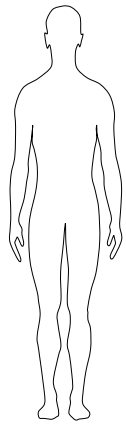# Vector Immunity as Barrier to Stem Cell Engraftment & Survival

John A. Zaia

Beckman Research Institute
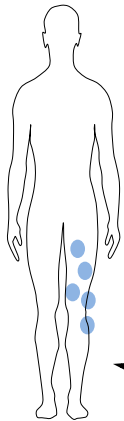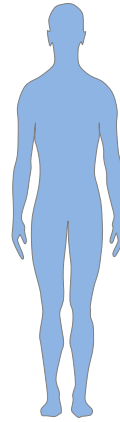
City of Hope

October 24, 2011

HSC transplant
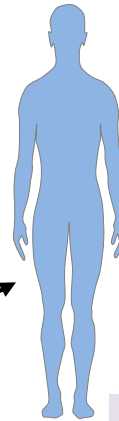CCR5Δ32 donor

Berlin patient

Patients' own
cells are treated
with ZFNs

CCR5(-)

AIDS lymphoma
patient

HIV "selection" for
CCR5(-) cells?

Courtesy of P. Cannon

Getting ZFNs to work in HSC
(goal: transient expression of the ZFN in the HSC to cause permanent gene disruption)

HSC source: CD34+ cells from cord blood

Nucleofection - DNA

Nucleofection - mRNA

Adenovirus [1]

Adeno-associated virus

Non-integrating lentivirus

0   5   10   15   20   25

% CCR5 disruption

	neg.	DNA	mRNA	Ad	AAV	NIL
	0	16	27	19	4	5

Cel1 Assay

Courtesy of P. Cannon/M. Holmes
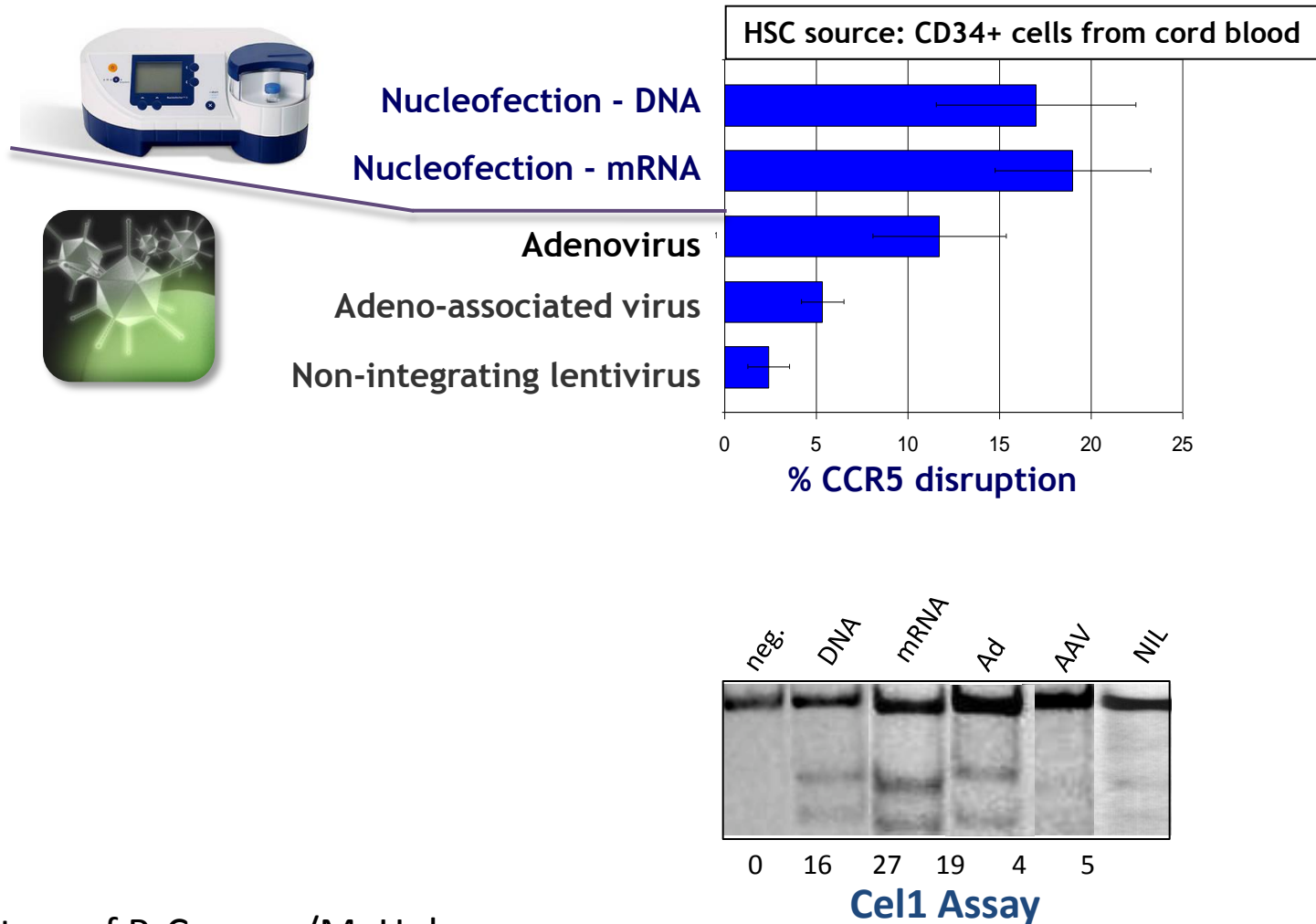
AIDS Lymphoma Clinical Platform

