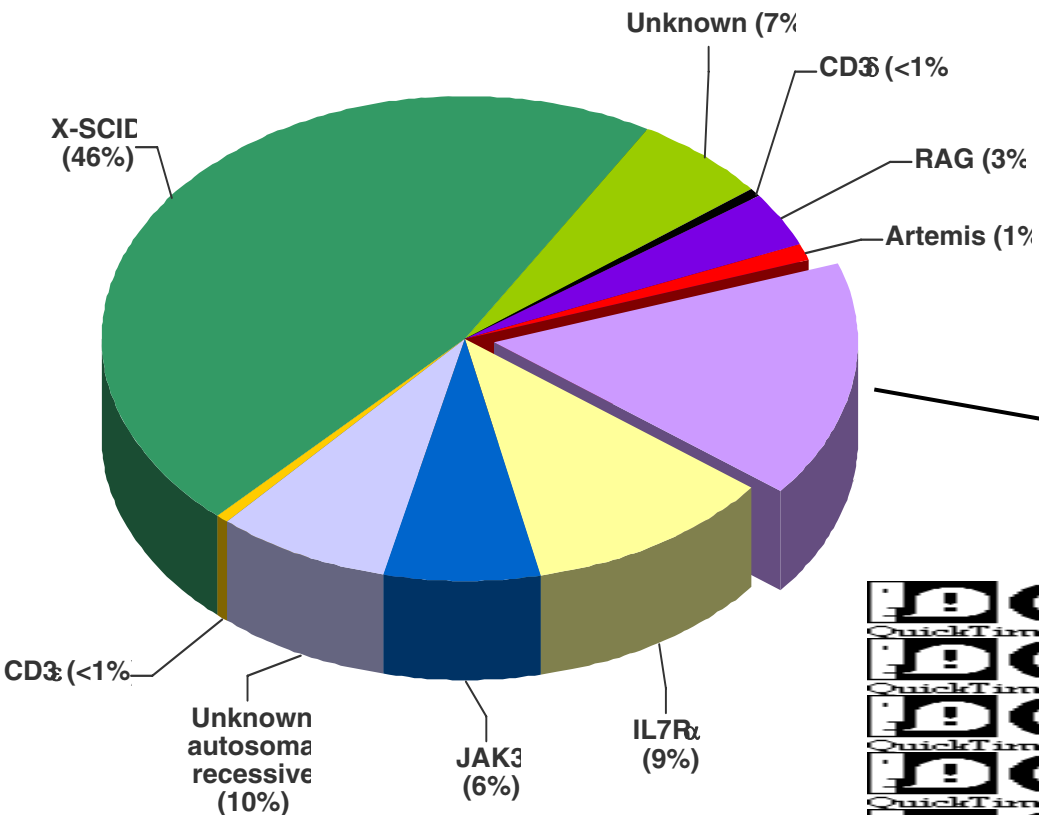
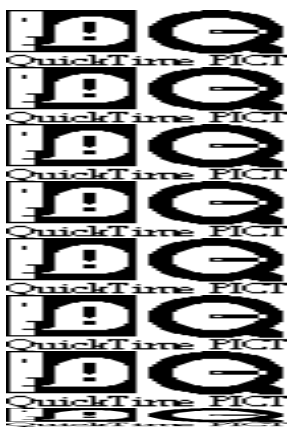
Severe Combined Immunodeficiencies



ADA Deficiency (16%)

- Autosomal Recessive
- Skeletal, G.I., Endocrine, SNC abnormalities
- dAXP-mediated lymphotoxicity
- T^-B^- SCID (early onset)
- CID (delayed/adult onset)



Buckley RH,
CTGTAC FDA, Bethesda, MD
March 2005

ADA deficiency - Conventional Treatments

Allogeneic Bone Marrow Transplantation

Lancet 2003, 361:553

- HLA-identical: 73% survival (n=18)
- Haploidentical: 23.5% survival (n=25)

