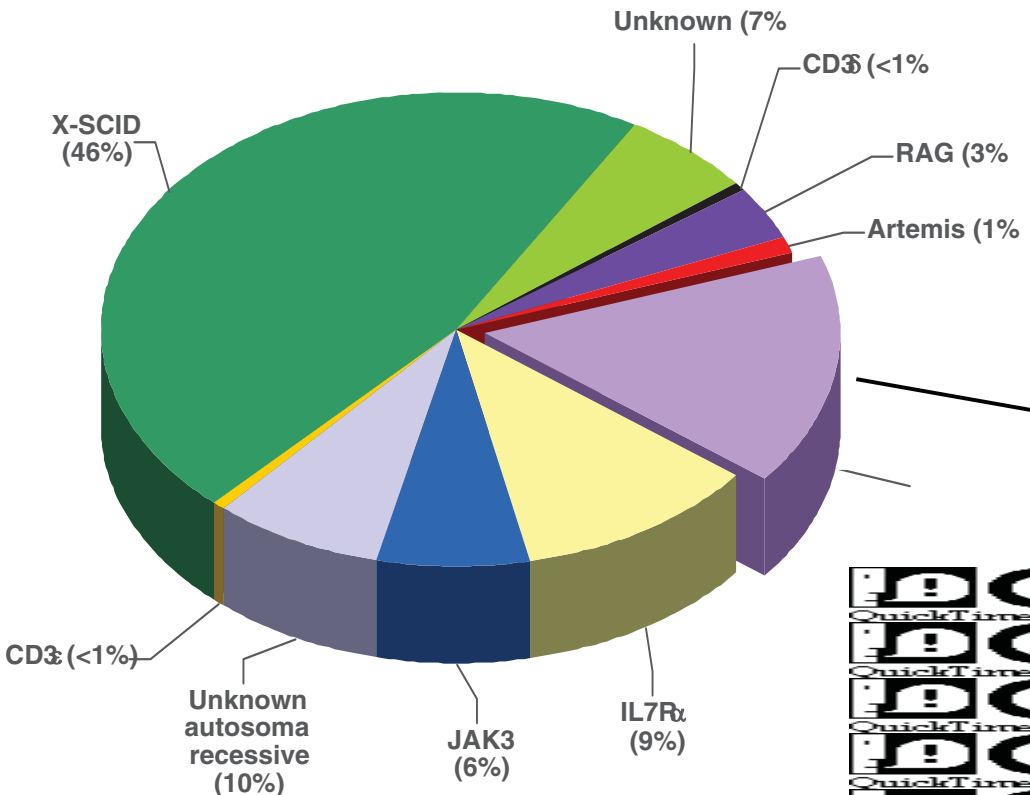
Severe Combined Immunodeficiencies



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

ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
T lymphocytes	USA	1990	2
T lymph (+BM-HSCs)	Italy	1992	6
Cord blood HSCs	USA	1993	3
BM-HSCs	France/UK/The Netherlands	1993	3
T lymphocytes	Japan	1995	1
BM-HSCs	Italy	2001	10
BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

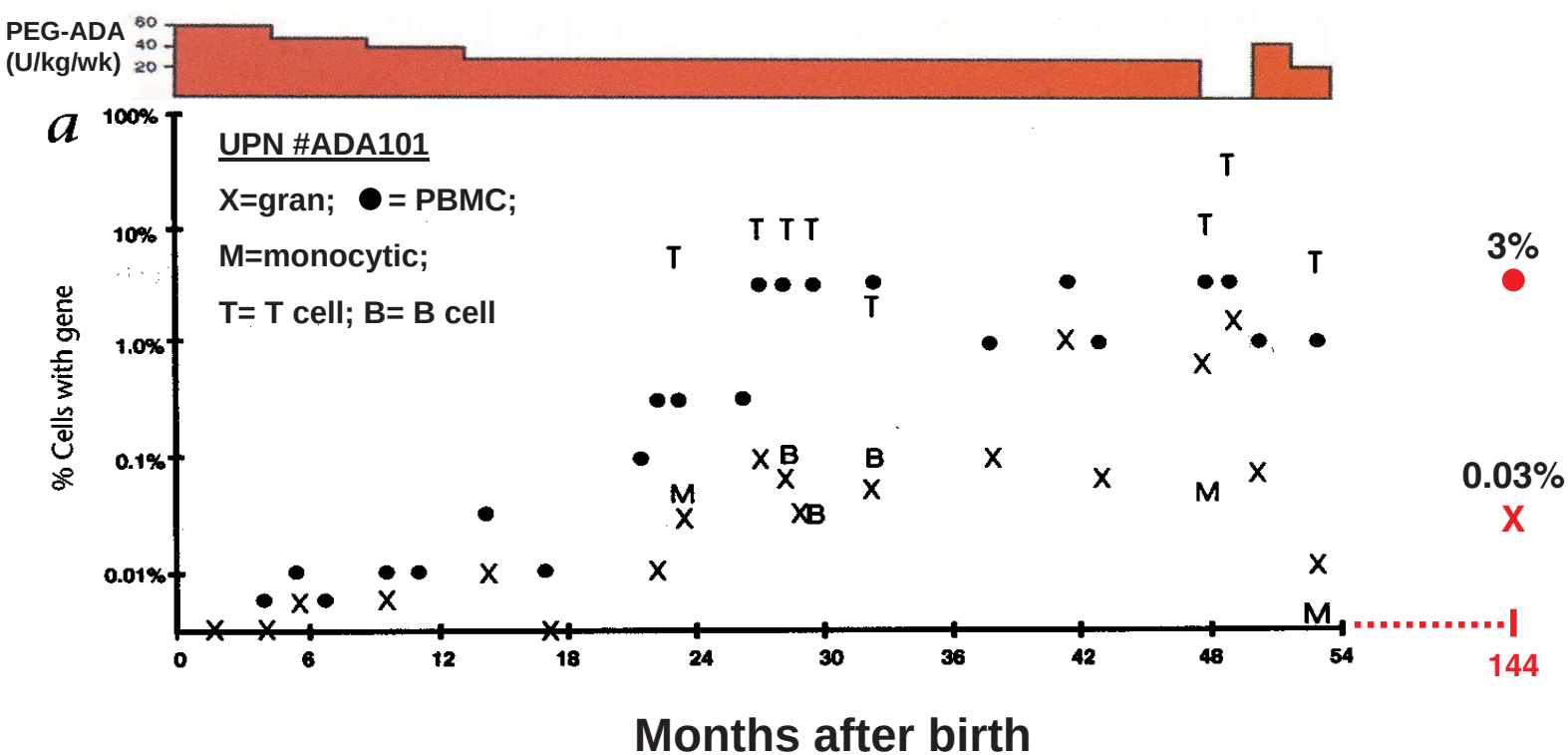
ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
T lymphocytes	USA	1990	2
T lymph (+BM-HSCs)	Italy	1992	6
Cord blood HSCs	USA	1993	3
BM-HSCs	France/UK/The Netherlands	1993	3
T lymphocytes	Japan	1995	1
BM-HSCs	Italy	2001	10
BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

Protocol details/results

- Three rounds of transductions in IL-3, IL-6, SCF
- ~1% gene-marked PBMC 4 years after treatment
- Unsuccessful withdrawal PEG-ADA in one patient
- No lymphoproliferative adverse events

Frequency of Gene-Containing Leukocytes



Kohn et al, *Nat Med* 4:775-780, 1998 and D.B. Kohn personal communication, February 2005

ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
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BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
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BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
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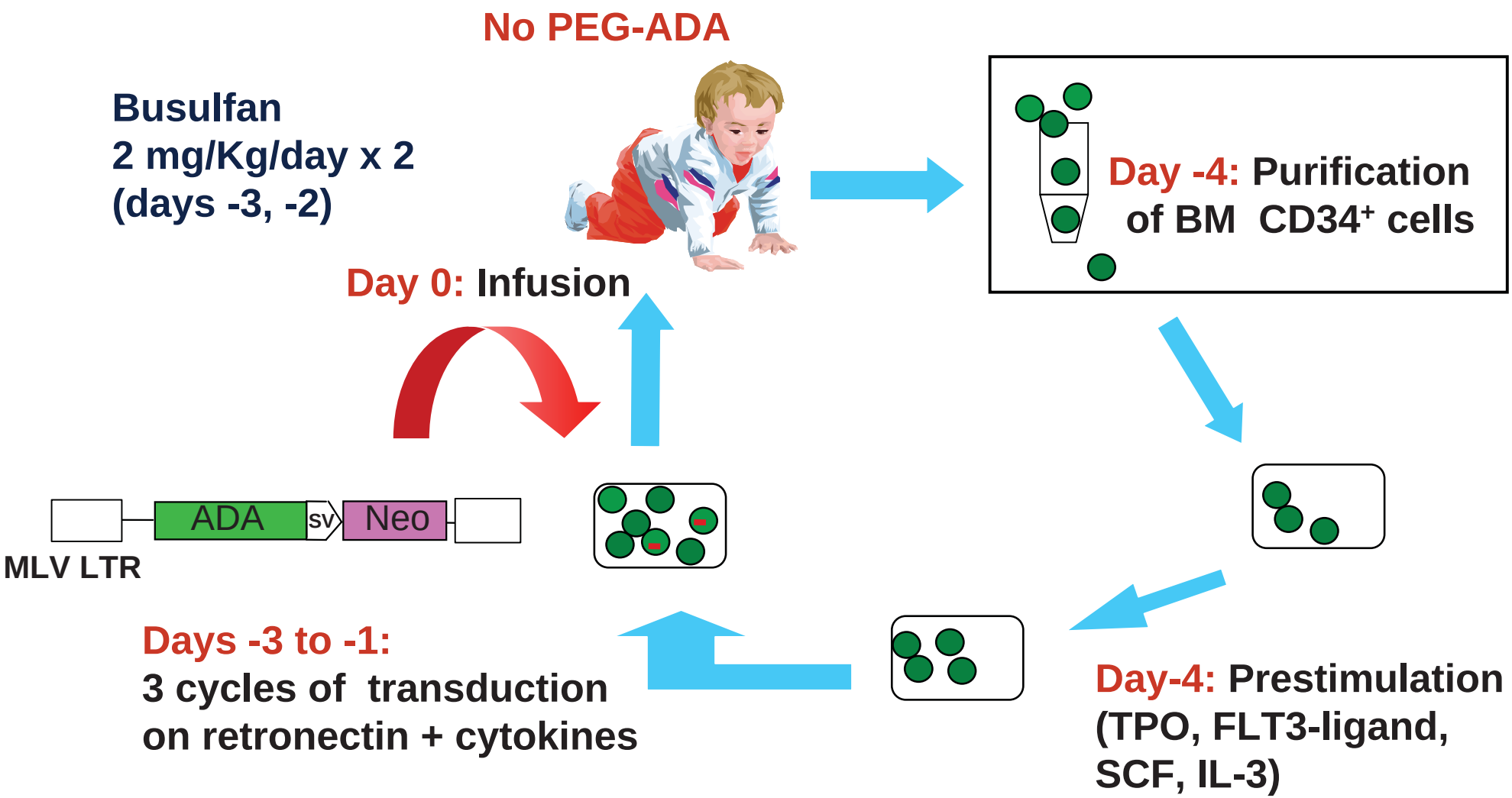
BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

