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

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

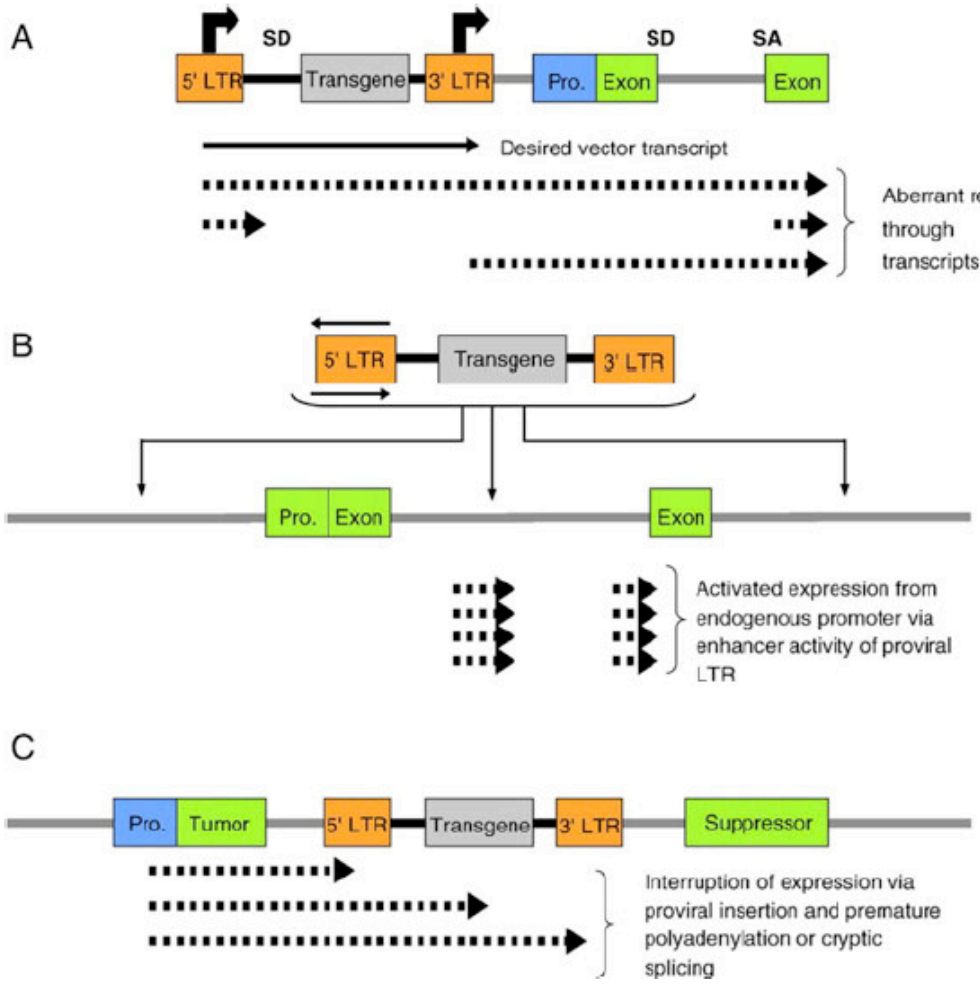
Research article

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

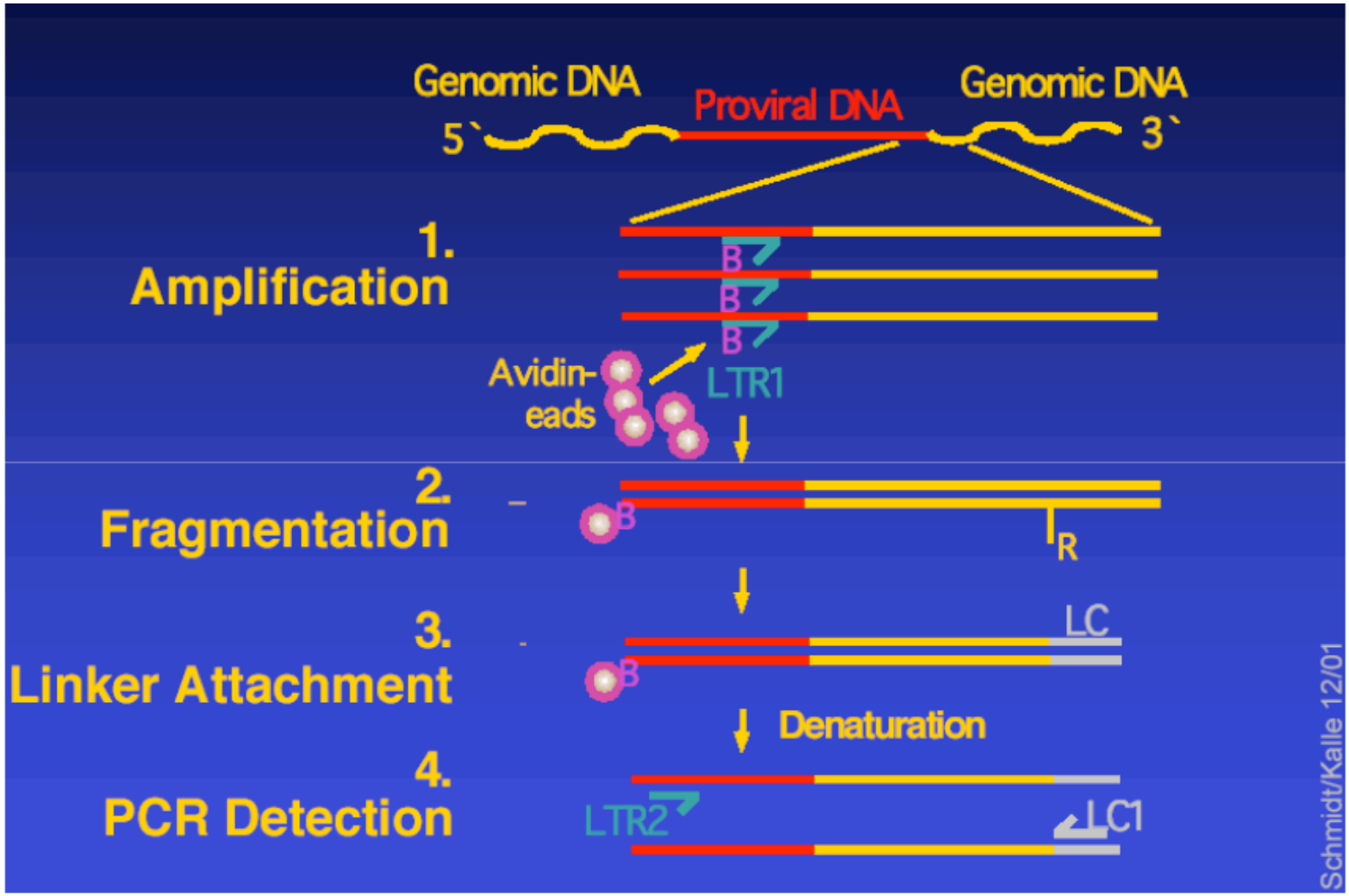


