

Institute of Emerging Diseases and Innovative Therapies



Inserm



Division of Genetics
Department of Medicine
Brigham and Women's Hospital / Harvard Medical School



GenetiX Pharmaceuticals

**Conversion to transfusion independence with
HMGA2 activation after lentiviral gene therapy
for severe human β -thalassemia**

(Clinical PIs: E. Gluckman, M. Cavazzana Calvo)

P. Leboulch – RAC – Bethesda, MD, 2009

History of the regulatory oversight

- Patient "PLB" is the first thalassemia gene therapy patient transplanted without injection of back-up cells (**June 2007**) under the regulatory authority of AFSSAPS, the French governmental agency
- One year post-transplant, conversion to transfusion independence but evidence of partial clonal dominance (< 10%) with activation of the HMGA2 gene
 - ➔ No adverse event but voluntary disclosure and formal presentation to AFSSAPS (**April 2009**)
 - ➔ Voluntary temporary hold while further evaluating hematopoietic homeostasis and evolution of the clone
- After > 6 months of further observation and evaluation, formal presentation to AFSSAPS in **November 2009**
 - ➔ AFSSAPS' Gene Therapy Advisory Committee unanimously voted to resume inclusion of patients

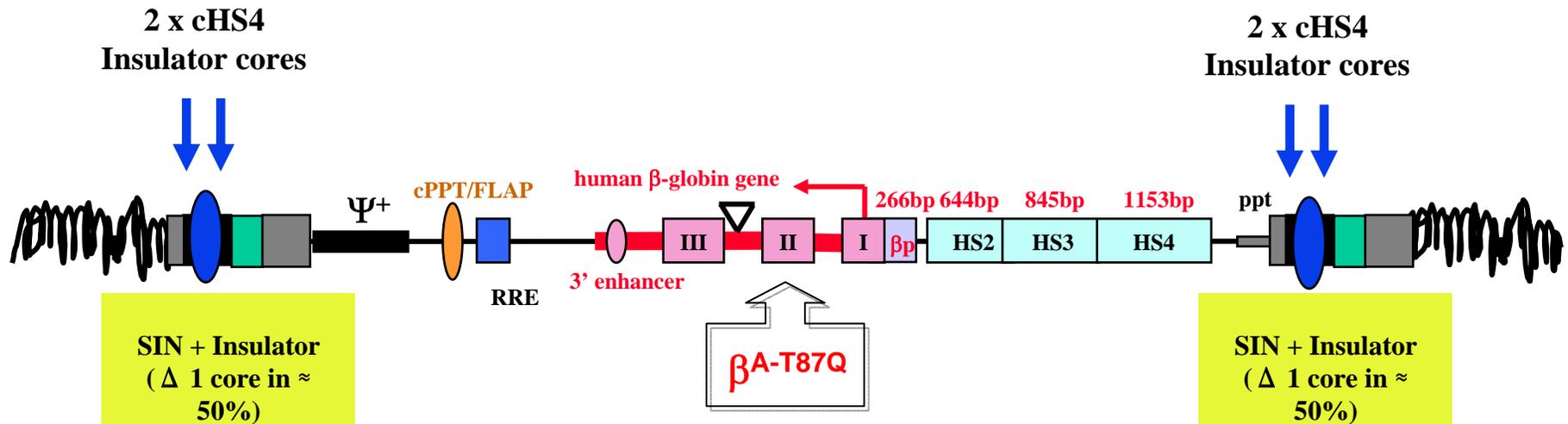
Pre-transplant clinical history of Patient "PLB"

- **Then 18 year old male with severe β^E/β^0 -thalassemia and no HPFH or α mutation.**
- **Transfusion dependent since age 3 (> 225 ml RBCs /kg/year for Hb > 10 g/dl).**
- **Spontaneous Hb levels as low as 4.5 g/dl.**
- **Major hepato-splenomegaly (splenectomy at age 6) and growth retardation.**
- **Failure of Hydroxyurea therapy (between ages 5 and 17).**
- **Desferoxamine (5 days/week) since age 8, and oral Exjade since age 18 (although nausea). No liver fibrosis. Moderate iron overload by liver MRI (561 $\mu\text{mol/g}$).**
- **Only child. No related, genotypical HLA-matched donor. Match strict inclusion and exclusion criteria.**



Transplantation at age 19 on June 7, 2007

Lentiviral vector design with marked β -globin gene



Transduction / transplantation parameters

- Viral titer cGMP lot = 1.3×10^8 TU / ml during CD34⁺ cell transduction
- Fresh marrow for transduction and mobilized CD34⁺ cells for rescue (back up)
- Pre-transplant transduction efficiency = 0.6 vector copy / CD34⁺ cell (qPCR)
- Pre-transplant conditioning: BUSULFEX alone (at myeloablative dose)
- Dose of CD34⁺ cells injected = 3.9×10^6 CD34⁺ cells / kg

Percentages of vector bearing cells in blood and bone marrow (qPCR - 27 months post-transplant)

BONE MARROW (24 months)

All nucleated cells	13.5 %
CD34+	10.8 %
Erythroblastes (glycoA+ CD71+)	9.8 %

BLOOD (27 months)

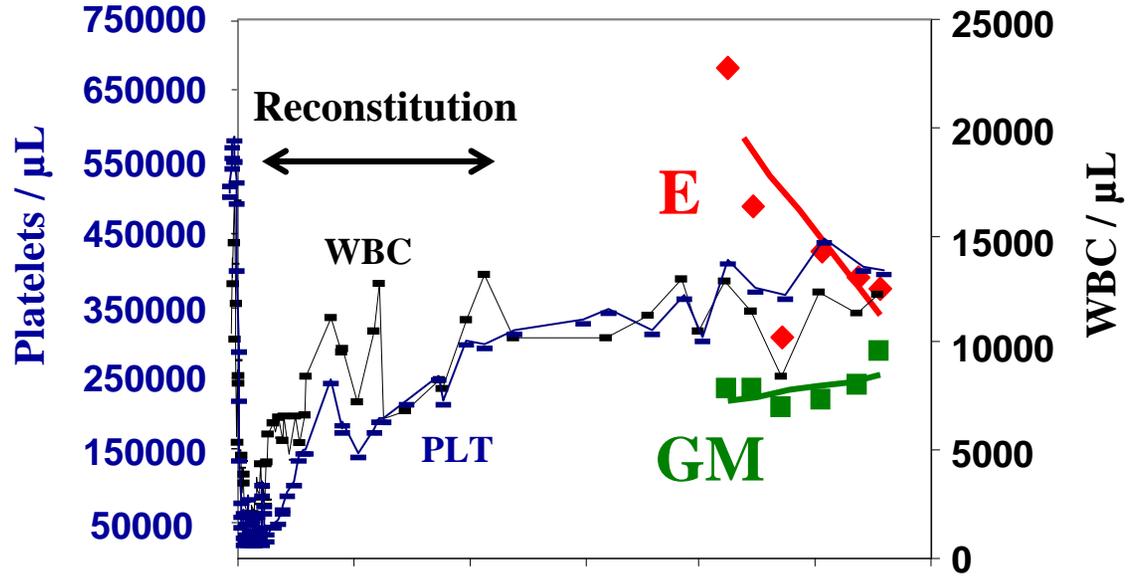
All nucleated cells	8.5 %
Granulo-Monocytes (CD15+)	17.2 %
B Lymphocytes (CD19+)	9.3 %
T Lymphocytes (CD3+)	1.3 %
Erythroblastes (CD45- CD71+)	1.8 %

