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Gene therapy deserves a fresh chance

Initial interest in gene therapy waned after the technology failed to live up to expectation. Progress made since has received little attention, but suggests that the pervading sense of disillusionment is misplaced.

A rationale for gene therapy of lifethreatening primary immunodeficiences

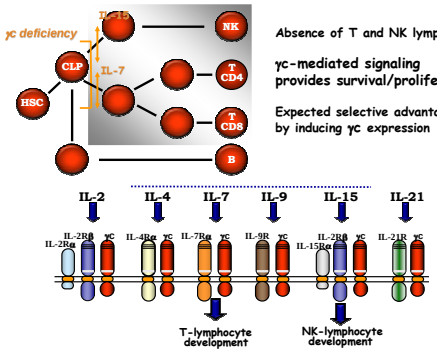
- Many PID are lethal diseases (SCIDs, WAS, HLH, CGD,…)
- Allogeneic hematopoetic stem cell transplantation (HSCT) can be an effective therapy
- HSCT is associated with significant serious adverse events (GVHD, …)
  (66-69 % 3 year-survival haplo-identical/URD HSCT in SCID
  > 2 000 in Europe)
- Most PID display Mendelian inheritance

  To add a normal copy ot the mutated gene in hematopoietic progenitor cells can correct the deficiency

A rationale for considering SCIDs as a first target for gene therapy

- A selective advantage of T cell precursors, conferred by transgene expression is expected
- Once differentiated, T cells are long lived (>… 20 years)

SCID-X1. a deficiency in the γc cytokine receptor



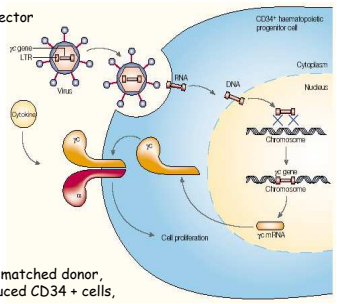
Absence of T and NK lymphocytes

γc-mediated signaling provides survival/proliferation

Expected selective advantage conferred by inducing γc expression

Ex vivo gene therapy of SCID-X1

amphotropic MFGB2 vector



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

SCID-XI gene therapy trial (April 1st, 2010)

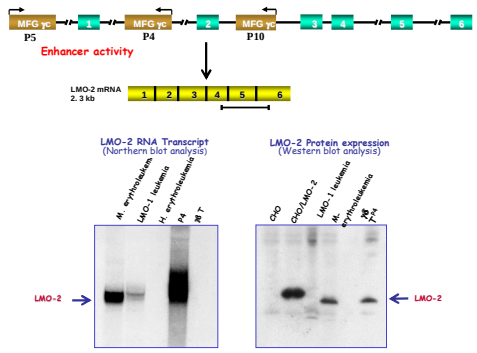
Patient	F.U. (year)	γc expression	T	B	Clinical status
			Immunity		
1	11.0	+++	++	+	A.W.
2	10.9	+++	++	+	A.W.
3	.7	+	-	-	A.W. (BMT)
4	(4.9)	+++	++	++	Died, "leukemia"
5	10.1	+++	++	+	A.W.,"leukemia",C.R.
6	8.9	+++	++	-	A.W.
7	8.7	+++	++	+	A.W.,"leukemia",C.R.
8	8.4	+++	++	++	A.W.
9	(3.1)	++	+	-	Died, infection (BMT)
10	8.0	+++	++	+	A.W.,"leukemia",C.R.

Median follow-up 8.9 y, 8 patients alive and well

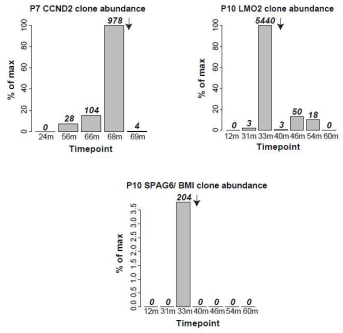
SCID-X1 gene therapy trial: characteristics of the 4 serious adverse events

