# Gene Therapy for Primary Immunodeficiency

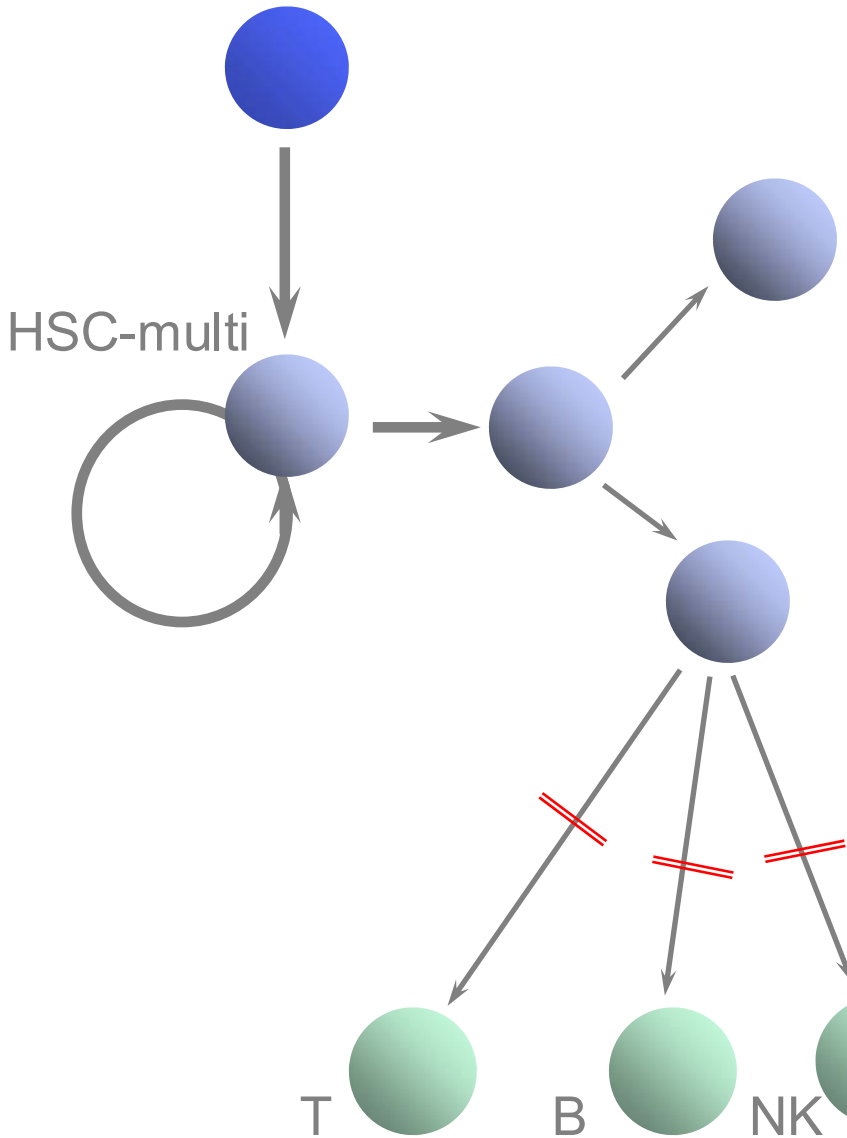
## Phase I/II studies at ICH/GOS

- X-SCID (9+1 patients)
- ADA SCID (1 patient)
- X-CGD (2 patients)

# X-linked severe combined immunodeficiency (SCIDX1)

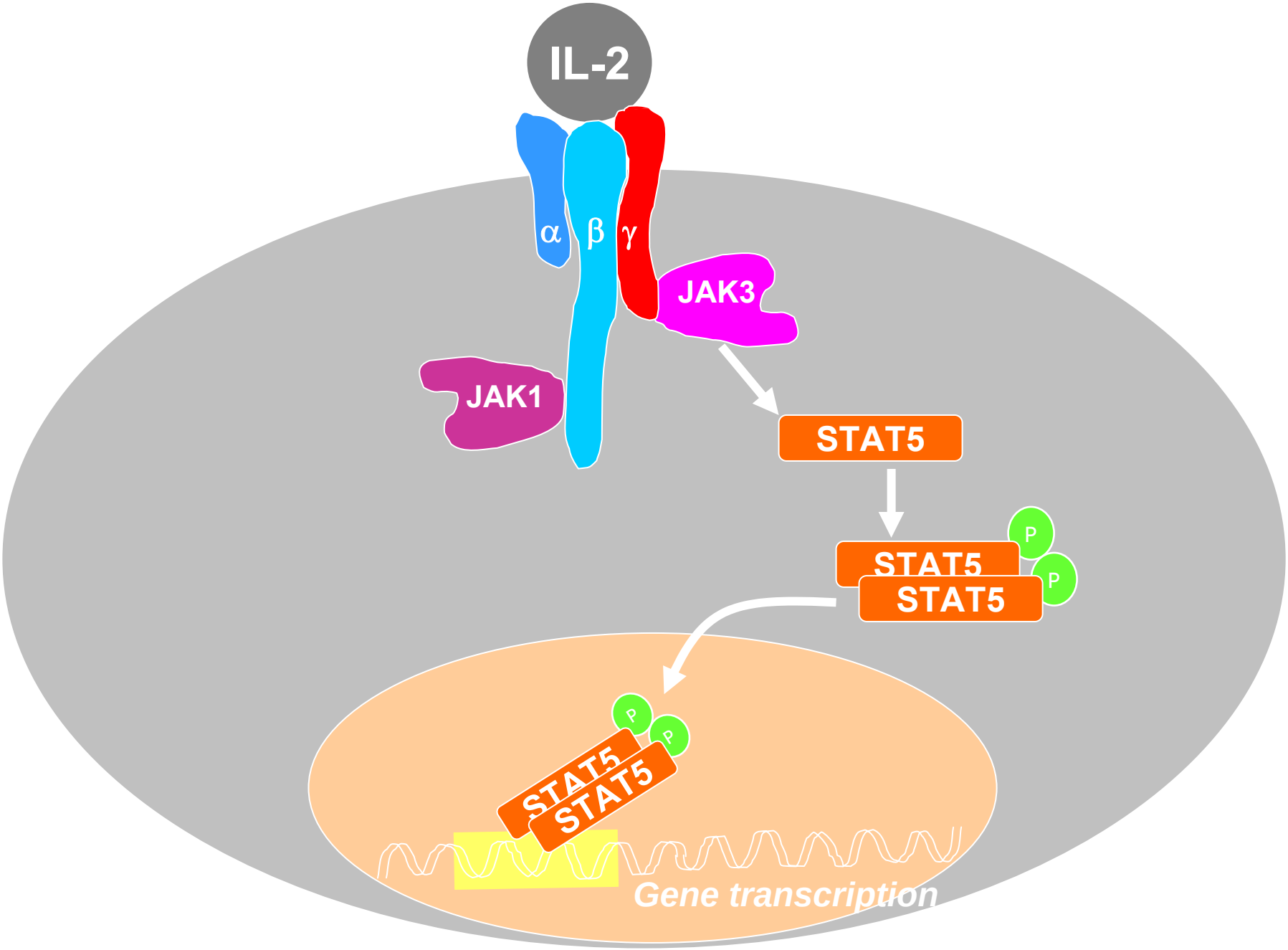

1 in 50-100,000 live births
Major form of SCID

Severe diarrhoea, pneumonia, septicaemia, fungal infection, failure to thrive, death usually within first year of life.

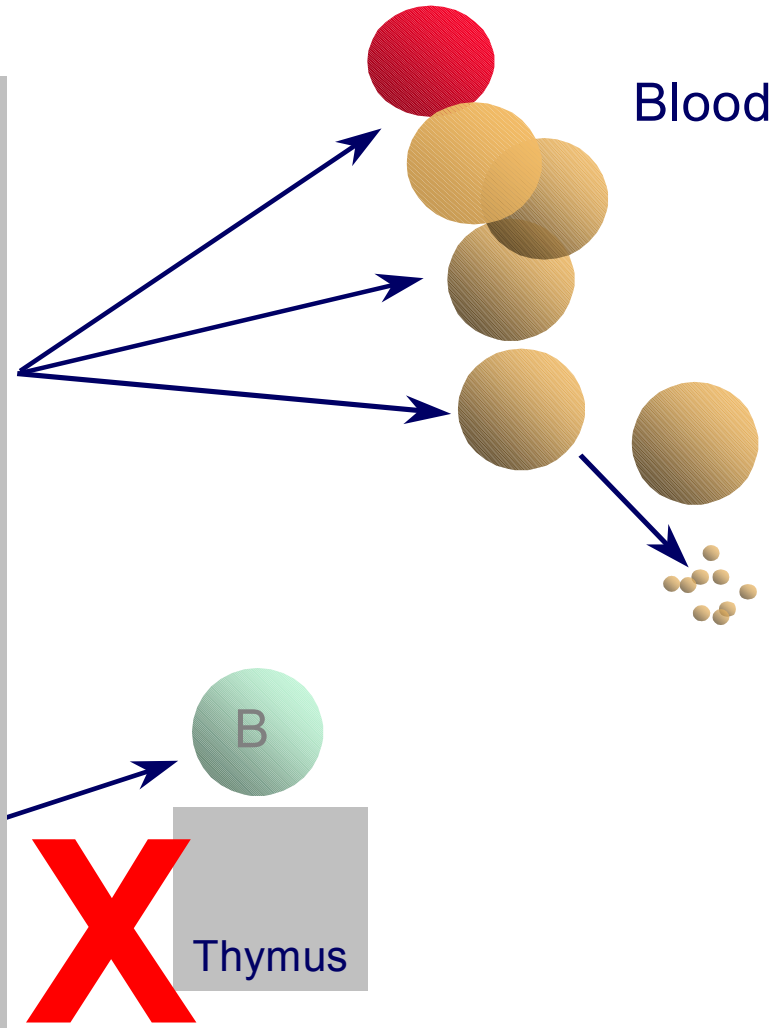



SCID/CID

γc, IL7Rα, JAK3, ZAP-70
RAG1/2, artemis, ligase IV
Cernunnos
ADA, PNP
MHC I/II, CD3, CD45