Gene Rx

Multipotent Progenitor Cell

CFU-Blast

Lymphoid stem cell

NK-Pre

Pre-B

Pre-T

NK cell

B cell

Plasma Cell

T cell

CFU-GEMM

CFU-GM

BFU-Meg

BFU-E

CFU-G

CFU-M

Monocyte

PMNL

Platelets

Retic

RBC

NSG: NOD/SCID/IL-2Ry^{null}

150 cGy

NSG neonate

CD34+ cells

2 mths

Successful engraftment of human cells?

65   bone marrow

SSC / CD45

83   thymus

CD8 / CD4

21   33   spleen

CD8 / CD4

33   GALT

SSC / CD3

Gated on human CD45
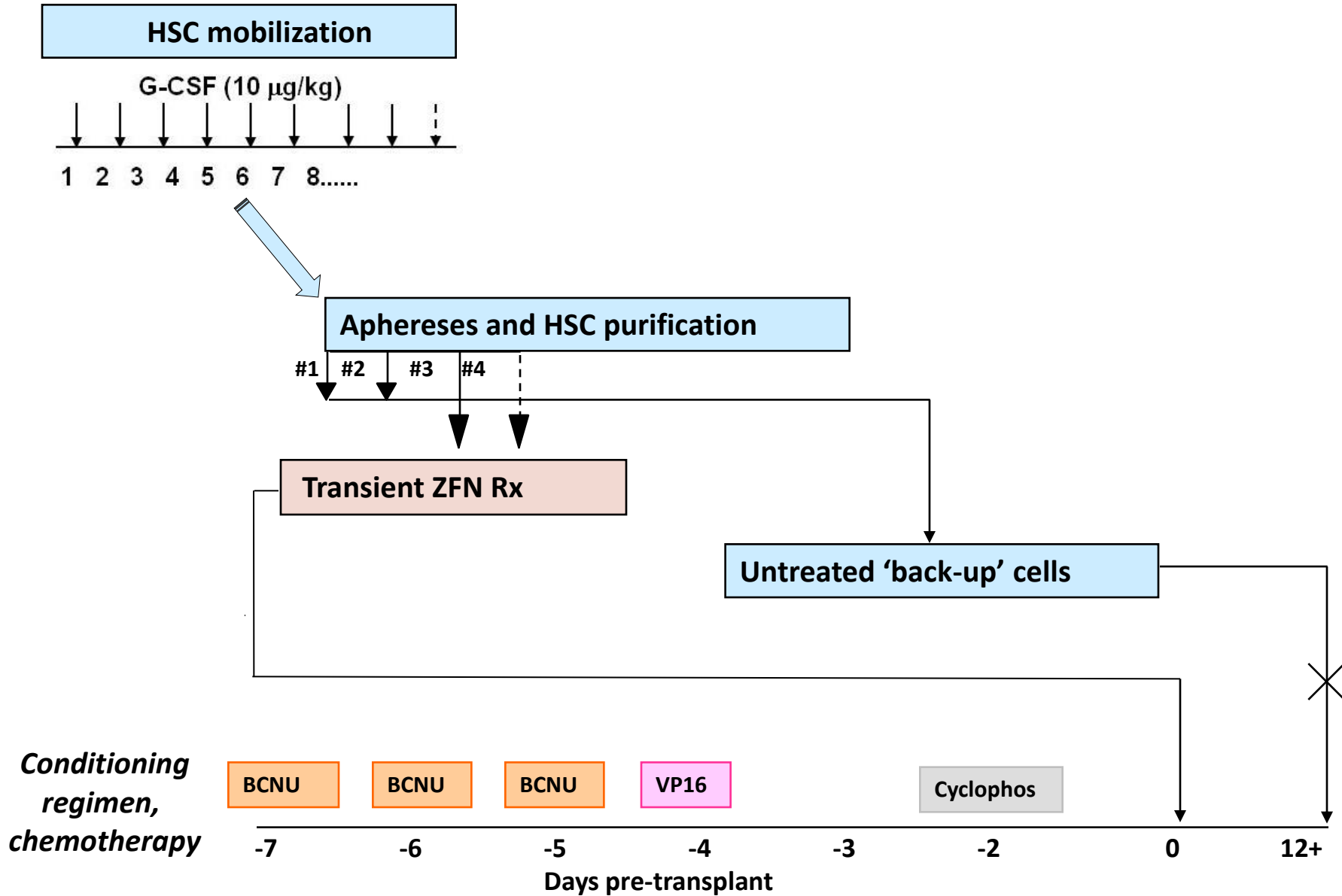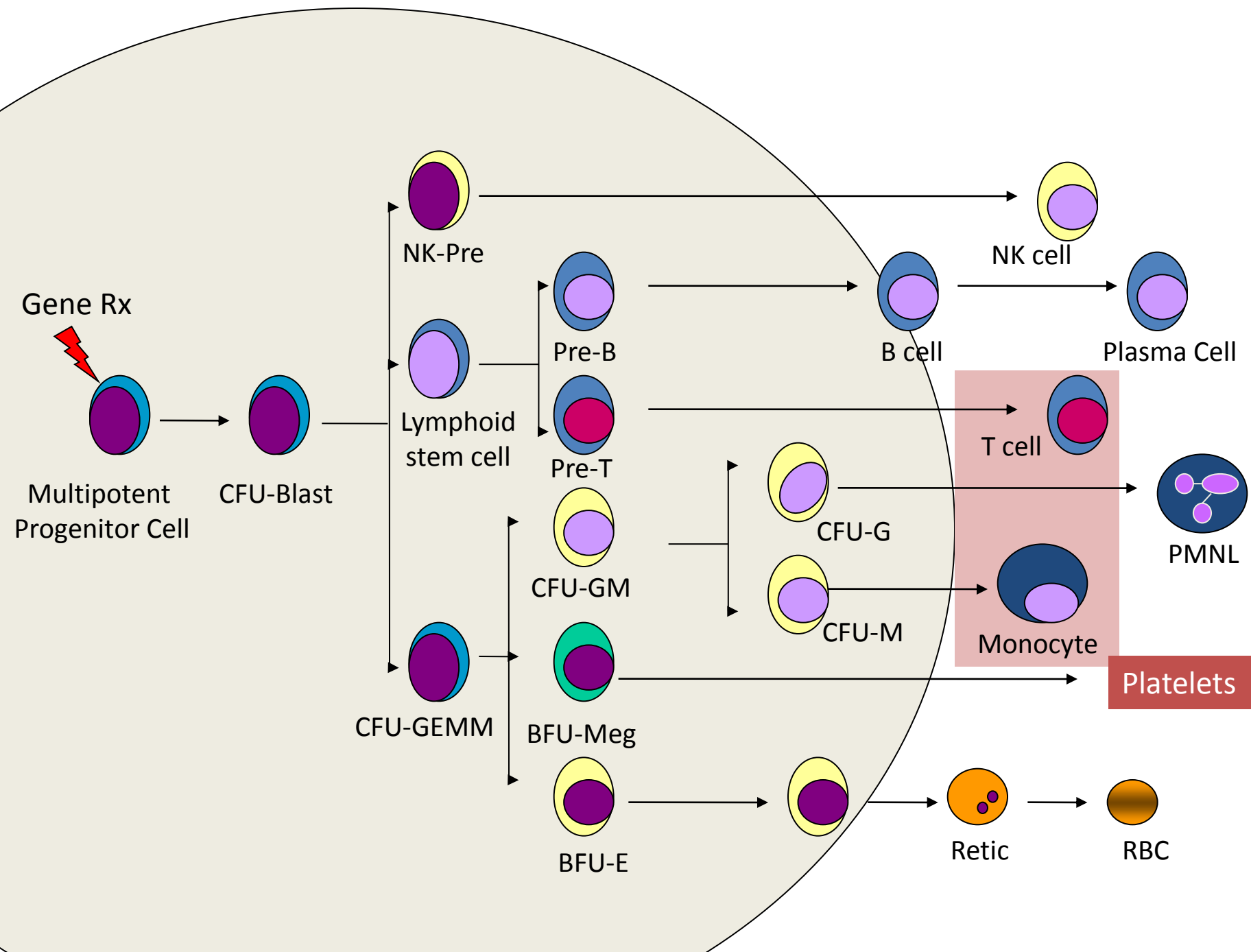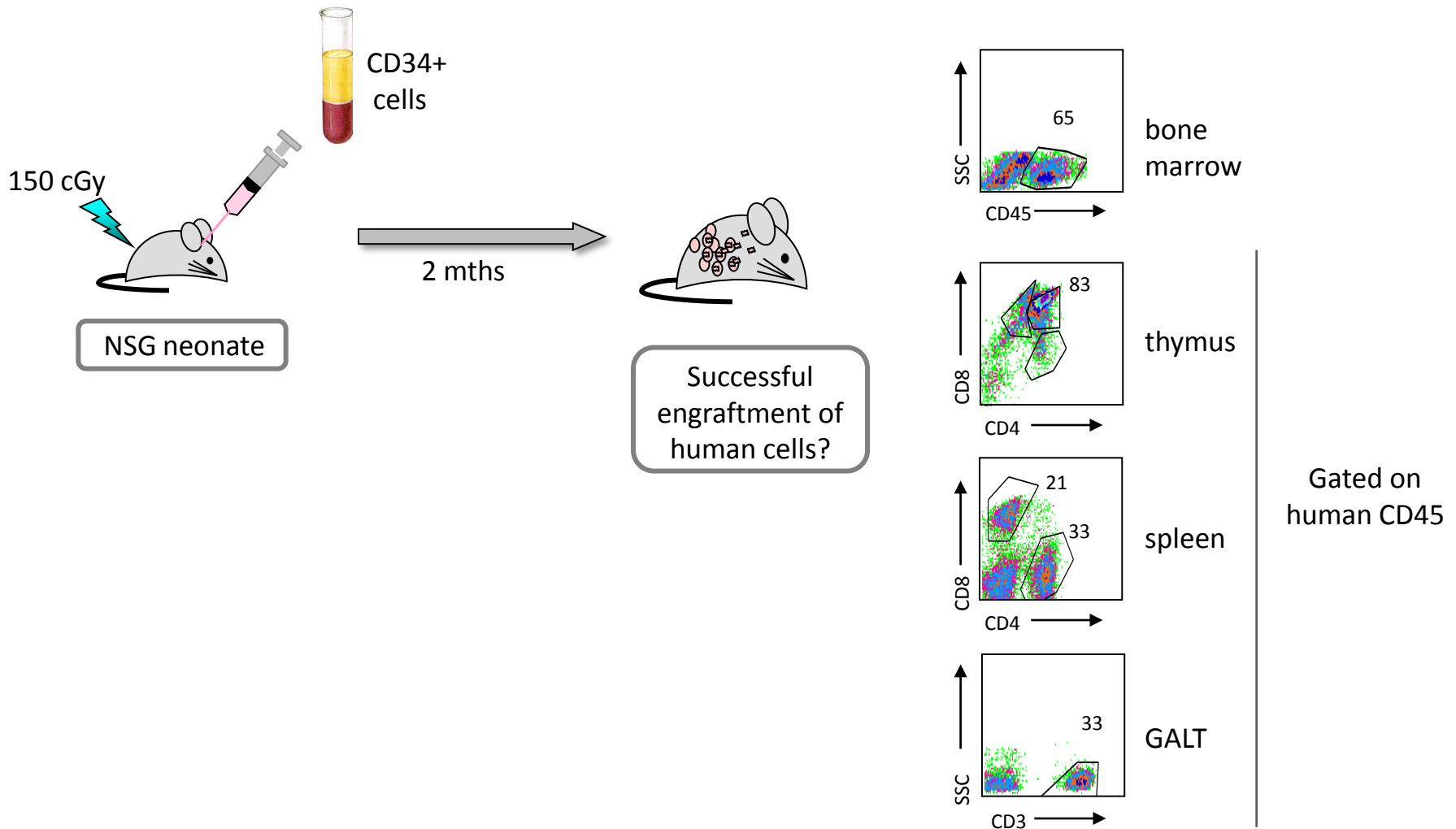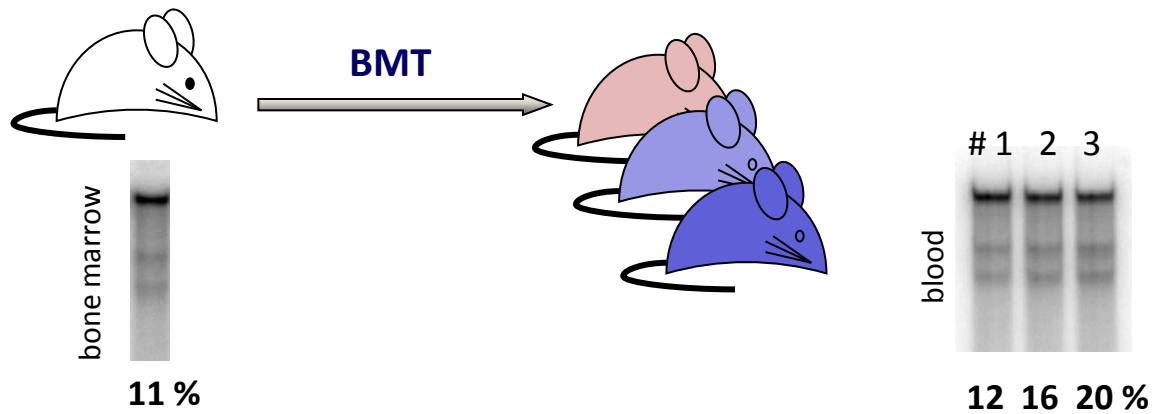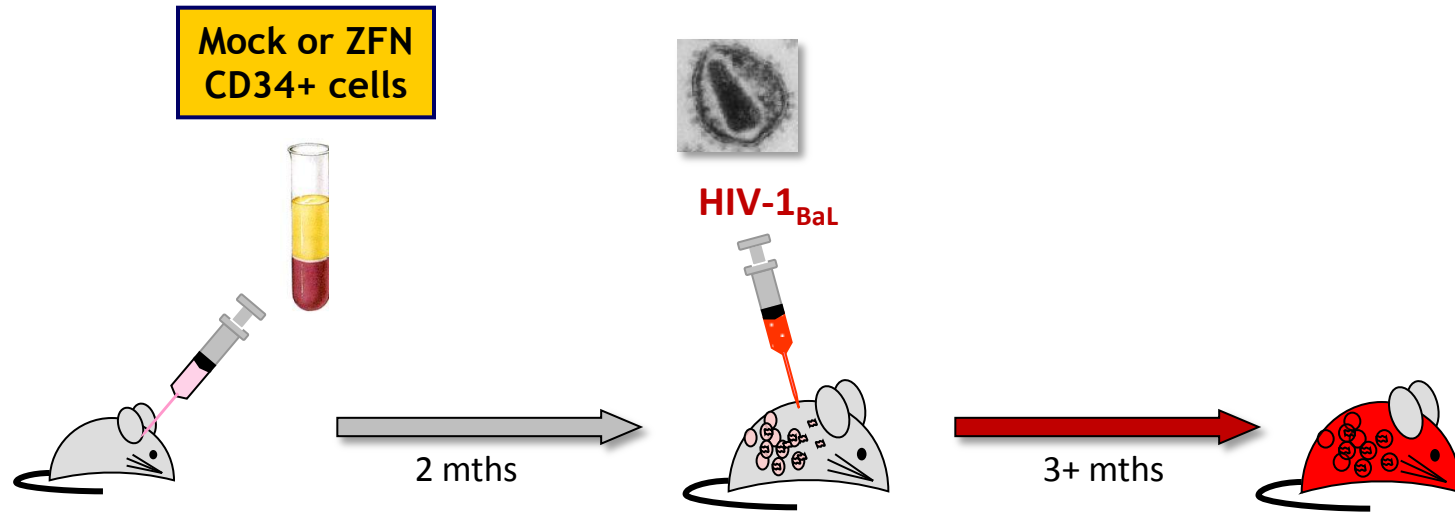
Adapted from Holt et al Nat Biotech 2010; 28: 839-47

