Treatment of Subjects With Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID) with Autologous Bone Marrow CD34+ Stem/Progenitor Cells after Addition of a Normal Human ADA cDNA by the EFS-ADA Lentiviral Vector

Donald B. Kohn, M.D.	UCLA
Fabio Candotti, M.D.	NHGRI
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Prior Trials
EFS-ADA Vector
Proposed Trial
Responses to RAC

Adenosine Deaminase (ADA)-Deficient SCID

ADA-deficiency causes ~15% of human SCID.

It was the first genetic form of human SCID where the biochemical and genetic bases were determined.

ADA-deficient SCID patients have profound pan-lymphopenia due to lymphotoxicity from accumulated adenine metabolites.

Results of HSCT, other than from matched sibling, have been poorer than for other genetic forms of SCID.

ADA enzyme replacement therapy (PEG-ADA) can sustain clinically-protective immunity. However, immune function is sub-normal and ADA-GEN is expensive ($200-400,000 /pt /yr).

CHLA and NIH 2nd Clinical Trial of Gene Therapy of ADA-SCID (1999)

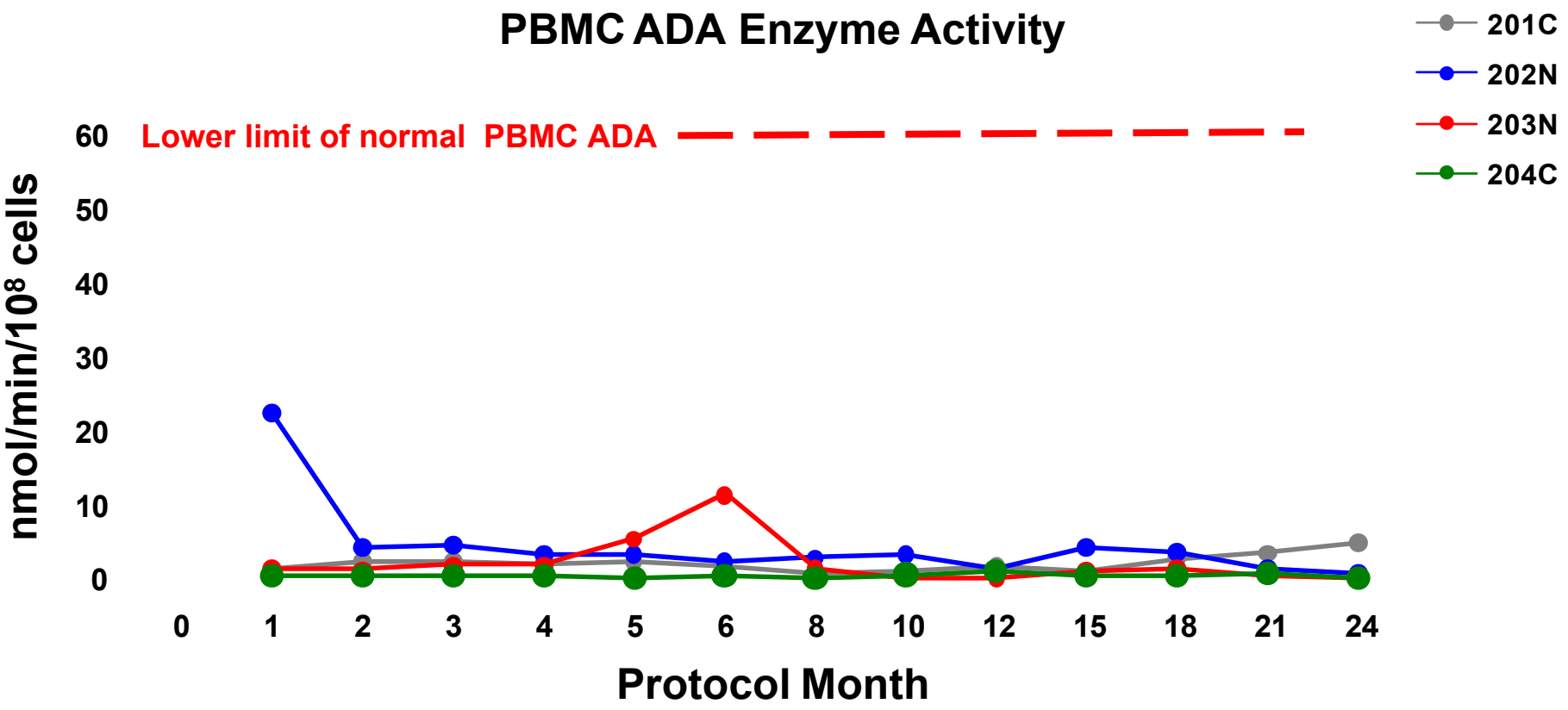
1. Phase I/II study, testing safety (and efficacy).
2. n=10 ADA-deficient SCID subjects.
3. CD34+ cells from UCBC (neonates) or BM (children).
4. Ex vivo transduction with two ADA retroviral vectors using c*kit* ligand, flt-3 ligand and TPO on rFBN CH-296.
5. PEG-ADA may be withdrawn if gene marking in T cells >1/1,000 at 1 year.
6. Two year active phase follow-up.

Clinical trial performance sites:

Childrens Hospital Los Angeles – Clinical BMT unit

The Clinical Center of the National Institutes of Health

The CHLA/NIH Study – Subjects Treated in 2001-2002



Results of initial gene therapy trial:

1. Gene therapy strategies appear safe
2. No significant clinical benefit

Clinical Trial of ADA Gene Therapy
Hospital San Raffaele, Milan Italy
Aiuti et al *Science* 2002.

Reported initial results for two ADA-deficient SCID infants treated by retroviral-mediated ADA gene transfer to bone marrow CD34+ cells.

- given non-myeloablative dose of busulfan prior to transplant to increase engraftment.
- did not receive PEG-ADA ERT to not blunt selective advantage for survival of ADA-corrected lymphocytes.

Immune restoration occurred, with increase numbers and function of T and B cells. Patients remained well.

Reported extended follow-up in 10 subjects (NEJM 1/09).

CHLA / NIH Clinical Trial of ADA Gene Therapy

Amendment to the CHLA/NIH trial for ADA-SCID (protocol and consent) developed in summer of 2002 [a la Milan] to withhold PEG-ADA and give busulfan.

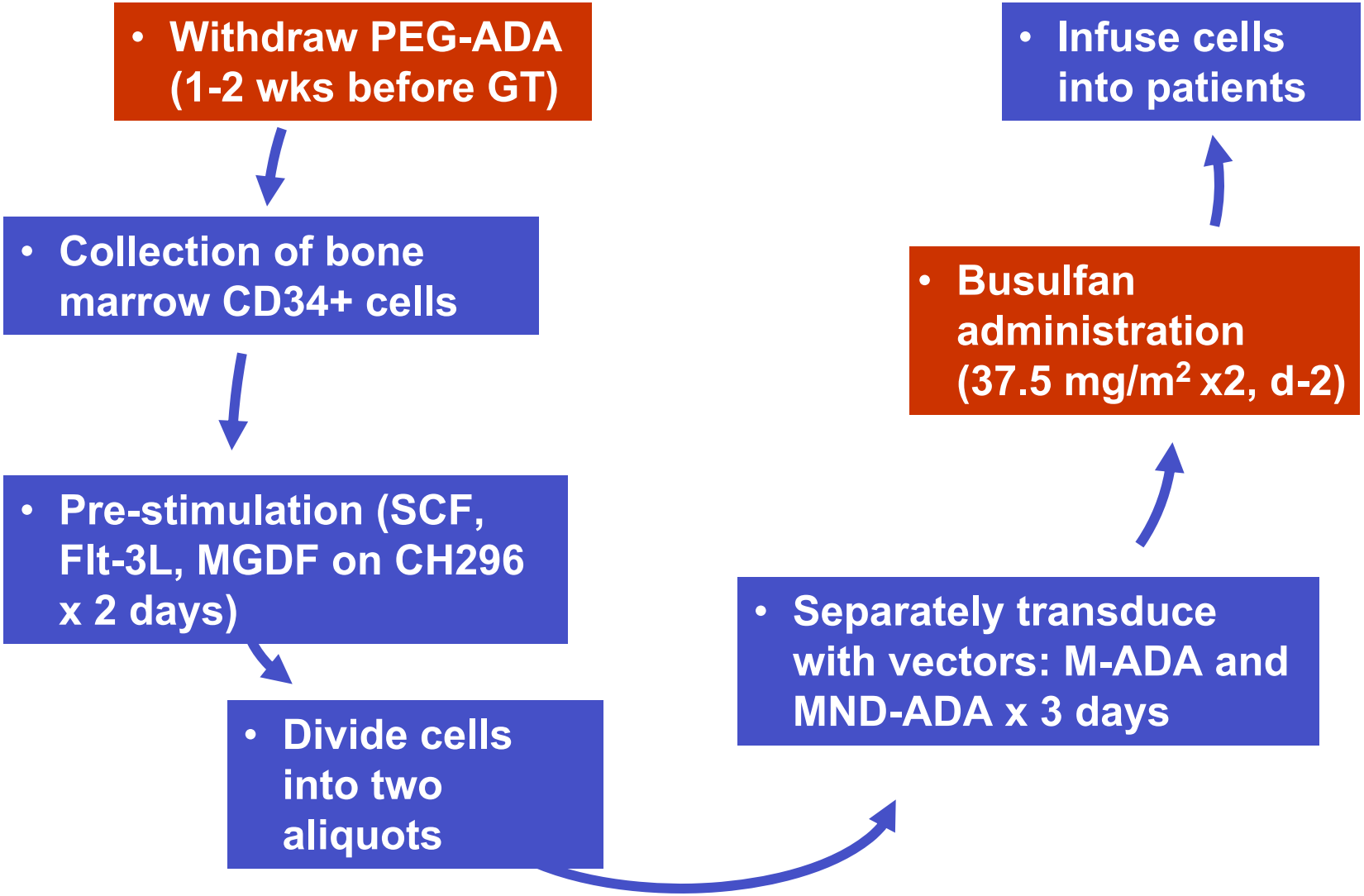
Then, reports from XSCID trials with SAE of T LPD.

On "clinical hold" or under regulatory review of amendment from September 2002 until June 2005. (2.5 years).

Protocol amended raising lower age from neonate to 6 m/o.

Trial (+Bu/-PEG) re-opened to accrual 7/05.

