References:

- Hartwell et al.: GENETICA dall'analisi formale alla genomica
- Alberts and Johnson: Molecular Biology of the Cell

- Saggio L'età se esiste
- Bencivelli de Ceglia Comunicare la scienza
- Meldolesi E l'uomo creò l'uomo. CRISPR e la rivoluzione dell'editing genomico

- Ppt via elearning

www.saggiolab.com

valutazione

Modalità di valutazione

- Valutazione di pezzi di comunicazione della scienza e della medicina con tutoraggio in aula.
- Redazione di un articolo, in data di appello.

Per tutti la verbalizzazione sarà in data di appello.

Science is a beautiful gift to humanity; we should not distort it. ...

Punto su festival della scienza

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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.

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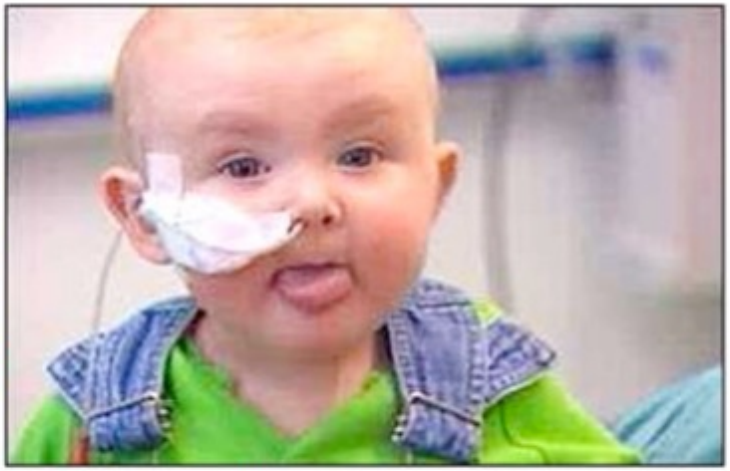
Technology
Health

Talking Point

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"

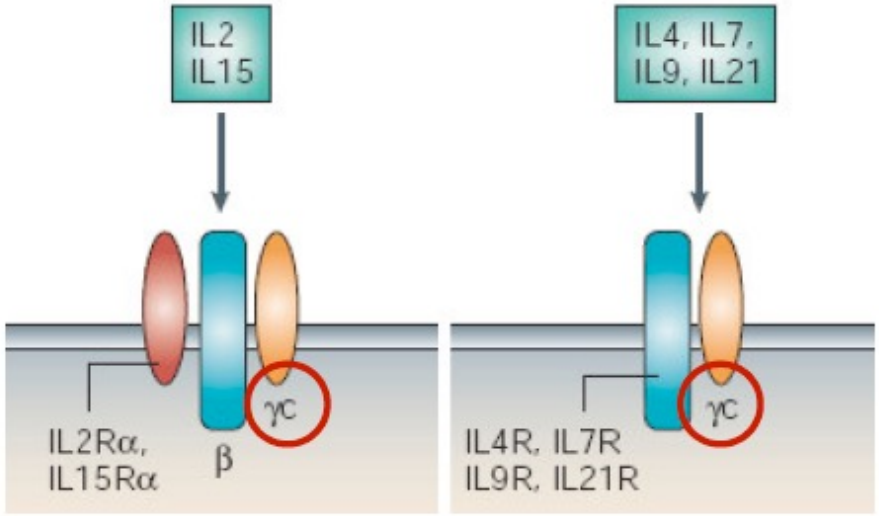
See also:

- 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'

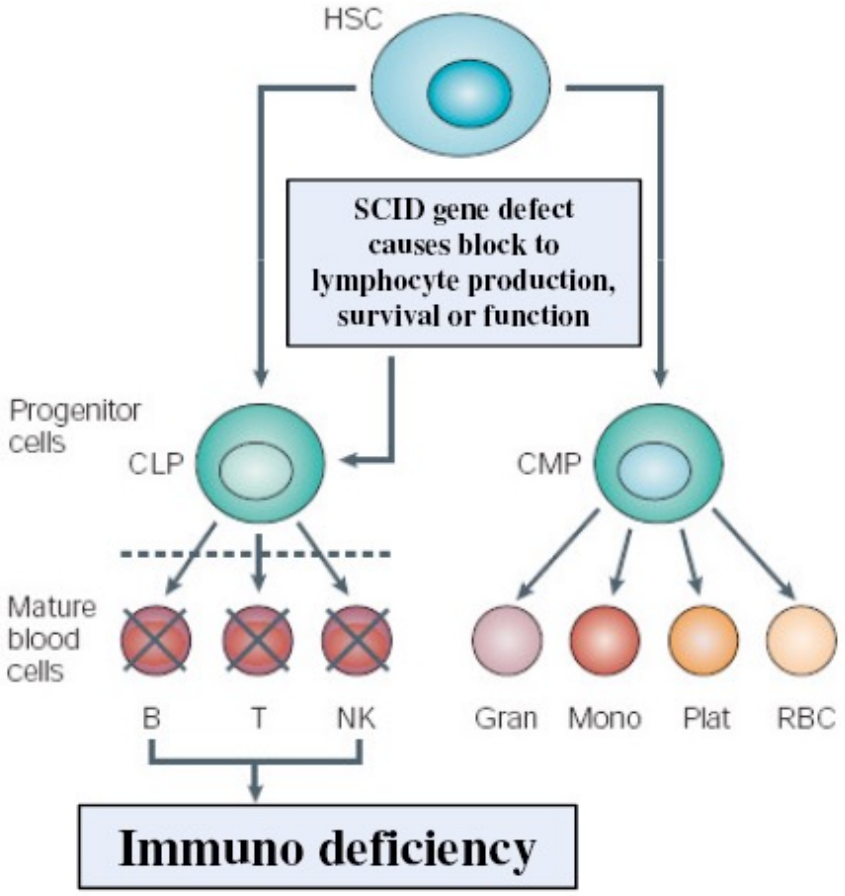
SCIDX1 (50% SCID cases)

