A PILOT STUDY OF TEMOZOLOMIDE AND O^6-BENZYLGUANINE FOR TREATMENT OF HIGH-GRADE GLIOMA, USING AUTOLOGOUS PERIPHERAL BLOOD STEM CELLS GENETICALLY MODIFIED FOR CHEMOPROTECTION

NCI Protocol # 7036

RAC Protocol # 699

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Background: High-Grade Glioma

- Primary brain tumors are now the leading cause of cancer death in children < 15 yrs of age
- High-grade gliomas (anaplastic astrocytoma or glioblastoma multiforme) are associated with particularly poor prognosis:
  - 5-yr progression-free survival of 19%
  - Median overall survival of 18-42 months

Treatment for High-Grade Glioma

1. Surgical resection
   - Important, but not expected to be curative
2. Focal radiation
   - Prolongs disease-free survival
3. Nitrosurea-based chemotherapy
   - May modestly improve survival
   - Limited by:
     - cumulative heme toxicity (delays, dose reductions, etc)
     - life threatening non-heme toxicity (pulmonary toxicity)
     - tumor cell resistance

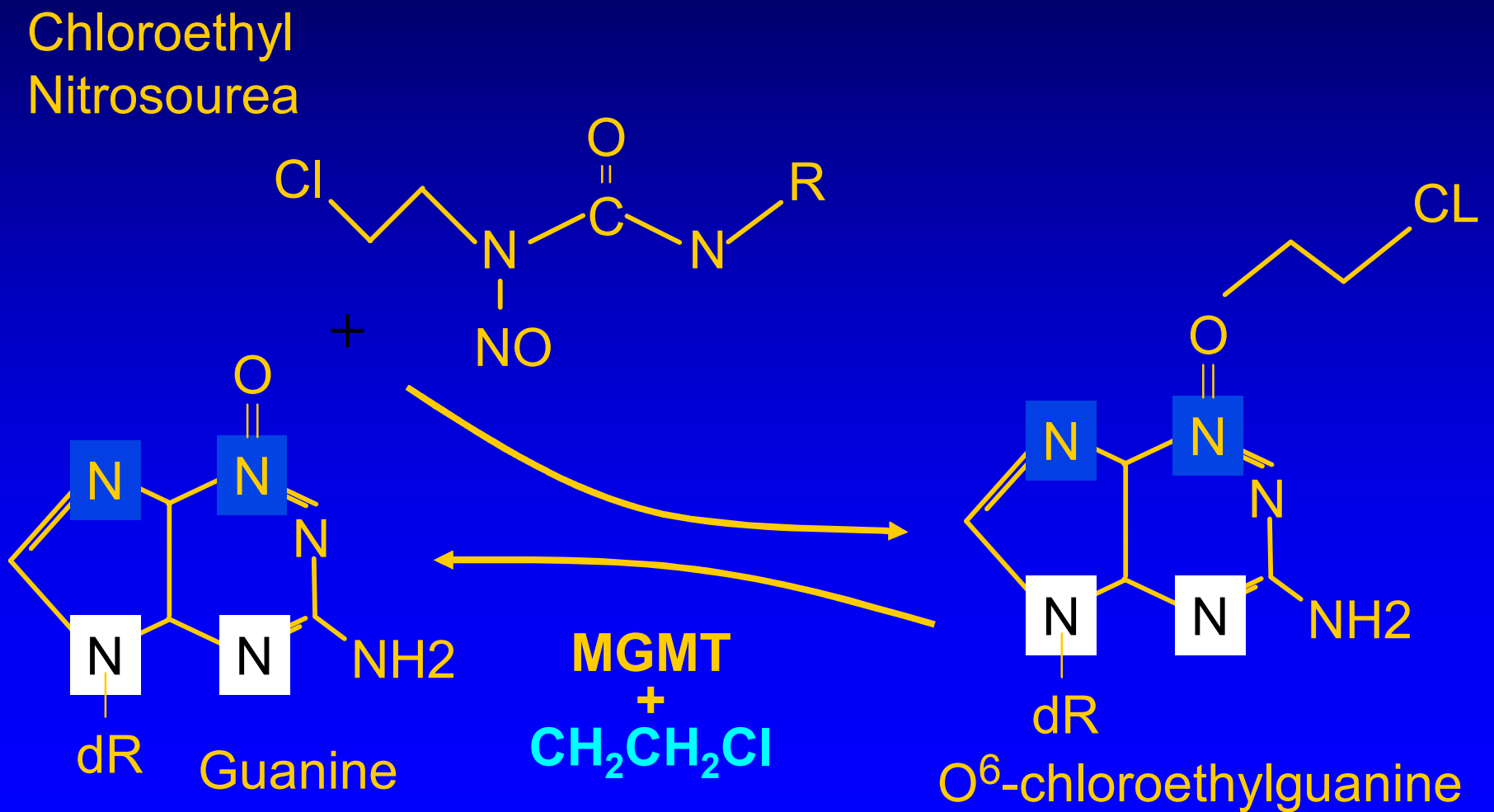
Use of Temozolomide

- Oral methylating agent with more limited non-hematologic toxicity profile
- TEM used post-surgery concurrent with and following radiation significantly improved survival compared with XRT alone
- Becoming new “standard of care” for adult GBM based on limited toxicity spectrum, oral administration; currently being evaluated in pediatric patients
- However, 2-yr overall survival rates of 26% indicate TEM is still not curative for majority of newly-diagnosed patients with glioblastoma multiforme

Overcoming Tumor Cell Resistance

- Methylguanine-DNA methyltransferase (MGMT) is primary mechanism of resistance to TEM
- MGMT removes adducts placed at O^6-guanine by TEM or BCNU

MGMT Selection: DNA Repair



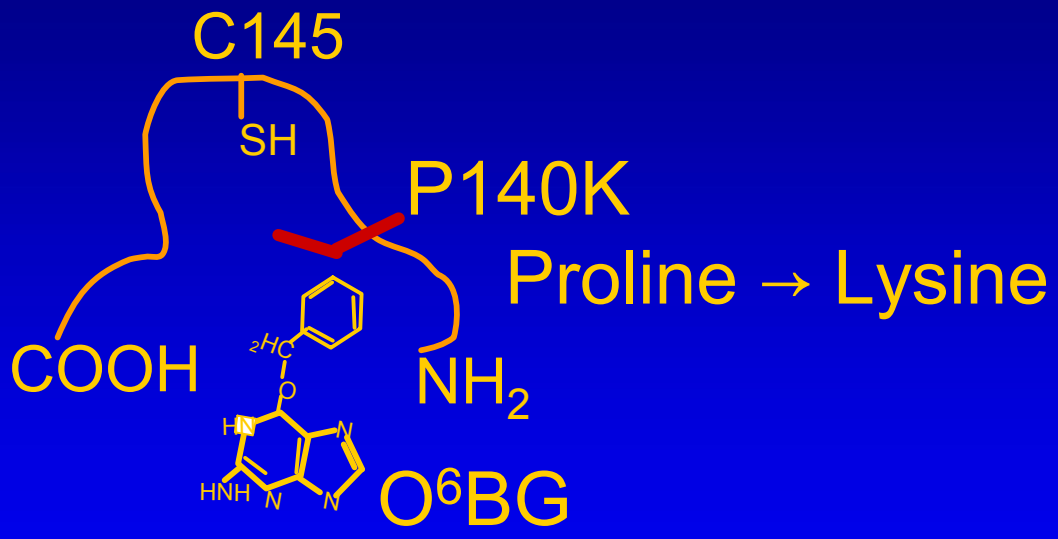
MGMT is a Relevant Therapeutic Target

- Expressed in 90% of pediatric gliomas, and 75% of adult gliomas
- Expression correlates with response to TEM in patients with high-grade glioma
- An available drug (O^6-Benzylguanine) safely and effectively inactivates MGMT in tumor tissue and increases sensitivity of tumor cells to TEM

Use of O^6-Benzylguanine to Reverse MGMT

- Phase I trials of TEM + BG in kids and adults shows exacerbation of hematologic toxicity, due to inactivation of low (but protective) levels of MGMT in HSC
- This requires dose reduction of over 50% for TEM in Phase I trials
  - 200 $\rightarrow$75 mg/m^2/d x 5 (pediatric study)
  - 1000$\rightarrow$ 472 mg/m^2 x 1 (adult study)
- Phase II study of BG + reduced-dose BCNU showed poor activity
- Optimization of TEM + BG may require a method to circumvent hematolgic toxicity

$MGMT^{P140K}$ is Resistant to Both Temozolomide and BG



$MGMT^{P140K}$

Dolan et al., Cancer Res. 51:3367.
Prog. Exp. Tum. Res. Basel, Karger, 1999, vol 36. pp 65-81.