2005 Clinical Trial Design

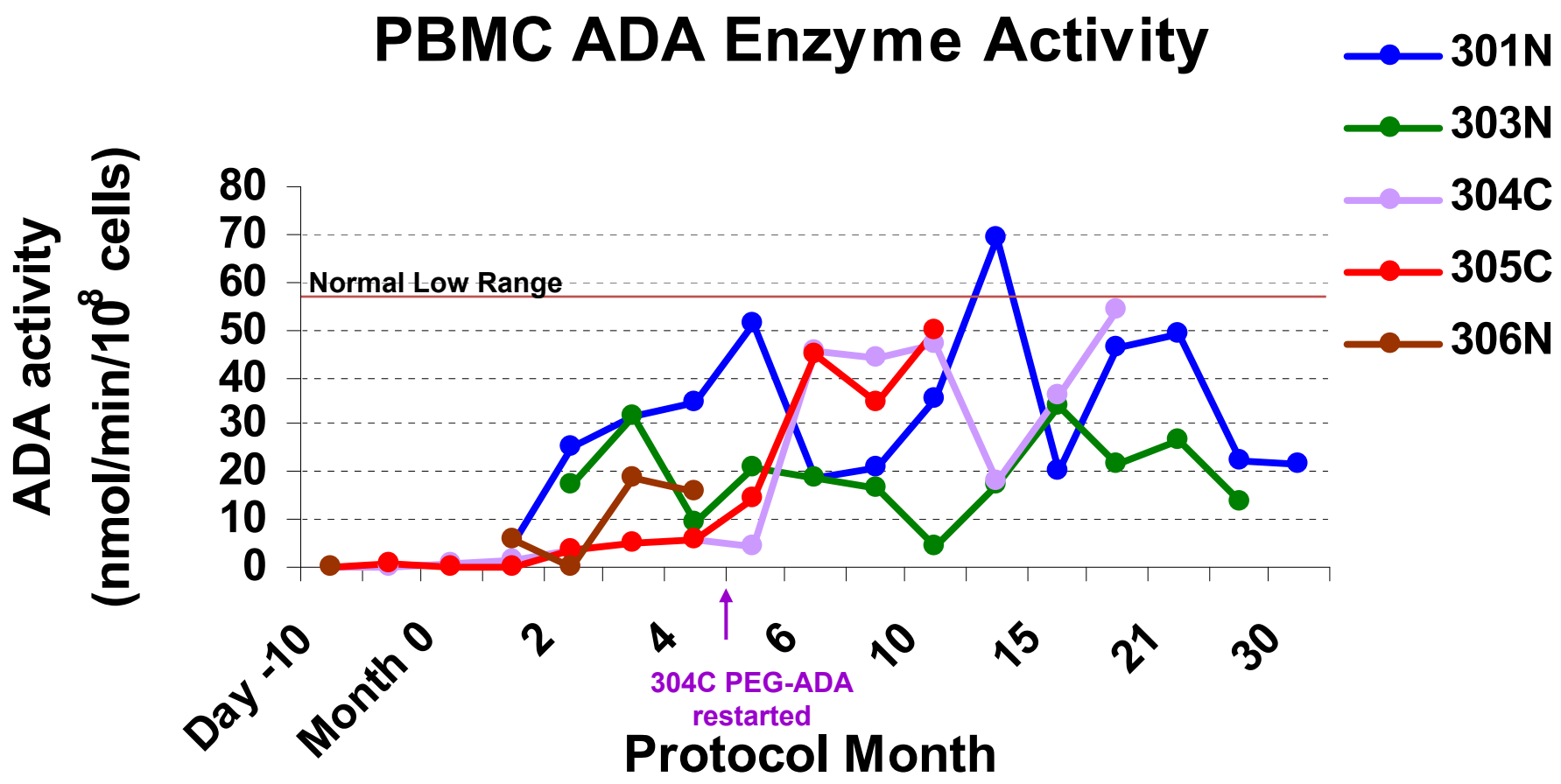


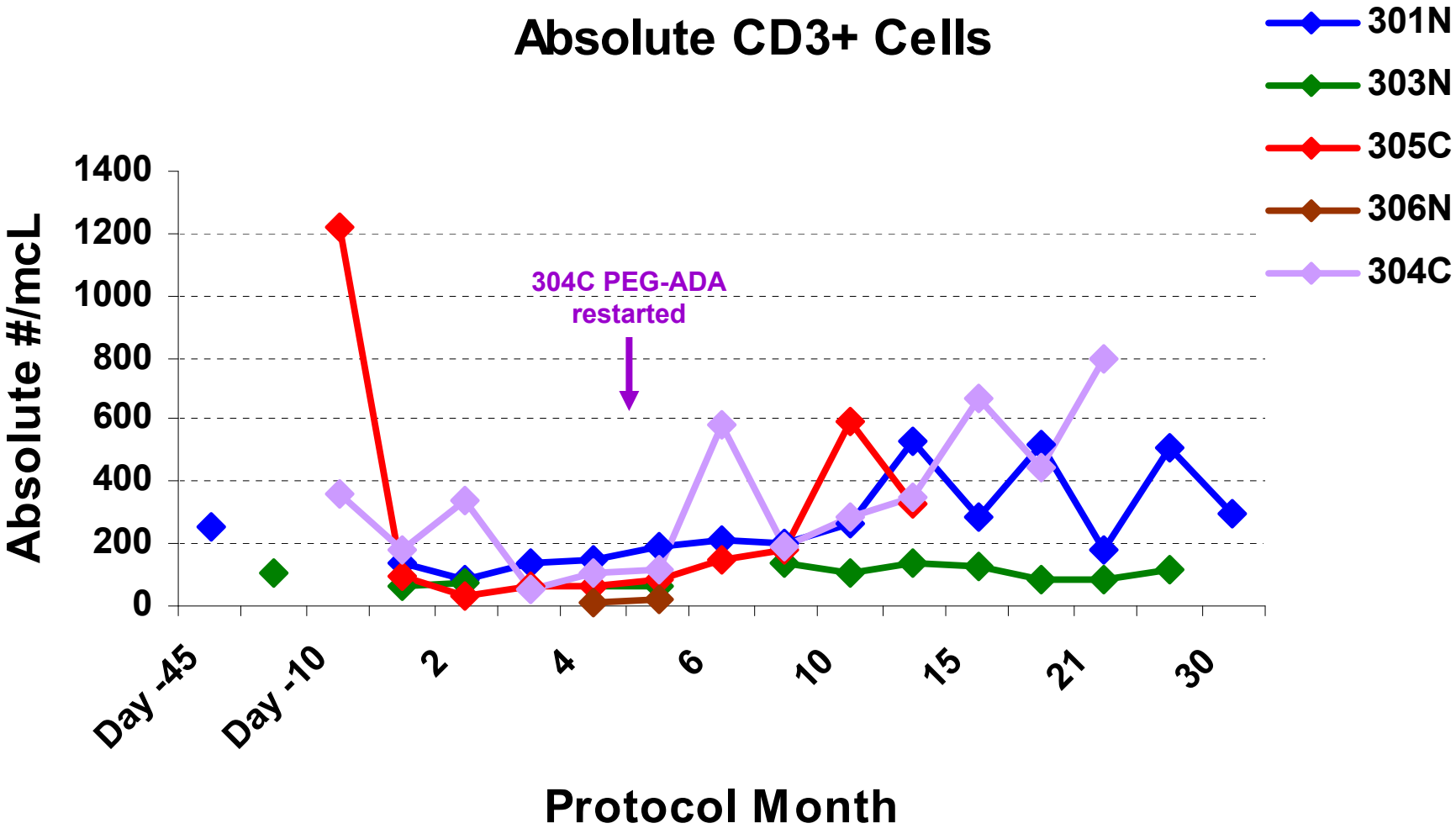
Subjects Enrolled

Subject ID	Age at Entry	Sex	PEG-ADA ERT	Busulfan (mg/m^2)	Cell Dose (CD34+/kg)	Length of Follow-up
301N	2 y	M	withdrawn	75	4.77×10^6	35 mo
302C	2.8 y	F	withdrawn/ MUD BMT	75	1.5×10^5	N/A*
303N	15 mo	F	withdrawn	75	1.9×10^6	31 mo
304C	9 y	F	withdrawn/ restarted**	64.6	1.6×10^6	25 mo
305C	20 mo	M	withdrawn	90	9.75×10^6	14 mo
306N	22 mo	M	withdrawn/ restarted**	90	9.5×10^6	7 mo

* 302C had trisomy 8 mosaicism and failed gene therapy (Engel, BC *et al.* Blood, 2007)

** restarted at 4-6 months post-infusion of gene-modified cells


PBMC ADA Enzyme Activity
ADA activity
(nmol/min/10^8 cells)
80
70
60
50
40
30
20
10
0
Normal Low Range
Day -10
Month 0
2
4
6
10
15
21
30
304C PEG-ADA
restarted
Protocol Month
301N
303N
304C
305C
306N


Absolute CD3+ Cells
301N
303N
305C
306N
304C
Absolute #/mcL
1400
1200
1000
800
600
400
200
0
304C PEG-ADA
restarted
Day -45
Day -10
2
4
6
10
15
21
30
Protocol Month

CHLA/NHGRI Trial of Gene Therapy for ADA-Deficient SCID - Phase I Trial

Outcome:

4 - no conditioning/on PEG-ADA (2001-2002):
low long-term marking in 2 (4 and 5 y/o);
short-term only in 2 (15, 20 y/o).

6 - with conditioning/off PEG-ADA (2006-2009):
3- immune reconstitution.
Off PEG-ADA @ 35, 31, 14 mo.
1- T8 mosaic→MUD BMT. Well.
2- Infxn @ 4 months→ PEG-ADA resumed.

UCLA/NHGRI Trial of Gene Therapy for ADA-Deficient SCID - Phase II Trial

Type of Vector: murine γ-RV (GALV): MND-ADA

Conditioning: busulfan 90 mg/m2 (~4 mg/kg).

Inclusion: no matched sibling donor.

Exclusion: Poor organ function. Active infection.
PB cytogenetic abnormalities.

End-points: 1° -safety.
2° -gene marking, immune reconstitution

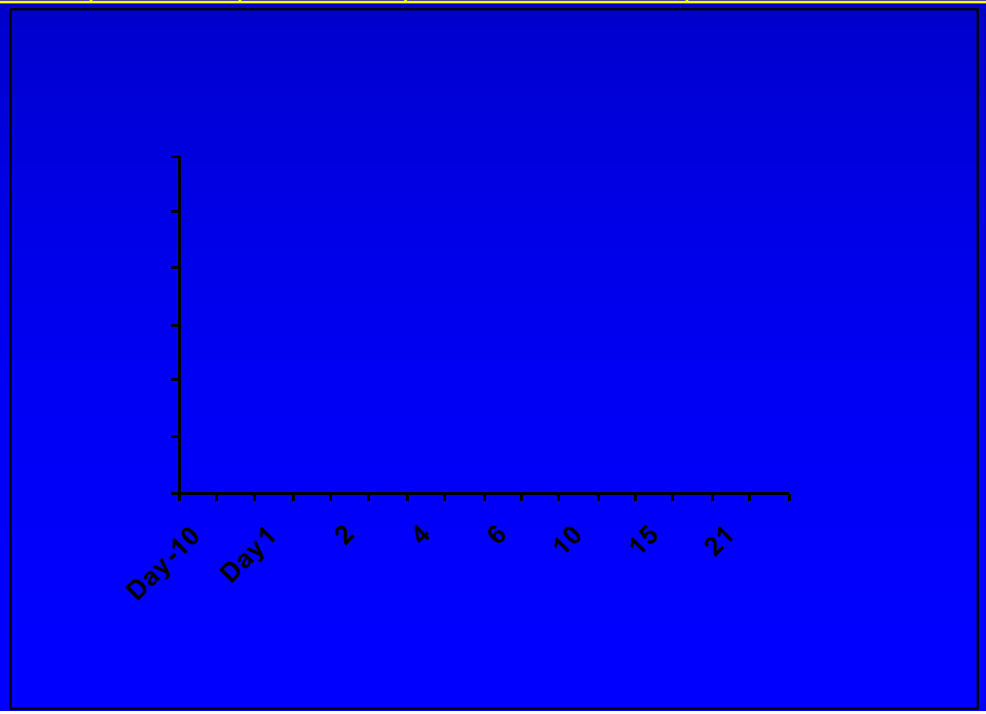
Open: **UCLA- March 2009; NHGRI- Oct 2009**

ClinicalTrials.Gov # NCT00794508

UCLA/NHGRI Trial of Gene Therapy for ADA-Deficient SCID - Phase II Trial

Subjects Enrolled

UPN	Age at Entry	Sex	ADA ERT	Busulfan (mg/m^2)	Cell Dose (CD34+/kg)	Length of Follow-up
401U	15 yr	M	Off	90	0.6×10^6	5 mo
402U	4 mo	F	Off	90	1.6×10^6	3 mo
403N	8 yr	M	Off	90	1.4×10^6	0.5 mo