Genotype

Mutations in gamma c gene mapped to chromosome Xq13



Phenotype



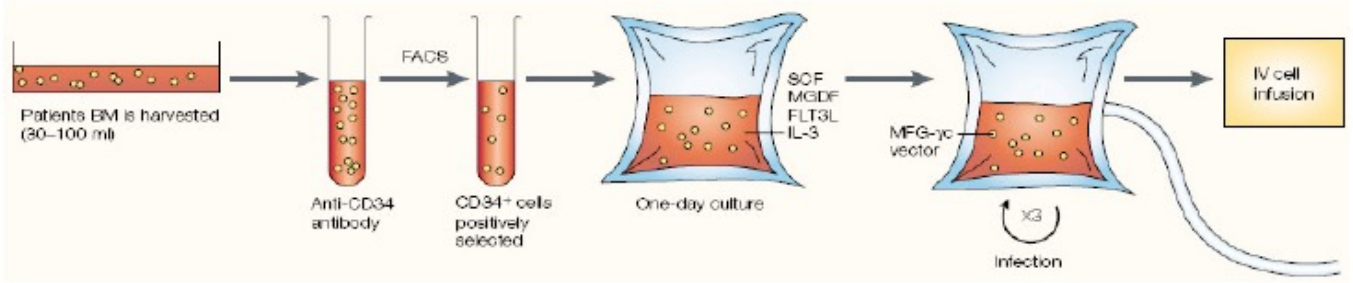
Ex-vivo Retrovirus-med. gene therapy: SCIDX1 trial 1998, A. Fisher France

- 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.

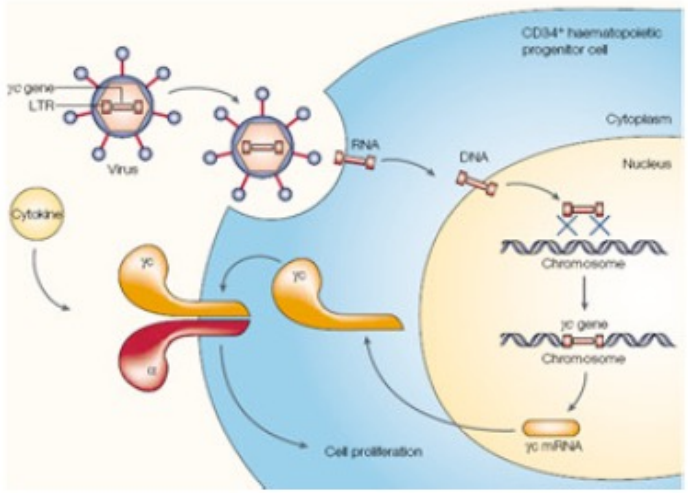
PROTOCOL



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]
ephanie 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

