Retroviral and Lentiviral Vectors Symposium
RAC-Clinigene Meeting
Washington Dec 9-10 2010

iget
TELETHON INSTITUTE
FOR GENE THERAPY

Gene Therapy for ADA-SCID: vector integration studies

Alessandro Aiuti

HSC gene therapy approach for ADA-SCID

Day -4: Purification of BM CD34$^+$ cells

Day-4: Prestimulation (TPO, FLT3-ligand, SCF, IL-3)

MoMLV-ADA

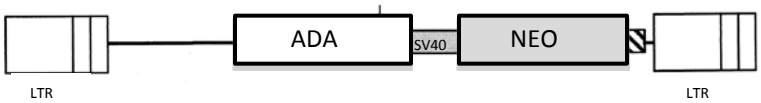
Days -3 to -1: 3 cycles of transduction on retronectin + cytokines

Busulfan 2 mg/Kg/day x 2 (Days -3, -2)

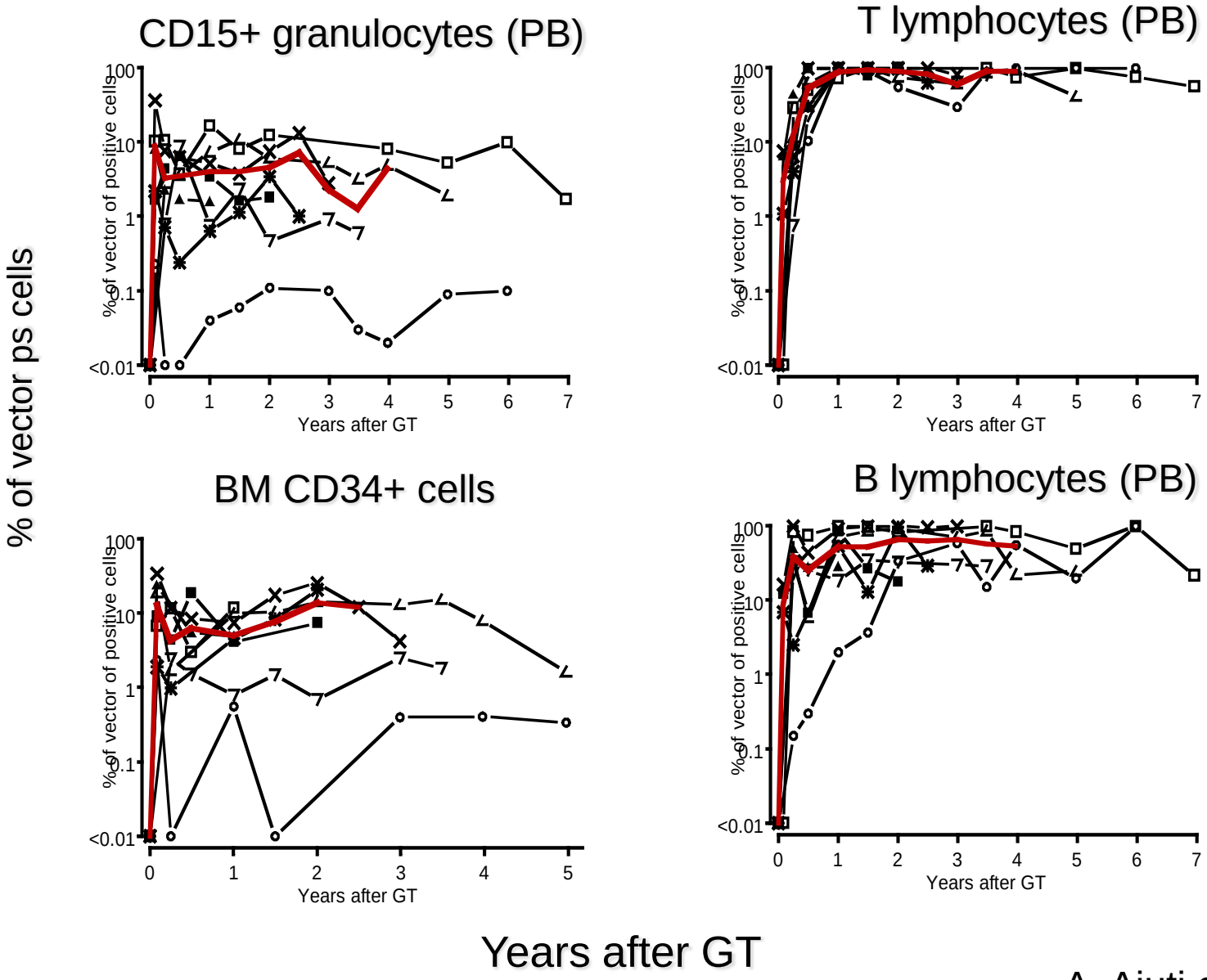
No PEG-ADA

Day 0: Infusion

Moloney MLV vector (MoMLV)