readthrough

new expression

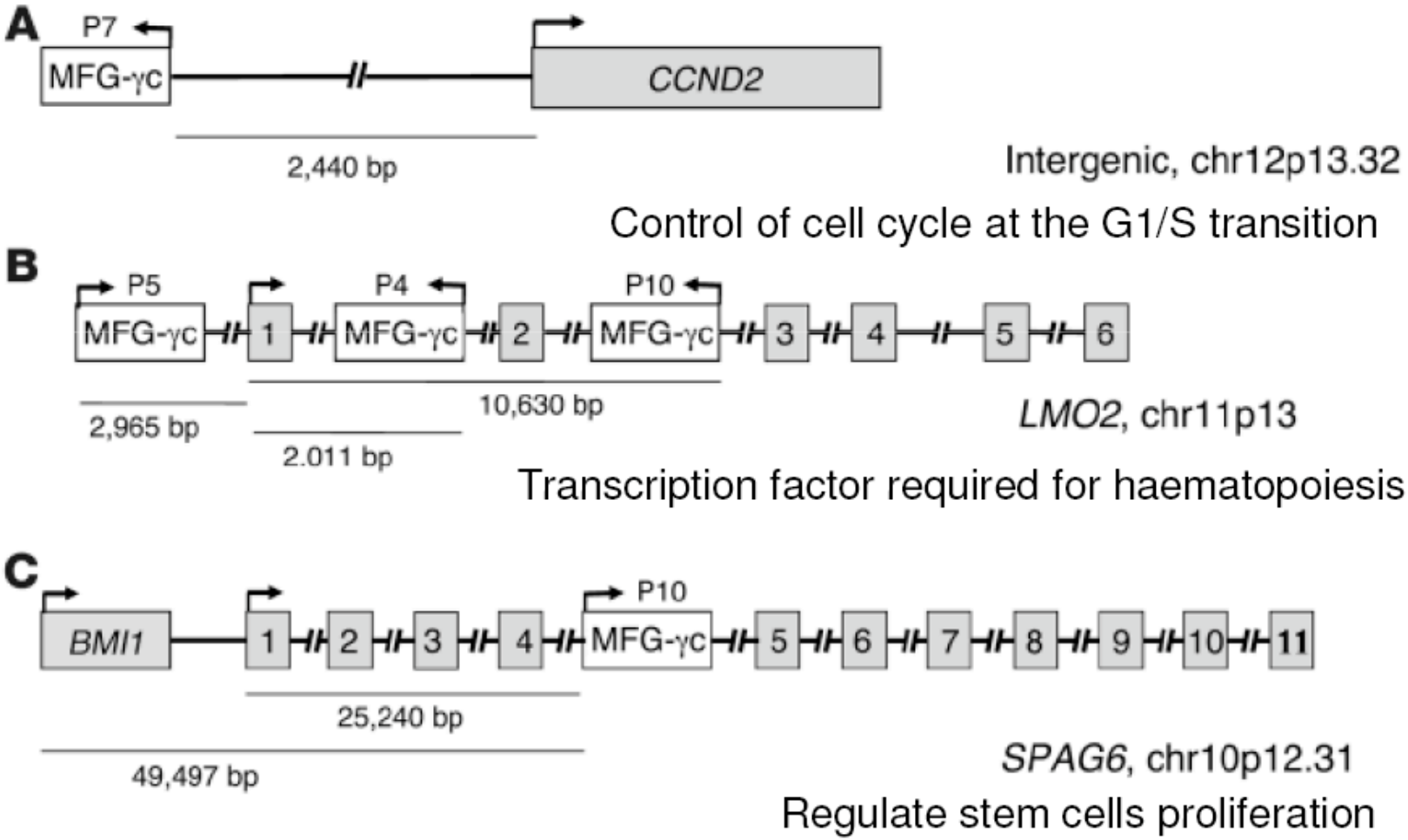
gene interruption

Linear Amplification (LAM) PCR strategy



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

Integration sites



Further oncogenic rearrangements

Patient characteristics