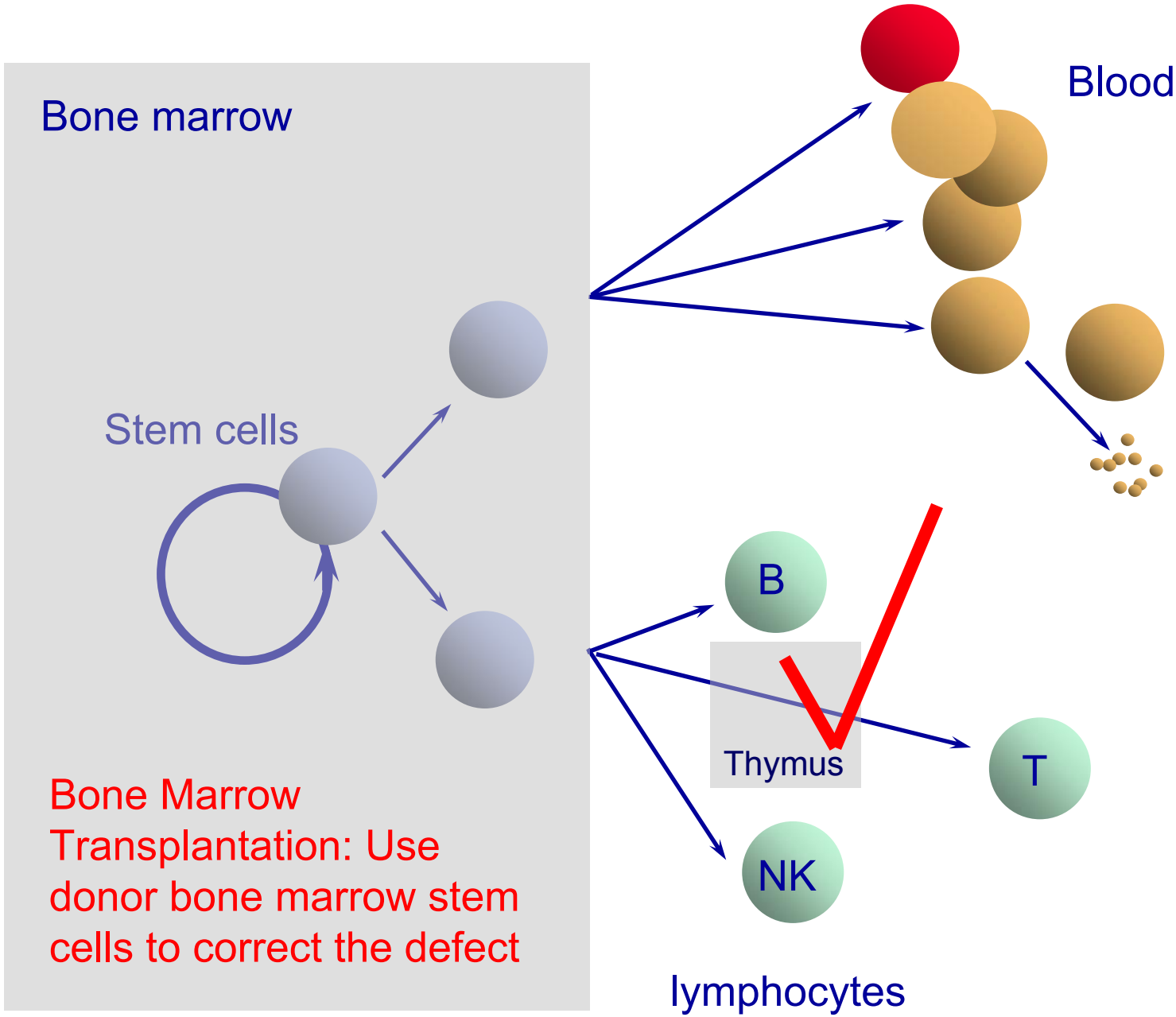

IL-2
α
β
γ
JAK3
JAK1
STAT5
P
STAT5
P
STAT5
P
P
STAT5
STAT5
Gene transcription

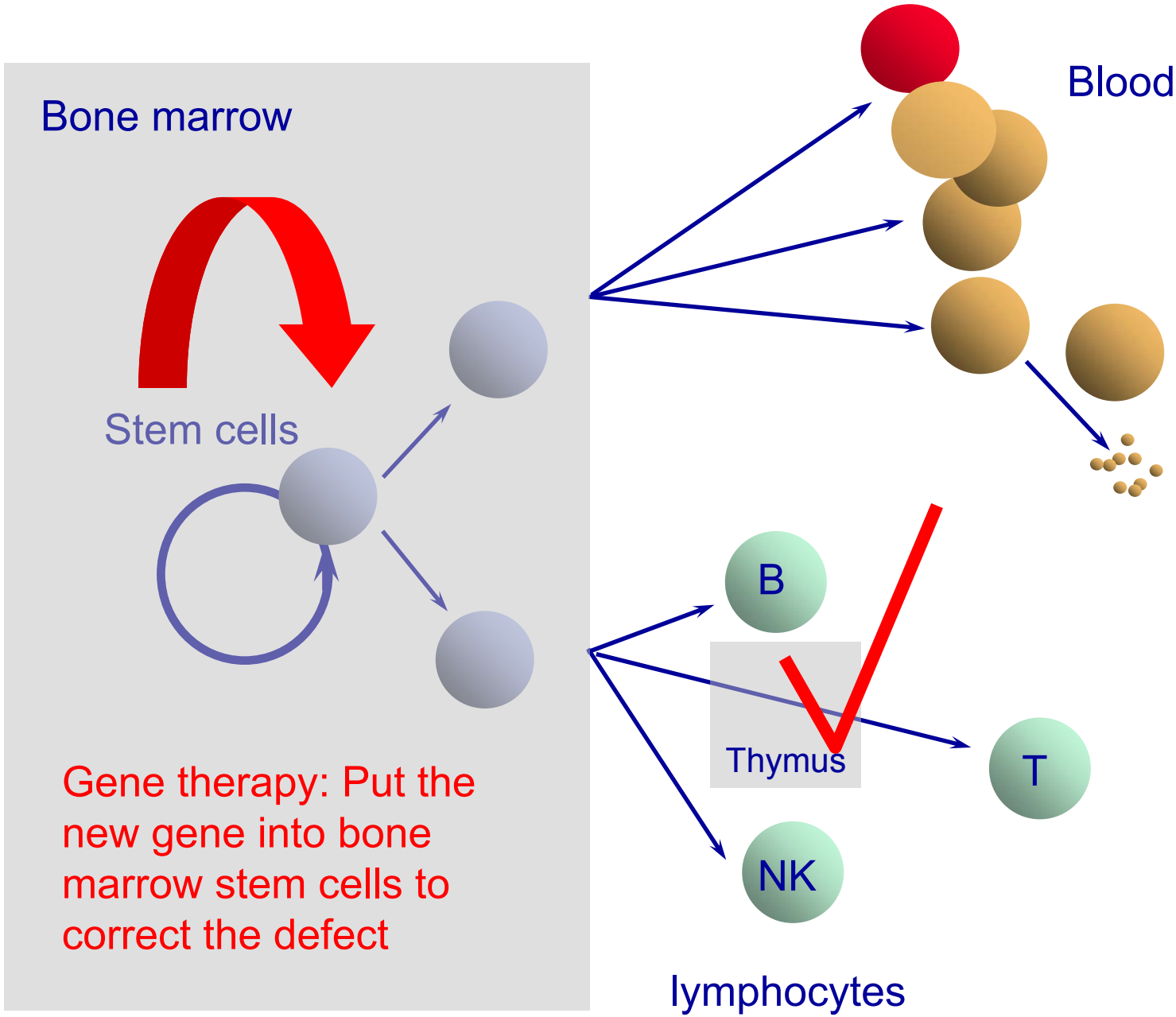
Bone marrow




In X-SCID growth of lymphocytes is blocked

lymphocytes

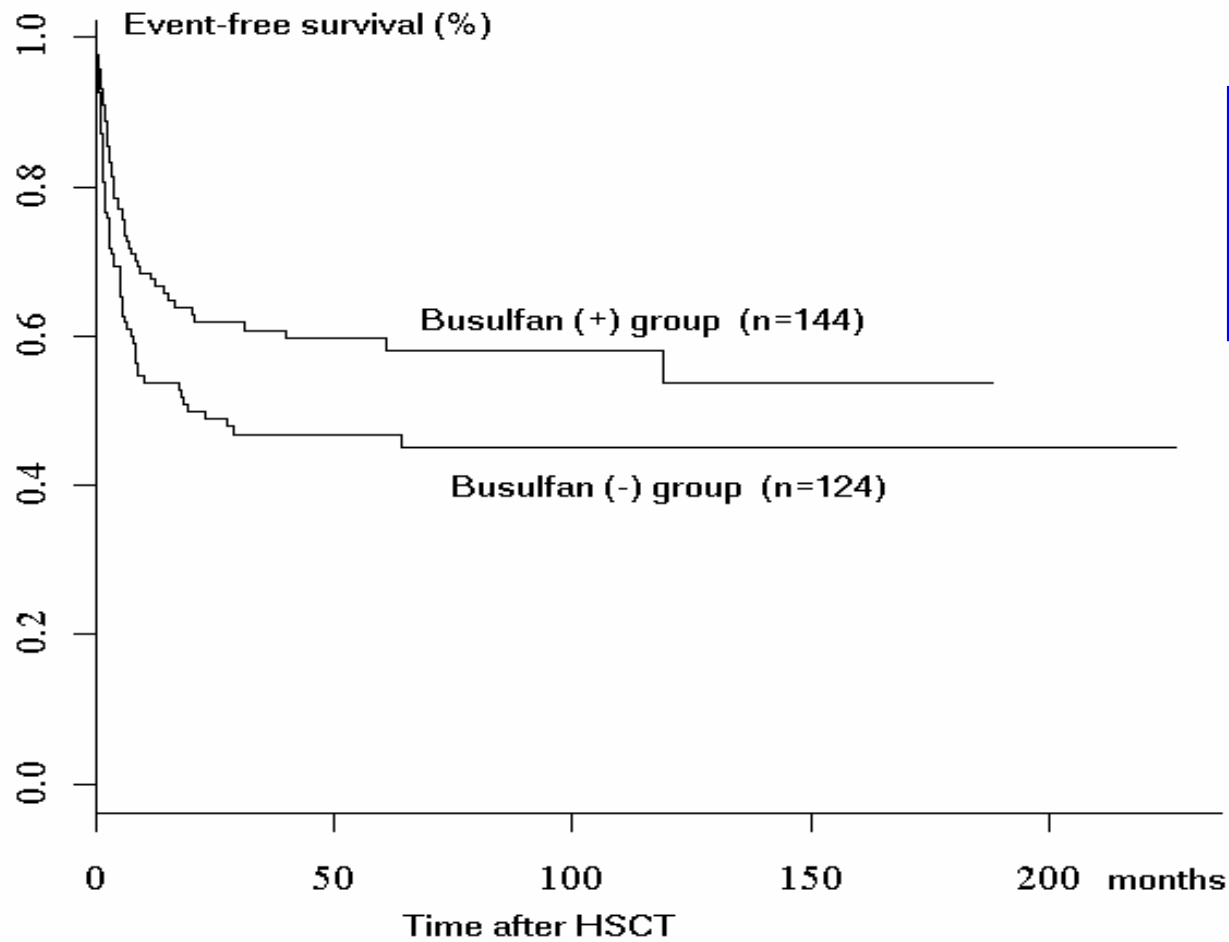

Bone marrow
Blood
Stem cells
B
Thymus
T
NK
lymphocytes
Bone Marrow Transplantation: Use donor bone marrow stem cells to correct the defect


Bone marrow
Stem cells
Blood
B
Thymus
T
NK
lymphocytes
Gene therapy: Put the new gene into bone marrow stem cells to correct the defect

# Treatment by bone marrow transplantation

- Use of matched donors successful in 90% of cases
- for 60% of cases, no matched donors have to use mis-matched donors, reduces success rate to <60%
- need to improve success for children with no matched donor

# Cumulative probability of survival after related HLA-mismatched HSCT in SCID patients




3 years survival rate
45.2%
53.8%

# SCIDX1 morbidity and mortality following HLA-mismatched transplantation…

20% 1 year mortality

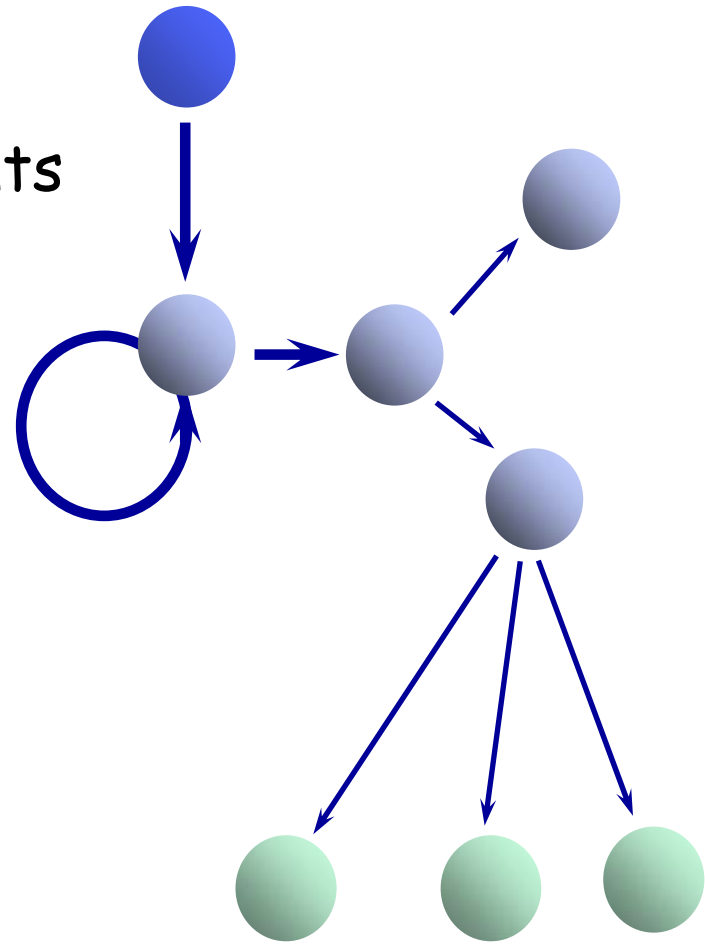
Long term effects related to chemotherapy, usually with alkylating agents (growth, fertility, secondary malignancy, neuropsychological, hypodontia)