**Correction
dyserythropoiesis**

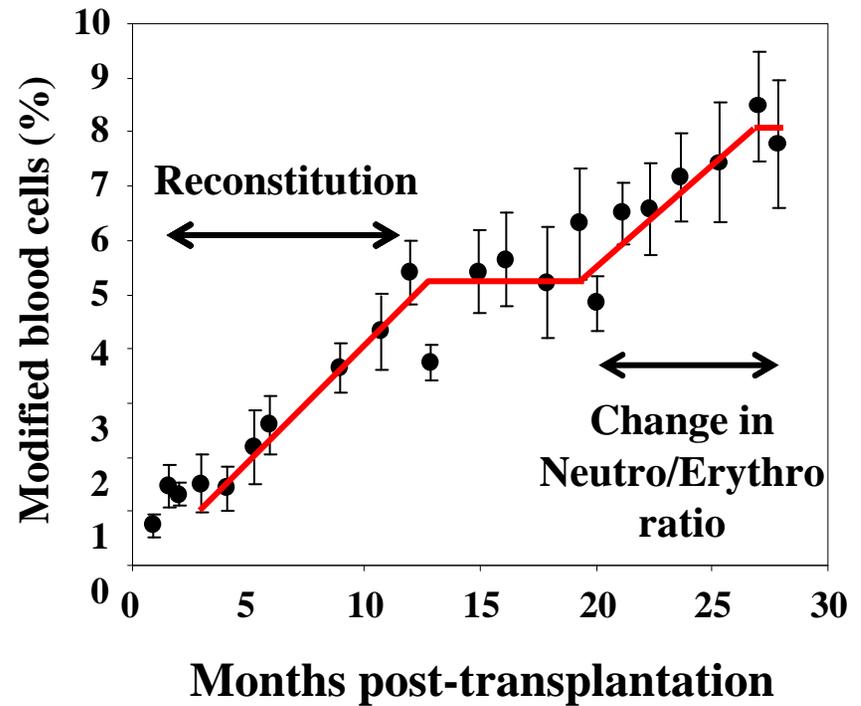
**Memory T cells
not exposed**



Hematopoietic reconstitution with change in blood Granulo-Mono (GM) / Erythro (E) ratio

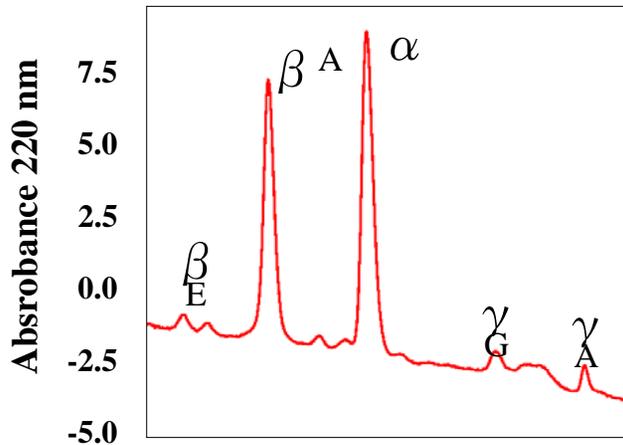


Kinetics of blood vector-bearing cells

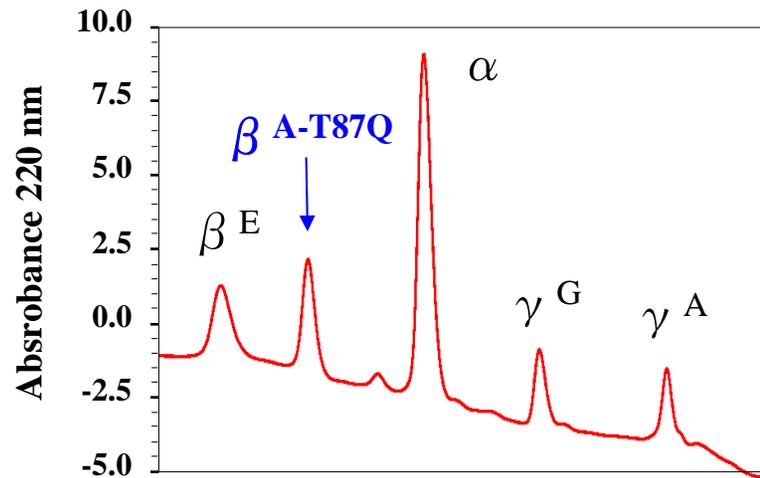


HPLC analysis of globin chains in whole blood (3 to 27 months post-transplant)