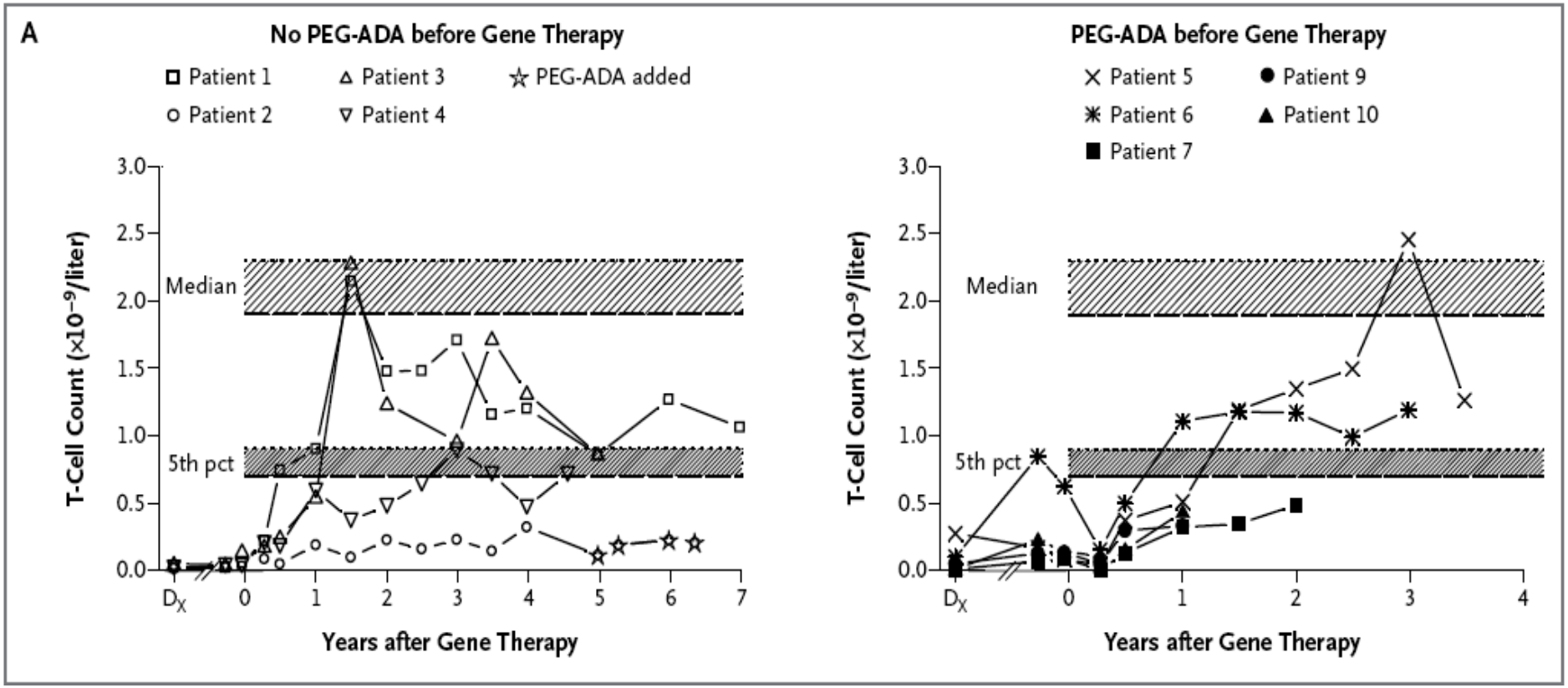


Long-term engraftment of gene corrected cells



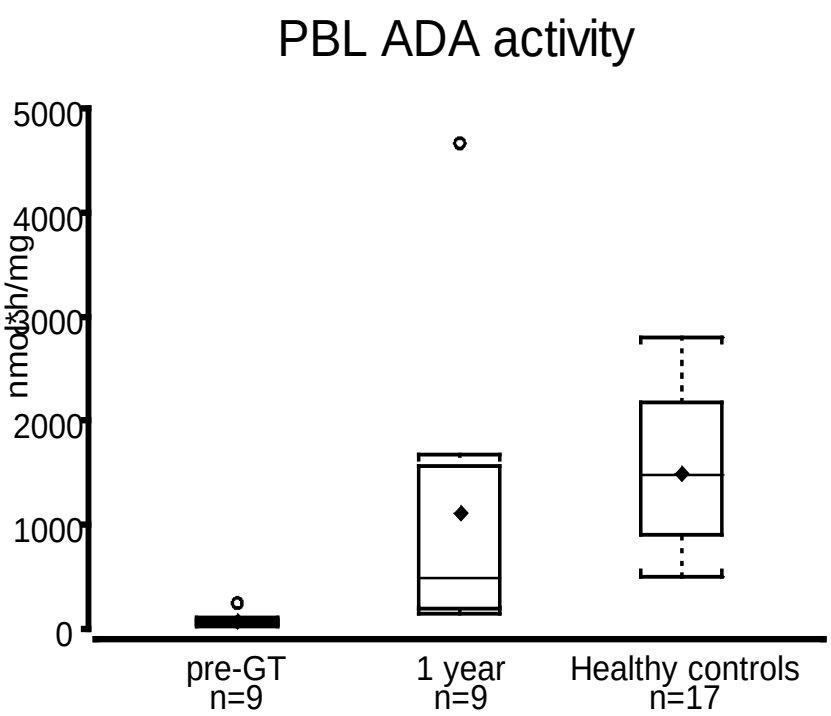
A. Aiuti et al. NEJM, 2009

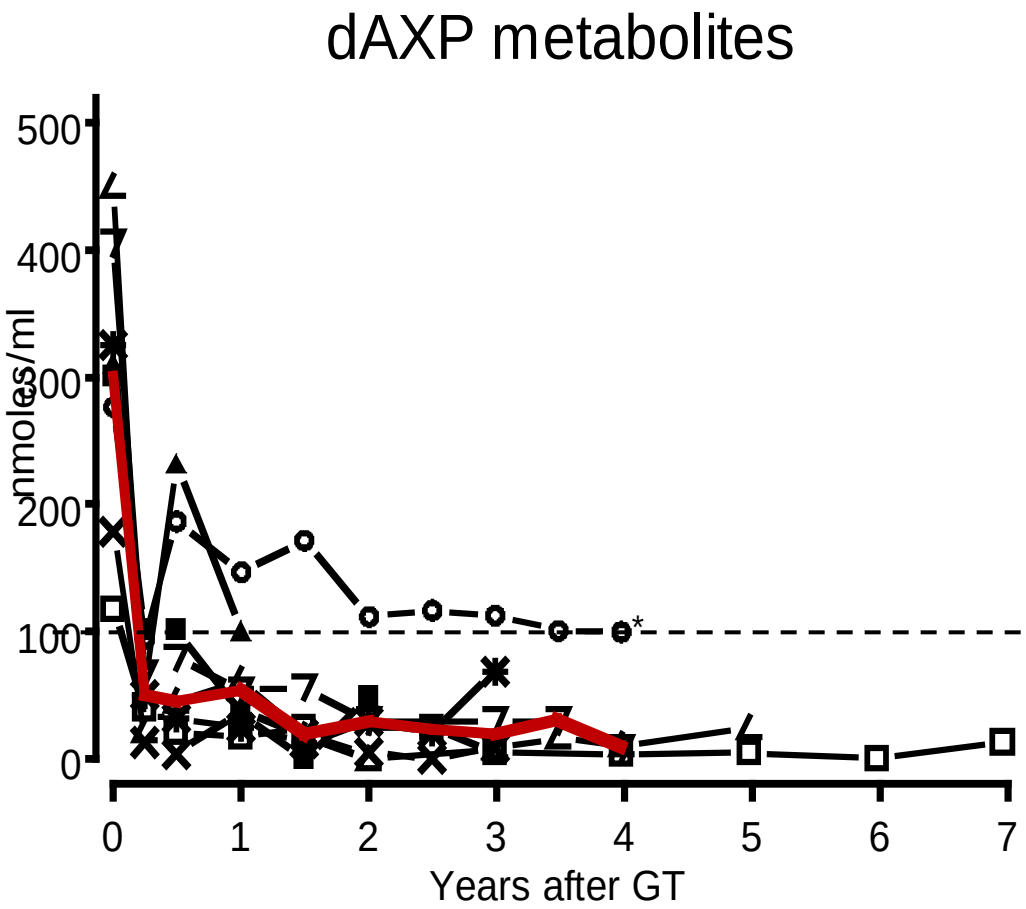
T-cell reconstitution after gene therapy



A Aiuti et al., NEJM, 2009

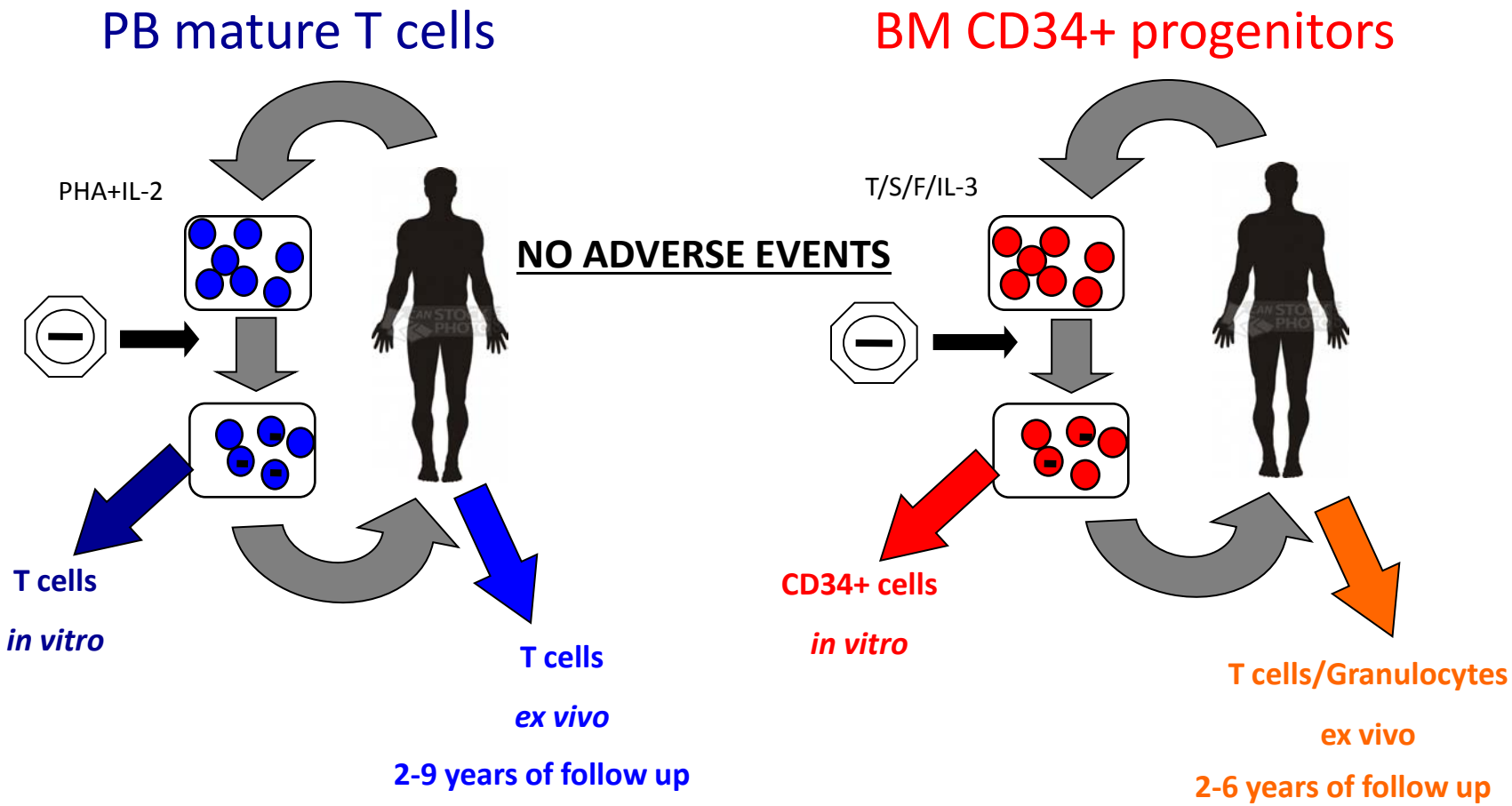
ADA expression leads to systemic detoxification





