Children's Hospital Boston

Interim Analysis of SIN-gamma C retrovirus vector trial in SCID-X1

NIH Recombinant DNA Advisory Committee

David A. Williams
Transatlantic Gene Therapy Consortium

12/11/14

DANA-FARBER CANCER INSTITUTE
Children's Hospital Boston
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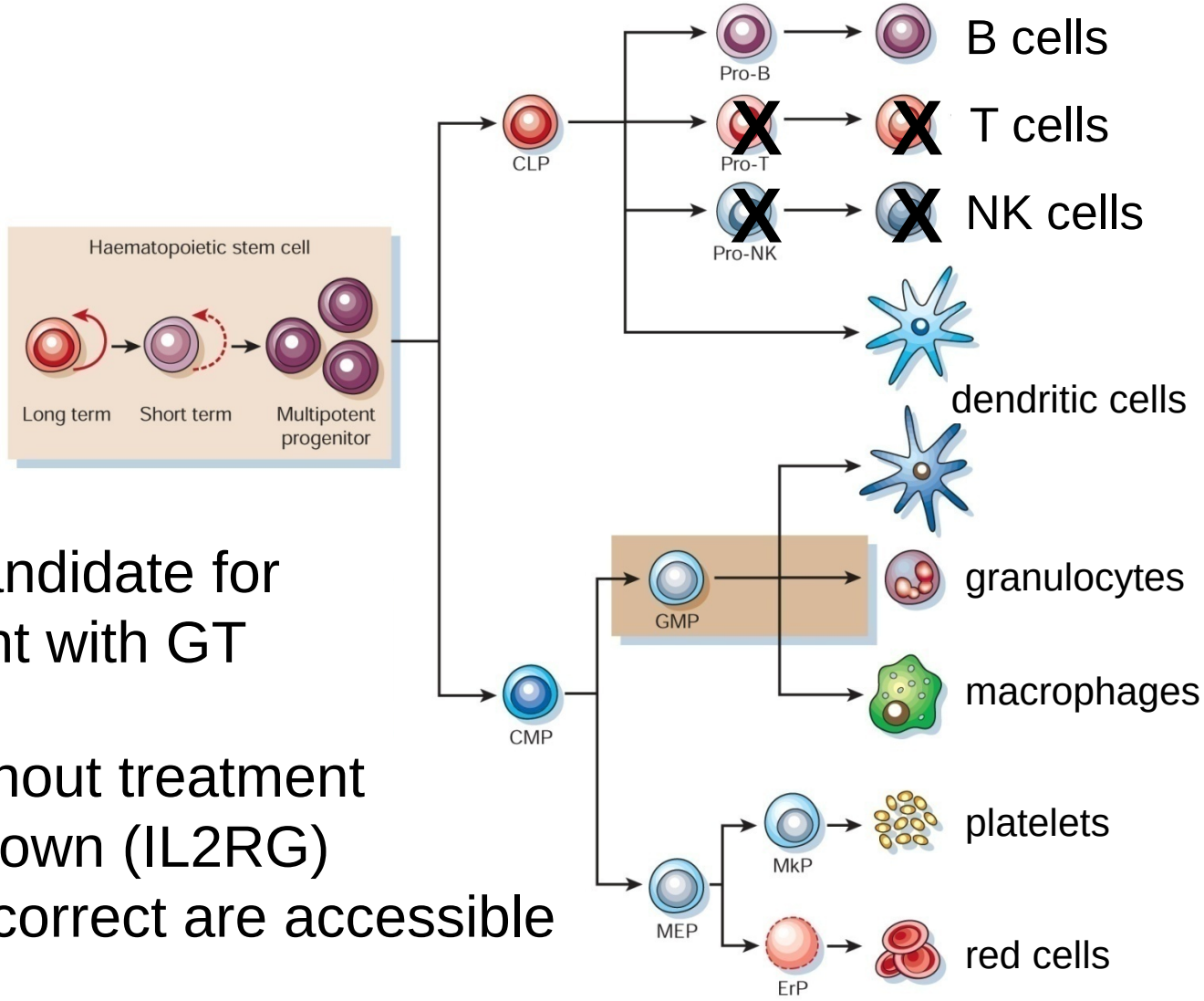
Current and planned trials-BCH/DFCI

	X-linked SCID	Wiskott-Aldrich	Childhood Cerebral Adrenoleukodystrophy	X-linked CGD	CD19 CAR T cells (relapsed B-ALL)	Sickle cell anemia
Vector	SIN γ-RV	SIN-LV	SIN-LV	SIN-LV	SFG-19-28z	LV shRNA target-BCL11A
Promoter	Internal EF1α	Internal hWAS 1.6 kB	Internal MND	Chimeric CatG/cFes	LTR	HSII.III-beta
Conditioning	No	Bu 12/Flu 120	Bu 16/Cy 200	Bu 12	Cy 1500 mg/m2	Bu 8-12
Sponsor	Williams	Williams	bluebird bio	Kohn	MSKCC	Williams
Boston PI/ co-I	Notarangelo/ Pai	Pai/Notarangelo	Williams/Duncan	Williams/Newburger/Wang	Silverman	Esrick, Heeney, Lehmann
US centers	Boston, Cincinnati, Los Angeles	Boston	Boston, Minnesota, Los Angeles	Los Angeles, Boston, NIH	New York, Boston	Boston
European centers	Paris, London	Paris, London	Paris, London	London, Frankfurt, Zurich	-	-
Vector production	Cincinnati	Genethon	bluebird bio	Genethon	MSKCC	TBD

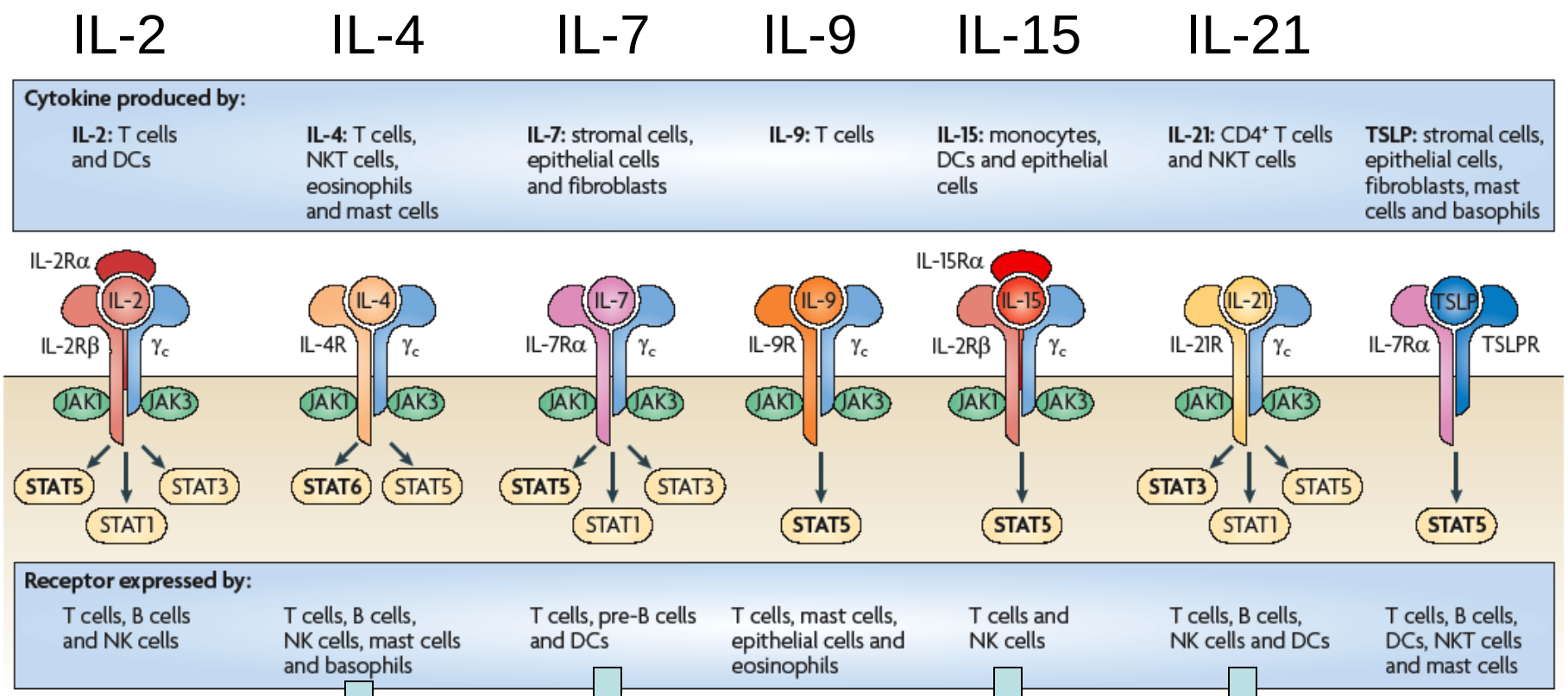
X-linked SCID results in the absence of T and NK cells



Good candidate for treatment with GT

- fatal without treatment
- gene known (IL2RG)
- cells to correct are accessible

γc (IL-2 receptor gamma, IL2RG) is required for many cytokine receptors and immune cell development



