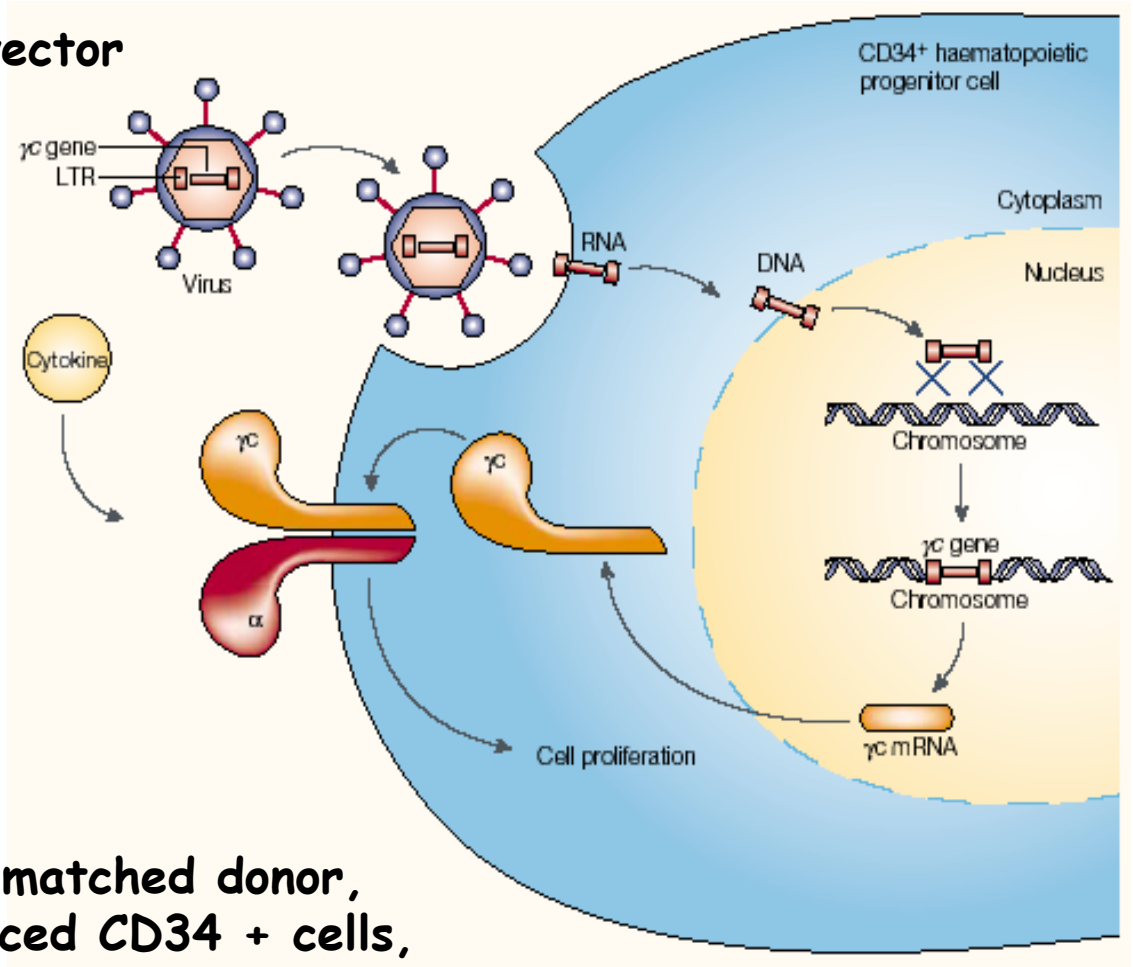
Lethal conditions, allogeneic HSC* transplantation can be curative but is associated with significant adverse events (GVHD)

** HSC : hematopoietic stem cell*

Ex vivo gene therapy for SCID-X1

Amphotropic MFGB2 vector

(1999-2002)

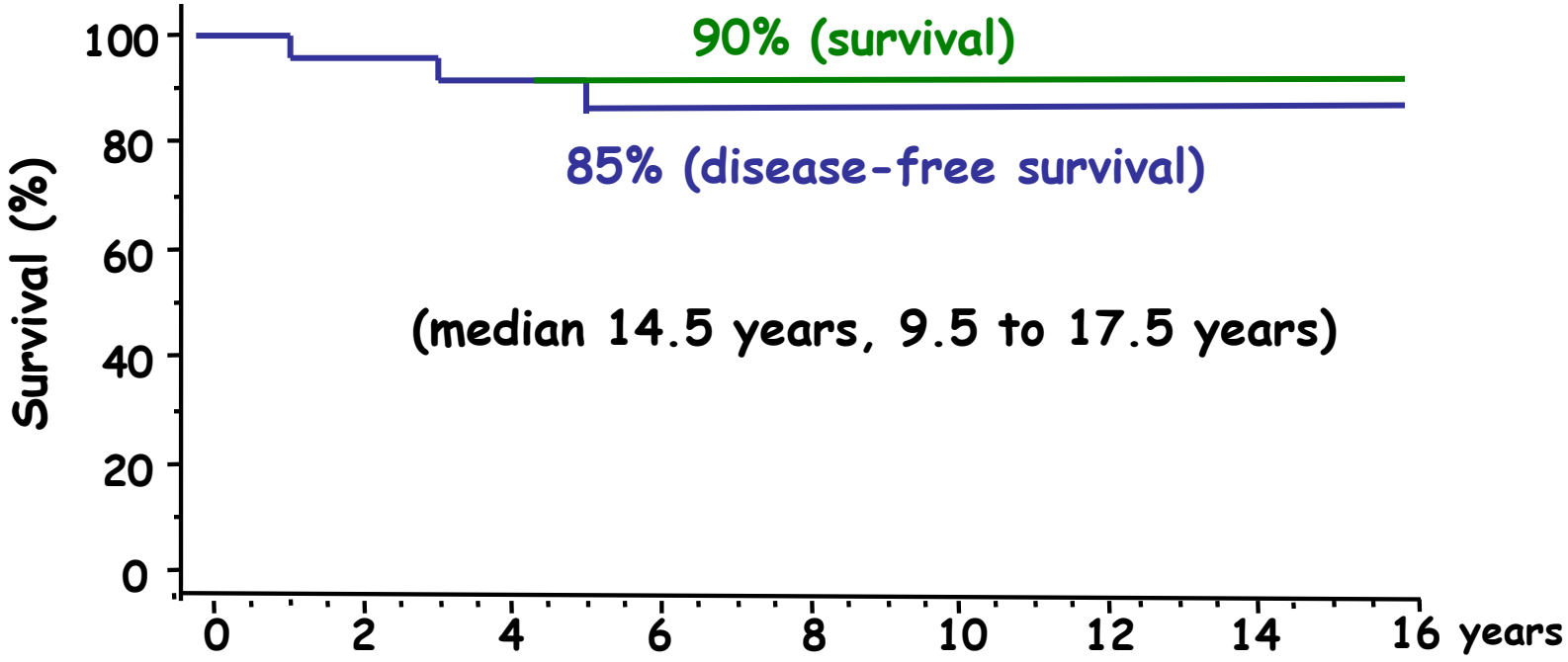


Patients with no HLA matched donor, reinjection of transduced CD34 + cells, no chemotherapy