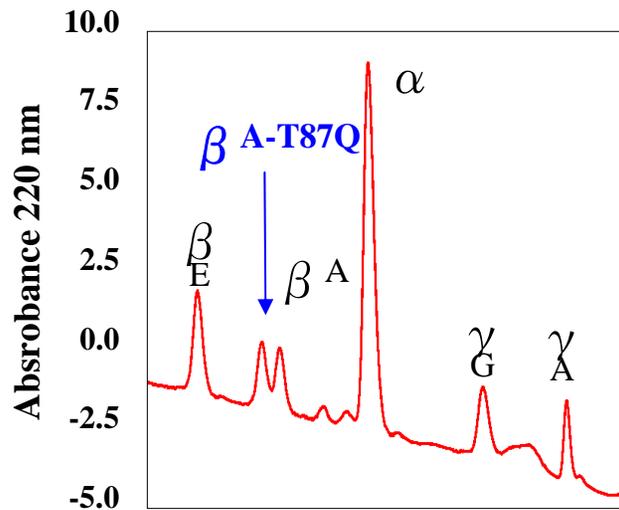
3 months



27 months

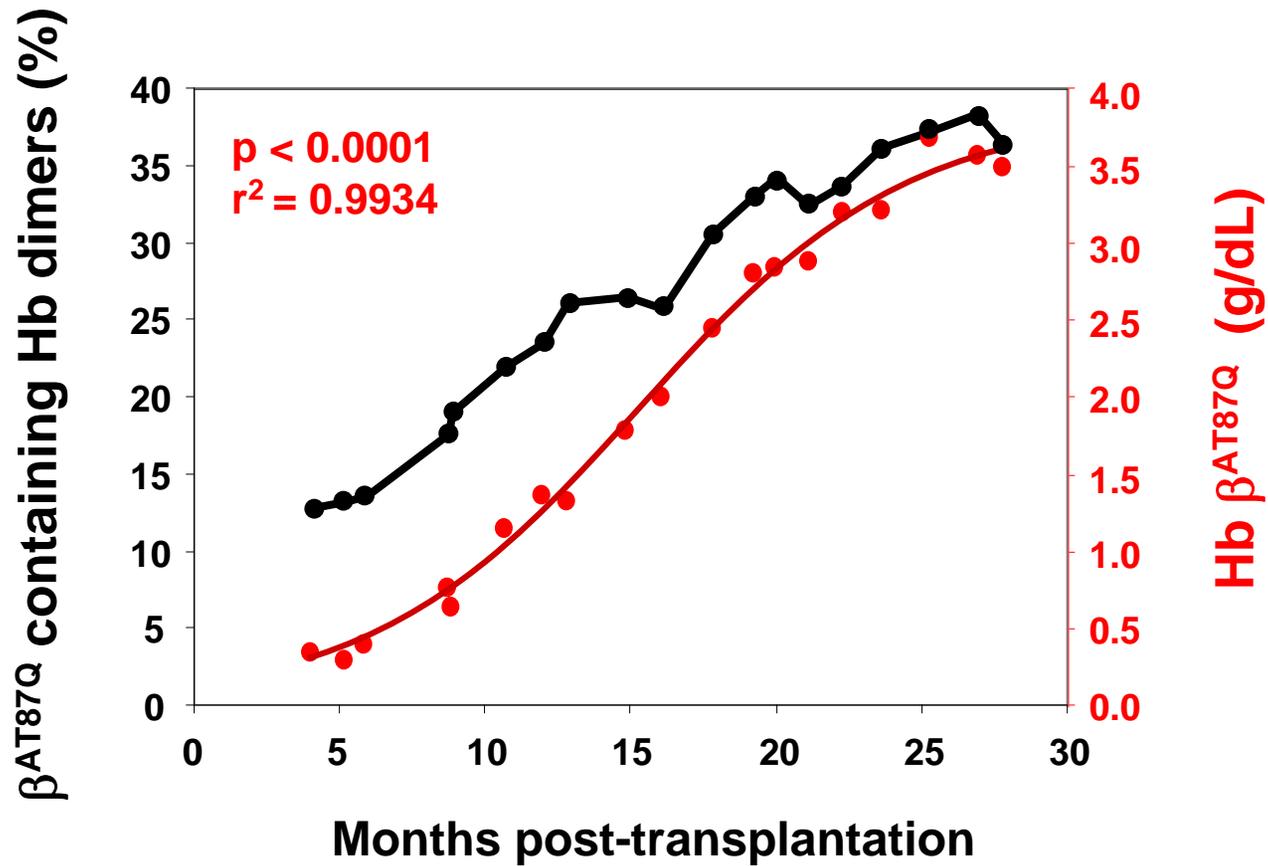


12 months

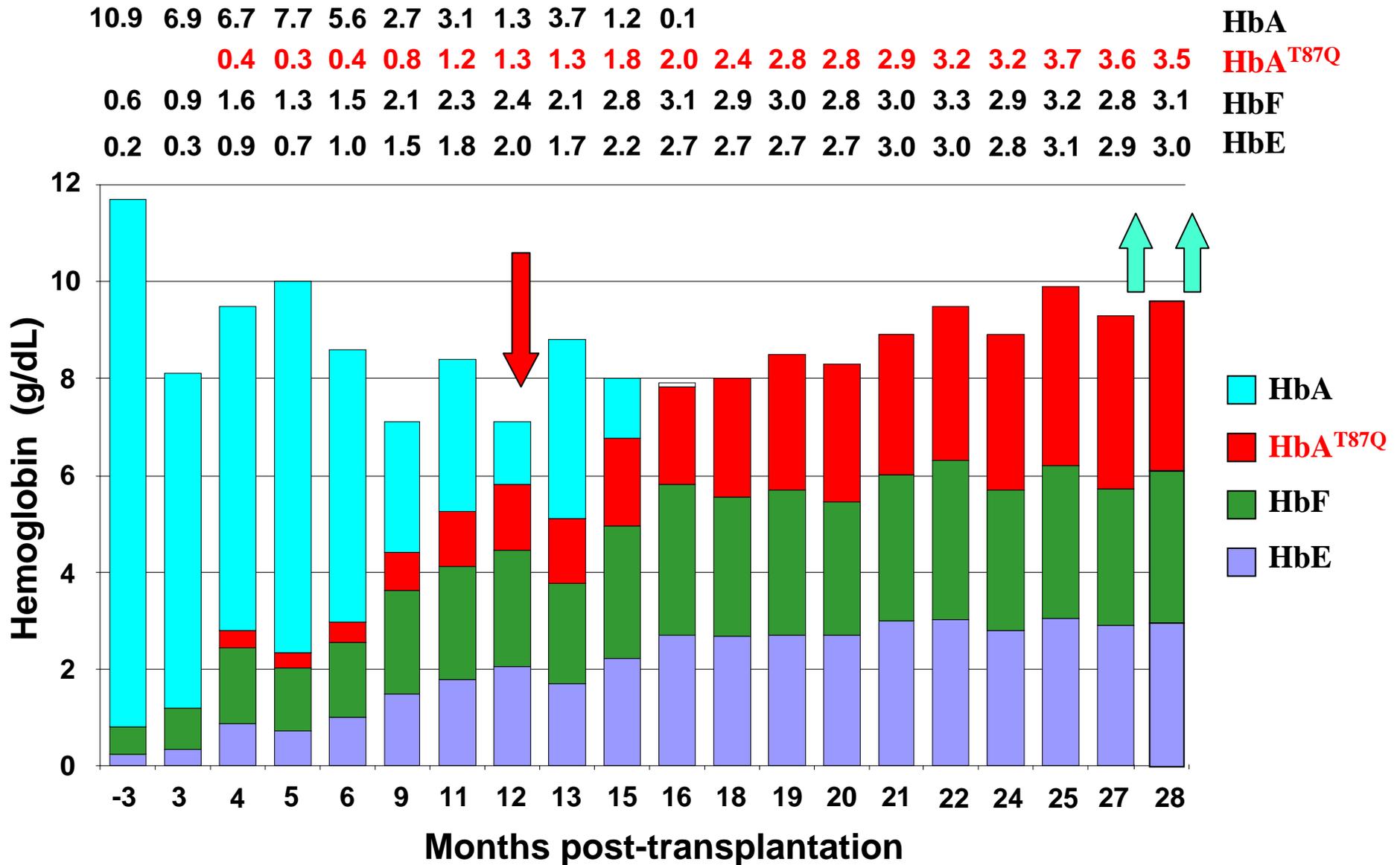


$$\frac{\beta^A\text{-T87Q}}{\Sigma (\text{all } \beta + \gamma)} \times 100 = 34 \%$$

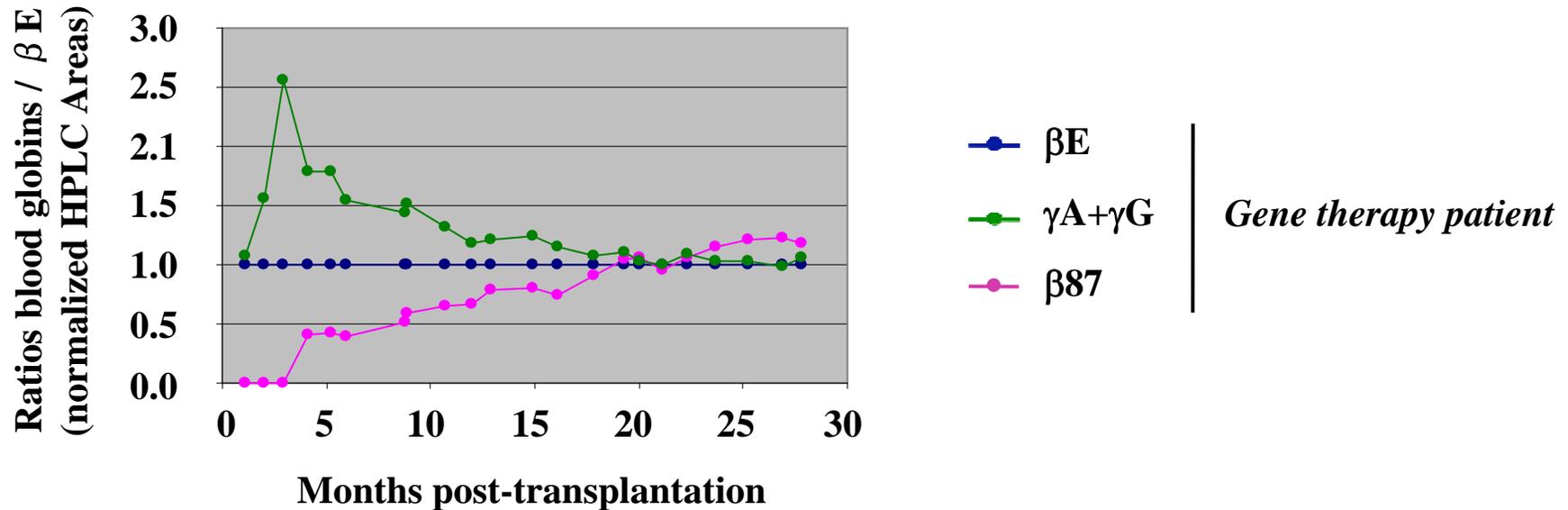
Expression of β^{A-T87Q} - globin in whole blood (HPLC analysis)



Conversion to transfusion independence (II)



Kinetics of γ -globin expression



- Homogenous γ -globin expression by HPLC in single BFU-E colonies regardless of vector integration sites (HMGA2 or not)
- Stable expression of BCL11A (RT-qPCR) throughout



Gene therapy with unmarked γ -globin vectors will be difficult to interpret

Decreased dyserythropoiesis and increased RBC lifespan

Decrease circulating erythroblast count > **3-fold**

Major increase in circulating RBC lifespan > **9.7-fold**

Near normal endogenous level of β^{A-T87Q} -globin expression in circulating RBCs on a per gene basis

MCH correction:

Patient's MCH 28 months post-transplant = **28.4 pg** (within normal range 27-32 pg)