	P4	P5	P7	P10
Age of therapy (month)	1	3	8	8
Occurrence of SAE (month)	30	34	68	33
Clonal T cell proliferation	γδ mature T	αβ matureT	Immature T	Cortical thymocyte
Oncogene	LMO2	LMO2	CCND2	LMO-2, BMI-1
2nd genetic modifications	t6:13	SIL-TAL notch mut, p16 del.	p16 del.	notch mut, p16 del.
Sensitivity to treatment	-	+	+	+

LMO-2 gene structure and integration of the provirus



Kinetics of abnormal clones



Characteristics of retrovirus integration

- Frequently within genes (promotor,exons/introns)
- Within active genes
- Many protooncogenes are transcribed in progenitor cells (including LMO-2)

Increased probability of integration in protooncogenes
Risk of transactivation (LTR enhancer)

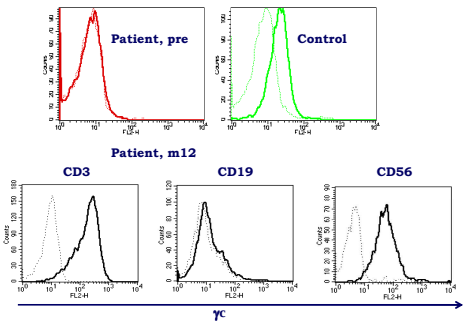
SAE as a function of SCID disease

	n=	SAE
γc	19	5
ADA	19	0

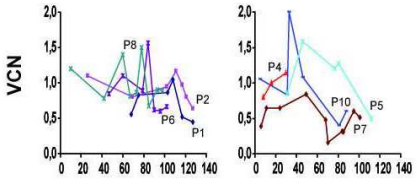
Same pattern of integration sites (LMO-2,…)

? A role for the disease → direct / indirect

γc expression (patient 4, m12)



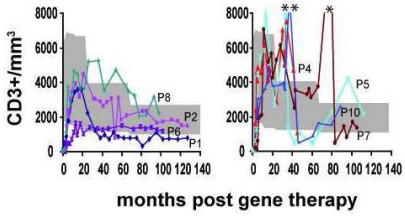
SCID X1 gene therapy trial - vector copy number



Patients without SAE | Patients with SAE

SAE: serious adverse event

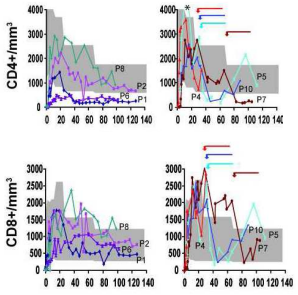
SCID X1 gene therapy trial – T cells recovery



Patients without SAE | Patients with SAE

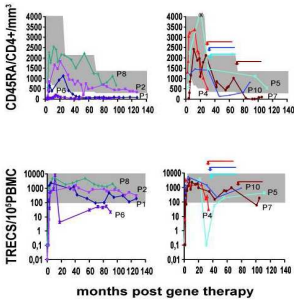
SAE: serious adverse event

SCID X1 gene therapy trial – T cells recovery



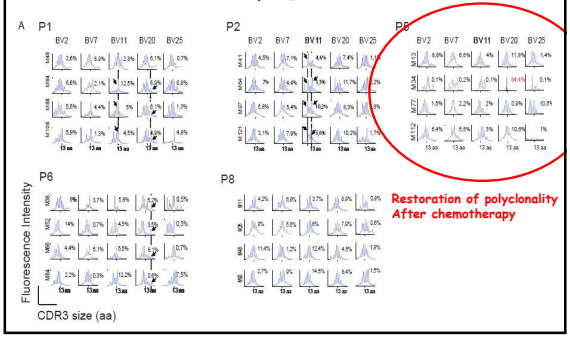
SCID X1 gene therapy trial - thymopoiesis