A. Aiuti et al. NEJM, 2009

VECTOR INTEGRATION STUDIES IN ADA-SCID: PBL-GT vs HSC-GT



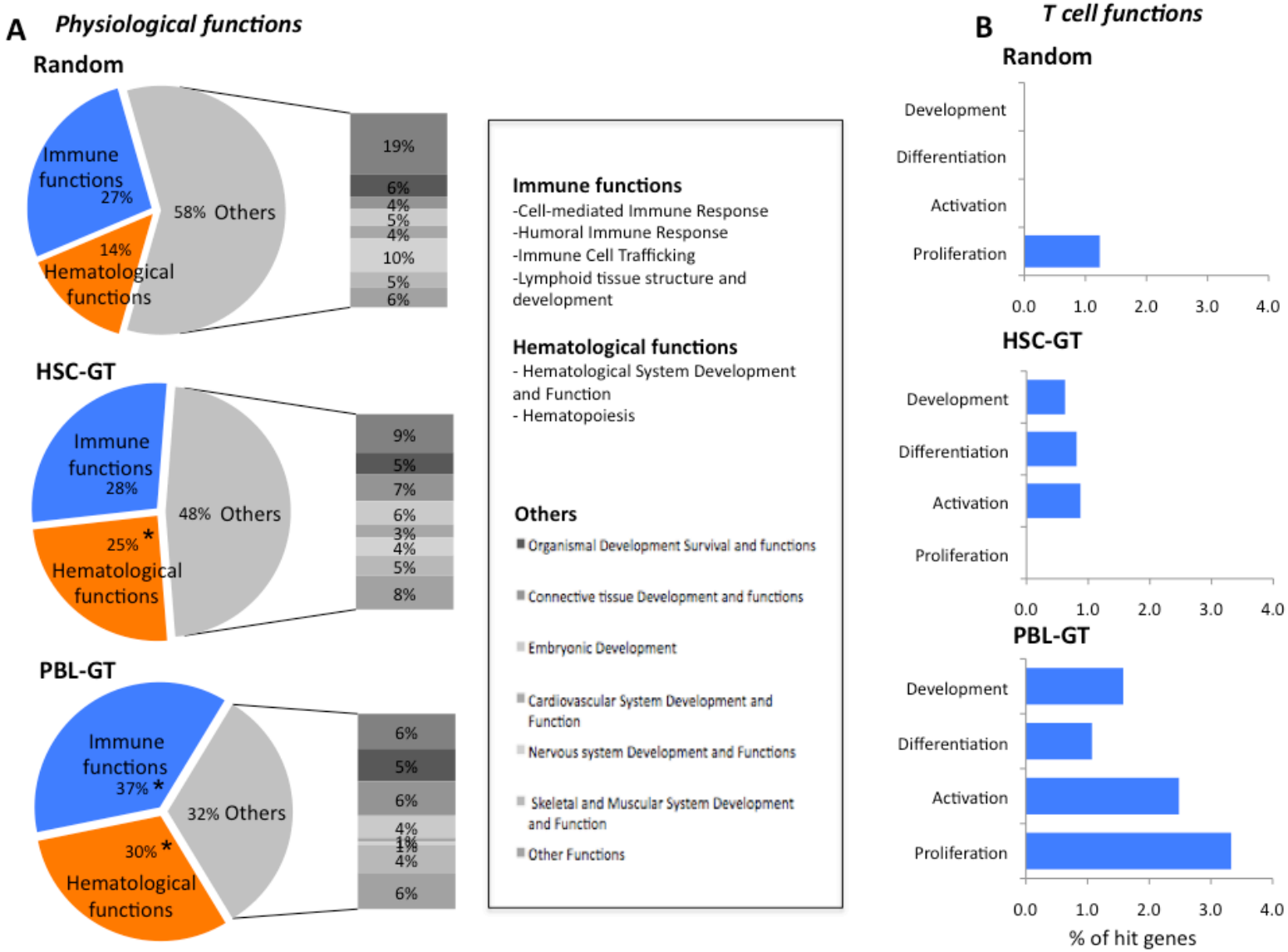
Genomic distribution of RIS in PBL-GT **(2198 unique integrations)** or HSC-GT **(1959 unique integrations)** in vitro and ex vivo

Patient samples	*Inside gene (%)*	*Outside gene (%)*	*10Kb window on TSS (%)*
PBL-GT pre-GT CD3+	55.9	44.1	27.6
PBL-GT post-GT CD3+	51.3	48.7	23.6
PBL-GT total	**54.1**	**45.9**	**26.1**
HSC-GT pre-GT CD34+	52.6	47.4	20.0
HSC-GT post-GT CD3+	40.7	59.3	23.0
HSC-GT post-GT CD15+	40.6	59.4	23.8
HSC-GT total	**46.1**	**53.9**	**21.8**
Randomly-generated RIS *	40.7	59.3	5.2
Human genome	35.5	64.5	N.D.

* 100.000 in silico generated integrations

L. Biasco et al, EMBO Mol Med, 2010

Integrations hit different physiological functions in PBL-GT vs HSC-GT



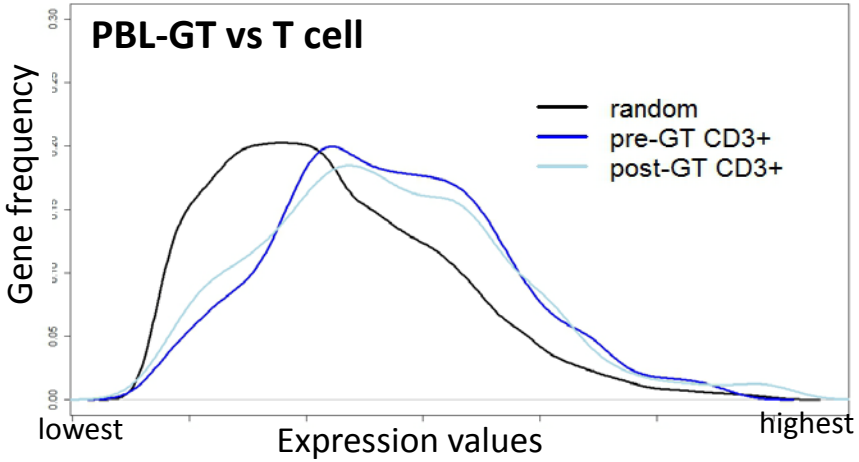
Differential expression profile of target cells drives RIS preferences

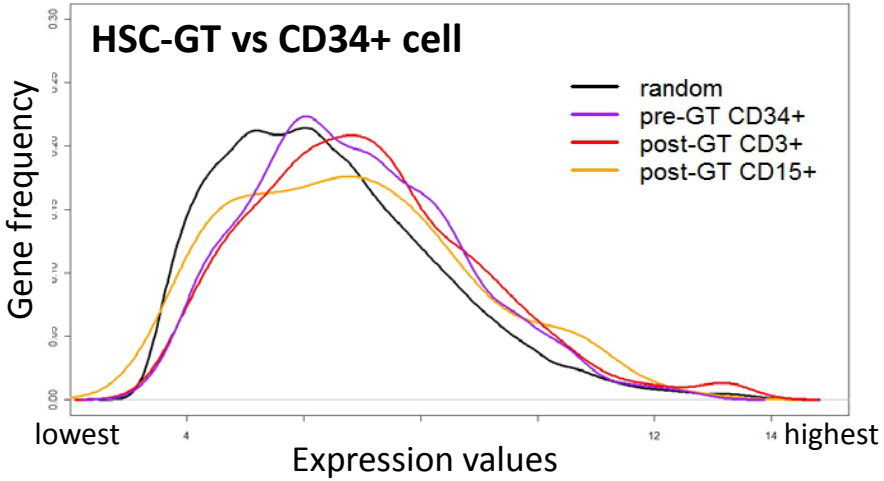
Microarray analysis at the time of transduction on:

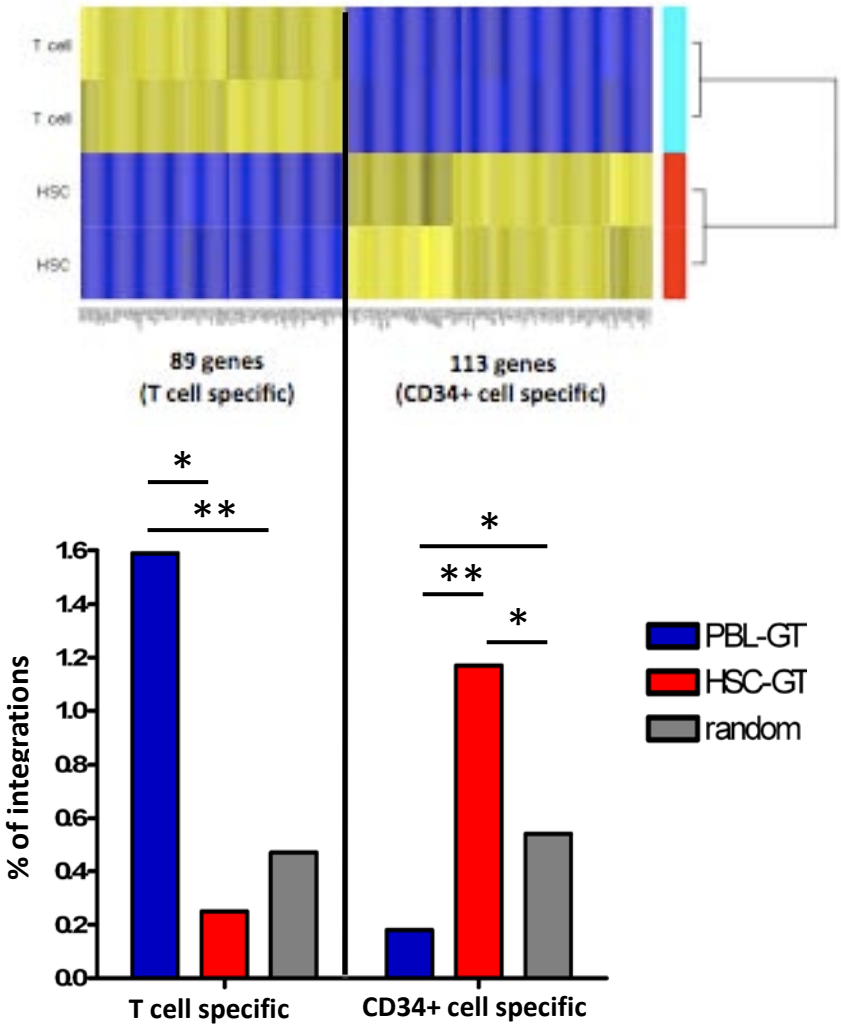
- T cells

- CD34+ cells

(HG-U133A affymetrix chip)

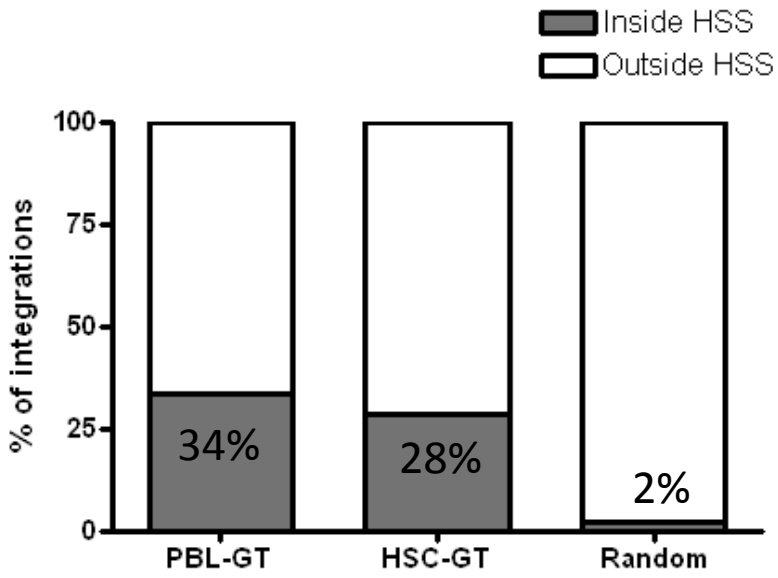


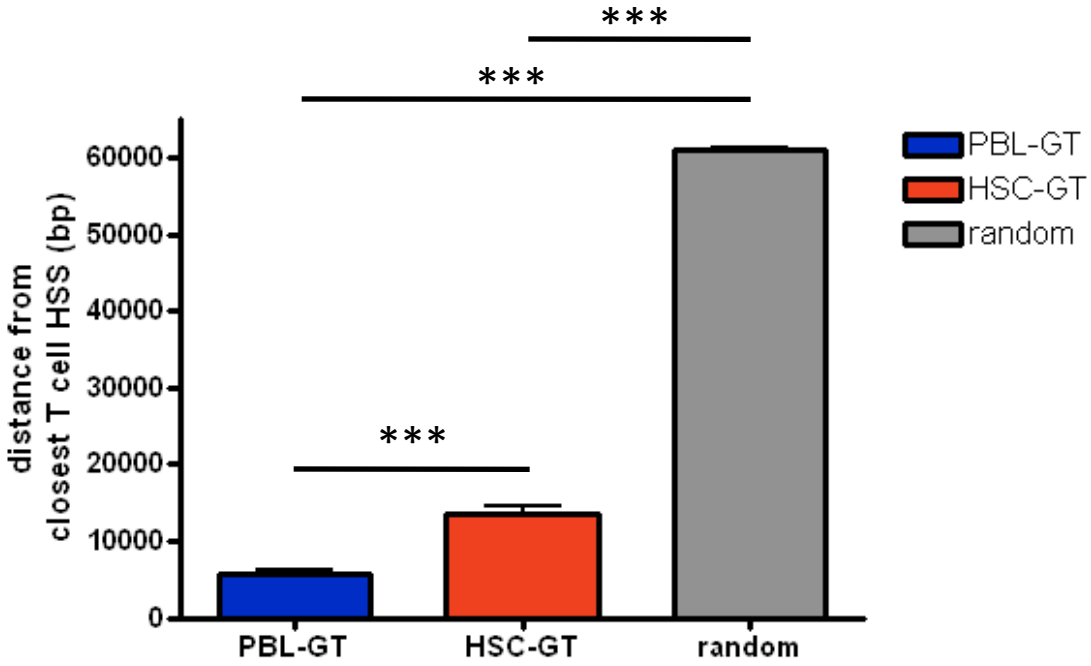




(*p<0.05, **p<0.005 Test on proportions, Holm correction)