Incomplete immunological reconstitution

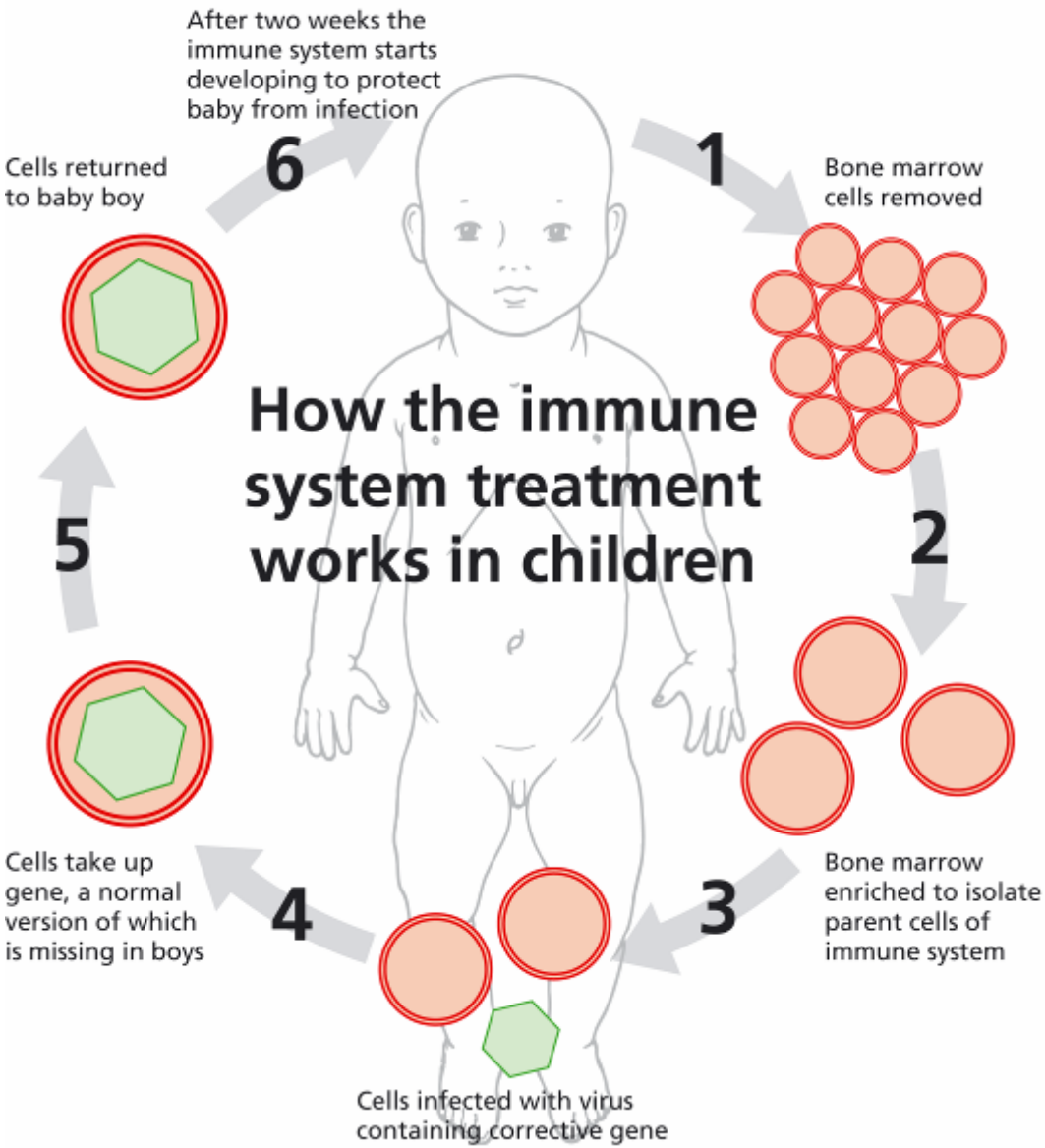
# X-SCID: major selective growth and survival advantage for corrected cells….

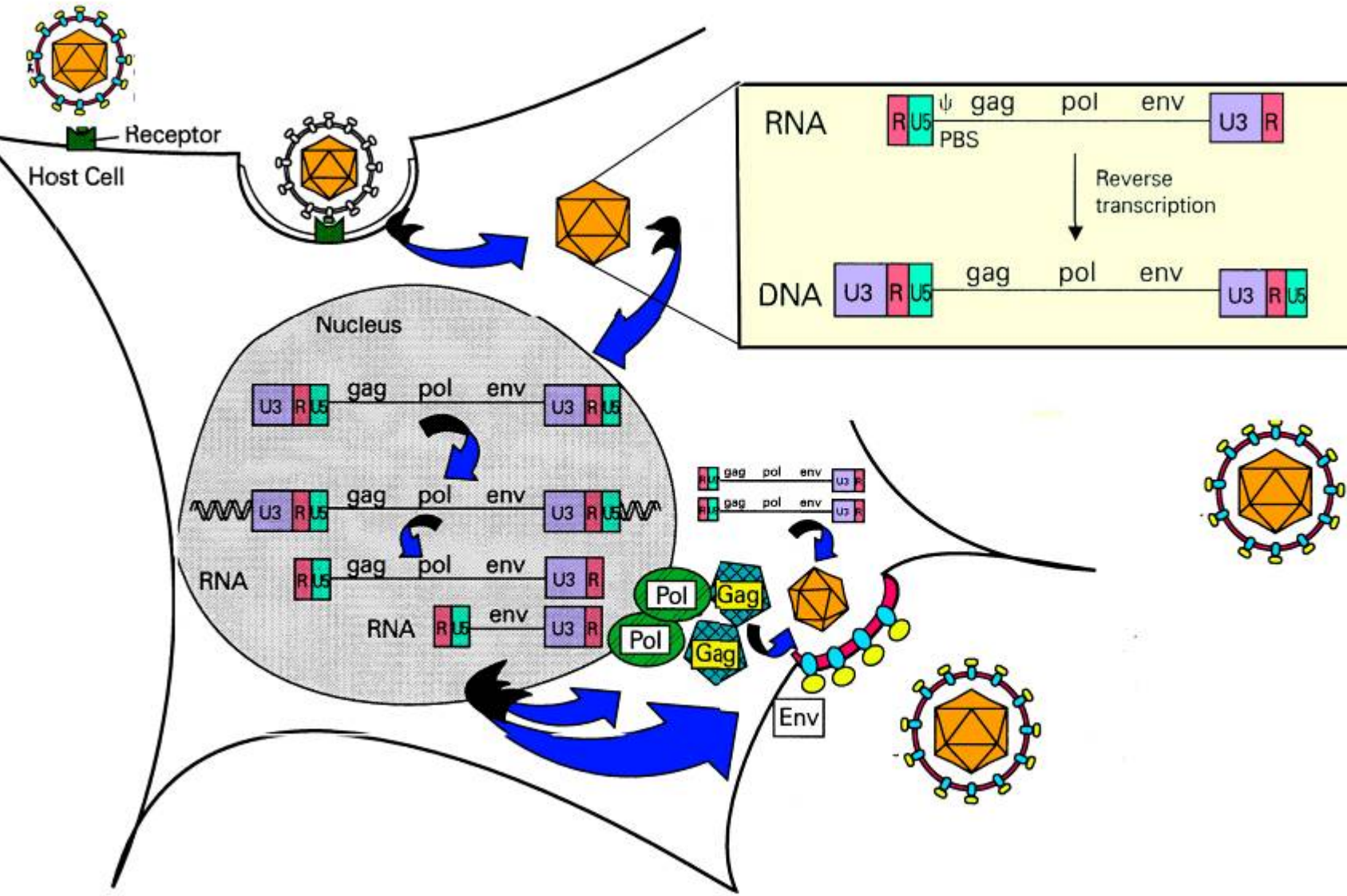
Somatic reversion events

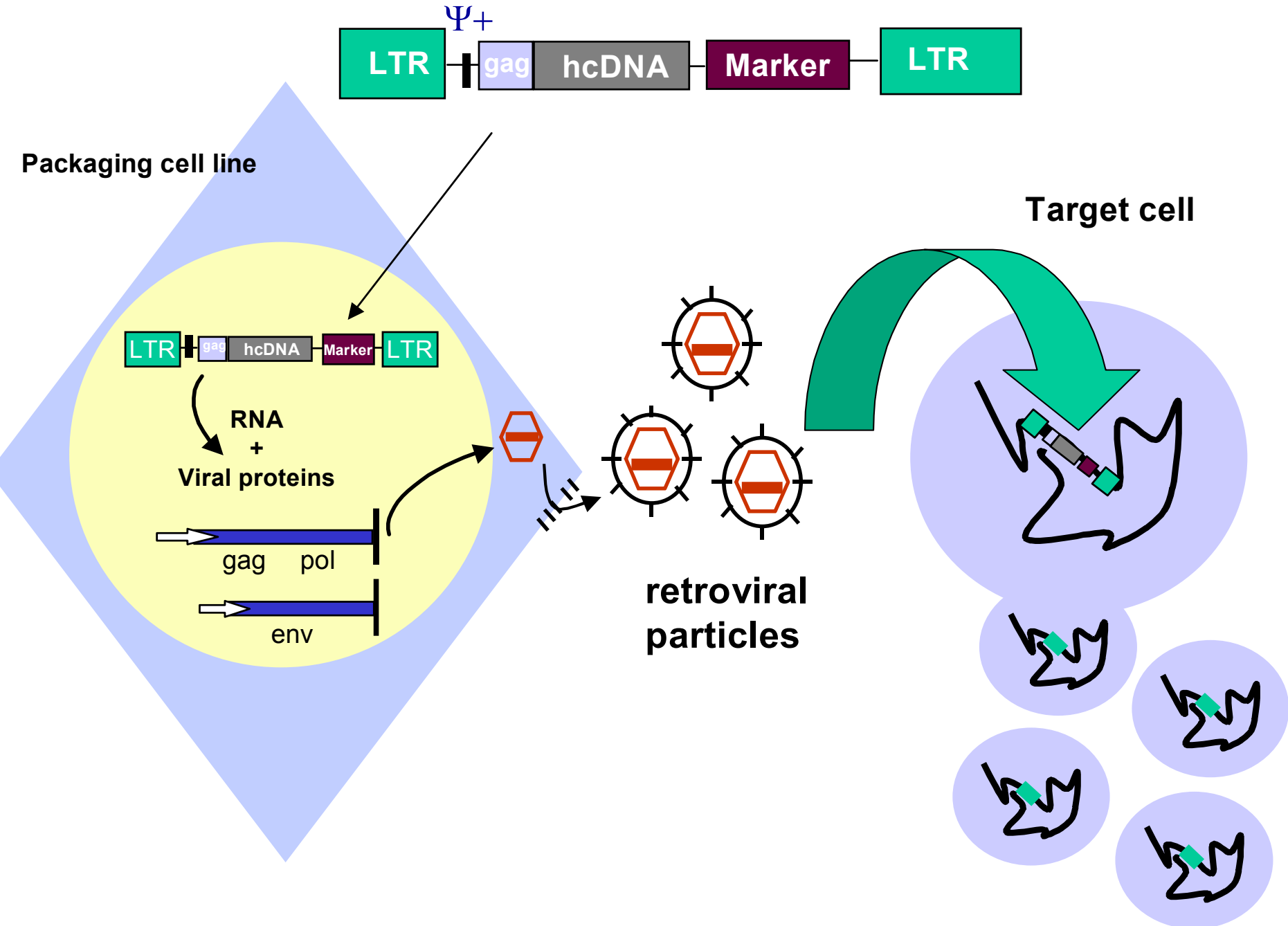
Animal models



# Gene Therapy

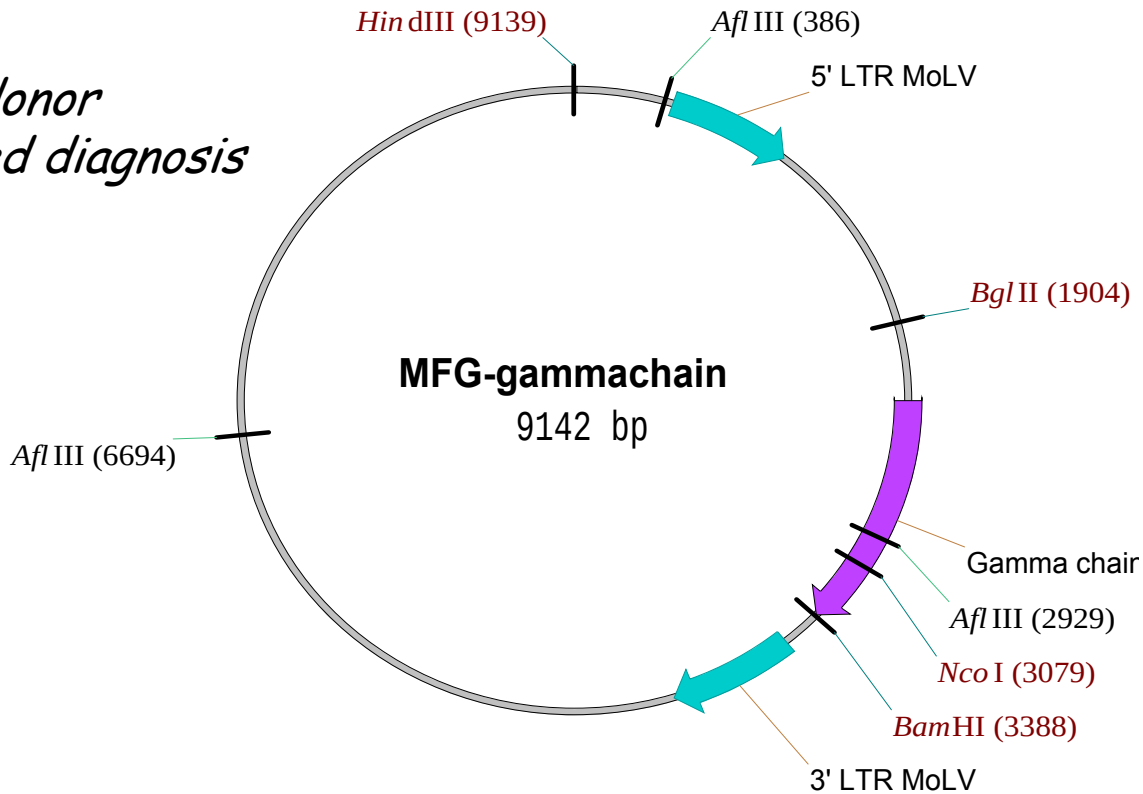



Receptor
Host Cell
Nucleus
RNA
gag
pol
env
U3
R
U5
ψ
PBS
Reverse
transcription
DNA
Pol
Gag
Env


Ψ+
LTR
gag
hcDNA
Marker
LTR
Packaging cell line
LTR
gag
hcDNA
Marker
LTR
RNA
+
Viral proteins
gag
pol
env
retroviral
particles
Target cell