Naïve T cells



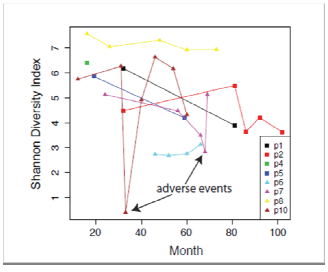
SCID X1 gene therapy trial

TCR Vβ repertoire

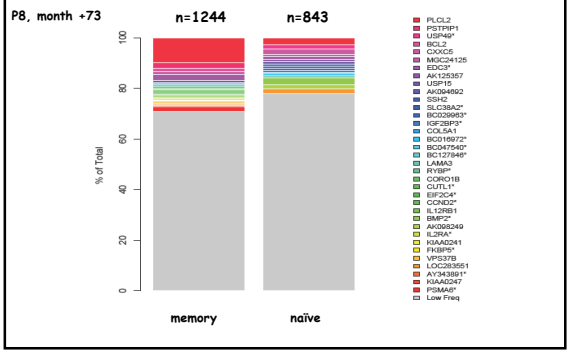


Restoration of polyclonality
After chemotherapy

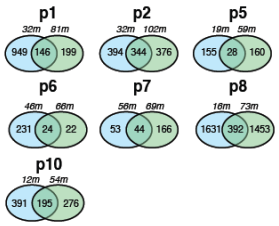
Diversity of the number of integration sites as a function of time



Diversity and abundance of integration sites. Comparison of naive and memory CD4 T cells

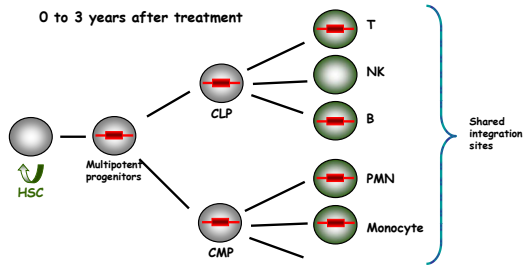


Diversity of integration sites found at different time points

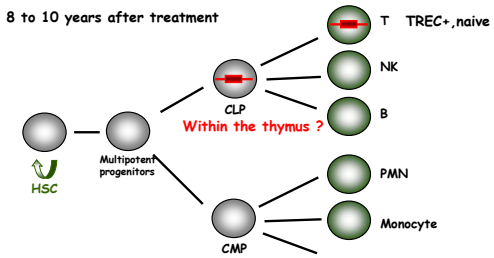


Subsequent / intermittent contribution of clones to the circulating T cell pool ?

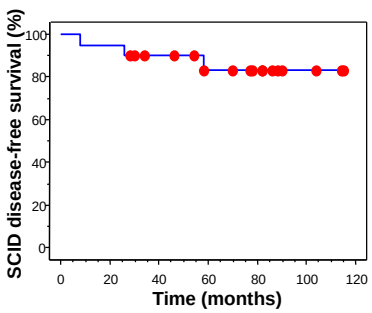
Evidence of transduction of multipotent progenitors



Long term assessment of transduced cells



Outcome of gene therapy trials in 20 patients with SCIDX1 (Paris-London data combined)



How to improve safety ?

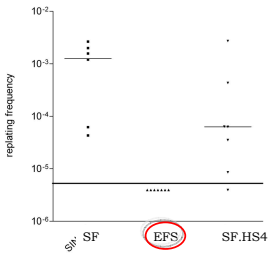
- Usage of self inactivated (SIN) LTR:

LTR-driven retroviral vector: MFG γc

New retroviral SIN vector

New trial

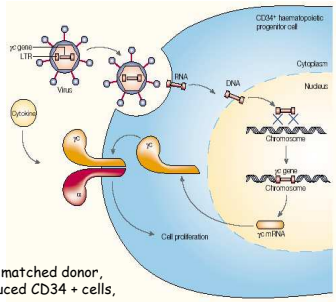
EF1α promoter is less mutagenic



In vitro clonogenicity assay (C. Baum's lab.)