ZFN-engineered HSC support secondary transplantations

BMT

bone marrow

11 %

blood

1 2 3

12 16 20 %

Courtesy of P. Cannon/M

HIV Challenge Model

Mock or ZFN CD34+ cells
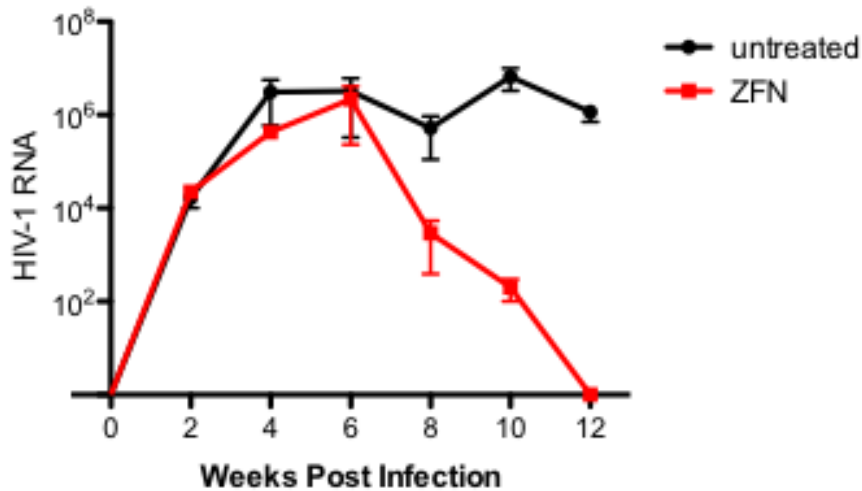
HIV-1$_{BaL}$

2 mths

3+ mths

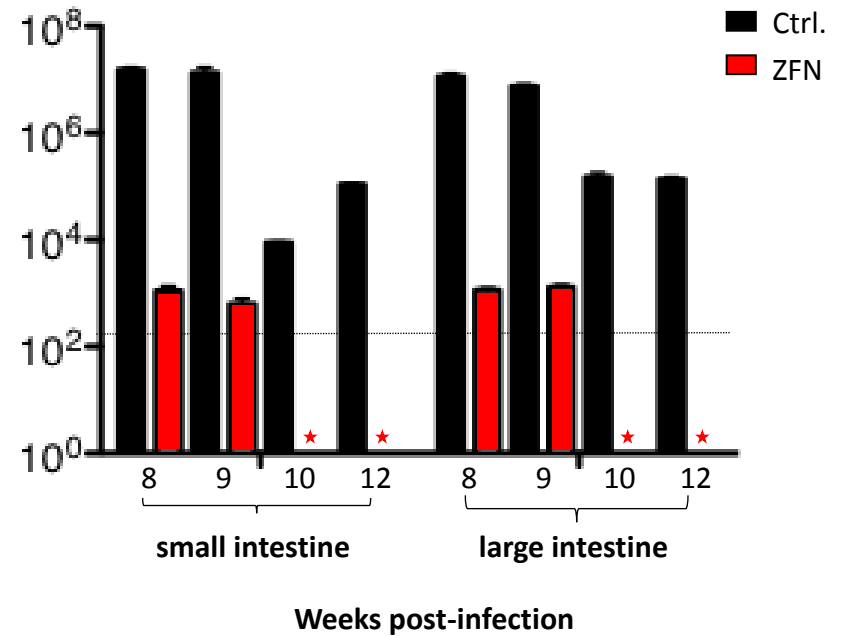Adapted from Holt et al Nat Biotech 2010; 28: 839-47