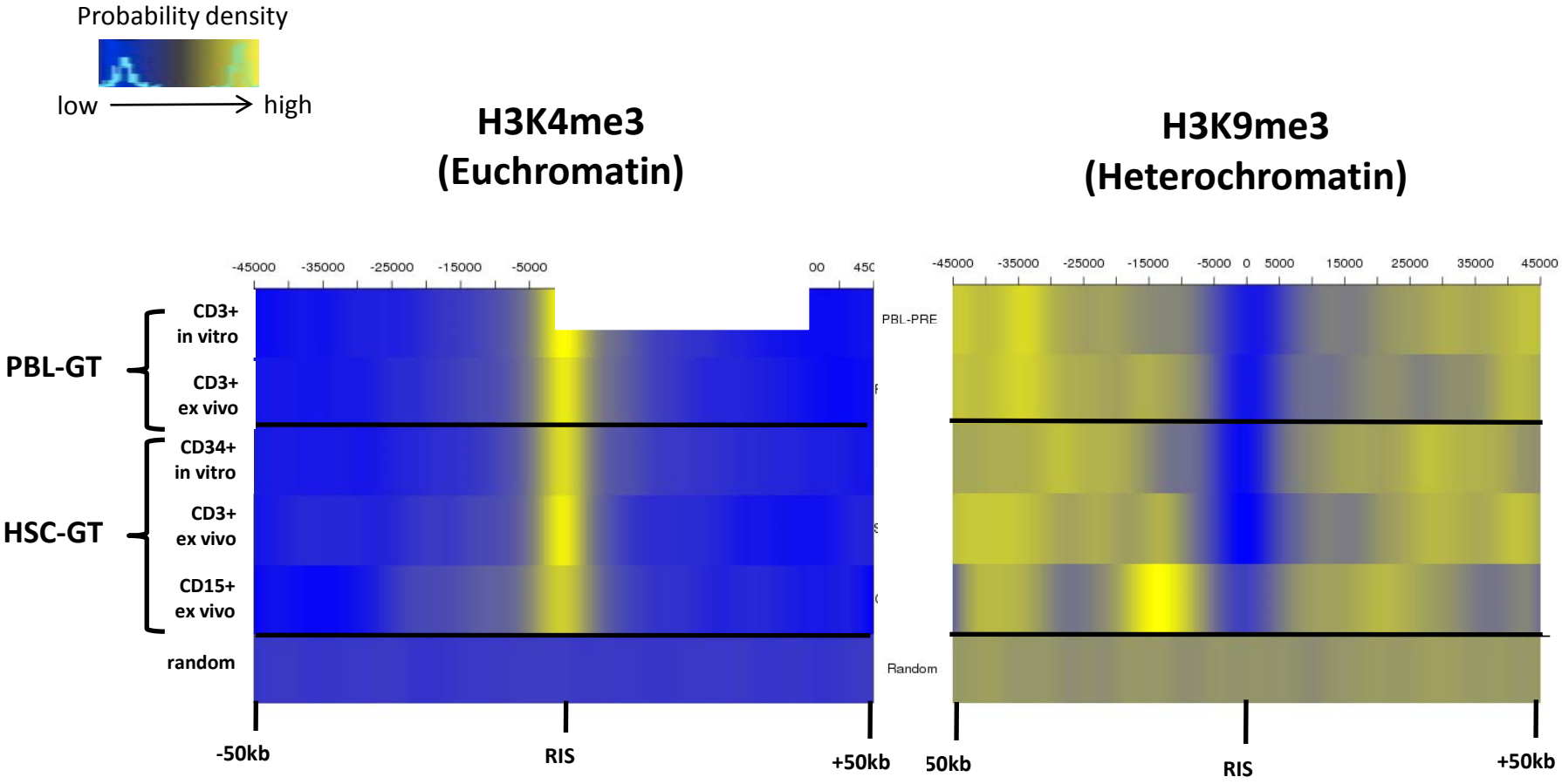
T-cell HSS are differentially hit by insertions in PBL-GT vs HSC-GT