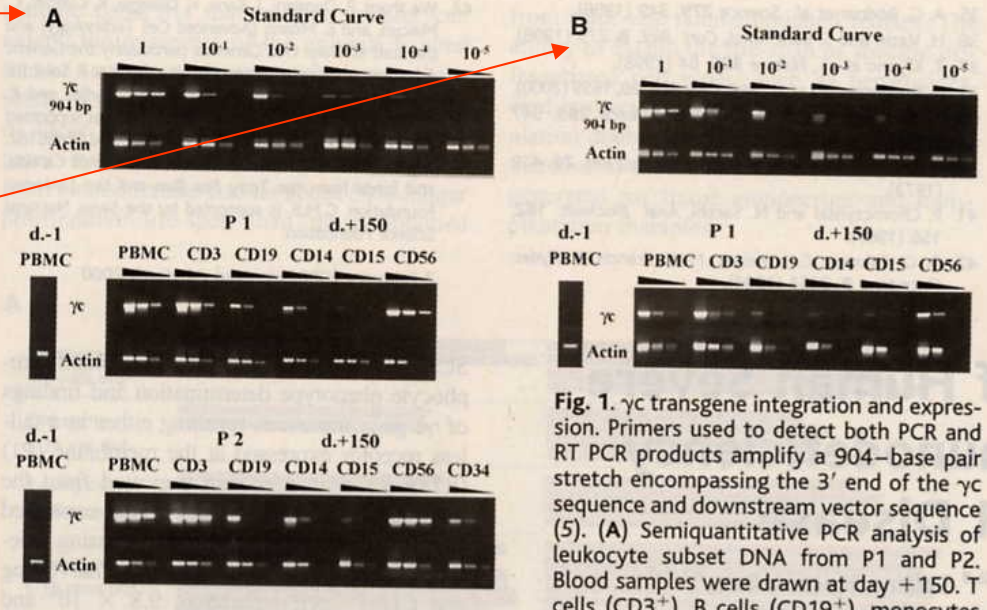


Fig. 1. γc transgene integration and expression. Primers used to detect both PCR and RT PCR products amplify a 904–base pair stretch encompassing the 3′ end of the γc sequence and downstream vector sequence (*5*). (**A**) Semiquantitative PCR analysis of leukocyte subset DNA from P1 and P2. Blood samples 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.

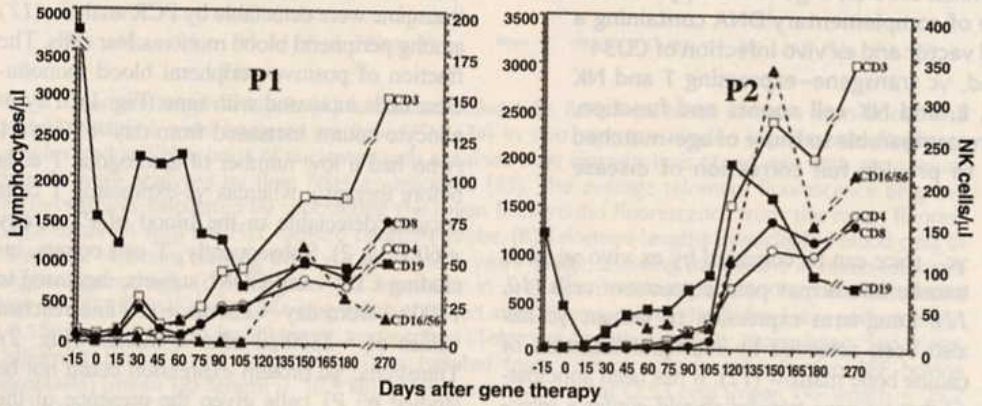
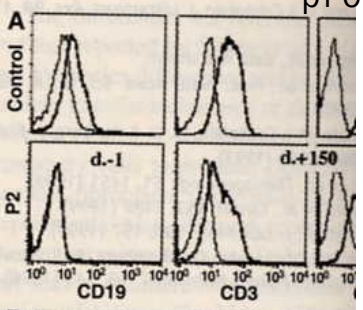


Fig. 2. Longitudinal study of lymphocyte subsets from patient 1 (P1) and patient 2 (P2). Absolute counts of T cells ($CD3^+$, $CD8^+$, and $CD4^+$), B cells ($CD19^+$), and NK cells ($CD16^+CD56^+$) are shown as a function of time. Day 0 is the date of treatment. The scale for NK cells is on the right-hand side of each panel.



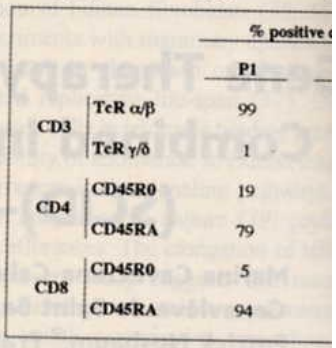
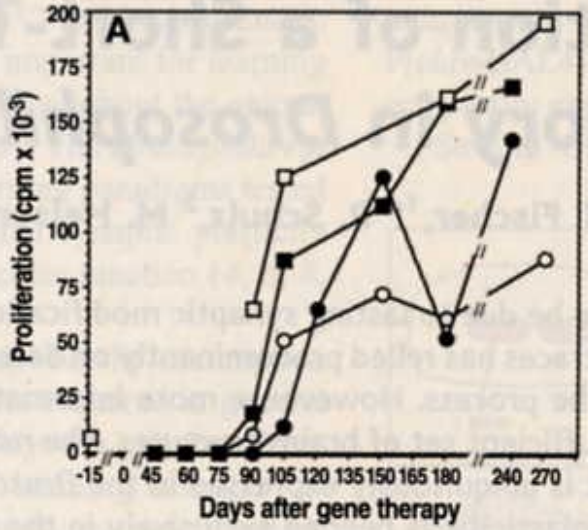


Fig. 3. γc protein expression and ly
subsets. (**A**) γc protein detection at t
of lymphocyte subsets from a contro
P2 obtained at day +150. γc expres
cells from P2 after treatment was un
(not shown). The *y* axis depicts the re
number, and the *x* axis shows the log
arbitrary immunofluorescence units.
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
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.	Dipht. Tox.	Polio virus I	II	III
		mg/dl		(iu/ml)		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.