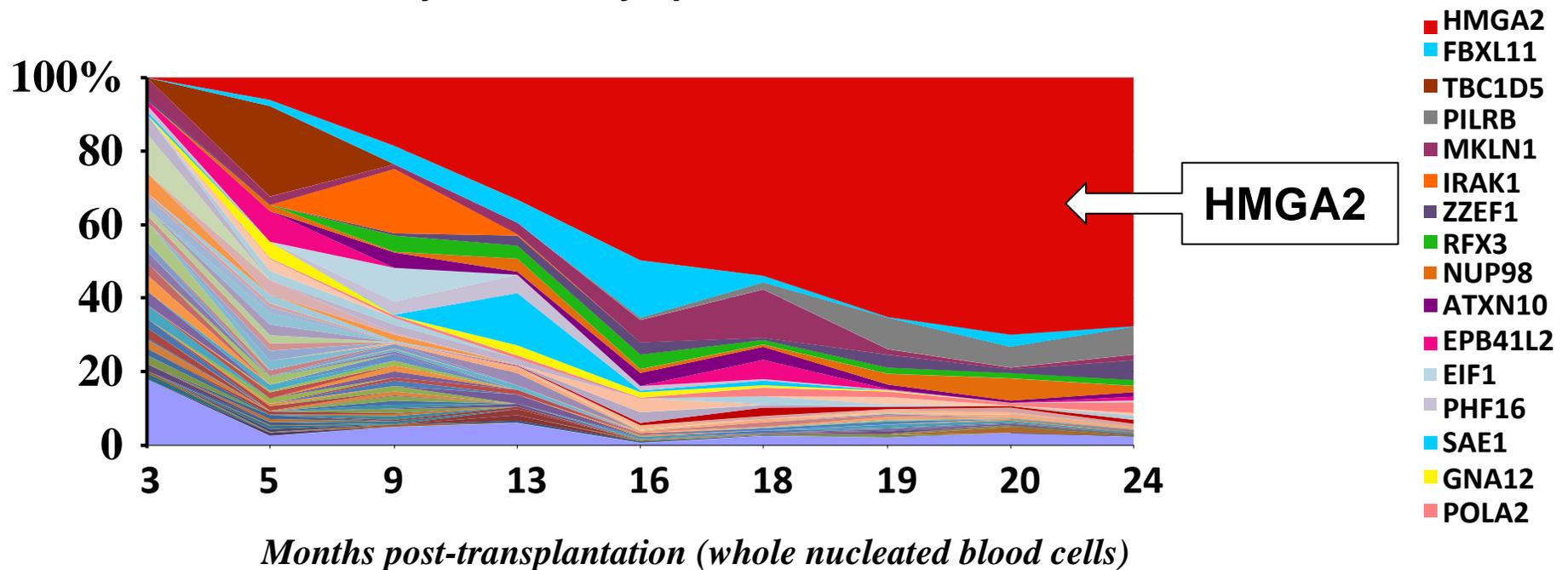
Average MCH for β^E/β^0 -Thal patients = **19 pg**

$$\frac{\beta^{A-T87Q}\text{-globin}}{\beta^A\text{-globin (normal)}} = \frac{\text{MCH}_P \times \text{Hb}^{A-T87Q} (\%) \times 2}{\text{MCH}_N} = \mathbf{0.67}$$

- Proportion of corrected RBCs = **100 – 67 %**
- Gene expression output on a per gene basis = **67 – 100 %**

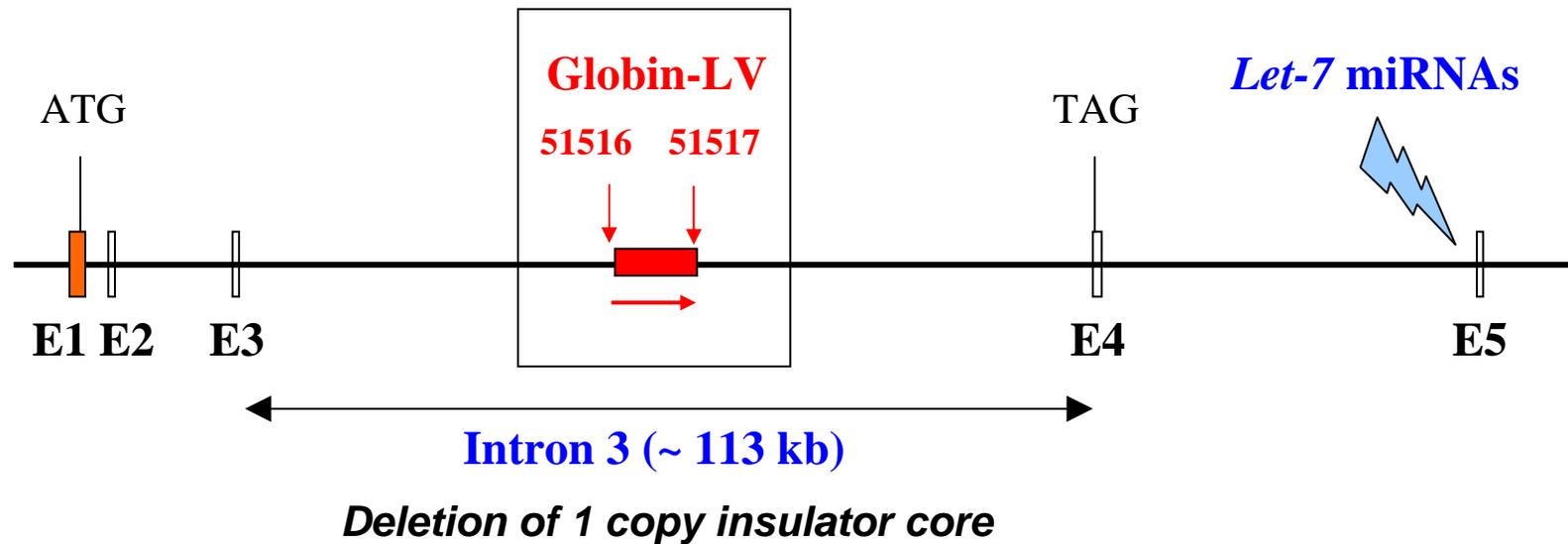
Integration site (IS) analysis by LM-PCR and DNA pyrosequencing (whole nucleated blood cells and purified sub-populations)

- Low total number of different IS (< 300)
In actively transcribed regions, similar to generic HIV vector
- 24 IS both myeloid and lymphoid



- Relative dominance of IS at the HMGA2 locus
(dominance relative to other IS, but > 85 % cells remain untransduced)

Vector integration in the third intron of the HMGA2 gene

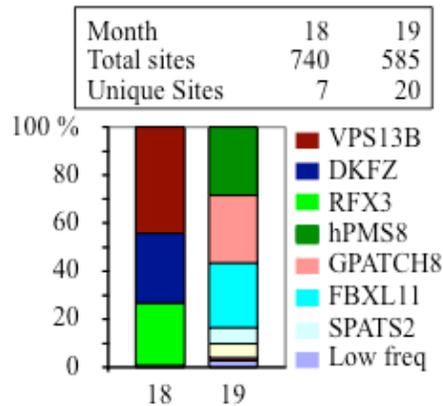


Physiological regulation of HMGA2 expression

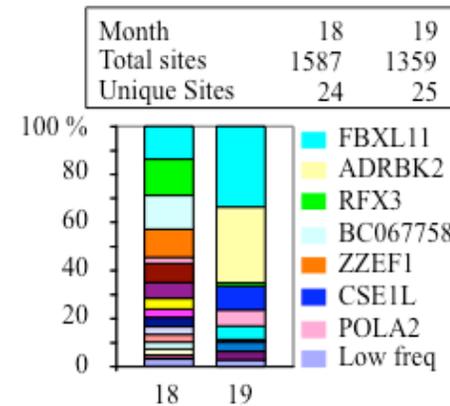
- Expression largely restricted to embryonic and stem cells
- Normal degradation of RNA by *Let-7* miRNAs (multiple targets in E5)
- Decreased expression in HSCs correlated with aging

Integration site (IS) analysis by DNA pyrosequencing (Blood cell subsets)