Overcoming Hematologic Toxicity

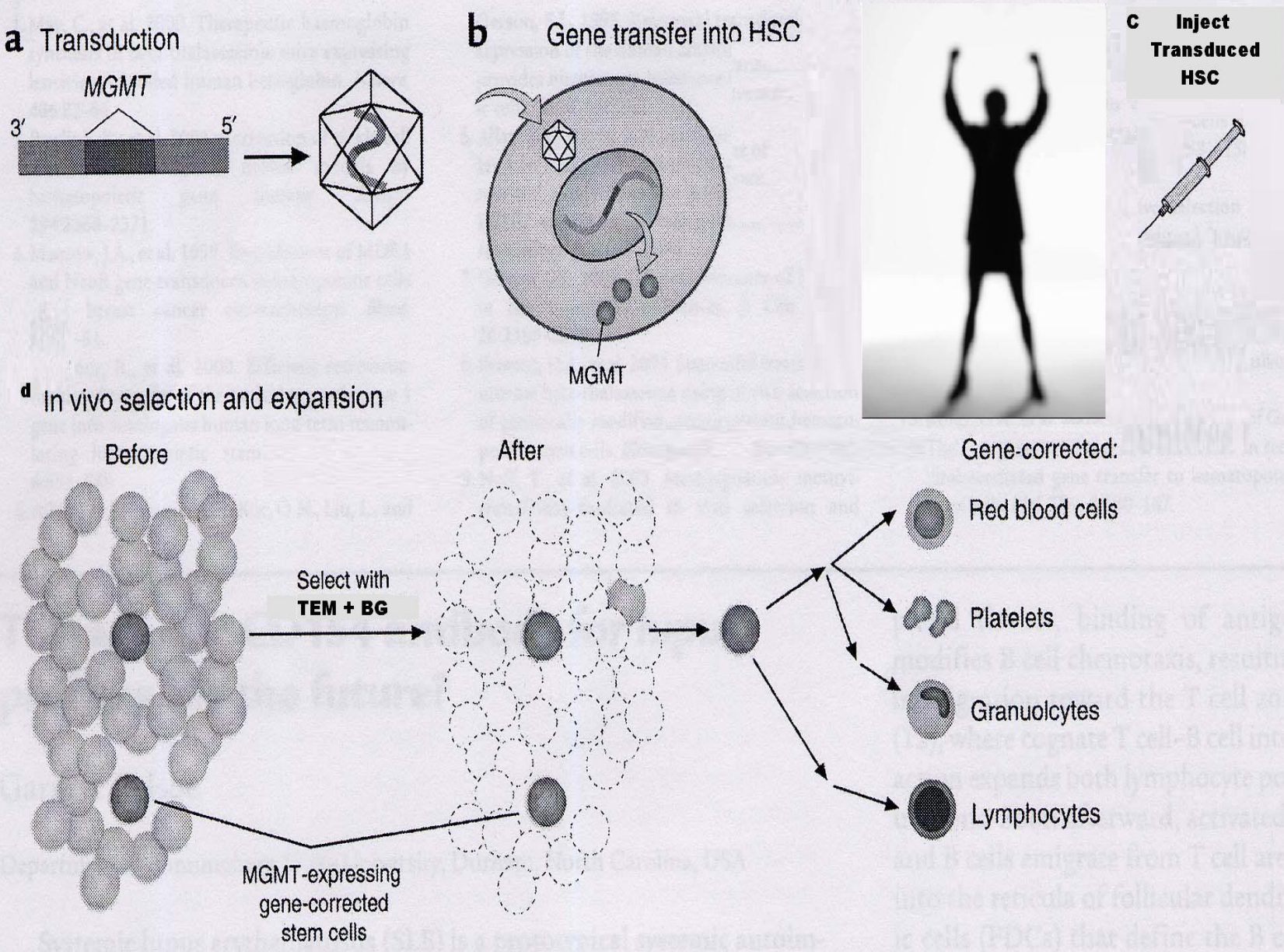
- Transduction of hematopoietic stem cells (HSC) with altered MGMT gene (*MGMT*^{P140K}) affords protection against both TEM AND BG
- Repeated doses of TEM + BG after transduction kills nontransduced HSC but not protected cells, thereby increasing the relative percentage of protected cells ("enrichment" effect) and causing less myelosuppression
- This enrichment effect also helps overcome initially low transduction efficiency
- Overcoming HSC toxicity allows for increasing doses of TEM → superior anti-tumor effect in xenograft model

Summary of Preclinical Studies Using *MGMT*^{P140K}

1. Protection from chemotherapy toxicity using both mouse and human HSC
   - Improvement in blood counts with continued therapy
   - Durable chemoprotection even when doing serial transplants into another mouse

2. Higher tolerated dose intensity, resulting in greater activity in xenograft model

2. Durable multilineage protection and enrichment in allogeneic canine model (15→98% in granulocytes)
   - Increase in donor chimerism (55→ 95%)


a Transduction
MGMT
3′
5′
b Gene transfer into HSC
MGMT
c Inject Transduced HSC
d In vivo selection and expansion
Before
After
Gene-corrected:
Select with
TEM + BG
Red blood cells
Platelets
Granuolcytes
Lymphocytes
MGMT-expressing
gene-corrected
stem cells

Pilot Study Objectives

- Demonstrate feasibility and safety of gene transfer and proposed chemotherapy regimen
- Assess efficiency of gene transfer and durability of MGMT expression
- Assess degree of chemoresistance and ability to do intrapatient dose escalation of TEM, using extent of transgene expression as a criteria for dose escalation
- Assess responses in confines of a small pilot study

Study Population

Inclusion Criteria

- ≥ 5 years of age with newly-diagnosed anaplastic astrocytoma or GBM
- Stable neuro status, adequate organ function and performance status

Exclusion criteria

- Gross total resection
- Oligodendroglioma or oligoastrocytoma
- Extracranial disease
- Tumor arising in the brainstem

Study Design

1. "Standard of care"

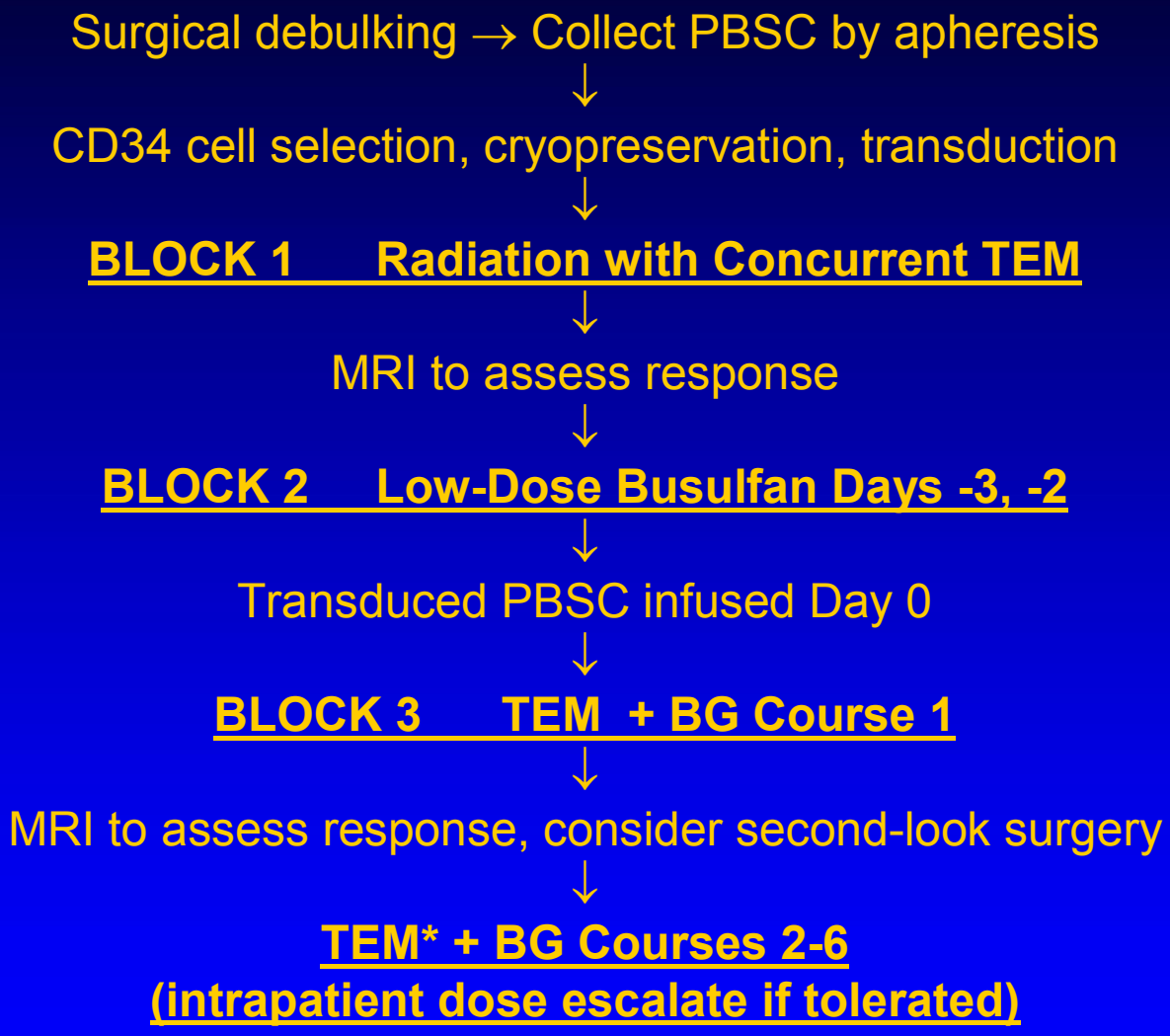
   Maximum tolerable surgical resection followed by
   focal external beam RT, concurrent with
   low-dose daily TEM (75 mg/m^2/day) x 6 weeks