PEG-ADA

M.S. Hershfield, Duke University Medical Center, NC, USA

- >160 pts worldwide
- 80% protective immunity; 20% minimal improvement
- >50% require IVIG
- 10% develop neutralizing antibodies
- 74% survival

HSCs Gene Therapy for ADA-SCID

	Year	No. Treated Pts.	Long-Term Marking	Clinical Benefit
Milan-1	1992	2	YES	NO
CHLA	1993	3	YES	NO
F/UK/NL	1993	3	NO	NO
Milan-2	2000	13	YES	YES
CHLA/NIH-1	2000	4	YES	NO
Japan	2003	2	YES	YES
London	2003	5	YES	YES
CHLA/NIH-2	2005	4	YES	YES
TOTAL		34	26	19

The Journal of Clinical Investigation http://www.jci.org Volume 117 Number 8 August 2007

Multilineage hematopoietic reconstitution without clonal selection in ADA-SCID patients treated with stem cell gene therapy

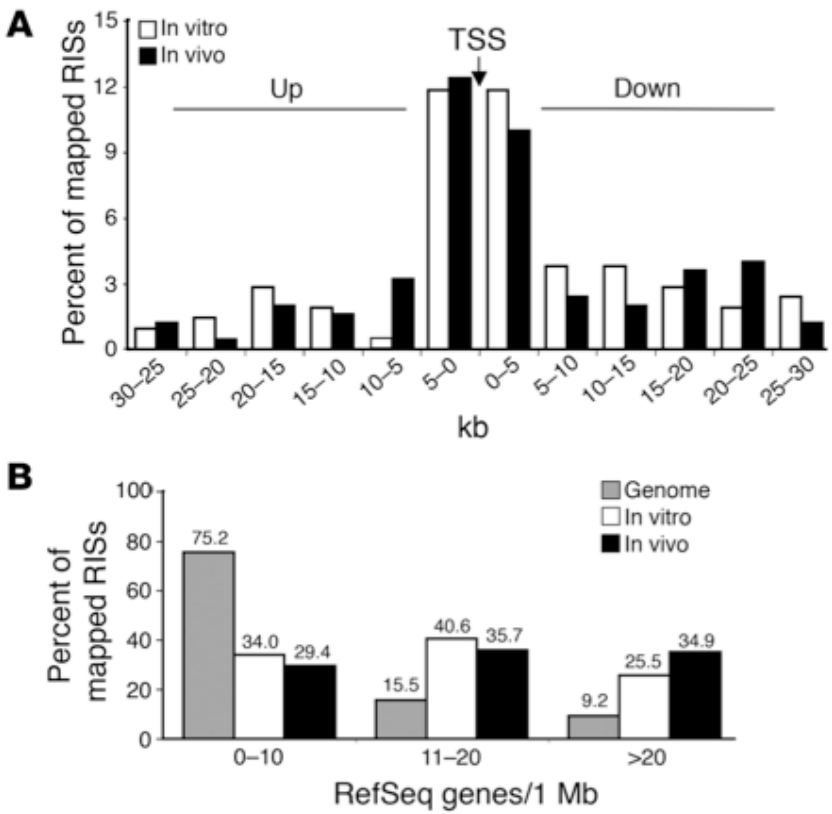
Alessandro Aiuti,[1] Barbara Cassani,[1] Grazia Andolfi,[1] Massimiliano Mirolo,[1] Luca Biasco,[1] Alessandra Recchia,[2] Fabrizia Urbinati,[2] Cristina Valacca,[1] Samantha Scaramuzza,[1] Memet Aker,[3] Shimon Slavin,[3] Matteo Cazzola,[1] Daniela Sartori,[4] Alessandro Ambrosi,[5] Clelia Di Serio,[5,6] Maria Grazia Roncarolo,[1,6] Fulvio Mavilio,[2,4] and Claudio Bordignon[1,6]

