Gene Therapy for Primary Immune Deficiencies

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Human Gene and Cell Therapy Program

Broad Stem Cell Research Center

Jonsson Comprehensive Cancer Center

The Wooden Centeer

Hypothesis:

Gene therapy using **autologous HSC** that are corrected with the normal gene will have beneficial effects on blood cell production or function, without the immunologic complications of allogeneic HSCT.

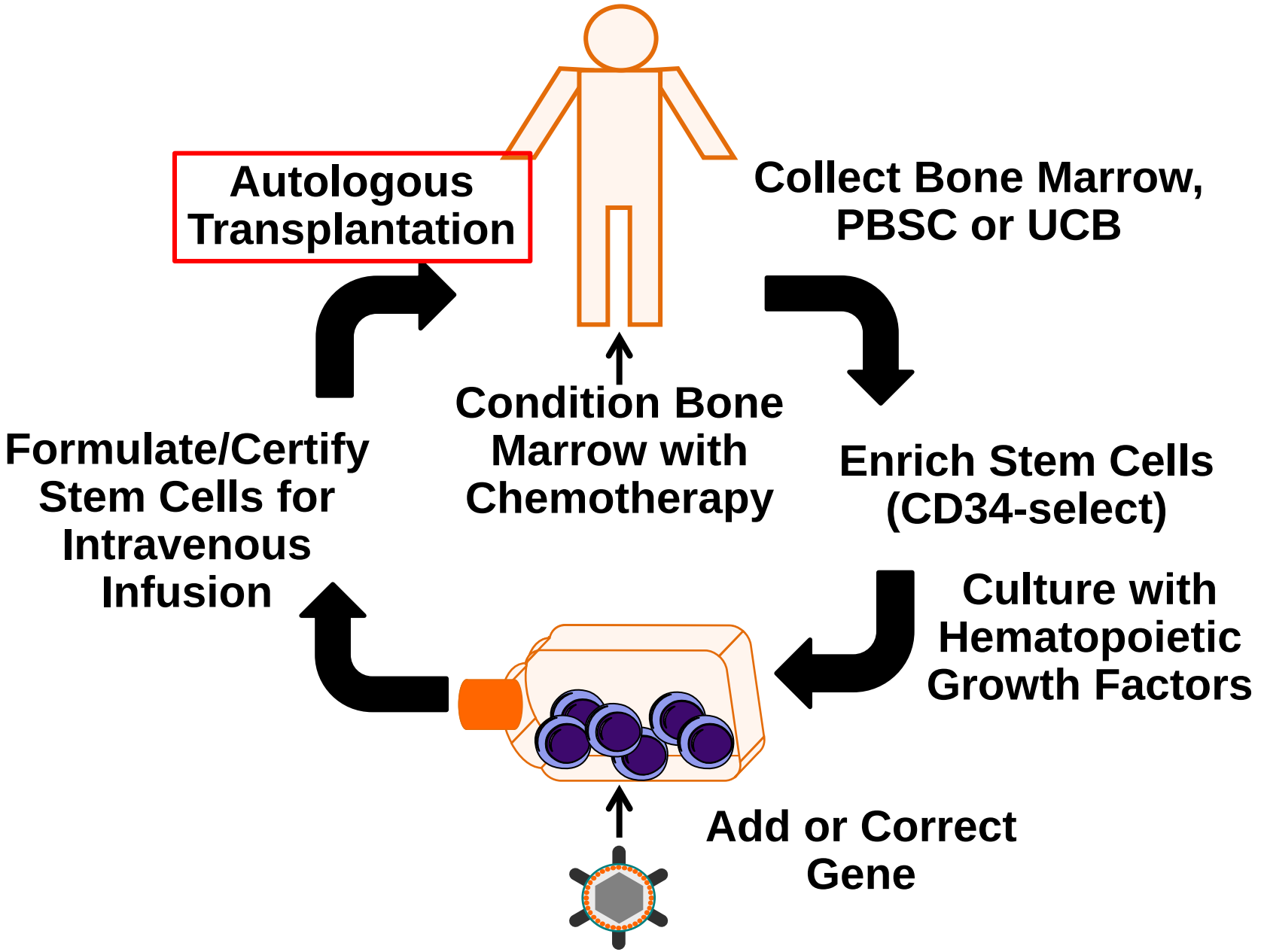
May also use same techniques to genetically engineer HSC to produce blood cells with:

a) resistance to HIV infection (shRNA, CCR5 k/o)

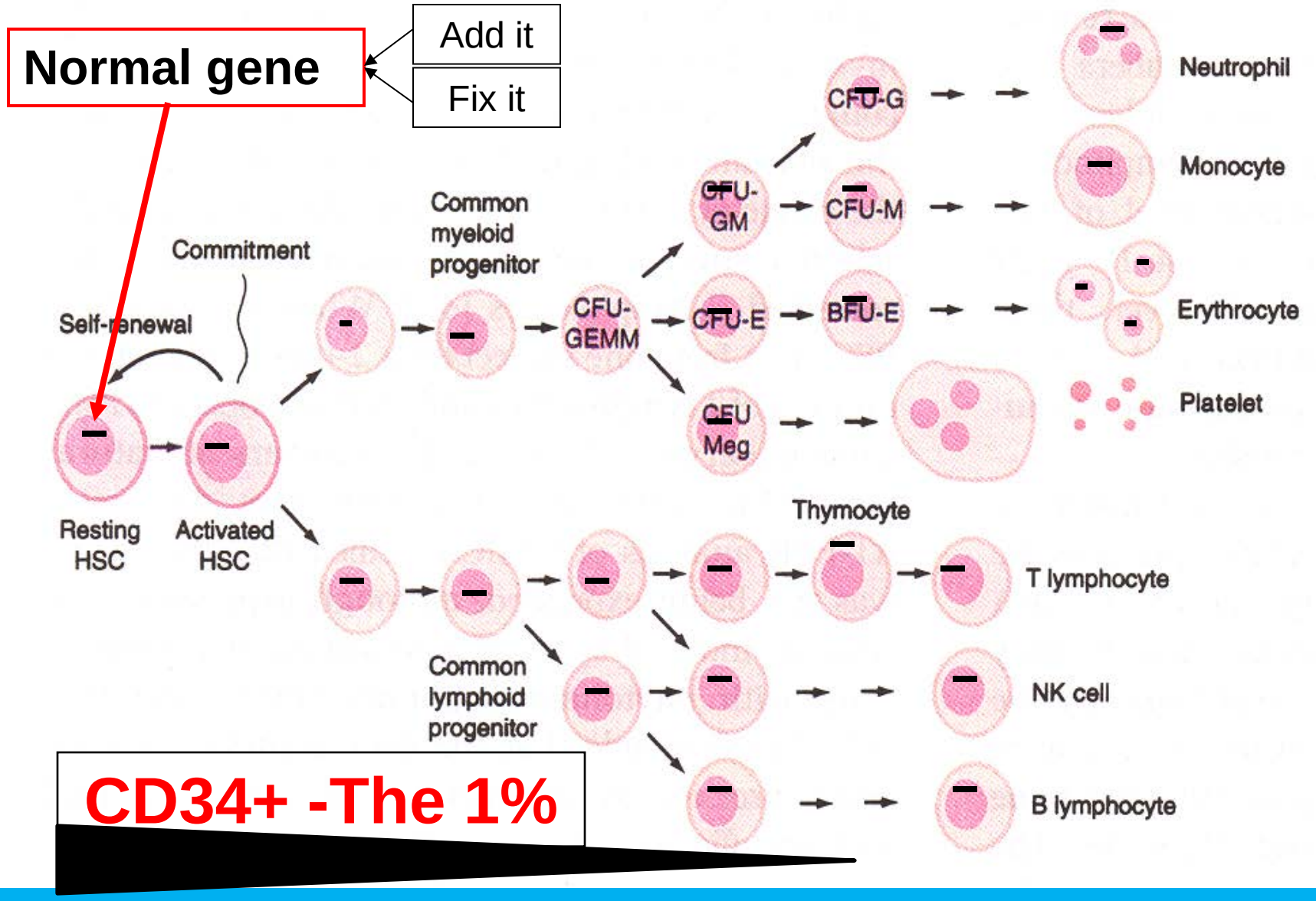
b) specific anti-cancer reactivity (TCR, CAR)

c) reduced suppression by chemotherapy (MGMT, DHFR)

Gene Therapy = Autologous Transplant



Gene Therapy Using Hematopoietic Stem Cells



Adenosine Deaminase (ADA)-Deficient Severe Combined Immune Deficiency (SCID)

Genetic deficiency of ADA enzyme activity is the underlying cause of 10-15% of human SCID.

In the absence of ADA, lymphocytes accumulate high levels of adenosine and deoxyadenosine metabolites (esp. dATP) which are toxic, leading to pan-lymphopenia ($T^-/B^-/NK^-$)

Frequency of ADA-SCID is ~1.7-2.5/million births or approx. 7-10/yr in US + Canada.

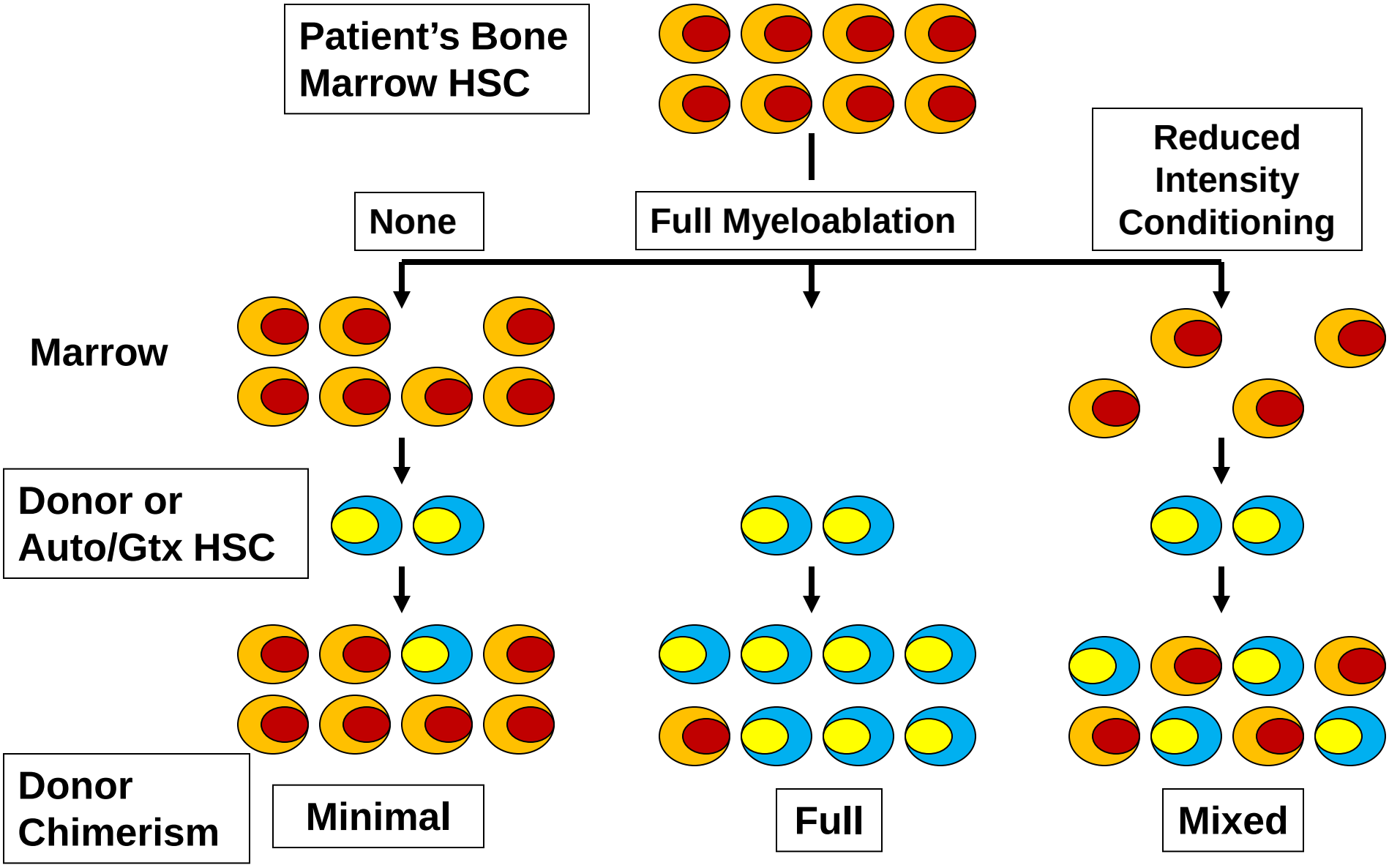
Treatments options include allogeneic HSCT (MSD, MUD, haplo), PEG-ADA enzyme replacement therapy (ERT) or autologous transplant of gene-corrected HSC (gene therapy).