Patient	Age at therapy (mo)	T-ALL (mo)	Follow-up (mo)	Infection before gene therapy	CD34+γc+ cells infused (×10⁶/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.

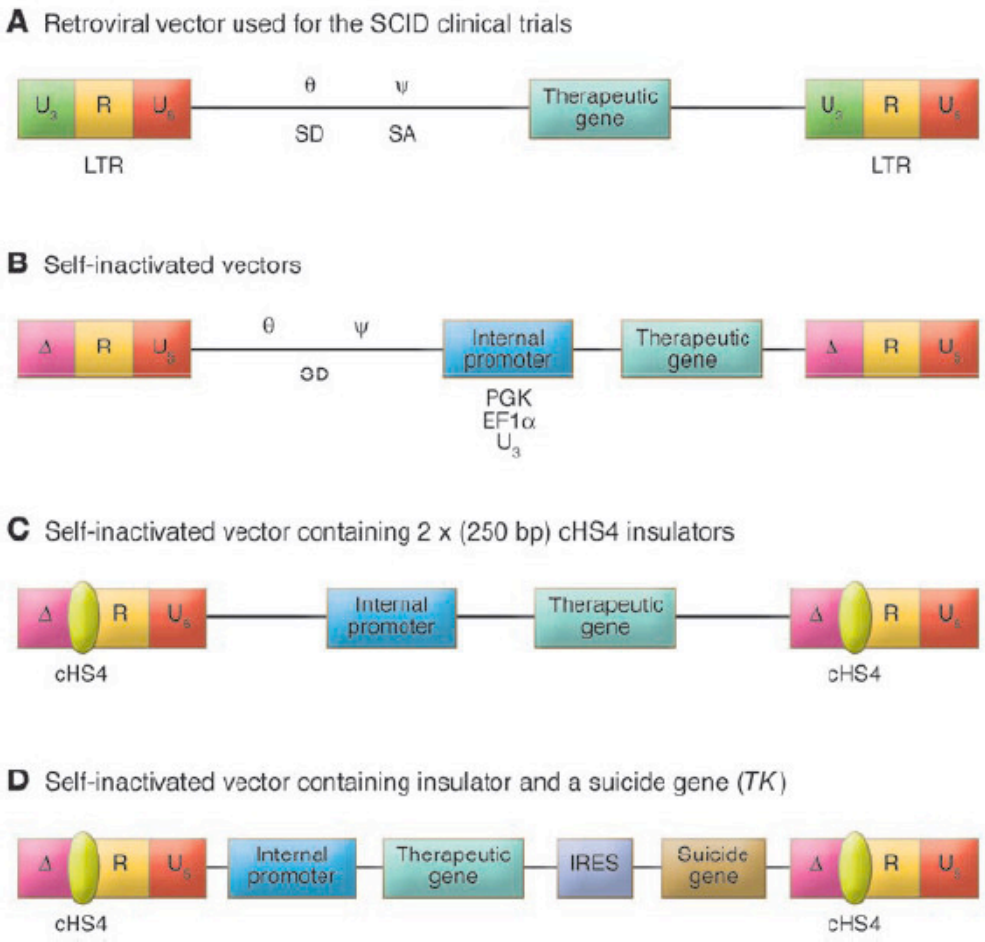
SCIDX1 background favors oncogenesis?