B cells lack function

no T cells

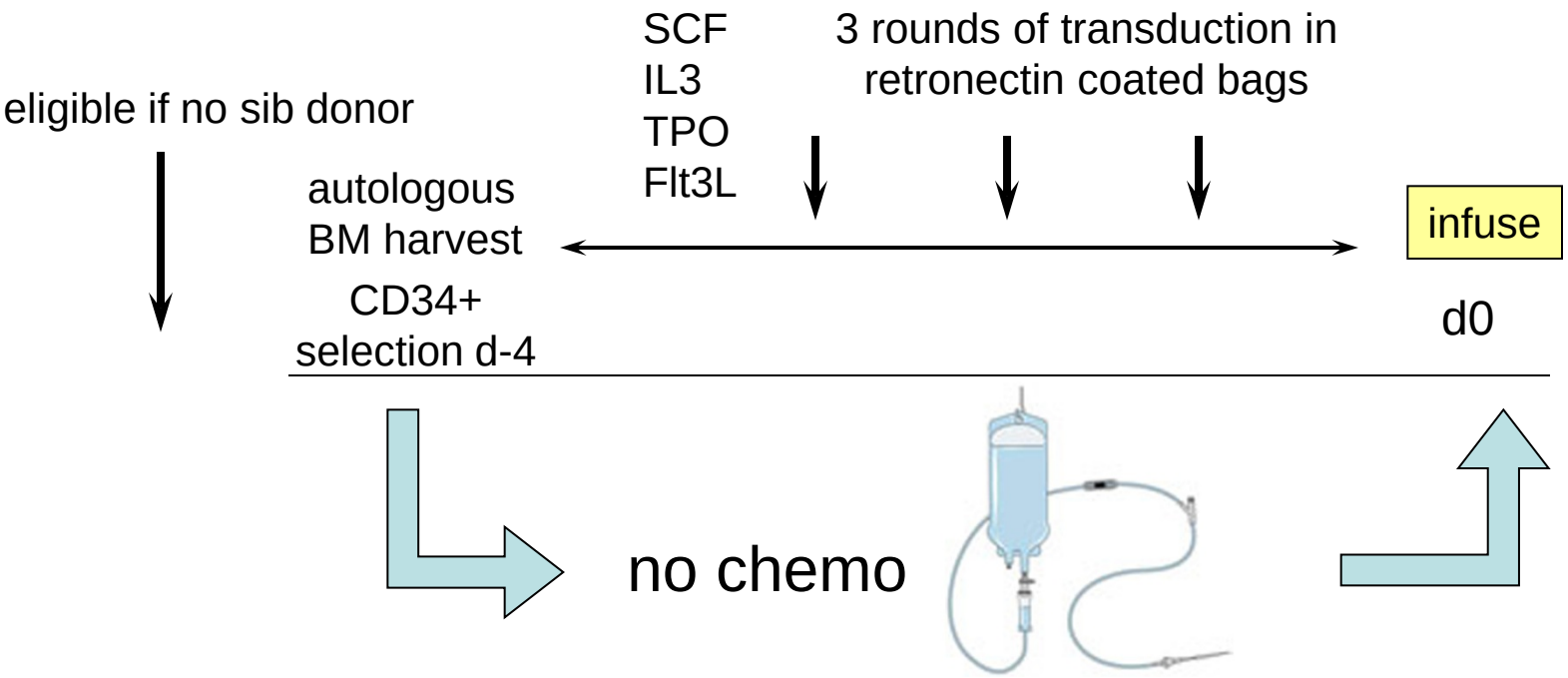
no NK cells

B cells lack function

modified from Rochman et al Nat Rev Immunol 2009

Vector and protocol used in Paris and London

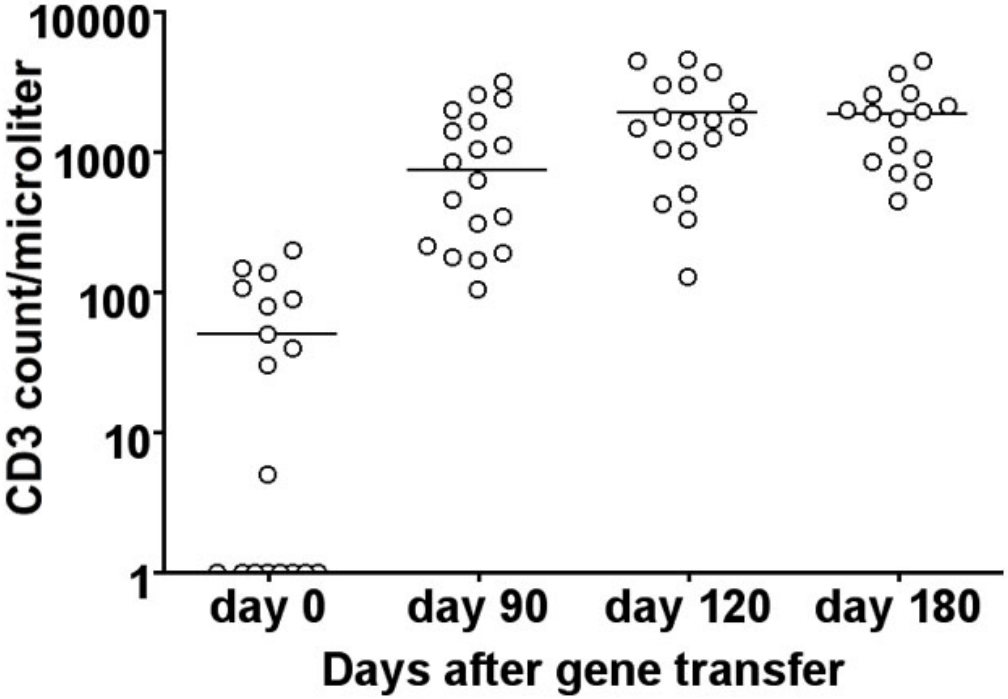
LTR-driven gammaretroviral vector: MFG γC



Hacein-Bey-Abina et al NEJM 2002; Gaspar et al Lancet 2003; Hacein-Bey-Abina et al Science 2003; personal communication Cavazzana-Calvo, Thrasher

Successful T cell reconstitution in patients after GT

(18 of 20)

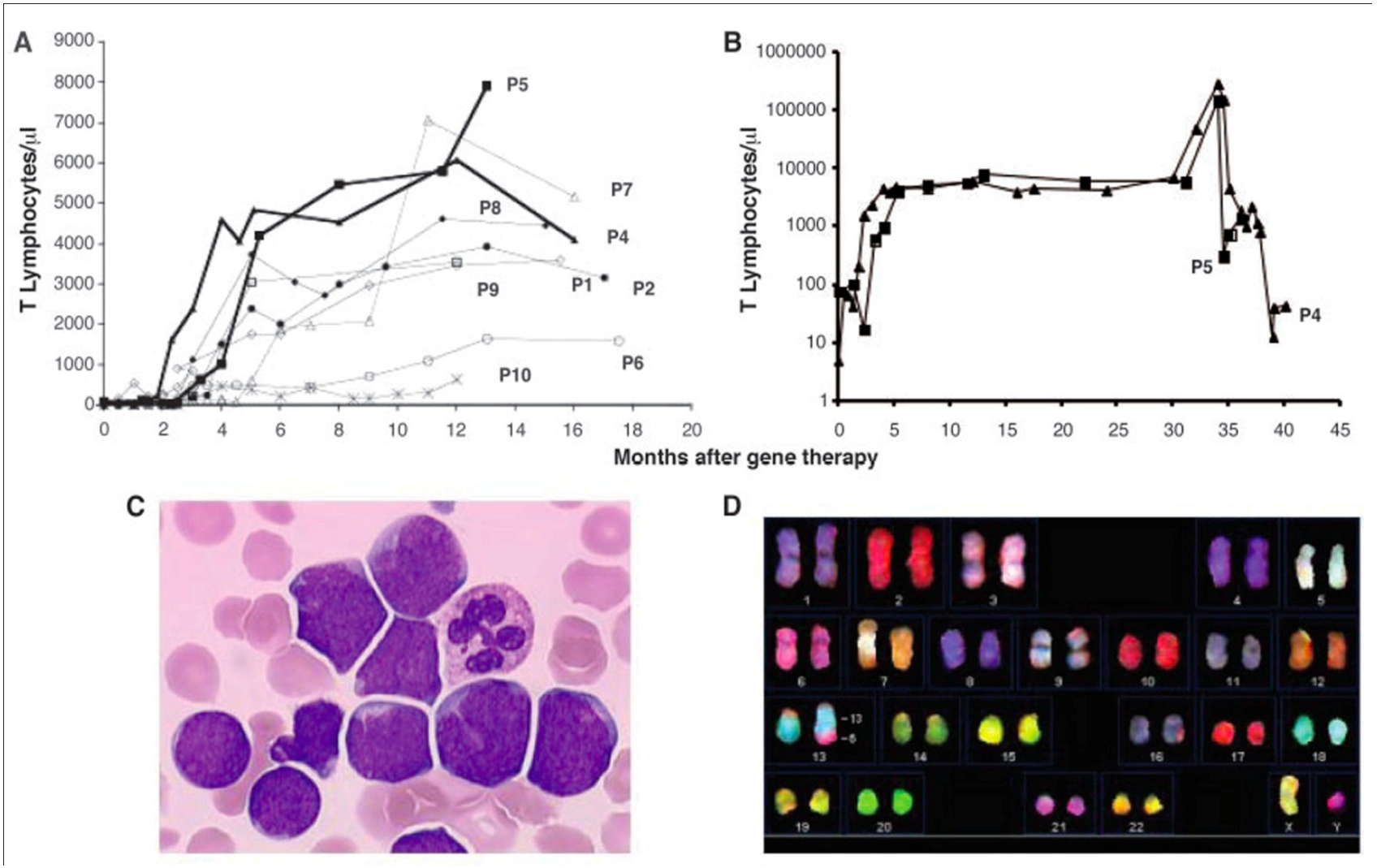


P3 in Paris received a low dose of transduced cells and failed to reconstitute (well after BMT)

P9 in Australia had low reconstitution, did not clear infections (died after BMT)

unpublished data courtesy of Salima Hacein-Bey and Adrian Thrasher

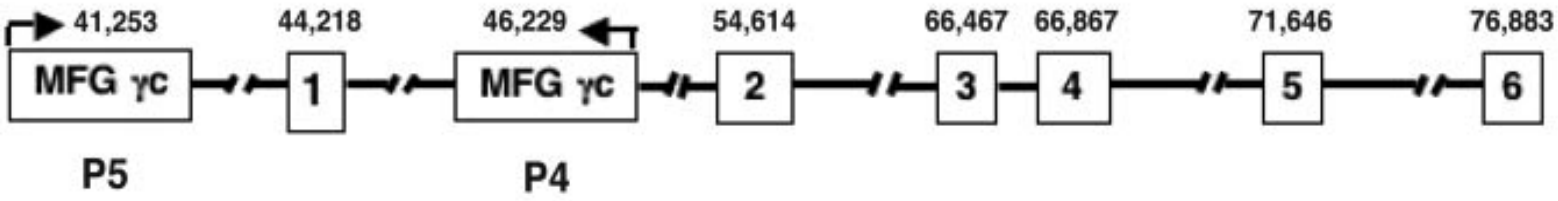
Leukemia in X-linked SCID patients post GT