Patients #1-5 (47-1.5 mos after GT)

212 RIS from pre-transplant CD34+ cells
496 RIS from circulating patients' cells

Preferential integration in: TSS
Gene-dense regions
Highly expressed genes

Similar integration pattern before and after transplantation



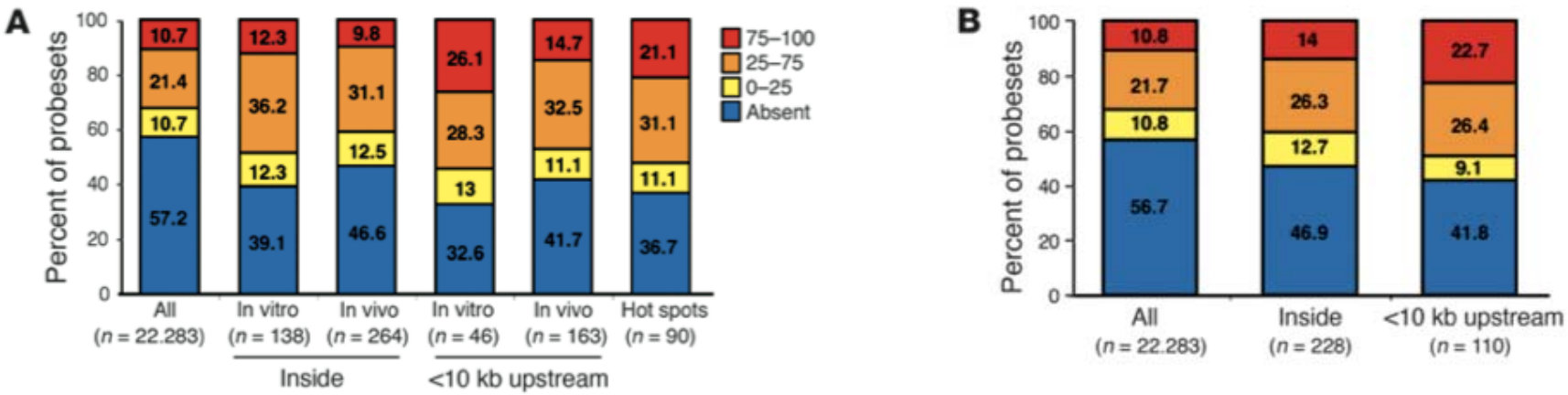


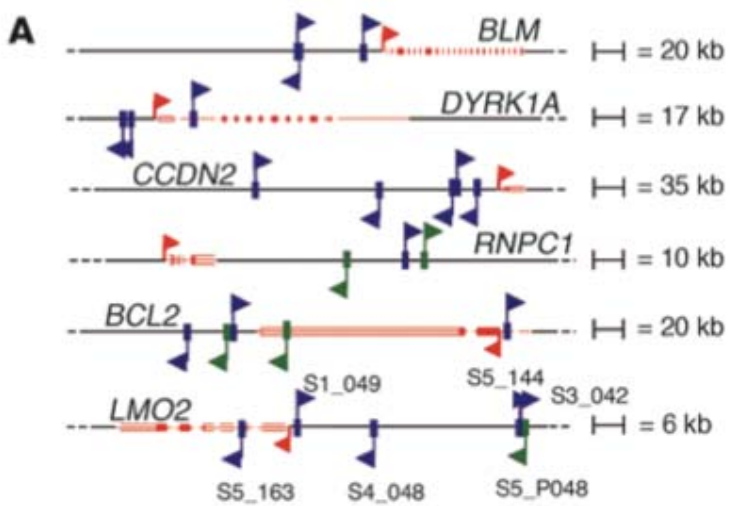
Table 1

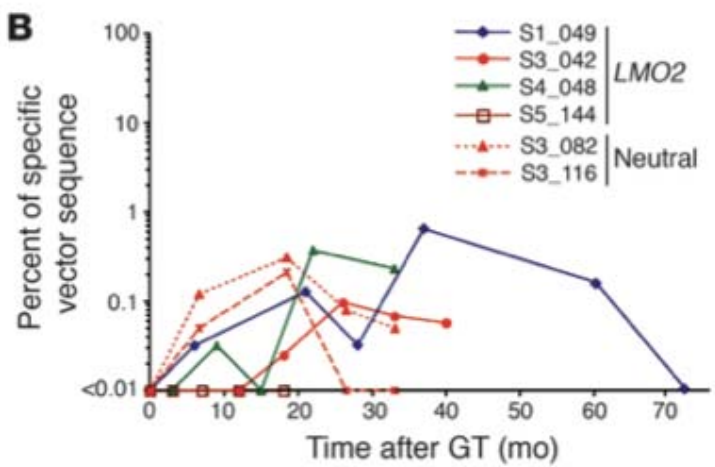
RIS distribution in hematopoietic cells from ADA-SCID patients

	Intragenic (%)	Intergenic (%)	<30 kb upstream (%)	<10 kb upstream (%)	<30 kb downstream (%)	±5 kb from TSS (%)
In vitro (pretransplant) CD34+ cells	50.9[A]	49.1	19.4	12.3[B]	4.3[C]	23.6[B]
Ex vivo follow-up	41.3[D]	58.7	25.6[B]	19.6[B,E]	12.7	28.8[B]
Total	44.3	55.7	23.7	17.4	10.2	27.3
Expected in the human genome[F]	35.5	64.5	ND	ND	ND	ND
Expected from random library[G]	37.9	62.1	14.5	4.2	10.5	6.1

Distribution of RISs unambiguously mapped in CD34+ HSCs from 4 ADA-SCID patients before transplantation (in vitro, pre-transplant CD34+ cells, 212 hits), from BM- or PBL-derived hematopoietic cells from 5 ADA-SCID patients 1.5 to 47 months after GT (496 hits), or total in vitro and ex vivo integrations (708 hits). Integrations were distributed as inside (intragenic) or outside (intergenic) RefSeq genes, at a distance of <30 kb or <10 kb upstream or <30 kb downstream from genes, and at a distance of ±5 kb from TSSs. ND, not determined. [A]$P < 0.00001$ versus human genome; $P < 0.01$ versus random; $P < 0.05$ versus ex vivo. [B]$P < 0.001$ versus random. [C]$P < 0.05$ versus random; $P < 0.001$ versus ex vivo. [D]$P < 0.01$ versus human genome. [E]$P < 0.05$ versus in vitro. [F]Values expected for the human genome (NCBI35 annotation). [G]Values obtained from a collection of 398 sequences randomly cloned from a library of *Pst*1/*Mse*I-restricted, LM-PCR–amplified human DNA.