Ex vivo gene therapy of SCID-X1

GALV-SIN RV

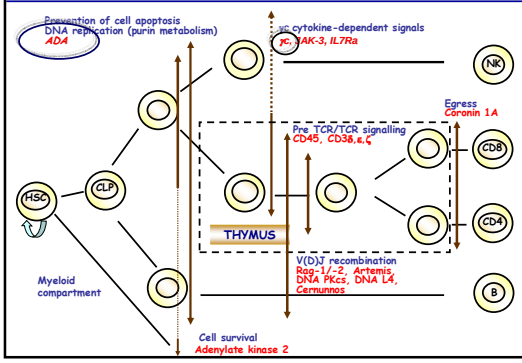


patients with no HLA matched donor,
reinjection of transduced CD34 + cells,
no chemotherapy
serious infectious complication ongoing

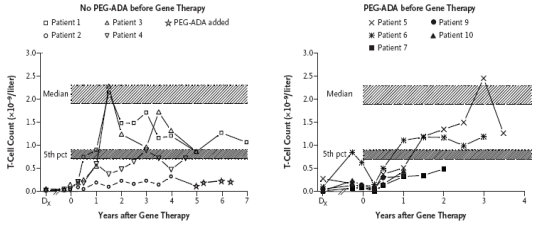
Conclusion

- Proof of principle of efficacy provided in a favorable setting with a sustained benefit (>10 years)
- Oligoclonal lymphopoiesis. Physiological ?
- A surprinsingly variable set of T cells detected in blood as a function of time. Physiological ?
- Safety to be improved by preventing the enhancer activity of the vector
- Likely extension to the treatment of other severe immunodeficiencies

Gene therapy of Severe combined immunodeficiencies



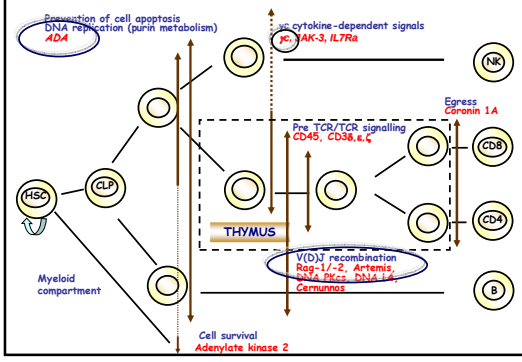
Gene therapy of adenosine deaminase deficiency T-cell reconstitution



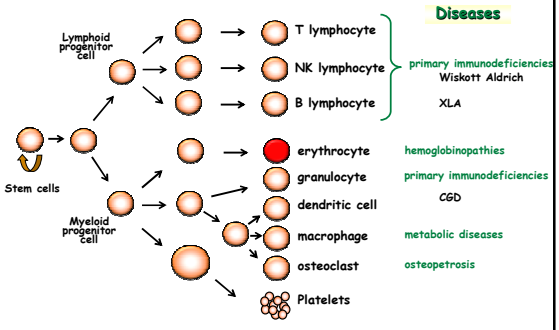
A. Aiuti et al. 2009

3 trials, 27 patients, all alive, 19 with disease correction (off ADA substitution)
(.5-9 years follow-up, median 3y.)

Gene therapy of severe combined immunodeficiencies



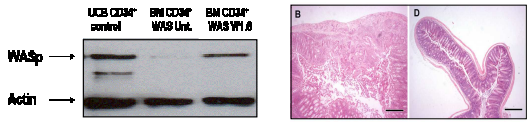
Gene therapy ⇨ hematopoietic stem cells



Gene transfer of WASP in WAS mice

Ex vivo lentiviral-mediated gene transfer in bone marrow stem cells

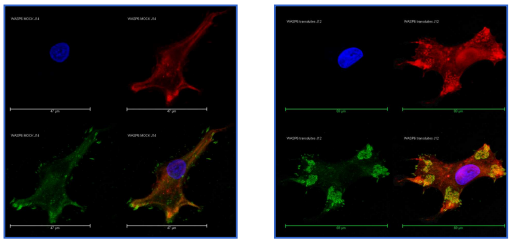
Expression of WASp **Colitis improvement**



No toxicity and long term efficacy

A.Galy

Restoration of cytoskeleton by WASP gene transfer



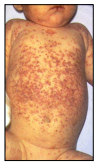
- WASP **+ WASP**

Actin/Vinculin/Nucleus

Gene therapy for WAS

Severe phenoype
No HLA compatible donor

CD34 progenitor cells



myeloablation

ex vivo gene transfer
SIN LV-WAS
(under WASP promoter)