Hacein-Bey-Abina et al, Science 2003

Retroviral insertion near oncogenes drove overexpression and leukemia

Out of 20 patients, 5 (4 in Paris, 1 in London) have developed T acute leukemia



<u>Insertion site</u>

P4	LMO2
P5	LMO2
P7	CCND2
P10	LMO2, BMI1
P8	LMO2

<u>Clinical outcome</u>

1 patient died (P4)
4 patients treated successfully now have normal T cell number and diversity

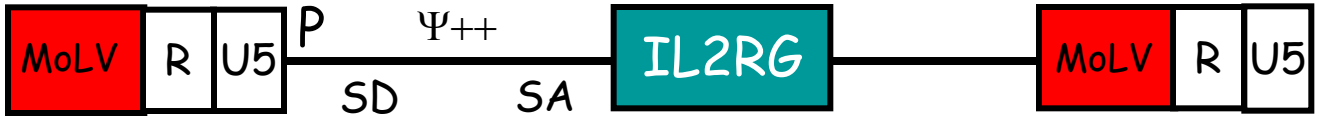
Hacein-Bey-Abina et al Science 2003; Hacein-Bey-Abina et al JCI 2008; Howe et al JCI 2008; Hacein-Bey-Abina et al NEJM 2010

Transatlantic Gene Therapy Consortium

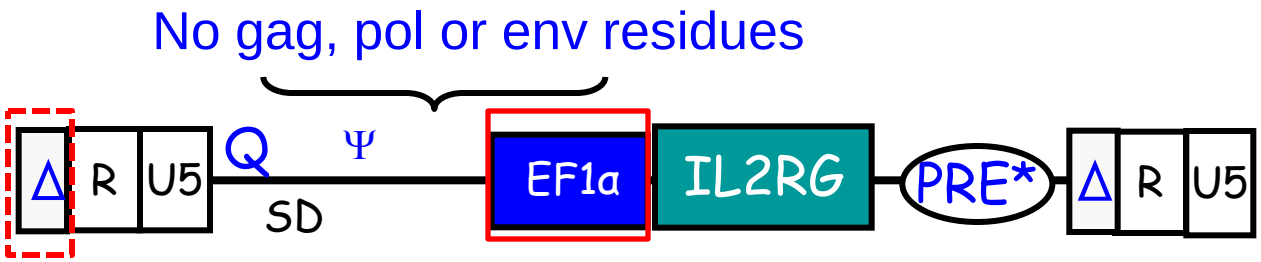
- Children’s Hospital Boston (Boston)
- Great Ormond Street (London)
- Hannover Medical School (Hannover)
- Cincinnati Children’s Hospital (Cincinnati)
- German Cancer Institute (Heidelberg)
- Mattel Children’s Hospital (Los Angeles)
- Georg-Speyer-Haus (Frankfurt, Germany)
- Genethon (Paris)
- CIEMAT (Madrid)
- Lund University (Lund)

Safer vector designed to reduce insertional mutagenesis

LTR-driven gammaretroviral vector: **MFG γC**



New gammaretroviral SIN vectors: **SRS11**



Key modifications compared to Paris/London MFG vector:

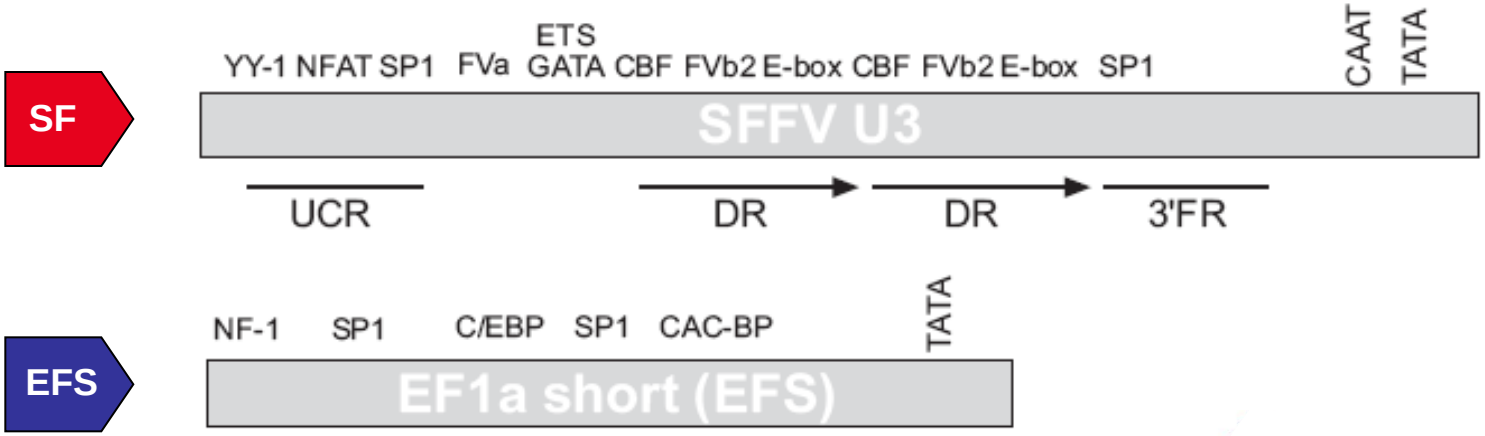
-Removal of MoLV U3 regions to reduce transactivation of neighboring genes

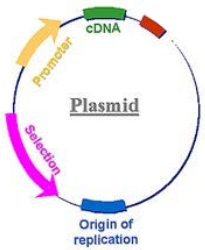
-Cellular EF1α promoter to drive transgene expression in an internal position

•Other minor changes: cellular EFS (EF1α) promoter to drive transgene expression in an internal position, modification of PRE (posttranslational regulatory element) to enhance expression, other modifications to improve titer

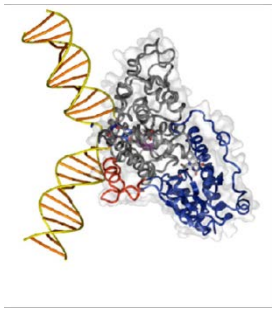
PRE-CLINCAL DATA

Assays to analyze the transactivating potential of different vector configurations and internal promoters