SCIDX1 trial results: Science 2000

- Clinical parameters ok (immune response, T cells counts)
- Biological parameters ok (transgene expression, infected T cells)
- **Importance of in vivo selection of transduced cells**

Year **2002**
A. Fisher SCID X1 trial

-Theoretical possibility of retroviral “oncogenic” integration = 10e-12
BUT
-2/11 patients developed leukemia
-3 patients had retrovirus insertion close to an oncogene (LMO-2) on chromosome 11. This gene was originally identified as a breakpoint of a translocation that causes a type of T-cell leukemia.

Year 2005: panel urges limits on X-SCID trials

Death of 1 of the two leukemia cases of the french trial

Third new leukemia case with insertion in site different from lmo2 in the french trial

One monkey developed leukemia with retrovirus transfer of marker genes at NIH

No cancer cases in ADA gene therapy

NEJM 2010 Hacein-Bey-Abina Hauer et al 2010, update XSCID X1 trial

In 2010 on New England Journal of Medicine, 10 year “follow up” (Hacein-Bey-Abina, Hauer et al. 2010)

- All patients ameliorated (the immune system)
- 7/9 the amelioration was long term
- 4/9 of the Necker patients developed leukemia
- 1/9 died of leukemia

Conclusions SCIDX1 trial

Curative results in several trials provides irrefutable proof-of-principle.

Use of cytoreductive conditioning to increase engraftment of transduced HSC will be essential to applications for disorders without the high selective advantage of SCID.

SAE in XSCID necessitates careful consideration of transgene-specific effects and development of improved, safer techniques.