2. Gene Transfer

   Low-dose Busulfan (2 mg/kg x 2 days)
   used to create space for transduced autologous PBSC

3. Enrichment/Dose escalation

   Chemotherapy with 6 monthly courses of TEM + BG,
   with **intrapatient** dose escalation as tolerated


Surgical debulking → Collect PBSC by apheresis
CD34 cell selection, cryopreservation, transduction
BLOCK 1 Radiation with Concurrent TEM
MRI to assess response
BLOCK 2 Low-Dose Busulfan Days -3, -2
Transduced PBSC infused Day 0
BLOCK 3 TEM + BG Course 1
MRI to assess response, consider second-look surgery
TEM* + BG Courses 2-6
(intrapatient dose escalate if tolerated)

Intrapatient Dose Escalation of TEM During Block 3

- Dependent on absence of DLT in previous course of TEM + BG, as well as "marked ANC" of ≥ 100/µl as assessed by flow cytometry before next course

- TEM increased by 35% per dose level (from 75 mg/m^2/day) as long as criteria are met

Dose Level	TEM Dose (mg/m^2/day x 5)
2	100
3	130
4	170
5	220
6	285

Assessment of Study Endpoints

Safety
- toxicity assessed by CTCAE criteria
- monitoring of blood and marrow for toxicity from gene transfer

Extent of gene transfer
- assessed by flow cytometry and real-time PCR

Efficacy of gene transfer
- MGMT activity and cytotoxicity assays with marrow progenitor cells
- ability to dose escalate during BLOCK 3

Antitumor activity
- response measured by serial MRIs during and following therapy

Potential Toxicities of Gene Transfer

- Secondary leukemia from insertional mutagenesis
  - Monitor blood/marrow by morphology, flow cytometry for clonality, and LAM-PCR for monoclonal vector insertion sites
- Development of replication-competent retrovirus
- Severe acute infusion-related toxicity
- Delayed engraftment
  - Use back-up stem cells

Stopping rule in place if any of first three toxicities occur

Potential Clinical Applications of Chemoprotection Approaches

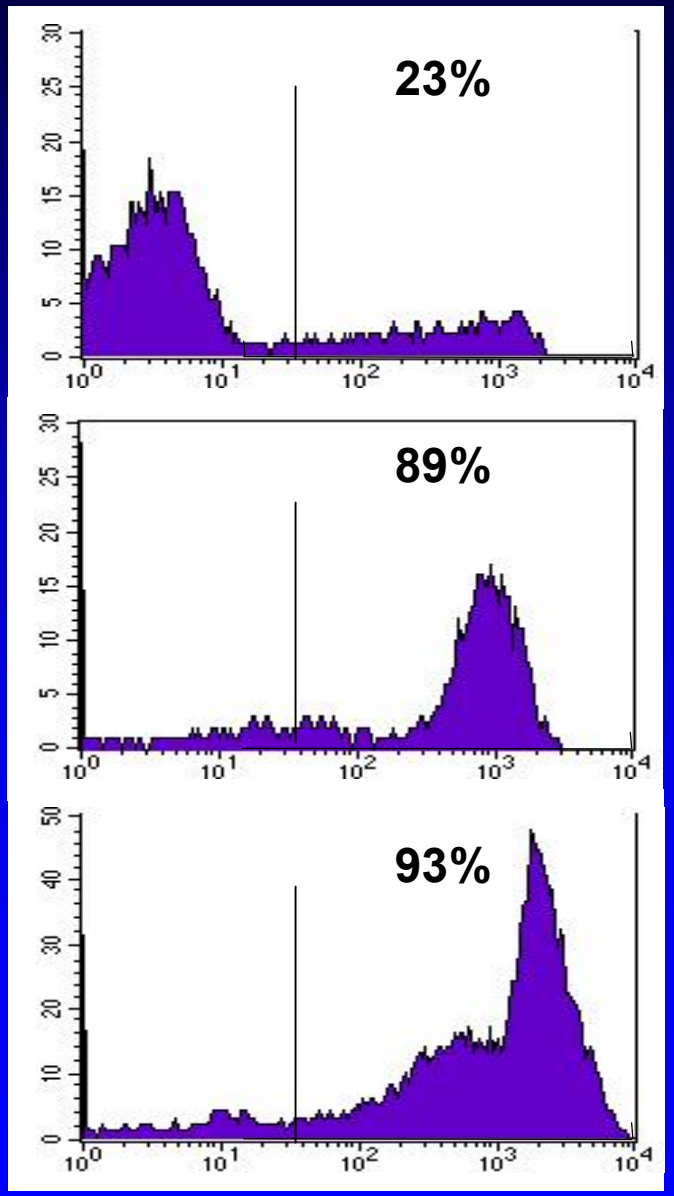
- Dose-intensification using TEM
  - Brain tumors
  - Metastatic melanoma
- Modifying chimerism following allogeneic transplantation to enhance GVL effect
  - High-risk leukemia
  - Metastatic melanoma
- Increasing transgene expression following gene transfer for single-gene disorders
  - Thalassemia