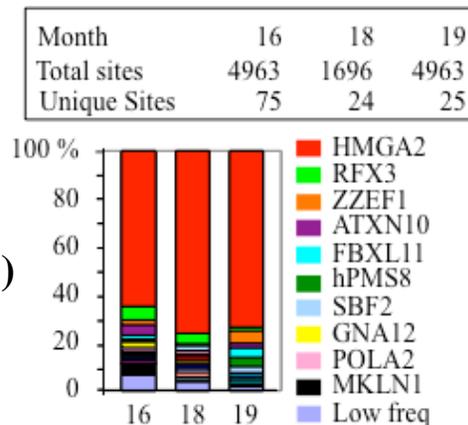
**T lymphocytes
(CD3+)**



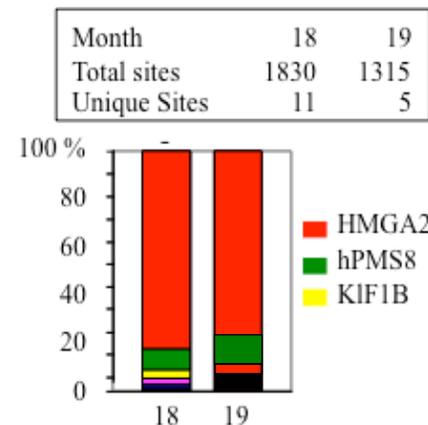
**B lymphocytes
(CD19+)**



**Granulocytes –
Monocytes (CD15+)**

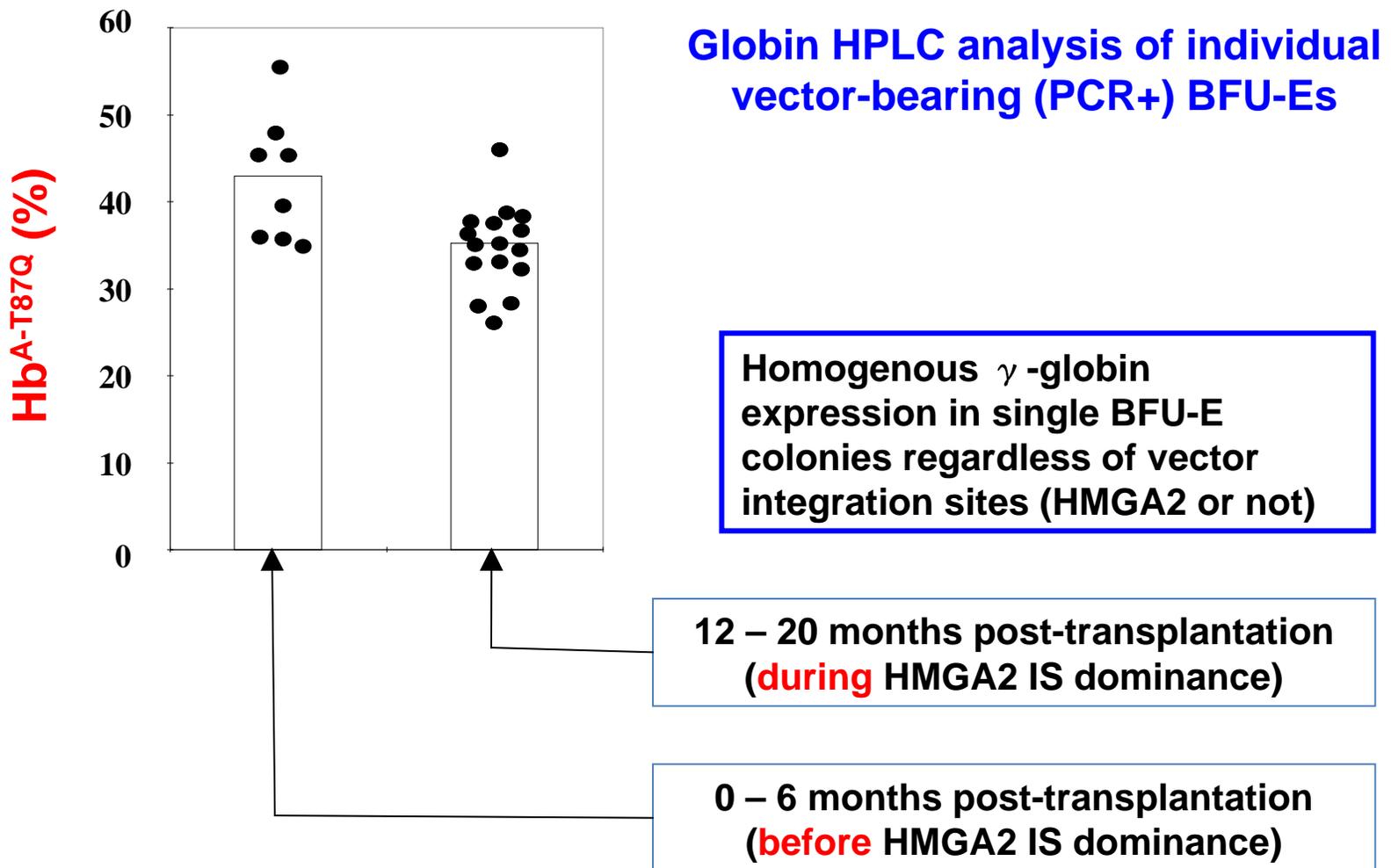


**Erythroblasts
(CD45-/CD71+)**



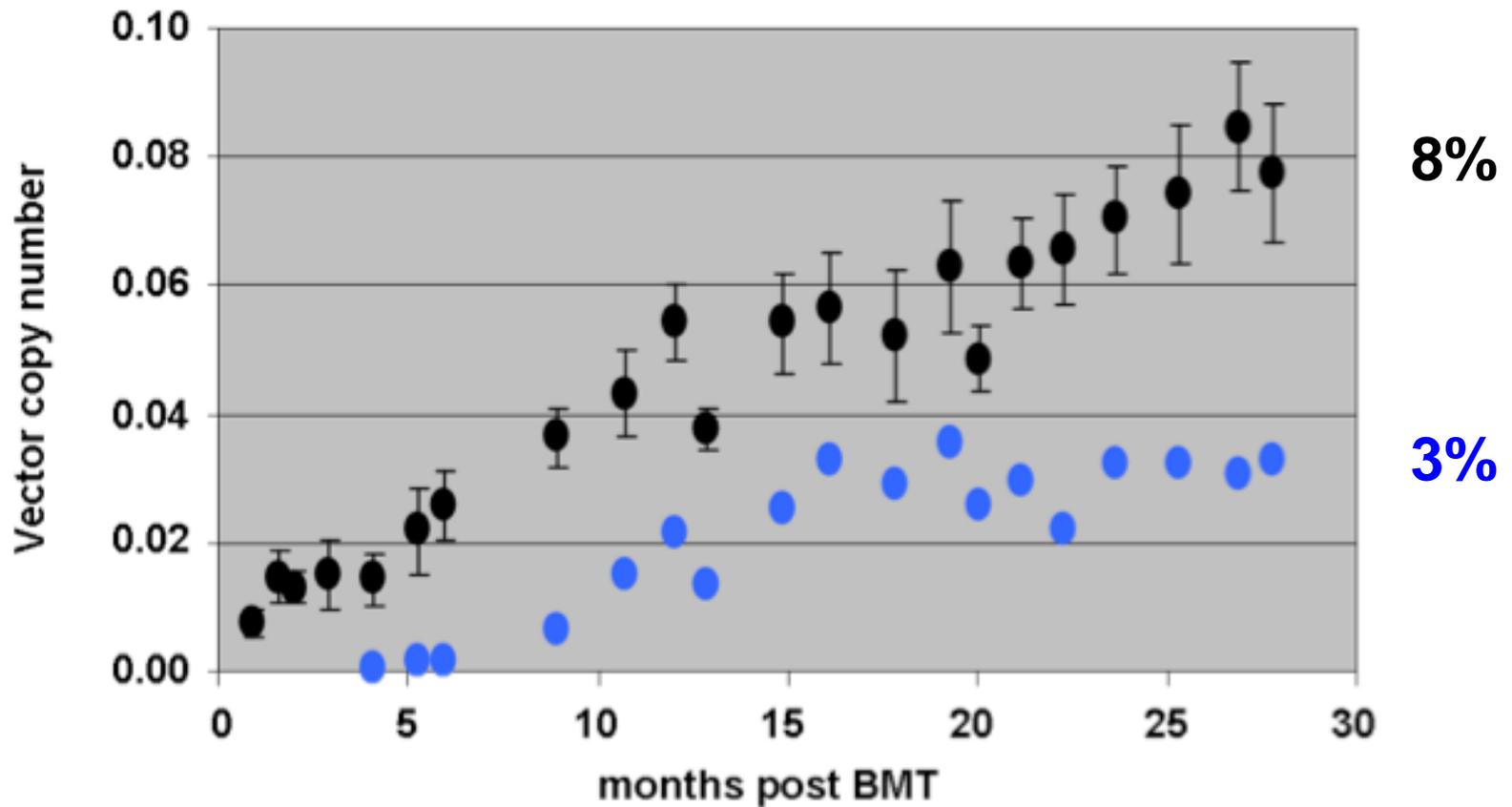
Dominance relative of the HMGA2 IS in both granulocytes-monocytes and erythroblasts, but not lymphocytes (negative qPCR after lymphocyte expansion)