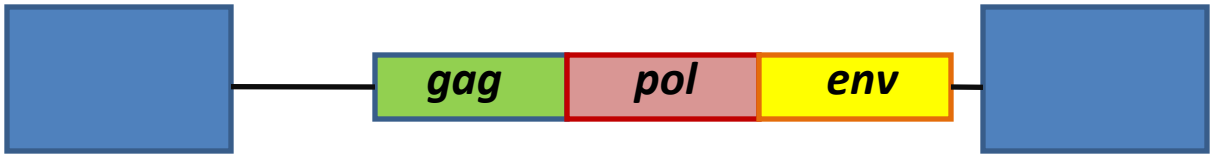
Summary of ADA-Deficient SCID Patients Retroviral Vectors–Milan/London/CHLA-NIH

Center	No. Patients	F/U (yrs)	Off Enzyme	Survival	DFS*
Milan	10	1.8 – 8.0	8/10	100%	80%
London	6	0.5 – 5.5	3/6	100%	50%
CHLA-NIH UCLA-NIH	6 3	0.5– 3 0.1-0.5	3/6 3/3	100% 100%	50% n.e.
TOTAL	**25**	**0.1 – 8.0**	**17/25**	**100%**	**67%**

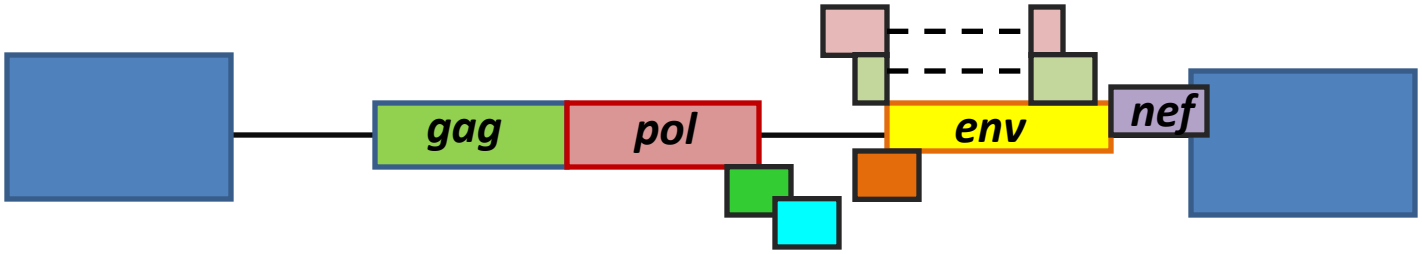
*DFS ≡ Alive without BMT or PEG-ADA re-start

The Retroviridae

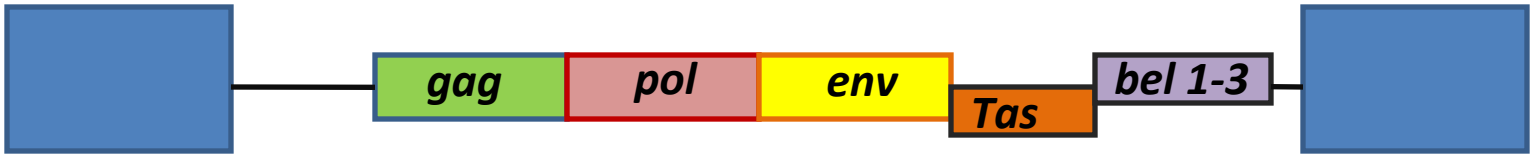
γ-Retrovirus



Lentivirus



Spumavirus (Foamy)



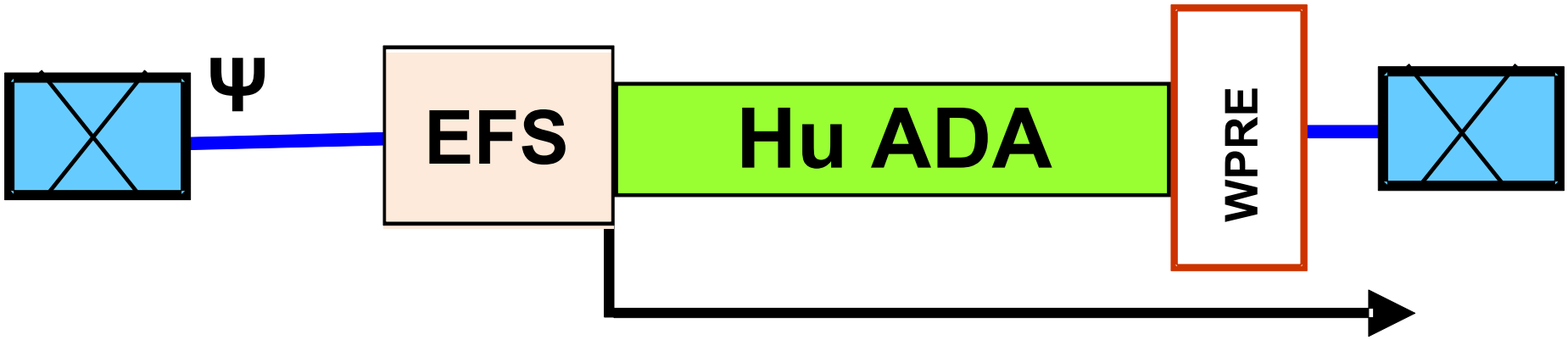
Lentiviral Vectors May Be Superior to MLV-Based γ-Retroviral Vectors

LV can transduce human CD34+ cells with shorter culture time than needed for RV (1-2d vs. 3-5d), and may transduce human HSC more effectively, leading to increased %HSC transduced/engrafted.

LV can carry larger inserts intact than RV, which may allow more sophisticated expression.

Integration site biases of LV may have lower risks for causing insertional oncogenesis than γ-RV.

The EFS-ADA Lentiviral Vector



SIN Lentiviral Vector (pCCL)
Human Elongation Factor1-α Promoter (EFS)
Human ADA cDNA (codon-optimized)
WPRE (ORF removed)
VSV-G pseudotype.
Titer: 0.5-1.0x 10^10 TU/ml (after ~1000X concentration)

The Genotoxic Potential Of Retroviral Vectors Is Strongly Modulated By Vector Design And Integration Site Selection In A Mouse Model Of HSC Gene Therapy

Montini…Naldini J Clin Invest. 2009 April 1; 119(4): 964–975.

Compared retroviral and lentiviral vectors for potential to accelerate lymphomagenesis in cdkn2a -/- mouse (p53 and Rb defects).

Vectors had retroviral LTR or cellular (PGK) promoters.

Conclusions

LTR were the major determinants of oncogenicity.

RV were slightly more genotoxic than LV (~3-10X). May reflect RV bias for integration near promoters, growth control/cancer genes.

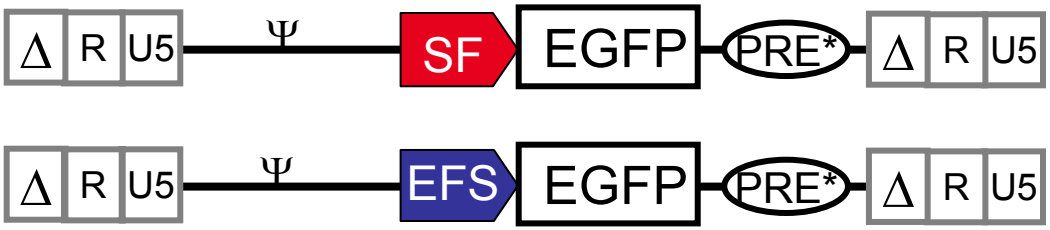
→Safest vector was SIN LV with moderately active internal promoter.

Physiological Promoters Reduce the Genotoxic Risk of Integrating Gene Vectors

Zychlinski F,…, Baum C. *Molecular Therapy* **16** 718–725, 2008

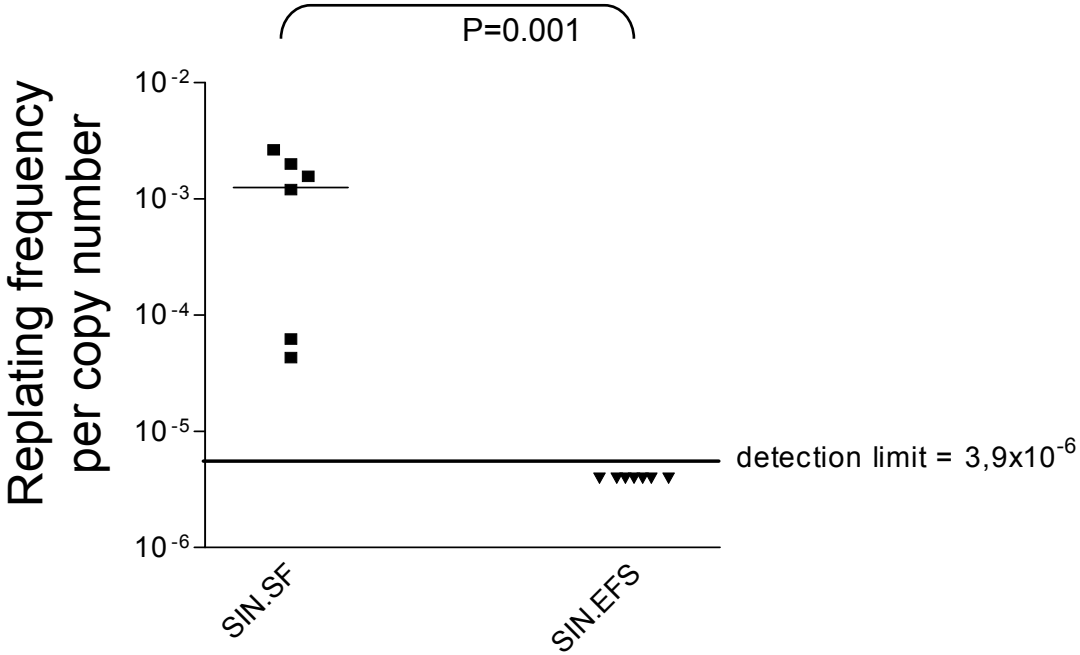
RV LTR promoter Sin.**SF**

Cellular promoter Sin11.**EFS**.P



EFS = human EF1-alpha gene promoter, lacking intron and enhancers

In Vitro "Clonal Dominance Assay"



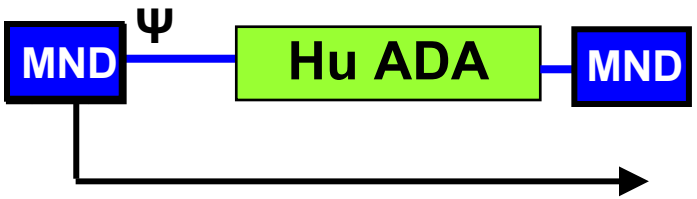
Conclusions:

- The EFS promoter showed low enhancer activity in reporter assays based on transient transfection or stable integration.

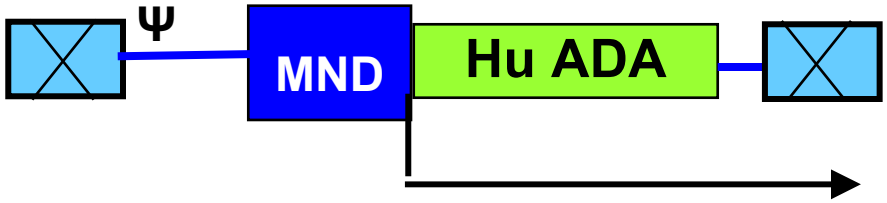
-SIN vectors containing EFS did not transform cells in the clonal dominance assay and did not activate Evi1 in mass cultures.