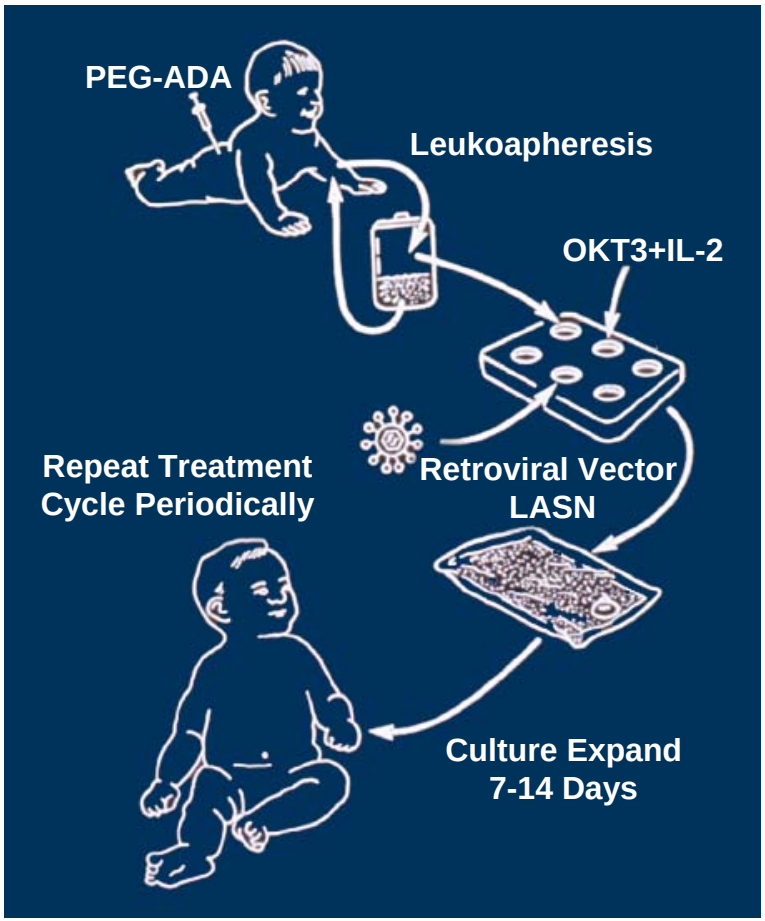
Integration Hotspot after HSCs Gene Therapy for ADA-SCID

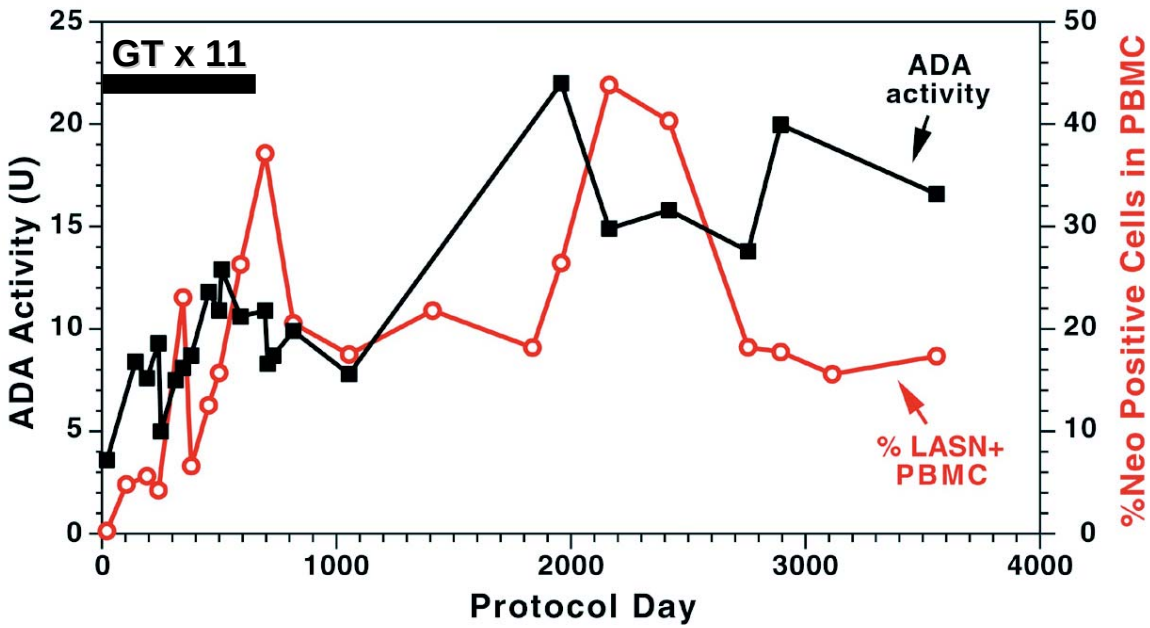




Aiuti A. et al. The Journal of Clinical Investigation http://www.jci.org Volume 117 Number 8 August 2007