1. Plasmid-based reporter assay

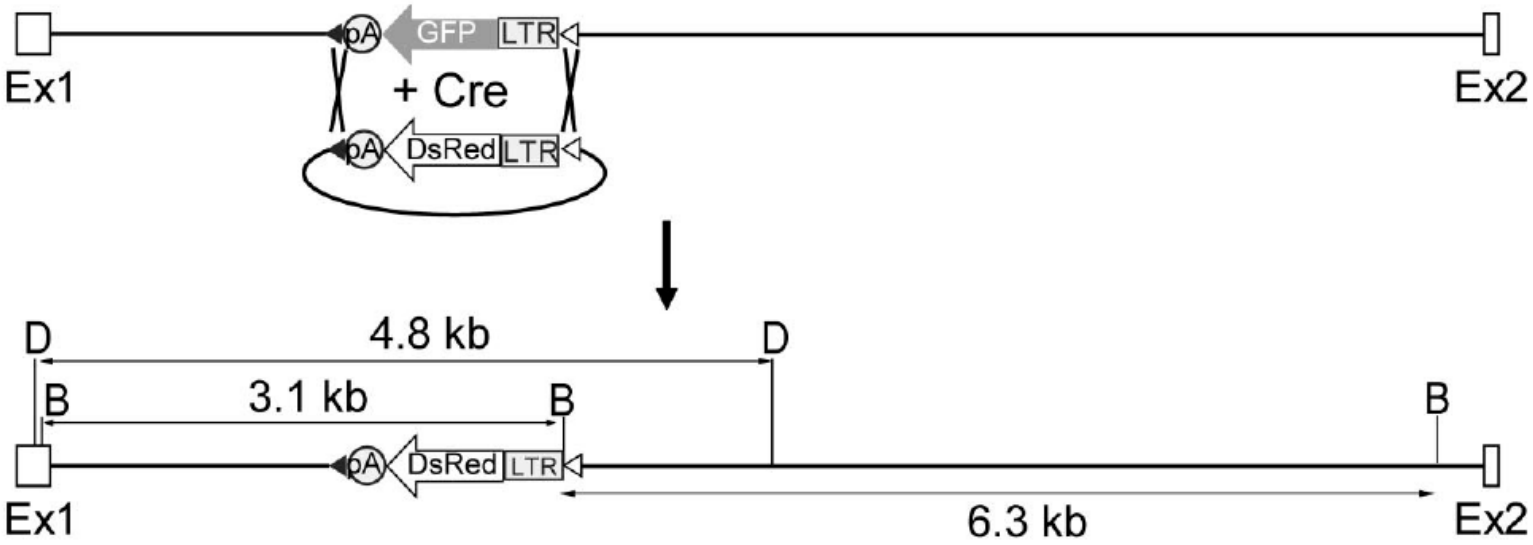


2. Stable, retrovirally transduced reporter assay

3. In vitro immortalization assay

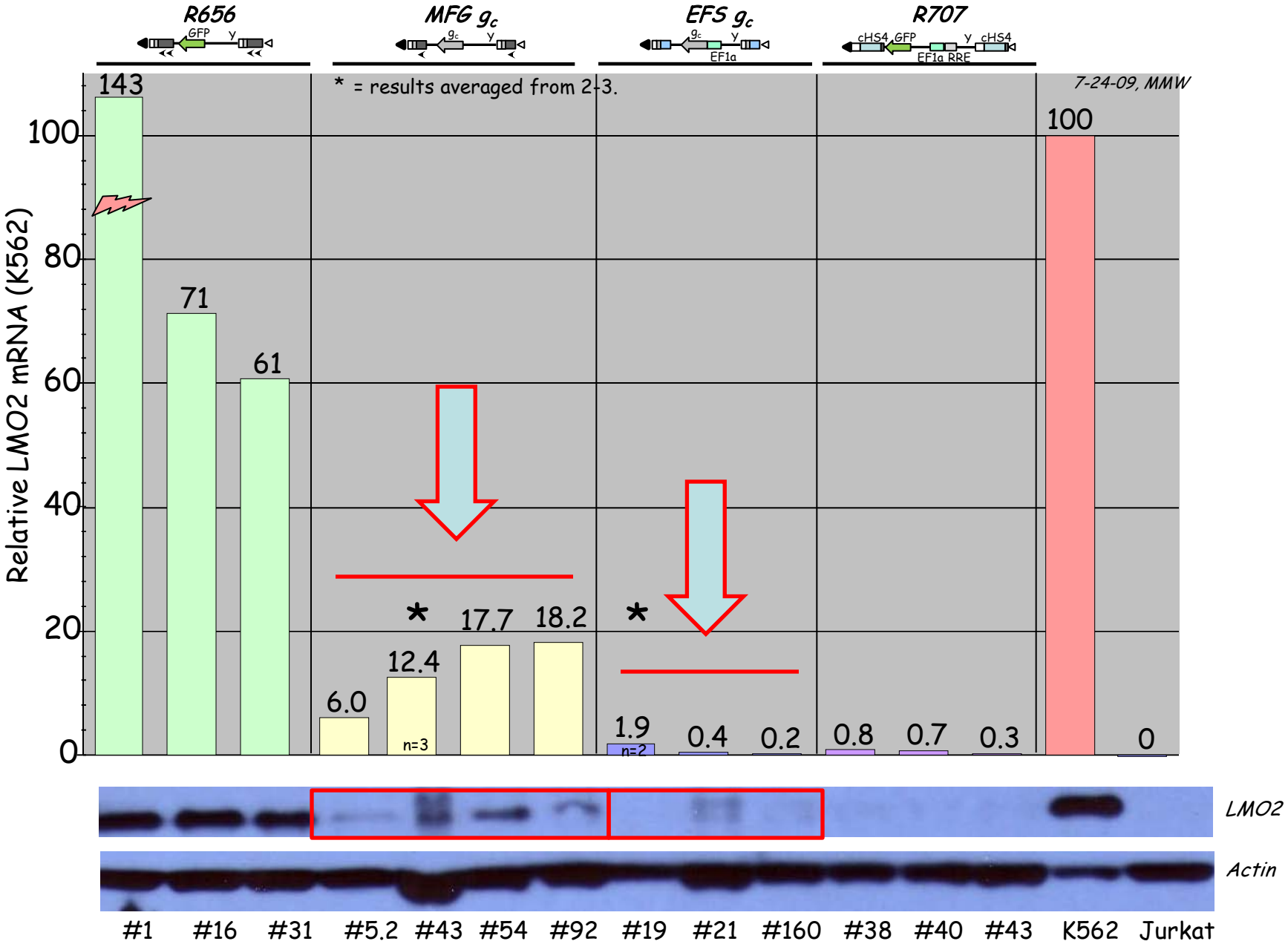
4. Clonal dominance assay

Plasmid-based reporter assay
Knock-in of SIN EFS vector is much less capable of activating LMO2

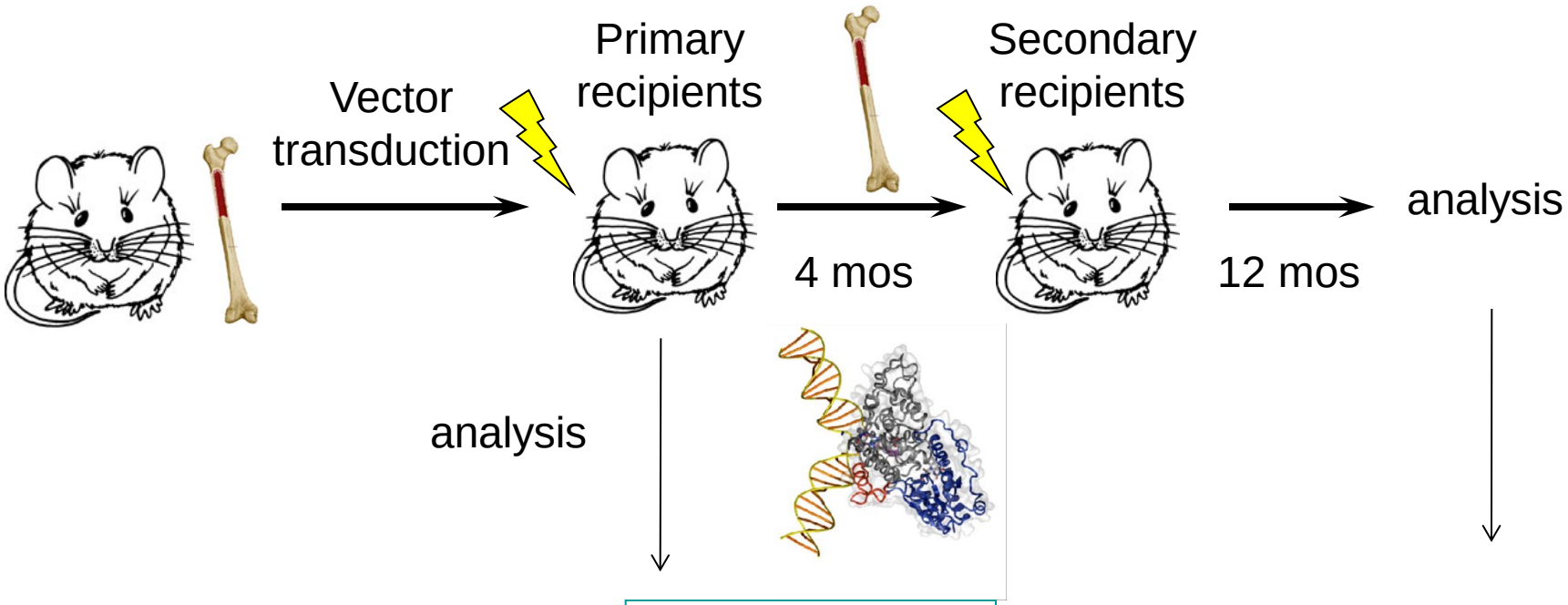


Ryu et. al. Blood 2008

Relative LMO2 expression (Jurkat cell line)



Clonal dominance assay



PB and BM,
deep sequencing

NO DONOR-DERIVED TUMORS (~100 MICE)

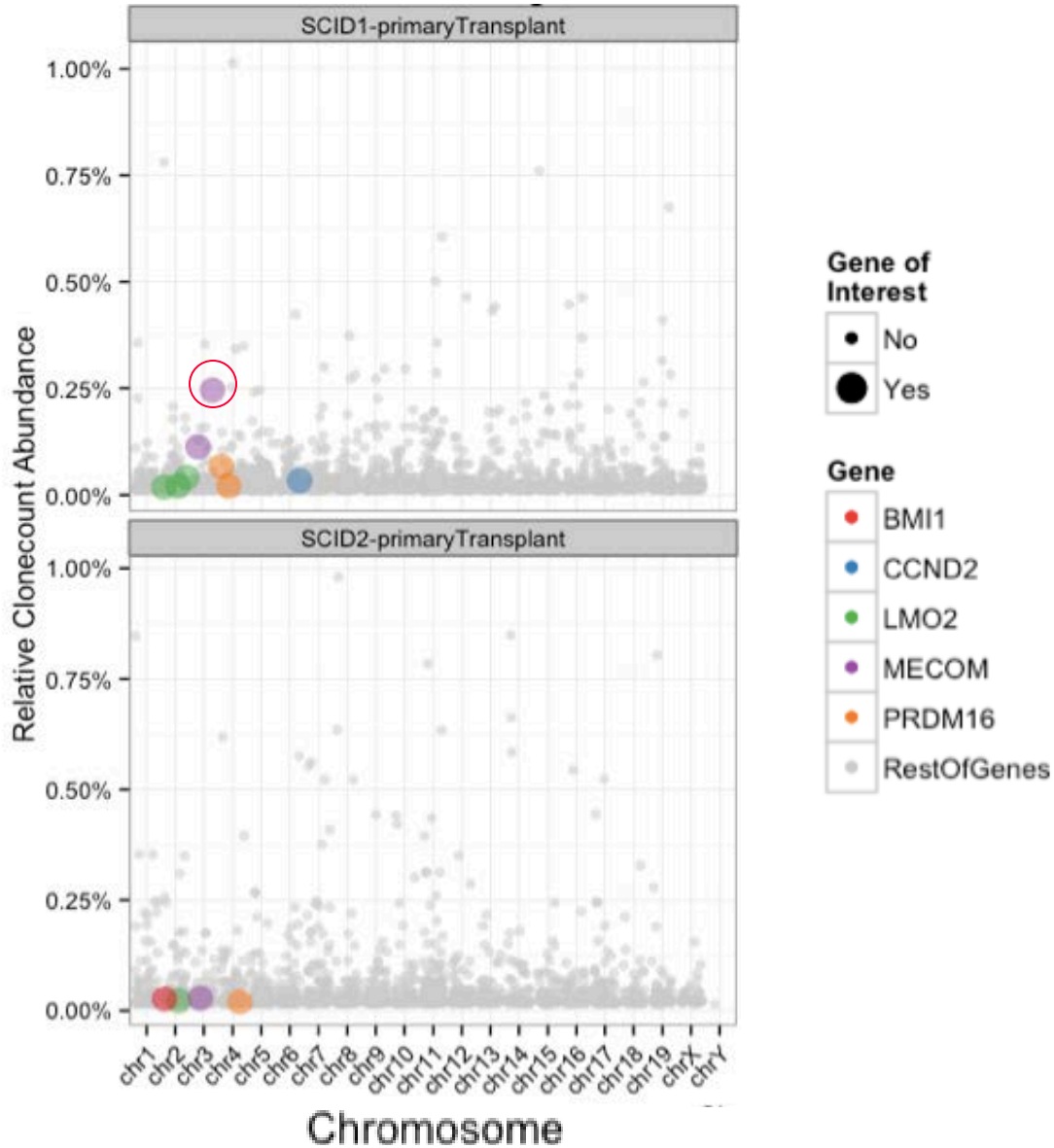
Chris Baum (HMS)
Chad Harris (CHB)
Martijn Brugman (HMS)

Preclinical data comparing SCID1 and SCID2 vectors

Harvest circulating blood cells. Sequence integration site populations to assess clonal expansion (~11,000 integration sites analyzed).

Some elevation of counts at two sites near MECOM/Evi1 (purple dots, circled in red, top).

These sites would not have been remarkable compared to the full population (grey dots) without specific examination and do not reach statistical significance.