Italian gene transfer protocol into autologous bone marrow CD34$^+$ cells



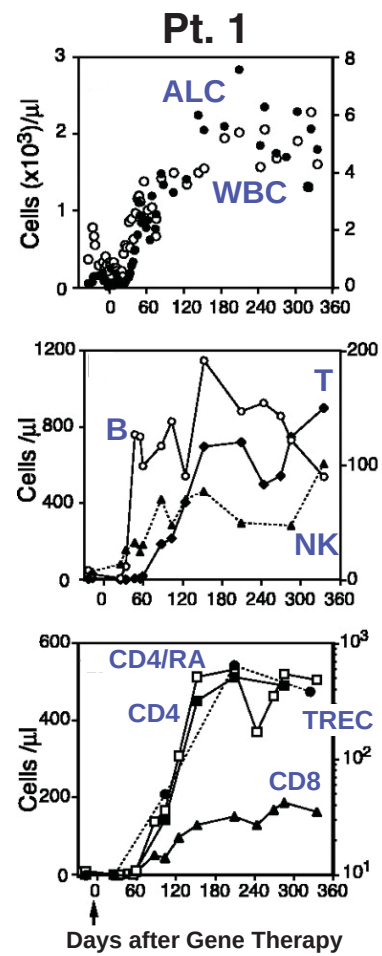
A. Aiuti, MG Roncarolo, C. Bordignon, 2002

Gene Therapy for ADA deficiency: Role of Preparative Chemotherapy

Correction of ADA-SCID by Stem Cell Gene Therapy Combined with Nonmyeloablative Conditioning

Alessandro Aiuti,[1] Shimon Slavin,[2] Memet Aker,[2] Francesca Ficara,[1] Sara Deola,[1] Alessandra Mortellaro,[1] Shoshana Morecki,[2] Grazia Andolfi,[1] Antonella Tabucchi,[3] Filippo Carlucci,[3] Enrico Marinello,[3] Federica Cattaneo,[1] Sergio Vai,[1] Paolo Servida,[4] Roberto Miniero,[5] Maria Grazia Roncarolo,[1,6] * Claudio Bordignon[1,6]*†

28 JUNE 2002 VOL 296 SCIENCE



Summary of ADA-SCID patients treated with CD34+ cells

	HSC-Pt	Age of onset (months)	Previous therapy	Age at GT (months)	Months of FU (March 1st 2007)
Hadassah	Pt1	1	None	7	79
HSR-TIGET	Pt1	2	Haplo-BMT	30	73
	Pt2	2	Haplo-BMT	12	59
	Pt3	5	Haplo-BMT PEG-ADA for 2 mo	22	54
	Pt4	2	PEG-ADA for 1.3 yr	19	36
	Pt5	1	PEG-ADA for 5 yr	67	28
	Pt6	1	PEG-ADA for 2.7 yr	34	13
	Pt7	1	PEG-ADA for 1 yr	18	16
	Pt8	3	Haplo-BMT PEG-ADA for 1yr	21	3
	Pt9	5	PEG-ADA for 10 mo	15	5

HSR-TIGET, CONFIDENTIAL

SUMMARY OF RESULTS

- Persistent, stable, engraftment of transduced cells in myeloid (0.1-8%) and lymphoid (20-100%) compartments (PB and BM)
- In all patients with >1 yr FU increase in T cells and functional response
- Five patients discontinued IVIg with proven antibody response to vaccinal antigens
- Good systemic metabolic detoxification (levels comparable to HLA-id BMT)

- No short-term or long-term toxicity
- No clonal expansion, no RCR

- 100% survival (median follow-up 32 months)
- Dramatic reduction in severe infections
- The first six patients treated are attending school regularly

A. Aiuti, MG Roncarolo, HSR-TIGET

ADA Gene Therapy Trials

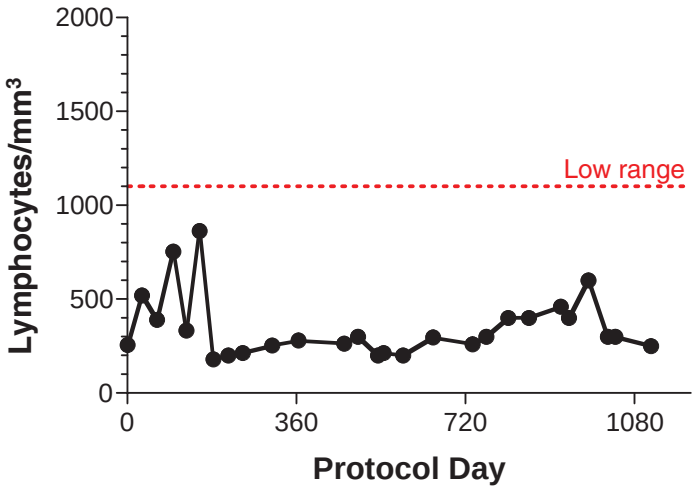
Target cell	Country	Year	No. of patients
T lymphocytes	USA	1990	2
T lymph (+BM-HSCs)	Italy	1992	6
Cord blood HSCs	USA	1993	3
BM-HSCs	France/UK/The Netherlands	1993	3
T lymphocytes	Japan	1995	1
BM-HSCs	Italy	2001	10
BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

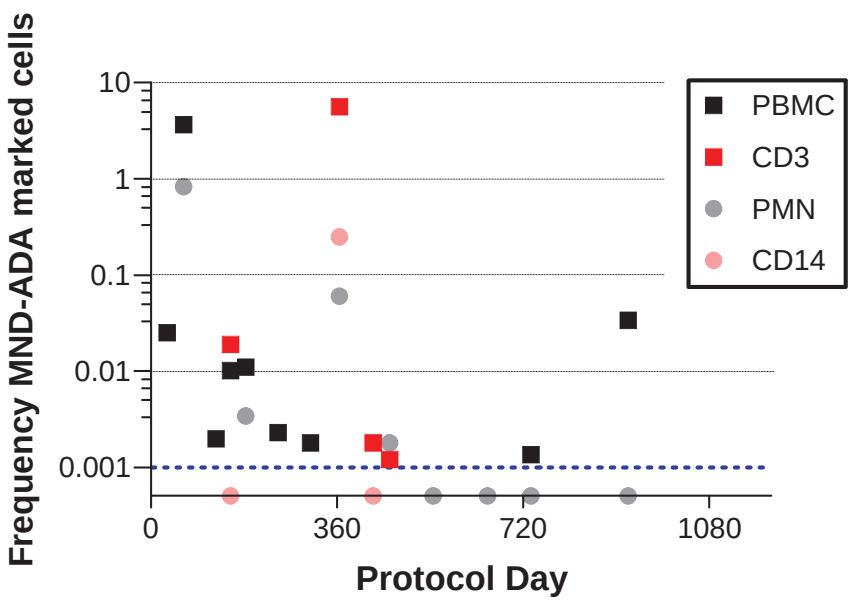
Comparison of Retroviral Vectors for Gene Therapy of ADA deficiency: 2001 Clinical Trial Design

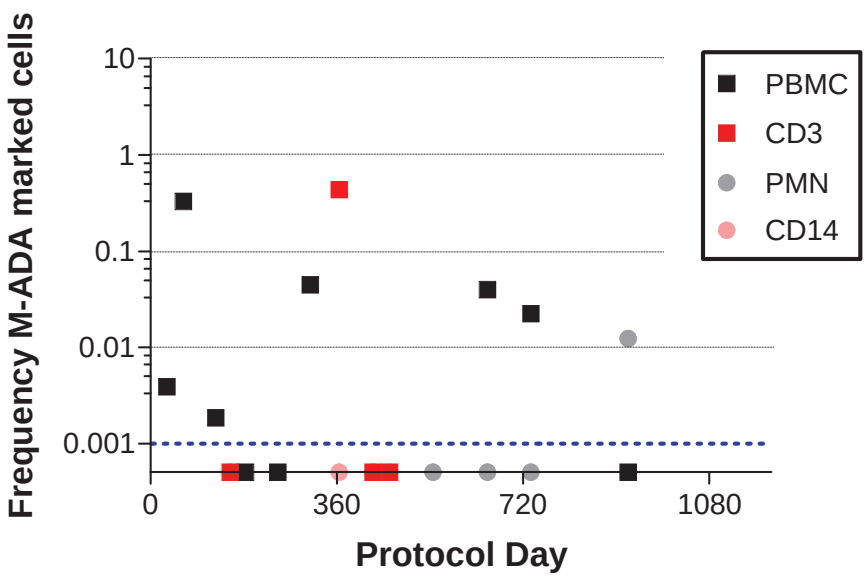
- Collection of cord blood or bone marrow CD34+ cells
- Pre-stimulation (SCF, Flt-3L, MGDF on CH296 x 2 days)
- Divide cells into two aliquots
- Separately transduce with GCsapM-ADA and MND-ADA x 3 days
- Infuse into patients (continue PEG-ADA)

Patient ADA202N: Transduction and Marking Data

				CD34+ Transduction		Vector-positive CFUs		ADA activity	
PIN	Sex	Age (yrs)	Infused $CD34^+$/Kg	MND	M-ADA	MND	M-ADA	MND	M-ADA
202	F	5	12×10^6	40%	66%	35%	62%	76 U	235 U