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.

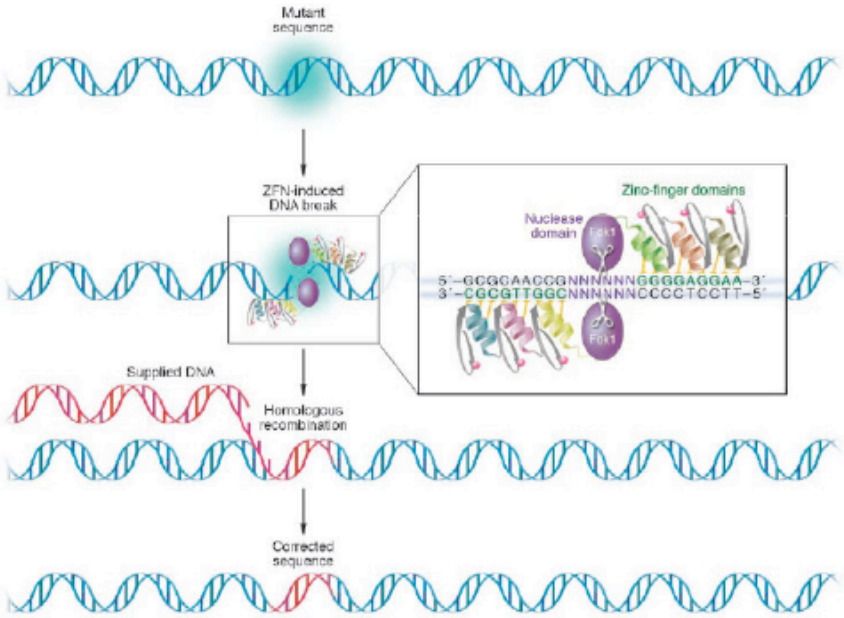
They received high dose of transduced cells.

Improvements



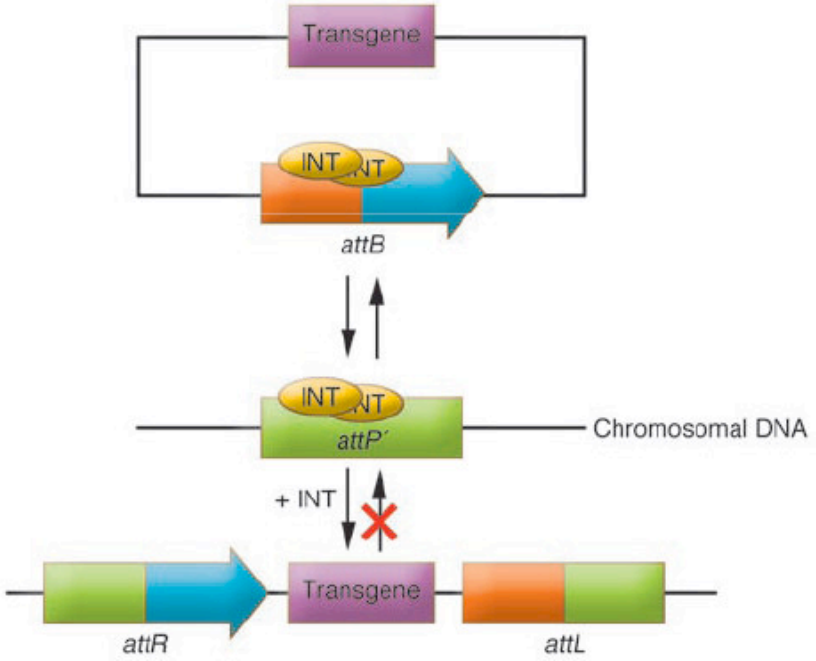
Improvements

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).