MND-ADA γRV and EFS-ADA LV

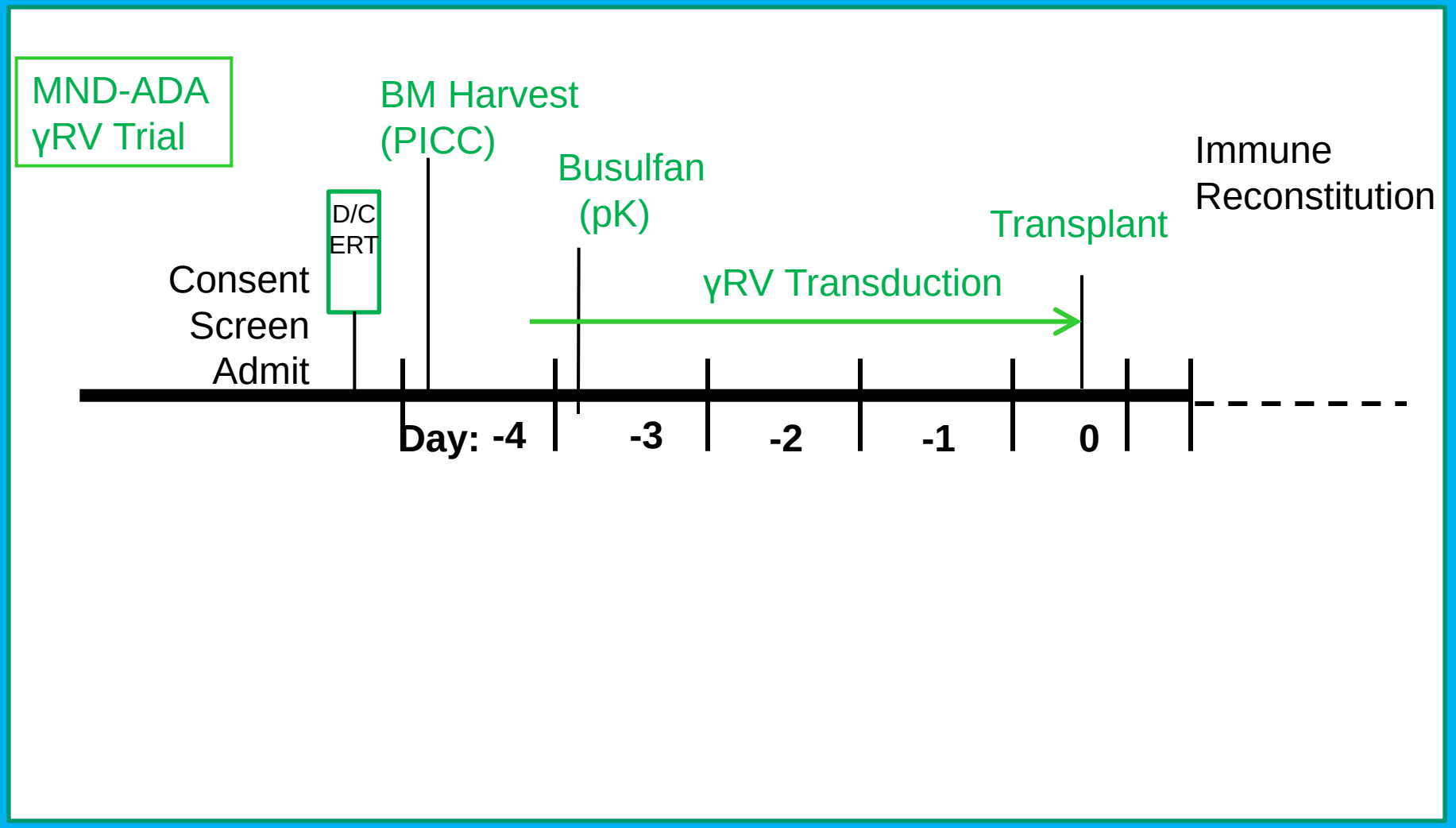
MND-ADA γRV from PG13 clone (GALV).
MPSV γRV LTR drives hADA cDNA.
Titer 3-5 x10^6 TU/ml (unprocessed).

EFS-ADA LV from 293T transient (VSV-G).
Hu EF1α gene short (EFS) promoter drives
codon-optimized hADA cDNA with WPRE, SIN LTR
Titer 3-5 x10^9 TU/ml (TFF concentrated).

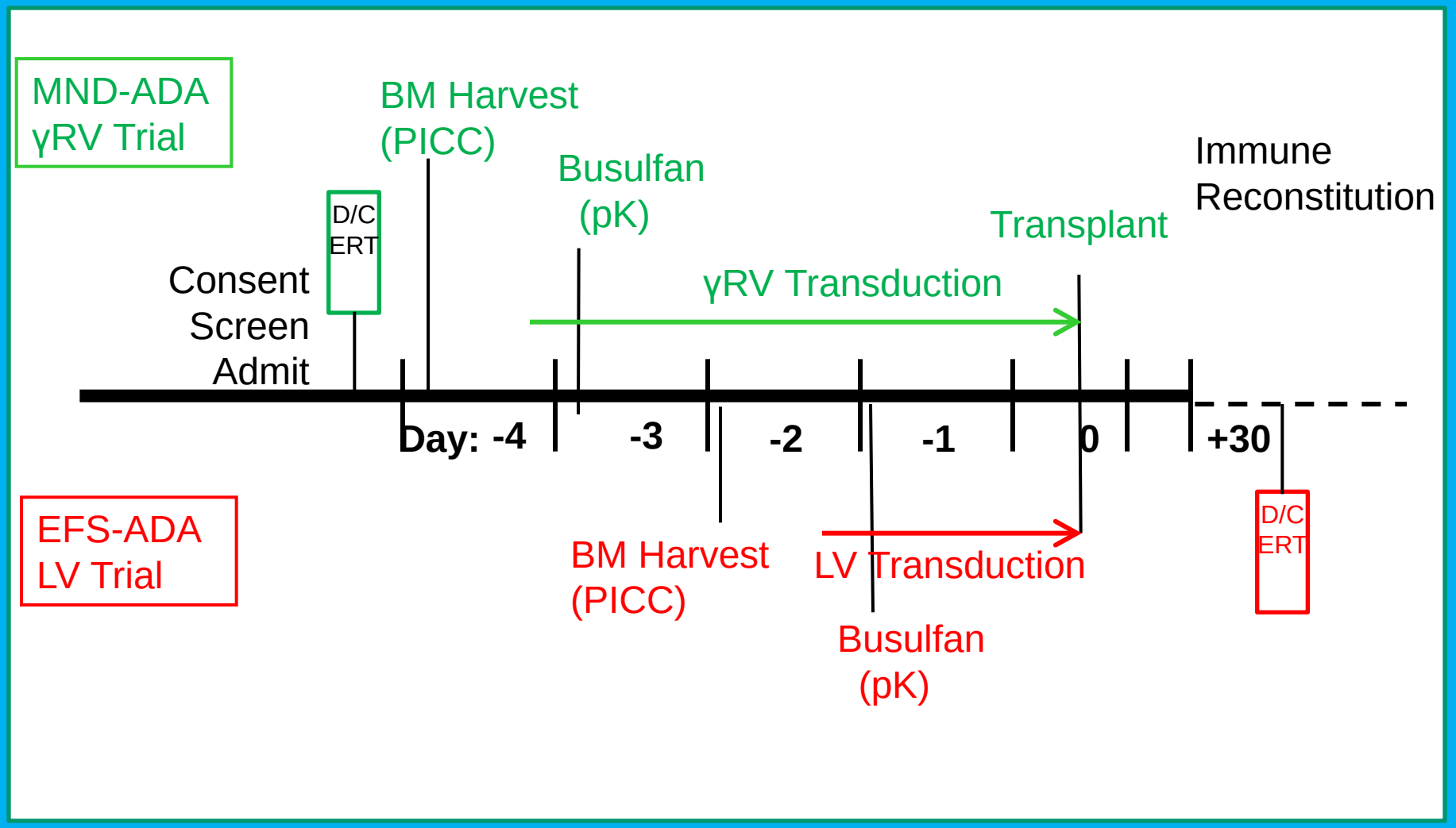
Outcome after HSCT with Full, Partial or No Myeloablative Conditioning