SCID-X1 gene therapy

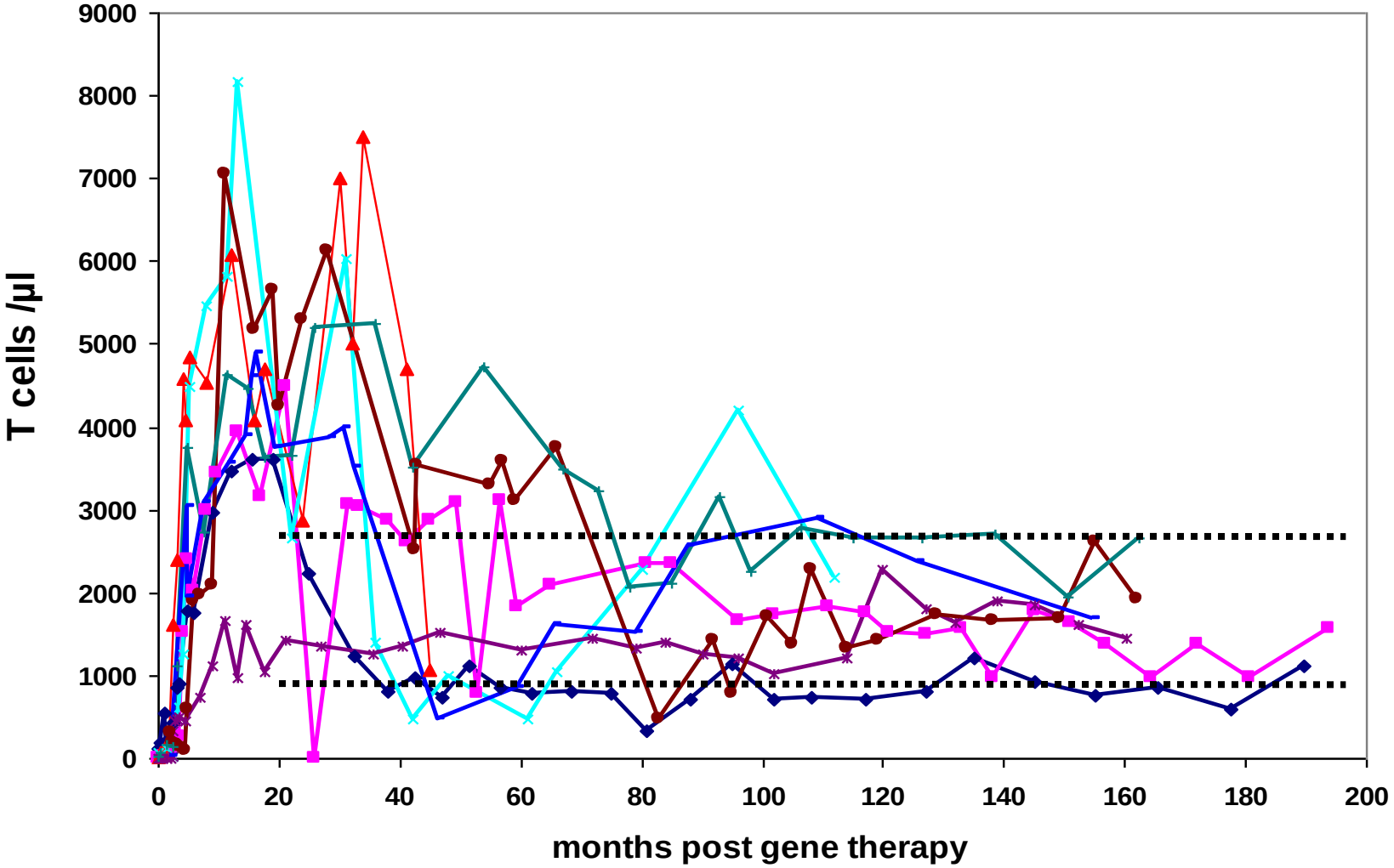
Paris + London data

First results: efficacy



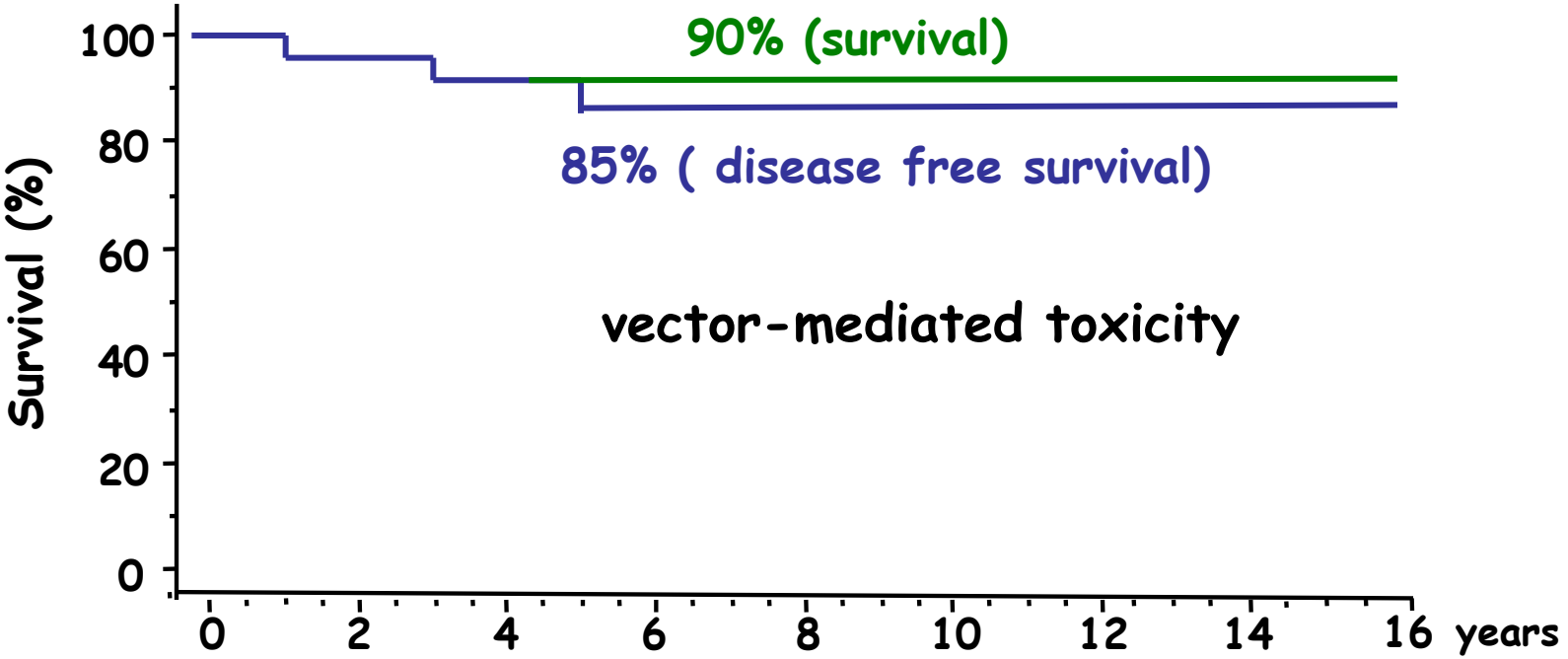
Correction of T cell-mediated immune functions, normal quality of life
Some require Ig substitution

SCIDXI trial 1: sustained T cell detection



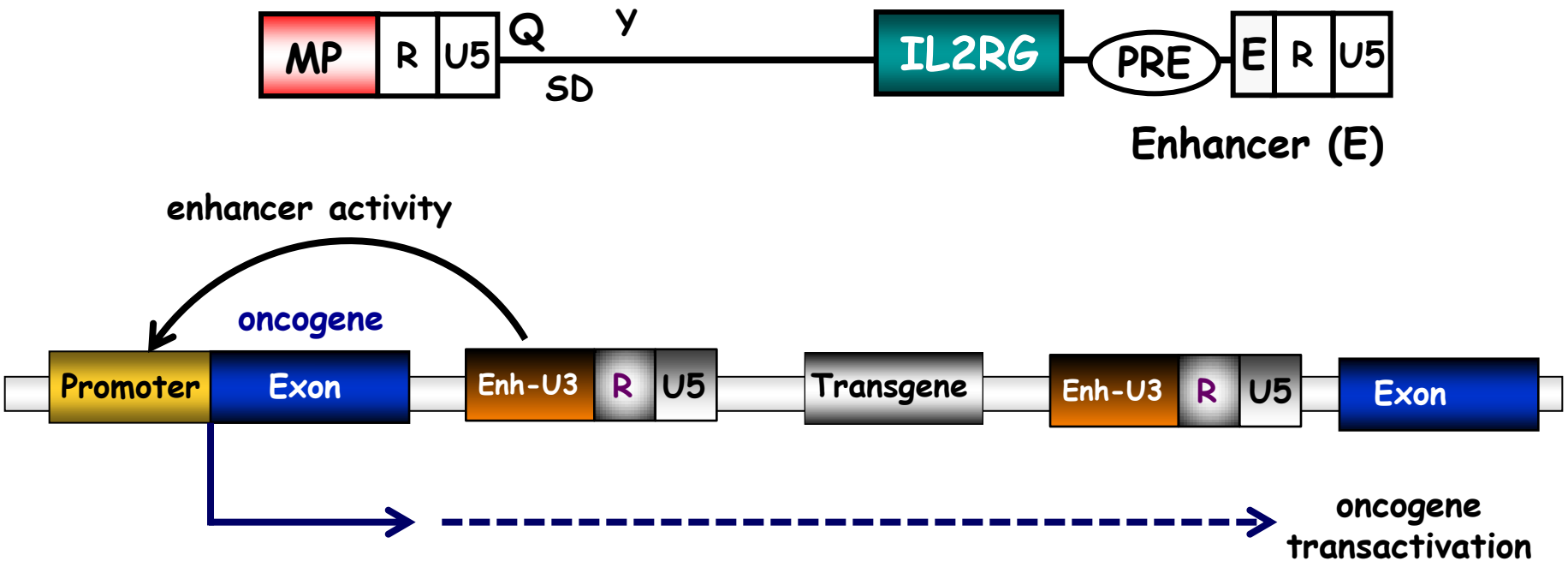
Gene therapy of SCID-X1

First results: safety issue



Occurrence of serious adverse events (T cell leukemias)
fatal outcome in 1
Interruption of clinical trials