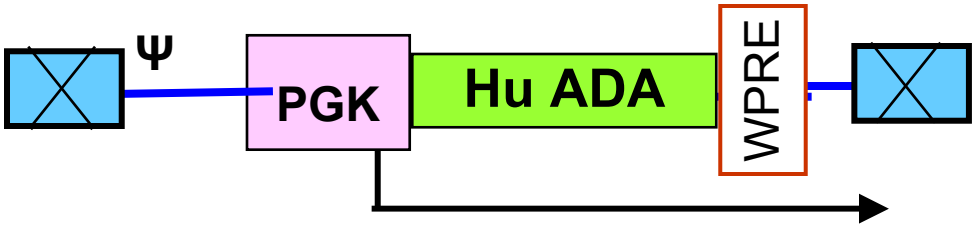
ADA cDNA Retroviral and Lentiviral Vectors

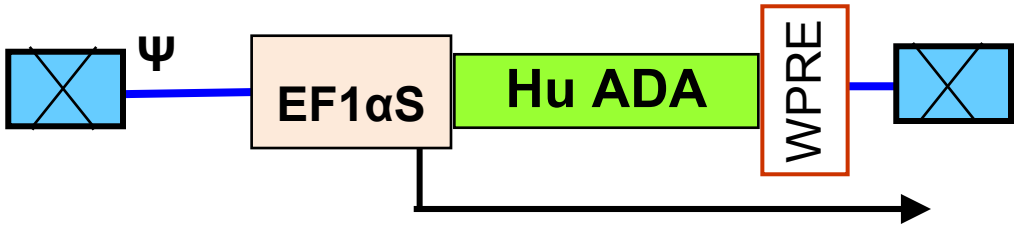
Retroviral Vector:



Lentiviral Vectors

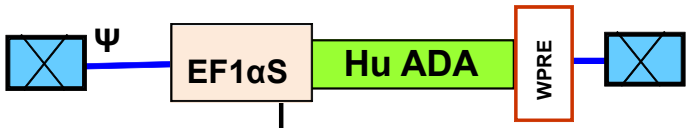




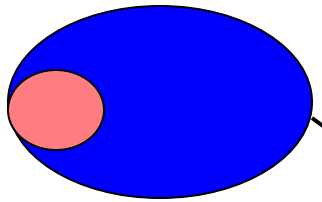


Transduction and ADA Expression by Human CD34+ Cells in Short-term Culture

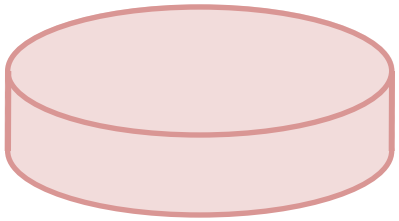
The EF1αS-ADA Lentiviral Vector



LV: pre-stim O/N. Hit x 1d.
RV: pre-stim 2day. Hit x 3d.



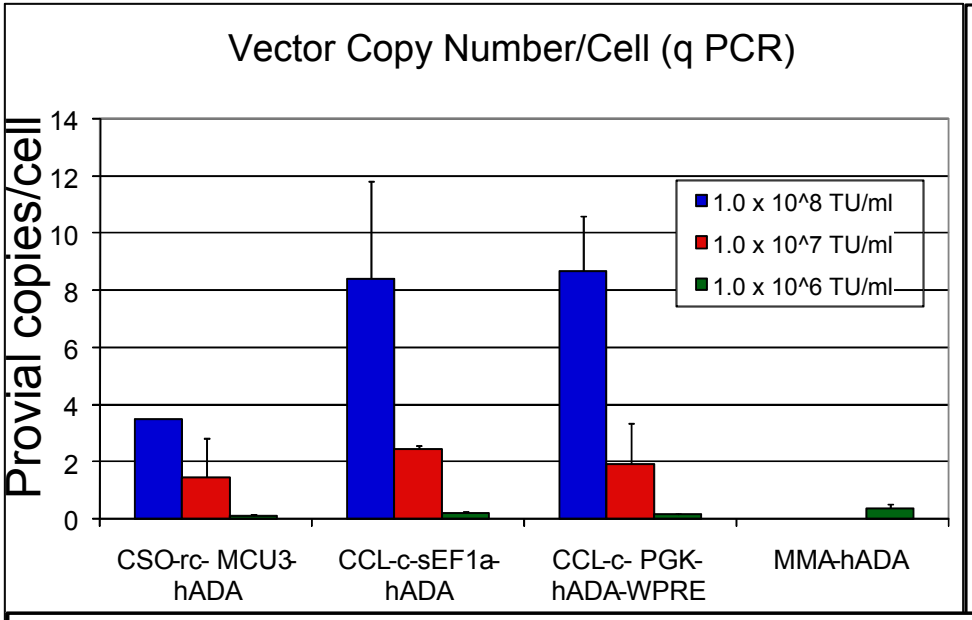
Human CD34+ HSC

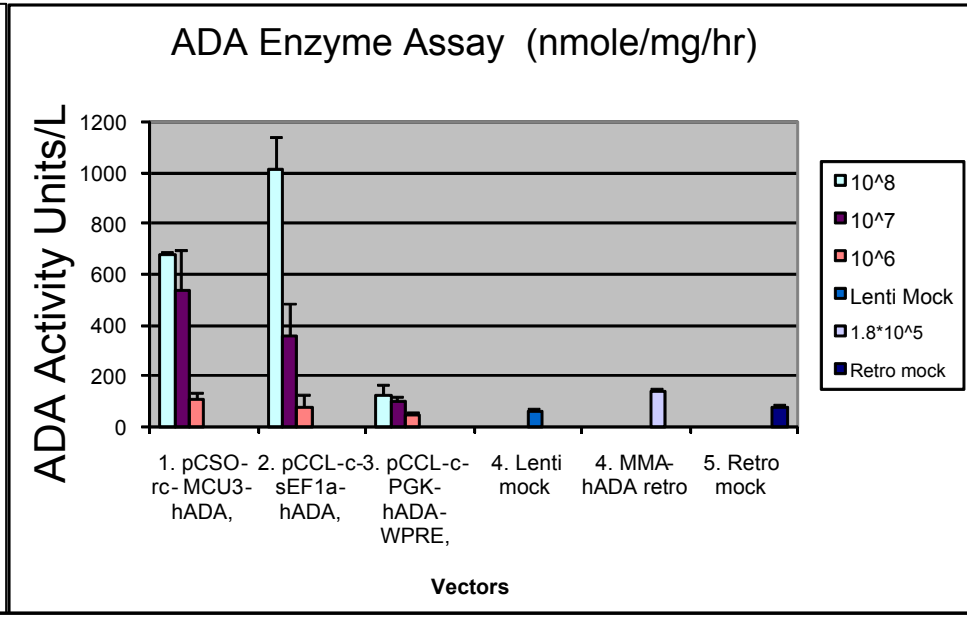


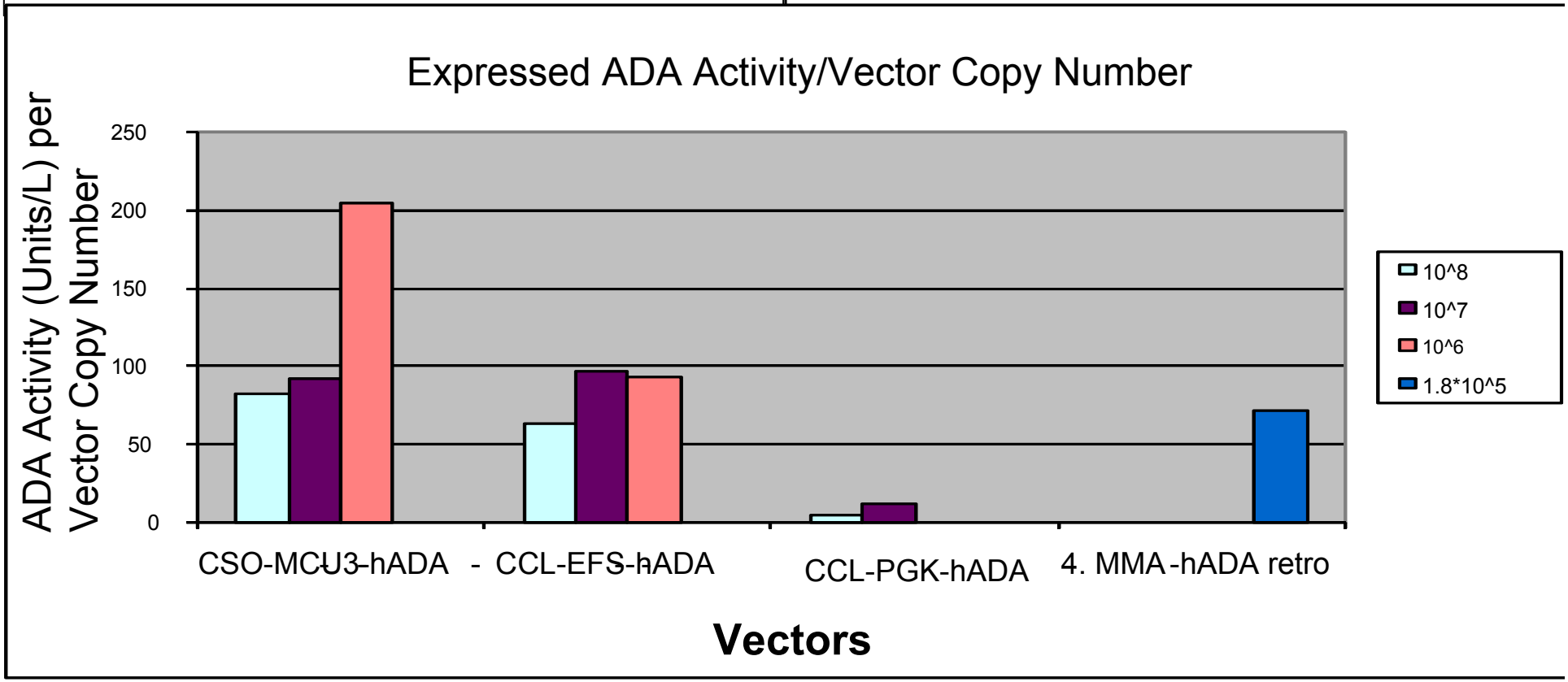
Short-term
In vitro
Culture
(2-3 weeks)

<u>Analyze:</u>
Mixed myeloid cells

<u>Measure:</u>
Vector Copy/Cell
ADA Enzyme Activity
ADA/VC

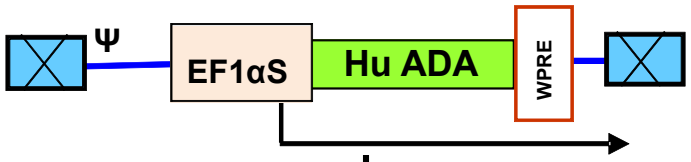

Vector Copy Number/Cell (q PCR)
Provial copies/cell
14
12
10
8
6
4
2
0
1.0 x 10^8 TU/ml
1.0 x 10^7 TU/ml
1.0 x 10^6 TU/ml
CSO-rc- MCU3-hADA
CCL-c-sEF1a-hADA
CCL-c- PGK-hADA-WPRE
MMA-hADA