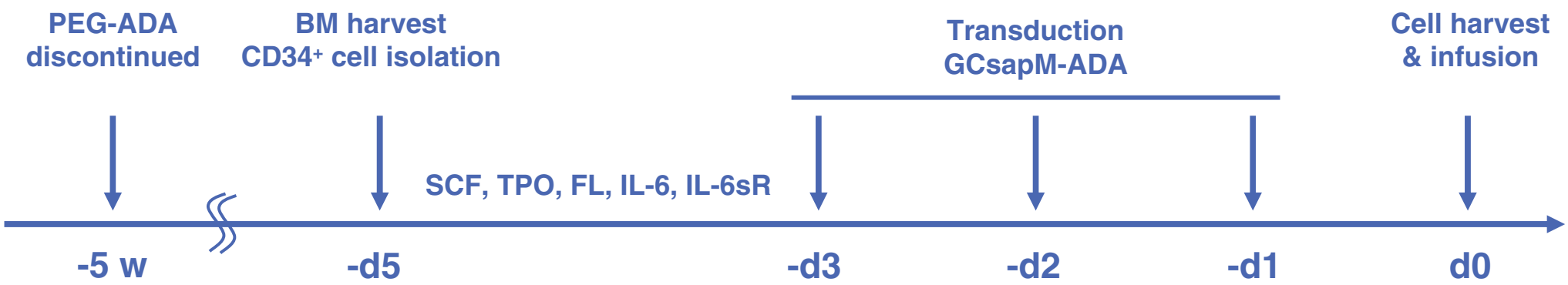
Summary

- Low level of multi-lineage marking
  - ≥ 1 year in 2 patients
  - < 6 months in 2 patients
- Higher marking in younger patients
- Higher marking in lymphoid cells
- No adverse events, no clinical benefit

ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
T lymphocytes	USA	1990	2
T lymph (+BM-HSCs)	Italy	1992	6
Cord blood HSCs	USA	1993	3
BM-HSCs	France/UK/The Netherlands	1993	3
T lymphocytes	Japan	1995	1
BM-HSCs	Italy	2001	10
BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

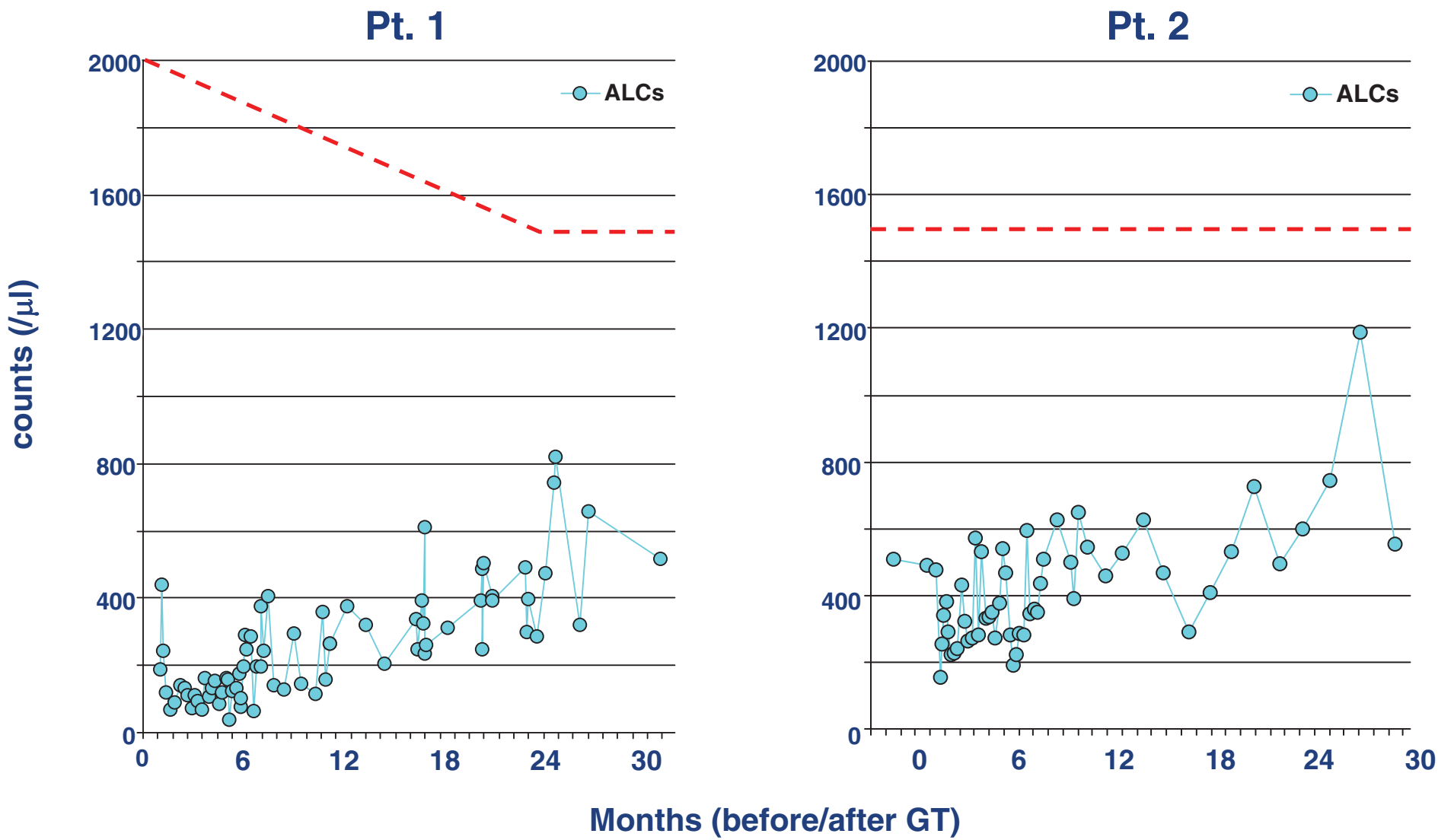
Japan Gene transfer protocol and treatment details



	Age	Infused CD34+ cells/kg	Vector-positive CFU (%)	ADA activity (U/min)
Pt. #1	4	1.38×10^6	40	318.2
Pt. #2	13	0.92×10^6	50	299.4

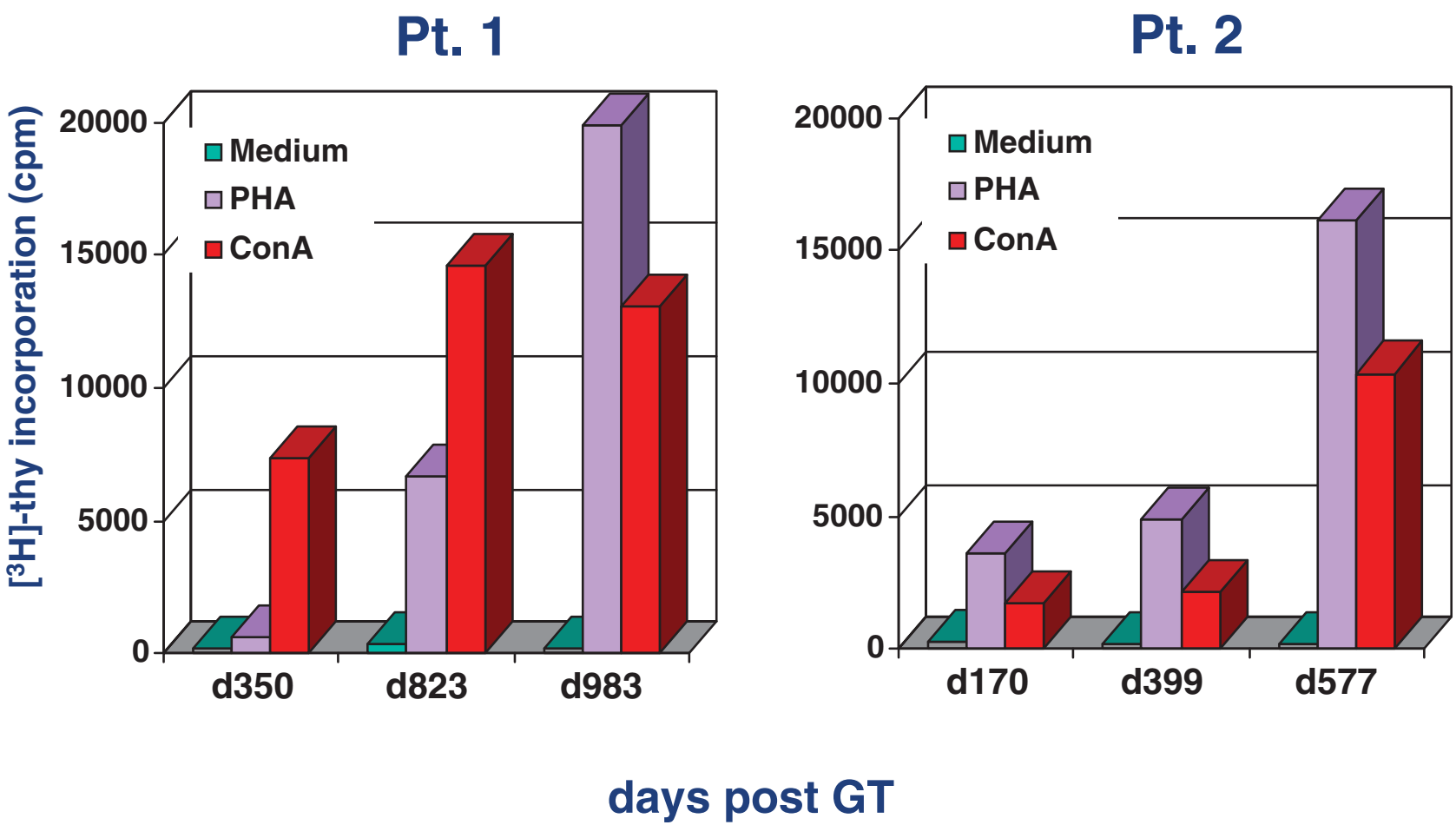
M. Otsu, personal communication

Hematopoietic reconstitution (PB lymphocyte counts)