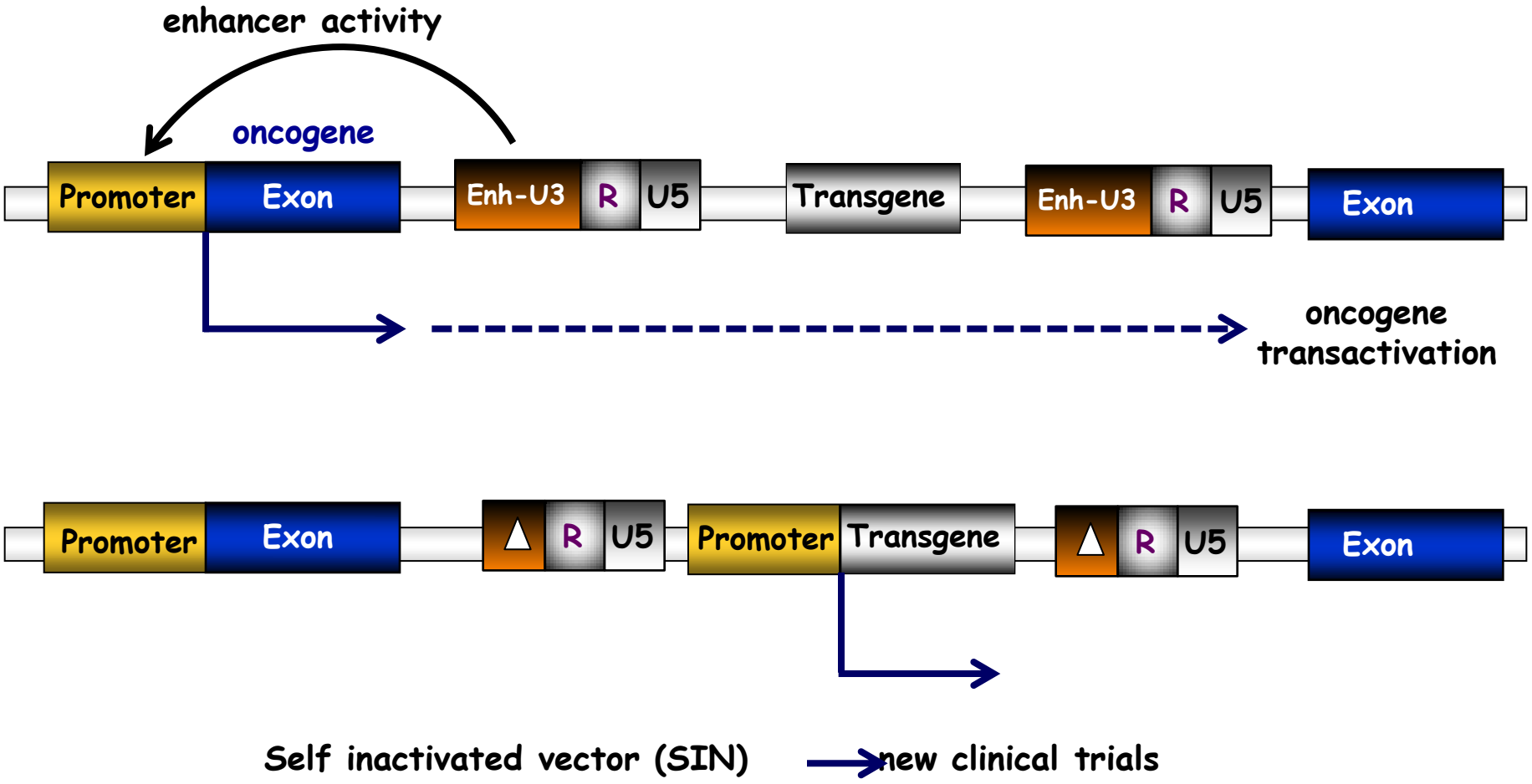
Insertional mutagenesis



Protooncogenes expressed in hematopoietic progenitor cells :
LMO-2, CCND2, ..

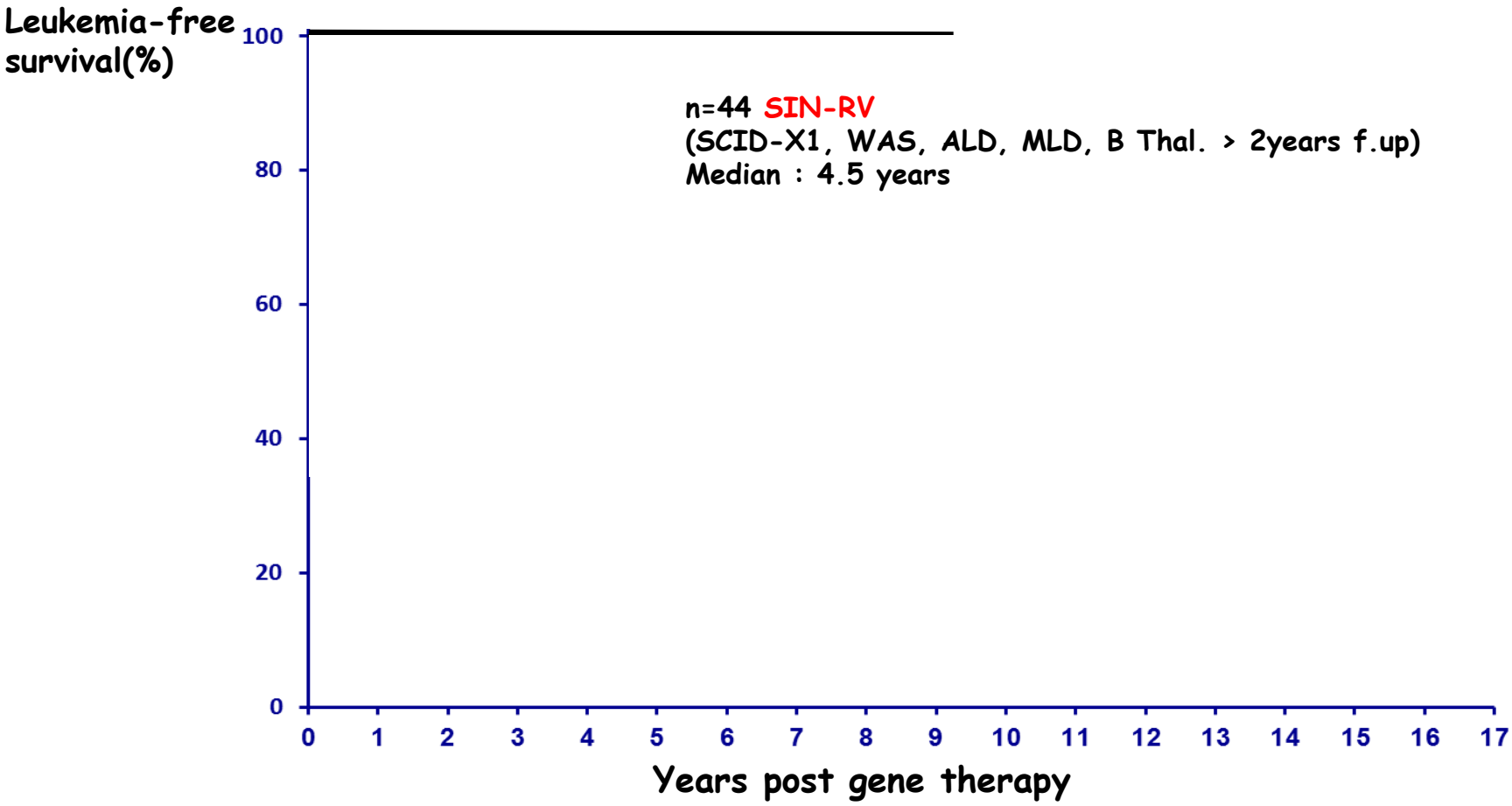
Insertional mutagenesis

How to prevent it ?