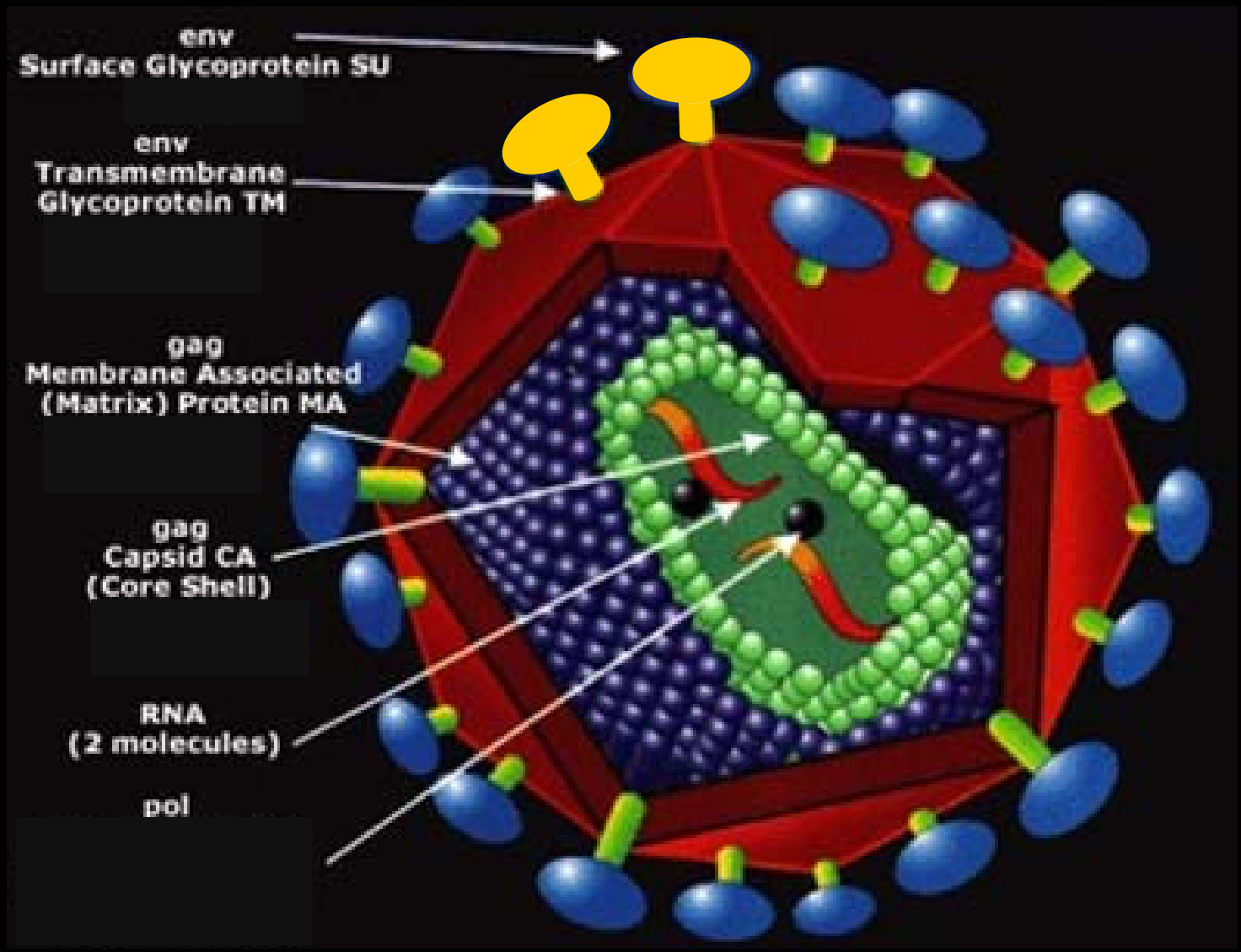
# Gene therapy for X-SCID: phase I study

Criteria for entry:
*No matched sibling donor*
*Molecularly confirmed diagnosis*




Common gamma chain vector:
*PG 13 producer cells (GALV envelope)*
*titre approximately 1x10e6 transducing units per ml*

# Retrovirus particle - pseudotyped



# Transduction protocol – SCIDX1

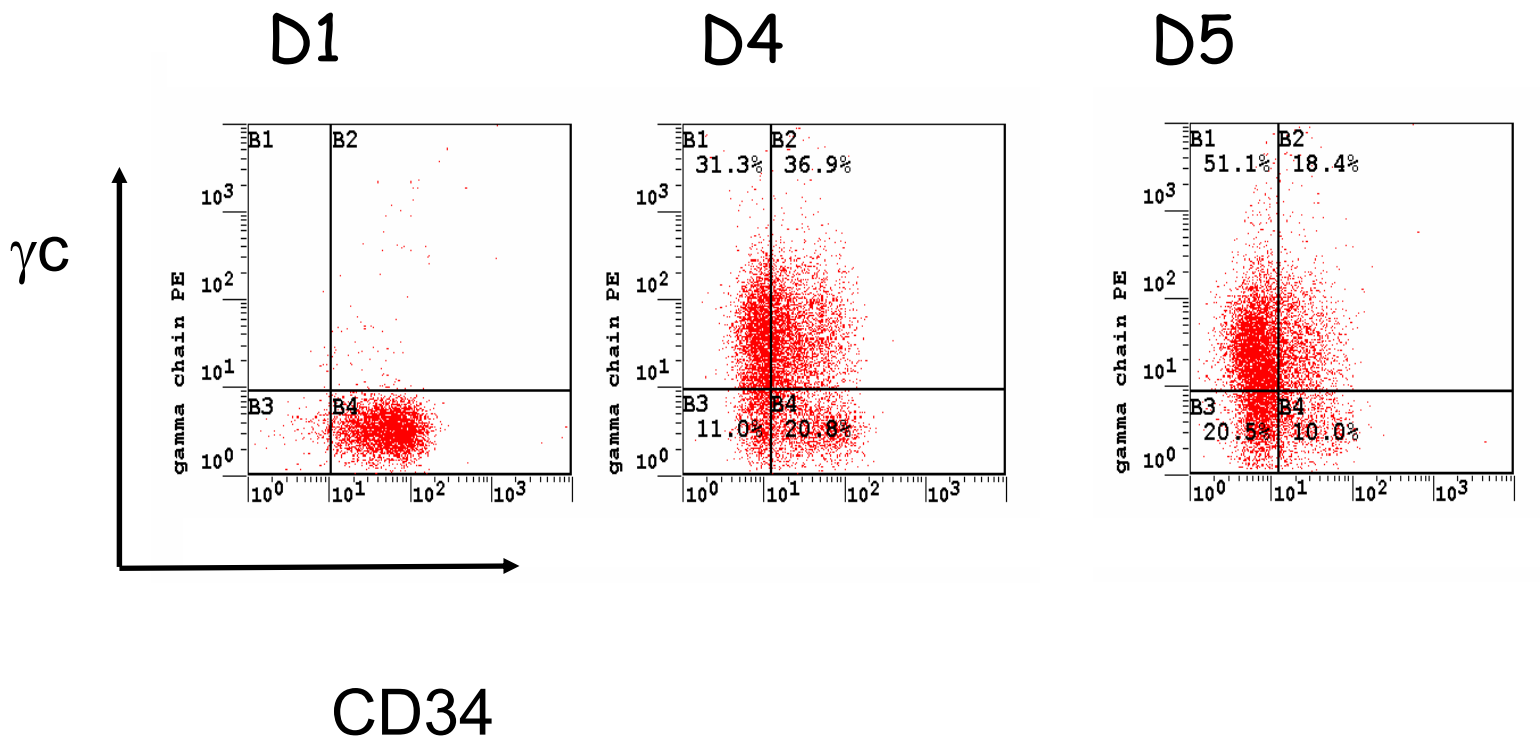
Harvest
CliniMacs CD34+ bone marrow

Pre-activation (40hours)
X-Vivo10 (serum free)
SCF 300ng/ml, FL 300ng/ml, TPO 100ng/ml, IL-3 20ng/ml

Transduction (3 cycles over 72 hours)
Nexell gas permeable flexible containers
Retronectin coating
Virus pre-loading

Infusion

# P2: Transduction process (days 1-5)

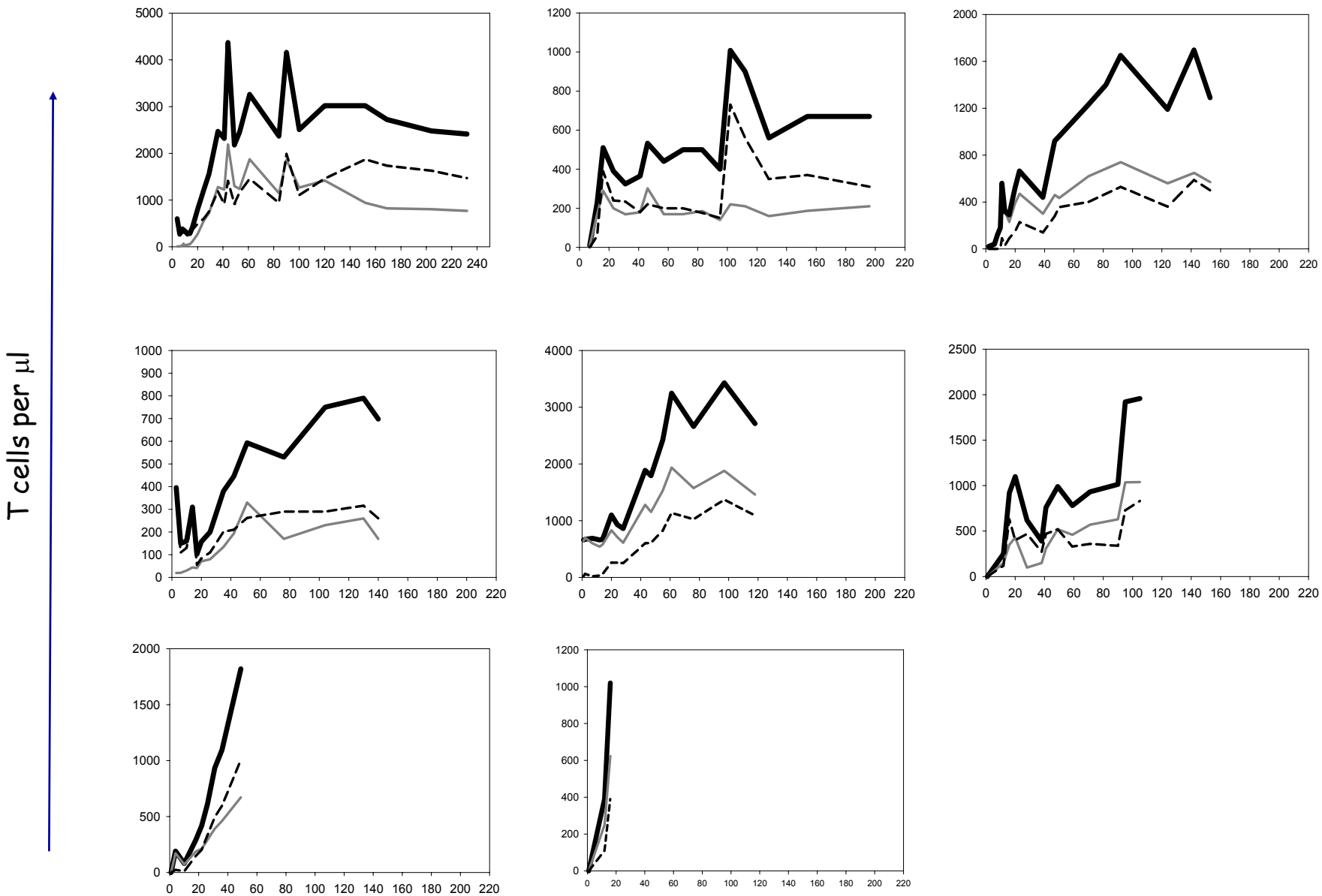



envair

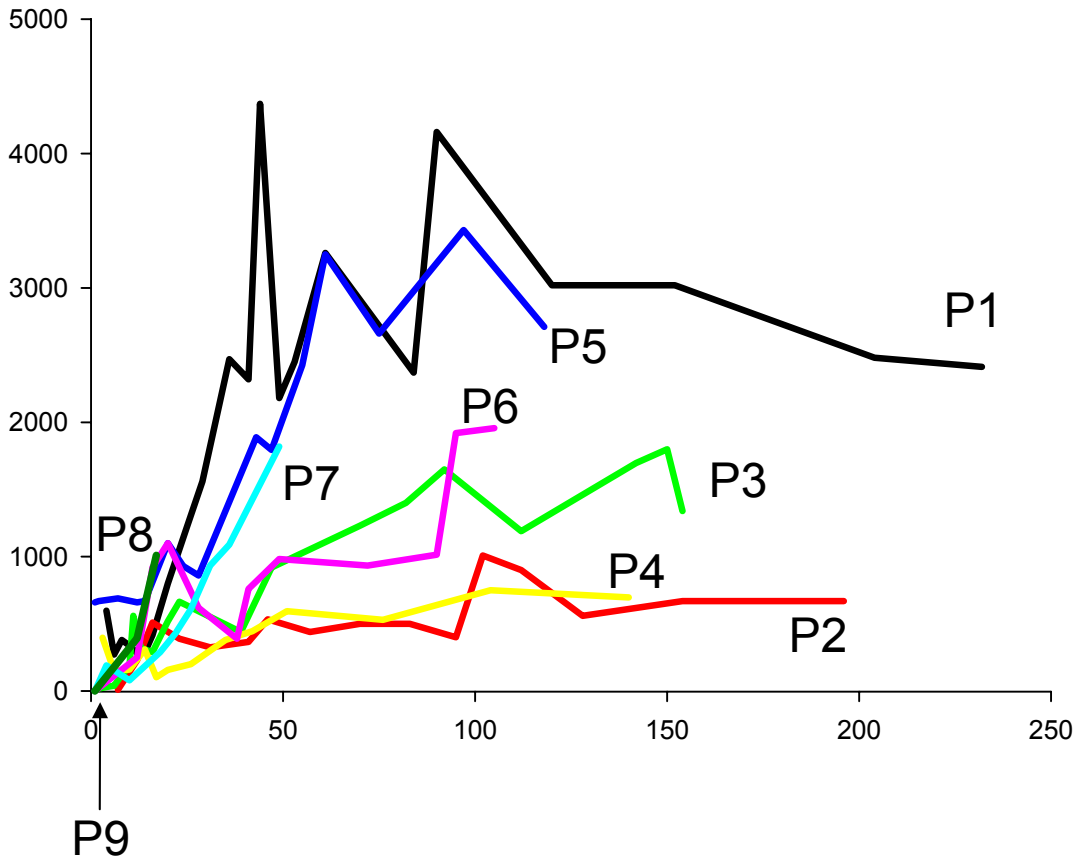
# Patient details (April 2006)