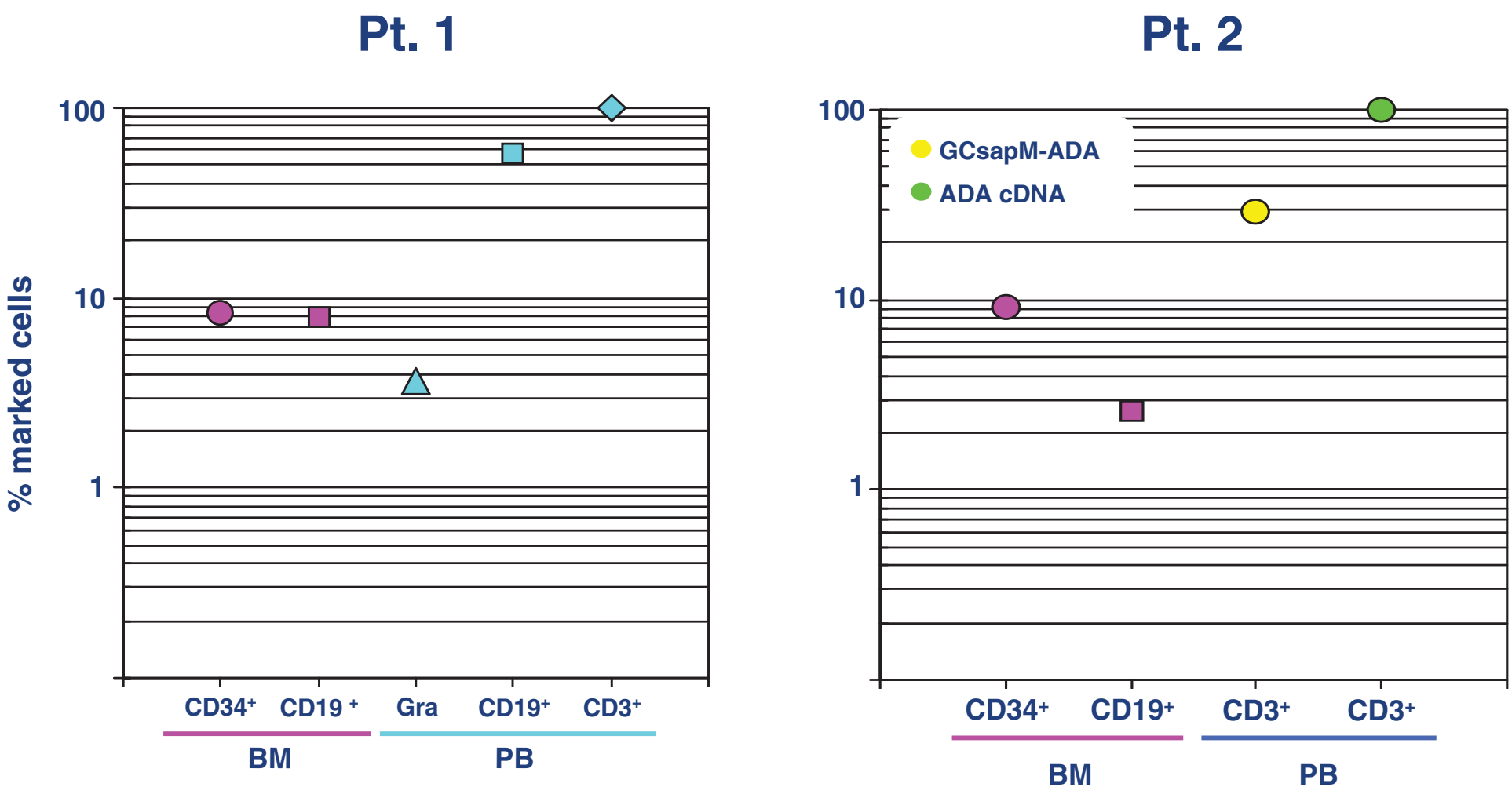
M. Otsu, personal communication

Mitogen-stimulated T cell proliferation



M. Otsu, personal communication

Analysis of gene-marking levels



M. Otsu, personal communication

ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
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T lymphocytes	Japan	1995	1
BM-HSCs	Italy	2001	10
BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

Protocol details and results

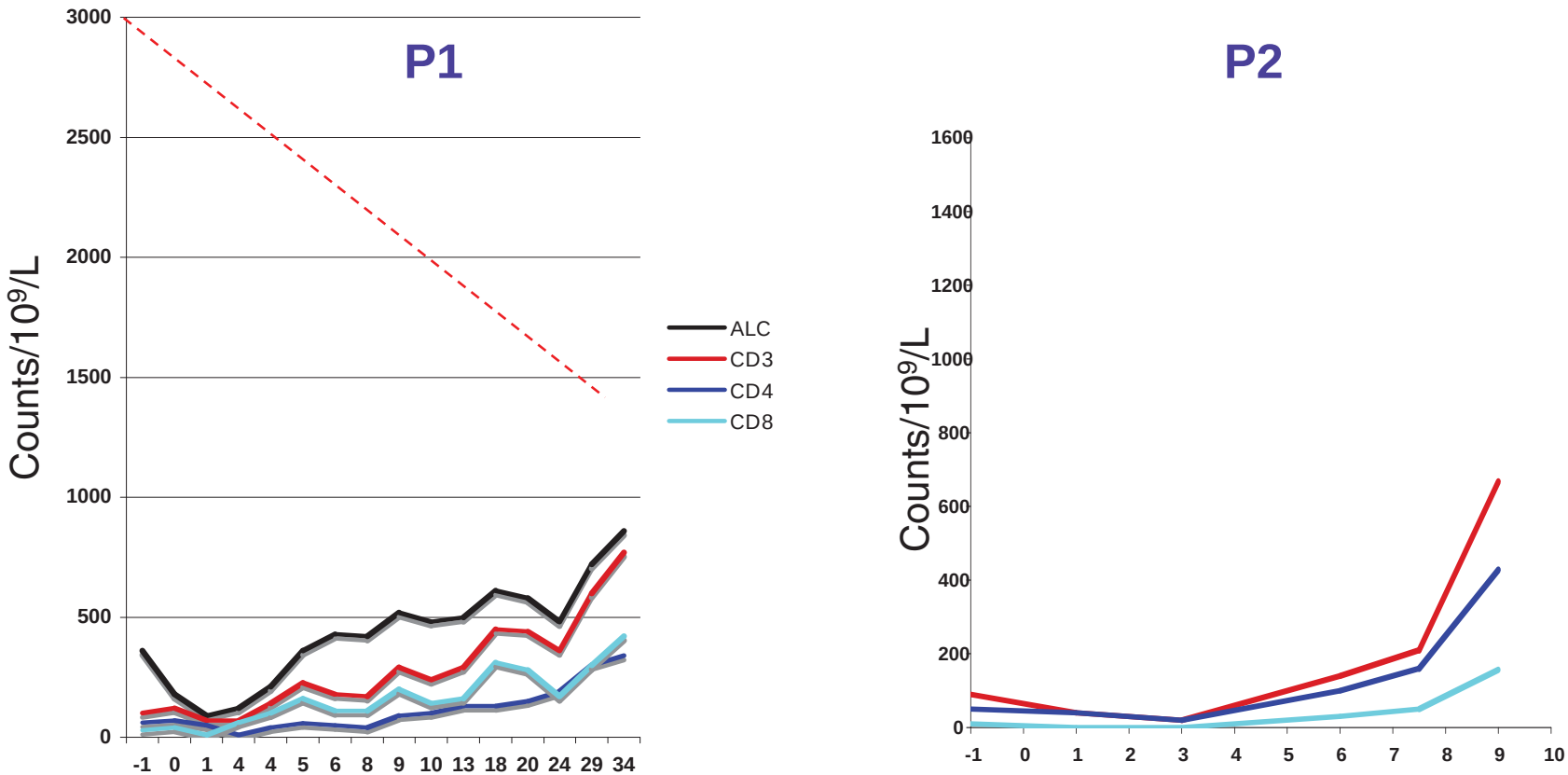
- Stop PEG-ADA 4 wks before treatment, Melphalan (140 mg/m^2) on day -2
- Three rounds of transductions in IL-3, SCF, TPO, Flt3-L after ~2 days of pre-activation
- P1 ~50% gene-marked T and NK cells, 10% B cells, 0.1% myeloid cells

Patient details (March 2007)

	Age at treatment	Mutation	dATP at Dx (umol/l)	PEG-ADA (duration)	Total CD34+ cells (10e6/kg)	% progenitor cell transduction
P1	3yrs 3mths	Q3X	1531	✓ (3yrs)	1.3	~25%
P2	11 mths		1218	✓ (6mths)	5.8	~50%
P3	2yrs 9mths		597	✓ (6mths)	<0.5	~28%

B. Gaspar, personal communication

Lymphocyte recovery post GT



Months post-gene therapy

B. Gaspar, personal communication

UK ADA GT - Summary

Well tolerated protocol
Good levels of gene transduction achieved

Effective medium/long term immune recovery
and metabolic correction in P1 and P2

In P3 – obtaining sufficient cells was difficult
No immune reconstitution 6mths after GT
Restarted PEG-ADA 6mths post GT (as per protocol)