Selection of MGMT-transduced Hematopoiesis by BCNU/O^6-BG

Primary Recipient
w/o Selection

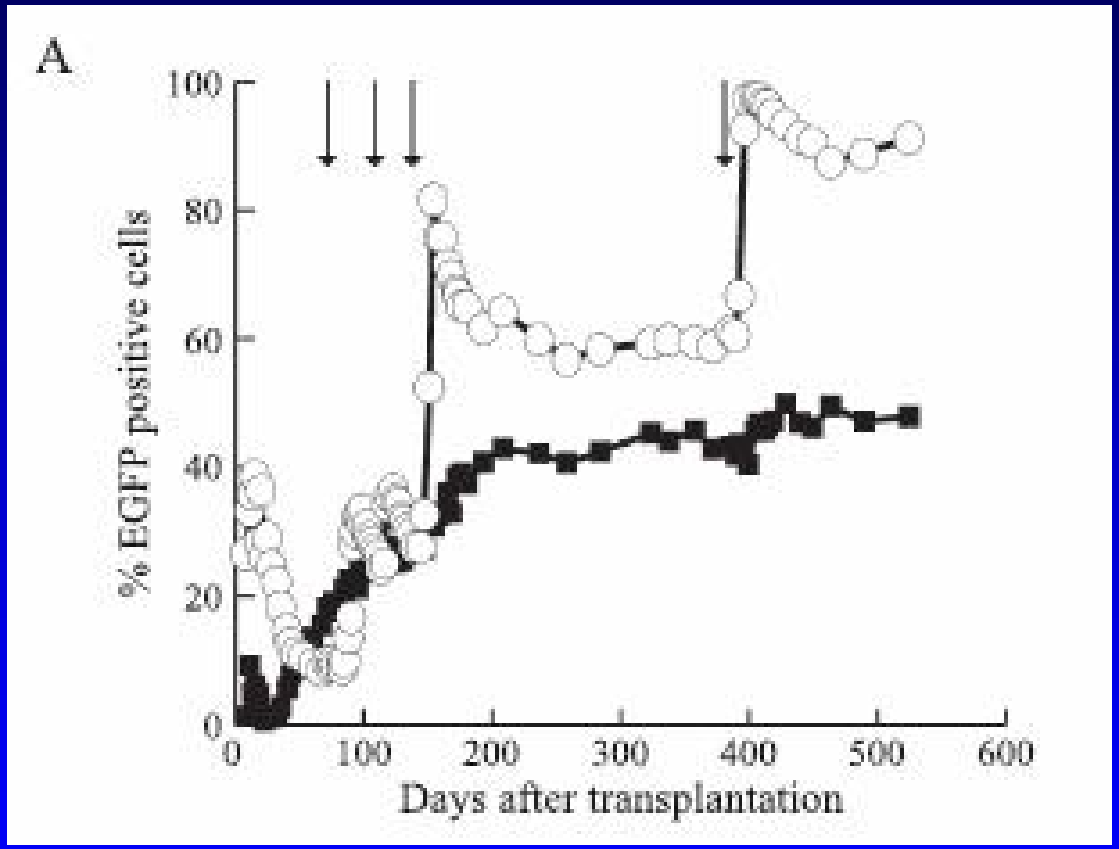
Primary Recipient
after 2 x BCNU/O^6-BG

Secondary Recipient
w/o Selection



Ragg *et al*, Cancer Res 2001;
Pollok *et al*., Mol Ther 2004

Canine Stem Cell Selection *In Vivo* by BCNU/O6 BG

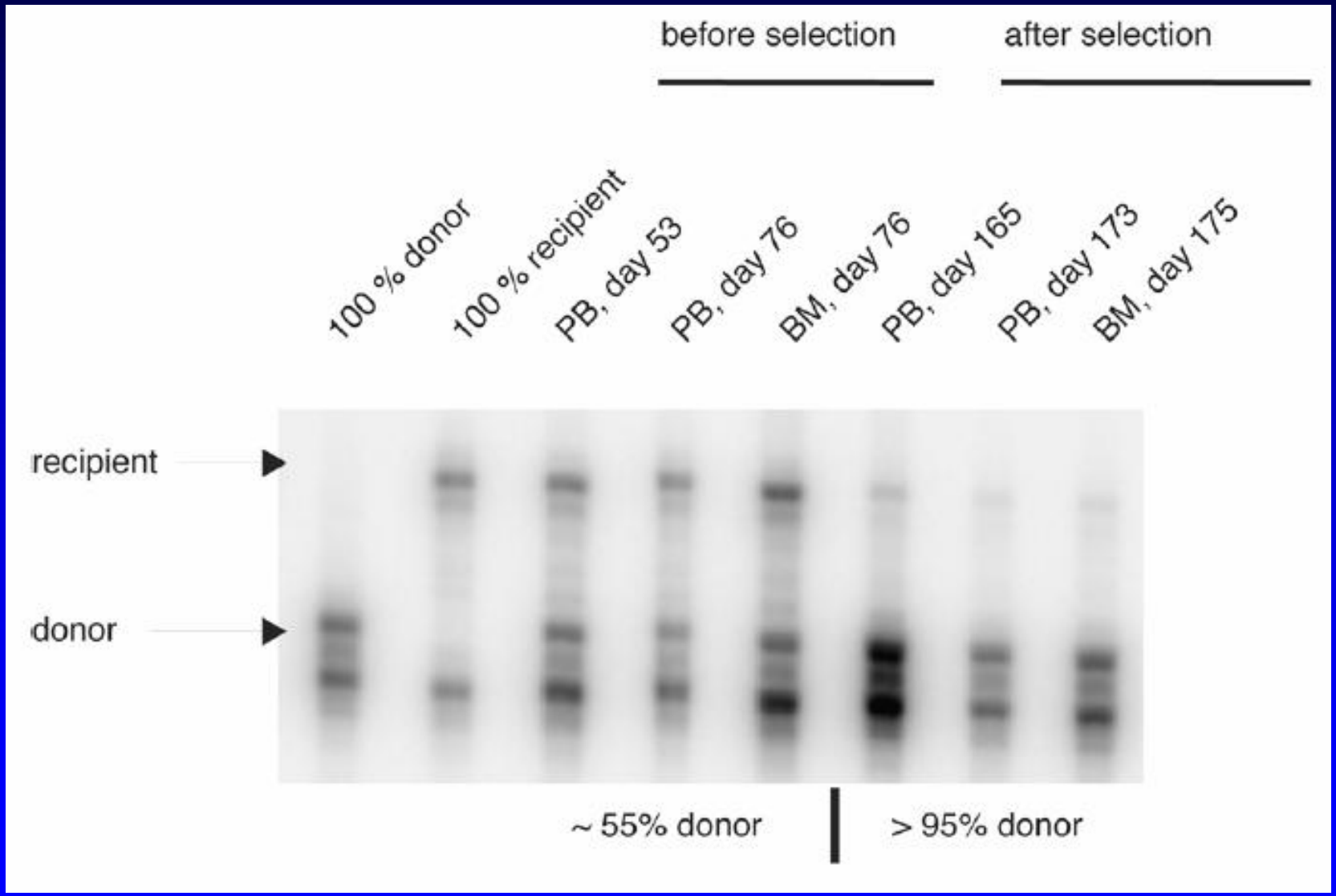


MGMT P 140K as a Therapeutic Transgene:

- Chemoresistance
- *In vivo* Selection
- Chimerism Control and Chemotherapy during Leukocyte Recovery

Neff *et al*., JCI 2003, Horn *et al*., Mol Ther 2004

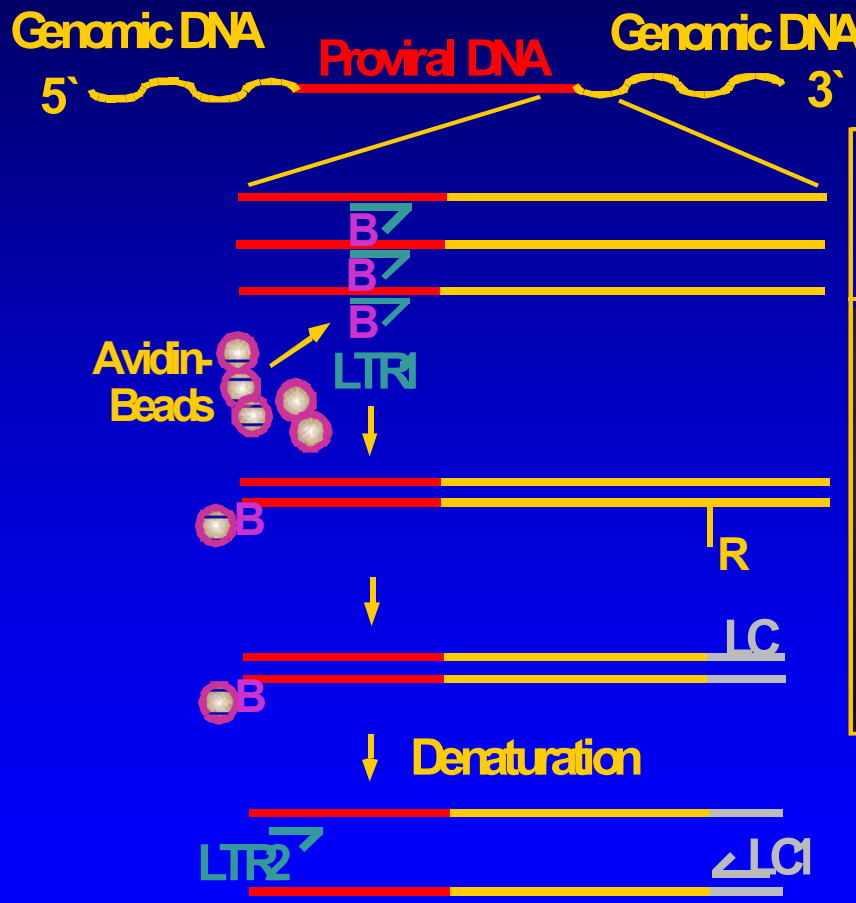
Regulating Donor Chimerism by Selection Pressure on Retrovirus Transgenes

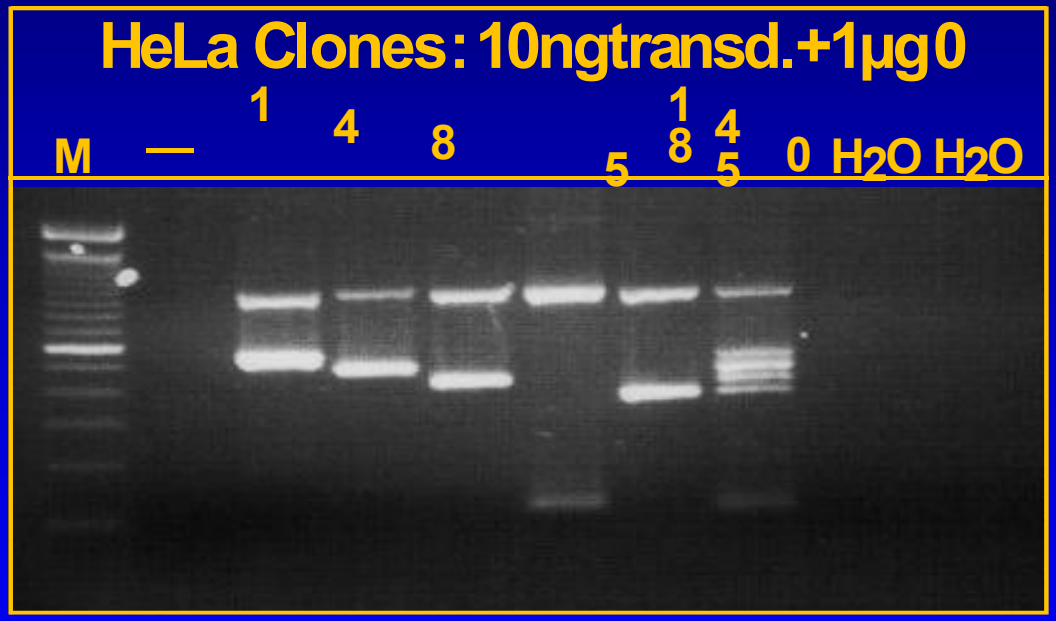


Insertion Site Analysis: Dose Finding in Gene Therapy

- Stem Cell Clonality
- Insertion Site Distribution Analysis
- Cell Type Dependence of Insertion
- Genomic Side Effects of Insertion
- Active Modification of Insertion Behavior