Chris Baum (HMS)
Chad Harris (BCH)
Martijn Brugman (HMS)

Gene transfer for SCID-X1 using a self-inactivating (SIN) gammaretroviral vector

A multi-institutional phase I/II trial evaluating the treatment of SCID-X1 patients with retrovirus-mediated gene transfer

Sites:
Great Ormond Street Hospital, UK
Hôpital Necker Enfants Malades, France
Children's Hospital Boston, US
Cincinnati Children's Hospital Medical Center, US
Mattel Children's Hospital, Los Angeles, US

Scientific questions:

1. Can change in vector configuration improve safety?
2. Can 'weaker' internal promoter maintain efficacy?
3. Does the deletion of U3 region lead to any differences in integration sites *in vivo*?

CLINCAL DATA

Gene transfer for SCID-X1 using a self-inactivating (SIN) vector

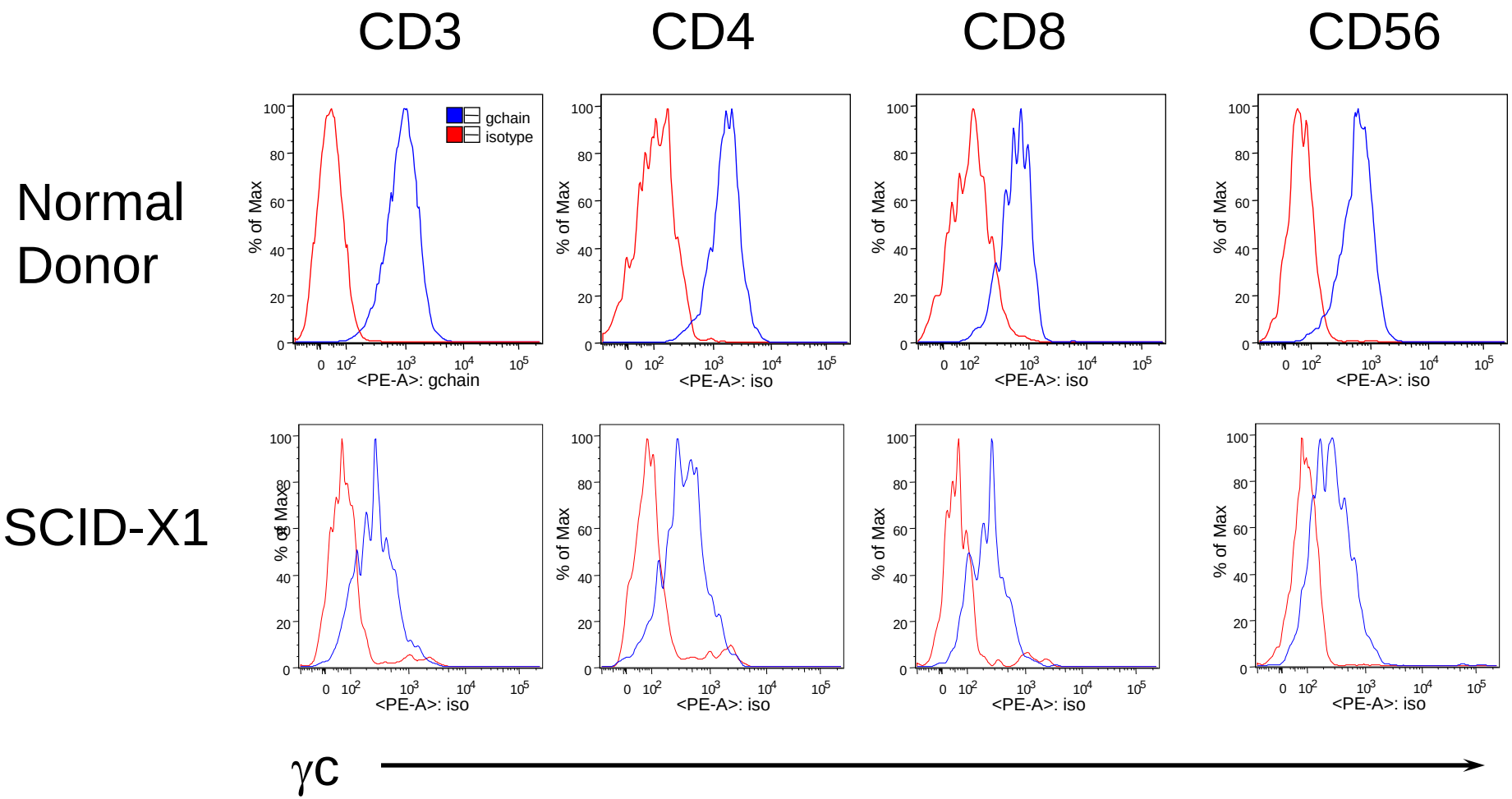
- Status
  - Open in Paris, London, Boston, Cincinnati, Los Angeles
  - 12 patients enrolled
  - Good transduction, cell yields and early engraftment data for majority
  - No SAEs related to investigational reagent to date

Data Summary

- At 12-38 months f/u, 8/9 subjects are surviving.
  - Pre-existing infections resolved in all but 1 subject
  - One patient died of overwhelming adenoviral infection prior to reconstitution with genetically modified T cells.
- Median dose of CD/34+ cells was 7.8 X 10^6 /kg (3.7-11.7).
- VCN ranged from 0.25-2.92 and was stable over up to 3 years post infusion of gene modified cells.
- In 8 evaluable patients, CD3+, CD4+ and CD8+ T cell recovery was achieved and sustained in 6 patients
  - 2 failures to reach primary endpoint at d120.
  - Both remained healthy, one received a cord blood transplant; one received a second infusion of transduced cells at 17.5 months following DSMB/FDA/IRB review.

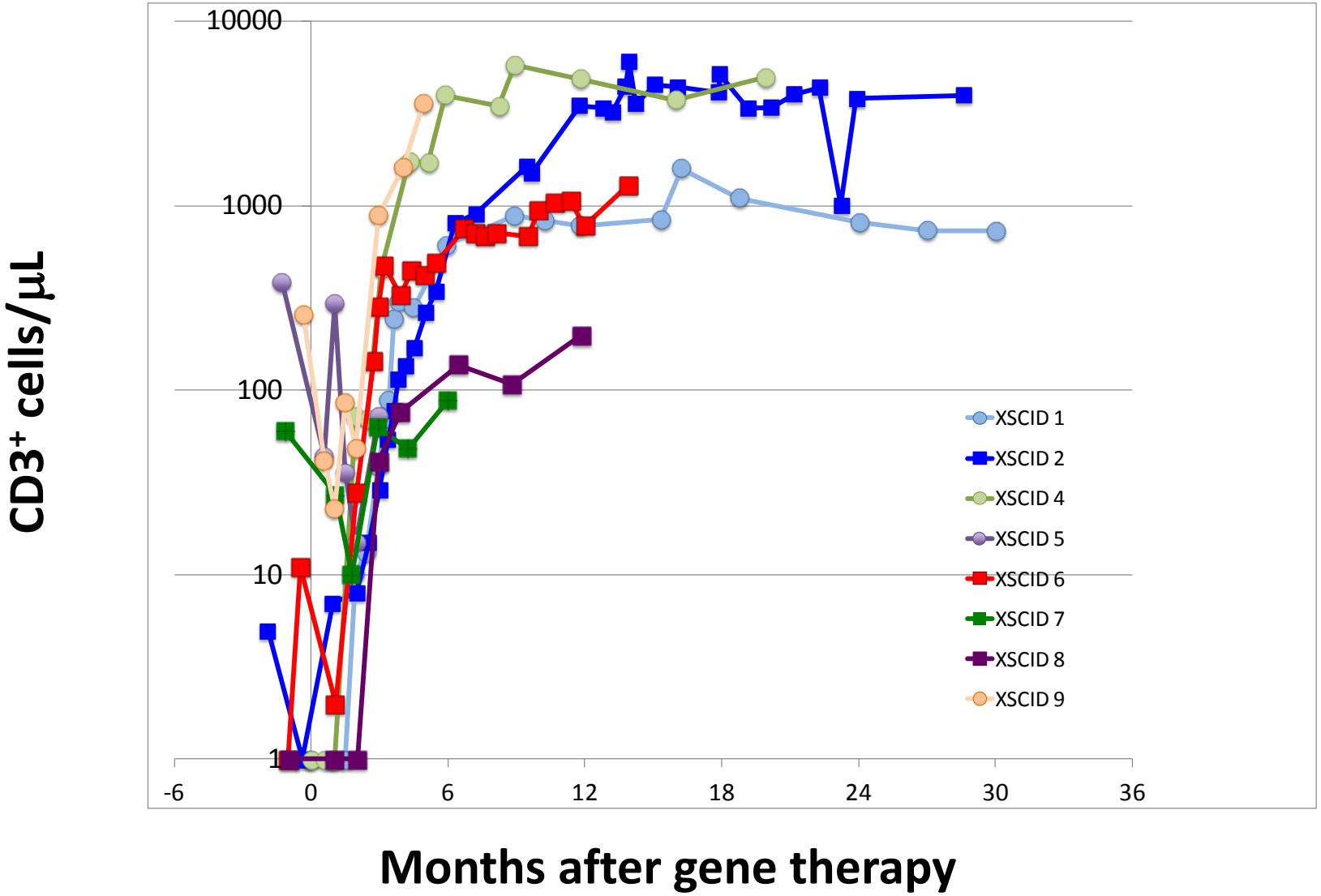
Hacien-Bey-Abina S, Pai S-Y et al. NEJM 371: 1407-1417, 2014.