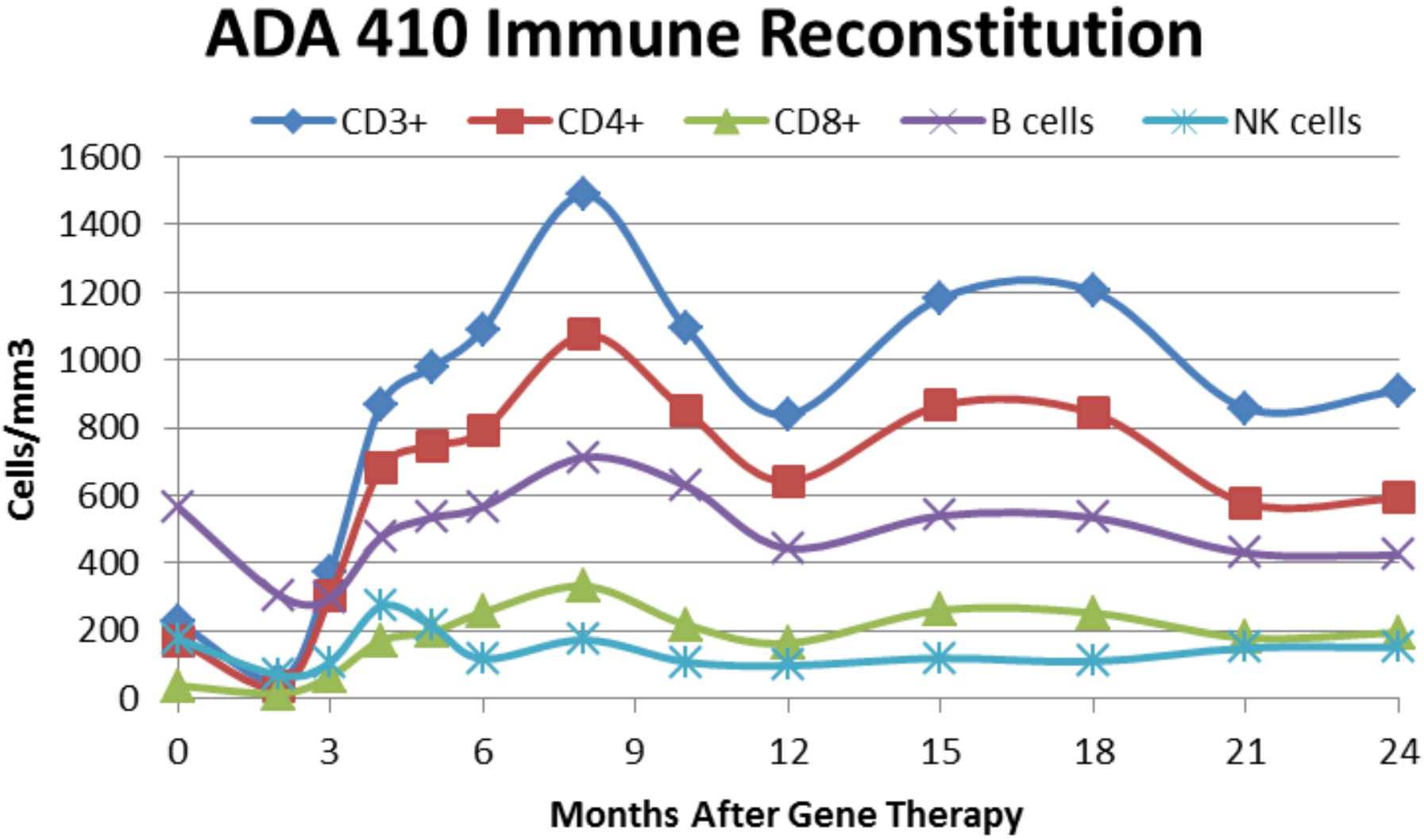


Clinical Trial Experimental Time-Line

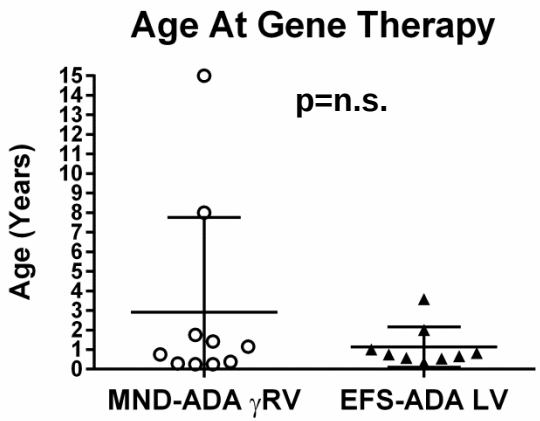


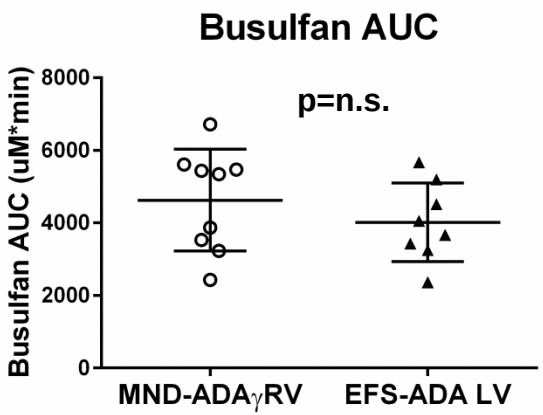
Clinical Trial Experimental Time-Line

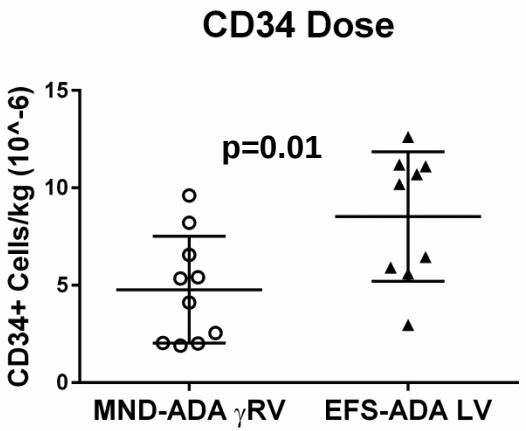


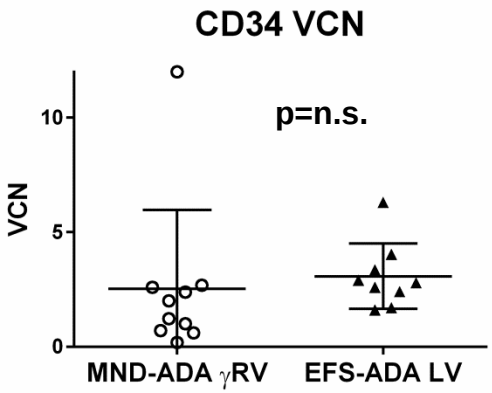

ADA 410 Immune Reconstitution
CD3+
CD4+
CD8+
B cells
NK cells
Cells/mm3
1600
1400
1200
1000
800
600
400
200
0
0
3
6
9
12
15
18
21
24
Months After Gene Therapy

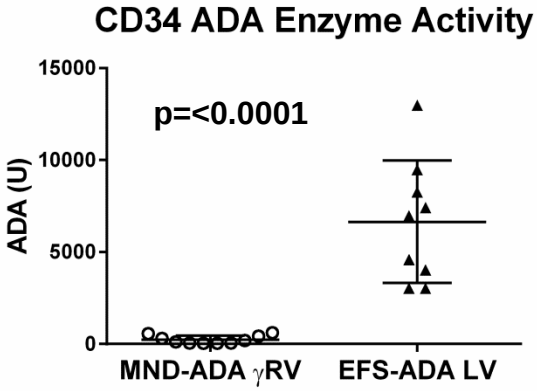
Comparison of Grafts Between MND-ADA γRV and EFS-ADA LV