| | Clinical data | Age at therapy (months) | Maternal graft | Mutation | Gamma chain expression | Total cells infused (x10e6) |
|---|---|---|---|---|---|---|
| P1 | | 10 | ++ | R289X | ++ | 180 |
| P2 | | 10 | ++ | S238N | - | 180 |
| P3 | | 4 | - | Y125C | +/- | 78 |
| P4 | | 3y | - | R289X | ++ | 115 |
| P5 | | 10 | - | R222C | ++ | 200 |
| P6 | | 10 | - | PolyA | - | 200 |
| P7 | | 6 | - | M1i | - | 84 |
| P8 | | 13 | - | C182Y | - | 207 |
| P9 | | 7 | - | S108P | - | 160 |

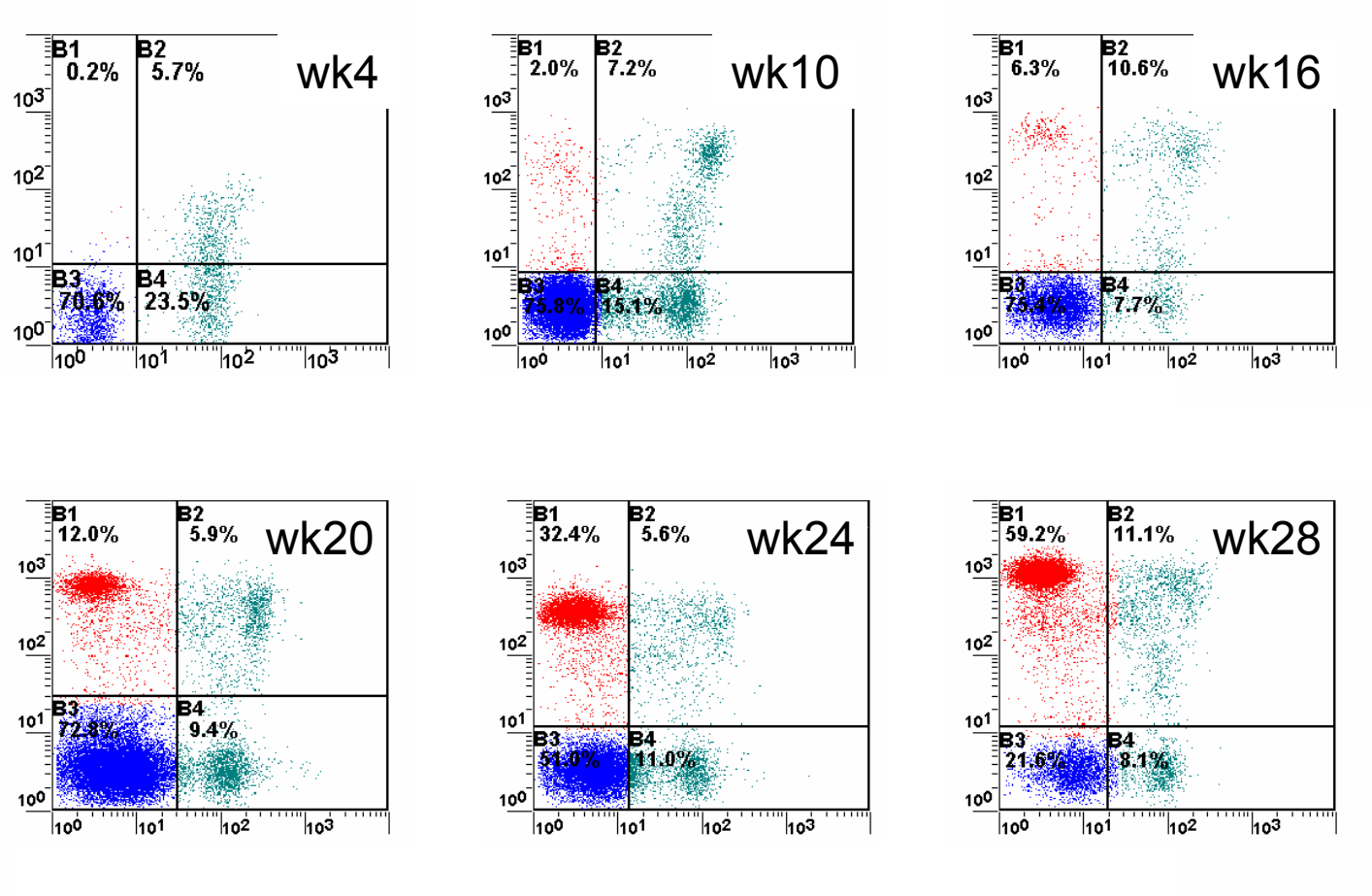
# Lymphocyte recovery CD3/4/8 (weeks, April 2006)



# Lymphocyte recovery CD3 (weeks, April 2006)



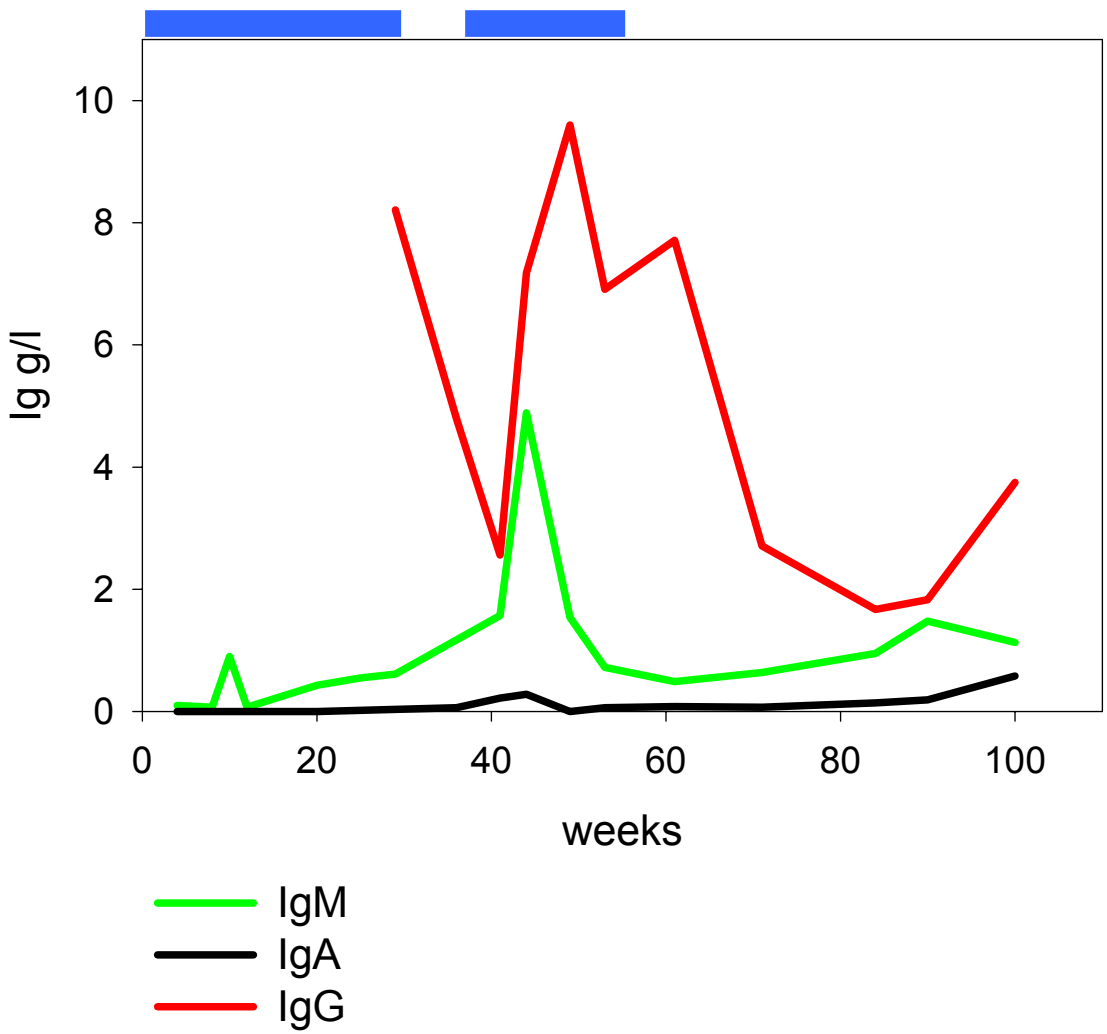
# Flow cytometric analysis of CD45RO-CD27+ naïve T cells in P1