γc expression in T and NK cells day +90 after GT Boston SCID-X1 00001



Sung-Yun Pai

Longitudinal enumeration of CD3+ T cells of first 9 subjects shows efficacy



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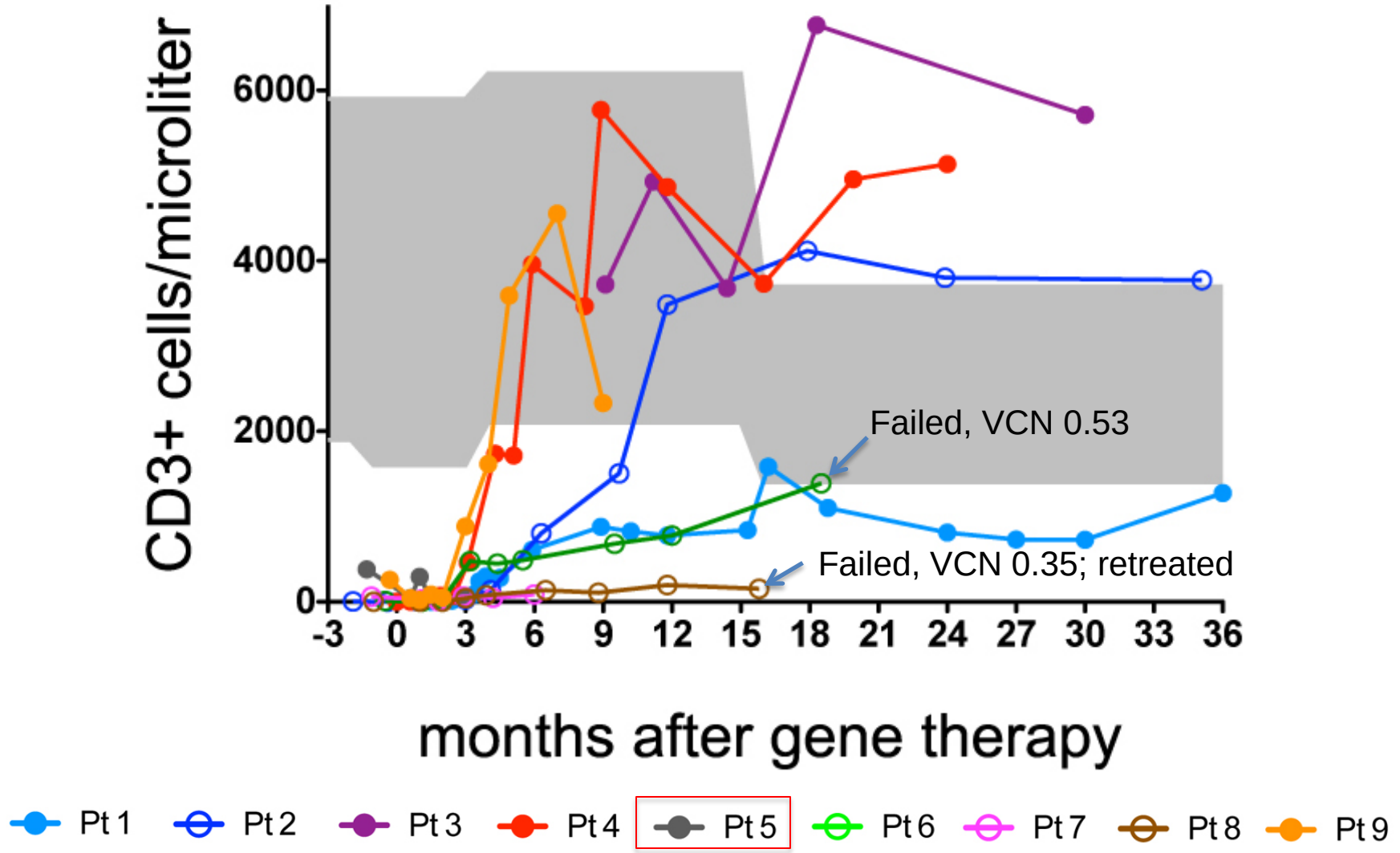
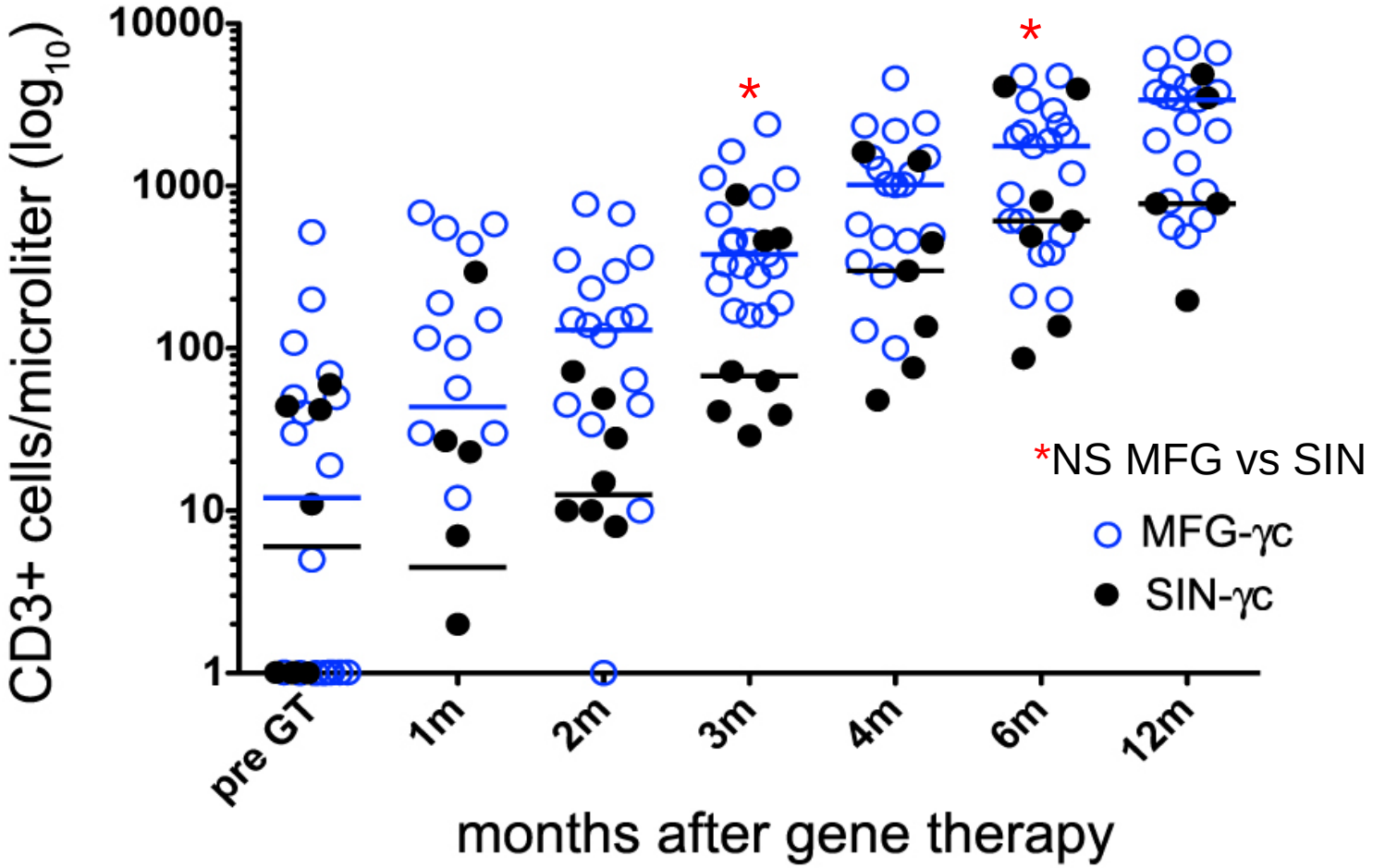
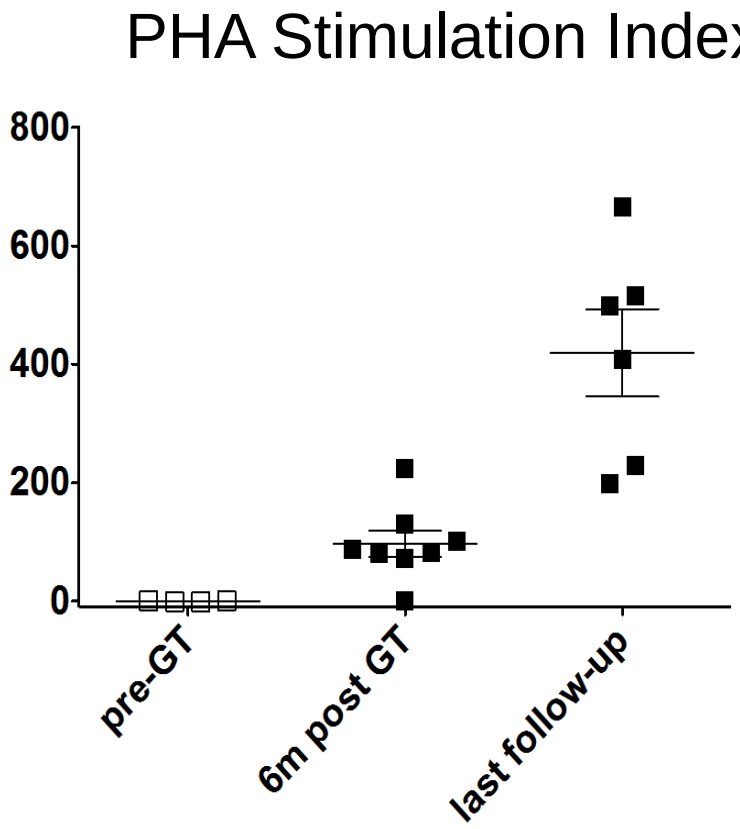