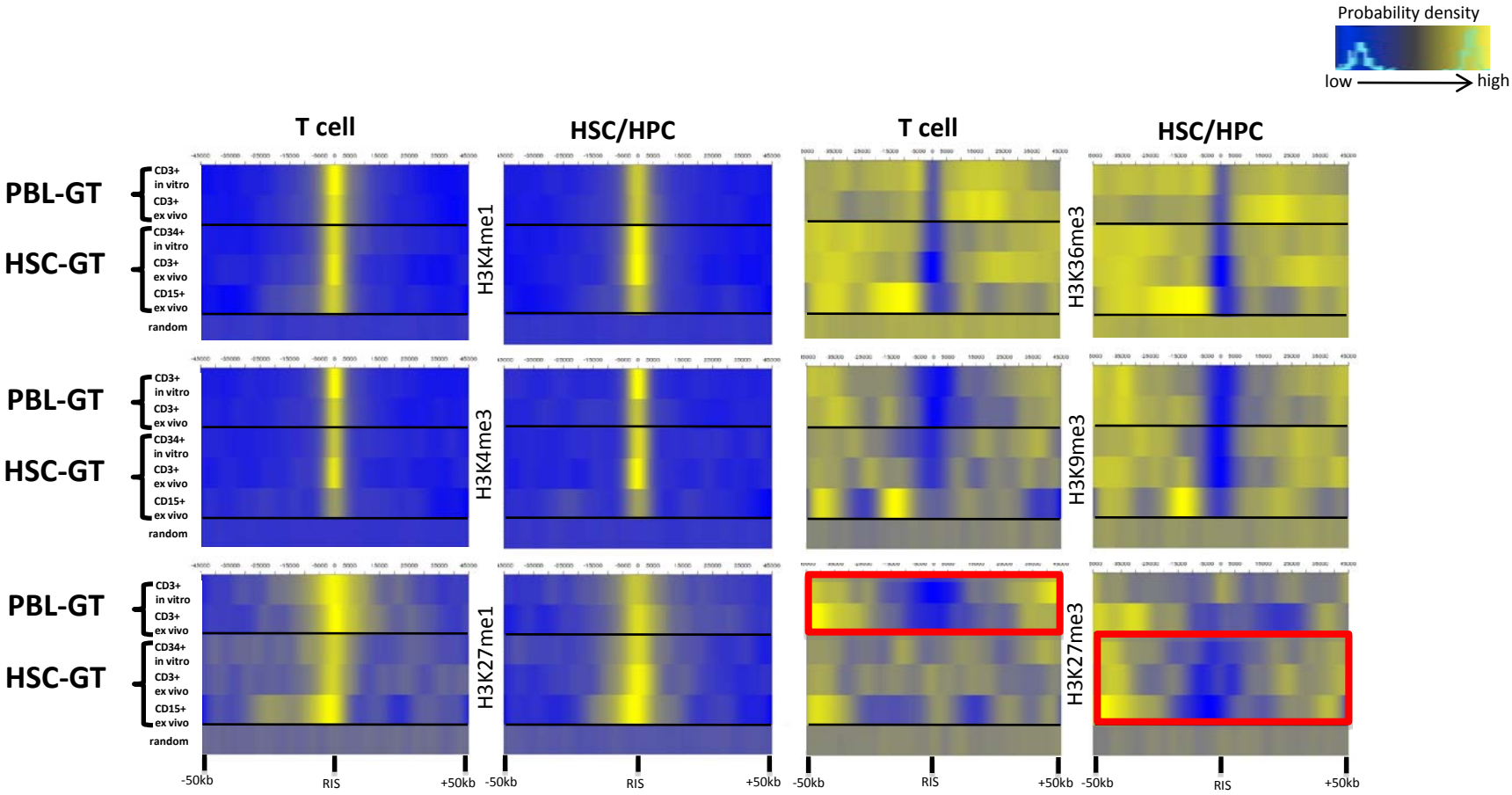


Mean and SEM
*** p<0.0001 Mann-Whitney t-test

Density distribution of histone methylations surrounding RIS



Density distribution of histone methylations surrounding RIS

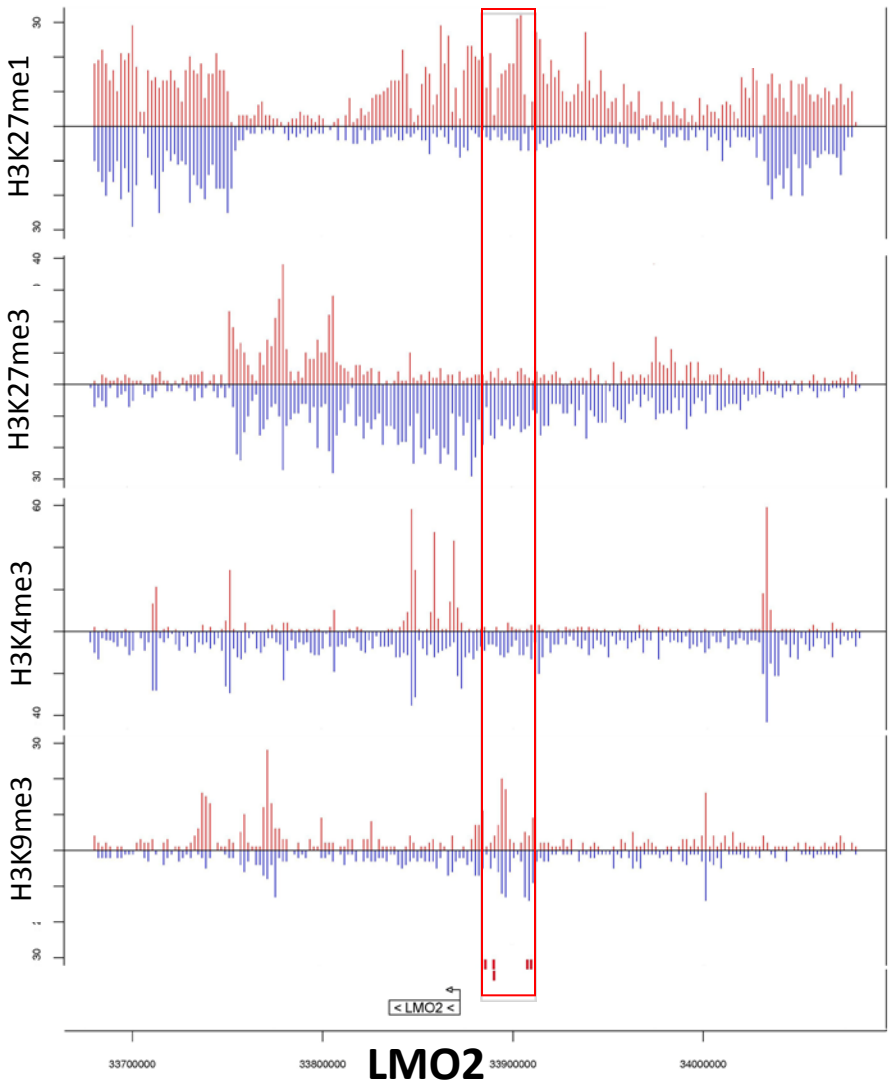


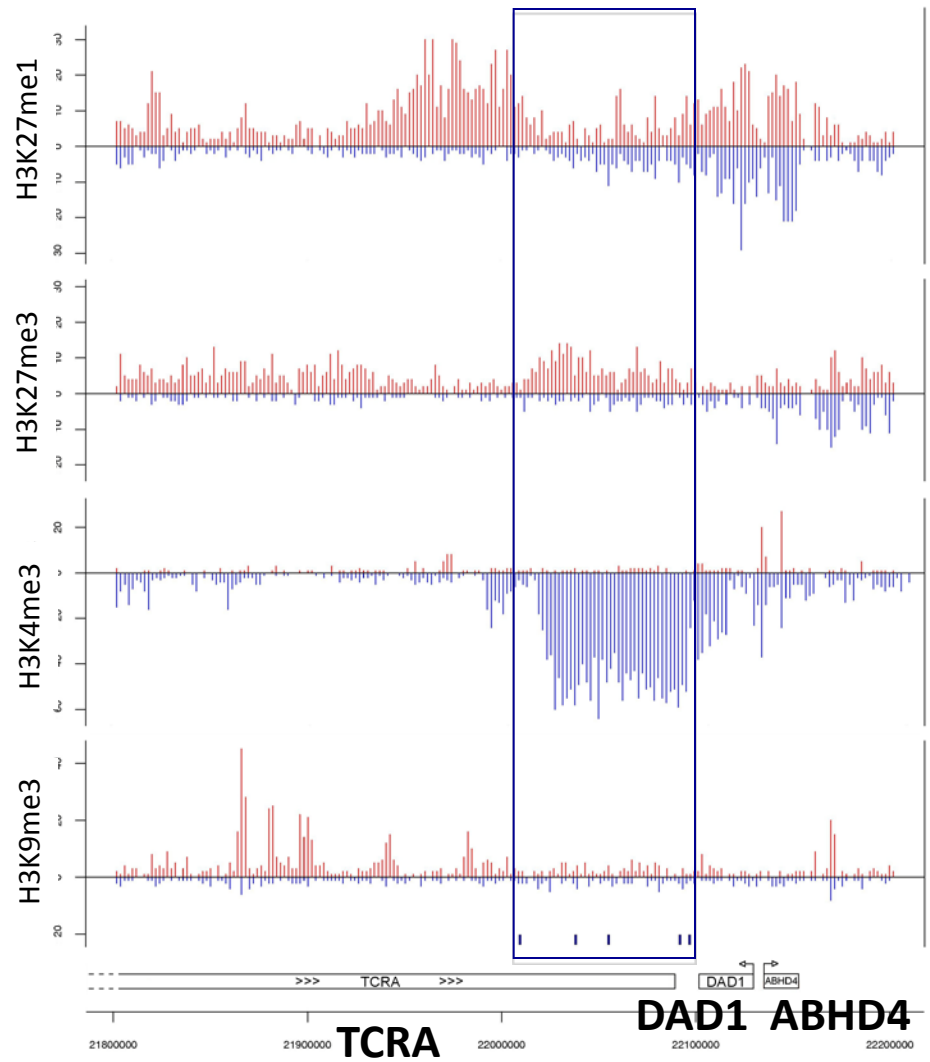
Histone methylation marks influences differential cl

Histone methylations mapped on:

- **CD4+ T cells (Barsky et al.)**
- **CD133+/CD34+ HSC/HPC (Cui et al '**

H3K4me3, H3K27me1 → *Euchromatin*
H3K9me3, H3K27me3 → *Heterochromatin*





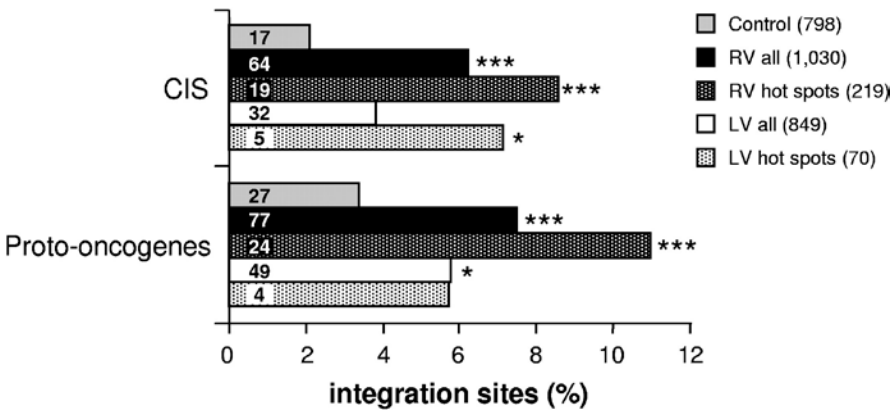
Controversies: CIS detection and proto-oncogenic hits

- *Cattoglio et al. Blood 2007,* Hot spots of retroviral integration in human CD34^{+} hematopoietic cells

  <u>CISs and proto-oncogenes are overrepresented in RV integrations and integration hot spots "in vitro"</u>.

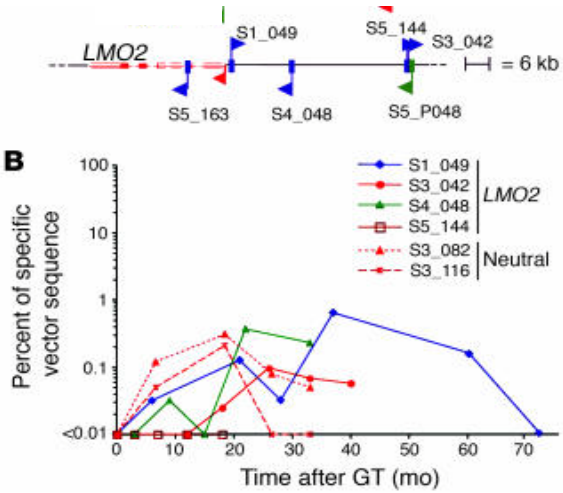
  Cattoglio et al. Blood 2010, (high resolution mapping of integrations)

- MLV "hyper cluster" target genes are functionally linked in networks involved in the hematopoietic system development and function (including LMO2, AML1/RUNX1, LMO2, EVI1/MDS1, RUNX2).