Lymphocyte Gene Therapy for ADA deficiency





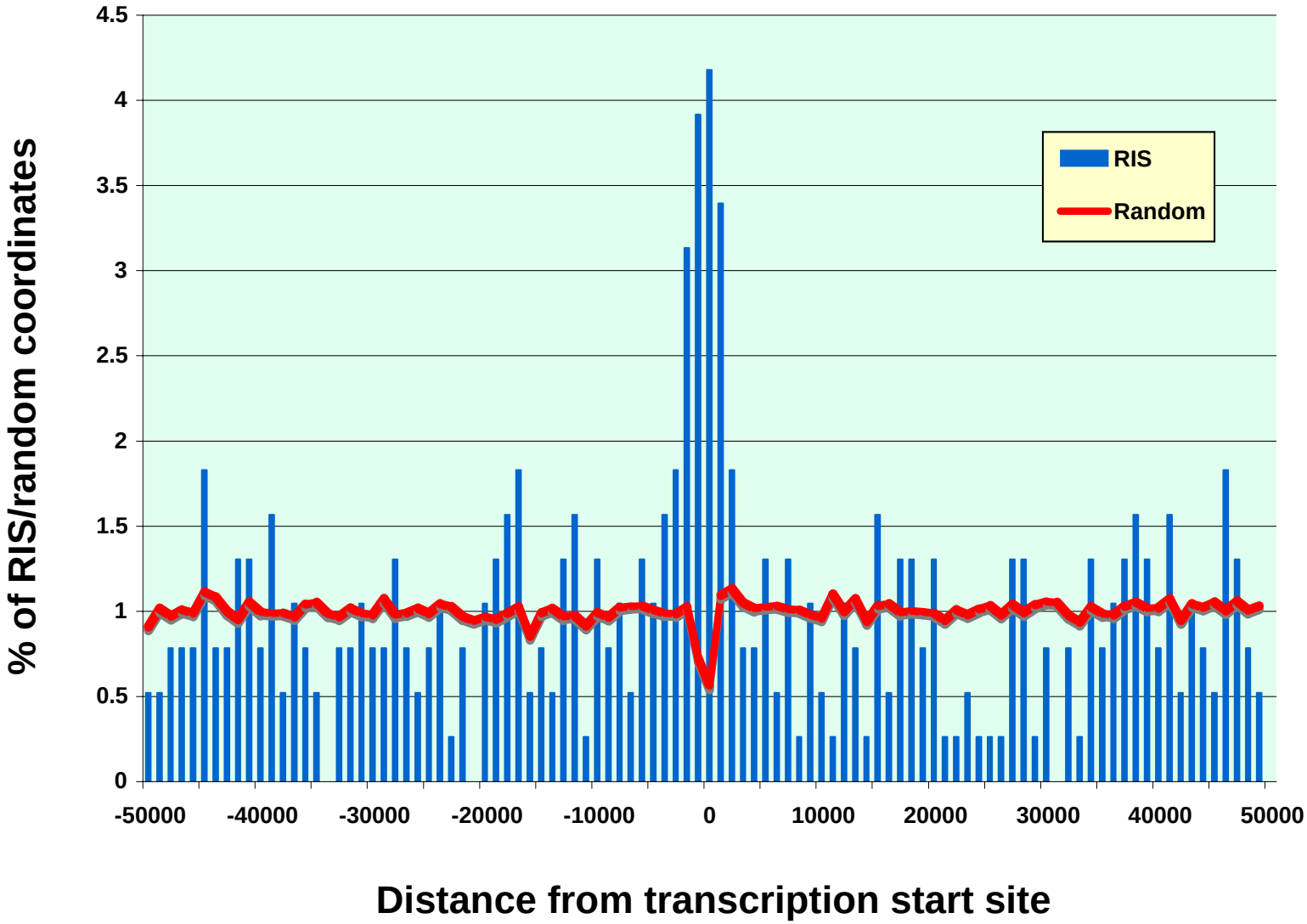
Blaese et al. *Science* 20 October 1995 - Vol. 270. no. 5235