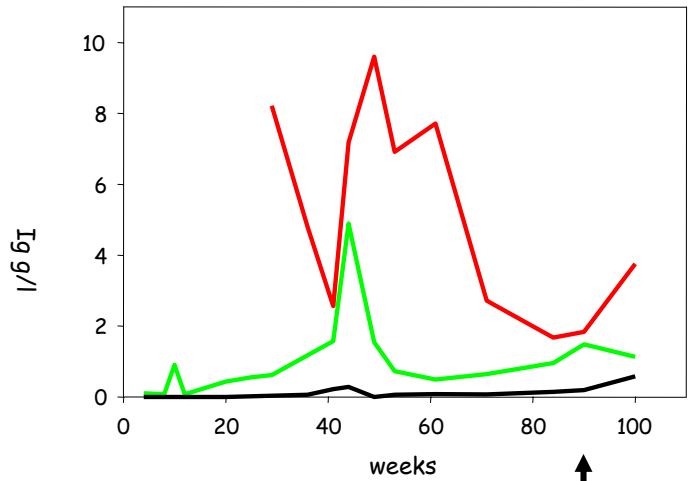

Good evidence for recent thymic emigrants

# P1 Immunoglobulin levels……

scIg



# Immune responses to challenge…

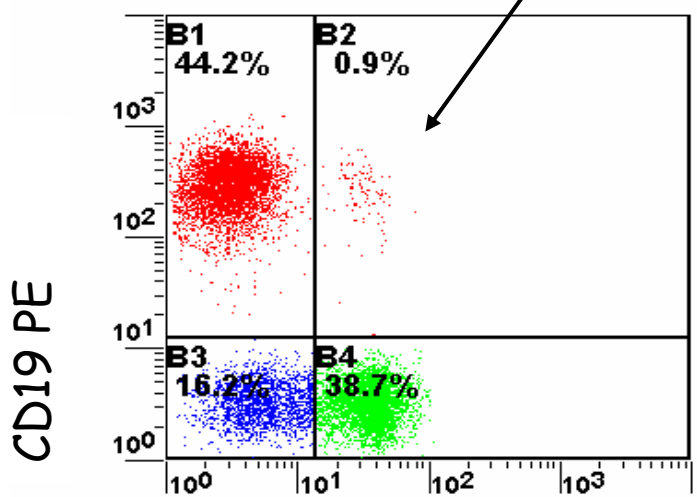



VZV

IgG and IgM positive

Vaccine antigen positive

Somatic mutation +

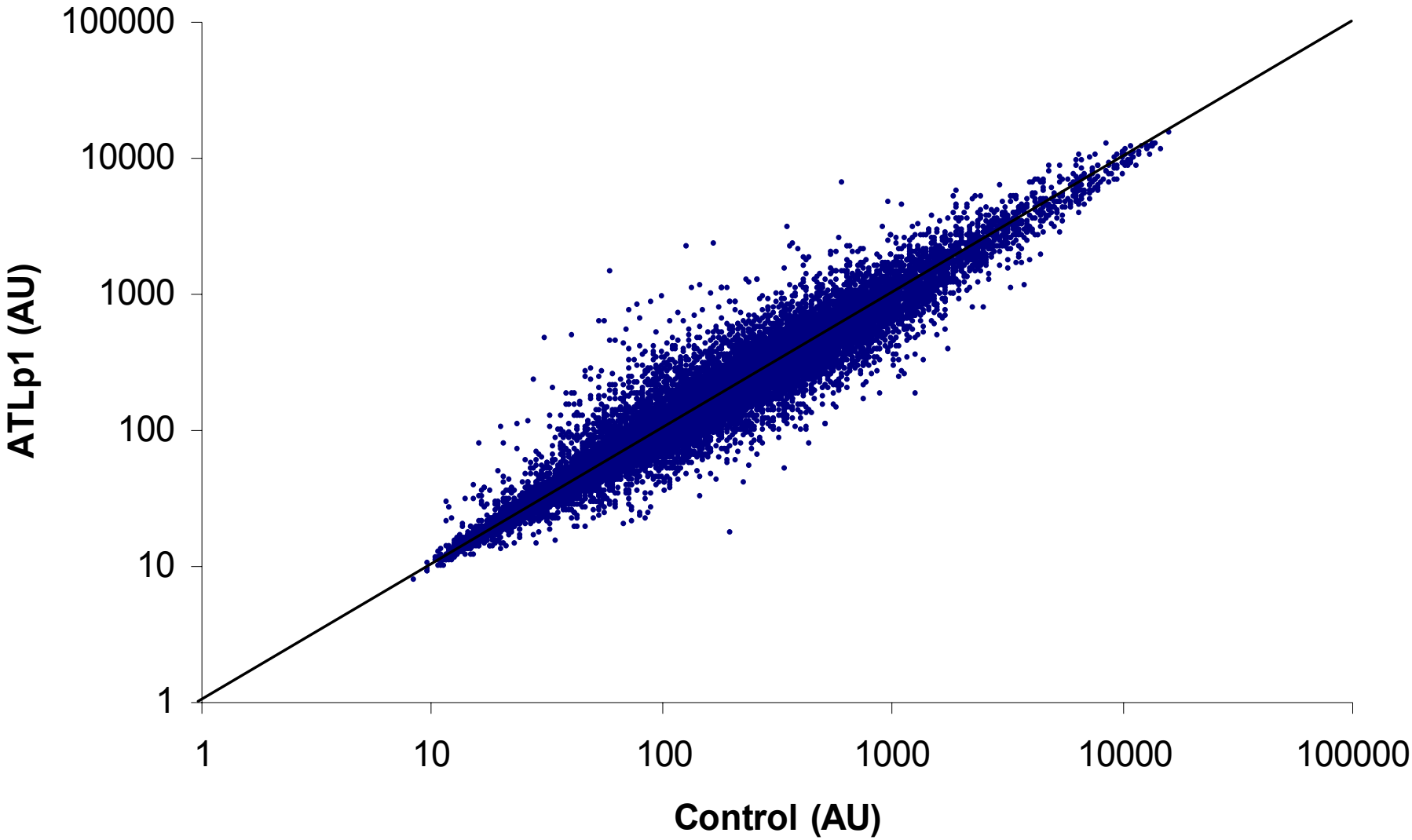



Development of antigen-experienced B cells (CD19+CD27+)

# Lymphocyte proliferation responses

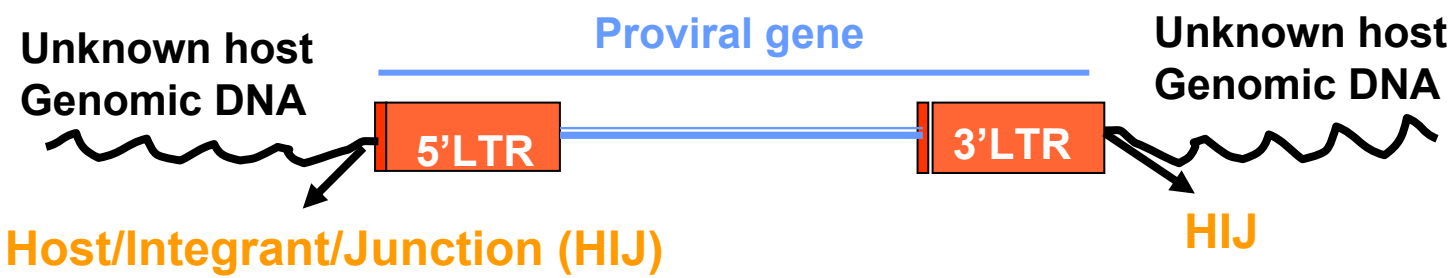
| Patient | CD3/CD28 | PHA | SEB | Con A | PWM | Candida | MLR |
|---|---|---|---|---|---|---|---|
| **P1** | ✓ | ✓ | ✓ | ND | ND | ~ | ND |
| **P2** | ✓ | ✓ | ND | ND | ND | ✓ | ND |
| **P3** | ✓ | ✓ | ND | ✓ | ✓ | ✓ | ✓ |
| **P4** | ✓ | ✓ | ND | ✓ | ✓ | ND | ✓ |

# Gene expression in CD3/CD28 stimulated CD4 cells of ATLp1 vs control



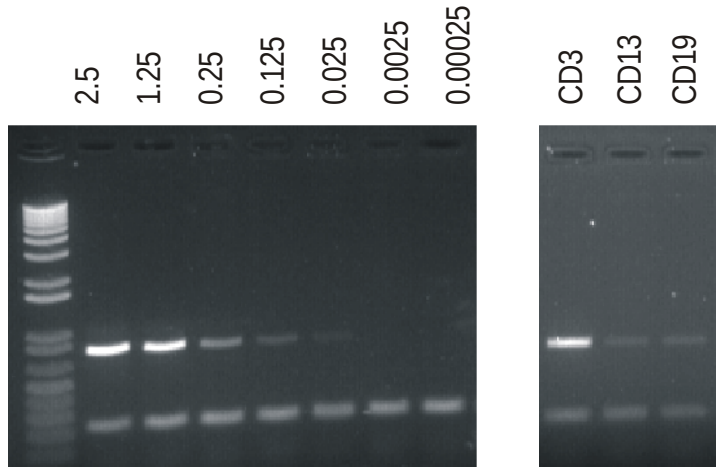
# Integration analysis
## - efficiency and quality of transduction

- RT-PCR, linker adaptor mediated (LAM)-PCR and related techniques
- Copy number
- Numbers of integration sites
- Integration destinations

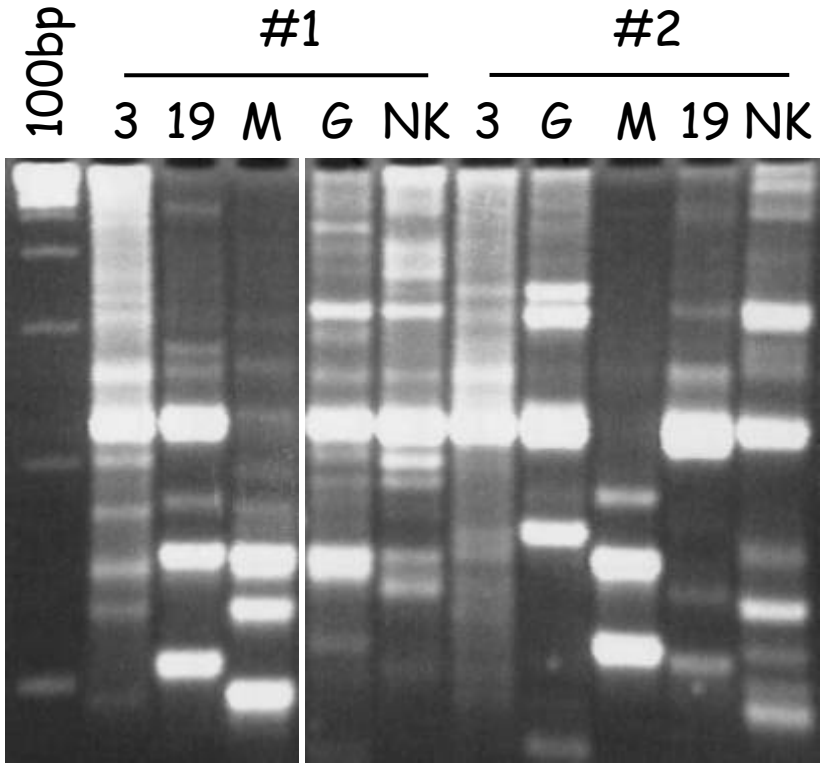


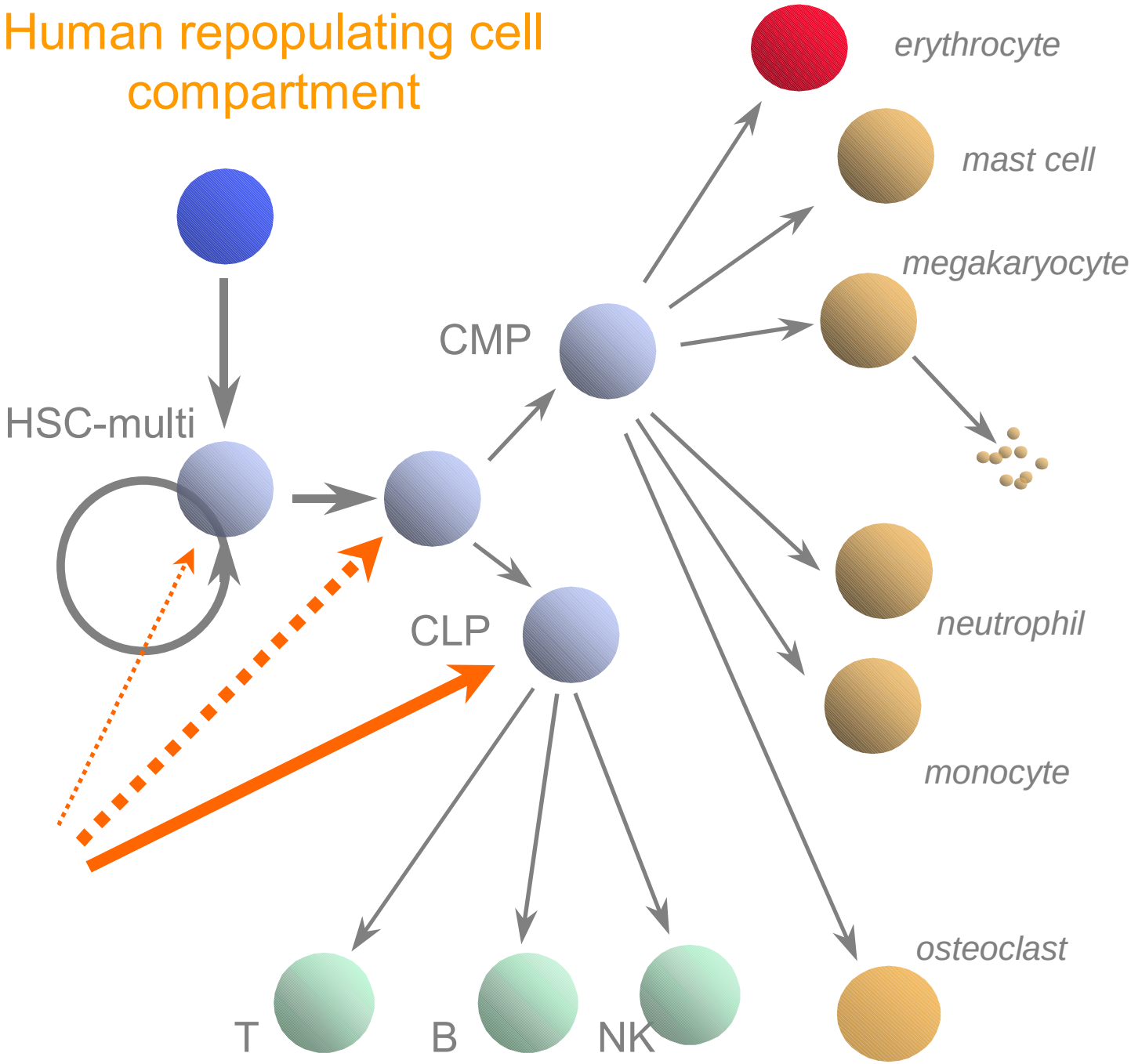
# Efficiency of gene transfer…..

| | |
|---|---|
| CD3 | <3 copies |
| CD19 | 0.01-0.2 copies |
| CD33 | 0.001-0.1 copies |
| CD34 | +ve (P1, P2, P3) |



# LAM-PCR analysis...




Human repopulating cell compartment
erythrocyte
mast cell
megakaryocyte
CMP
HSC-multi
neutrophil
CLP
monocyte
osteoclast
T
B
NK

# Summary of results:

- Immunological reconstitution ✓
- Immune cell function ✓
- Immunisation ✓
- Children at home, off therapy ✓

# Adult patient details

| | Clinical data | Age at therapy | Details at treatment | Follow up (months) | Current status |
|---|---|---|---|---|---|
| **P5** | Bronchiectasis<br>Liver disease | 20y | Previously undergone mismatched transplant, judged to be failing, previous donor no longer available | 17m | No change |

Similar case with another adult patient in Paris

# Paris study - update

11 children treated (1 atypical)
Good response in 10 patients

***However:***

**Patients 4, 5 and 10 – Serious adverse event**

Full immune reconstitution but developed monoclonal T cell lymphoproliferation
- CD3+ leukemia

All presented at between 30-36 months post-treatment
All treated by chemotherapy & BMT