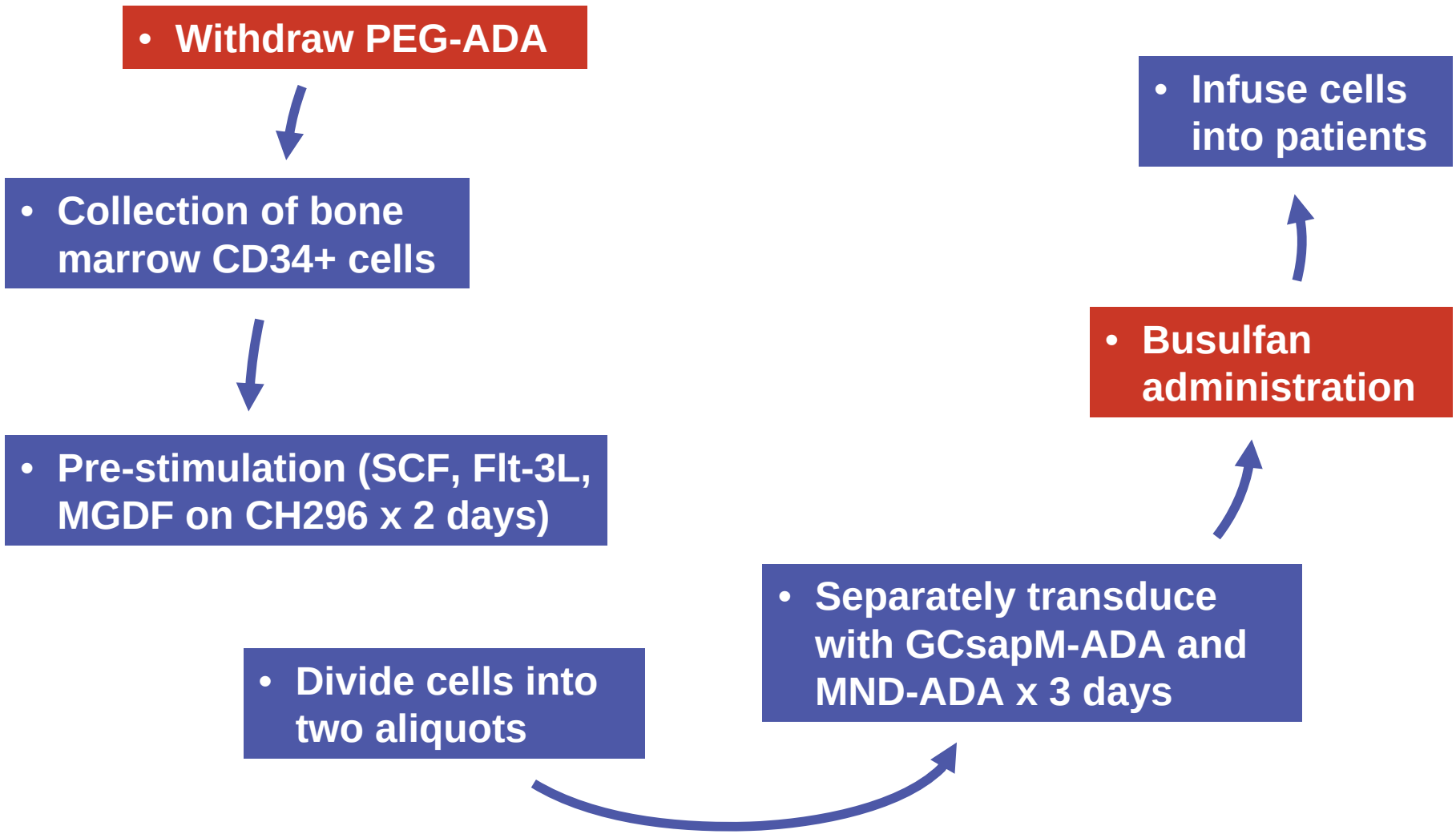
No severe adverse events seen

B. Gaspar, personal communication

ADA Gene Therapy Trials

Target cell	Country	Year	No. of patients
T lymphocytes	USA	1990	2
T lymph (+BM-HSCs)	Italy	1992	6
Cord blood HSCs	USA	1993	3
BM-HSCs	France/UK/The Netherlands	1993	3
T lymphocytes	Japan	1995	1
BM-HSCs	Italy	2001	10
BM-HSCs	USA	2001	4
BM-HSCs	Japan	2003	2
BM-HSCs	UK	2003	3
BM-HSCs	USA	2005	2

Comparison of Retroviral Vectors for Gene Therapy of ADA deficiency: 2005 Clinical Trial Design



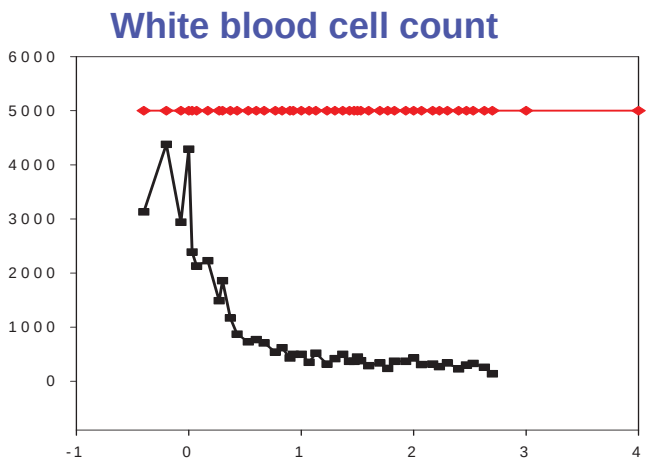
HSC Gene Therapy for ADA deficiency: Summary

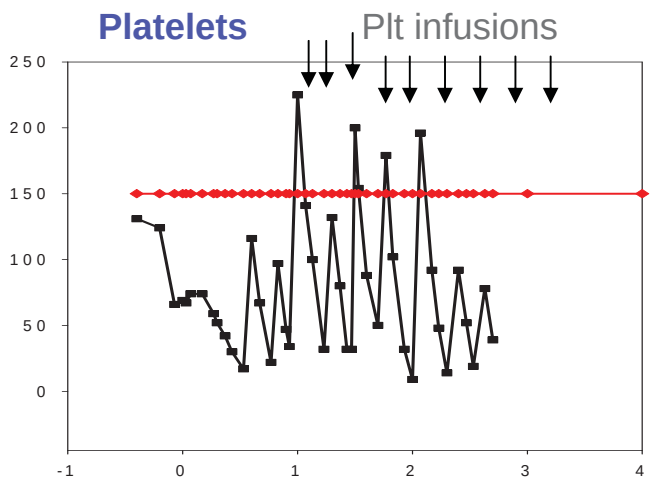
- 29 patients treated
  - 26 infants/children
  - 2 adolescents
  - 1 adult
- 12 patients with gene marking for ≥30 months
- 12 patients showed clinical benefit
- No lymphoproliferative complications