Self inactivated vector (SIN) → new clinical trials

Improvement in the safety of retroviral vectors



Gene therapy for PIDs (modern era)

I. First generation of γRV

	n=	alive	successful	median f.up (y.)	range (y.)
SCID-X1*	20	18	17	14.5	9.5-17.5
SCID ADA	42	42	31	8	2-15
total	62	60	48 (77.4%)		

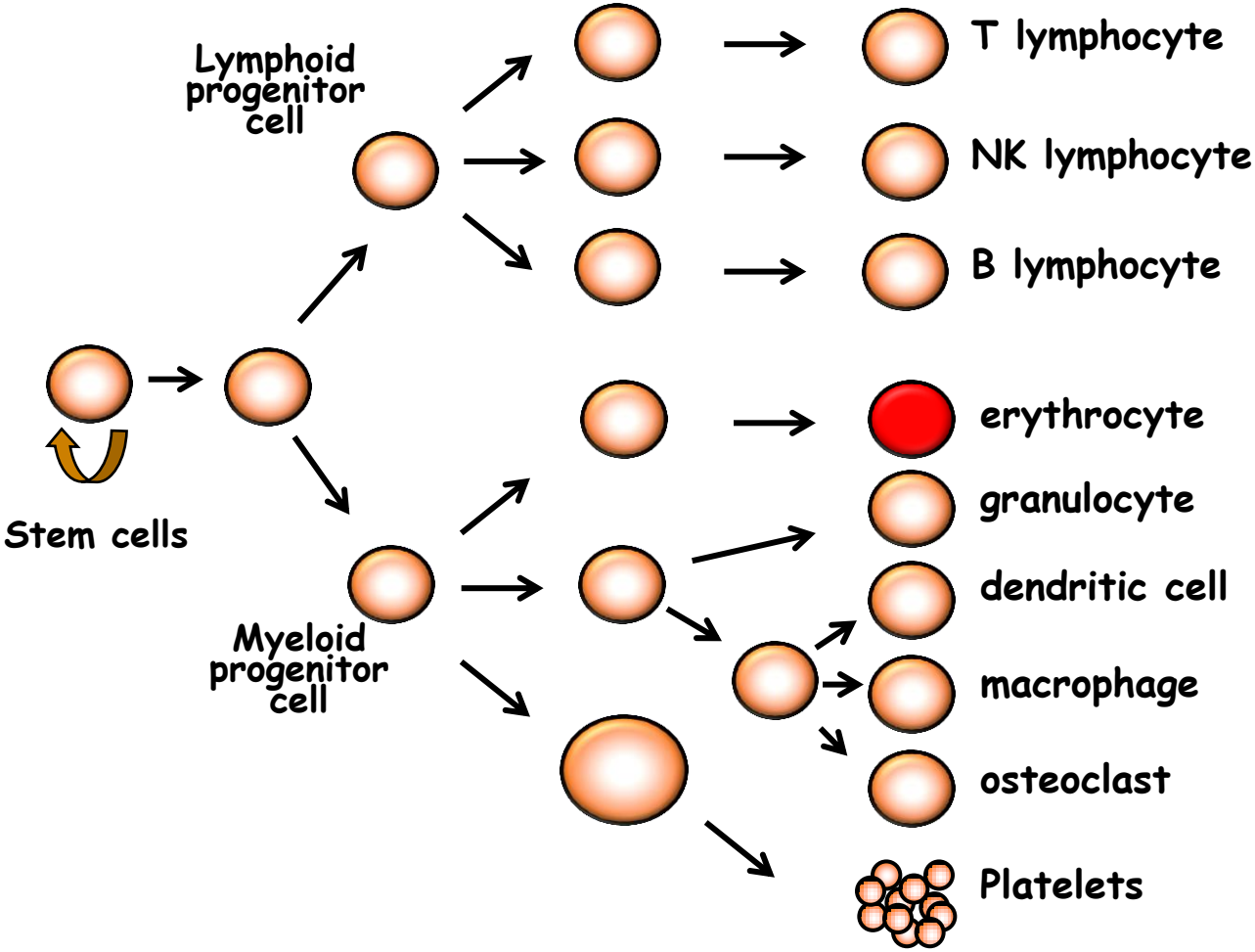
II. 2nd generation of vectors – SIN RV (→RV+LV)

SCID-X1*	13	12	10	4.2	1-5.5
SCID ADA	33	33	32	1.5	.5-4.5
WAS	21	20	20	2.7	.5-6
total	67	65	62 (92.6 %)		