ZFN-treated mice control HIV-1
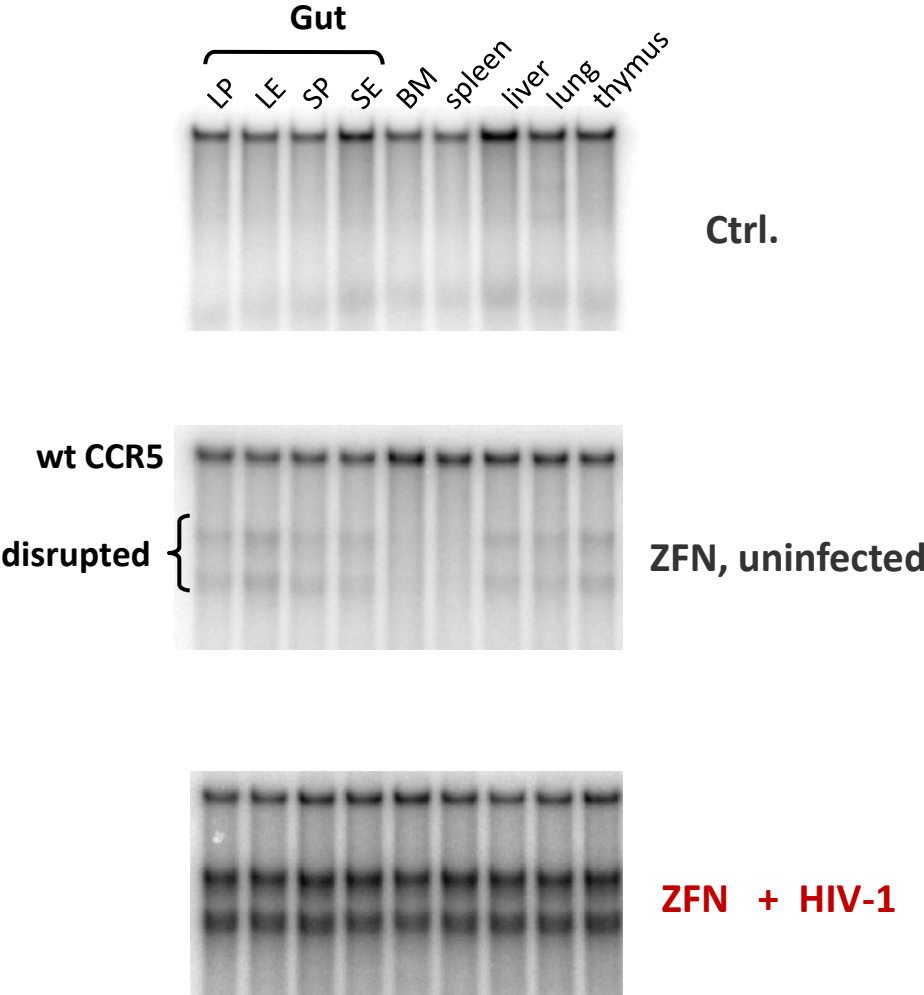
HIV-1 in blood

HIV-1 in gut mucosa

Adapted from Holt et al Nat Biotech 2010; 28: 839-47

HIV infection appears to select for CCR5(-) cells

Adapted from Holt et al Nat Biotech 2010; 28: 839-47

Vector Issues

- Immediate immune reaction: e.g. TLR; danger signal-responses; AdV direct injection
- Delayed response to vector or to neoantigen: e.g. neo selection; Factor XI/AAV
- In vitro transduction might mitigate effect of immune recognition of residual vector; e.g. lentivirus transduction of HSCs leads to long term engraftment and expression
- Non-integrating modification might mitigate immune recognition of continual expressed transgene; e.g. AdV-ZFN

Experiment

- Does anti-AdV alter engraftment in the NSR model?

- Passive transfer of human Ig = neutralizing titer

- Comparison of engraftment potential as measured by % CD45+ cells in marrow/spleen

Ad Neutralization pilot study

	control mouse 1	control mouse 2	human serum	human serum	50 ul IgG	50 ul IgG	100 ul IgG	100 ul IgG	Pos Serum Ctrl
EC50	0.2592	0.7865	2.033	2.568	5.600	5.191	10.35	7.632	41.58