Absence of major position effect variegation (PEV) regardless of integration site (IS) dominance



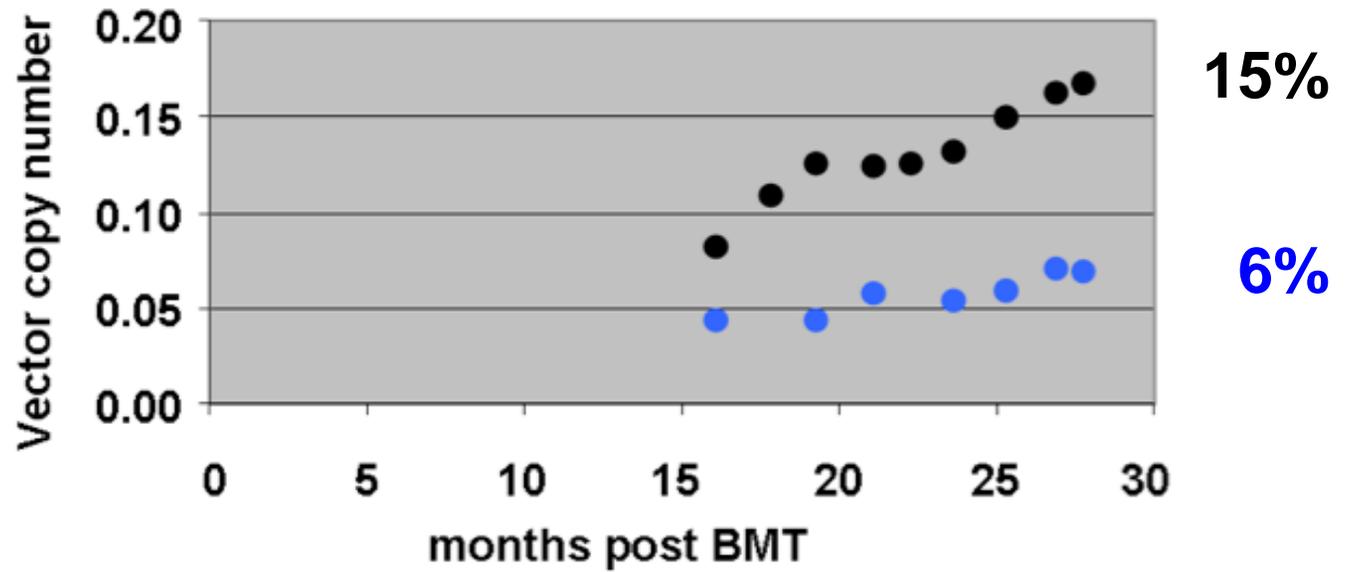
Quantification by qPCR of the number of copies of vector provirus in circulating blood cells (overall and HMGA2 IS)
(qPCR for whole nucleated blood cells and purified sub-populations)

Whole nucleated blood cells

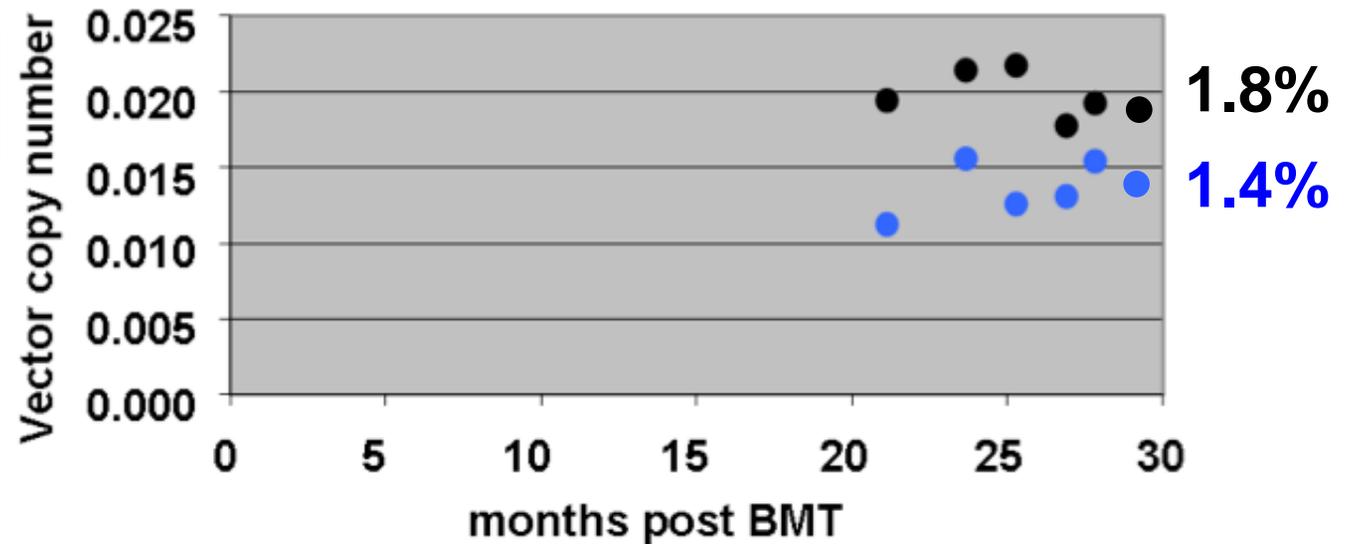


Total vector copy number (black) and copy number at specific HMGA2 site (blue)

**Blood CD15+
cells (GM)**

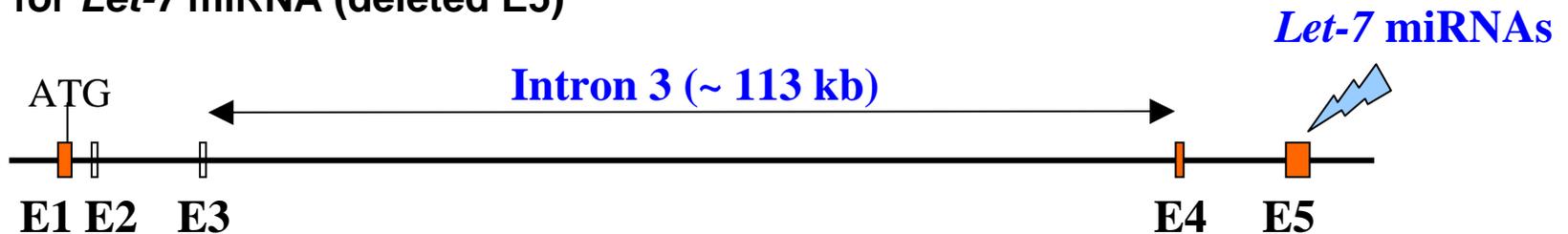


**Blood CD45-/CD71+
cells (E)**



Main mechanism of activation of HMGA2 correlated with benignity e.g., lipomas and Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Expression of truncated HMGA2 mRNA (E1 – E3) upon rearrangement within the long Intron 3 (with or without translation into fusion protein)
- Amplification of the expressed truncated mRNA by loss of target sites for *Let-7* miRNA (deleted E5)