Boston, London, Los Angeles, Milan, Paris

* rescue therapy post HSCT, atypical cases excluded

Gene therapy ⇨ hematopoietic stem cells



Diseases

hemoglobinopathies

no selective advantage
tissue restriction

metabolic diseases
ALD, MLD

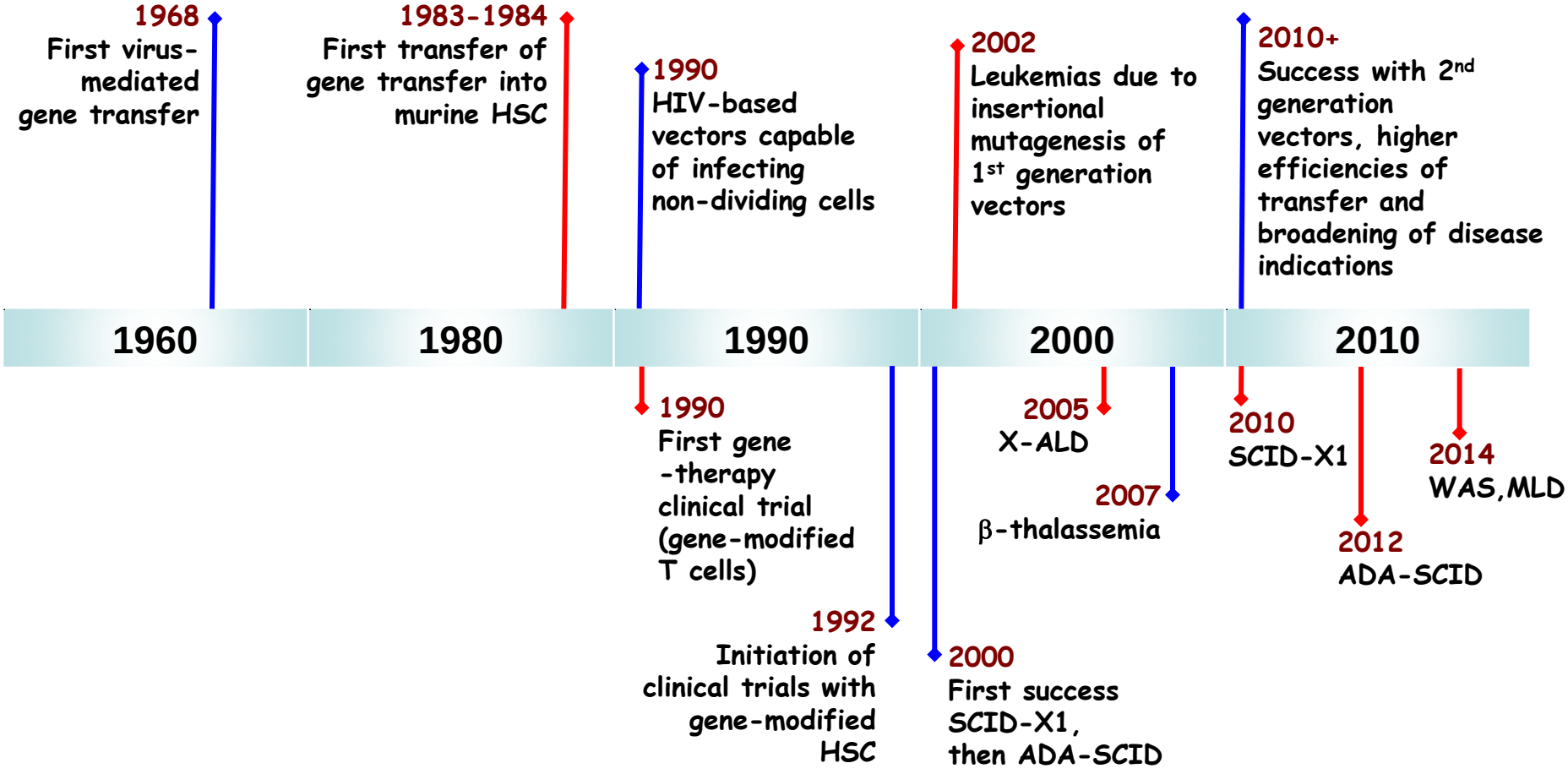
no selective advantage

Clinical development of gene therapy for Hb disorders

Indication	Sponsor	Vector	Clinical development	Date initiated, current status, clinicaltrials.gov ID, references
Sickle cell disease	UCLA	Lenti/βAS3-FB (anti-sickling globin)	Phase 1	2014, recruiting NCT02247843 Ref. 28
	bluebird bio	Lentiglobin human β-A(T87Q)-globin	Phase 1,2	2013, recruiting NCT02151526, NCT02140554
	Children's Hospital Cincinnati	Lentivirus, γ-globin	Phase 1,2	2014, recruiting NCT02186418
Thalassemias	bluebird bio	Lentiglobin human β-A(T87Q)-globin	Phase 1,2	2013, recruiting NCT01745120, NCT02151526,
	San Raffaele	Lentivirus GLOBE vector (human β-globin)	Phase 1,2	2015, recruiting NCT02453477
	Sloan Kettering	Lentivirus with human β-globin	Phase 1	2012, active not, recruiting NCT01639690

C.T. Scott & L DeFrancesco, Nature Biotechology 2016

HSC gene therapy timeline



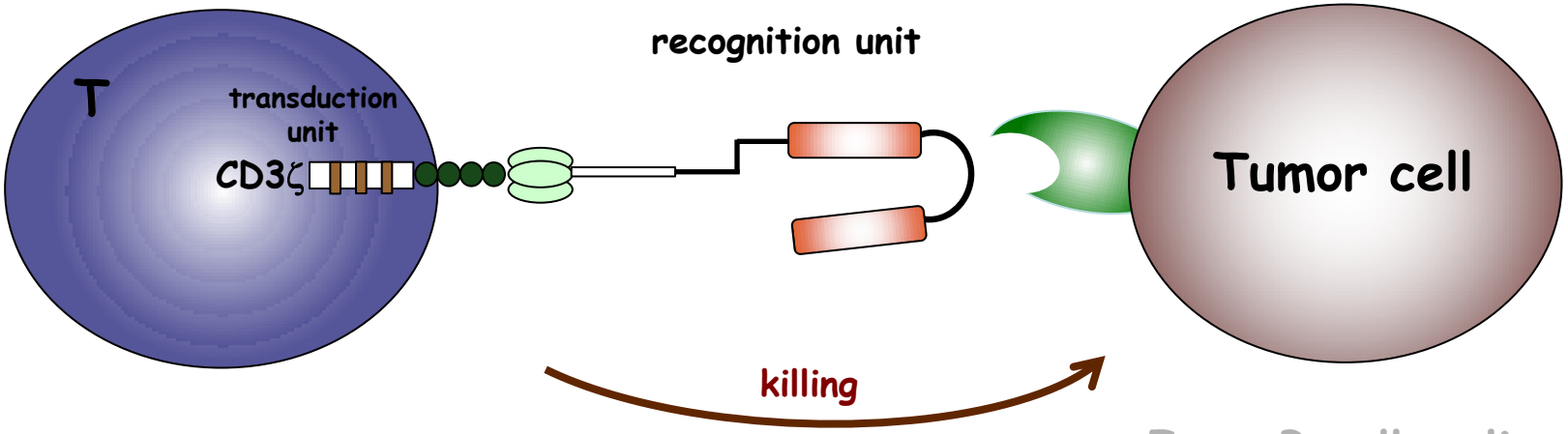
modified from C.T. Scott & L DeFrancesco, Nature Biotechology 2016

Target cells for gene therapy

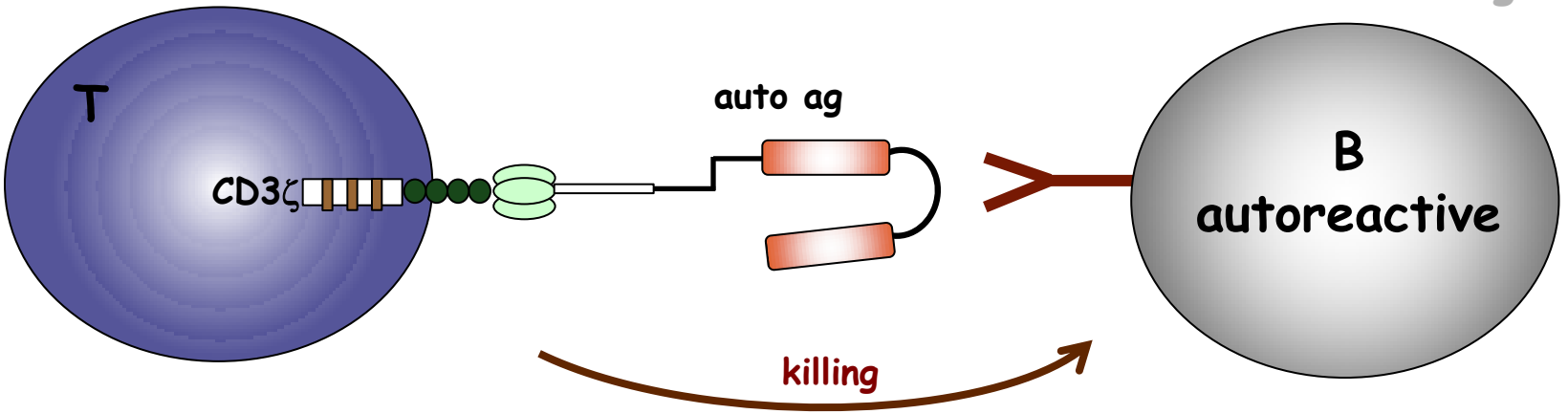
- **Dividing cells**
  - bone marrow,T cells
  - skin (epidermodysplasia bullosa)
- Post mitotic cells
  - hepatocytes (hemophilias)
  - nervous system (lysosomal storage diseases)
  - pigmented layer of the retina (R. pigmentosa)
  - Muscle (myopathies)

Engineering of T lymphocytes to fight cancer or autoimmune diseases

chimeric antigen receptors ("CAR")



From B cell malignancies

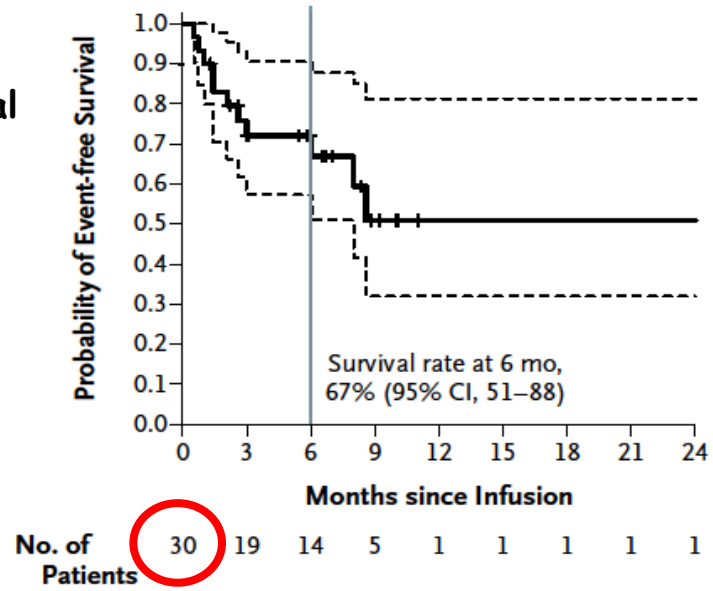


Treatment by antiCD19 CAR of acute B lymphoblastic leukemia

Proof of concept

- CD19 : B cell surface molecule
- Patients in relapse ~ 1 à $20x10^6$/kg CAR αCD19