SCIDX1 trial - problems

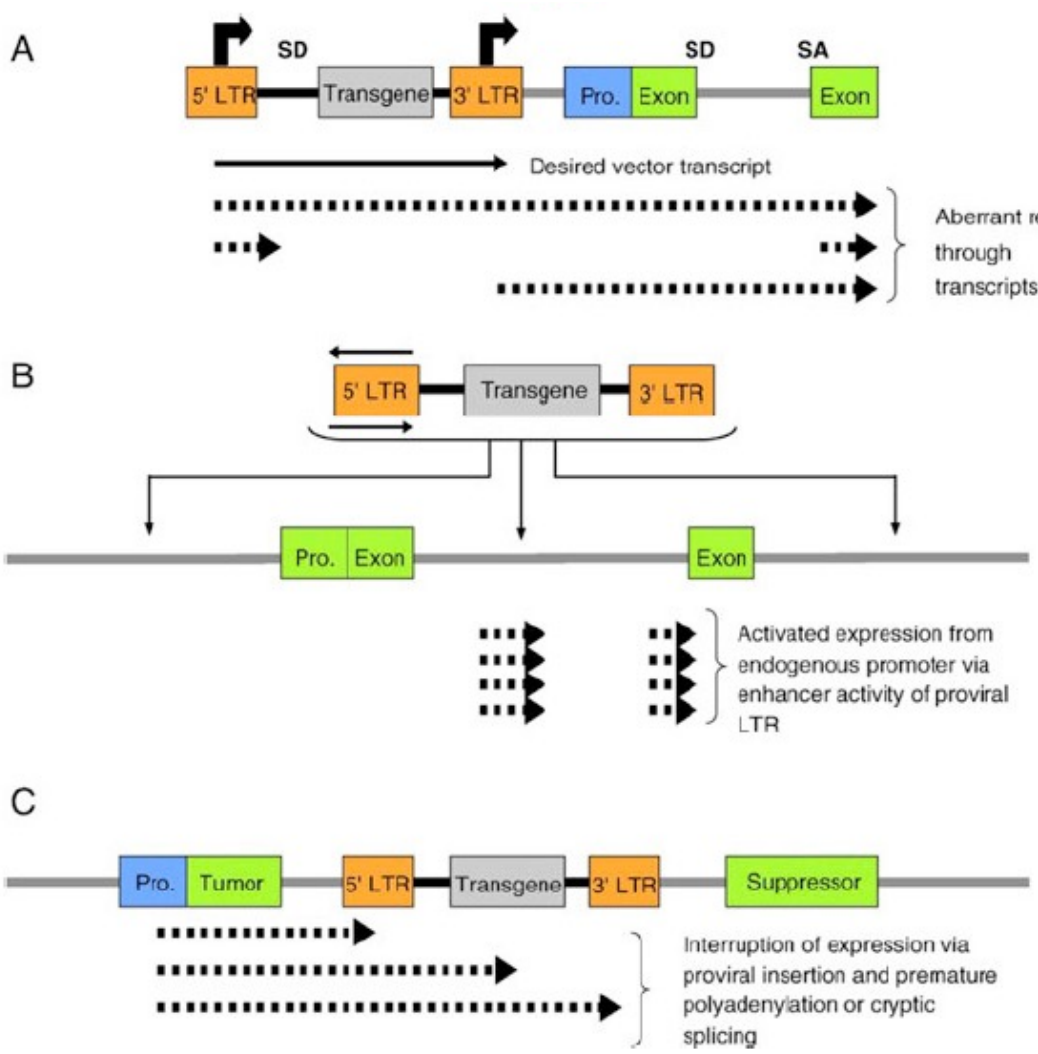
Research article

Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1

Salima Hacein-Bey-Abina,[1,2] Alexandrine Garrigue,[2] Gary P. Wang,[3] Jean Soulier,[4] Annick Lim,[5] Estelle Morillon,[2] Emmanuelle Clappier,[5] Laure Caccavelli,[1] Eric Delabesse,[6] Kheira Beldjord,[7,8] Vahid Asnafi,[7,8] Elizabeth MacIntyre,[7,8] Liliane Dal Cortivo,[1] Isabelle Radford,[8] Nicole Brousse,[9] François Sigaux,[4] Despina Moshous,[10] Julia Hauer,[2] Arndt Borkhardt,[11] Bernd H. Belohradsky,[12] Uwe Wintergerst,[12] Maria C. Velez,[13] Lily Leiva,[13] Ricardo Sorensen,[13] Nicolas Wulffraat,[14] Stéphane Blanche,[10] Frederic D. Bushman,[3] Alain Fischer,[2,10] and Marina Cavazzana-Calvo[1,2]