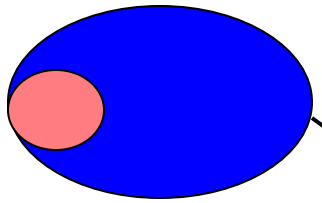

ADA Enzyme Assay (nmole/mg/hr)
ADA Activity Units/L
1200
1000
800
600
400
200
0
10^8
10^7
10^6
Lenti Mock
1.8*10^5
Retro mock
1. pCSO-rc-MCU3-hADA,
2. pCCL-c-sEF1a-hADA,
3. pCCL-c-PGK-hADA-WPRE,
4. Lenti mock
4. MMA-hADA retro
5. Retro mock
Vectors


Expressed ADA Activity/Vector Copy Number
ADA Activity (Units/L) per Vector Copy Number
250
200
150
100
50
0
10^8
10^7
10^6
1.8*10^5
CSO-MCU3-hADA
CCL-EFS-hADA
CCL-PGK-hADA
4. MMA-hADA retro
Vectors

Transduction and ADA Expression by Human CD34+ Cells in Human T Cells

The EF1αS-ADA Lentiviral Vector





Human CD34+ HSC

NOD/SCID/γc$^{-/-}$ (NSG)
Neonate (150 cGy)

<u>Analyze:</u>
Thymocytes
PBL
Splenocytes

<u>Measure:</u>
Vector Copy/Cell
ADA Enzyme Activity
ADA/VC
Integration sites

Treatment of Subjects With Adenosine Deaminase (ADA)-Deficient Severe Combined Immunodeficiency (SCID) with Autologous Bone Marrow CD34+ Stem/Progenitor Cells after Addition of a Normal Human ADA cDNA by the EFS-ADA Lentiviral Vector

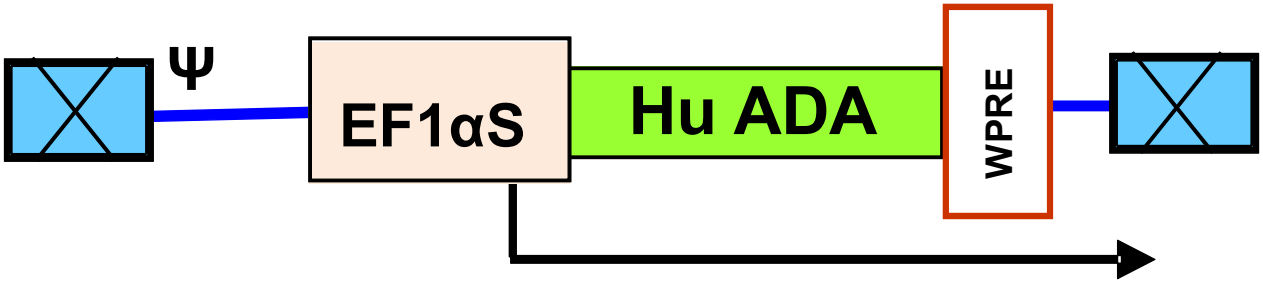
Sponsor and Protocol PI: Donald B. Kohn, M.D., U.C.L.A.

NHGRI NIH Site PI: Fabio Candotti, M.D.,

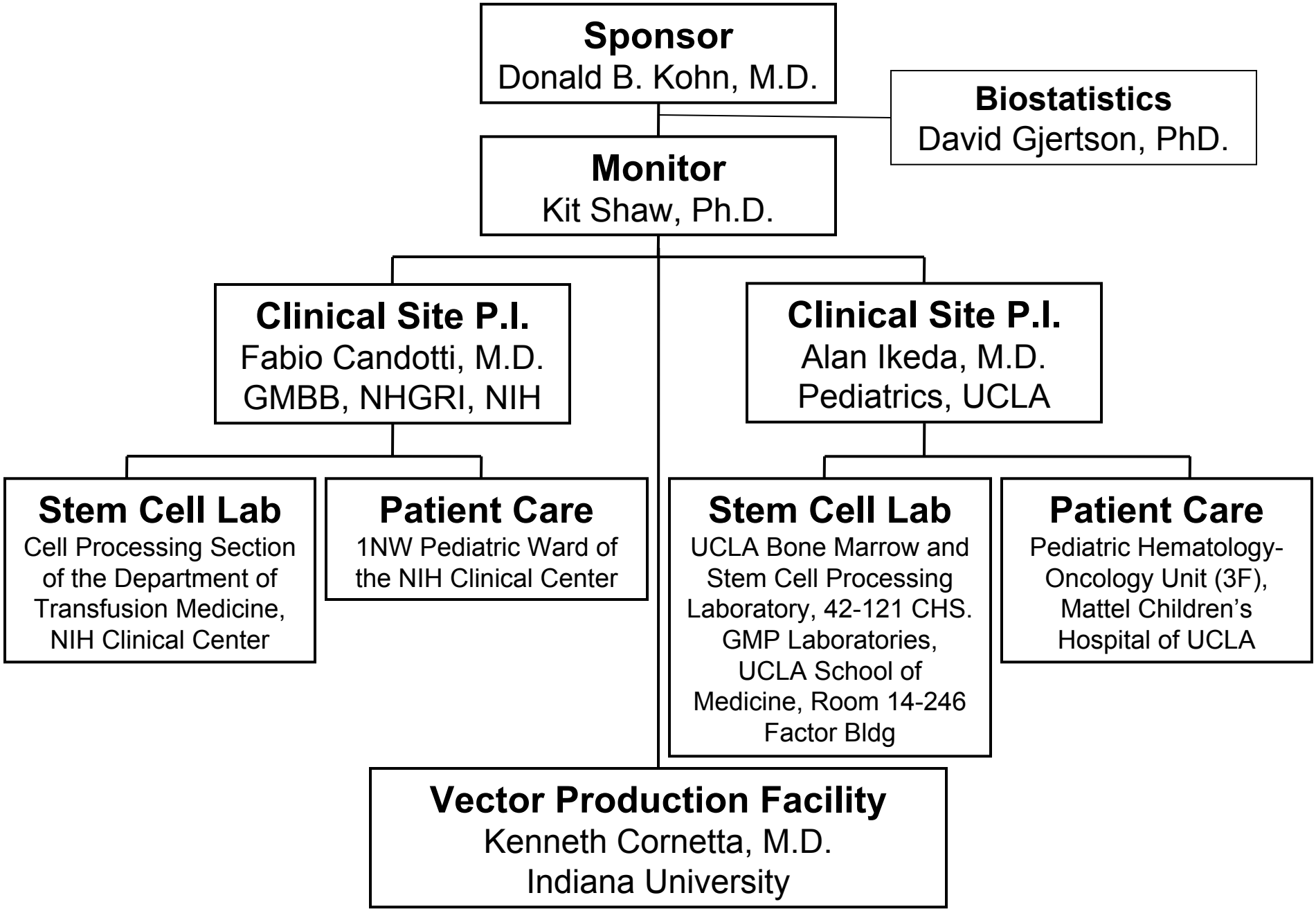
U.C.L.A. Site PI: Alan Ikeda, M.D., U.C.L.A.

Vector Production: Ken Cornetta, M.D., IUVPF

Associated U.K. Trial PIs: H. B. Gaspar and A. Thrasher



UCLA/NHGRI Trial Organization



Study Design

Prospective, non-controlled, non-randomized Phase I/II clinical trial to assess the safety and efficacy of autologous transplantation of CD34+ cells from the bone marrow of subjects with ADA-deficient SCID following human ADA cDNA transfer by the EFS-ADA lentiviral vector.

Following provision of informed consent, enrolled subjects will be screened to determine full eligibility for participation over 1-2 weeks.

Subjects will not be receiving PEG-ADA enzyme replacement and will receive cytoreduction with Busulfan (4 mg/kg).

Bone marrow will be harvested, CD34+ cells isolated and transduced with the EFS-ADA lentiviral vector and then be re-infused IV.

Peripheral blood samples at 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 21, and 24 will be analyzed to assess end-points.

Subjects will then be consented for a long-term follow-up study that consists of semi-annual to annual visits and lab draws over the subsequent 13 years.

Treatment of ADA(-) SCID with Bone Marrow CD34+ Stem/Progenitor Cells by the EFS-ADA Lentiviral Vector

Primary Objective

Examine the safety of autologous bone marrow transplantation for ADA-deficient SCID using a lentiviral vector to transduce CD34+ cells with a normal human ADA cDNA, which are transplanted after non-myeloablative conditioning with busulfan and withholding of PEG-ADA enzyme replacement therapy. Subjects (n=10) will be monitored at regular intervals by a clinician, as well as standard blood counts and chemistries.

Secondary Objectives

1. Determine the frequencies of peripheral blood leukocytes containing the transferred ADA cDNA using real-time PCR,

2. Assess ADA gene expression by measuring ADA enzymatic activity in peripheral blood leukocytes,

3. Examine the effects of ADA gene expression on the immune function by measuring complete blood counts, immunoglobulin levels, T, B, NK lymphocyte subsets, and T cell mitogen and proliferation assays.

Inclusion Criteria

I. Children with ADA-Deficient SCID ≥ 3.5 months of age.

II. Ineligible for matched sibling allogeneic bone marrow transplantation.

III. Written informed consent according to guidelines of the IRB (UCLA/NIH).

Exclusions

I. Organ dysfunction

II. Active opportunistic infections

III. Malignancy, other than DFSP

Response to RAC Questions - 1

Monoclonal expansion risks:

Q- Provide # of ADA(-) SCID gene therapy subjects with monoclonal expansion

A- None reported to date.

(We reported one subject with low level, oligo-clonal marking which was stable over 10 years –Schmidt Nat Med 2003)

Q- Consider stopping rule for monoclonal expansion, not just leukoproliferation.

A- Will monitor clonality by LAM-PCR. Plan will be developed with FDA, based on emerging findings e.g. from beta-thal trial.

Target cells:

Q-Consider use of CD34+/CD38- cells as targets.

A- CD34+/CD38- cells are contained within the bulk CD34+ population and attempts to purify would likely decrease their absolute numbers, does not increase their transduction, and would require clinical-grade anti-CD38 mAb and reagents/device.

Response to RAC Questions - 2

Ethics/risks of withholding PEG-ADA enzyme replacement therapy:

Q- Is withholding of PEG-ADA because it may undermine efficacy?

A – Yes. PEG-ADA has been shown to reduce selective amplification of ADA gene corrected lymphocytes (refs. cited)

Q- Withholding PEG-ADA as potential risk should be stated more distinctly in ICD.

A. Agree. Withholding of PEG-ADA leads to decline in lymphocyte counts for several months until effects of gene transfer occur.

May be an acceptable risk, based on prior beneficial results seen in 3 trials using γ-retroviral vectors.

Diagnostic bone marrow aspirates:

Q- Are 1-2 possible BMA purely for research? If yes, IRB may consider if they meet criteria for "minor increase over minimal risk.

A- May be important for assessing loss of ADA(+) cells, cytopenia, etc.

Prior IRB review of our γ-RV trials did code as 406.

Response to RAC Questions – 3

Busulfan risks:

Q- Busulfan may be given, but then cells not meet release criteria.
A- Need >24 to admin busulfan and allow "wash-out" prior to CD34+ cell re-infusion. Certified final cell product not stable over that time.

If busulfan given and transduced cells not available, protocol calls for infusion of cryopreserved back-up.

Q- Likelihood of transduced CD34+ cells not meeting release criteria?
A- Low, but possible. Kohn lab has prepared transduced CD34+ cells for 25 subjects in six trials without incident. NIH lab similar success.

NB: In allogeneic HSCT, full ablative conditioning is given with potential for unavailability of transplant d/t accident, illness, etc.

Q- Busulfan may pose risks for neurotox, decr. fertility, malignancy, even with low dose used. This should be stated more explicitly.
A. Wording added to ICD re: possible bu neurodevelop./fertility/Ca risks

Q4. Back-up infusion and PEG-ADA re-start criteria:
A. Proposed to stay with criteria of Day+42 ANC<200 and ADA not detected by 6 months, based on findings from prior trial.