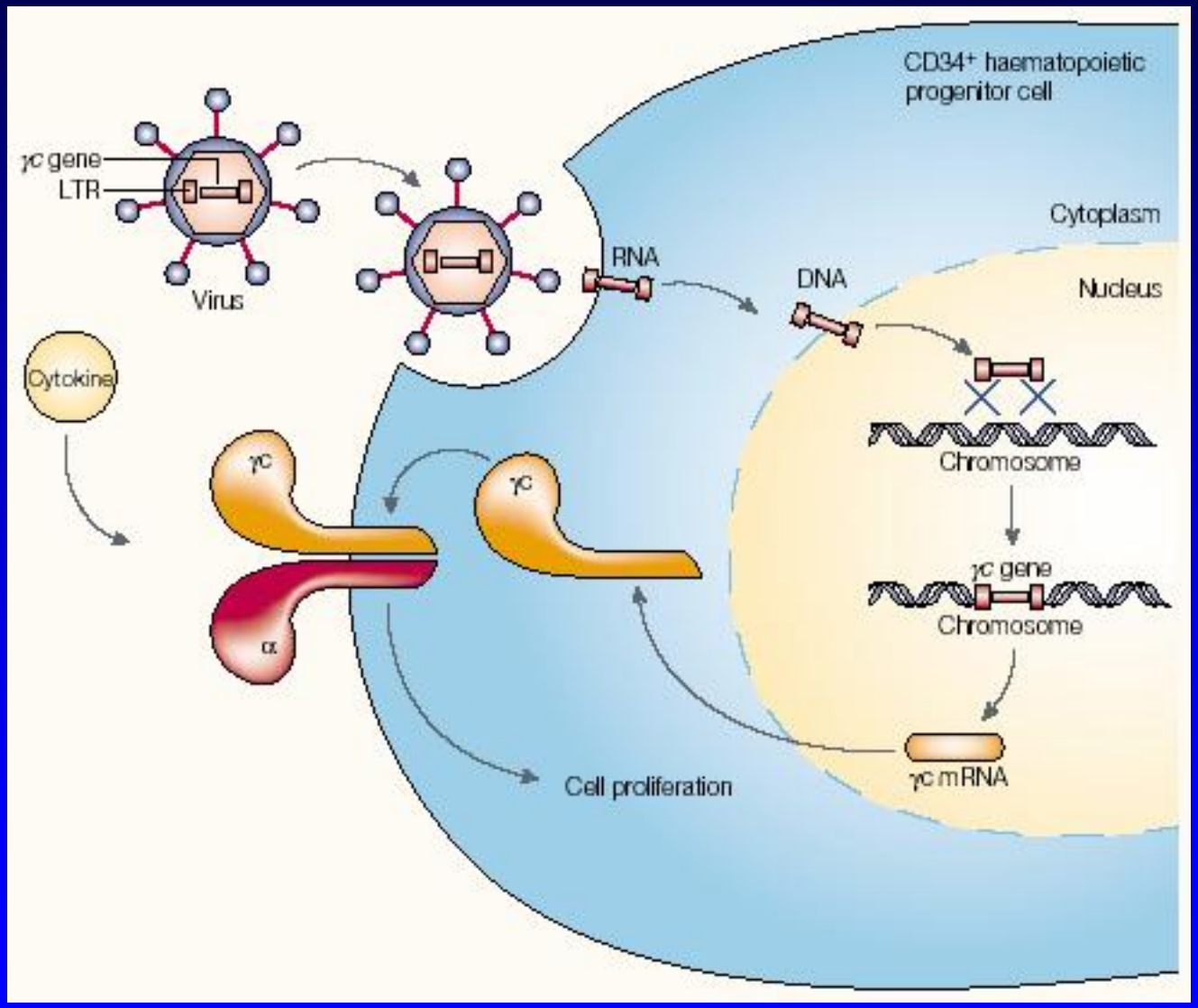
Integration Site Analysis by LAM-PCR



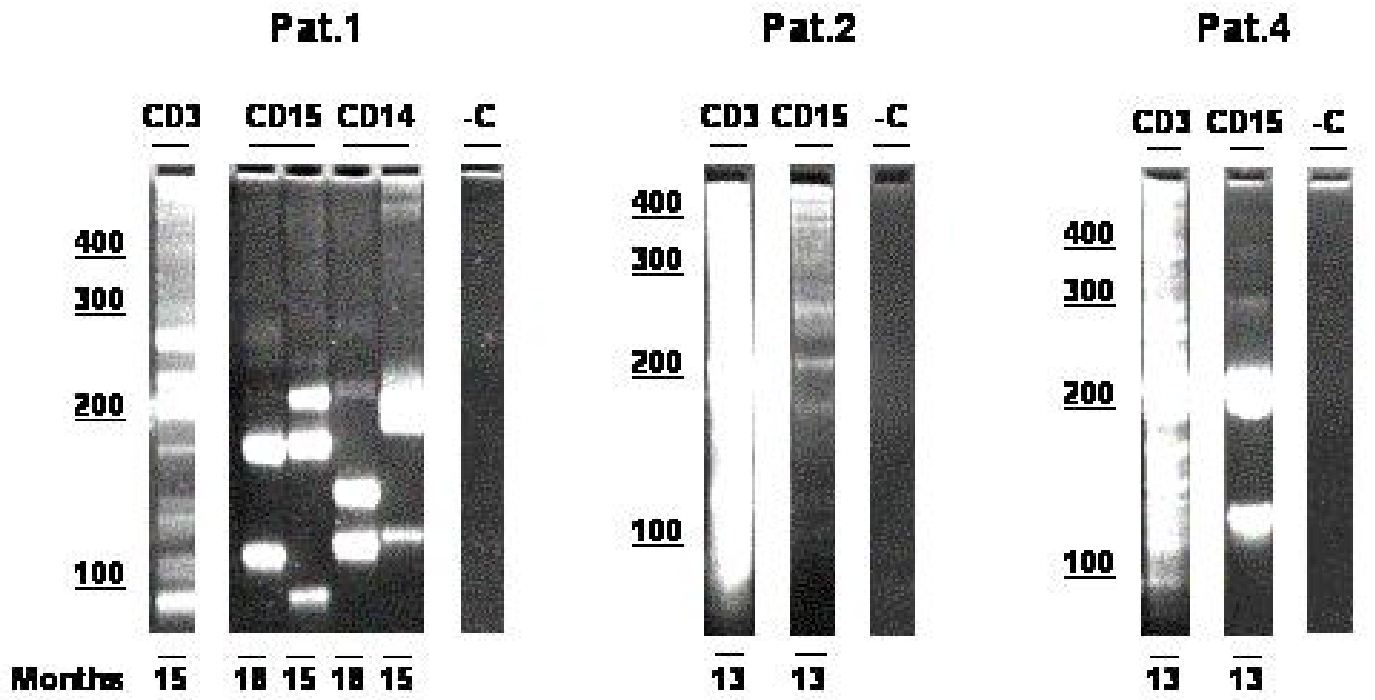


Schmidt *et al.*, Hum Gen Ther 2001
Schmidt *et al.*, Blood 2002
Ailles *et al.*, Hum Gen Ther 2002
Woods *et al.*, Blood 2003
United States Patent No. 06514706 B1

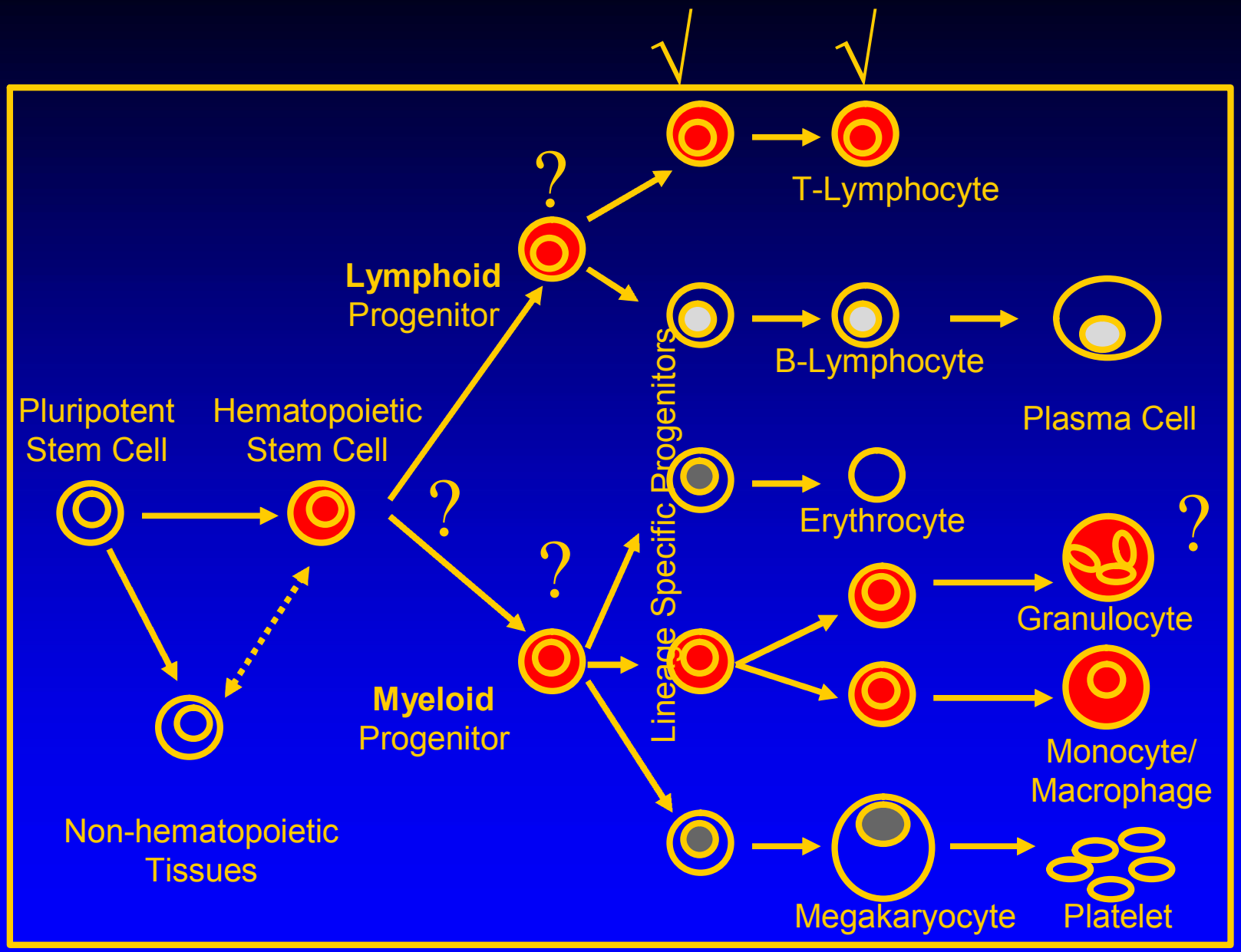
Ex vivo gene therapy of the SCID-X1 condition