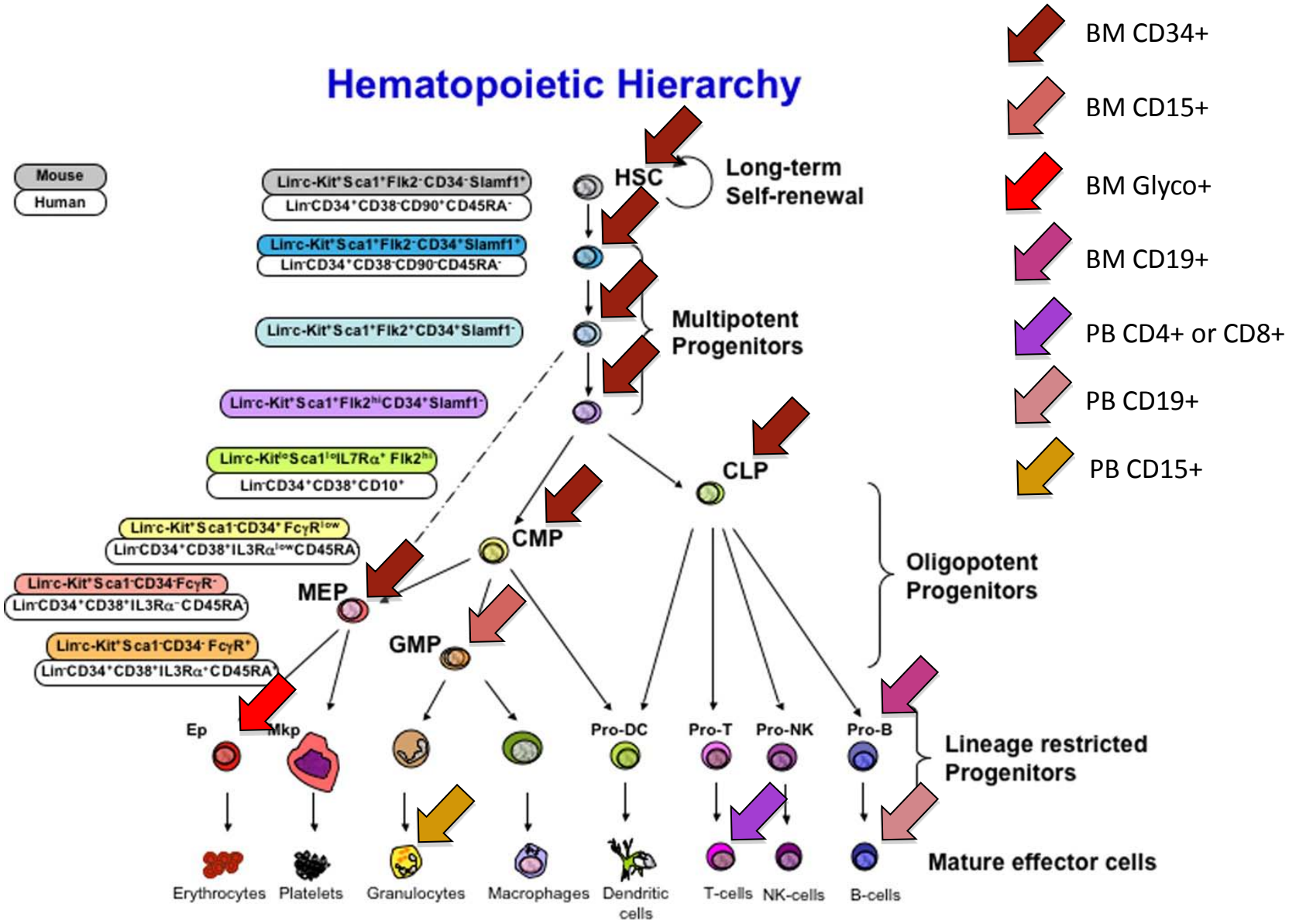


JCI 2007, Aiuti et al.
<u>LMO2 is a recurrent RIS in CD34^{+} cells, but is not associated with clonal selection in vivo</u>."

Integrations in protoncogenes are detected ex vivo but not expanded(frequency <1%)



Panel of hemopoietic integration sites in 4 HSC-GT patients



Summary

- MLV shows vector preference for
  - transcription start sites
  - active genes
  - accessible regions of chromatin (Histone methylations and HSSs)

- MLV-vector displayed a cell-specific integration profile on the basis of:
  - Active cell-specific functions/pathways (Ingenuity analysis)
  - Differential gene expression (Microarray expression profile)
  - Accessibility of chromatin (HSSs and Histone methylations)

    H3K27me3 differential distribution in target cells

- Integration profile of MLV-vector in vivo
  - Is multilineage and identifies core HSC integrants
  - Is dependent on vector position before infusion
  - Skewing for cell-specific features is mantained long-term in patients

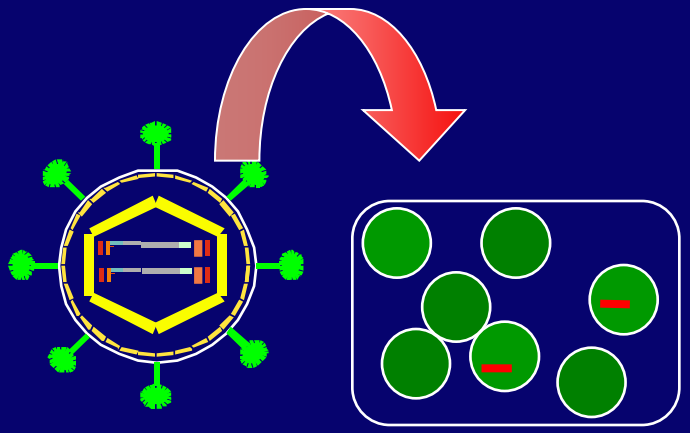
Potential explanation for differences with other RV studies

- ADA housekeeping gene, expressed in all hematopoietic cells
- Survival selective advantage by ADA-expressing cells (transgene may be selected in highly expressed sites)
- Different MLV promoter (weaker) and vector design
- Targeted progenitor populations and disease background

Gene therapy for

Wiskott-Aldrich Syndrome

LENTIVIRAL VECTOR GENE THERAPY FOR WAS



HIV derived, self-inactivating system

Non replicating

- Efficient gene transfer of HSC
- Stable, robust expression of therapeutic gene
- WAS physiological (1.6 Kb) promoter

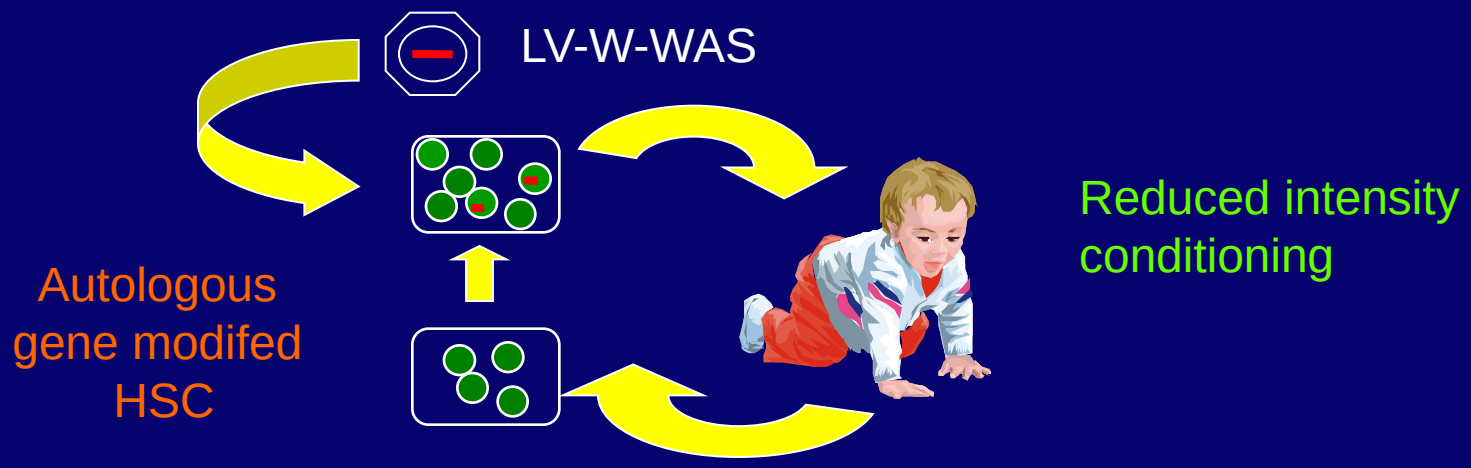
- Preclinical studies
  - In vitro human CD34+ cells and cell lines
  - Transplant of murine Lin- cells in WAS KO mice
  - In vivo biodistribution of human CD34+ cells in immunodeficient mice

Preclinical data for WAS GT

- Safety and efficacy in the WAS KO animal model
- Selective advantage for gene corrected cells
- Efficient gene transfer in human CD34+ cells differentiating in vitro and in immunodeficient mice
- Restored WASp expression in multiple lineages
- Lack of toxicity in vitro
- Lack of vector shedding and germline transmission in immunodeficient mice

Duprè et al., Mol Ther 2004; Dupre et al., Hum Gene Ther 2006; Marangoni et al., 2005; Charrier et al., Gene Ther 2007; Marangoni et al. Mol Ther 2009; Scaramuzza et al., in preparation.