2008. J Clin Invst 118(9):3132.

Genotoxicity: possibilities

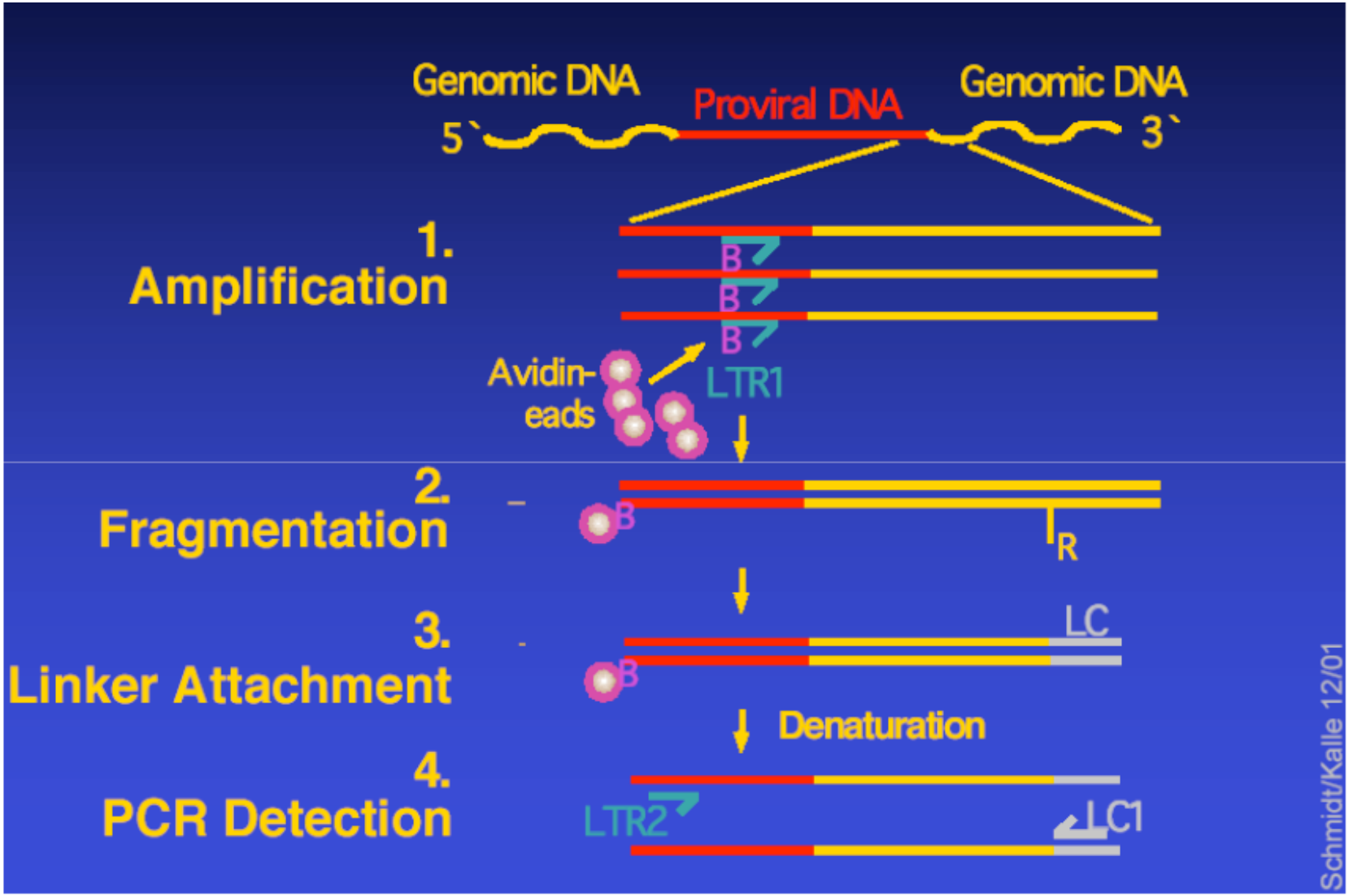


readthrough

new expression

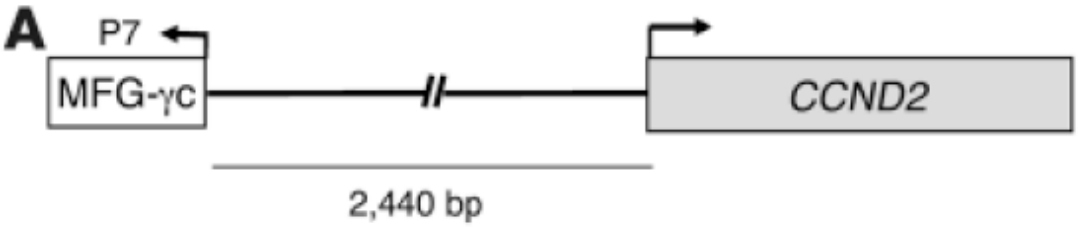
gene interruption

Linear Amplification (LAM) PCR strategy



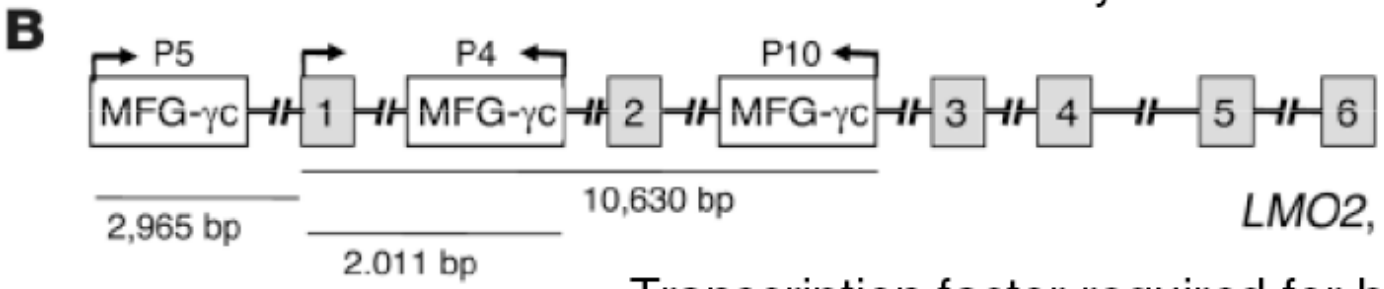
Schmidt M, von Kalle C et al. 2007. Nat Methods. 4(12):1051.