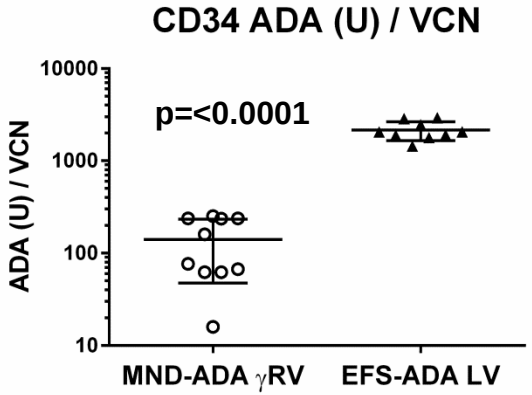




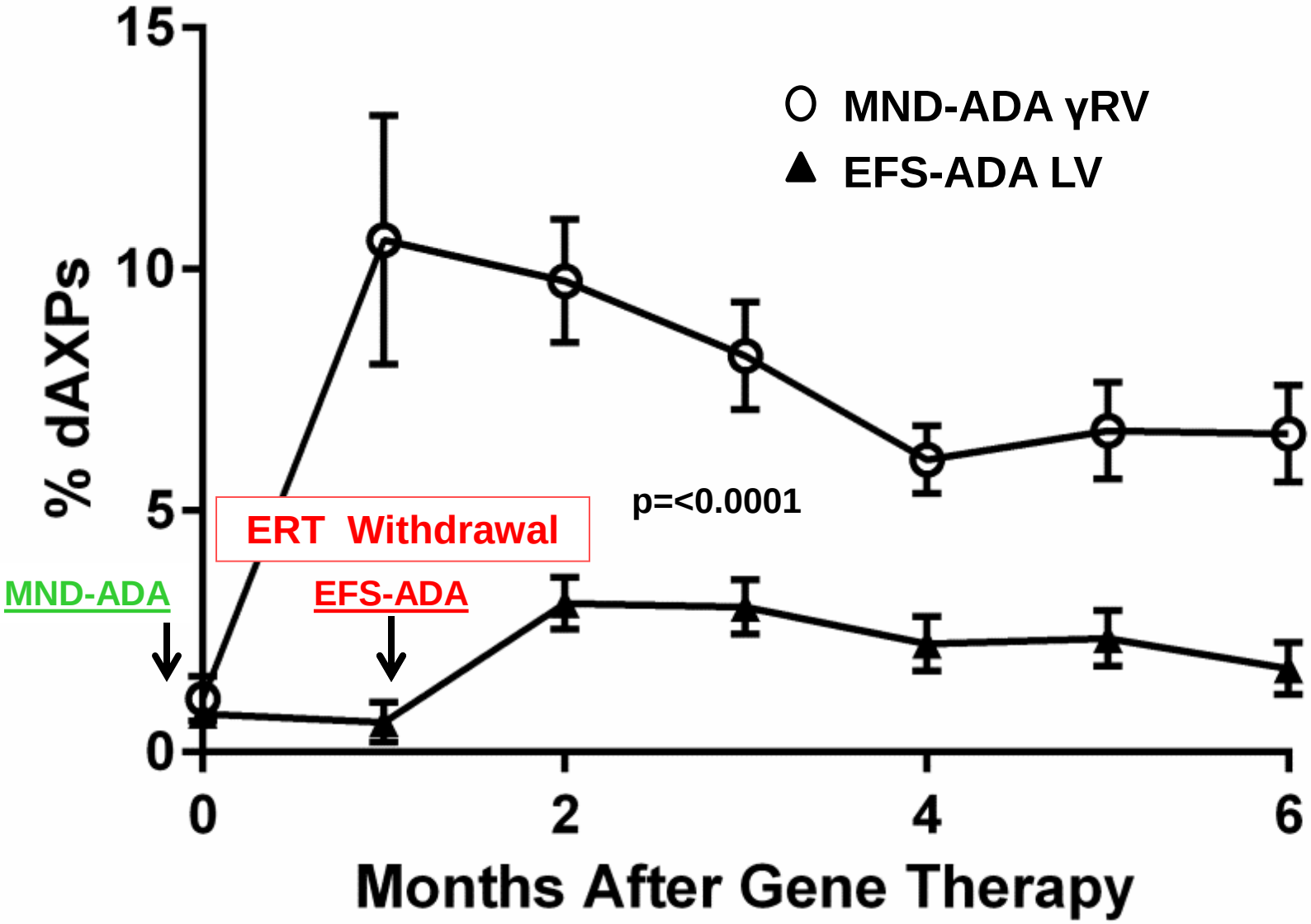


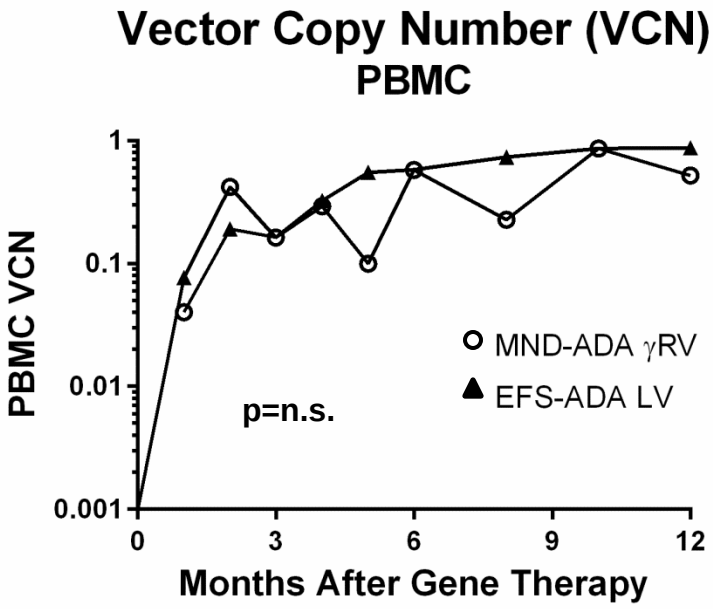


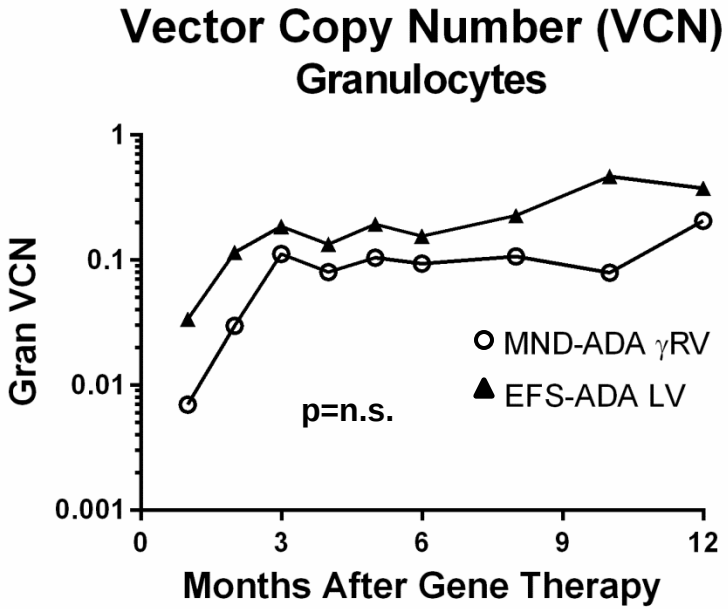


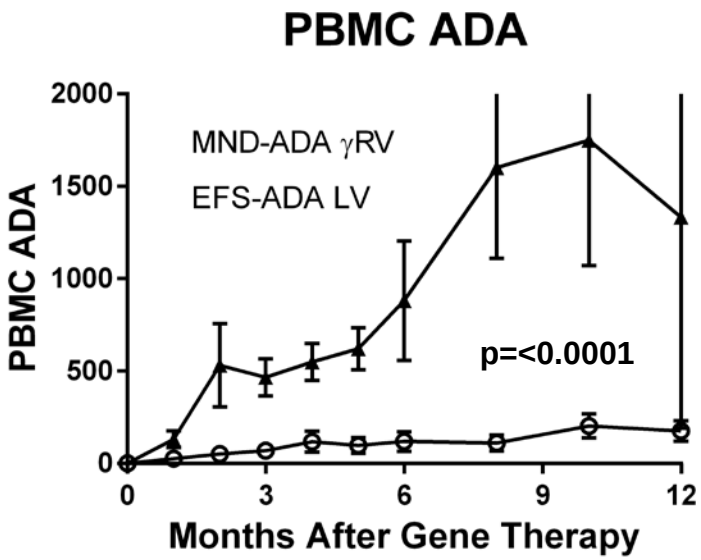


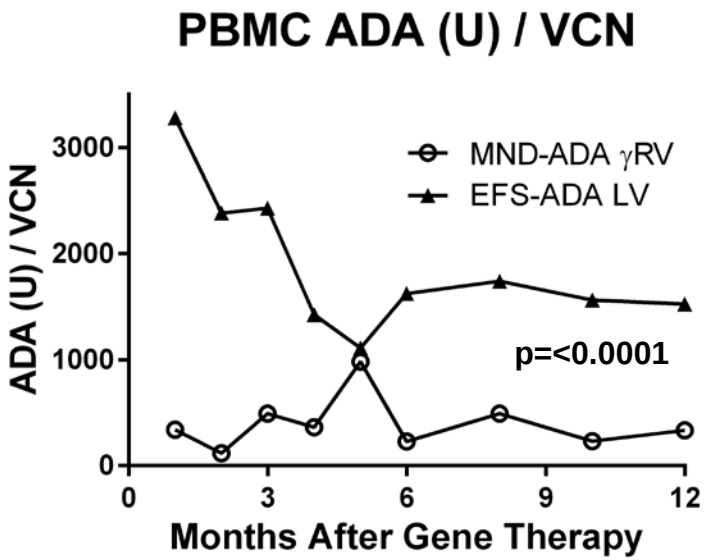
Comparison of Deoxyadenosine Nucleotides (dAXP) in RBC After Gene Therapy and ERT Withdrawal



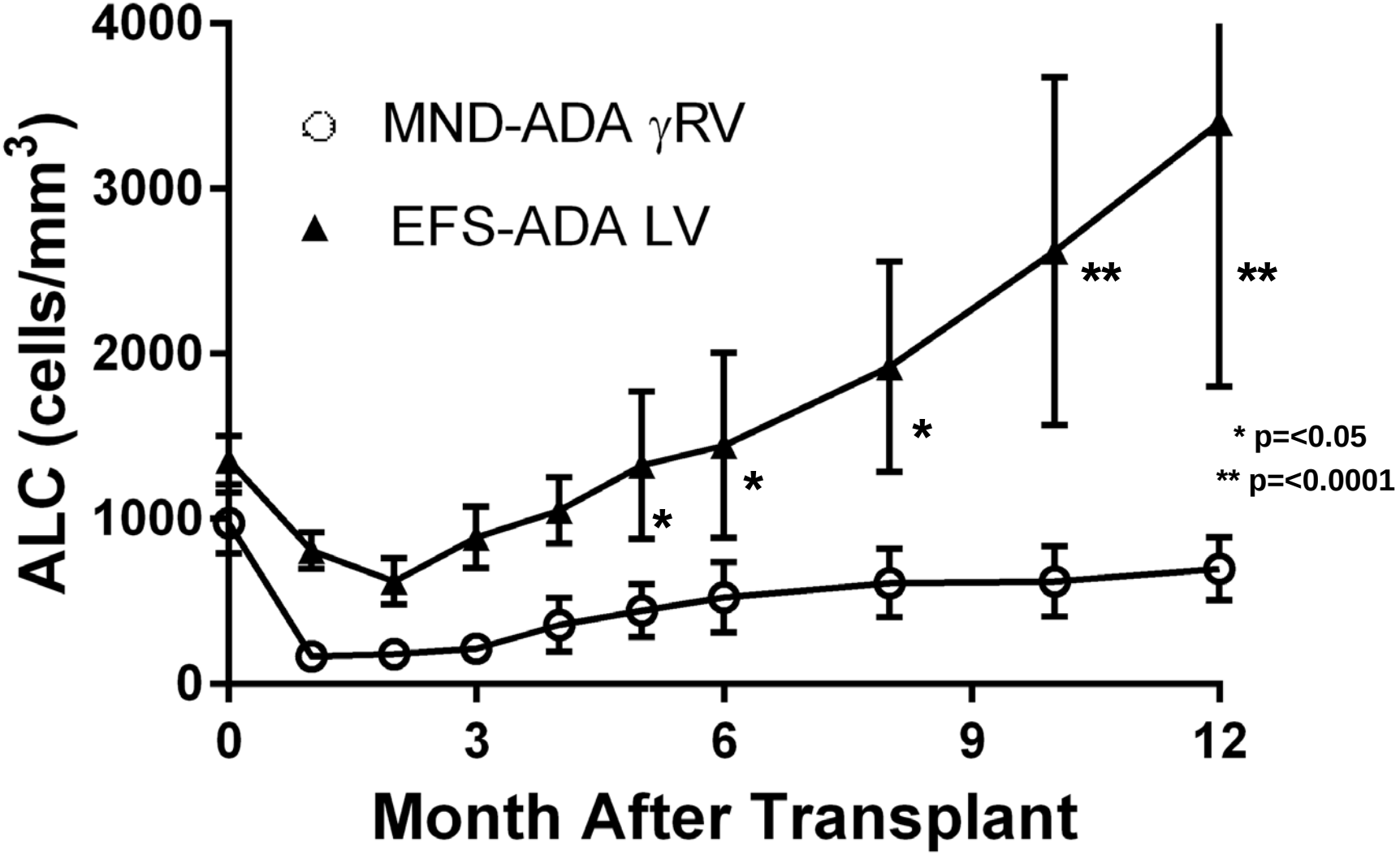

Vector Copy Number (VCN)
PBMC
PBMC VCN
1
0.1
0.01
0.001
0
3
6
9
12
Months After Gene Therapy
MND-ADA γRV
EFS-ADA LV
p=n.s.


Vector Copy Number (VCN)
Granulocytes
Gran VCN
1
0.1
0.01
0.001
0
3
6
9
12
Months After Gene Therapy
MND-ADA γRV
EFS-ADA LV
p=n.s.