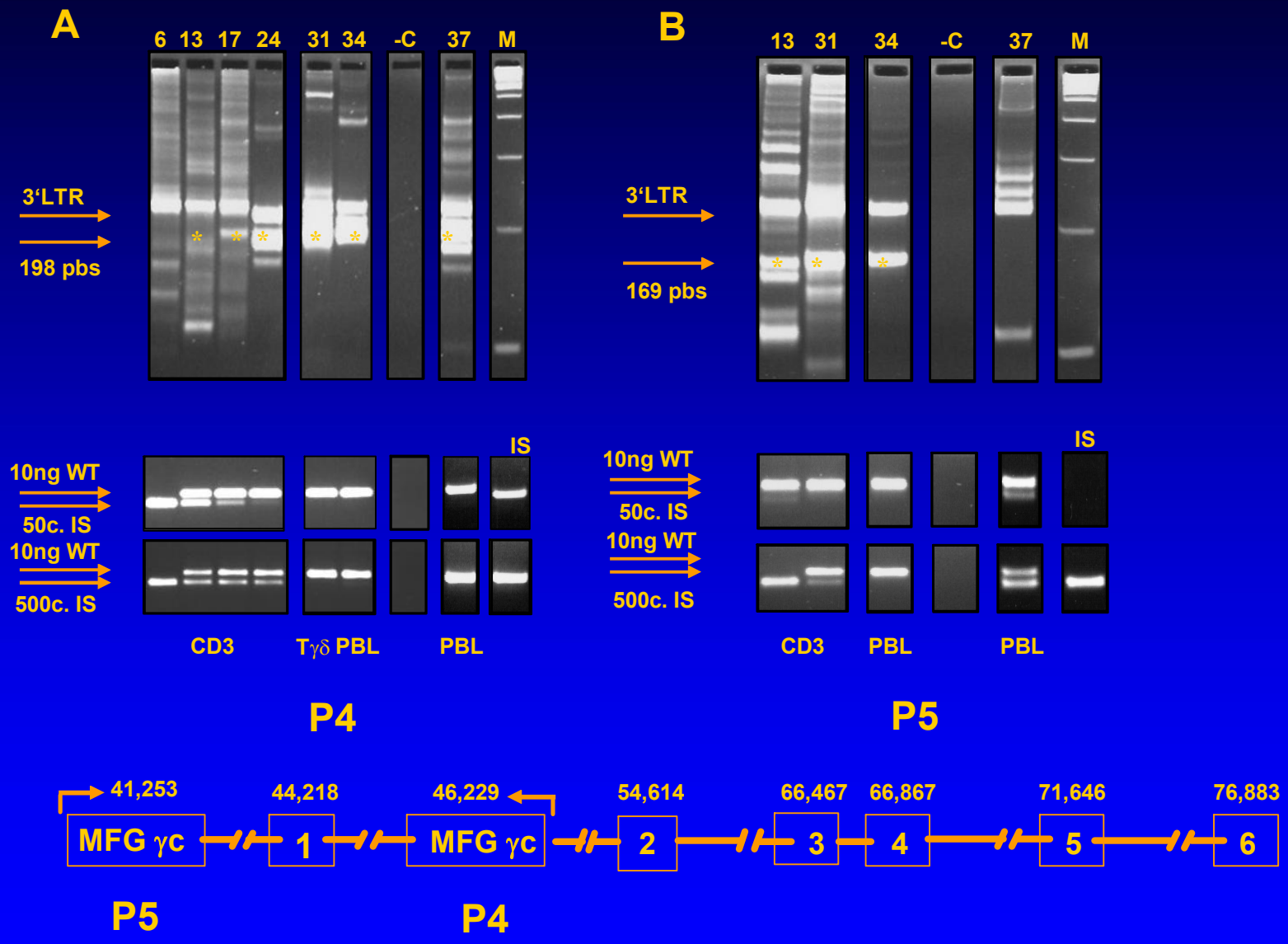
SCID-X1 Gene Therapy (A. Fischer *et al*., A. Thrasher *et al*.) In vivo Clonality Analysis



Gaspar *et al.,* Lancet *in press*, Schmidt M *et al.,* Blood, in press

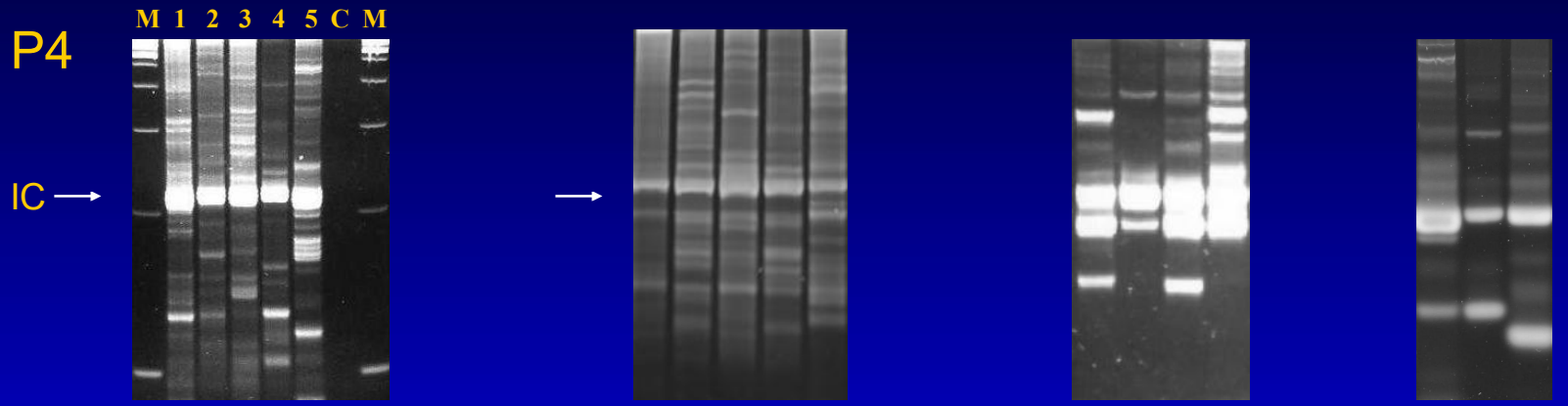

√
√
T-Lymphocyte
?
Lymphoid
Progenitor
B-Lymphocyte
Plasma Cell
Pluripotent
Stem Cell
Hematopoietic
Stem Cell
?
Lineage Specific Progenitors
Erythrocyte
?
?
Granulocyte
Myeloid
Progenitor
Monocyte/
Macrophage
Non-hematopoietic
Tissues
Megakaryocyte
Platelet

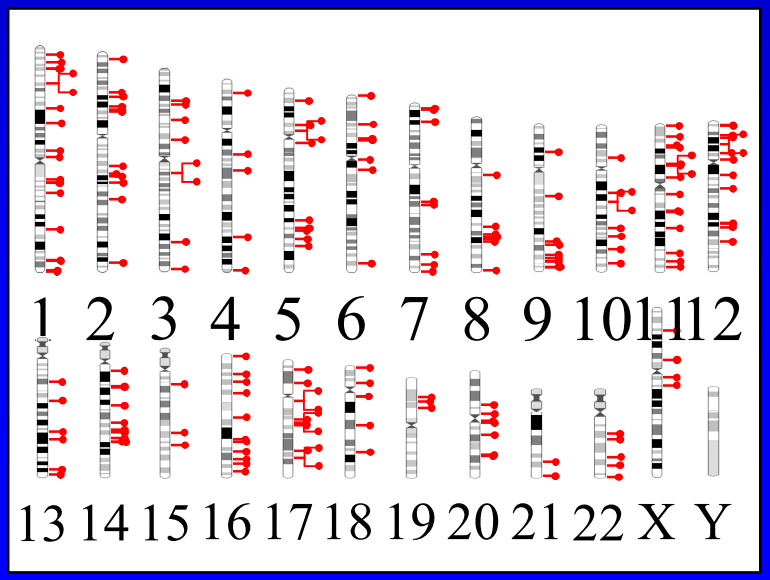
Development of Monoclonality in Human Hematopoiesis