Integration sites



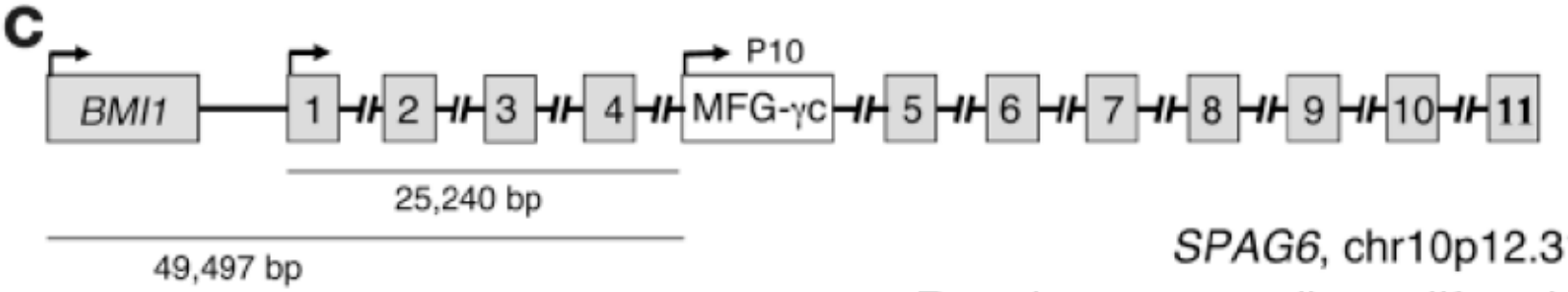
Intergenic, chr12p13.32

Control of cell cycle at the G1/S transition



LMO2, chr11p13

Transcription factor required for haematopoiesis



SPAG6, chr10p12.31

Regulate stem cells proliferation

Further oncogenic rearrangements

Patient characteristics

Patient	Age at therapy (mo)	T-ALL (mo)	Follow-up (mo)	Infection before gene therapy	CD34$^+\gamma c^+$ cells infused ($\times 10^6$/kg)	Clinical status	Insertion sites	Chromosomal abnormalities	Notch mutation (aa residue)	*CDKN2A* deletion
P4	1	30	60	–	18	Died	*LMO2*	t(6,13)	–	+
P5	3	34	99	–	20	AW, CR	*LMO2*	*SIL-TAL*, trisomy10	1593F/S	–
P7	11	68	84	Lung, B-LPD	4.3	AW, CR, chemotherapy	*CCND2*	0	–	+
P10	8	33	73	Lung, gut	11.3	AW, CR	*LMO2*, *BMI1*	0	1707A/P	–

Collectively, these data fit with multistep oncogenesis of T-ALL , in which oncogenes were first activated by vector insertional mutagenesis, followed by accumulation of secondary genome rearrangements, including point mutations as well as gene deletions and amplifications.

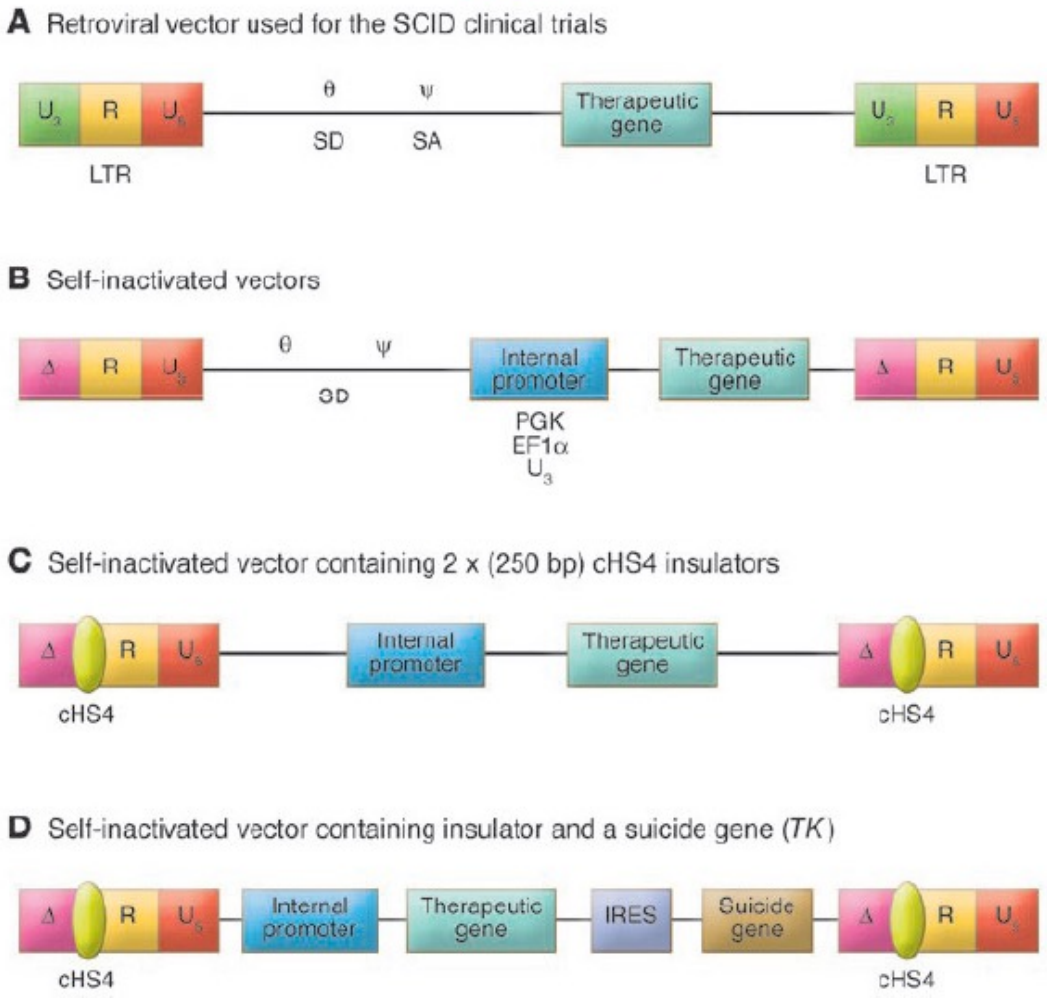
SCIDX1 background favors oncogenesis?