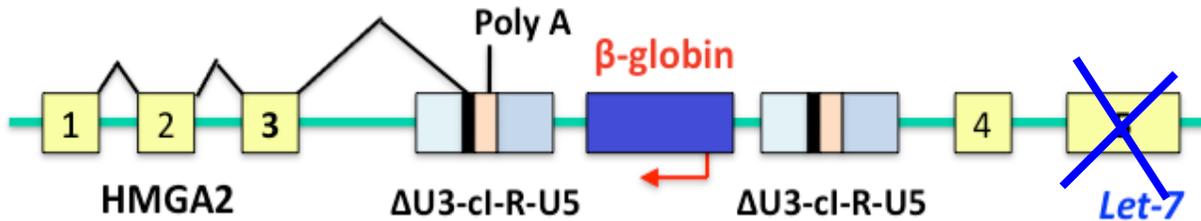
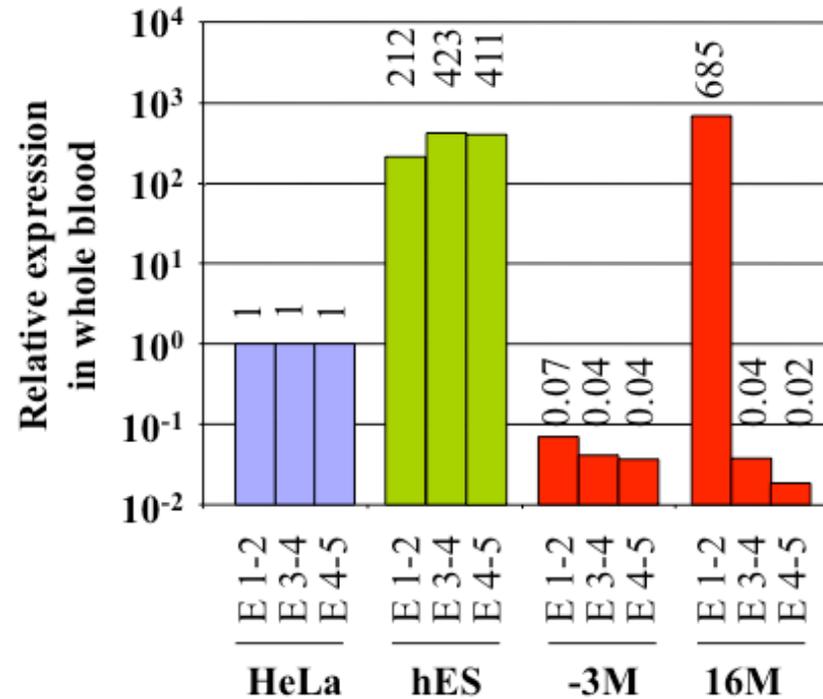


Main mechanism of activation of HMGA2 correlated with malignancy

- Expression of full-length HMGA2 mRNA by loss of expression of *Let-7* miRNAs
- Possible epiphenomenon since *Let-7* miRNAs also control the degradation of multiple oncogenic mRNAs (e.g., *Myc* and *Ras*)
- HMGA2 was NOT identified as an oncogene in the genome-wide N. Copeland (NCI)'s mouse oncogene screen

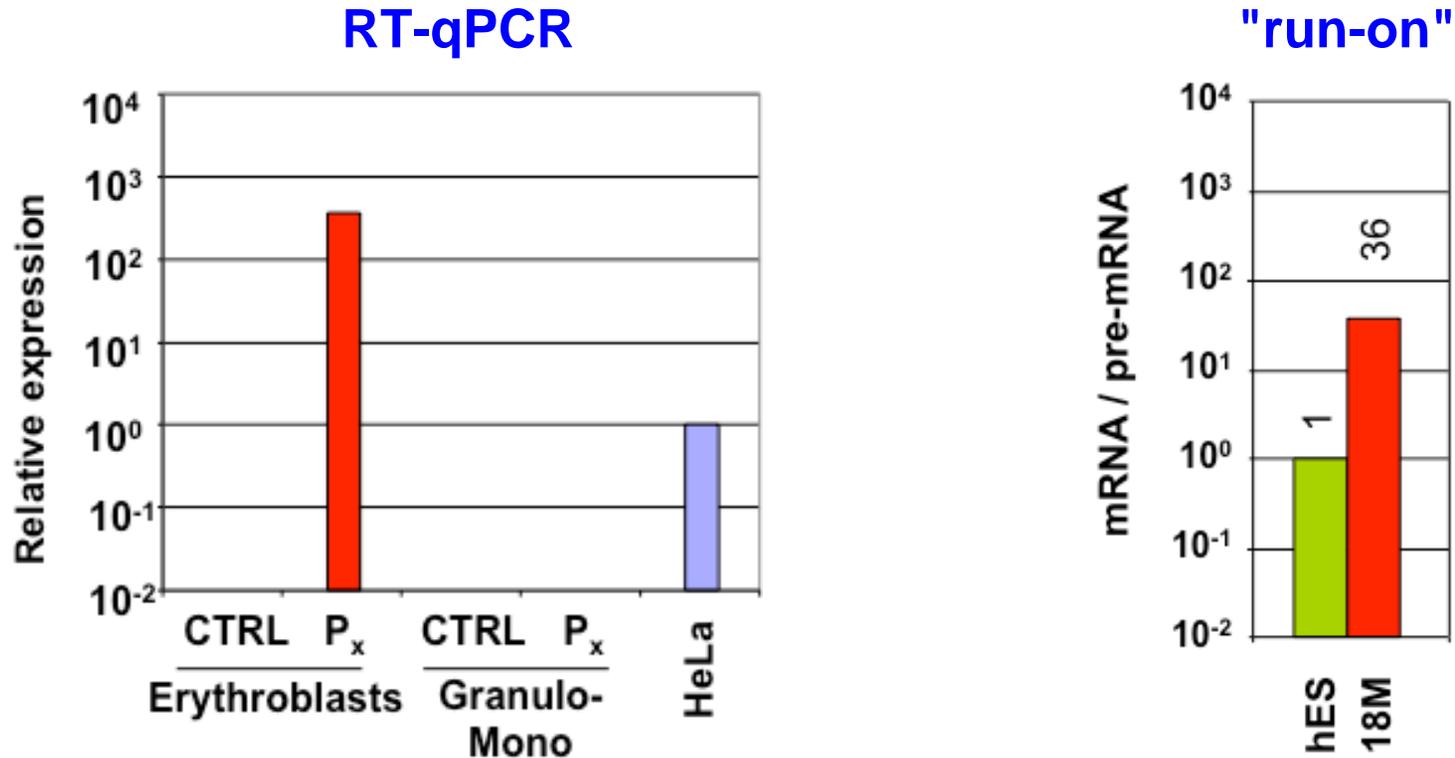
Is partial clonal dominance linked to HMGA2 activation ?

Evidence of truncation of the main HMGA2 transcript (E1 – E3) by staggered RT-qPCR



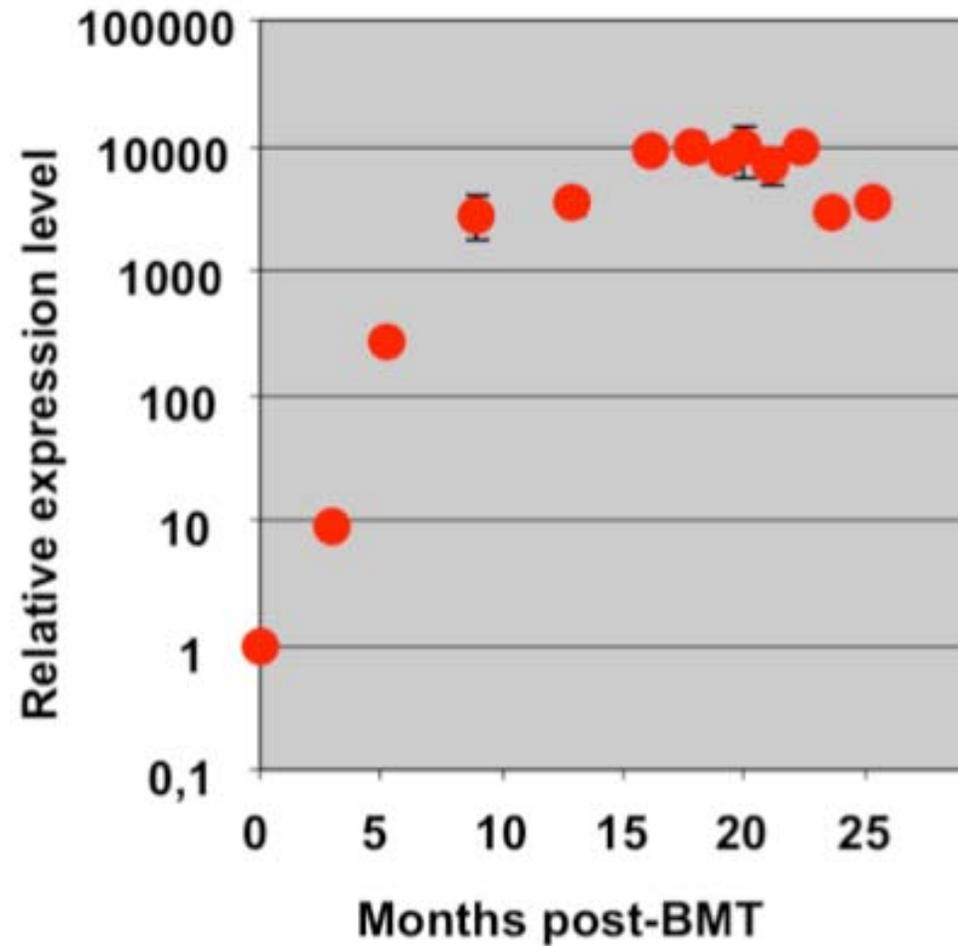
Sequencing of the main HMGA2 transcript:
Aberrant splicing within the vector insulator + polyadenylation

LCR-mediated effect vs. loss of Let-7 miRNA targets?