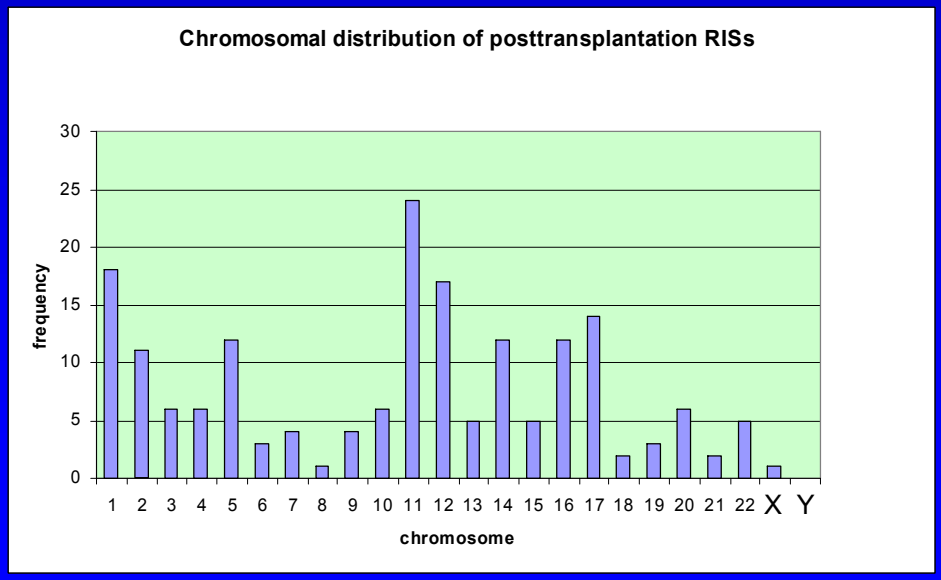


Hacein-Bey, Kalle, Schmidt et al., Science 2003

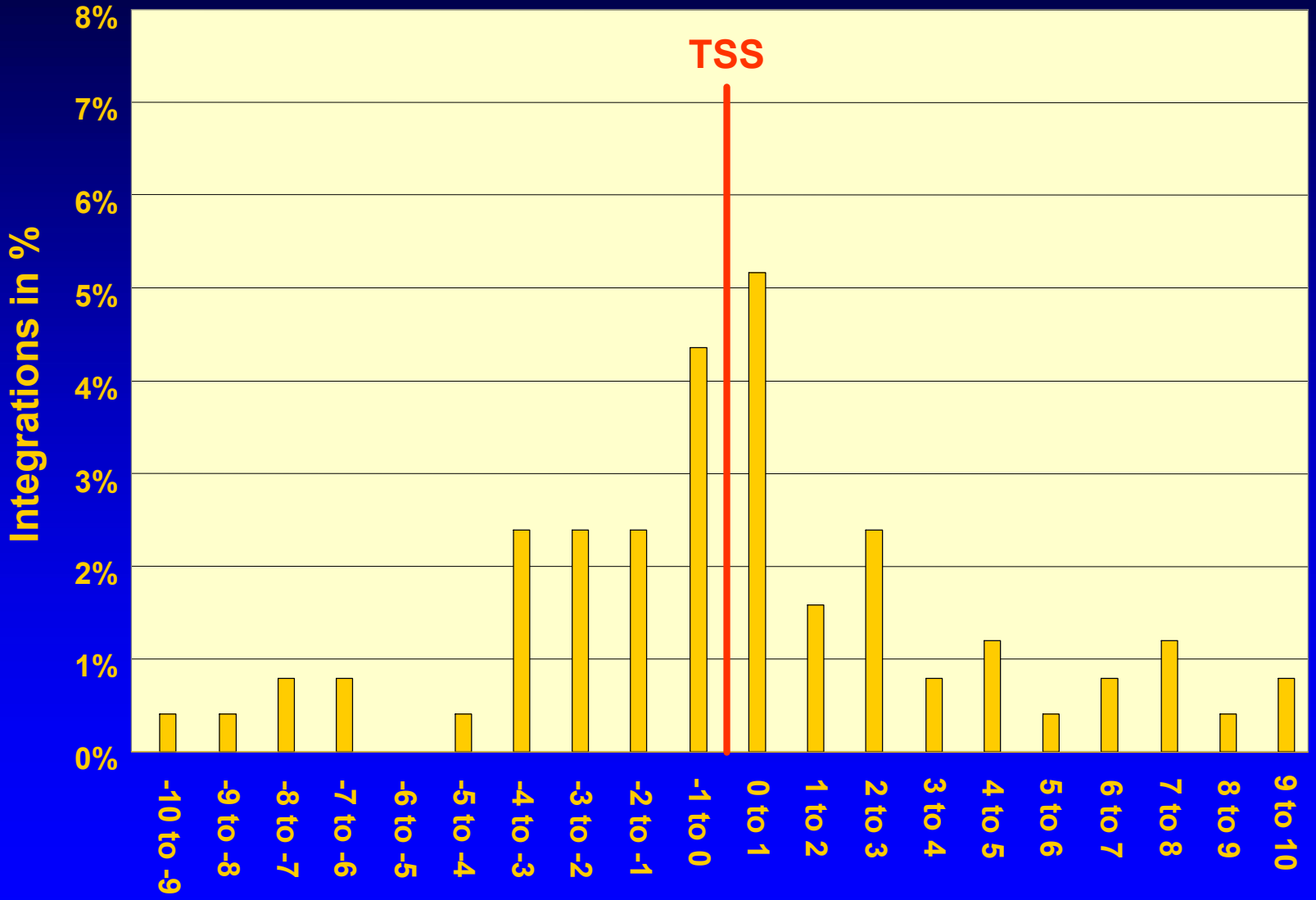
LAM Characterization of Post-Transplantation Hematopoiesis







Insertions around Transcription Start Sites



Gene Ontology Analysis (NCBI)

Molecular Function

Level	Category	List Hits	Fisher Exact
2	Kinase activity	14	0.000534
2	Transferase activity	19	0.00173
3	Protein kinase activity	10	0.00403
4	Phosphotransferase Activity	12	0.00134
4	Protein-tyrosine kinase Activity	5	0.00632

Total number of RefSeq genes in the list: 136

MLV Insertion Summary

- Preferred integration of MLV-based vectors
  within or close to genes:

  59% within or close to genes,
  39% within the gene itself,
  ~50% of these within in the first 20% of the gene

- Insertion peaks around the start of transcription
  in a narrow segment (+/- 4 kb)

- Clustering in Common Insertion Sites

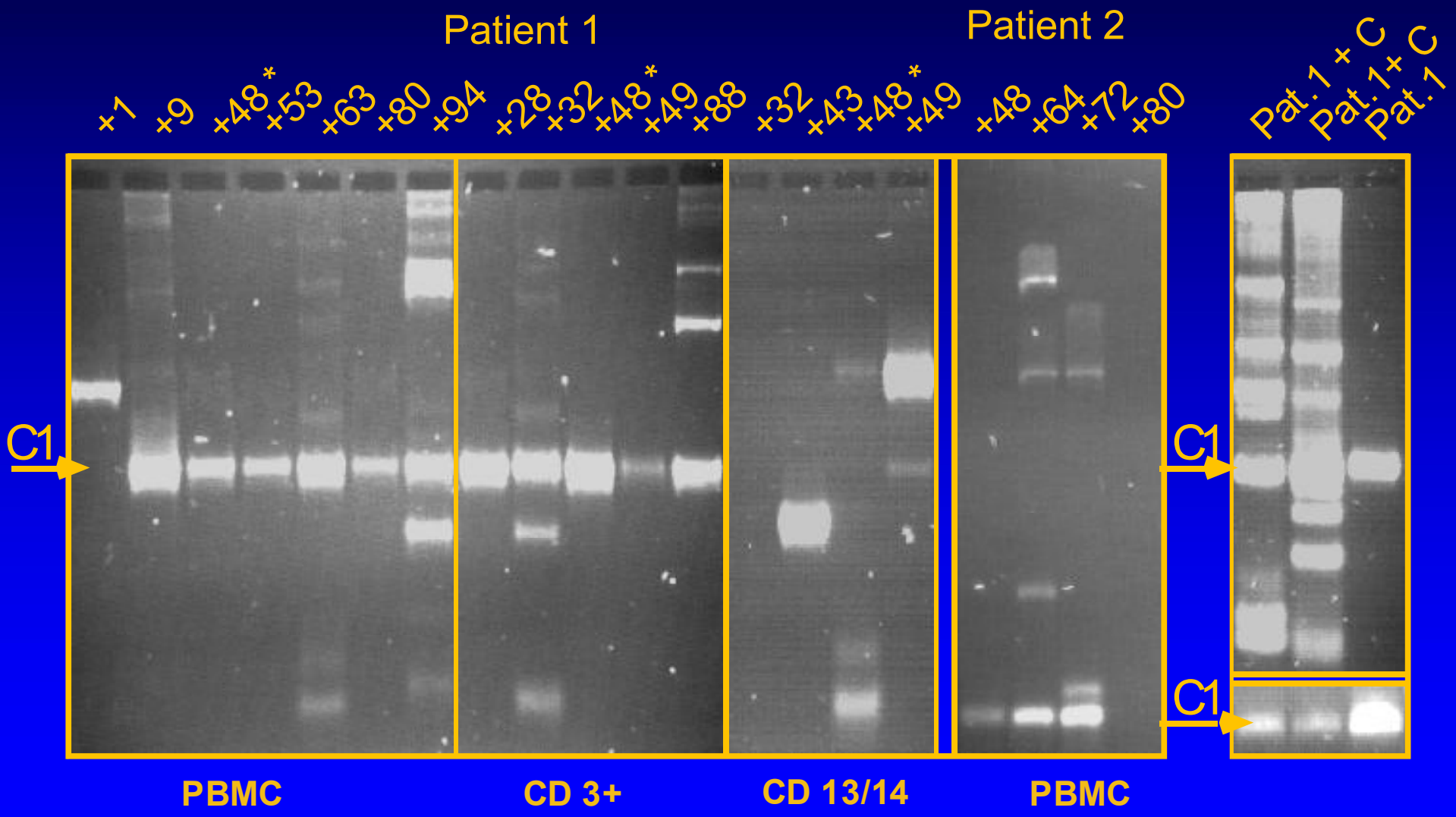
- Differences before and after engraftment