An expanded population of primitive progenitors highly prone to growth-promoting integration may be present because of the differentiation block .The strong proliferative advantage of γc-transduced lymphoid progenitors could predispose these cells to transformation.

The age of the patient at the time of treatment. It is thought that below the age of 1, the bone marrow stem and progenitor compartments have a higher proliferative capacity.

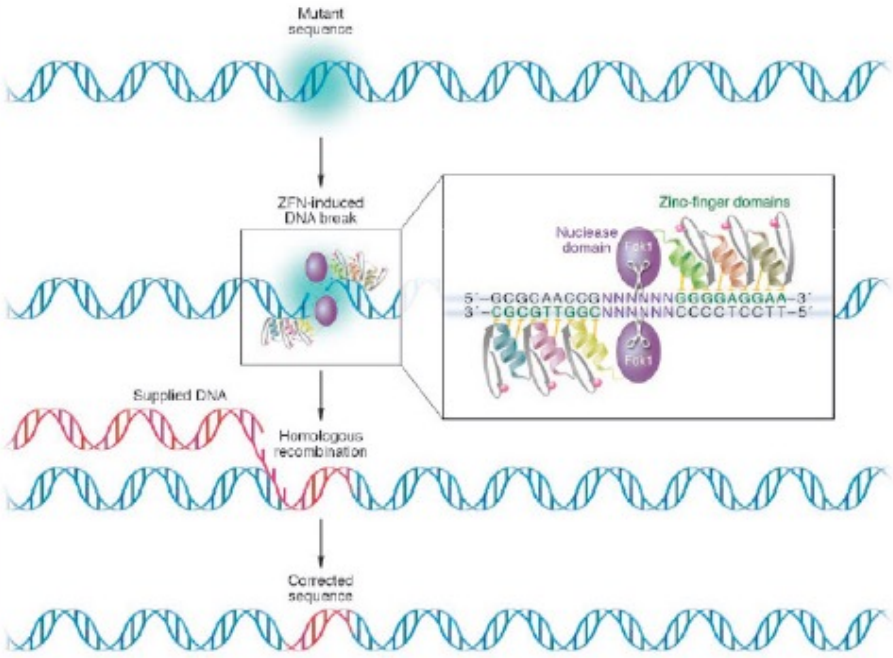
They received high dose of transduced cells.

Improvements



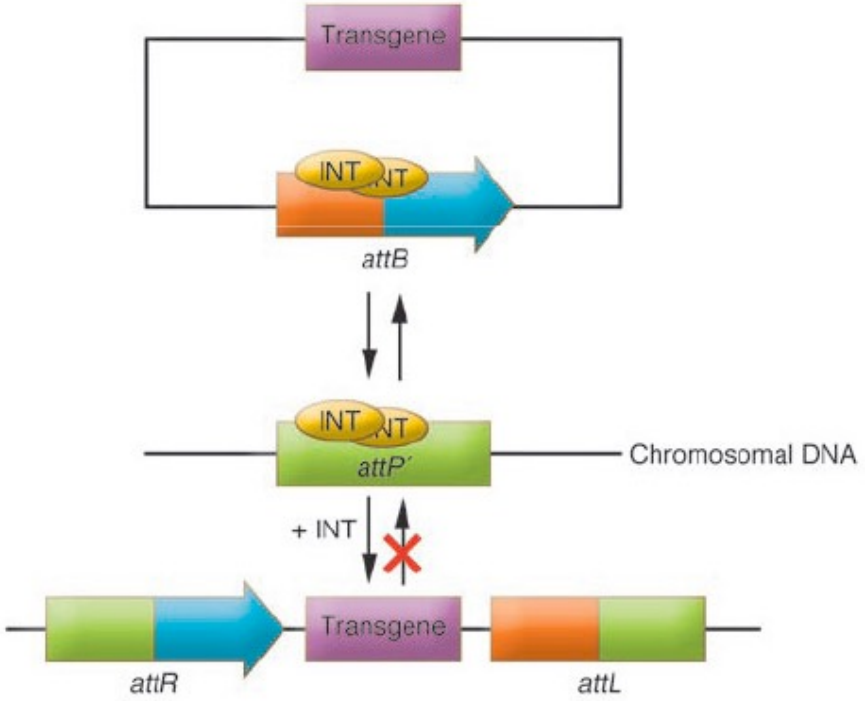
Improvements

ZFP gene correction



DNA binding and nuclease function= Zinc finger protein= highly specific genomic scissors

Site-specific integration



Phage integrase. It has been demonstrated previously that a plasmid expressing the integrase can mediate the integration of a co-delivered attB-containing plasmid into mammalian chromosomes at pseudo attP-sites (host sites sharing homology to attP, as recognized by phiC31).