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Kit Shaw, Ph.D.	UCLA
Kenneth Cornetta, M.D.	IUPUI

Prior Trials
EFS-ADA Vector
Proposed Trial
Responses to RAC

Halting Rules for the Protocol

1. If there is one death, or two grade 4 toxicities attributed to this study, not Busulfan-related.

2. Two subjects with prolonged pancytopenia, defined as at least any two of the following (ANC < 200, and/or platelets < 20,000, and/or Hb < 8.0) beyond day +42 after CD34+ cell infusion.

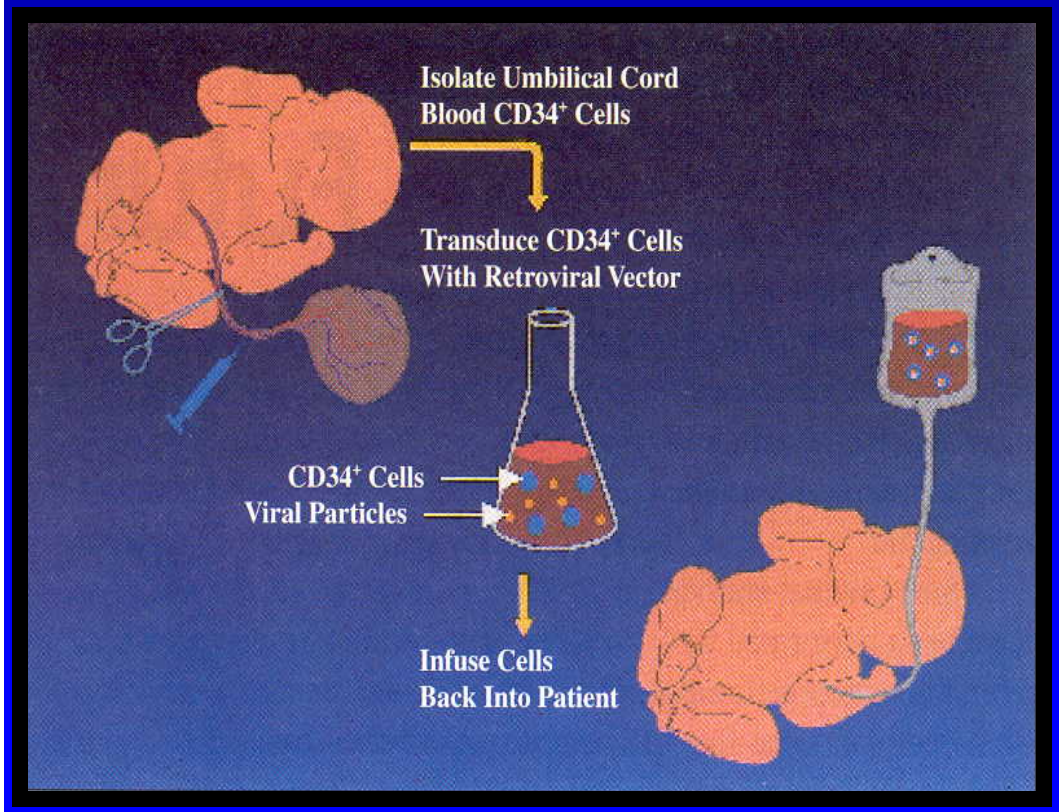
3. If 0 of 3 successive patients show evidence of engraftment of transduced cells by six months of follow-up.

4. If RCL is detected, and confirmed, in one subject.

5. If a subject in this study develops leuko-proliferative or malignant disease due to vector.

Clinical Trials of Gene Therapy for SCID

In 1993, we performed the first trial of gene therapy for three ADA-deficient SCID newborns, using their own umbilical cord blood as the source of HSC for gene therapy.



Outcome:

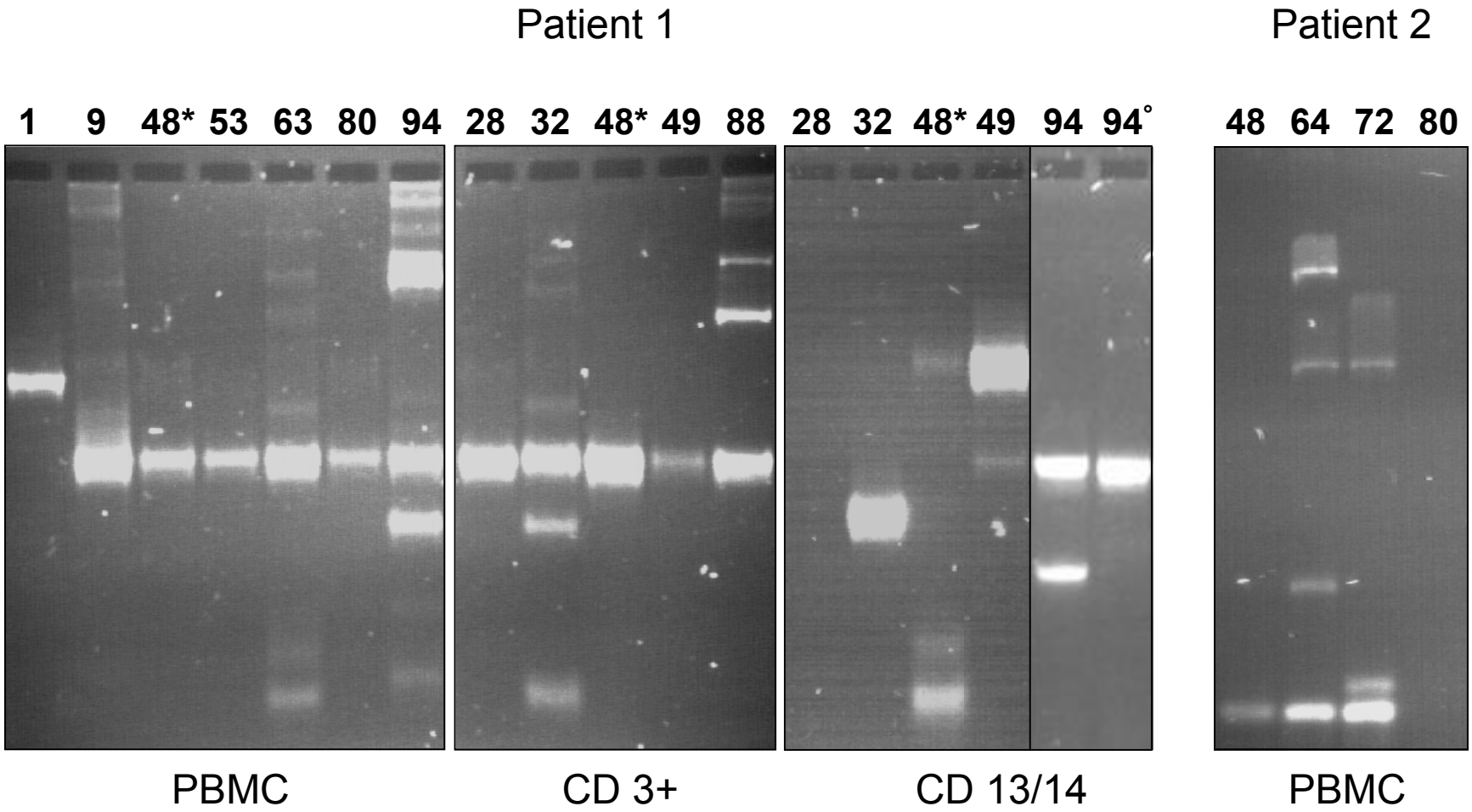
Low level of gene-corrected stem cells engrafted, with pauci-clonal marking.

Not sufficient to “cure” their immune deficiency.

Continue to get ADA enzyme replacement therapy.

Figure 3A. LAM-PCR analysis of PBMC, T cells and myeloid cells

From: Schmidt, Carbonaro…..Kohn, von Kalle. Nat Med April 2003.



Analysis of T Cell Clones for Vector Integrants and TcR Gene Rearrangement

T cell clones grown from peripheral blood 8 years after neonatal CD34+ cell gene transduction showed:

1. 13/220 (6%) contained LASN vector.
2. 10/13 (77%) had the predominant integrant.
3. Multiple TcR rearrangement patterns were seen among clones with predominant integrant.

Thus, a pre-thymic T cell progenitor was transduced.

The presence of the predominant vector integrant in both myeloid cells and a pre-thymic T cell progenitor cell demonstrates gene transduction of a pluripotent hematopoietic stem cell.