MUUL et al, BLOOD, 1 APRIL 2003 • VOLUME 101, NUMBER 7

Location of RIS in relation to annotated genes (± 5 Kb)

Year	Total no. of RIS	Unique BLAT Hits	Within Gene Range	5 Kb range			
				Exon	Intron	Upstream	Downstream
1991	134	58	45 (pŠ0.001)	1 (NS)	35 (pŠ0.004)	11 (p<0.001)	5 (NS)
1992	105	53	34 (p<0.001)	1 (NS)	27 (pŠ0.003)	7 (pŠ0.005)	4 (NS)
1995	101	42	26 (pŠ0.005)	0 (NS)	21 (pŠ0.014)	7 (pŠ0.003)	5 (pŠ0.022)
1998	96	56	39 (p<0.001)	1 (NS)	27 (pŠ0.002)	11 (p<0.001)	3 (NS)
2000	102	48	30 (pŠ0.001)	0 (NS)	24 (pŠ0.008)	6 (pŠ0.010)	3 (NS)
2003	122	42	25 (pŠ0.009)	0 (NS)	19 (pŠ0.055)	8 (p<0.001)	1 (NS)
2005	121	56	34 (p<0.001)	1 (NS)	24 (pŠ0.068)	8 (pŠ0.006)	5 (NS)
Total	781	326	199 (p<0.001)	6 (NS)	86 (NS)	52 (p<0.001)	25 (pŠ0.006)

Distribution of random coordinates and recovered RIS relative to gene transcriptional start sites



RIS located within a 5 kb range from validated or suspected cancer-related genes

	Chr	Gene Name	Location
1	1	CA6	Intron
2	1	CDC14A	Intron
3	1	ST7L	Intron
4	2	BRE	Intron
5	2	INPP5D	Intron
6	2	NCOA1	Intron
7	3	TNFSF10	Intron
8	4	ARGBP2	Intron
9	6	TFEB	Intron
10	6	FYN	Intron
11	6	SENP6	Upstream
12	6	LATS1	Intron
13	6	RPS6KA2	Intron
14	8	STK3	Intron
15	9	RAPGEF1	Intron
16	9	NTRK2	Intron
17	10	MLLT10	Exon
18	10	ADK	Intron
19	10	FER1L3	Intron
20	11	PICALM	Intron
21	11	MAML2	Intron
22	12	BLOC1S1	Upstream
23	12	NACA	Intron
24	12	RSN	Intron

	Chr	Gene Name	Location
25	12	LAG3	Downstream
26	12	DGKA	Upstream
27	12	ITGA7	Upstream
28	13	ABCC4	Intron
29	14	STRN3	Intron
30	14	PRKCH	Intron
31	15	SIN3A	Intron
32	15	AKAP13	Intron
33	15	TCF12	Intron
34	15	IQGAP1	Intron
35	15	IL16	Intron
36	16	PRKCB1	Intron
37	16	GSPT1	Intron
38	17	TADA2L	Intron
39	17	NF1	Intron
40	17	MYO18A	Intron
41	19	BAX	Downstream
42	19	RAB8A	Intron
43	19	DNMT1	Intron
44	20	PTPRA	Intron
45	20	PPP1R16B	Intron
46	20	SDBCAG84	Upstream
47	22	BCR	Intron
48	X	SH3KBP1	Intron

Cancer annotated genes

Genes possibly implicated in cancer

ADA-SCID: Is it the same?

- Similar RVV integration pattern, different outcome
- ADA is a survival factor, γ_c is a proliferation signal
- Different co-operation partners for the two gene products?
- Technical differences in gene transfer protocols

The future of gene transfer for ADA-SCID?

- Monitoring
- Lentiviral, Foamy, and ASLV vectors