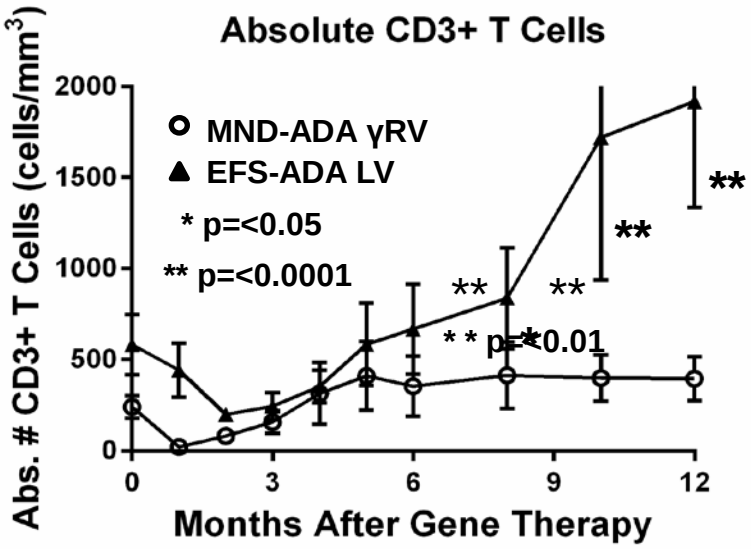

PBMC ADA
PBMC ADA
2000
1500
1000
500
0
0
3
6
9
12
Months After Gene Therapy
MND-ADA γRV
EFS-ADA LV
p=<0.0001

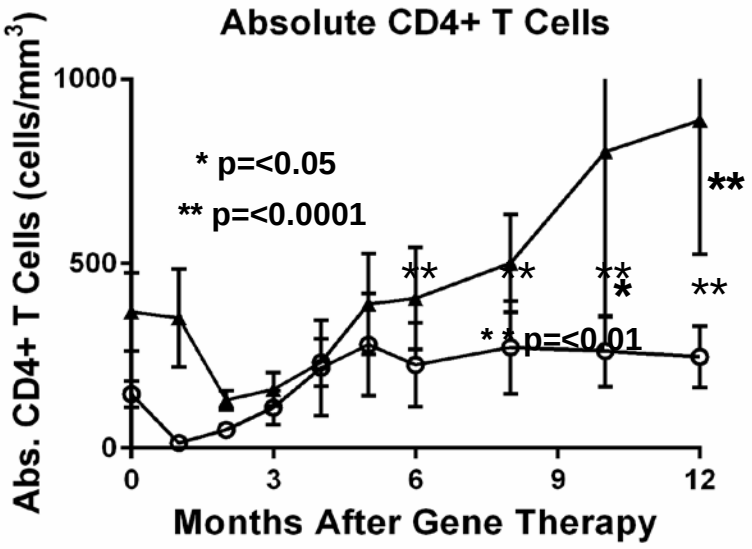

PBMC ADA (U) / VCN
ADA (U) / VCN
3000
2000
1000
0
0
3
6
9
12
Months After Gene Therapy
MND-ADA γRV
EFS-ADA LV
p=<0.0001

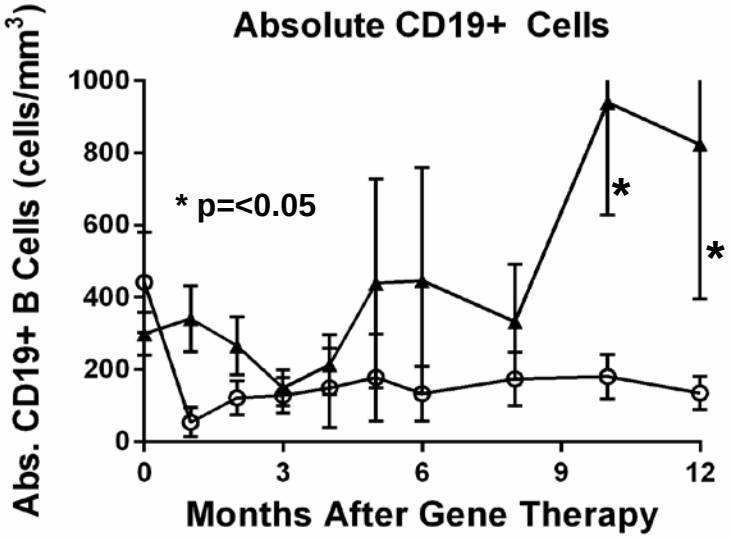
Absolute Lymphocyte Counts (ALC)

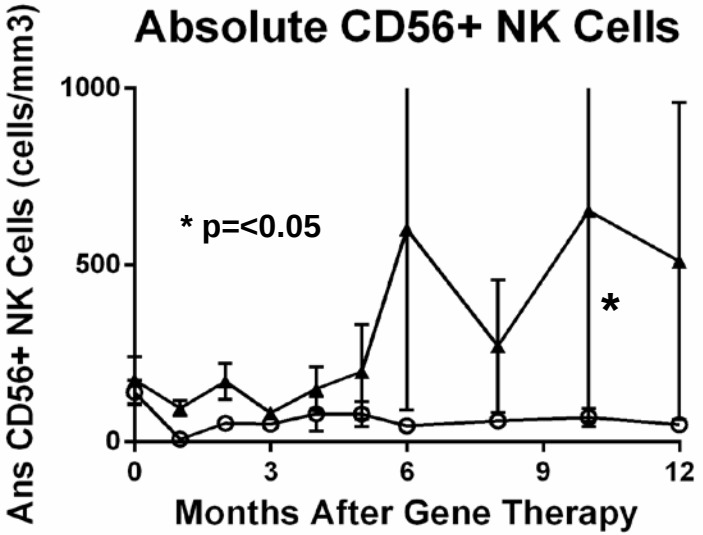


Lymphocyte Recovery After Gene Therapy









Application to FDA for Orphan Drug Designation

Submitted: 072414
Date Designated: 102114

EFS-ADA Phase II/III Clinical Trial(s)

Based on guidance from FDA and EMA, a Phase II/III clinical trial is being planned to be done at UCL/UK and UCLA/US.

Major end-point will be survival compared to historical controls with unrelated or haploidentical allogeneic HSCT

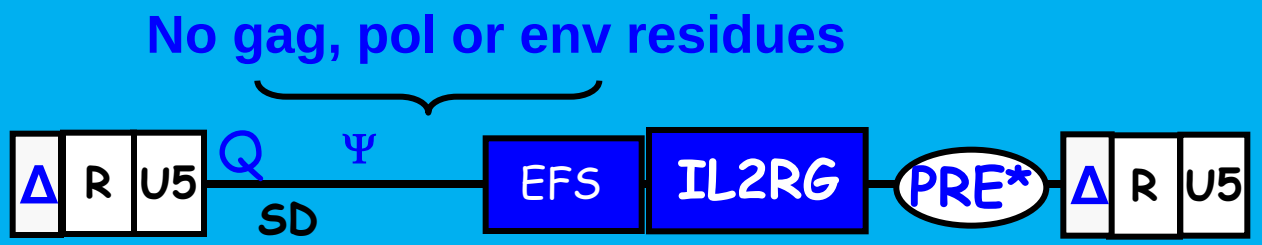
Will use BM for smaller or G-CSF mPBSC for older.

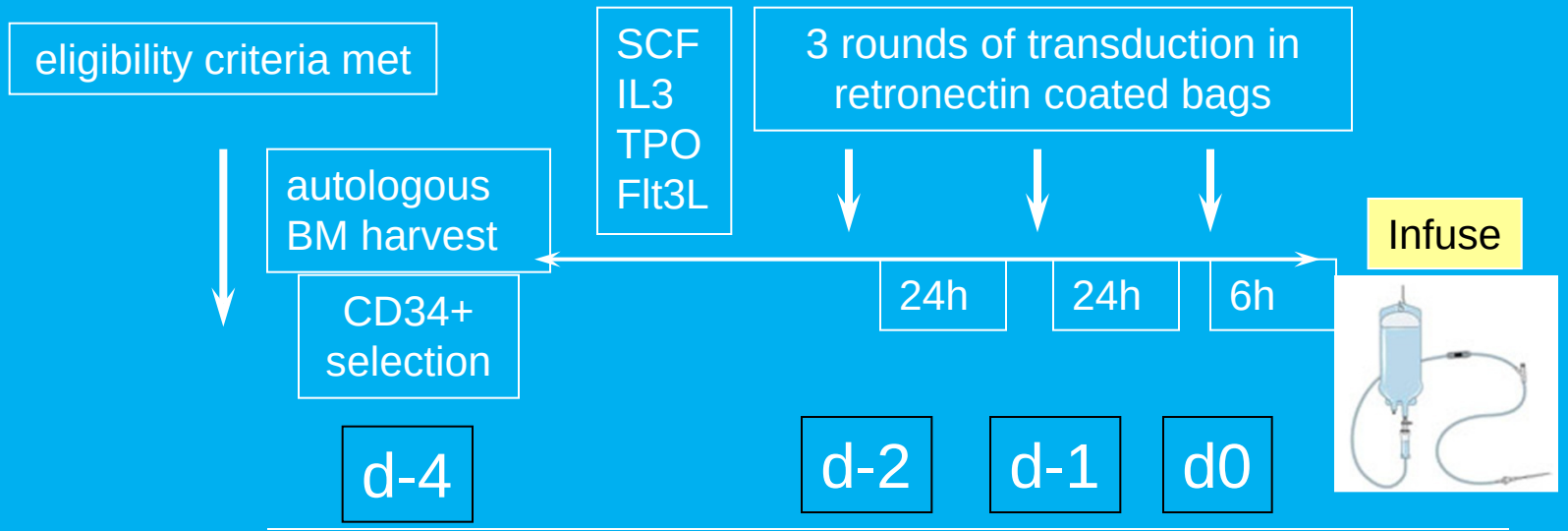
EFS-ADA LV transduced CD34+ final cell product will be cryopreserved and fully characterized prior to transplant.

Busulfan dose will be split into two, to allow subject-specific adjustments based on first dose pK to reach target net AUC = 4,900 uM*min = 20 ug/L*hr.