event free survival



S.L. Maude et al, NEJM 2014

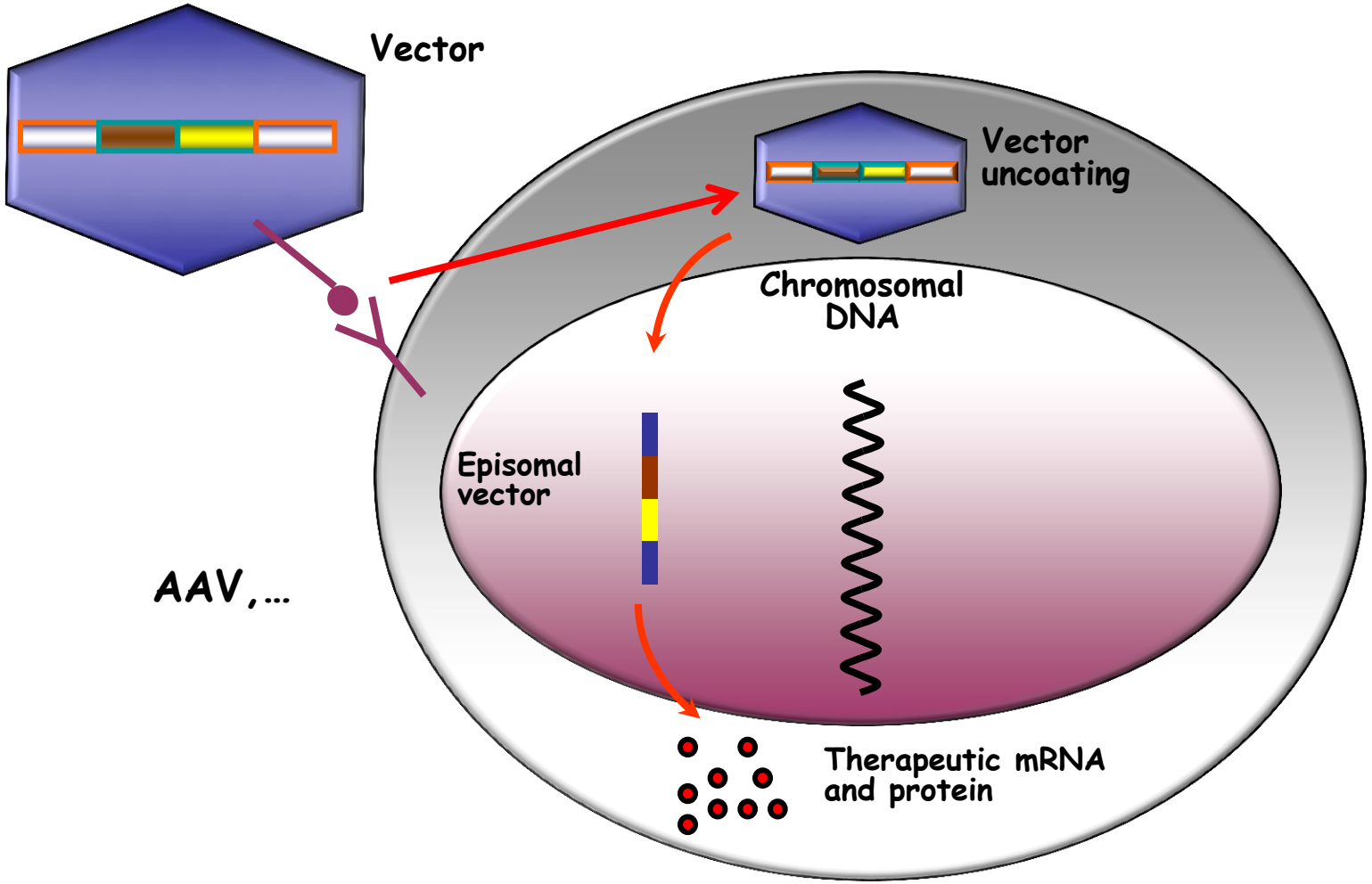
From B cell malignancies to solid tumors ?

Target cells for gene therapy

- Dividing cells
  - bone marrow,(T cells)
  - skin (epidermodysplasia bullosa)
- **Post mitotic cells**
  - **hepatocytes (hemophilias,..)**
  - **nervous system (lysosomal storage diseases,..)**
  - **retina (R. dystrophies)**
  - **Muscle (myopathies)**

In vivo gene therapy using non integrative adenoassociated viral (AAV) vectors

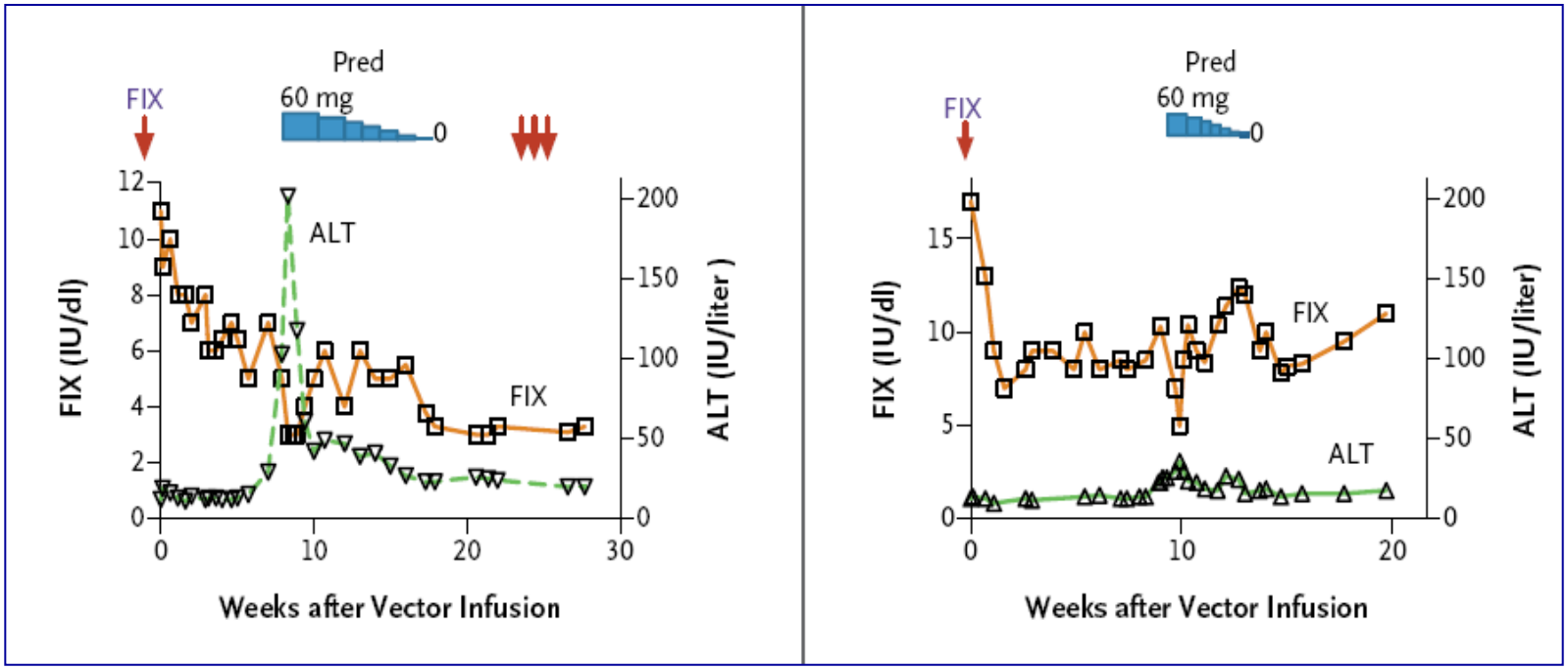
Gene delivery to post mitotic cells



Vector for gene delivery into postmitotic cells

	Adeno-associated virus
Tropism	Dividing & non dividing cells
Host genome	No integration
Transgene expression	Lost in dividing cells
Packaging capacity	~ 5kb
Advantages	High production yields
Disadvantages	Small packaging capacity immunogenicity