Data 1

From M. Holmes
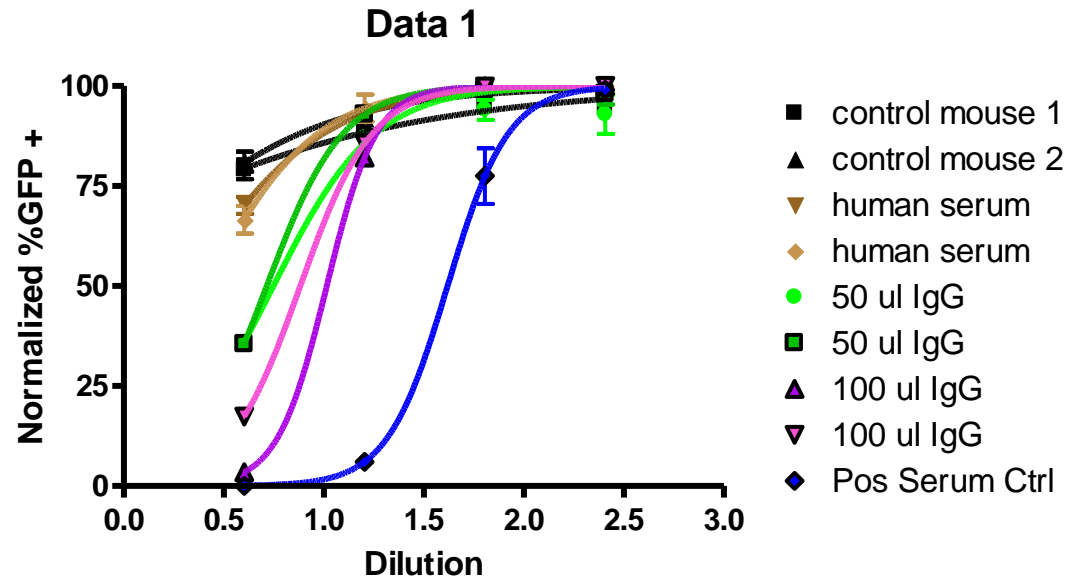
Anti-AdV Effect on Engraftment
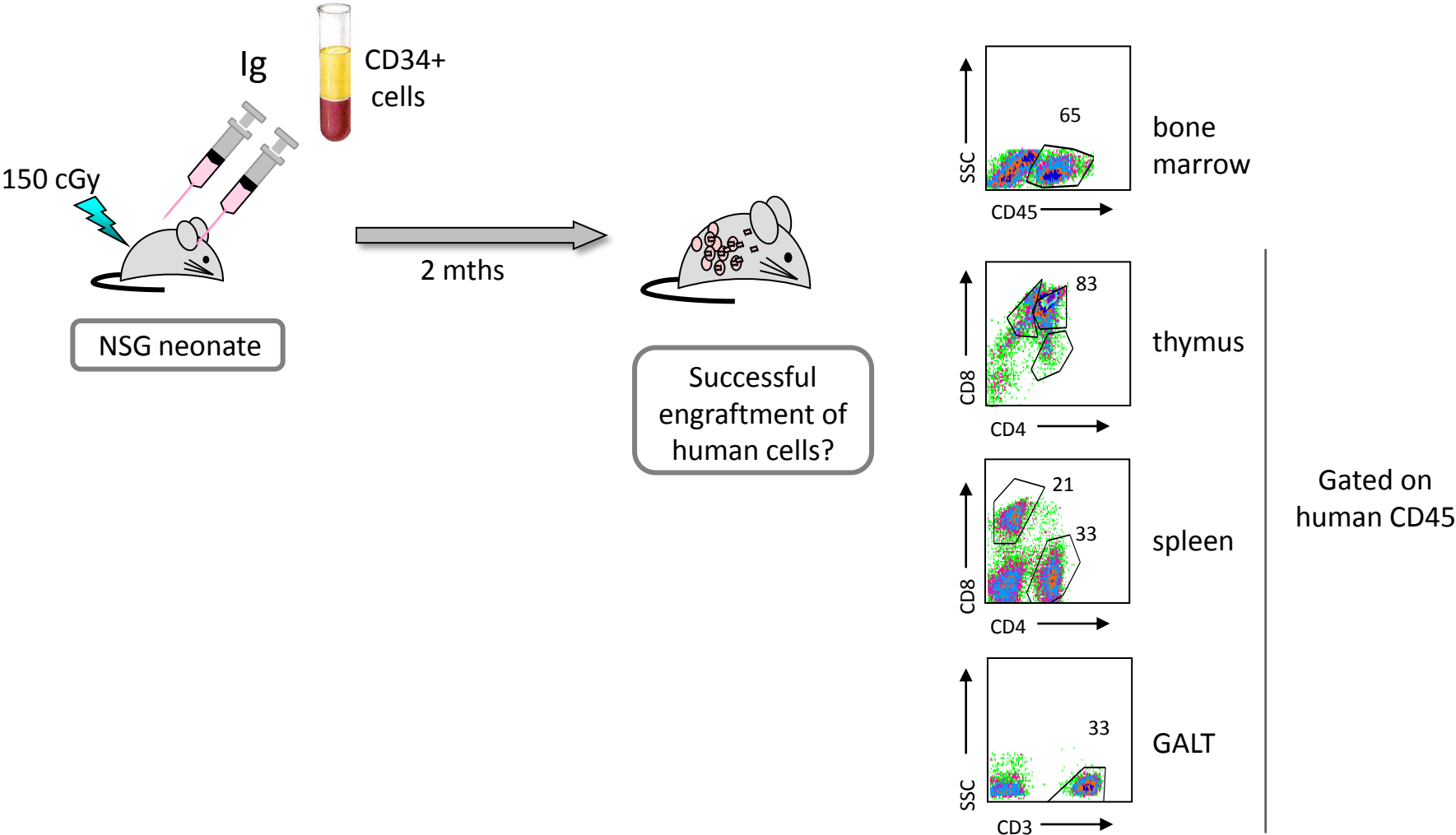
AIDS Lymphoma Patient Screening
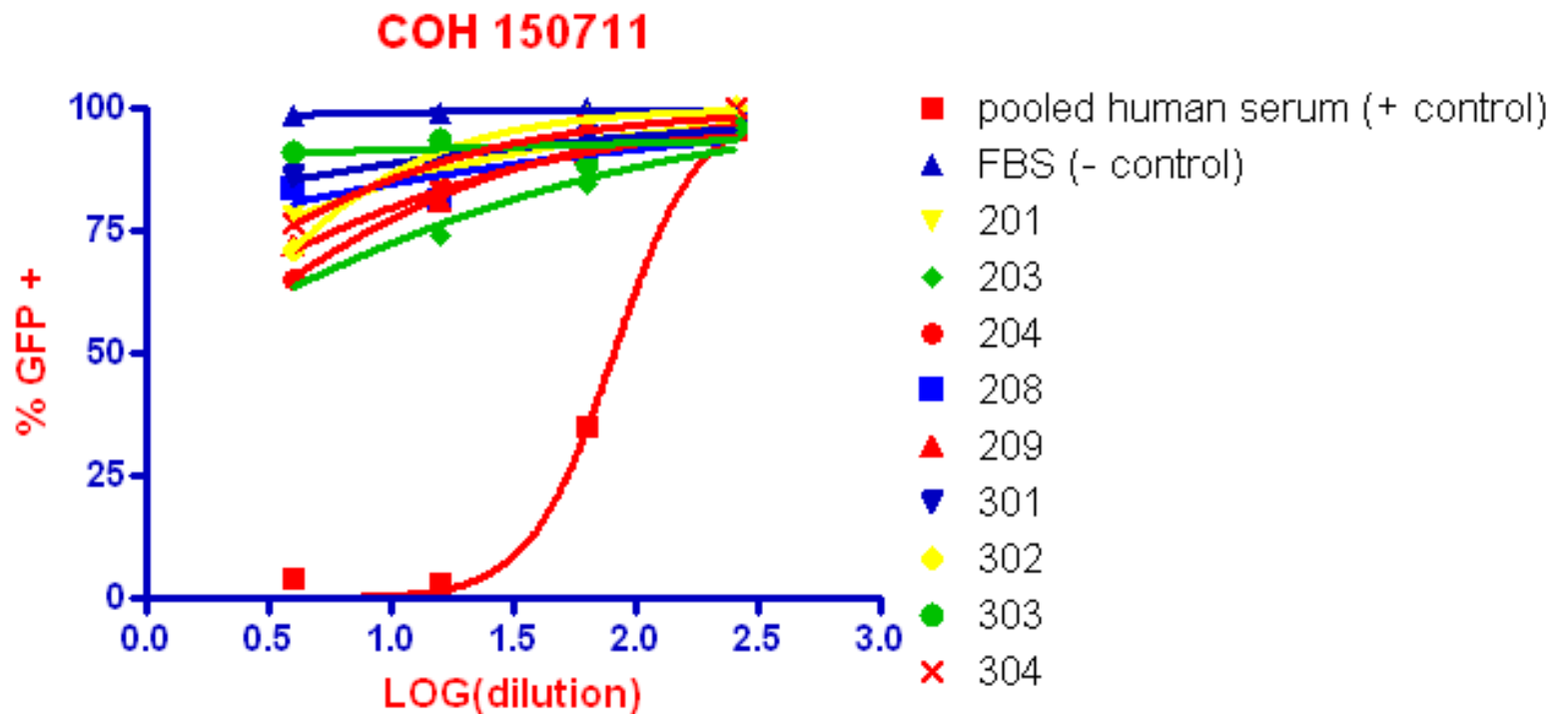
Sample	Normalized EC 50
Patient 1	0.4
Patient 2	1.4
Patient 3	1.7
Patient 4	0.0
Patient 5	0.8
Patient 6	0.0
Patient 7	1.9
Patient 8	0.0
Patient 9	0.8
Patient 10	2.3
Patient 11	32.8
Patient 12	37.8
Patient 13	30.6
Patient 14	8.4
Patient 15	1.5
Patient 16	0.7
Patient 17	3.4
Patient 18	0.0
Patient 19	9.1
50 ul IgG	13.4
100 ul IgG	15.7
Avg. Positve Control	94.7

Table 2. The EC50 values for the 19 plasma samples taken from AIDS Lymphoma patients just prior to HSPC infusion are listed. For comparison, the serum samples from mice receiving either 50 or 100 ul of purified human IgG are also listed. The average positive control value is derived from taking the average EC50 for pooled human serum from 3 independent studies.

Summary

- The NSG model is immunologically incomplete and any specific predictive value for vector immunity and engraftment is unknown

- Engraftment into an immunodeficient target population would mitigate any host anti-vector effect

- For blood stem cell transplantation, the option for backup, using untransduced stem cell, exists as a fall-back strategy