XSCID Gene Transfer Schema

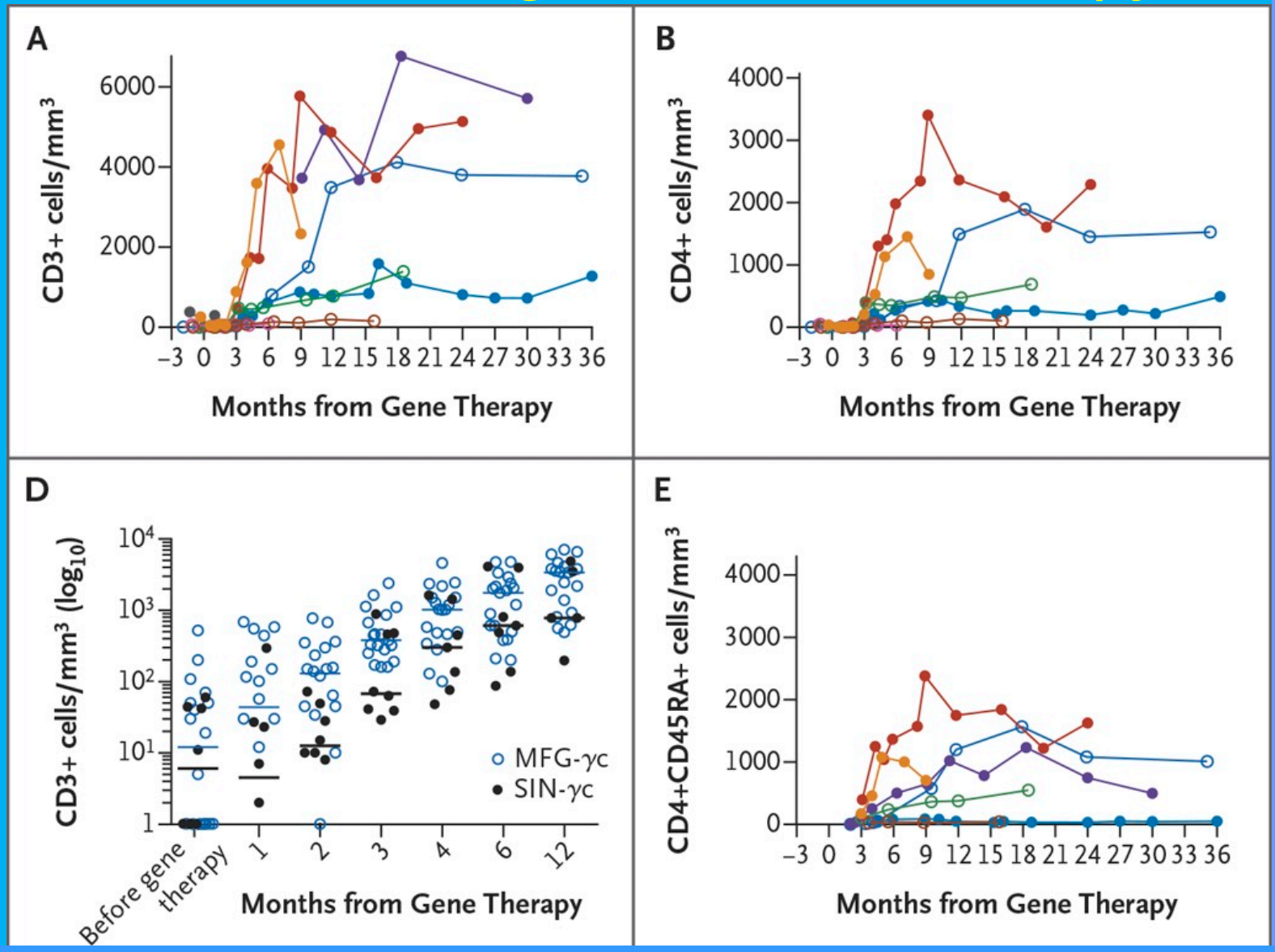
Self-inactivating gammaretroviral vector





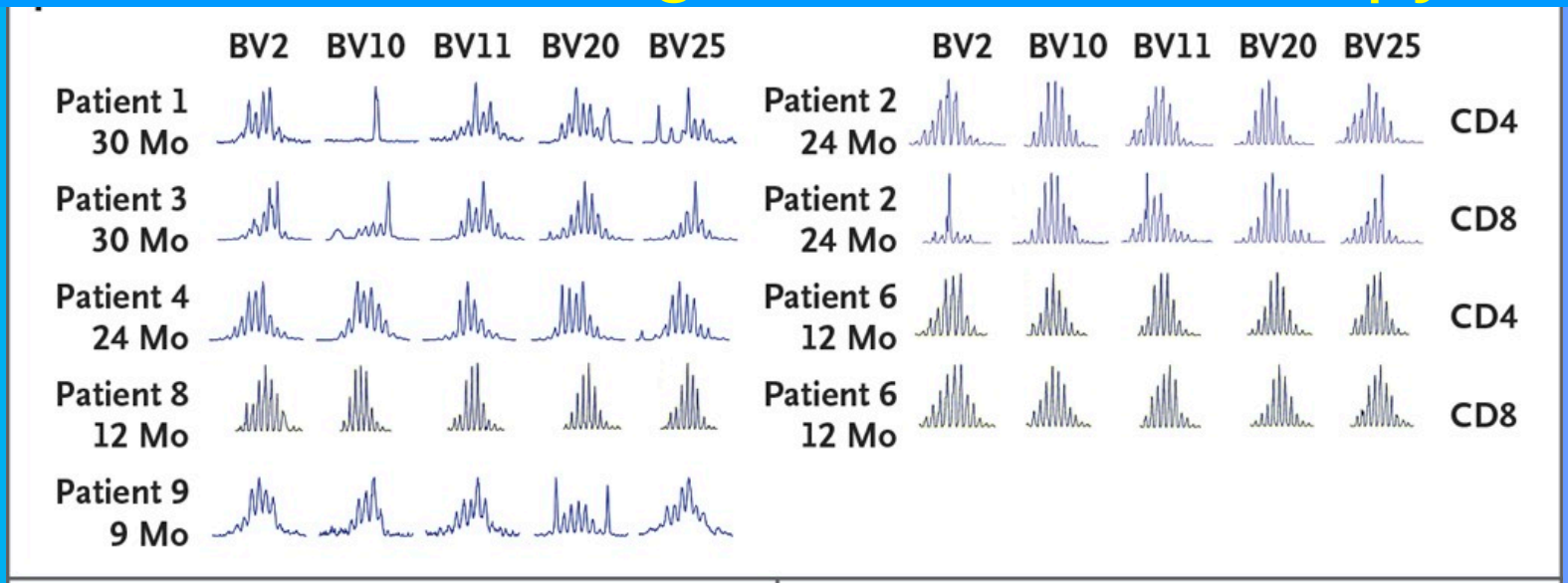
NO CONDITIONING

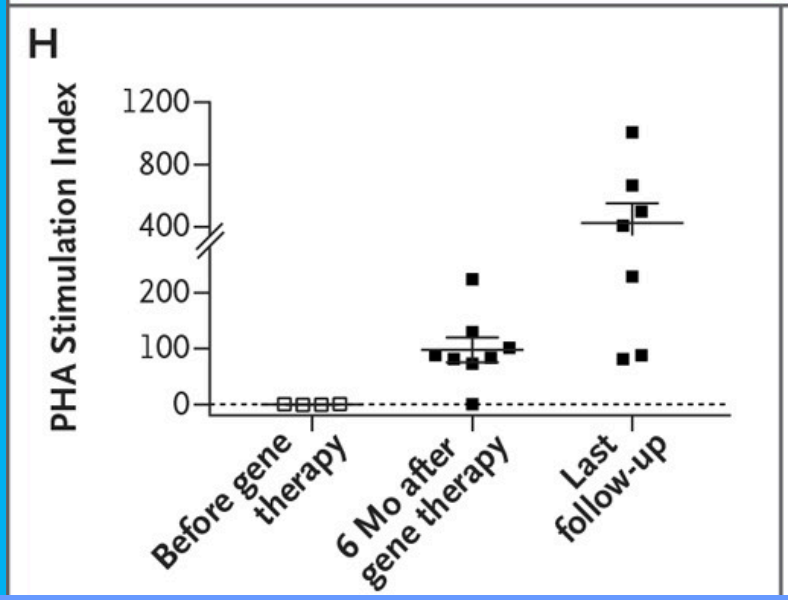
Immune Reconstitution and Gene Marking after Gene Therapy

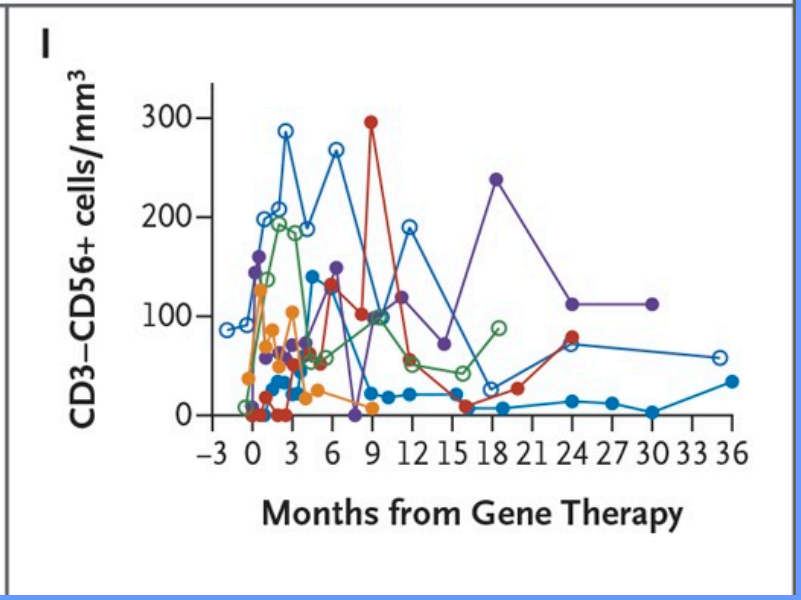


N Engl J Med 371(15):1407-1417, 2014

Immune Reconstitution and Gene Marking after Gene Therapy







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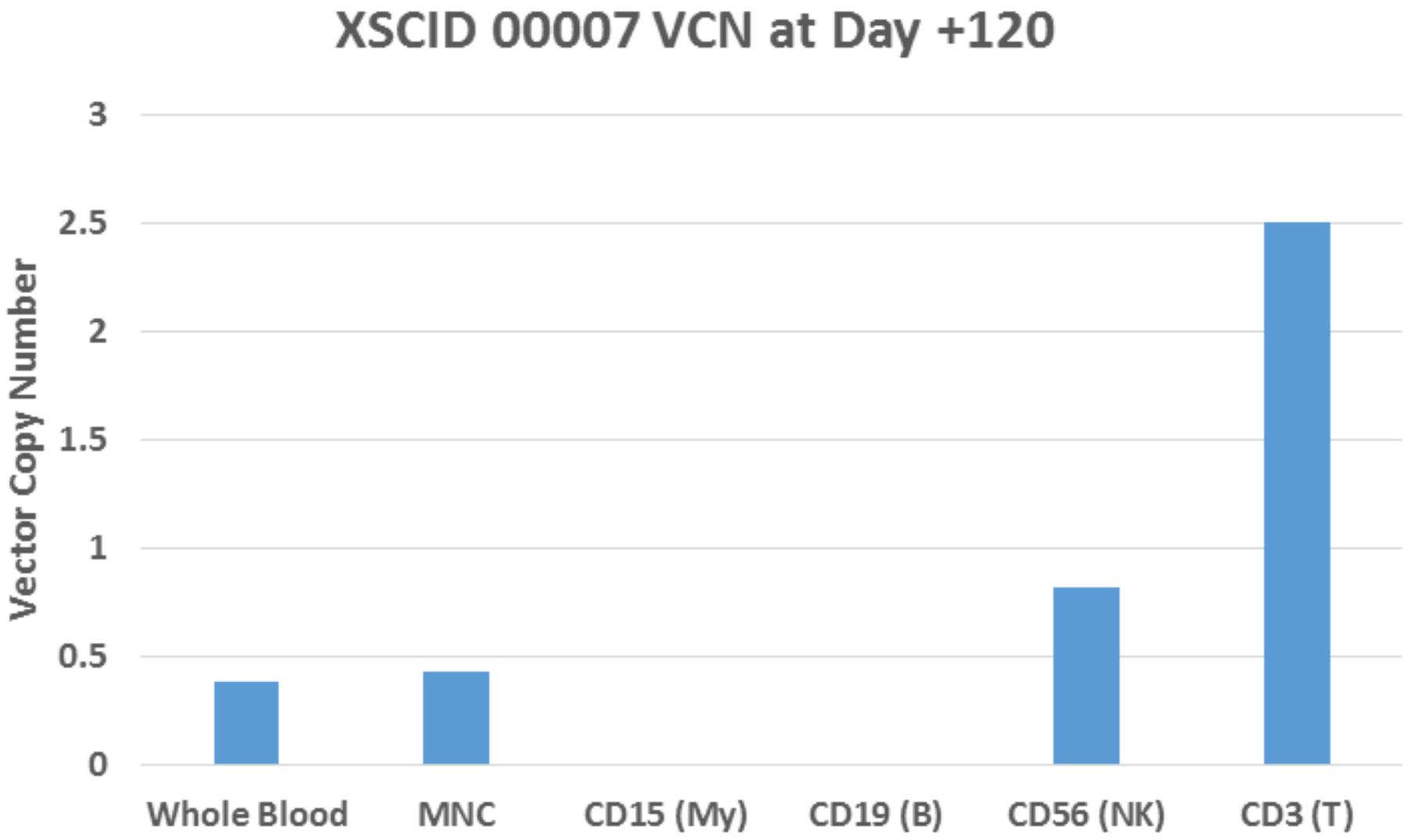
Average Vector Copies/Cell (VCN) by qPCR