Comparison of CD3+ T cell recovery between vectors



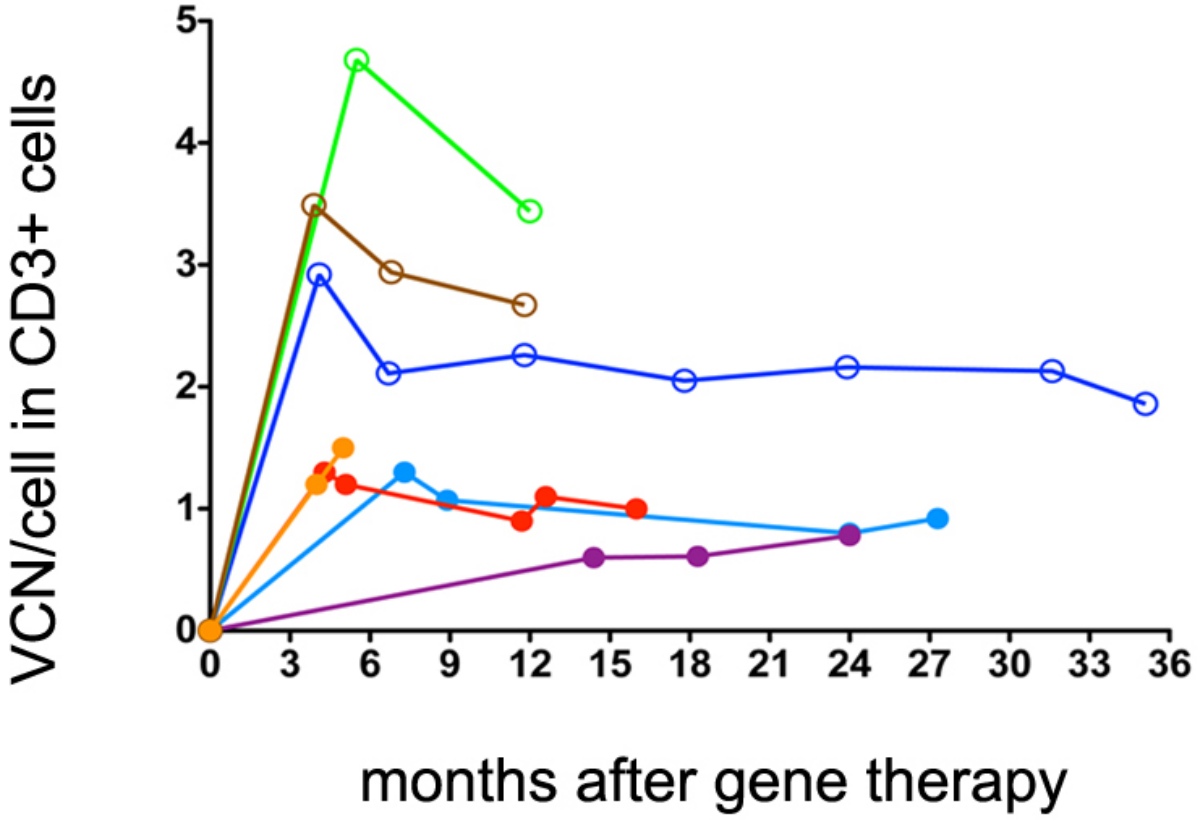
T cell function after engraftment of gene modified cells



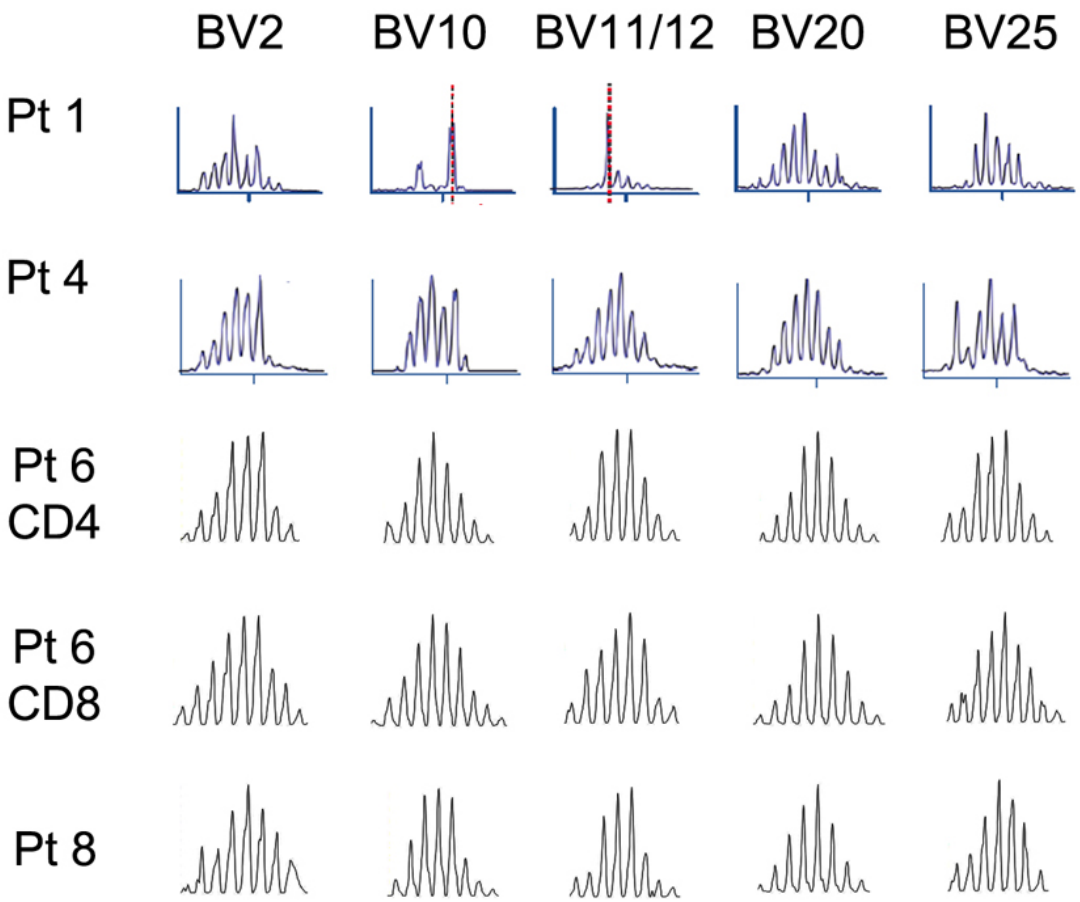
Months after gene therapy

SAFETY DATA

CD3 vector copy number



Diversity T cell reconstitution



Comparison of integration site distributions in subjects treated in the SCID1 and SCID2 trials

SCID 1: 5/20 subjects suffered acute T-ALL
SCID 2: 0/9 acute T-ALL

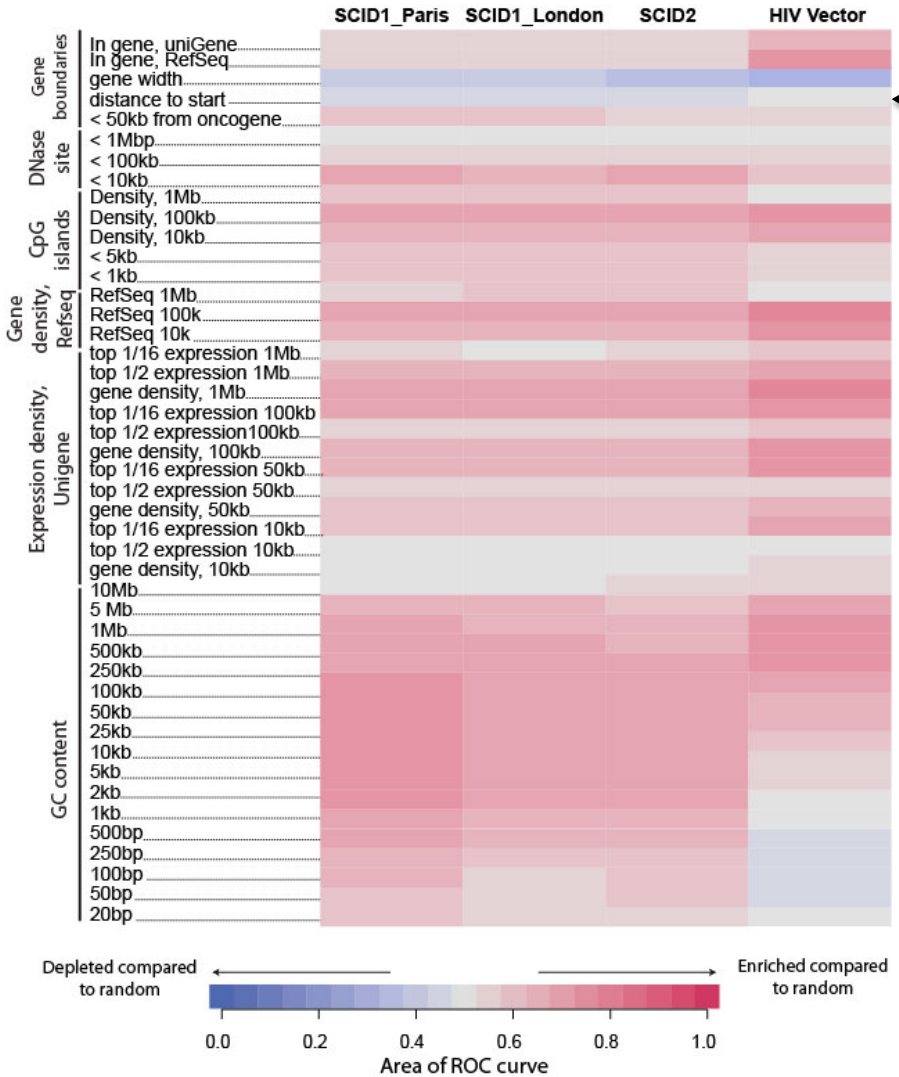
Compare behavior of cell clones as reported by integration site sequence data from circulating blood cells

SCID1 data:1.3×10^4 unique integration sites, 2.7×10^5 sequence reads.

SCID2 data: 2.9×10^4 unique integration sites, 2.8×10^5 sequence reads.

Thanks to Manfred Schmidt and Christoph Von Kalle for some of the SCID1 data

Comparison of SCID1 and SCID2 trials: global relationship to genomic features

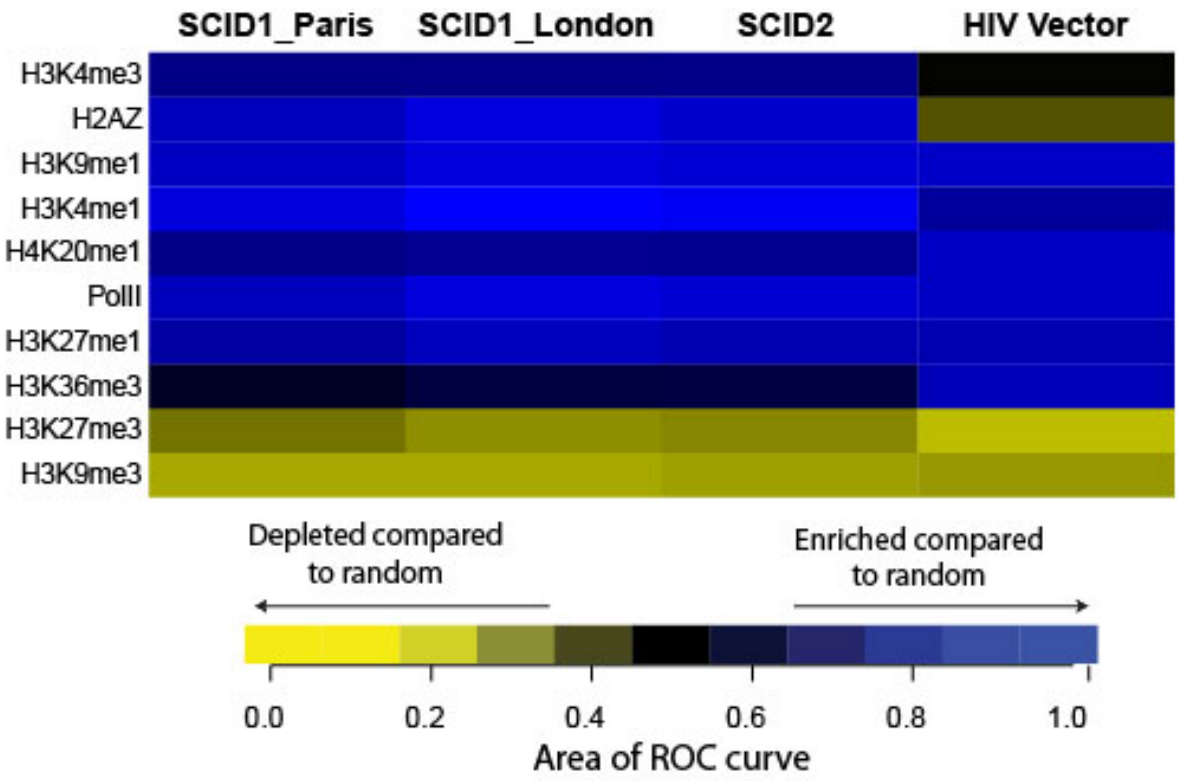


short distances to transcription start site favored by gammaretroviral vectors but not HIV-based vectors

Global patterns very similar between SCID trials.