***Conclusion:***

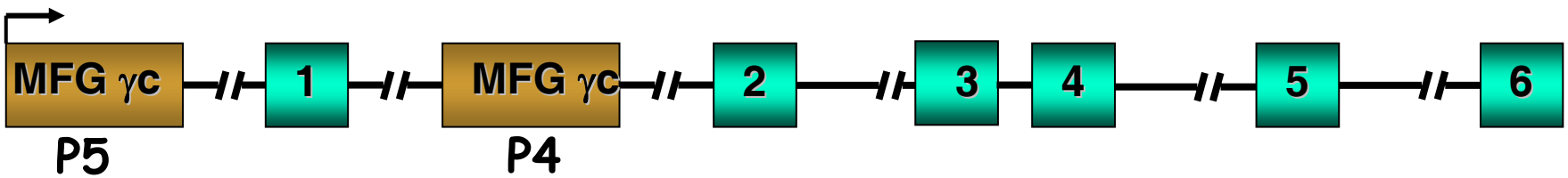
P5 AW
P10 AW
P4 relapsed and died

# Mechanism of leukaemogenesis?

***Insertional mutagenesis***

P4 & P5 - Single insertions into LMO-2 gene

(P4 in intron 1, P5 5' upstream, promoter region)




**LMO-2**

Oncogene, known to be expressed in T cell leukemia,
LMO-2 is highly expressed in leukaemic clones

***Other factors?***

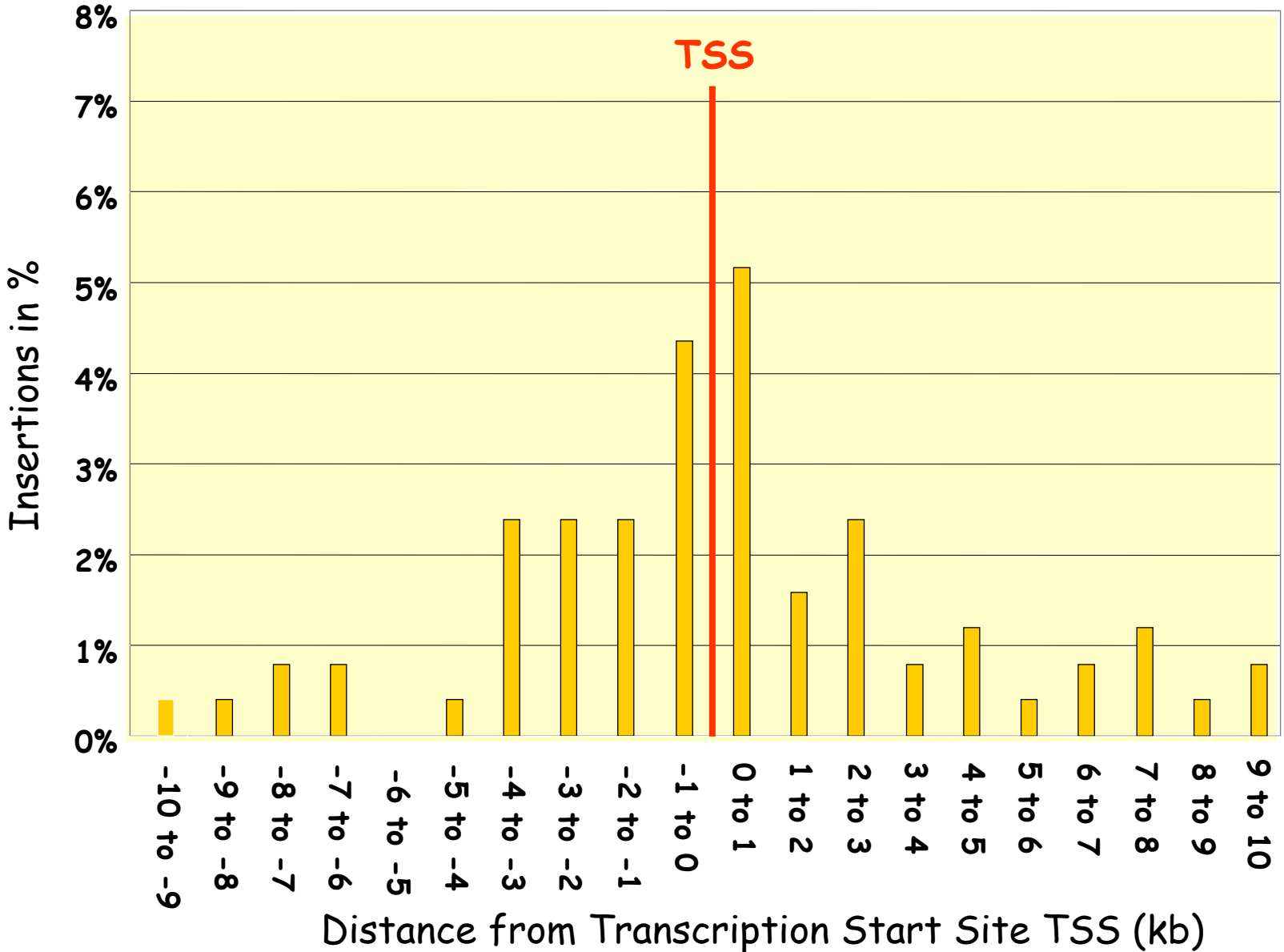
P10 - 3 different integration sites (Lyl-1, Bmi-1, ?)
Synergistic effect of γc + LMO-2?

# Patient follow up (April 2006)…

| | | Follow up months |
|---|---|---|
| P1 | AW* | 56 |
| P2 | AW | 52 |
| P3 | AW* | 47 |
| P4 | AW* | 39 |
| P5 | AW* | 27 |
| P6 | AW* | 24 |
| P7 | AW | 17 |
| P8 | AW | 4 |
| P9 | AW | 0 |

*off prophylactic therapy

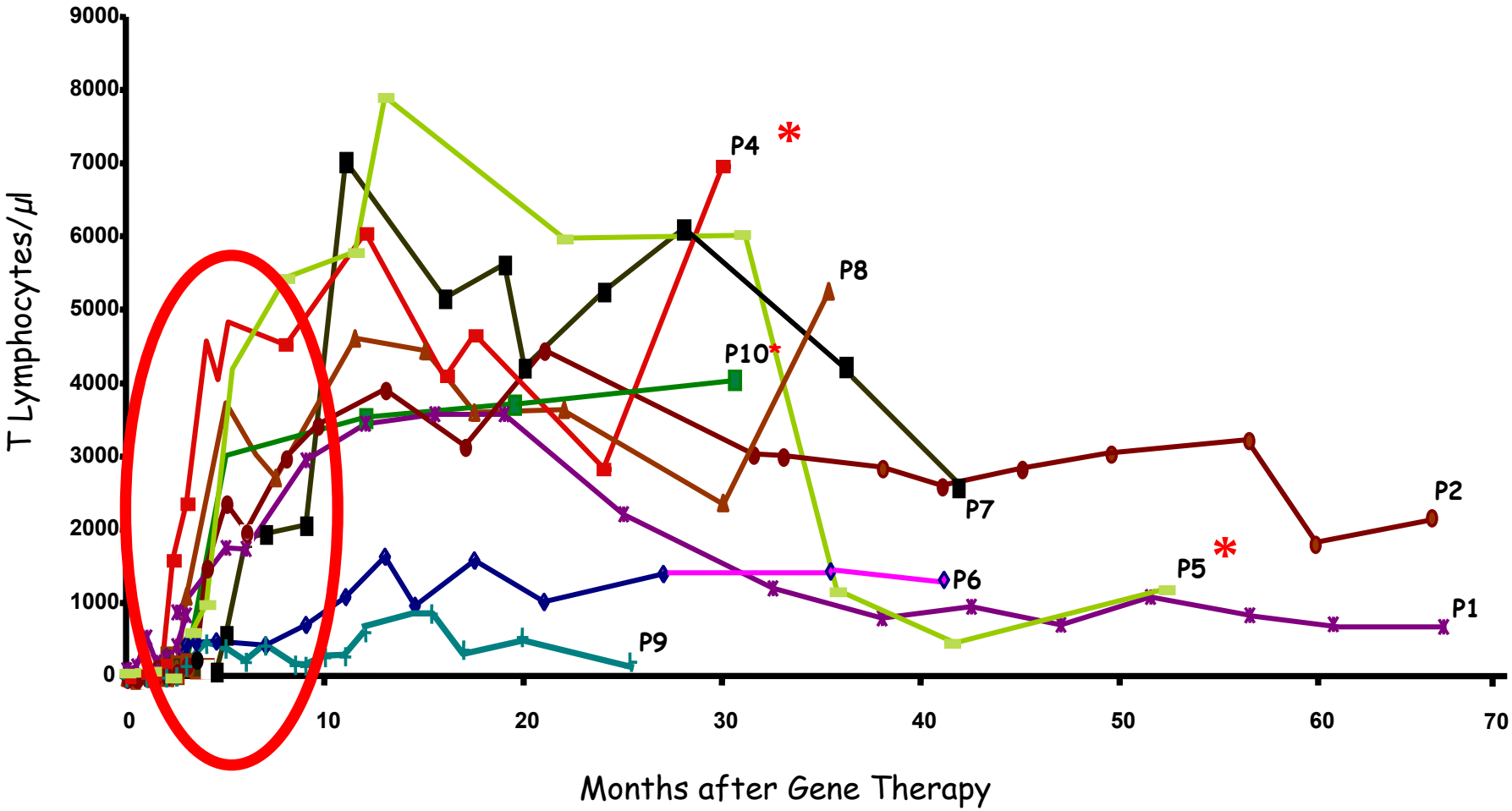
# Insertions around Transcription Start Sites



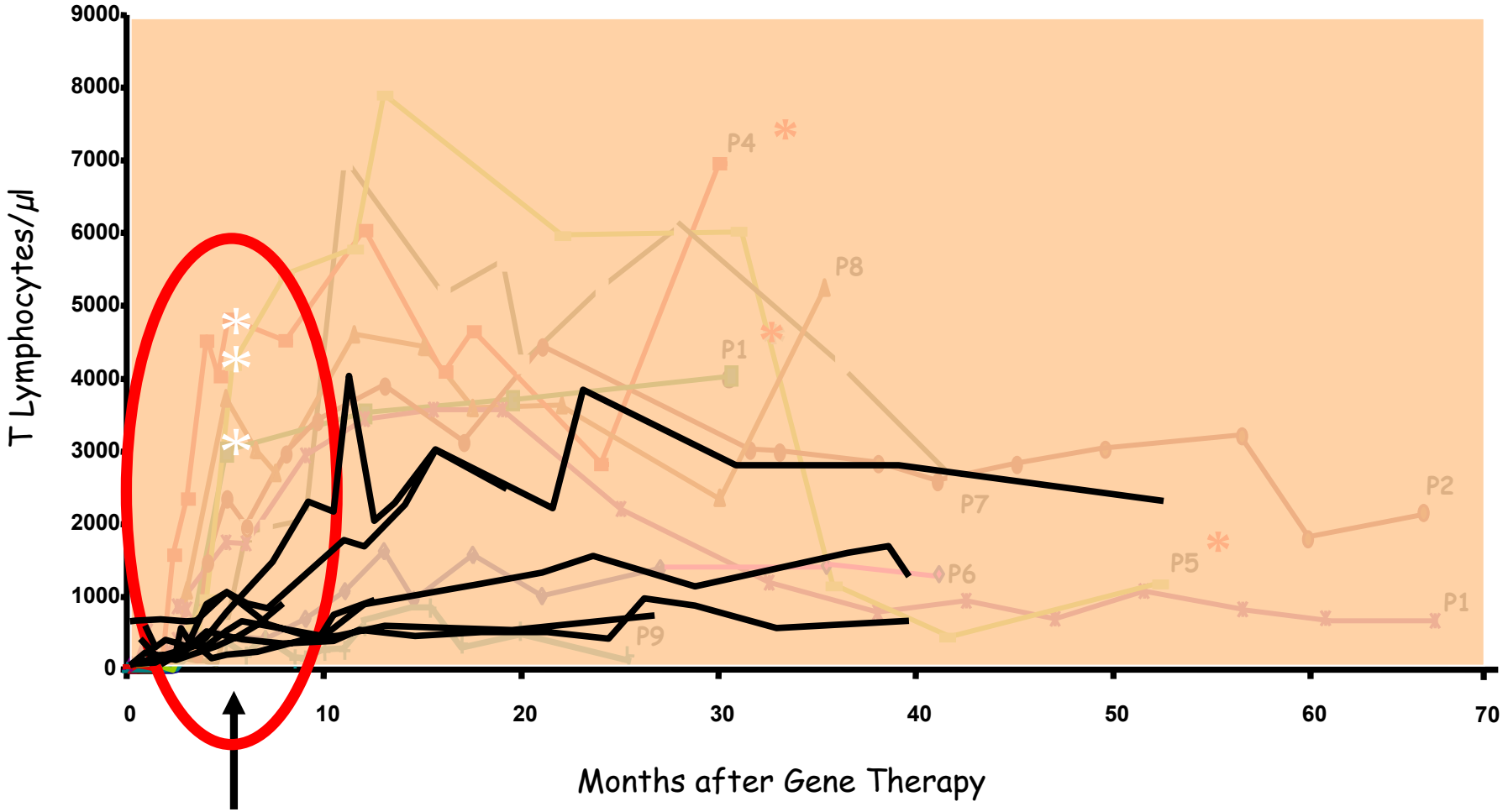
# Analysis of Retroviral Integration Sites (IS)

| | Post-Transplantation | |
|---|---|---|
| | Absolute | Percentage |
| Total number of integration sites (IS) | 344 | |
| Exactly mappable IS | 252 | 100% |
| IS in RefSeq genes | 99 | 39% |
| IS in RefSeq genes including the region 10 kb upstream and downstream of the gene | 148 | 59% |
| IS more than 10 kb away from RefSeq genes | 104 | 41% |
| IS within +/- 5 kb around transcription start site | 58 | 23% |