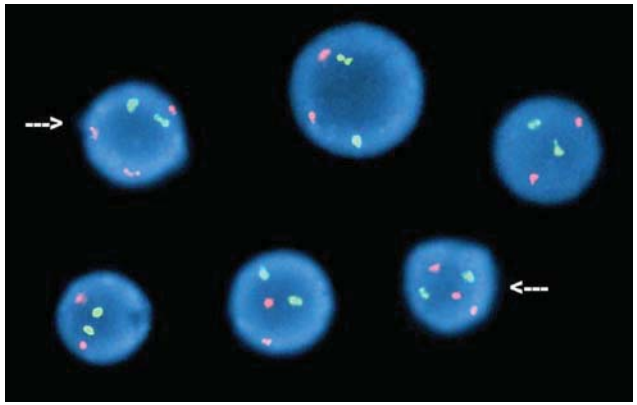
GT for ADA deficiency vs. X-linked SCID

- 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

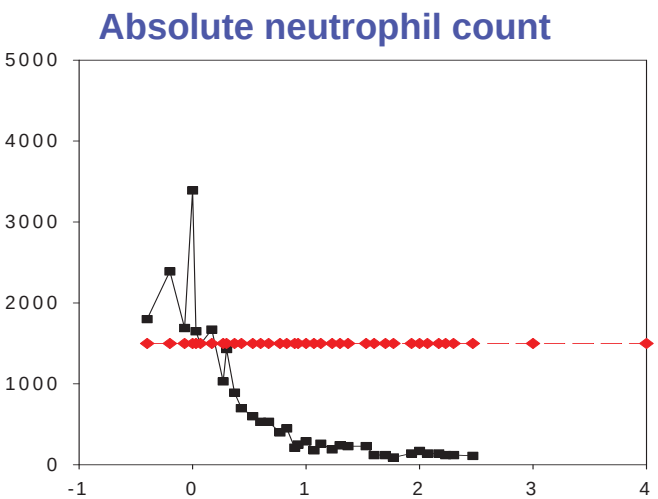
ADA 302C

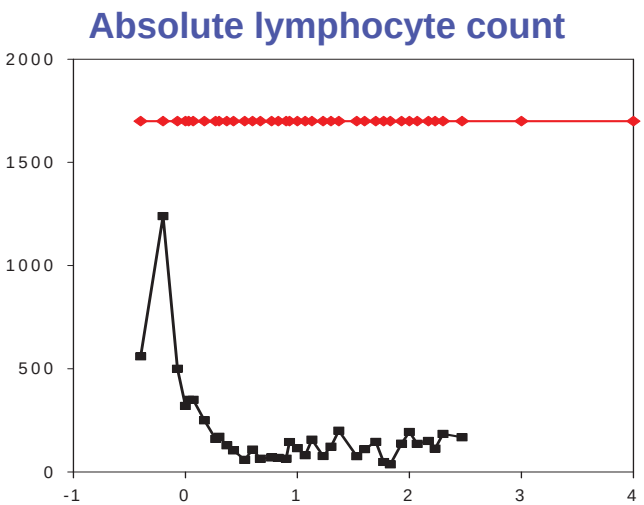


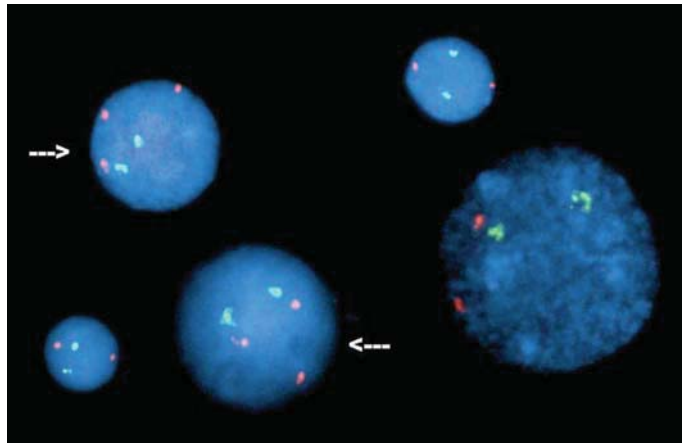




BM pre-GT: ~3% T8 cells







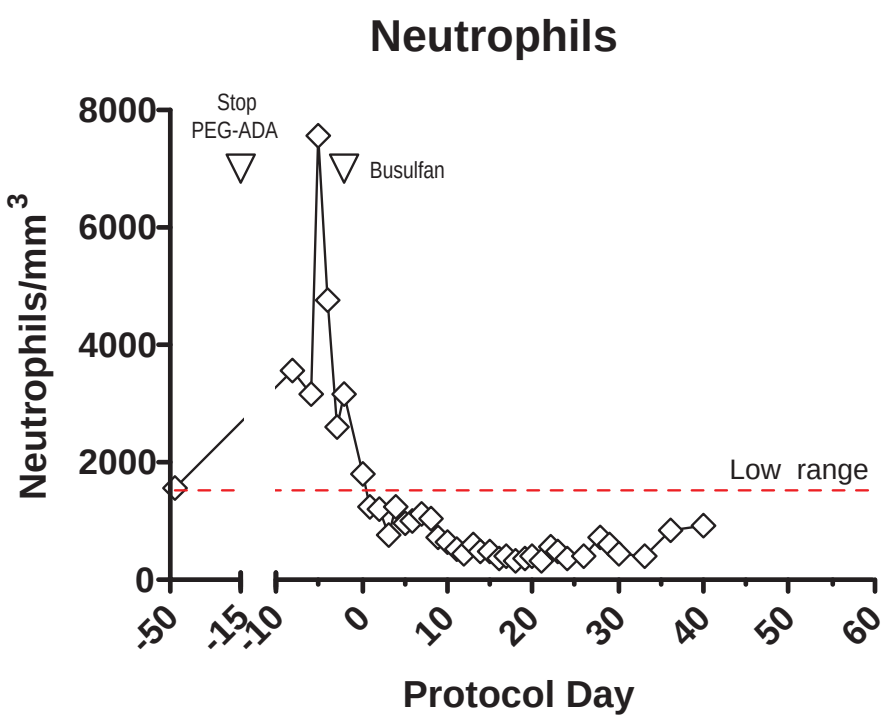
BM 3 mos post-GT: ~20% T8 cells

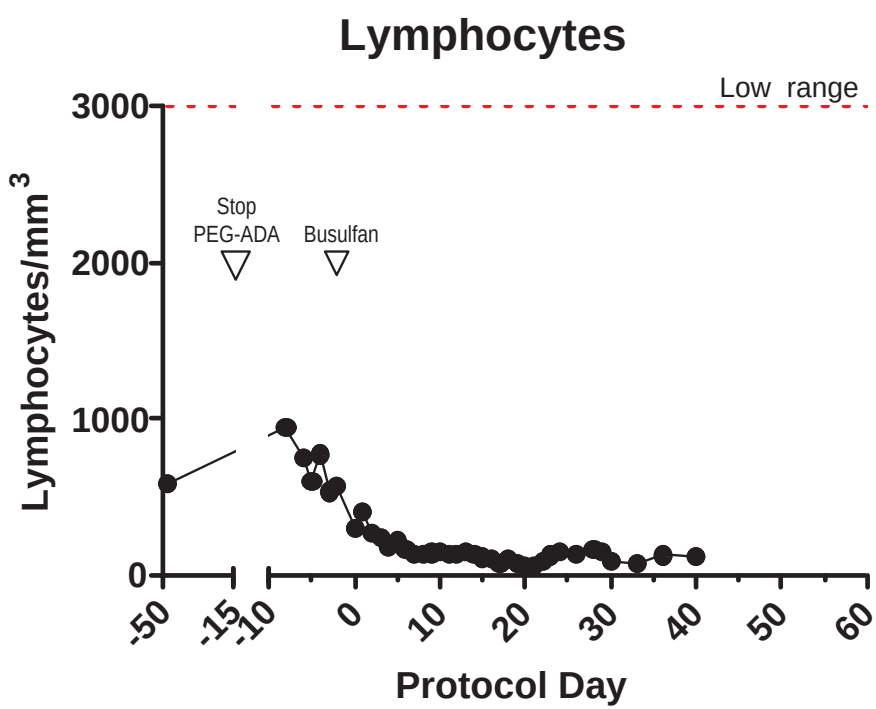
Months post treatment

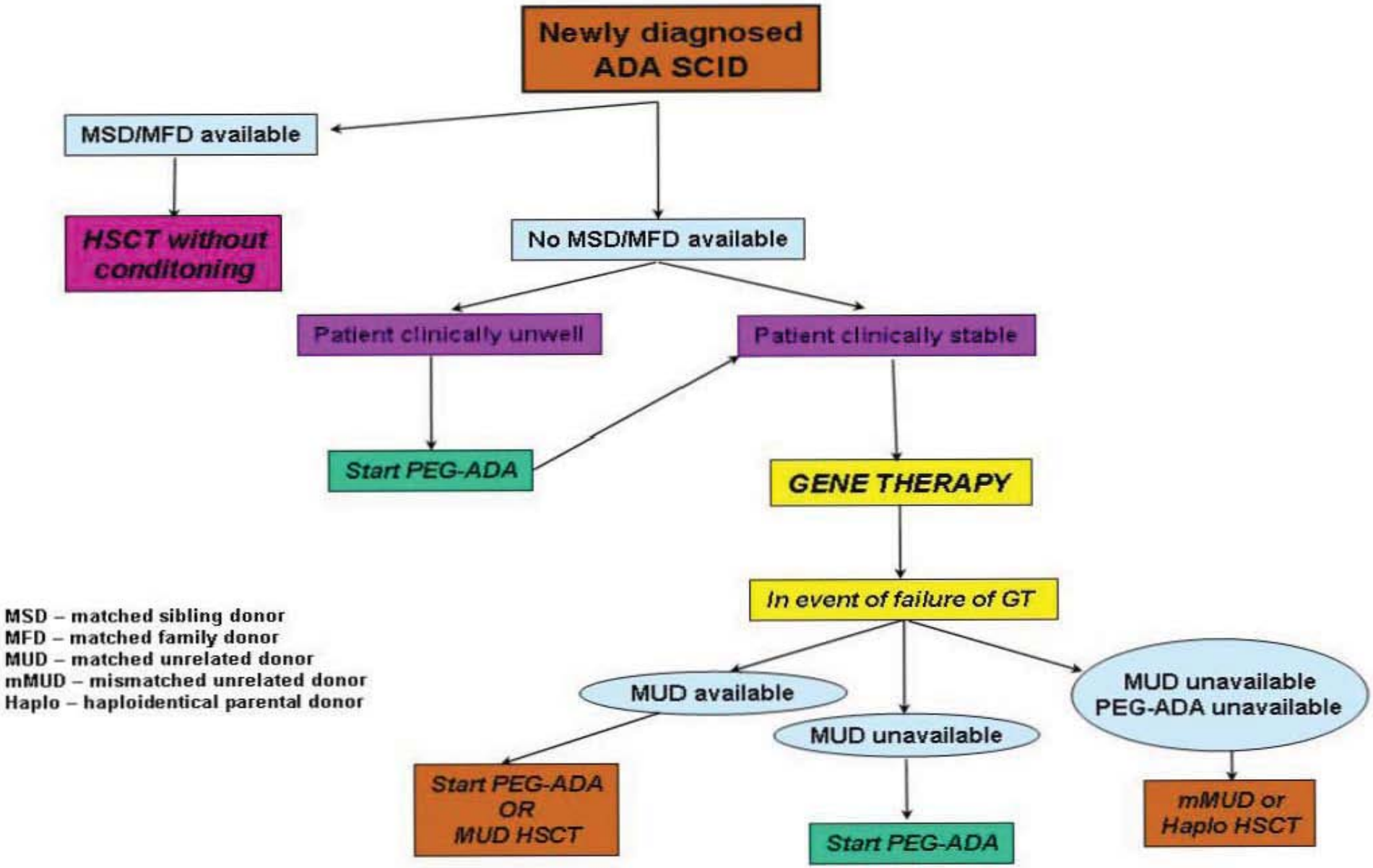
ADA 301N

(1/31/07)

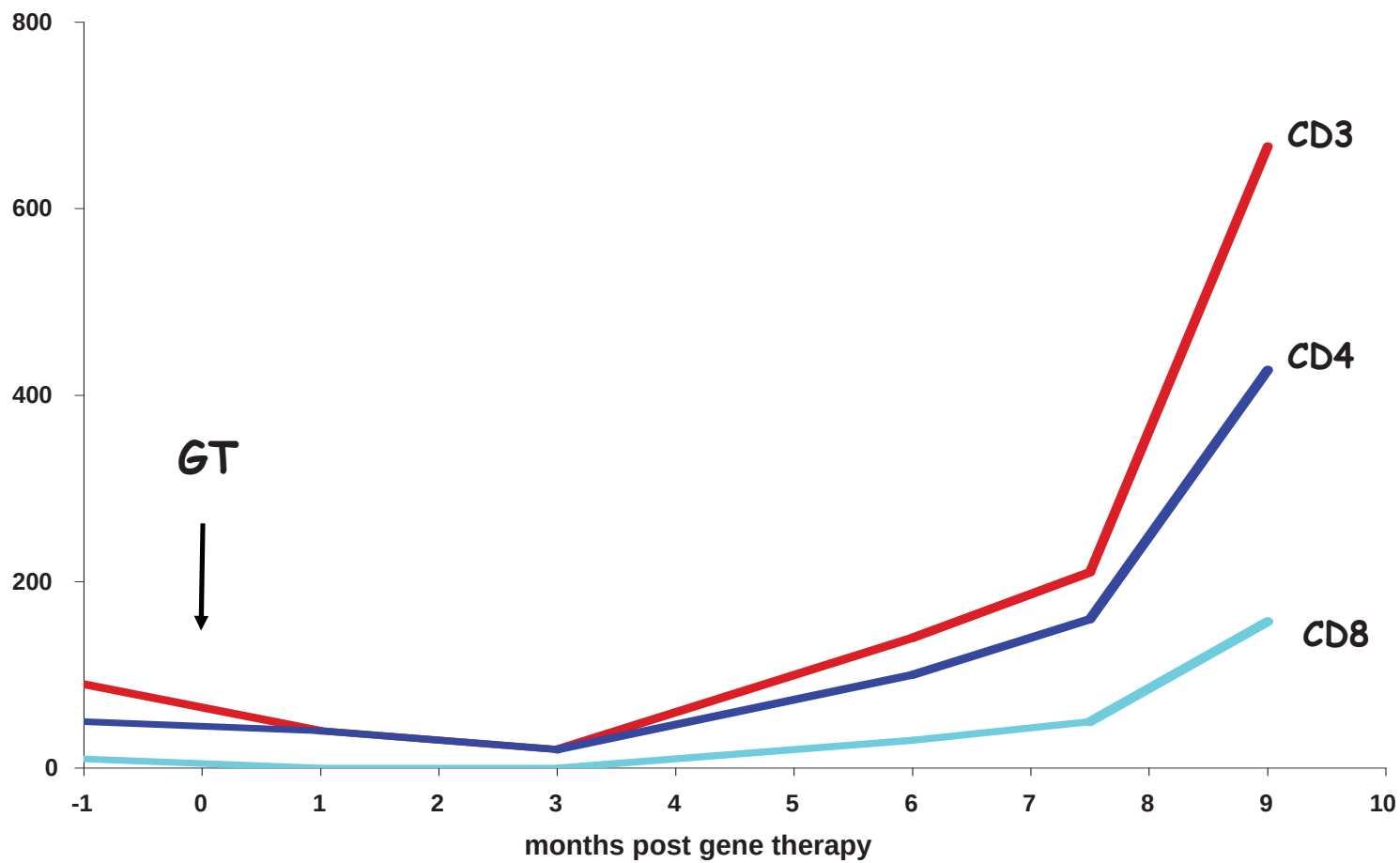
Age	Infused CD34+ cells/kg	ADA activity (U/min)
2	$5x10^6$	40-200






Newly diagnosed ADA SCID
MSD/MFD available
HSCT without conditoning
No MSD/MFD available
Patient clinically unwell
Patient clinically stable
Start PEG-ADA
GENE THERAPY
In event of failure of GT
MUD available
MUD unavailable
MUD unavailable PEG-ADA unavailable
Start PEG-ADA OR MUD HSCT
Start PEG-ADA
mMUD or Haplo HSCT
MSD – matched sibling donor
MFD – matched family donor
MUD – matched unrelated donor
mMUD – mismatched unrelated donor
Haplo – haploidentical parental donor