Protocol for Monitoring of Oligo/Monoclonality

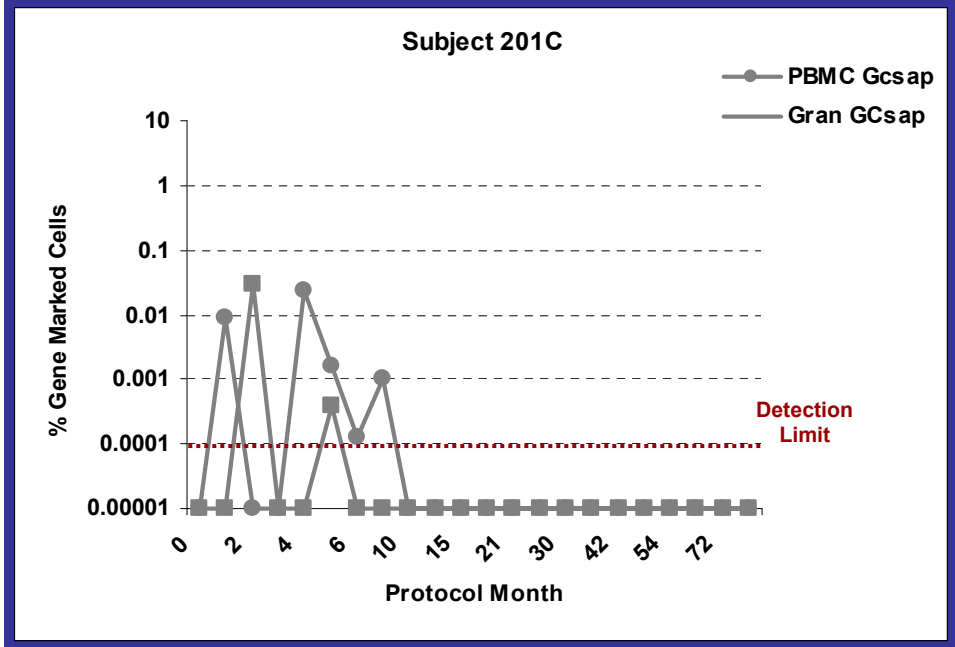
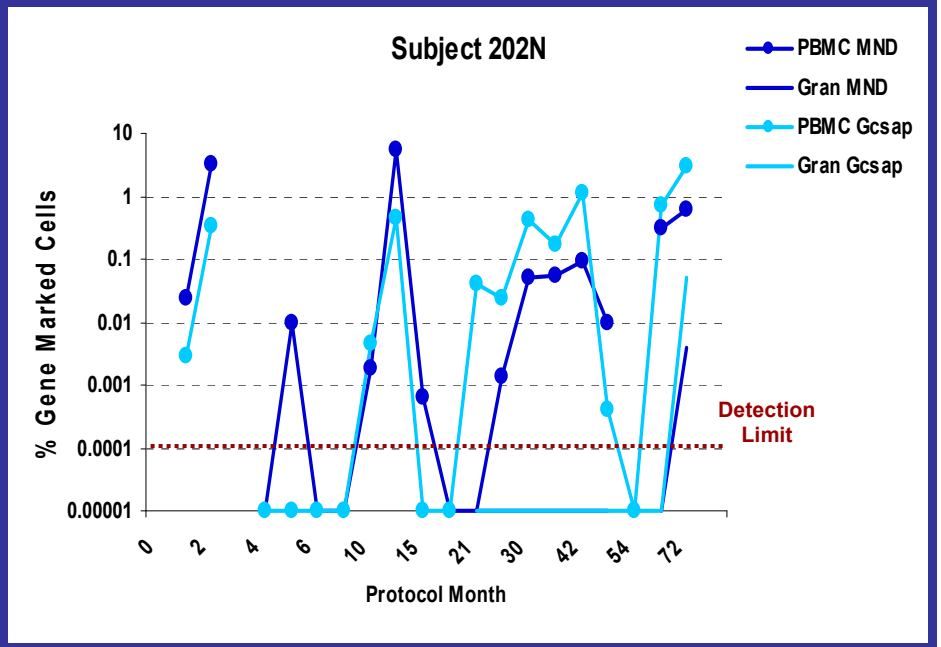
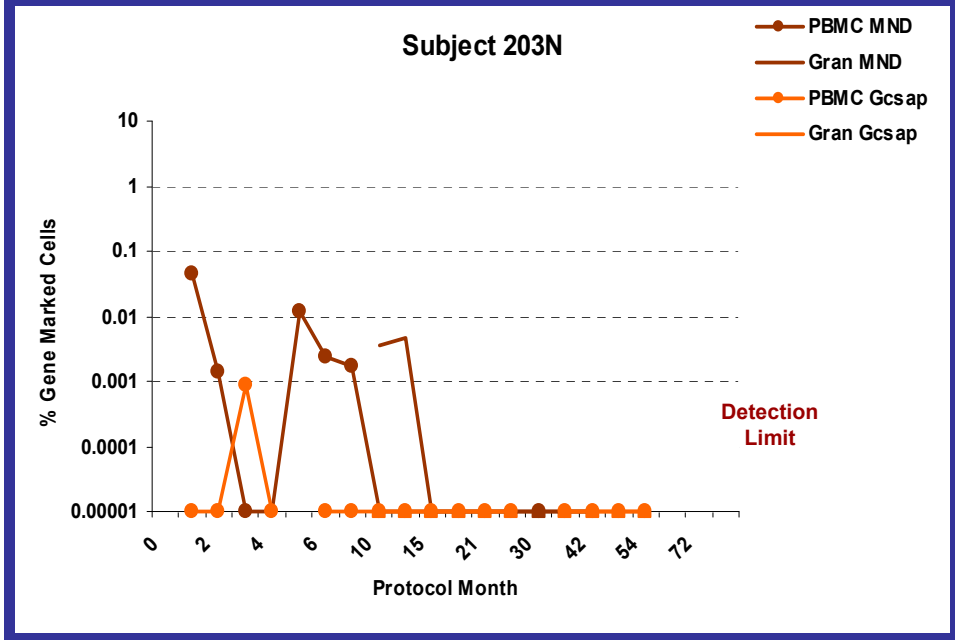
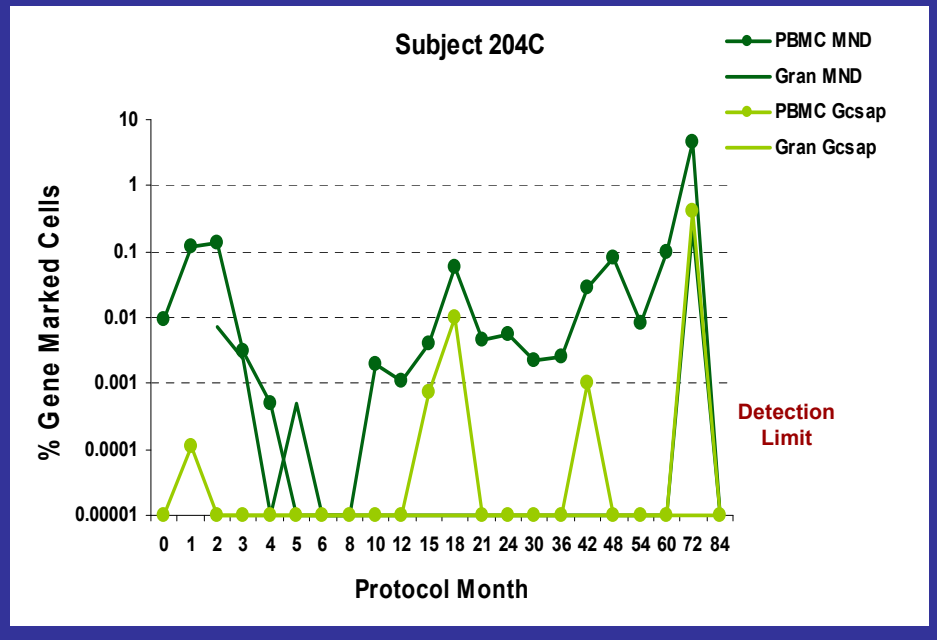
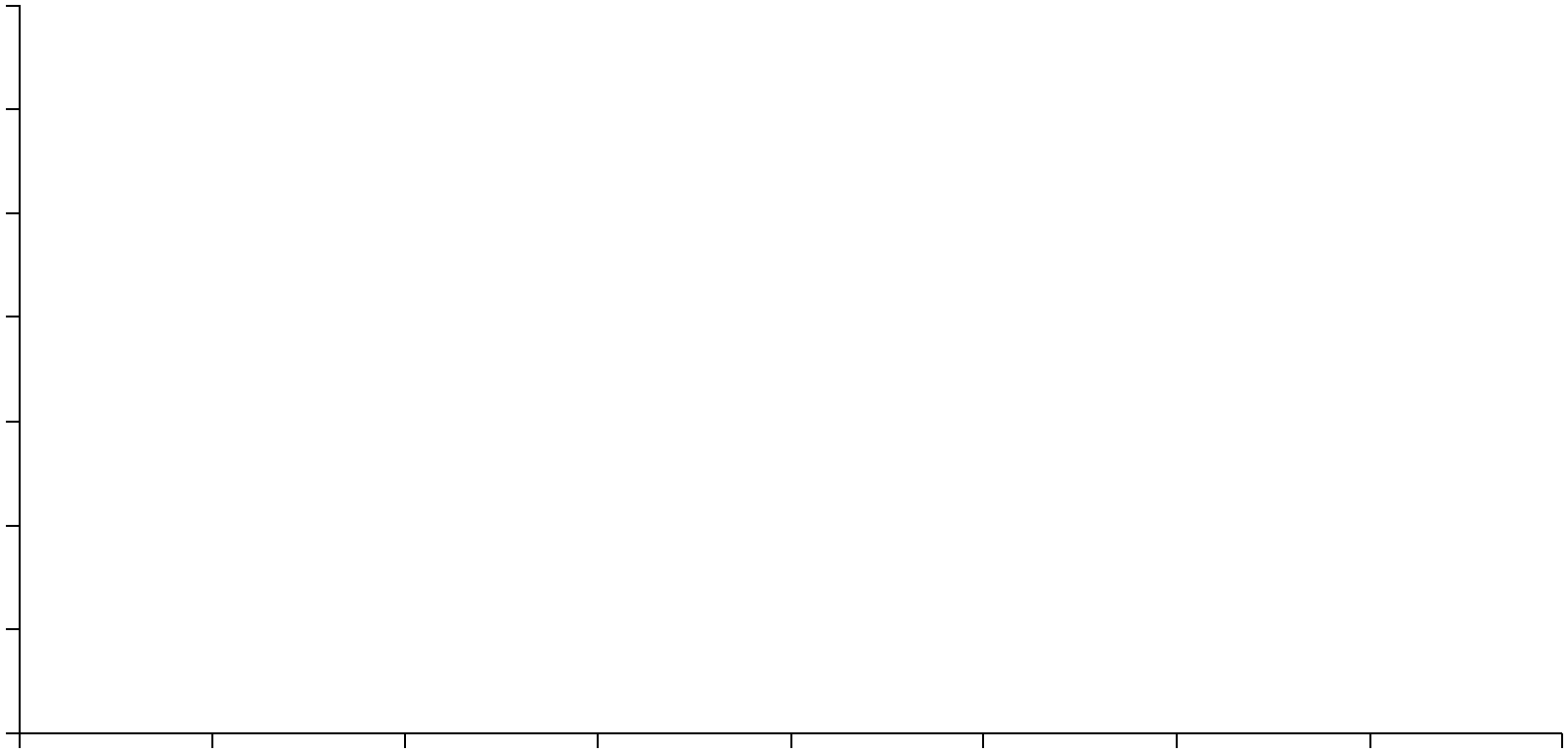
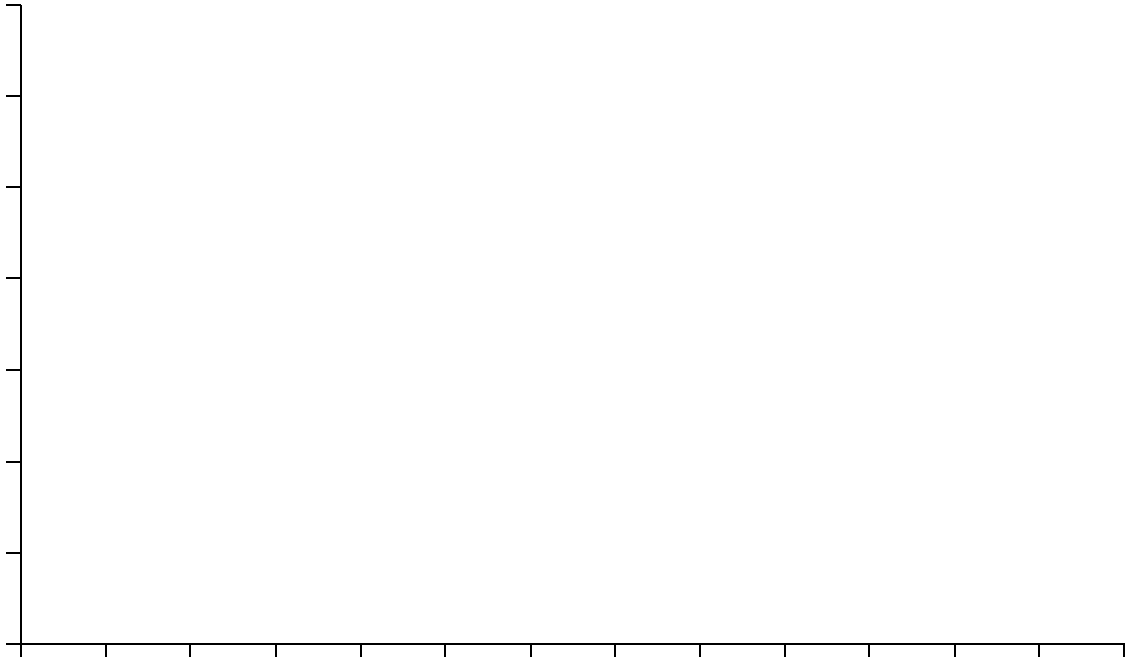
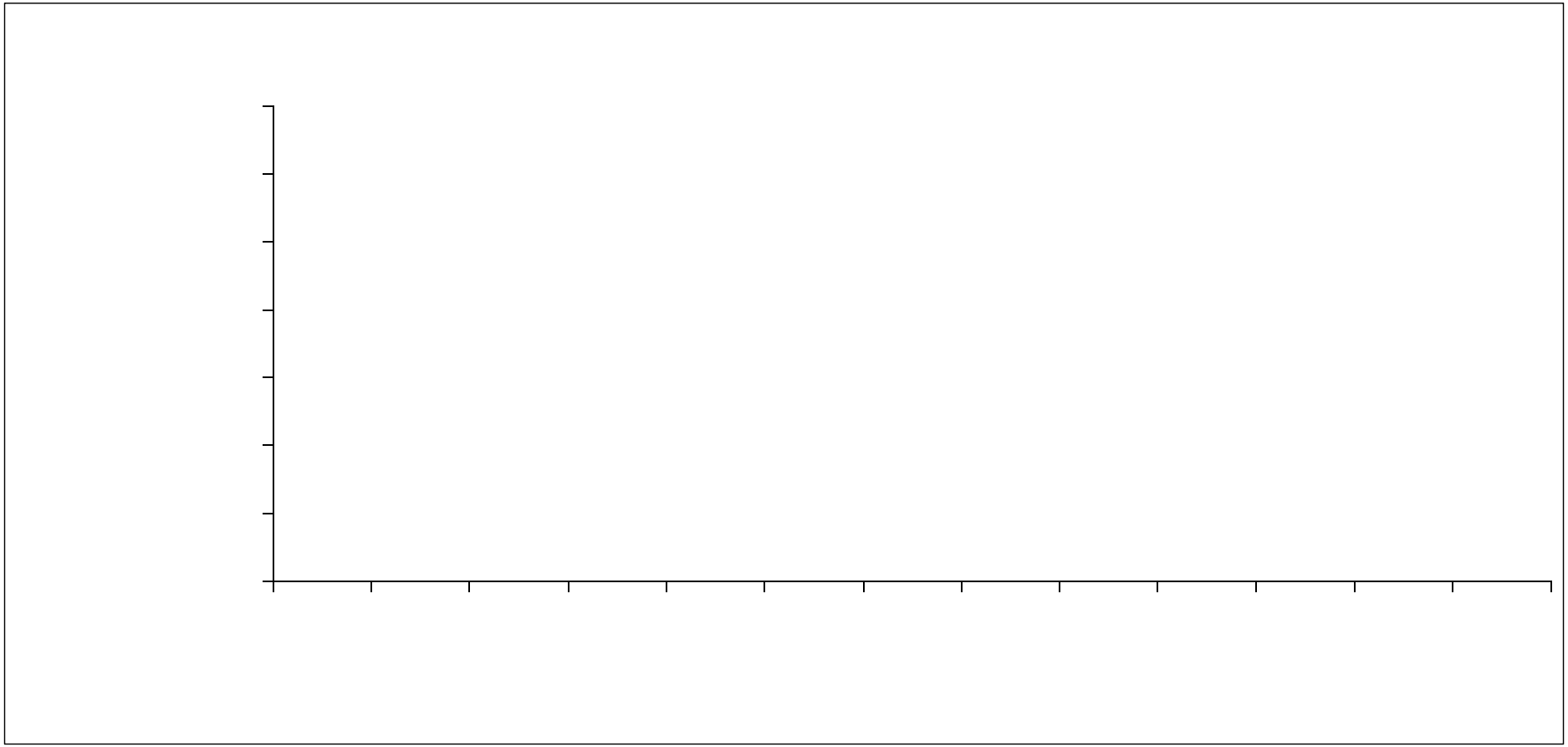
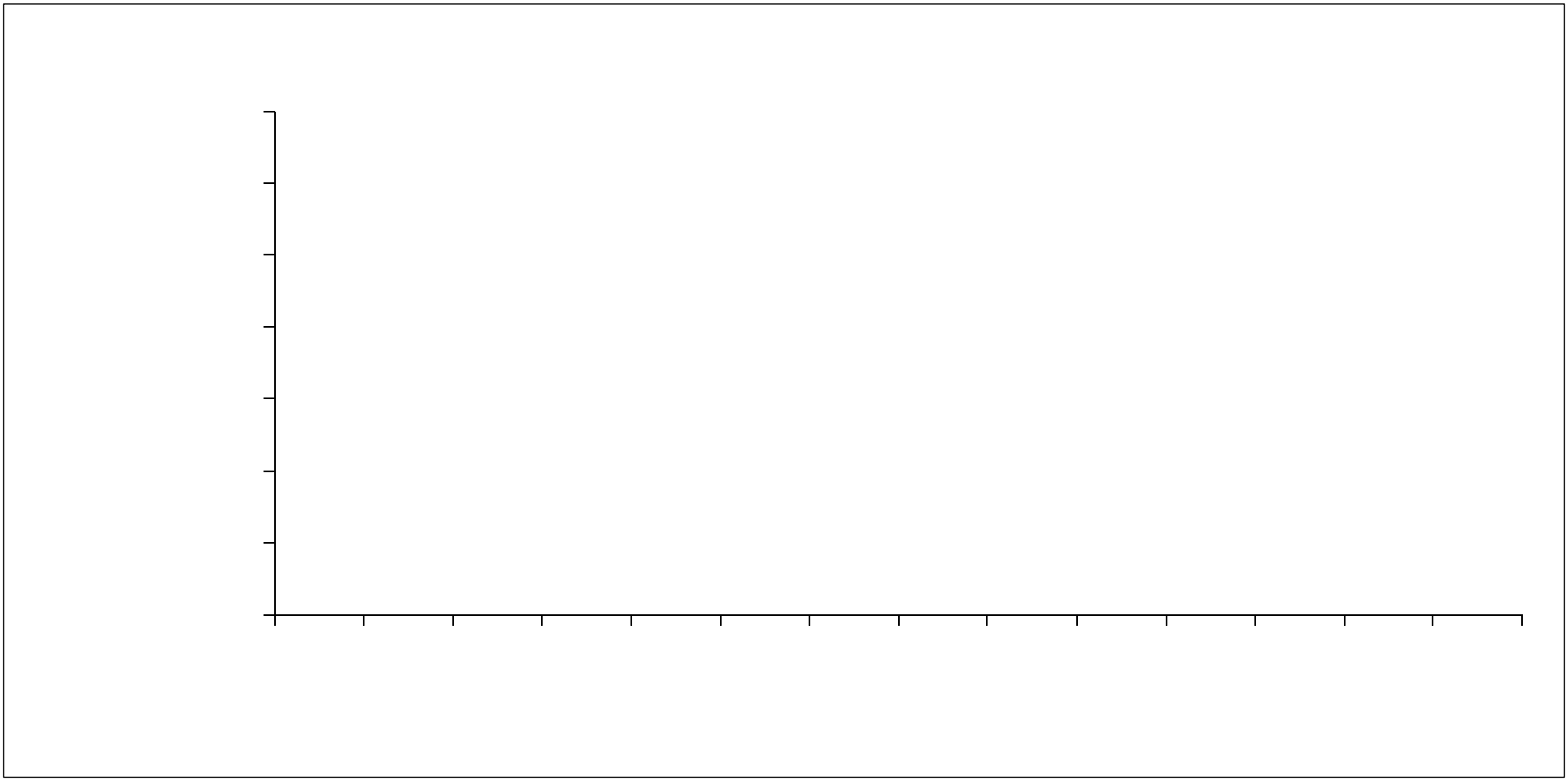
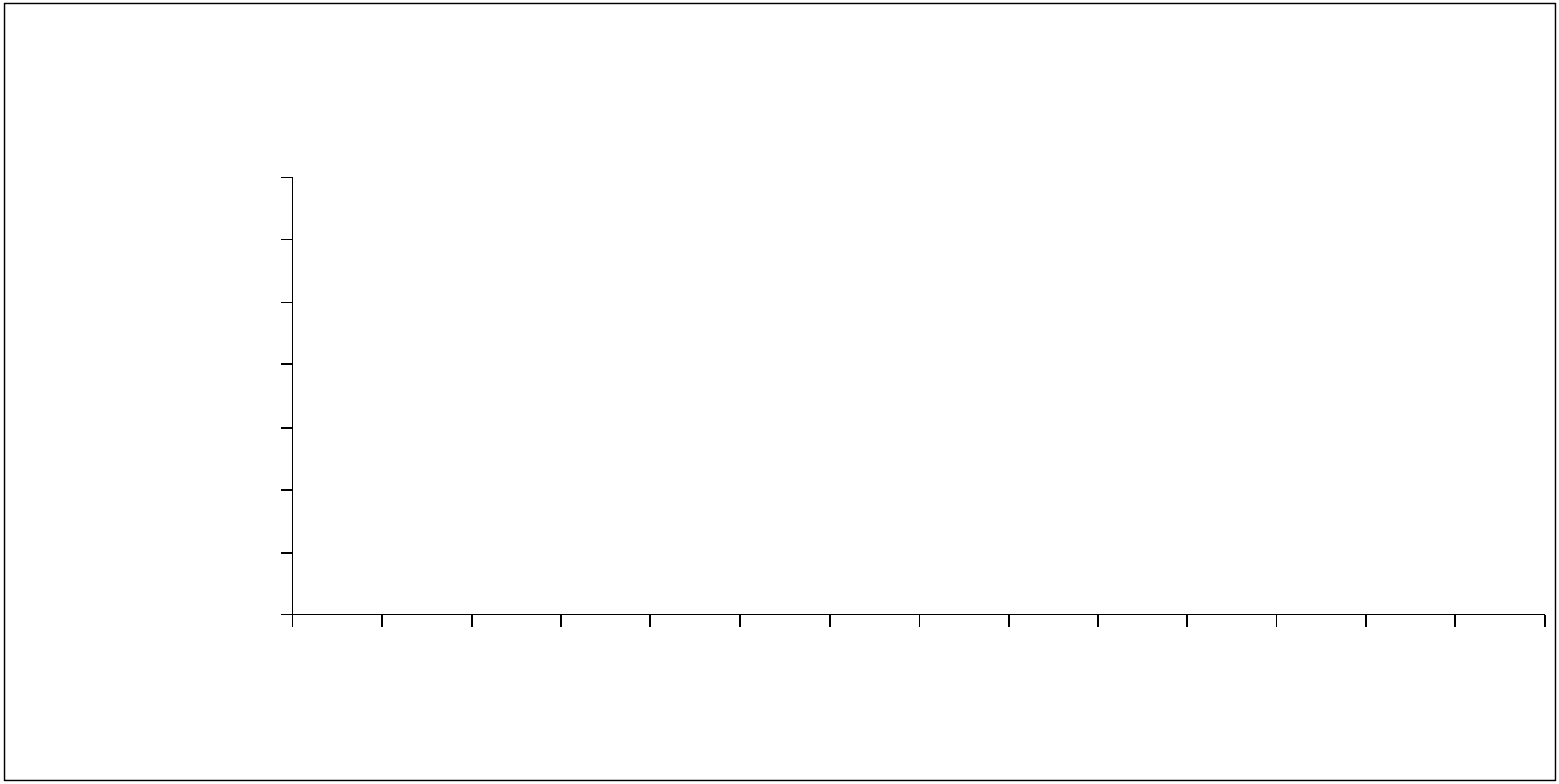
Q 6 Months x 5 years:

1. Clinical Assessment : Hx, P.E. and imaging studies(prn).
2. CBC/diff, lytes, BUN/Cr, LDH, uric acid, LFTs.
3. qPCR for the frequency of gene-modified PBMC, gran.
4. LAM-PCR, if frequency of gene-modified cells >1%.

If the LAM-PCR shows oligo/monoclonality, the following laboratory assessments will be performed, as applicable:

A. Immunophenotype and determination of abs. # T, B, NK, myeloid.

B. High resolution cytogenetic analyses of blood and/or marrow cells.

C. Identification of vector integration site(s)

The CHLA/NIH Study –Subjects Treated in 2001-2002