Hemophilia B : weakly immunogenic AAV vectors

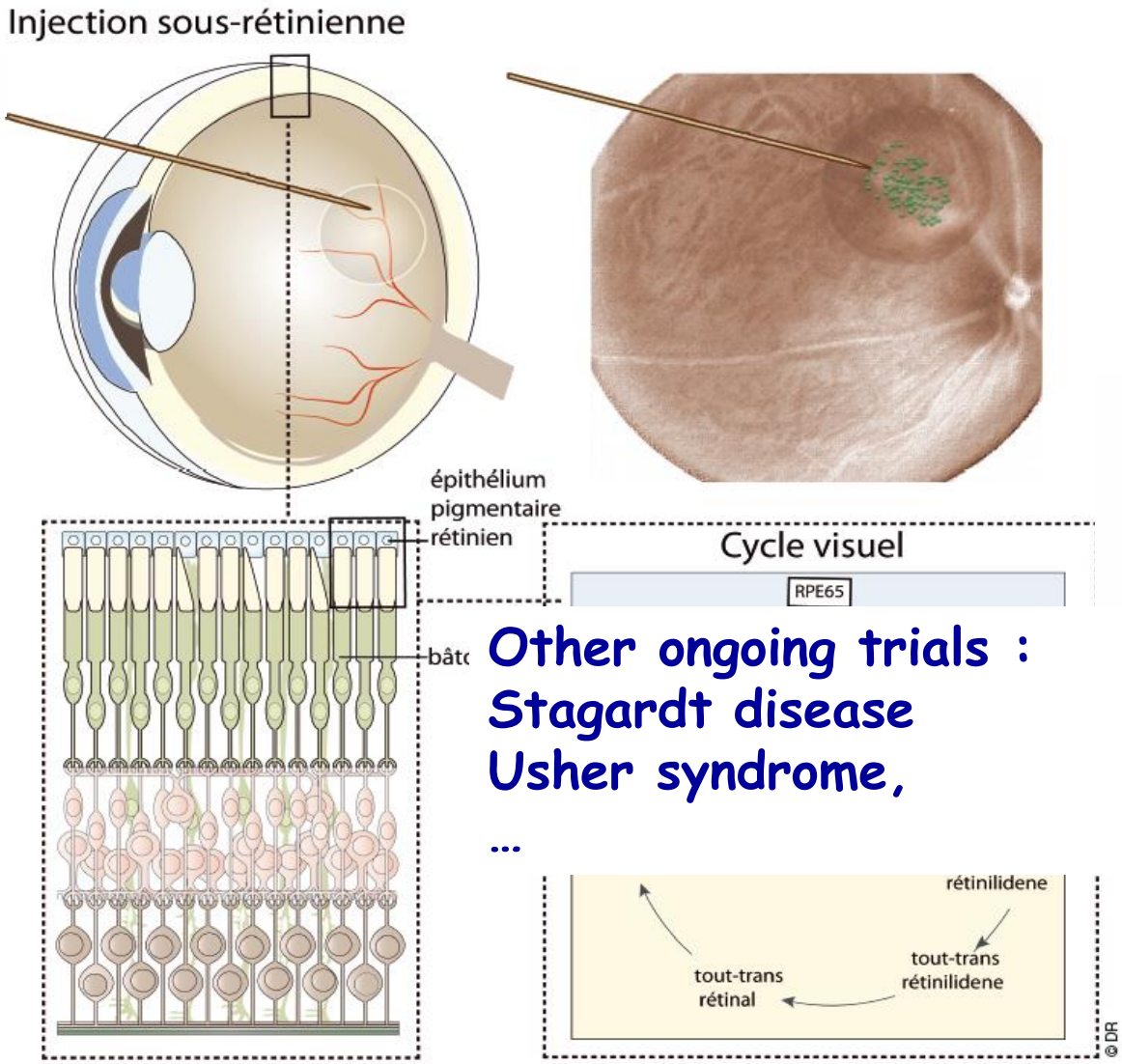


- Follow up > 30 months, n > 10 patients
- 1 to 6% factor IX in plasma
- Prophylaxis stopped in 2/3 patients
- Toward treatment of Hemophilia A

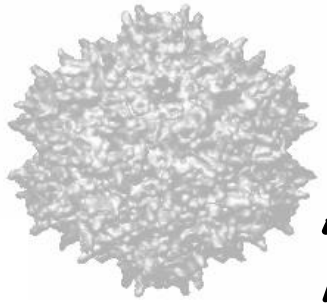
AAV 8

A.C. Nathwani et al

Gene therapy of Leber amaurosis



Représentation schématique d'une injection sous-rétinienne permettant le ciblage de l'épithélium pigmentaire rétinien. Le cycle visuel et l'implication de RPE65 en cause dans l'ACL.



AAV - RPE65

Le cycle visuel et l'implication de *RPE65* en cause dans l'ACL.

Other ongoing trials :
Stagardt disease
Usher syndrome,
…

J.A. Sahel

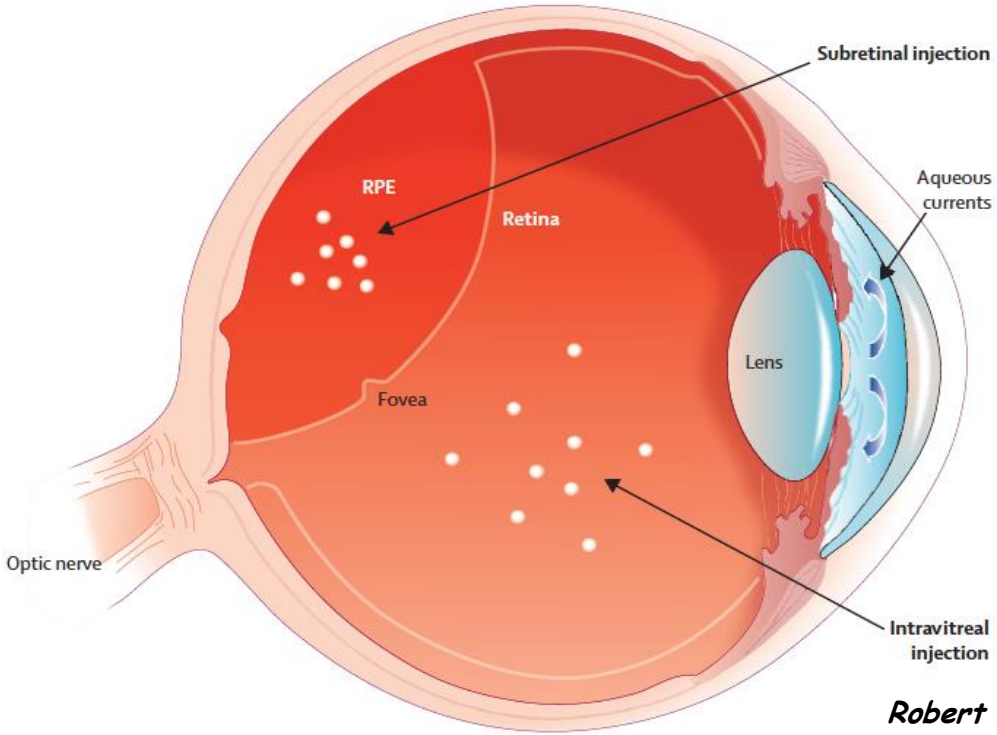
Benefits of gene therapy for both eyes

Leber's congenital amaurosis type 2, a blinding disease caused by deficiency of the *RPE65* gene in the retinal pigment epithelium.[3]

The other ten participants were followed up for up to 3 years and showed significantly improved navigational vision in the maze test and improved light sensitivity in their second eye after treatment.