The vector modifications, including the SIN deletion, did not make an obvious difference in integration site distribution

Comparison of SCID1 and SCID2 trials: global relationship to histone post-translational modification, Pol2, and H2AZ



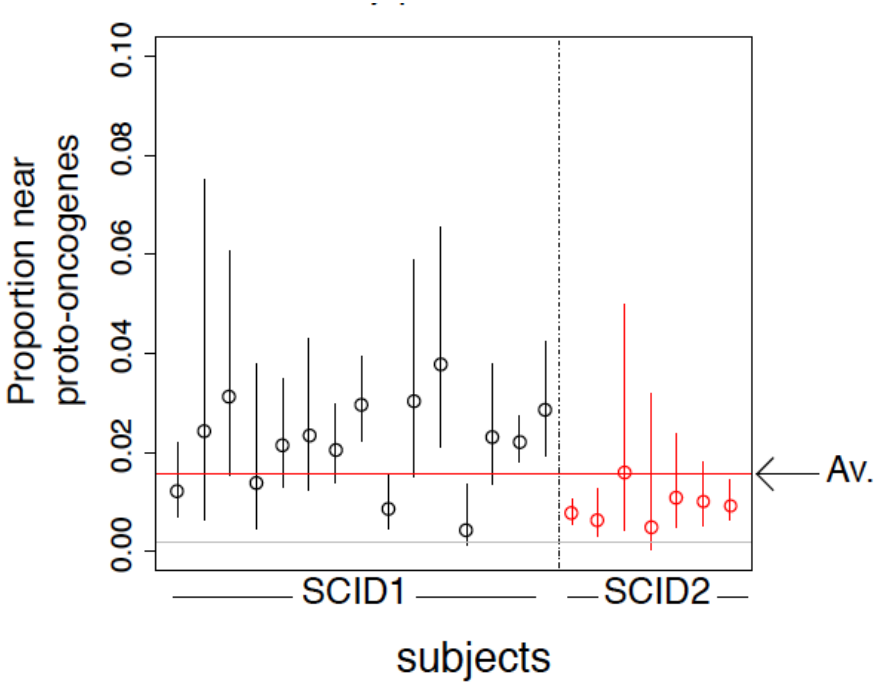
Integration favored near marks associated with active genes

Global patterns very similar between SCID trials.

The vector modifications, including the SIN deletion, did not make an obvious difference in global integration site distribution

Frequency of integration near major lymphoid proto-oncogenes

- Assembled list of 38 genes that are major bad actors in lymphoid cancer
- Determine proportion of integration sites within 50 kb of transcription start site of genes in this class



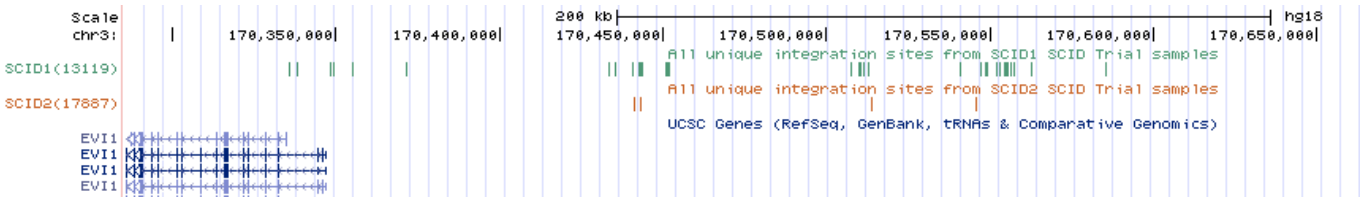
p=0.003

(patients with fewer than 100 integration sites were omitted)

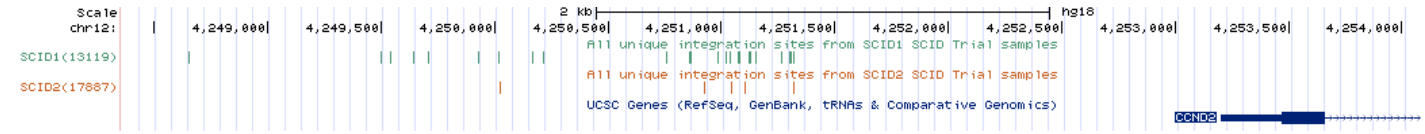
Taking each subject as a data point, the difference achieves significance, with more integration near lymphoid proto-oncogenes in SCID1

Clustering of integration sites differed between SCID1 and SCID2

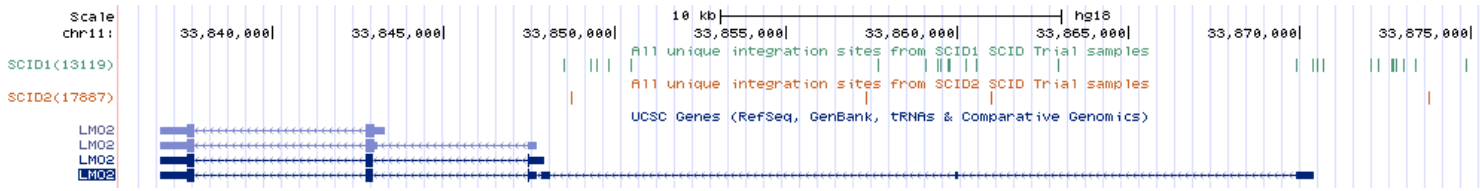
EVI1



CCND2



LMO2



- Devised method for identifying integration site “clumps” where clumps can be of different sizes (Berry et al., 2014). Significance tests involved permutation by patient.
- Identified 45 differential clumps (SCID1 vs SCID2) False discovery rate of 0.12. No convincing differences SCID1 British versus French subjects.
- The most extreme differential clumps were all clumps in SCID1. From most extreme, these involved: EVI1, CCND2, and LMO2.

Summary I

- Development of enhancer-deleted IL2RG vector with ‘improved safety’ characteristics in non-clinical models
  - Represents unique international collaboration with shared costs, developmental work and clinical trial
- Evidence of efficacy by T cell recovery
  - Higher vector copy number (VCN) correlated with successful T cell engraftment.
  - The 6 subjects with a VCN in the CD34+ graft of ≥0.7 copies/cell achieved T cell reconstitution, which was also heralded by an early rise in CD56+ NK cells.

Summary II

- The presence of the SIN deletion correlated with reduced integration near major lymphoid proto-oncogenes (taking each patient as a datapoint).
- Integration sites on chromosomes differed between the trials, with the presence of intact enhancer correlating with integration clumps at *EVI1, CCND2, LMO2* and other genes of concern.
- Since the use of SIN deleted gammaretrovirus vector was not associated with major alterations in global integration site distributions compared to the intact LTR-vector, these results are consistent with “vector driving” of cell growth or survival in SCID1.

Summary III

- In murine preclinical study, no statistically significant differences between SCID1 and SCID2 integration site data, however there were more integrations near *Evi1* in mice transplanted with cells transduced with the intact LTR-vector.
- The preclinical model did not predict the difference in performance of the two vectors.

Acknowledgments

Gene transfer for SCID-X1 using a self-inactivating (SIN) gammaretroviral vector

A multi-institutional phase I/II trial evaluating the treatment of SCID-X1 patients with retrovirus-mediated gene transfer

Children's Hospital Boston, Local PI: **Luigi Notarangelo,**
Co-PI: **Sung-Yun Pai**
Study Sponsor, US sites: David A. Williams

Overall Chief Investigator: Adrian Thrasher, GOSH
Co-Investigators: Alain Fischer, Marina Cavazzana-Calvo and Salima Abina Hacein-Bey (Hôpital Necker), Donald Kohn and Alan Ikeda (UCLA), Alexandra Filipovich and Punam Malik (CCHMC)
Collaborators: Chris Baum, Hannover Medical School, Germany
Rick Bushman, University of Pennsylvania Medical School

It takes a great team!

Gene Therapy Program Manager
Colleen H. Dansereau, RN (H/O)

HSCT Program
Leslie Lehmann, M.D. *(H/O)*

Data management (InForm) and Biostats
Clinical and Translational Investigation Program (H/O)
Wendy London PhD, Samantha Reilly, QACT

Consent Monitor
Stephen Brown,M.D.
(Radiology)

Research RNs
Brenda McKinnon, RN (CTSU)
Maggie Malsch, RN (CTIP H/O)
Cindy Williams, *(Patient Services)*

CRC
Megan Piaquadio (H/O)

Gene Therapy Program Administrative Assistant
Natasha Rossi

Pre-GMP SOP Development:
Myriam Armant, PhD; Les Silberstein M.D

Cell Manipulation*:* DFCI, Connell and O'Reilly Families Cell Manipulation Core
Jerry Ritz, M.D.
GMP: Grace Kao, M.D.
Quality control procedures
Olive Sturtevant

Insertional analysis:
Rick Bushman, Charles Berry, Nirav Malani,Frances Male, and Troy Brady, UPENN

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