XSCID 3

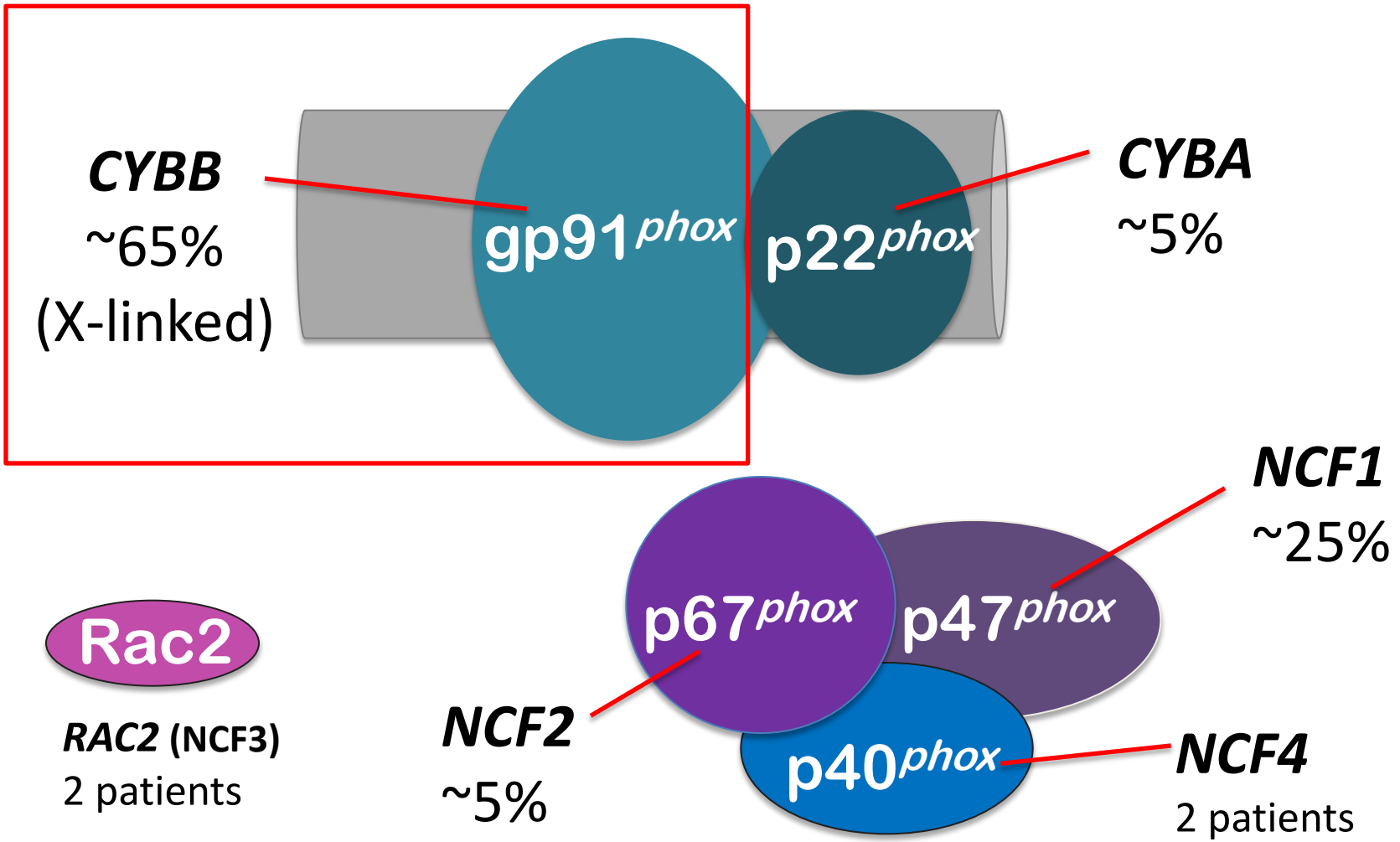
Thrasher - UCL/GOSH. London
Cavazzana - Hopital Necker Enfants Malade, Paris
Pai – Boston Children's Hospital
Filipovich - Cincinnati Children's Hospital
Kohn - Mattel Children's Hospital, UCLA

Lentiviral vector (EFS-gammaC)
– developed by Genethon, France.
David Williams – US IND Sponsor

Two arms:
a) Good health: busulfan 6 mg/kg (target 30 mg*hr/L)
b) Severe infection: no conditioning

UO1 To submit - Sept 2015

Genetic Defects in CGD



A PHASE I/II, NON RANDOMIZED, MULTICENTER, OPEN-LABEL STUDY OF G1XCGD (LENTIVIRAL VECTOR TRANSDUCED CD34+ CELLS) IN PATIENTS WITH X-LINKED CHRONIC GRANULOMATOUS DISEASE

IND Sponsor:
Donald B. Kohn, M.D.
University of California, Los Angeles (UCLA)

Primary Institution:
Mattel Children's Hospital, UCLA
Site PI: Donald B. Kohn, M.D

Collaborating Institutions:

Children's Hospital Boston
Site PI: David A. Williams, MD

National Institute of Allergy and Infectious Disease
Site PI: Elizabeth Kang, MD

Collaborating Investigators

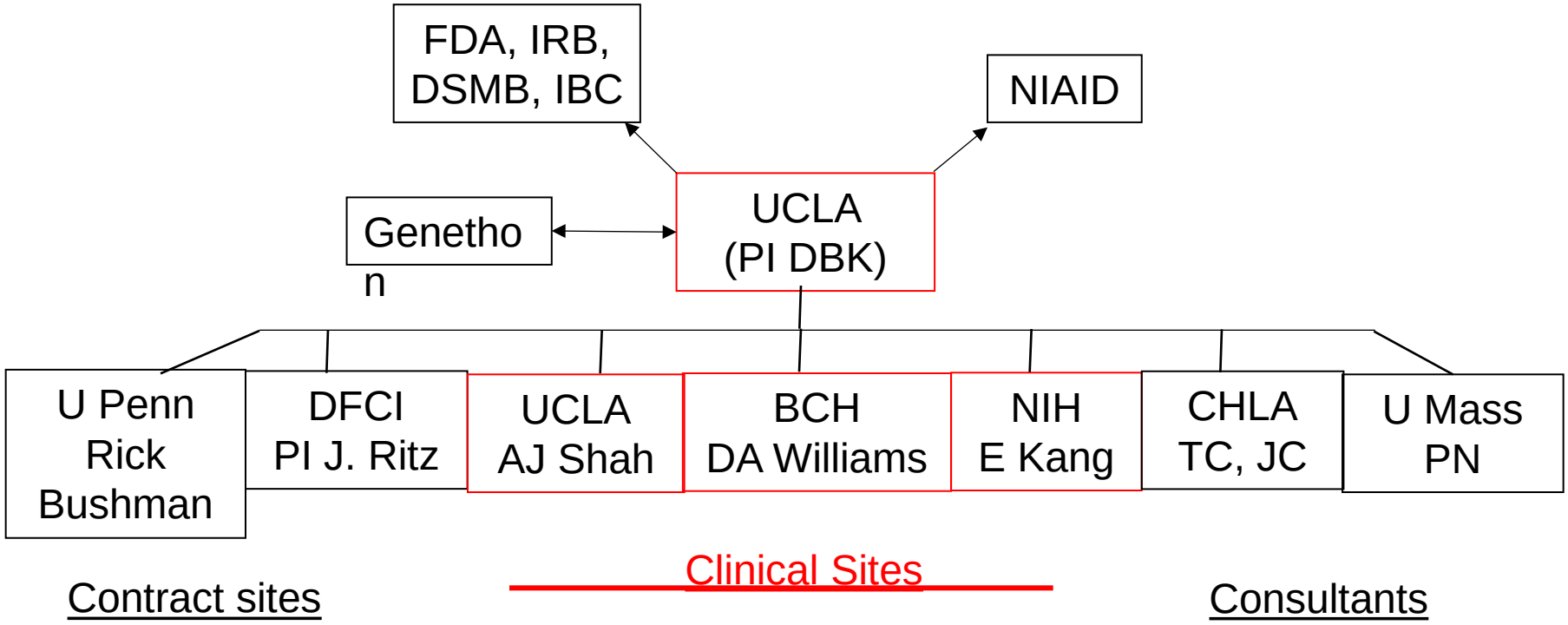
Caroline Kuo - UCLA
Ami Shah - UCLA
Harry Malech - NIAID, NIH
Suk See DeRavin - NIAID, NIH
Peter Newburger - U Mass, Worcester
Thomas Coates - Children's Los Angeles
Joseph Church - Children's Los Angeles

Vector Production / Scientific Coord.
GENETHON – EVRY - FRANCE
Anne Galy, PhD

Cell Processing Facilities
UCLA HGMP Laboratories
Dana Farber Cancer Institute Cell Manipulation Core
NIH Dept. of Transfusion Medicine

NIH RAC OBA # 1304-1223
UCLA IRB # 13-001875; CHB IRB # P00012407; NIH IRB # 2014-564
FDA IND # 16141 (approved 10/11/15); NCT02234934
Funding - pending

Clinical Trial Organization Chart



Diagnosis and Inclusion Criteria

-N=10. Male X-CGD patients >23 months of age.

-Molecular diagnosis confirmed by DNA sequencing and supported by laboratory evidence for absent or reduction > 70% of the biochemical activity of the NAHPD-oxidase.

-At least one prior, ongoing or refractory severe infection and/or inflammatory complications requiring hospitalization despite conventional therapy .

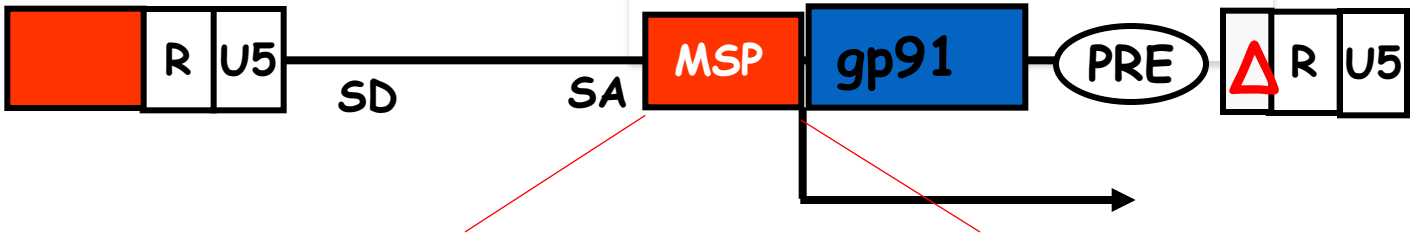
-No HLA-matched donor available after registry search.