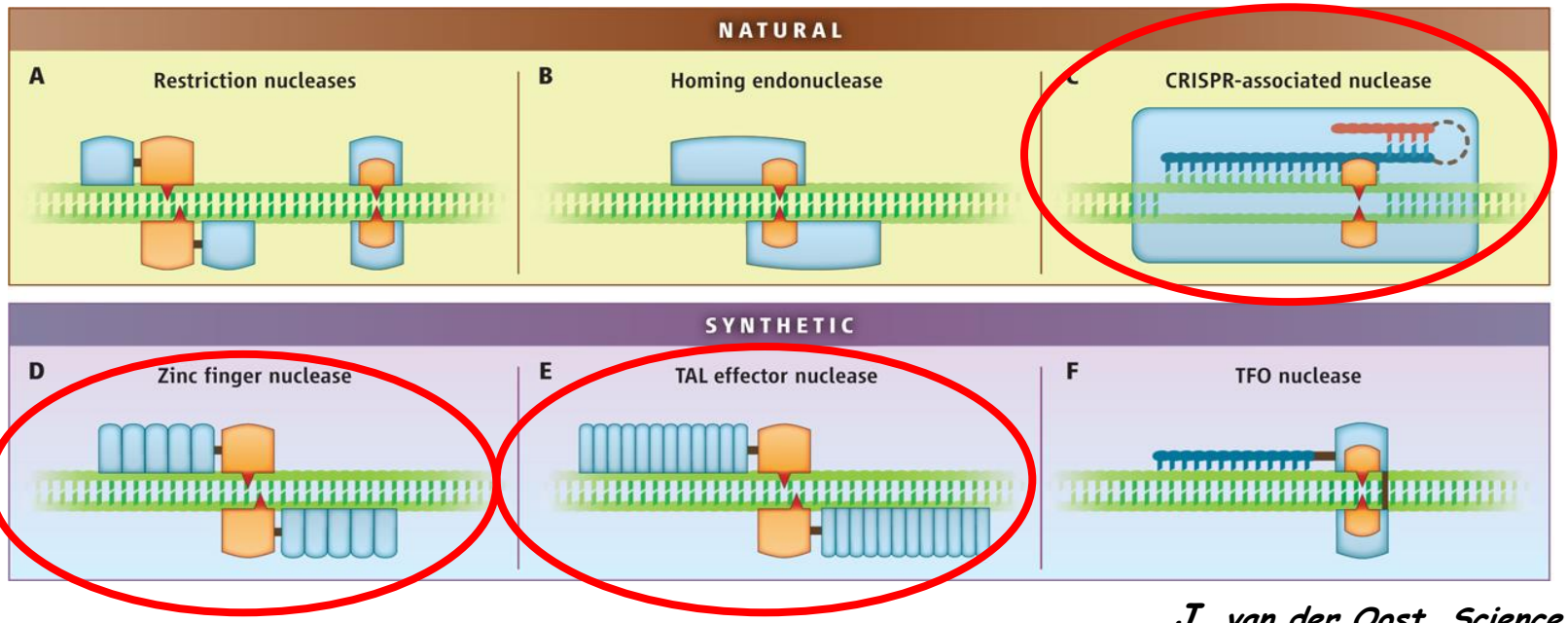
Phase III study

Bennett J et al, 2016



Robert E MacLaren, The Lancet 2016

Tools for genome editing



J. van der Oost, Science 2013

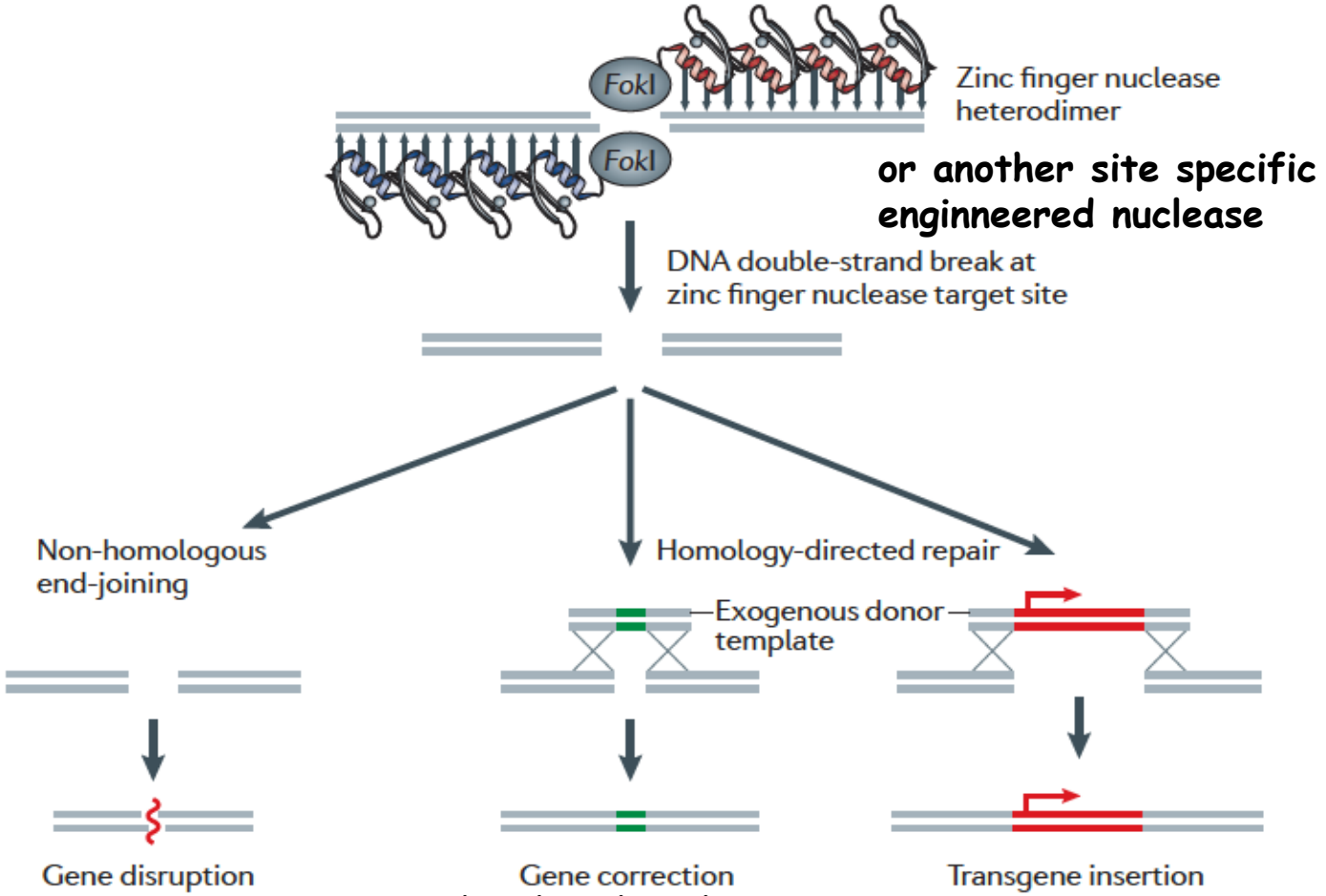
Gene inactivation by NHEJ* : to silence a pathogenic gene, (exon)

Gene repair by homologous recombination, with a designed template

Genome editing in a given (safe) locus: e.g. the albumin locus

** NHEJ non homologous end joining*

Genome editing



Resistance to virus infection
Resistance to rejection:
(universal CAR T cells ?)
Mutated exon disruption

physiological regulation

efficacy rate (non dividing cells)
off targets,… delivery

Modified from L. Naldini, Nature Reviews Genetics, 2011

Conclusions

- Proof of concept achieved in selected cases,
  based on pathophysiological studes of targeted diseases

- Extension of indications
  Engineering high rate of cell transduction
  Usage of less immunogenic vectors

- Long term monitoring /safety - stepwise advances

- Stable producing cell line of lentiviral vectors, AAVs

Perspectives

- Alternative technology: use of engineered nucleases, cell engineering
- Large scale production, toward automated manufacture
- Involvement of industry, from big pharmas (GSK, Novartis) to medium size (Biogen,..) and Biotechs (Bluebird bio, Spark therapeutics,..)
- Standardisation of preclinical studies
- First approved products (in Europe) : Strimvelis for ADA deficiency
- Cost

SCID X1

M. Cavazzana
S. Hacein-Bey-Abina
G. De Saint-Basile
F. Touzot
L. Caccavelli
J. Blondeau
E. Six
C. Picard
D. Moshous
B. Neven
S. Blanche
A. Garrigue
A. Lim (I. Pasteur)

A. Deichmann, M. Schmidt,
C. von Kalle (Heidelberg)
G. Wang,T. Brady,
N. Malani, C. Berry, R. Bushman (Philadelphia)

A. Schambach, C. Baum (Hannover)
A.Thrasher, H.B. Gaspar (London)
D. Williams, S.Y. Pai,
L. Notarangelo (Boston)
P. Malik, A.H. Filipovich (Cincinatti)
D.B. Kohn (UCLA)

WAS

A. Galy, S. Charrier
F. Mavilio
B.P. Noquiez-Hellin,
O.W. Merten (Genethon)
A. Thrasher,
H.B. Gaspar (London)

ALD

N. Cartier
P. Aubourg

Inserm
Institut national
de la santé et de la recherche médicale

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS
NECKER - ENFANTS MALADES

PARIS DESCARTES

INSTITUT imagine DES MALADIES GENETIQUES

COLLÈGE DE FRANCE 1530