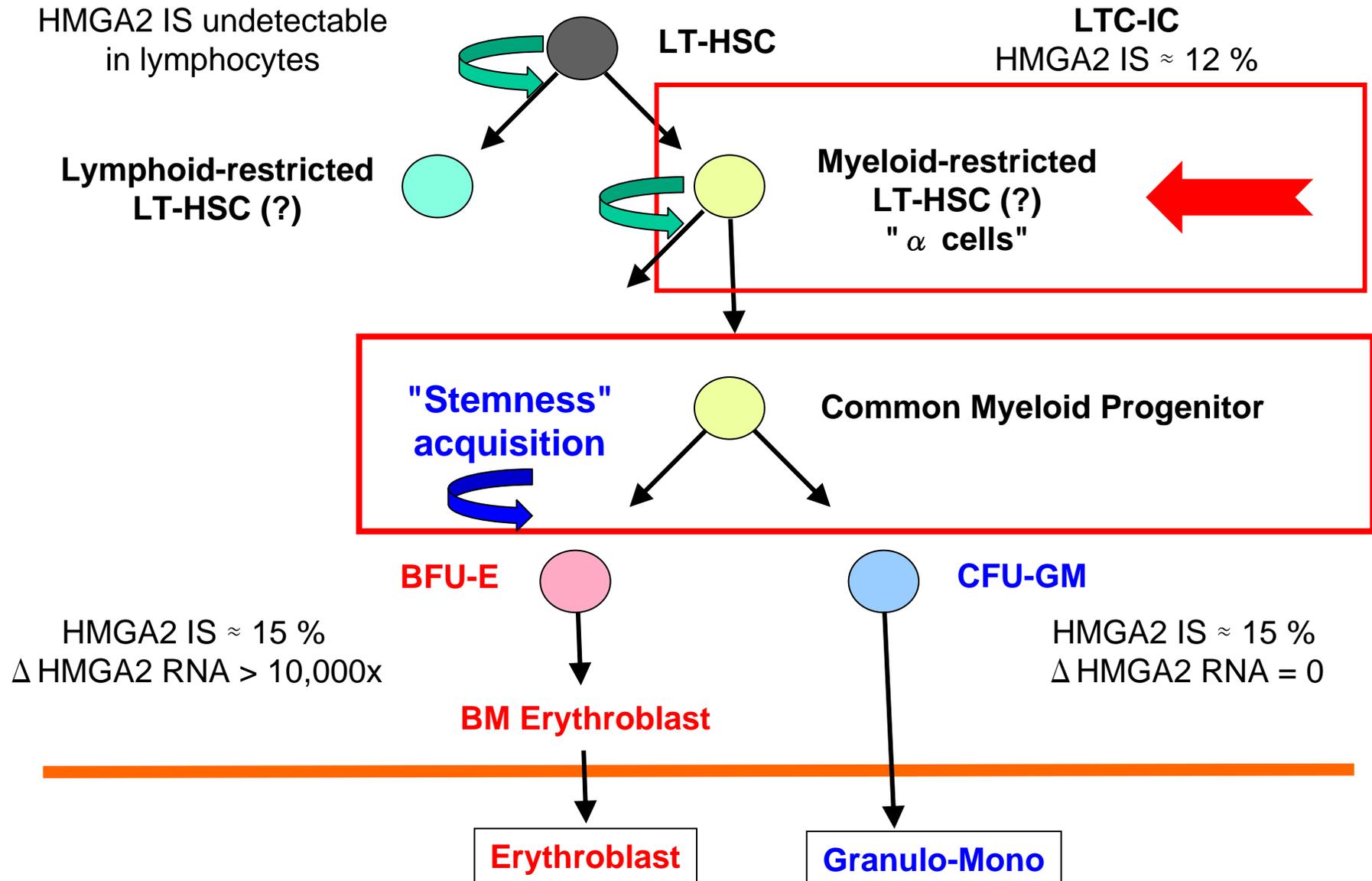
- Only expressed in erythroid cells (not granulo-mono) in spite of similar percentage vector at HMGA2 IS (qPCR HMGA2/LV junction)
- Increased transcription = 371 fold (likely LCR-driven with insulator failure)
Increased RNA stability = 36 fold (likely loss of Let-7 miRNA targets)

Stabilization of HMGA2 mRNA expression in whole blood (erythroblasts) – RT-qPCR analysis



Red dots: RNA was extracted from whole blood before (0) and after transplantation.

Proposed target of Globin LV-HMGA2 IS clone formation



Is hematopoietic homeostasis maintained ?

- Normal blood and bone marrow cytology – Normal cytofluometry analysis
- Normal karyotype and high resolution CGH-array chromosomal analysis – No Trisomy 8 with specific probe
- Lack of cytokine-independence in CFU-C assays
- Normal LTC-IC counts
- Asymptotic stabilization of the clone relative dominance (in agreement with mathematical modeling and the natural cases of PNH with HMGA2 dysregulation)
- Importance of the HSC dose:
 - In **deterministic** mathematical models, increasing transduced HSC dose results in increased risk with paradoxical increase in appearance of polyclonality
 - In certain **stochastic** mathematical models, increasing transduced HSC dose results in balanced co-suppression

Recent evidence of HMGA2 IS in other gene therapy trials ("hotspot" or evidence of homeostatic *in vivo* advantage?)

HMGA2 in X-SCID trial (γ -RV vector)

> 15 cluster IS in HMGA2 (aggregates of patients data):

- 12 in HMGA2 Intron 3
- 11 in same orientation - Increase abundance with time and then stabilize
- 2 (at least) with truncated RNA by aberrant splicing Intron 3 into vector

HMGA2 in ALD trial (LV vector)

1 IS in HMGA2 Intron 3 in patient P1:

- only in B lymphocytes and 1 time-point (9 months)

Interpretation of polyclonal patterns of IS (*ALD, ADA trials*)

True lack of genetic dysregulation resulting in growth advantage

"Peaceful co-existence" of cells with minor genetic dysregulation

Risk-Benefit Analysis

Recent statistical considerations for estimation of risk / benefit ratio in β -thalassemia

[Ann N Y Acad Sci](#). 2005;1054:40-7.

Survival and complications in thalassemia.

Borgna-Pignatti C, Cappellini MD, De Stefano P, Del Vecchio GC, Forni GL, Gamberini MR, Ghilardi R, Origa R, Piga A, Romeo MA, Zhao H, Cnaan A.

Department of Pediatrics, University of Ferrara, Ferrara, Italy. c.borgna@unife.it

... In a recent study from the United Kingdom, it was found that 50% of the patients had died before age 35. At that age, 65% of the patients from an Italian long-term study were still alive. Heart disease is responsible for more than half of the deaths...

Rationale to continue patients inclusion

- **Enhanced Benefit / Risk ratio derived from now known efficacy data**
- **No known vector improvements likely possible: splicing unavoidable – insulators without guaranty of improved safety (*E. Bouhassira, 2008*)**
- **Absence of clinical adverse event**
- **Stability of biological event (partial clonal dominance) – Similar to naturally occurring and benign (non-pre-leukemic) PNH**
- **No signs of breach of hematopoietic homeostasis**
- **Other cases of HMGA2 IS without malignancy: SCID-X1 (RV), ALD (LV)**
- **LCR shown to be < 200-fold less genotoxic than γ -retroviral LTR in transformation assays (*C. Baum and P. Malik, 2009*)**
- **Lack of preclinical detectability: No detection of clonal dominance and HMGA2 IS by pyrosequencing in mouse studies with same vector lot**
- **Necessity to further evaluate Benefit / Risk ratio in larger cohort**