P2 ADA GT – T cell recovery



B. Gaspar, personal communication

Gene Therapy for ADA deficiency - Protocol Comparison

	US	Italy	UK	Japan
Age	4-20 y	6 m-2.5 y	School-age	4 y, 13 y
PEG-ADA	ON	OFF	OFF	OFF
Conditioning	None	Busulfan	Melphalan	None
Clinical benefits	NO	YES	YES	Partial

Summary Patient Data

				CD34+ Transduction		Vector-positive CFUs	
PIN	Sex	Age (yrs)	Infused $CD34^+$/Kg	MND	M-ADA	MND	M-ADA
201	F	15	0.7×10^6	NA	11%	NA	14%
202	F	5	12×10^6	40%	66%	35%	62%
203	F	20	1.3×10^6	1.4%	0.8%	ND	ND
204	M	4	2×10^6	48%	25%	27%	13%

Gene Therapy for ADA deficiency: Role of Concomitant PEG-ADA Treatment

T lymphocytes with a normal ADA gene accumulate after transplantation of transduced autologous umbilical cord blood CD34+ cells in ADA-deficient SCID neonates

Donald B. Kohn[1], Michael S. Hershfield[2], Denise Carbonaro[1], Ann Shigeoka[3], Judith Brooks[1], E. Monika Smogorzewska[1], Lora W. Barsky[1], Raymond Chan[1], Felix Burotto[1], Geralyn Annett[1], Jan A. Nolta[1], Gay Crooks[1], Neena Kapoor[1], Melissa Elder[4], Diane Wara[4], Thomas Bowen[5], Edward Madsen[6], Floyd F. Snyder[7], John Bastian[8], Linda Muul[9], R. Michael Blaese[9], Kenneth Weinberg[1] & Robertson Parkman[1]

NATURE MEDICINE • VOLUME 4 • NUMBER 7 • JULY 1998 775

Immune reconstitution in ADA-SCID after PBL gene therapy and discontinuation of enzyme replacement

Alessandro Aiuti[1], Sergio Vai[1], Alessandra Mortellaro[1], Giulia Casorati[2], Francesca Ficara[1], Grazia Andolfi[1], Giuliana Ferrari[1], Antonella Tabucchi[3], Filippo Carlucci[3], Hans D. Ochs[4], Luigi D. Notarangelo[5], Maria Grazia Roncarolo[1] & Claudio Bordignon[1,6]

NATURE MEDICINE • VOLUME 8 • NUMBER 5 • MAY 2002 423

T-cell lines from 2 patients with adenosine deaminase (ADA) deficiency showed the restoration of ADA activity resulted from the reversion of an inherited mutation

Tadashi Ariga, Noriko Oda, Koji Yamaguchi, Nobuaki Kawamura, Hideaki Kikuta, Shoichiro Taniuchi, Yohnosuke Kobayashi, Kihei Terada, Hisami Ikeda, Michael S. Hershfield, Kunihiko Kobayashi, and Yukio Sakiyama

Blood. 2001;97:2896-2899

Adenosine deaminase deficiency with mosaicism for a "second-site suppressor" of a splicing mutation: decline in revertant T lymphocytes during enzyme replacement therapy

Francisco X. Arredondo-Vega, Ines Santisteban, Eva Richard, Pawan Bali, Majed Koleilat, Michael Loubser, Abdulaziz Al-Ghonaium, Mariam Al-Helali, and Michael S. Hershfield

Blood. 2002;99:1005-1013

Stem Cell Gene Therapy for ADA-SCID: Results of Three Clinical Trials

	Kohn et al.	Bordignon et al.	Valerio et al.
	3 Pts.	2 Pts.	3 Pts.
Transduction efficiency	PBMC: 1% T cells: 1-10% PMN: 0.01-0.03% BM cfu: 4-6%	PBL: 5-18% BM cfu: 0.8-8.5%	PBL: 0.001% BM cells: 0.001%
ADA expression	30-100U	200-500U	---
Immunity	↑ T cells ↑ blastigenesis	↑ T cells ↑ blastigenesis ↑ Ab production	No improvement
PEG-ADA	40-60U/kg/wk	12-15U/kg/wk	60U/kg/wk

Rationale for Future Directions

Preparative chemotherapy may be needed

- Evaluate risk/benefit of myeloablation

Gene therapy in the absence of PEG-ADA is likely to result in higher levels of marking

- Enroll patients off PEG-ADA

Stem Cells Gene Therapy for ADA-deficiency in the presence of PEG-ADA: The Lessons

Low levels of gene corrected cells

(Nat Med 1995, 1:1017; Science 1995, 270:470; Gene Ther 1996, 3:179)

Low ADA expression

(Nat Med 1998, 4:775)

Stem Cells Gene Therapy for ADA-deficiency in the presence of PEG-ADA: The Lessons

Low levels of gene corrected cells

(Nat Med 1995, 1:1017; Science 1995, 270:470; Gene Ther 1996, 3:179)

Improve gene transfer conditions

- ***SCF, Flt-3L, MGDF***
- ***Recombinant Fibronectin***
- ***Gibbon-Ape Leukemia Virus envelope***

Stem Cells Gene Therapy for ADA-deficiency in the presence of PEG-ADA: The Lessons

Low ADA expression

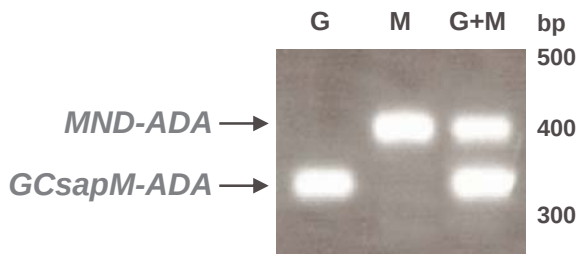
(Nat Med 1998, 4:775)

Improve ADA expression from retroviral vectors

- ***GCsapM-ADA***
- ***MND-ADA***

New ADA Vectors

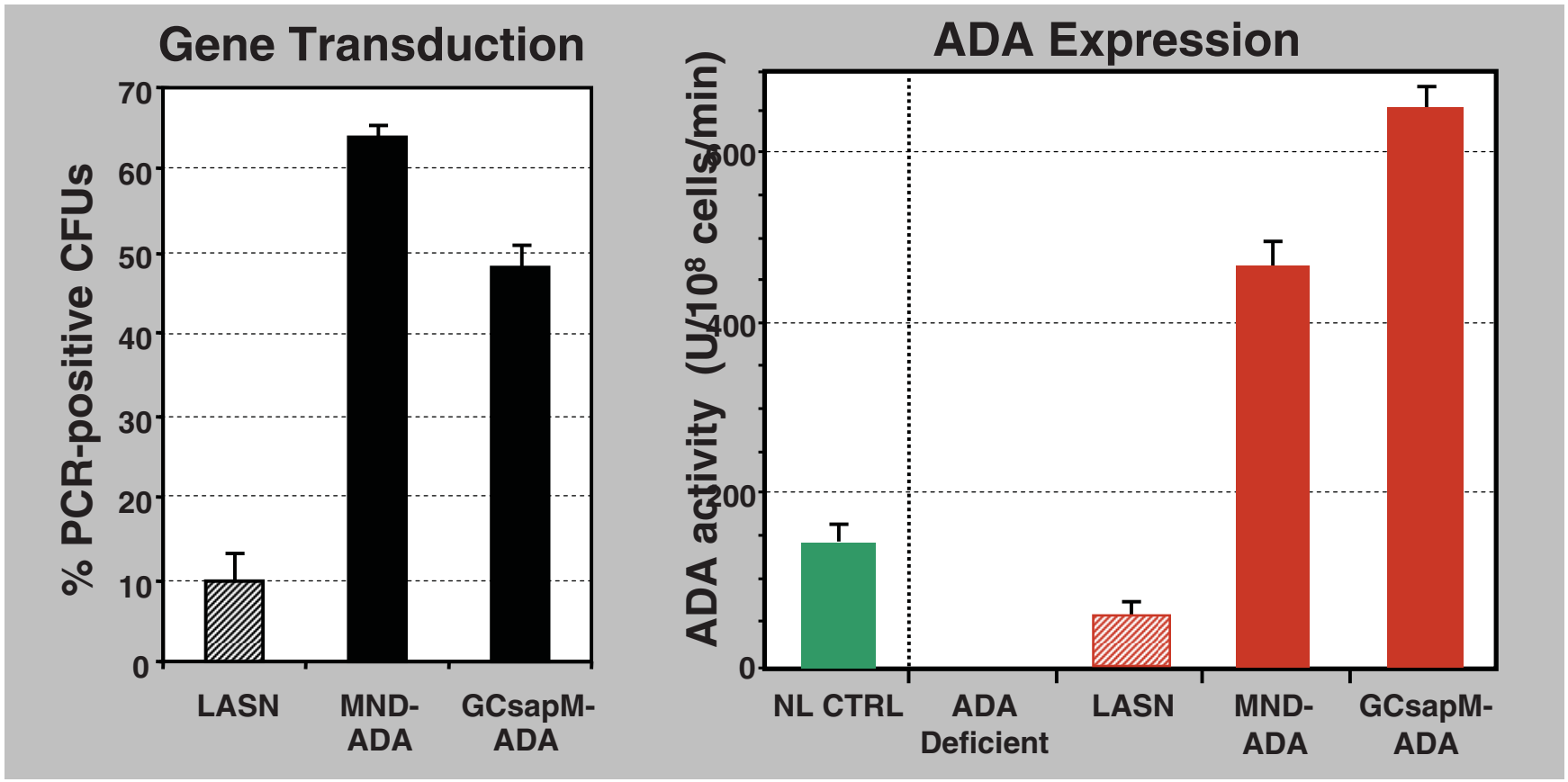
GCsapM-ADA



MND-ADA

MND: “M”=MPSV; “N”=ncr deleted; “D”=dl587rev PBS

Preclinical Testing of New ADA Vectors and Transduction Conditions



Comparison of Retroviral Vectors for Gene Therapy of ADA deficiency: 2001 Clinical Trial Design