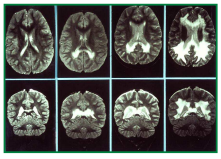
Transplantation

Adrenoleukodystrophy (ALD)

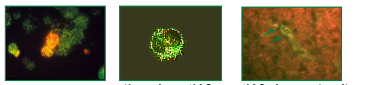
Demyelinating disease

Deficiency in the ALD protein (ABC transporter)

Bone marrow transplantation is efficient



Ex vivo correction of haematopoietic progenitor cells by using a lentivirus-ALDP



précurseur ALDP+ Macrophage ALDP+ ALDP+ human microglia in a mouse brain (xenogeneic transplantation in SCID/Nod mice)

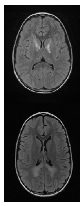
Benhamida et al; 2003

HSC gene therapy of ALD

- Two patients included

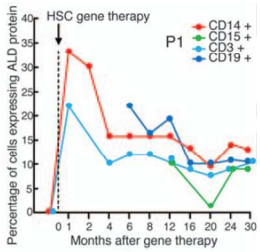
p1 : transplanted 11/09/2006
LS score = 2,25
gadolinium +
7 1/2 y, ALD protein -

p2 : transplanted 31/01/2007
LS score = 8
gadolinium +
7 y, ALD protein -

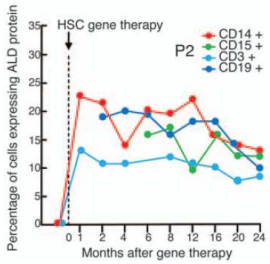


- No matched donor (geno id., URD.)
- Myeloablation (Bu.16/Cy 200mg/kg)
- Lentiviral vector
- Ex vivo transduction of CD34+ cells (cryopreserved)

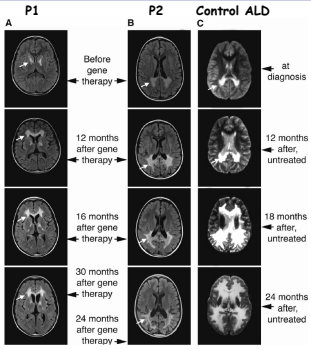
Expression of ALD protein following gene therapy



Expression of ALD protein following gene therapy



Brain MRIs from P1 (A) and P2 (B)



HSC gene therapy with lenti-ALD vector in X-ALD preliminary conclusions

- No safety concern so far
- Stable expression in 10-15 % of peripheral blood cells
  - ⇨ efficient transduction of self renewal haematopoietic progenitor cells
  - Short term clinical efficacy = HSCT
- Long term…

Gene therapy of PIDs

- Proof of principle provided (SCID-X1,ADA)
- Safety issue possibly solved by using SIN vectors
- Likely extension to diseases where selective advantage is less or absent (ALD exemple)
- Long term monitoring is essential
- Vector production issue
- Future advances…

Preclinical and clinical studies

M. Cavazzana-Calvo
S. Hacein-Bey-Abina
G. de Saint-Basile
I. André-Schmutz
E. Six
J.P. de Villartay
A. Durandy
C. Lagresle
J. Hauer
E. Morillon
J. Rivière
A. Lim (I. Pasteur)

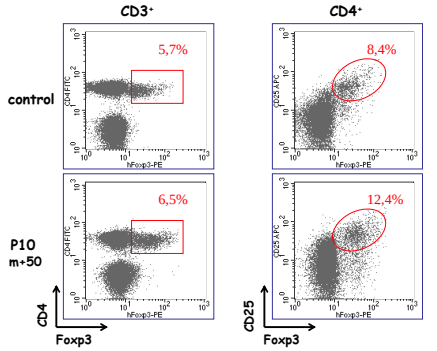
Integration sites

A. Garrigue
A. Deichmann,M Schmidt,C von Kalle(Heidelberg)
G. Wang, R. Bushman (Philadelphia)

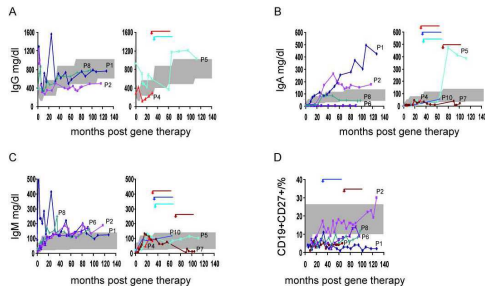
ALD
P. Aubourg (SVP hospital)
N. Cartier