# Lymphocyte recovery CD3 (Paris, March 2005).....



# Lymphocyte recovery CD3 (Paris and London, March 2005).....



# UK SCIDX1 protocol….

## (MFG, GALV-pseudotype, no B2 mutation)

Harvest
CliniMacs CD34+ bone marrow
Pre-activation (40hours)
**X-Vivo10 (serum free)**
SCF 300ng/ml, FL 300ng/ml, TPO 100ng/ml, **IL-3 20ng/ml**
Transduction (3 cycles over 72 hours)
Nexell gas permeable flexible containers
Retronectin coating
Virus pre-loading
**No protamine sulphate**
Infusion


# Will it happen to children treated in London?

We don't know, but:

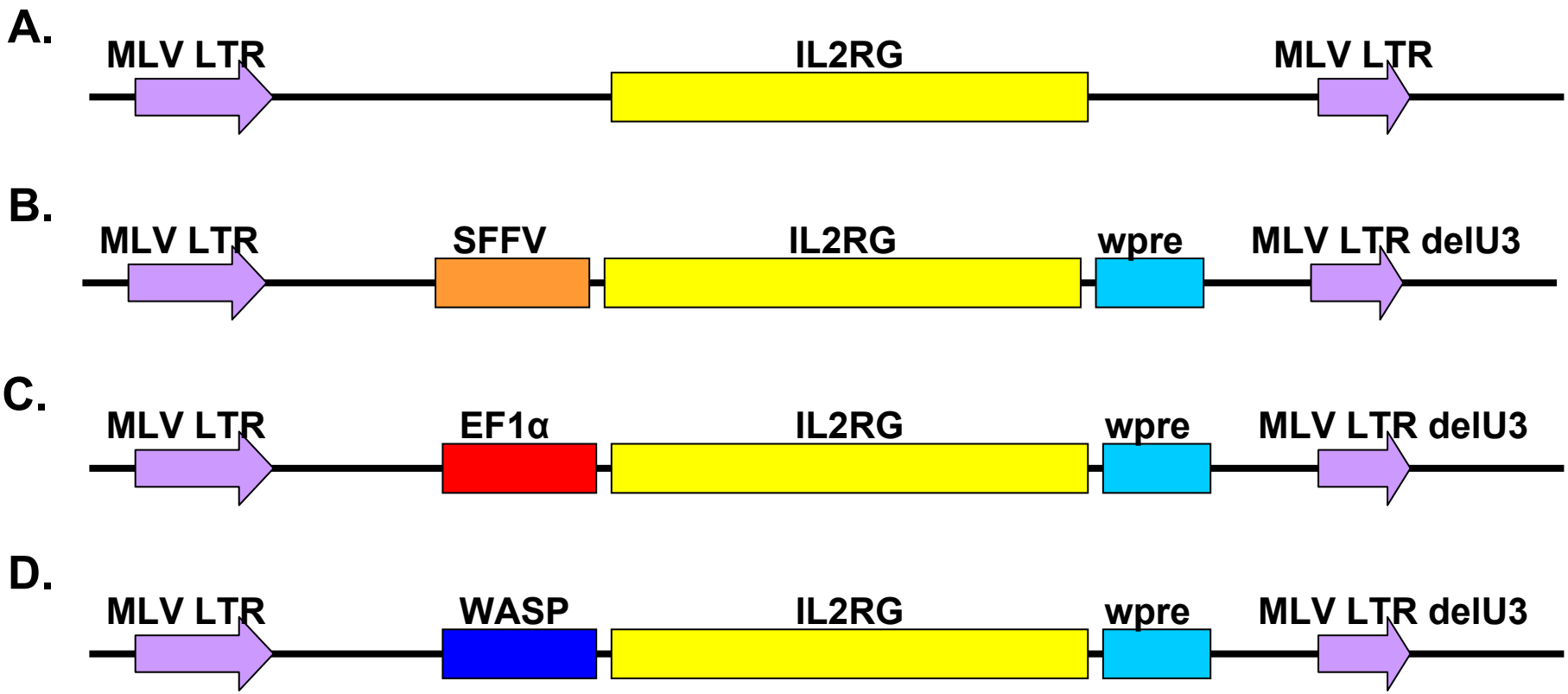
1. At least 4 of our patients are out of the "time-frame"
2. We used a slightly different virus
3. We used a different protocol

**Development of safer vectors**

# New Improved Retroviral Vectors

- **Self-inactivating (SIN) vectors**
  - promoter and enhancer element deletion in 3'LTR
  - should have no LTR-directed transcription
  - should reduce the risk of insertional mutagenesis

- **Tissue specific promoters**
  - viral promoters, SFFV LTR is active in stem and progenitor cells
  - constitutive eukaryotic promoters, EF1α
  - haematopoietic cell specific promoters, WASp




## Schematic representation of SIN vectors

(A) Vector used in our current clinical trial

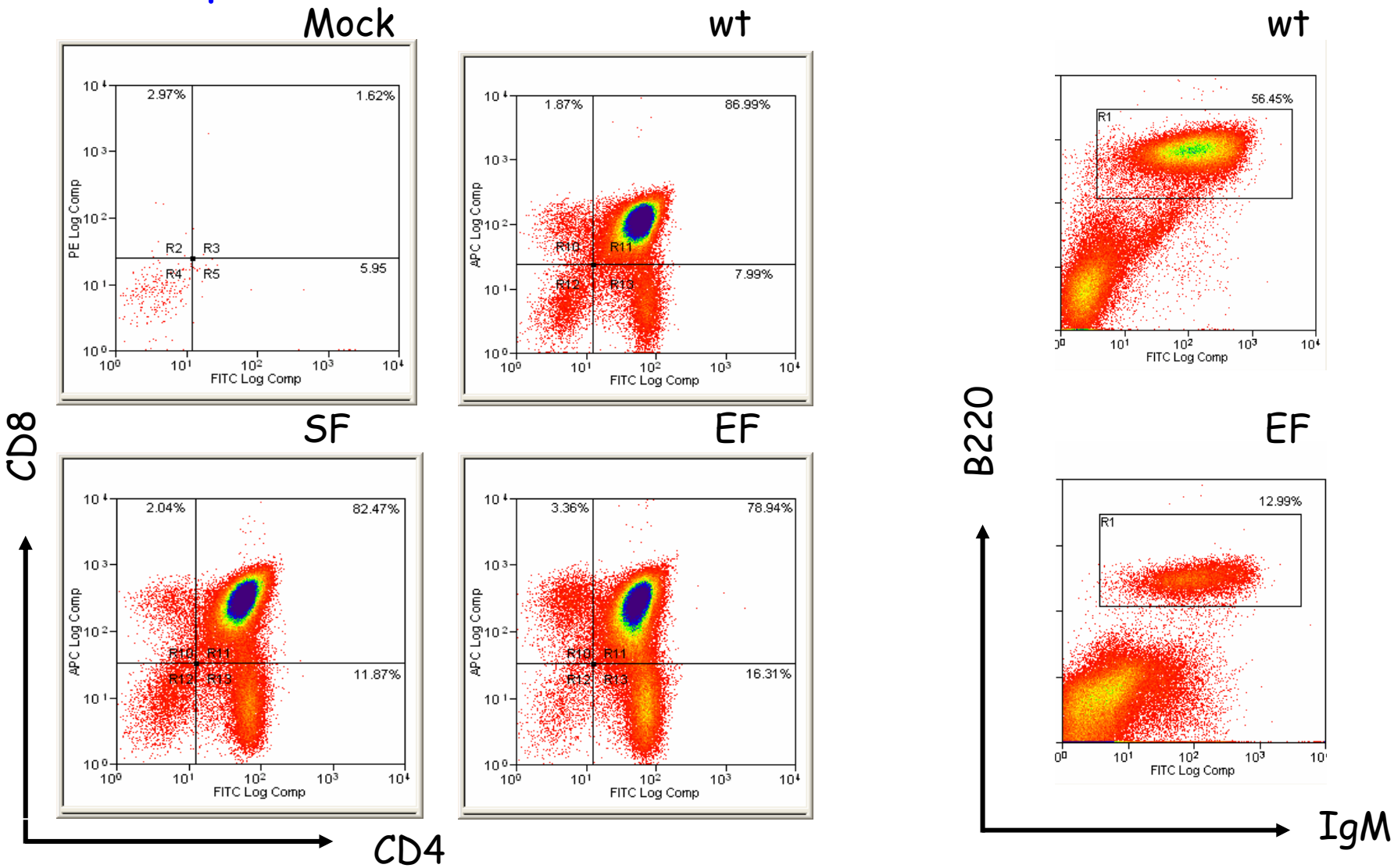
(B-D) SIN vectors in which the *IL2RGγc* transgene is regulated by internal regulatory sequences:

(B) spleen focus forming virus promoter (SFFV)

(C) elongation factor 1α promoter (EF1α)

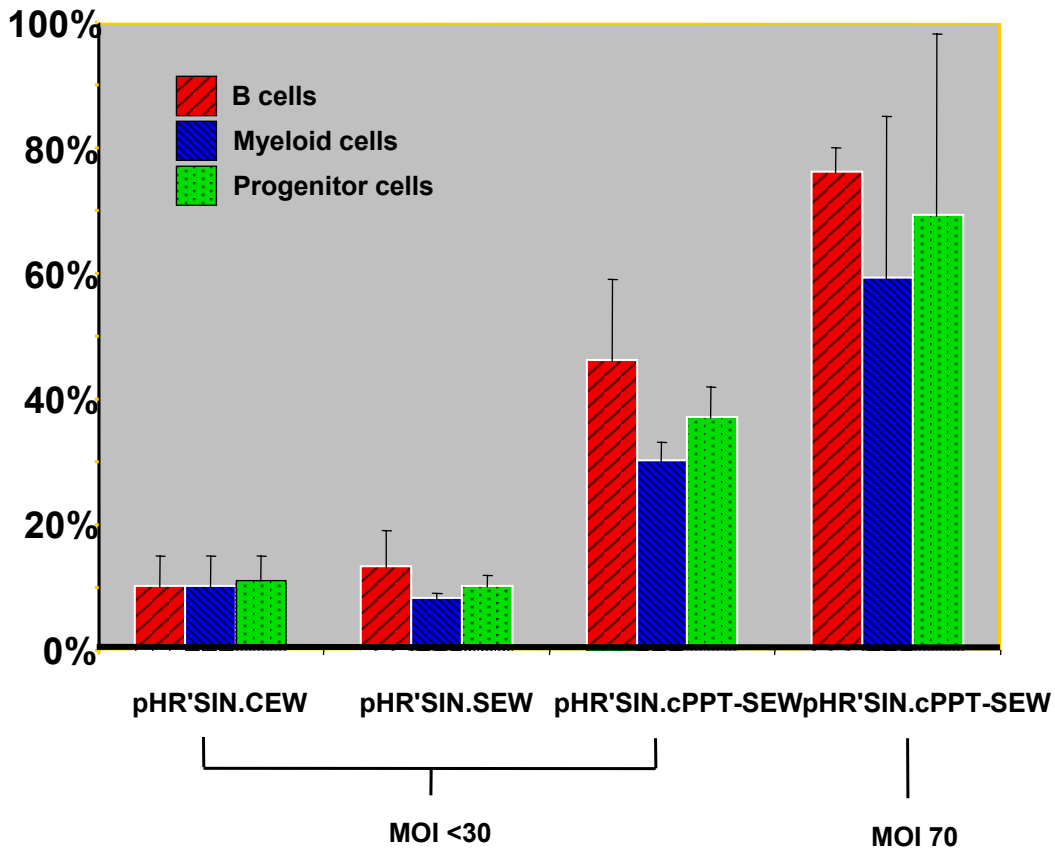
(D) Wiskott Aldrich syndrome protein promoter

# SIN gammaretroviral reconstitution of murine T and B cell compartments....




5 months after gene transfer

# GFP expression from modified lentiviral vectors



# Future gene therapy:

Other primary immunodeficiency disorders

-ADA-SCID (1 patient, 3 further planned)

-X-CGD (2 patients)

-WAS (hope to start 2007?)

Primary haematological disorders, including leukemia

Stem cell protection strategies

# The Molecular Immunology Unit at ICH

**ICH**
Kate Parsley
Kimberly Gilmour
Jo Sinclair
Steve Howe
Doug King
Suzy Bailey
Fang Zhang
Aris Giannakopoulos
Meera Ulaganathan
Mohammed Osman
Mike Blundell
Graham Davies
Christine Kinnon
Bobby Gaspar
Adrian Thrasher

**GOS**
Nursing Staff
Pharmacy Staff

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Department of Health,
CGD Trust, Primary Immunodeficiency Association, CF Trus
EU, LRF, ARC,
SPARKS
and others…

**EUFETS**
Klaus Khulke

**Cincinatti/ Freiberg**
Manfred Schmidt
Christof Von Kalle

**Frankfurt**
Manuel Grez
Stefan Stein

**Hannover**
Christopher Baum