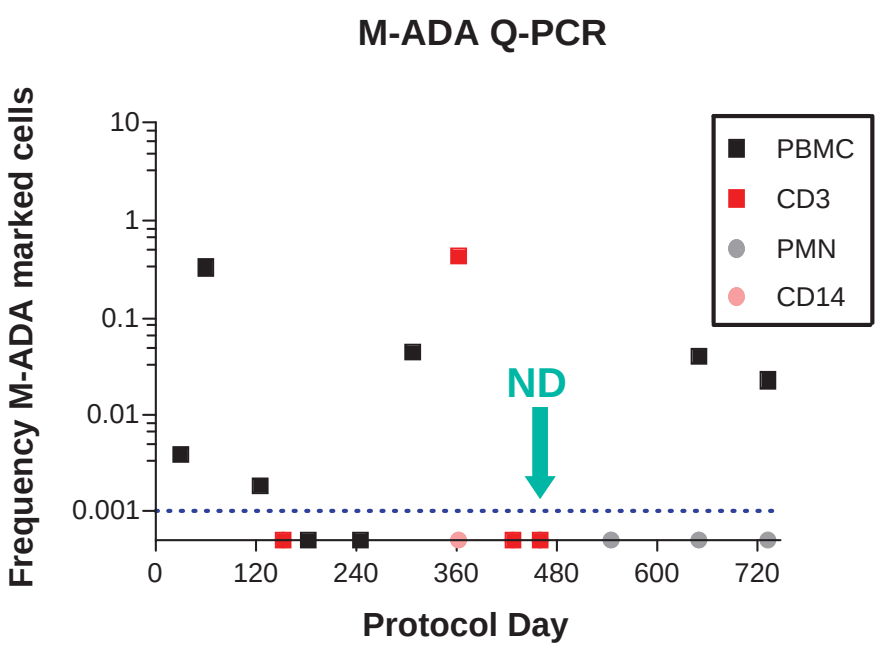
- Collection of cord blood or bone marrow CD34+ cells
- Pre-stimulation (SCF, Flt-3L, MGDF on CH296 x 2 days)
- Divide cells into two aliquots
- Separately transduce with GCsapM-ADA and MND-ADA x 3 days
- Infuse into patients (continue PEG-ADA)

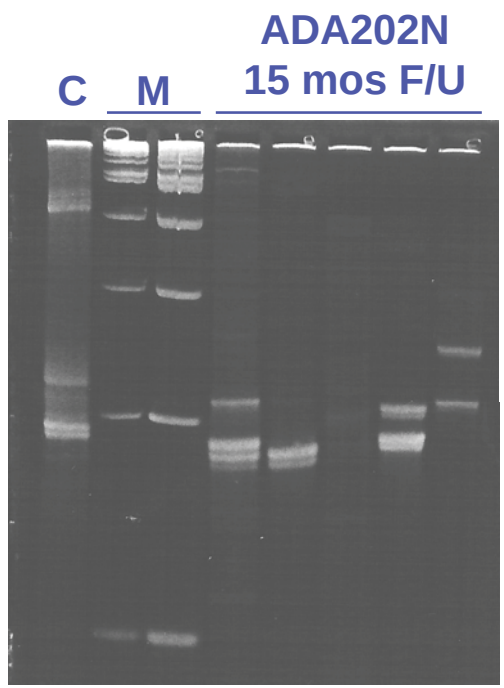
Monitoring of retroviral oncogenesis

If retroviral marking ≥ 1%, LAM-PCR analysis of clonality

- q6 months for first 5 years
- q12 months for the next 10 years

Clonal analysis of PBMCs and determination of integration sites



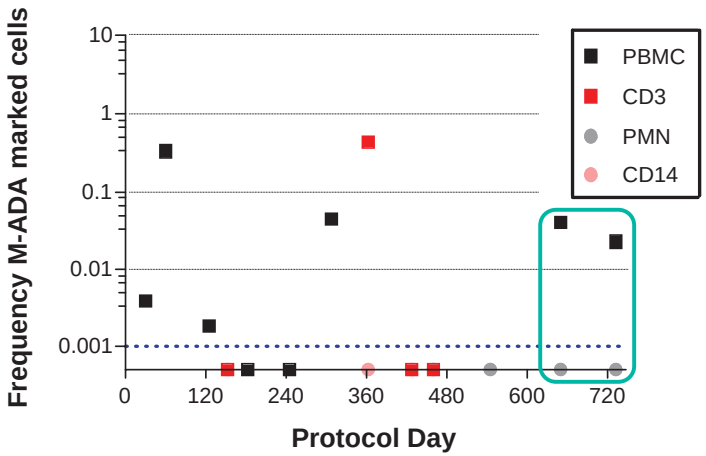


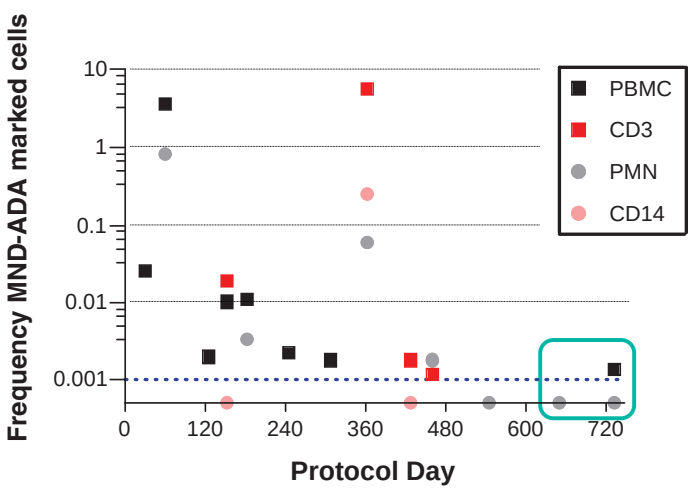
10 bands sequenced

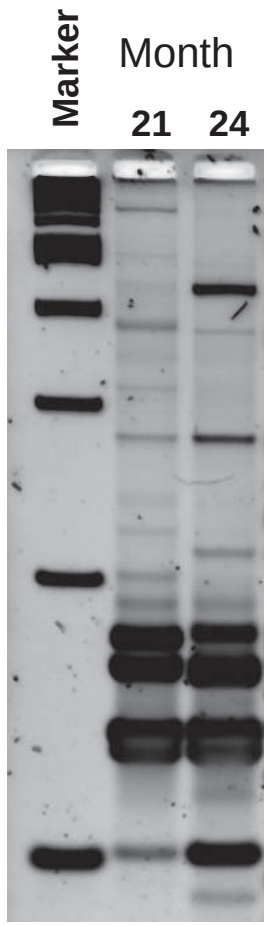
1 unique hit: intron 5 of SCHIP1 (schwannomin interacting protein 1)

6 no significant homology

Pt. 202N: Clonal analysis of PBMCs and determination of integration sites







9 bands sequenced

5 unique hits: SCHIP1, POLR2C, no gene (2p11.2, 2p25.1, 2q13)

4 no significant homology

Retroviral Integration: Challenging the Dogma

Cell, Vol. 110, 521–529, August 23, 2002, Copyright ©2002 by Cell Press

HIV-1 Integration in the Human Genome Favors Active Genes and Local Hotspots

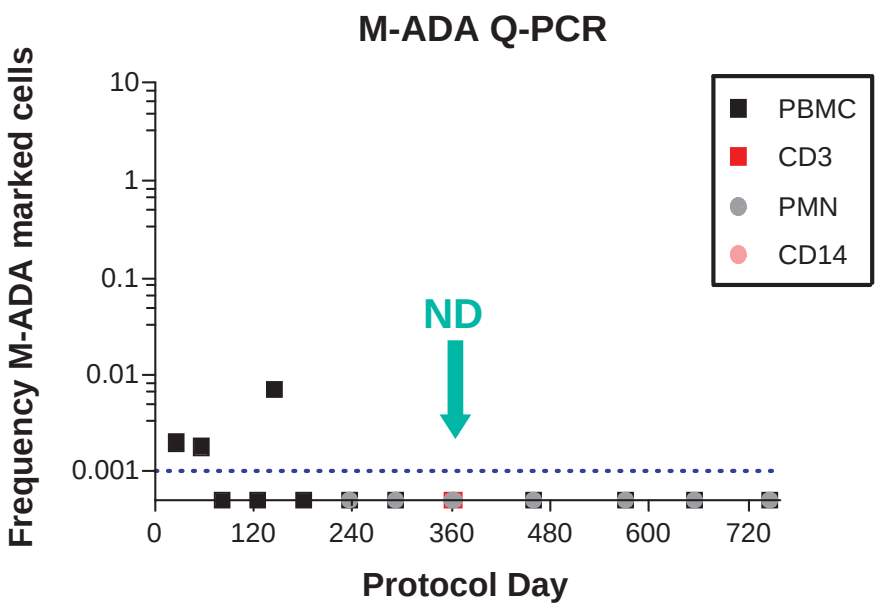
Astrid R.W. Schröder,[1] Paul Shinn,[2] Huaming Chen,[2] Charles Berry,[3] Joseph R. Ecker,[2] and Frederic Bushman[1,4]

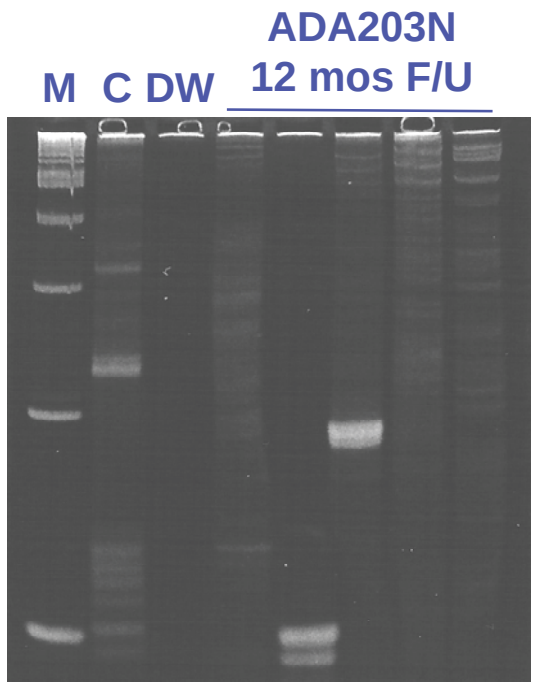
Transcription Start Regions in the Human Genome Are Favored Targets for MLV Integration

Xiaolin Wu,[1] Yuan Li,[2] Bruce Crise,[2] Shawn M. Burgess[1]*

SCIENCE VOL 300 13 JUNE 2003

Clonal analysis of PBMCs and determination of integration sites





5 bands sequenced

1 intron 2 of DACH (Drosophila dachsund homolog)
1 chromosome 19, no specific gene

3 no significant homology