- Differences between trials

Immunodeficiency Clinical Trials Summary

- French, British, Italian, German, Australian Trials: 27 Patients
- Survival: 26/27 Patients:
- Successful Correction: 24/27 Patients
- Reported SAEs: 3/27 Patients
- SAE Survival 2/3, Successfully Corrected
- Differences Between Trials

ADA Gene Therapy
In Vivo Clonality Analysis

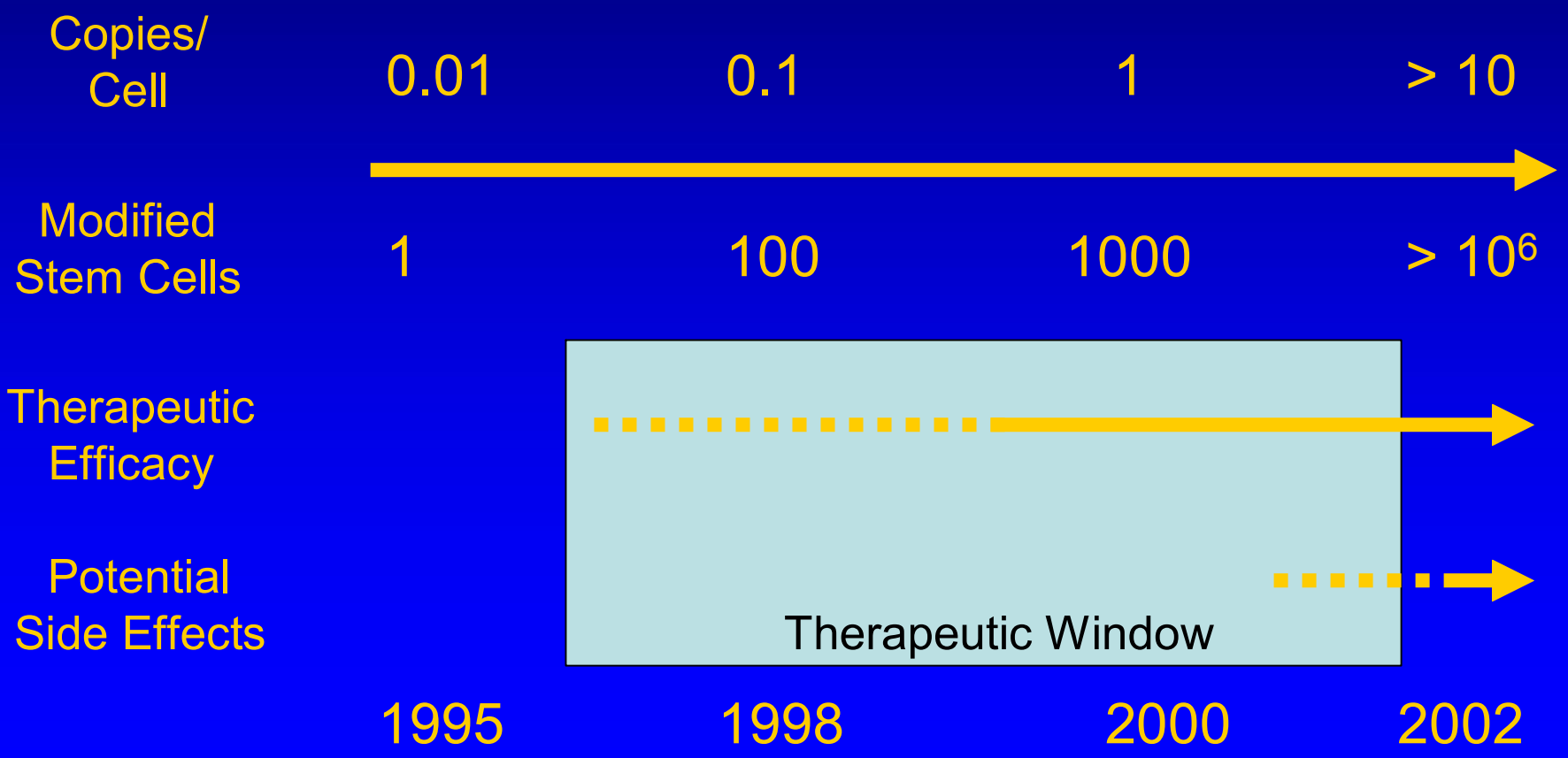


Schmidt M *et al.*, Nat Med 2003

Non-Random Insertion – Positive Results

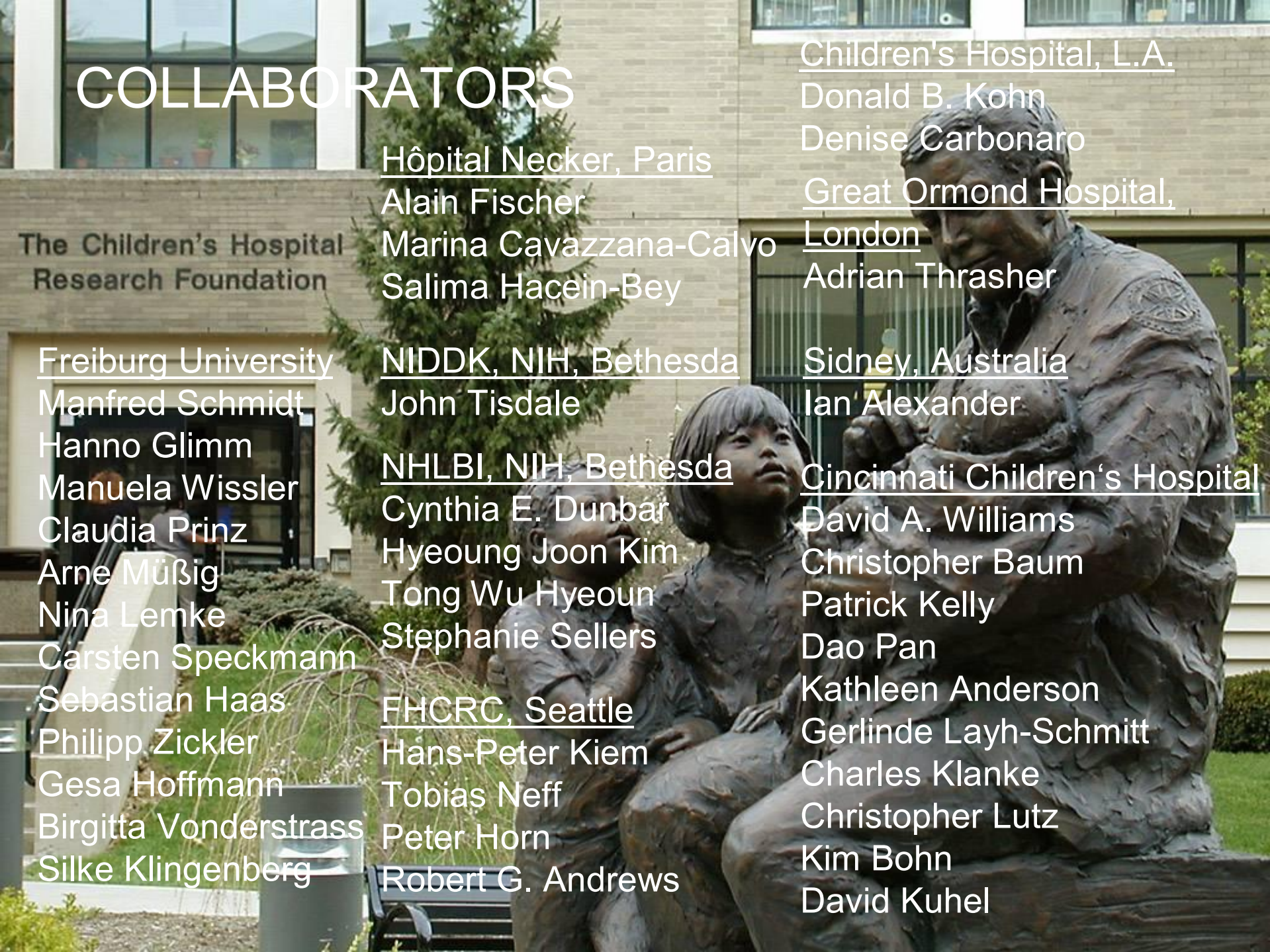
- Unprecedented Levels of Correction
  Even Prior to Expansion
- Therapeutic Benefit
- Likely Single and Double Copy Insertions
  - Continued Regulation
  - “Burning Out” is Conceivable
  - What would be Normal?
- Identification of Regulators of
  Long-Term Hematopoiesis
  No Leukemic Phenotype
- Prospective Screening of Insertions is Possible

Risk/Benefit Assessment of Clinical Applications For Insertional Vector Systems As Pharmaceutical Agents



Conclusions

- Stem Cells Can Be Genetically Modified and Effectively Selected
- Clarification of Insertional Side Effects
- Determination of Stem Cell Contributions at the Clonal Level Offers Unique Insights into *In Vivo* Hematopoiesis
- Gene Transfer Offers Unique Therapeutic Opportunities



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