Future SCID and WAS trials
A. Thrasher, H. Gaspar (London)
C. Baum (Hannover)
D. Williams (Cincinnati/Boston)
C. Galy (Genethon)

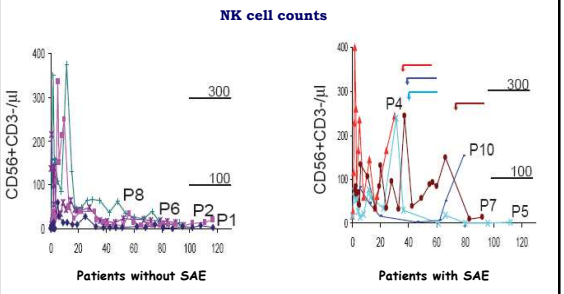
Regulatory T-cells reconstitution after γc gene therapy



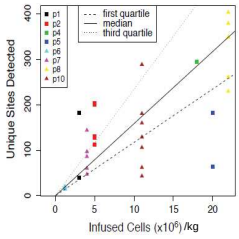
Immunoglobulins and memory B cells



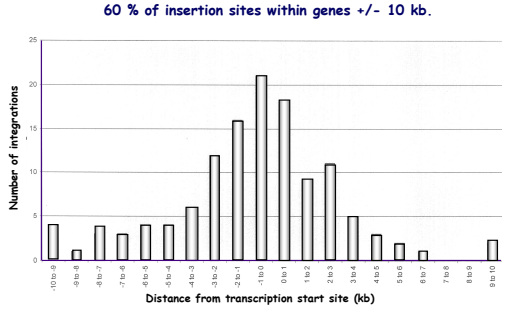
SCID X1 gene therapy trial



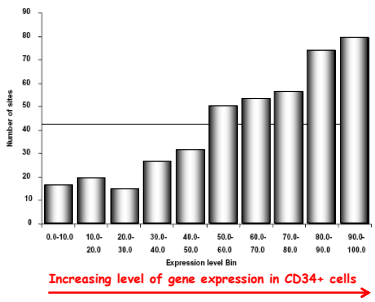
The number of integration sites is proportional to the number of infused cells



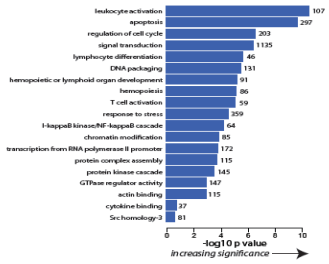
Insertions distributed around the transcription start site



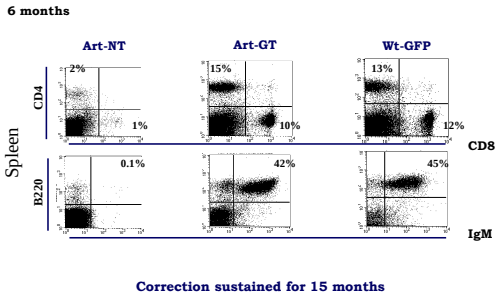
Association between vector integration and gene expression



Gene classes enriched near integration sites

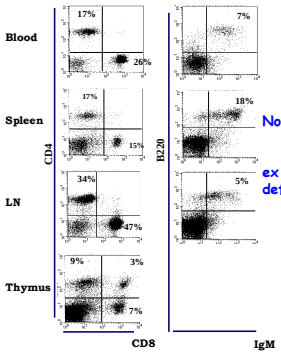


Correction of the immunodeficiency in Artemis-deficient mice after ex vivo lentiviral-mediated gene transfer



Correction sustained for 15 months

Secondary transplantation (10 months)



No vector-driven toxicity

ex vivo correction of CD34 Artemis-deficient cells

Gene therapy of T cell immunodeficiencies-projects