-No co-infection with HIV or hepatitis B or hepatitis C virus), CMV, adenovirus, parvovirus B 19 or toxoplasmosis.

-Written informed consent for adult patient or by parent/guardian and where appropriate child’s signed consent/assent.

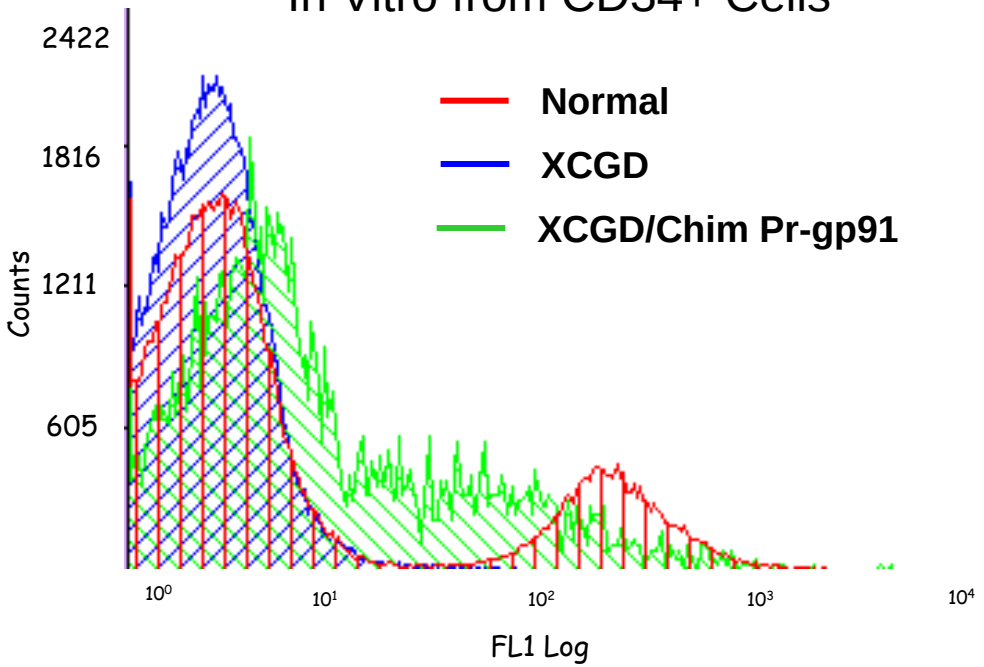
A novel chimeric promoter targets transgene expression to myeloid cells and efficiently corrects a murine model of X-CGD

G Santilli et al. London, UK; Madrid, Spain; Frankfurt, Germany. Mol Ther 2011



Cathepsin G/cfes promoter

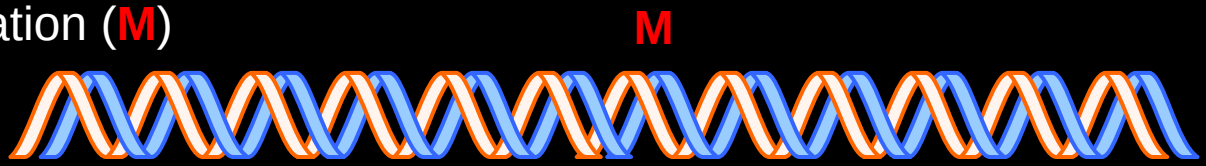
Expression in Human PMNs
In Vitro from CD34+ Cells



Increased expression with myeloid maturation in murine X-CGD model.

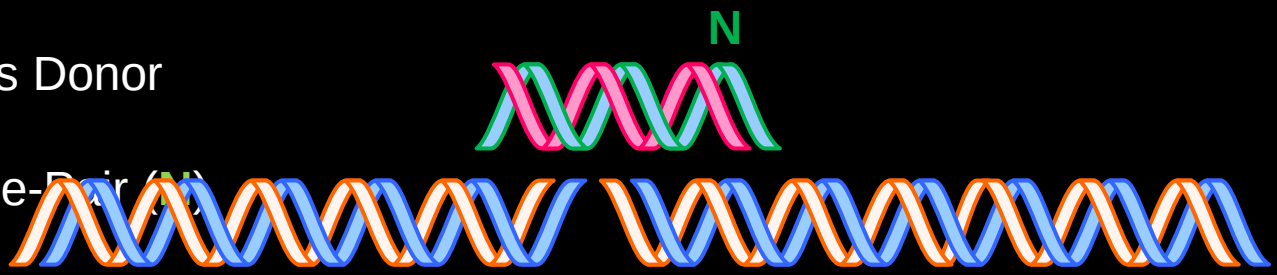
Site-Specific Gene Modification

Genomic Sequence with
Base-Pair Mutation (**M**)



Site-specific
Endonuclease
Creates DS DNA Break

Homologous Donor
with
Normal Base-Pair (**N**)



Edited Genome with
Normal Base-Pair **N**

Conclusions

In Phase I/II trials, both γRV and LV were effective and safe to transduce HSC and express ADA.

---100% survival. 0% GVHD. No vector-related AE.

---Fair to good immune reconstitution –
18/19 off ERT x 0.2-6 years.

---Better outcomes in infants than older children.

EFS-ADA LV expresses more ADA/VC than MND-ADA γRV.

Initial immune reconstitution may be more robust with EFS-ADA LV

Autologous HSCT with gene therapy provides a beneficial treatment option.

The Future of Gene Therapy for PID

Unlike allo HSCT, where one approach treats all SCID (or CGD, etc) genotypes, autologous gene therapy requires a genotype-specific therapeutic

Cost/time to develop for increasingly rare subset of disease is a challenge

Randomly integrating vectors, constitutively expressing transgene may be problematic, esp, for genes involved in signaling, etc

Risks from vectors must outweigh risks from GVHD, immune suppression with allo HSCT

The Future of Gene Therapy for PID

Site-specific gene correction under development using designer endonucleases to augment homologous recombination.

Would lead to physiologic expression of corrected endogenous gene

Efficiency currently low, but improving.

Apply to less severe but more common disorders, e.g. X-HIM, XLA, as benefit/risk ratio improves.

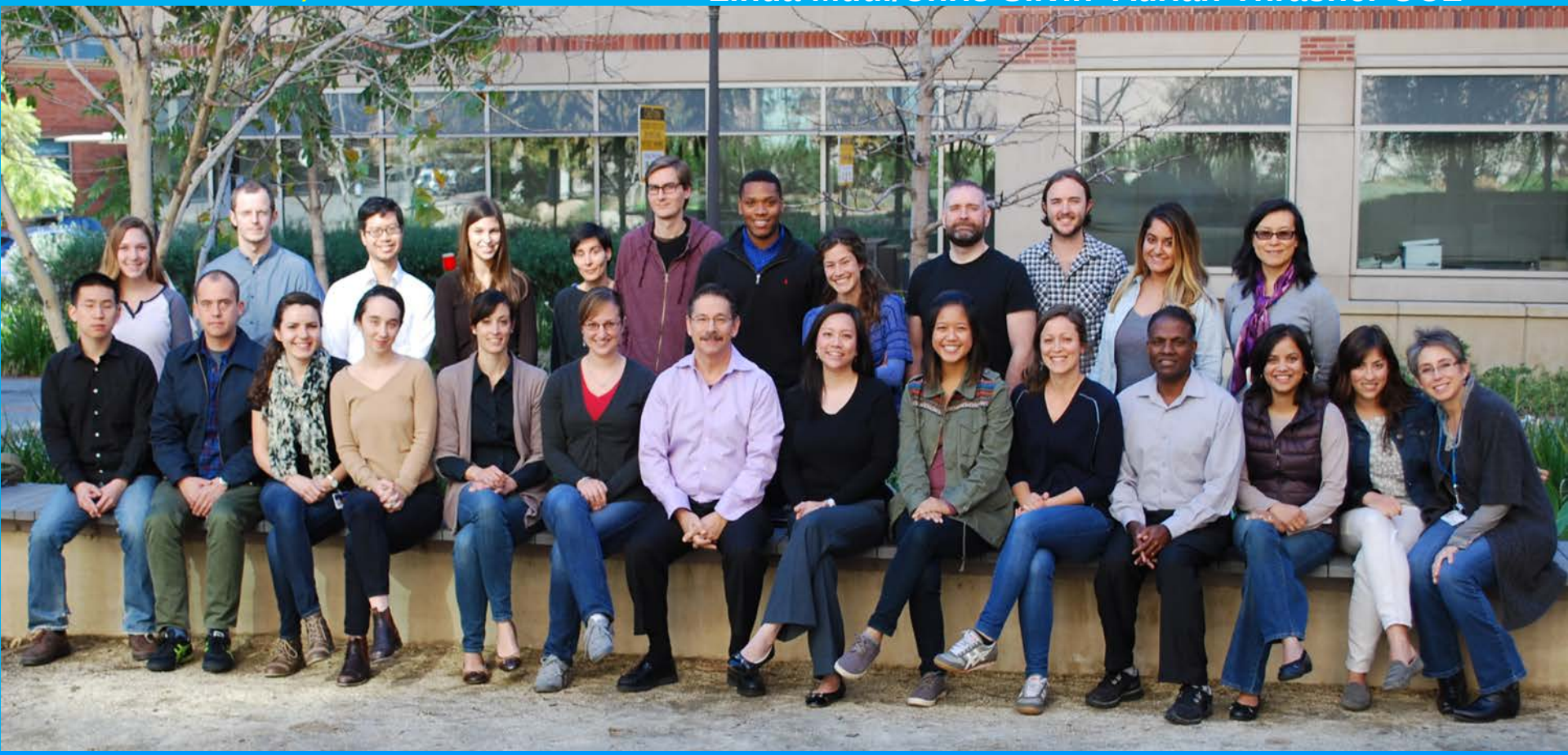
U.C.L.A. Stem Cell Gene Therapy Group

Funding:

Doris Duke Charitable Foundation
R01 FD003005; P01 HL073104;
U01 AI100801, NHLBI GTRP; NGVB
CIRM DR-06945; TR4-06823

ADA SCID Clinical Trial:

Fabio Candotti Ken Cornetta IUVPF
Rob Sokolic Lilith Reeves IUVPF
Elizabeth Garabedian Bobby Gaspar UCL
Linda Muul/Chris Silvin Adrian Thrasher UCL



UCLA BSCRC, JCCC, CDI, Depts. of MIMG and Pediatrics
Ami Shah, Satiro De Oliveira, Ted Moore, Gay Crooks
Sally Shupien, Erica Stanley, Dayna Terrazas, Jennifer Chung, Brenda Mueller