Rationale for the HSR-TIGET clinical protocol



-Lentiviral vector encoding WAS under physiological promoter

-Autologous procedure (No rejection/ GVHD)

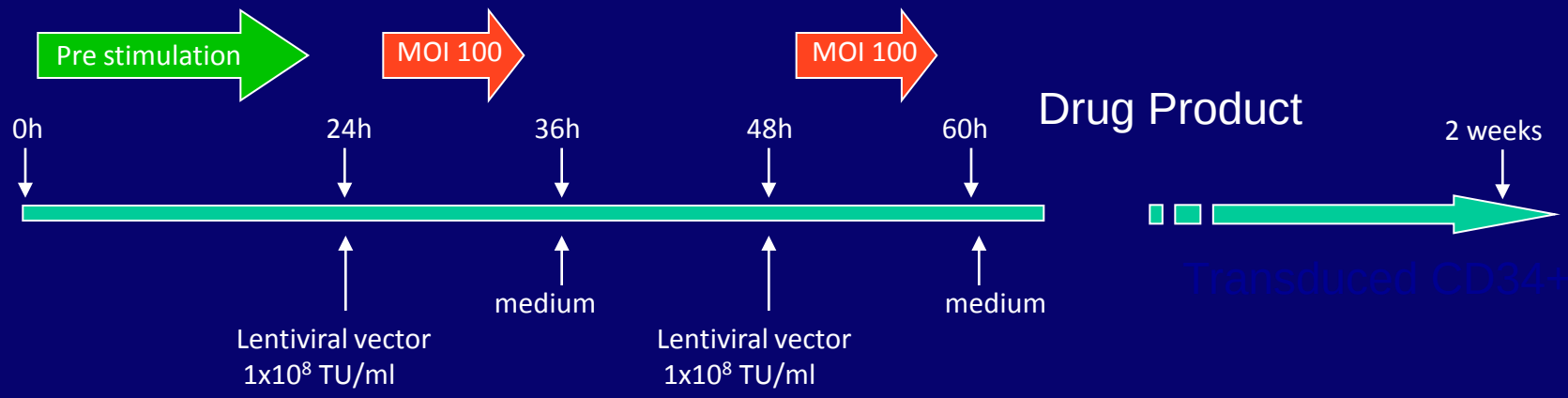
-Lower toxicity associated with reduced intensity conditioning

-Selective advantage in lymphoid cells and platelets

GENE TRANSFER PROTOCOL IN HUMAN CD34+ CELLS

BM / MPB CD34^{+} cells
1 x 10^{6}/ml

CULTURE CONDITIONS:
Serum free medium
SCF 300ng/ml, Flt-3L 300ng/ml,
TPO 100ng/ml, IL-3 60ng/ml
Retronectin coated bags



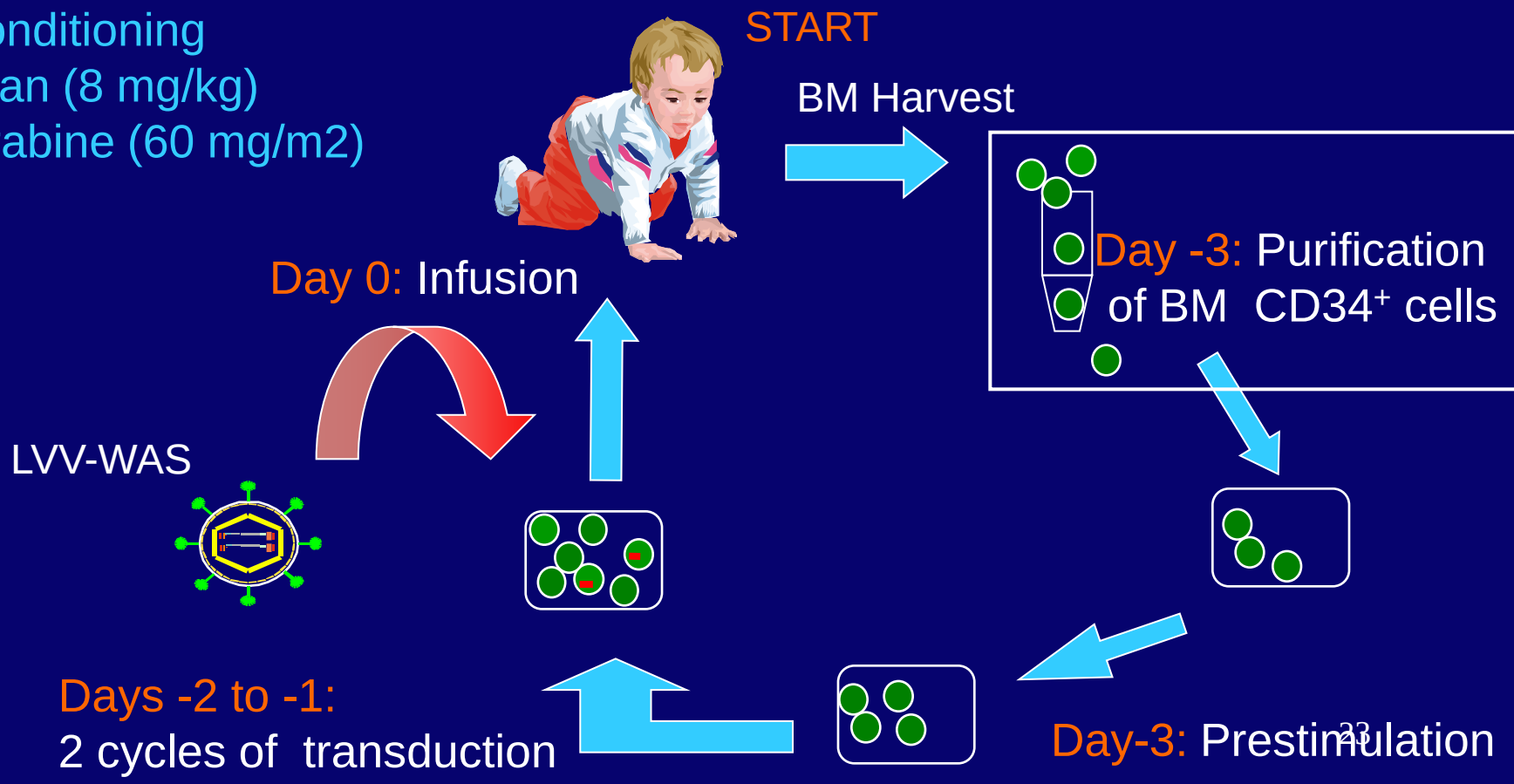
Highly purified, GMP grade large scale batches of lentivector

Treatment design

Preparation:
Collection of back up CD34+ cells

Pre-treatment: anti-CD20 antibody +/- ATG (if clinical autoimmunity)

Pre-conditioning
Busulfan (8 mg/kg)
Fludarabine (60 mg/m2)



San Raffaele Telethon Institute for Gene Therapy

I Brigida
B. Cassani
S. Scaramuzza
A. Ripamonti
L. Biasco
A. Sauer

M. Bosticardo
A. Villa

L. Naldini
M.G. Roncarolo

Pediatric Clinical Research Unit

S. Marktel
M.P. Cicalese
F. Ferrua
M . Casiraghi
A. Biffi
F. Bondardo
L. Callegaro

HSR BMT Unit

F. Ciceri

Hadassah
M. Aker
S. Slavin

MolMed S.p.A
M. Salomoni
M. Radrizzani
M. Dieci
C. Bordignon

Univ. of Siena
A. Tabucchi
F. Carlucci

All participating physicians worldwide

DKFZ
C. Bartholomae
M. Schmidt
C. Von Kalle

Univ. Bicocca
MG Valsecchi
S. Galimberti

WAS collaborators (WAS)
A. Thrasher (London)
A.Galy (Paris)