



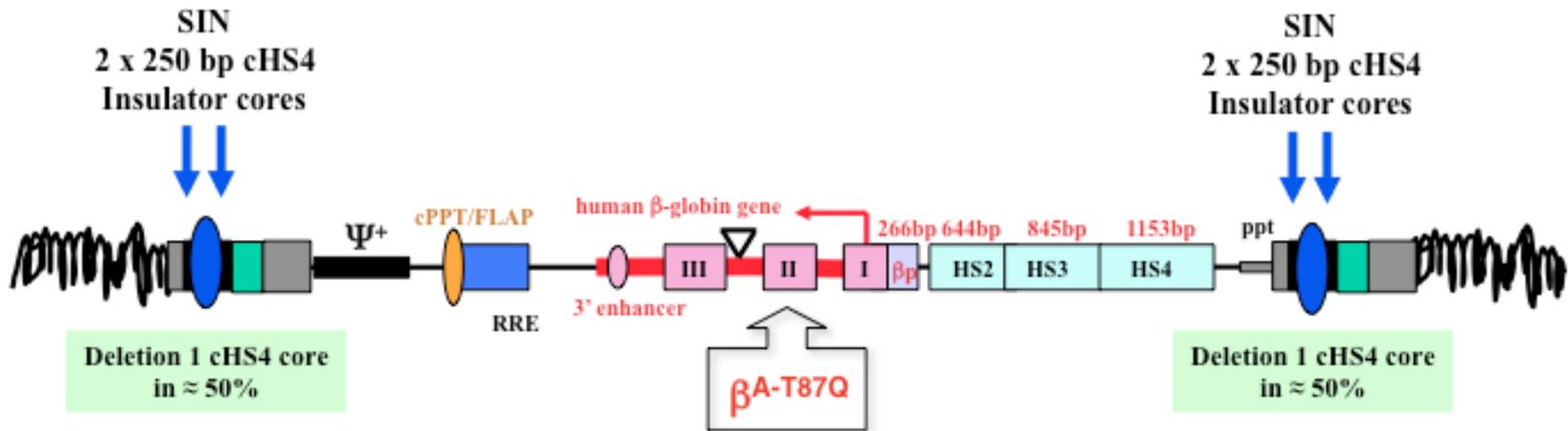
Transfusion independence with *HMGA2* activation after lentiviral gene therapy of human β -thalassemia

(UPDATE)

RAC - Bethesda, MD - December 2010 - P. Leboulch

Summary of patient and protocol history

- Severe β^E/β^0 -thalassemia with splenectomy at age 6, spontaneous Hb levels < 4.5 g/dL, life-long monthly transfusion-dependency
- Ex vivo lentiviral vector-mediated CD34+ cell transduction



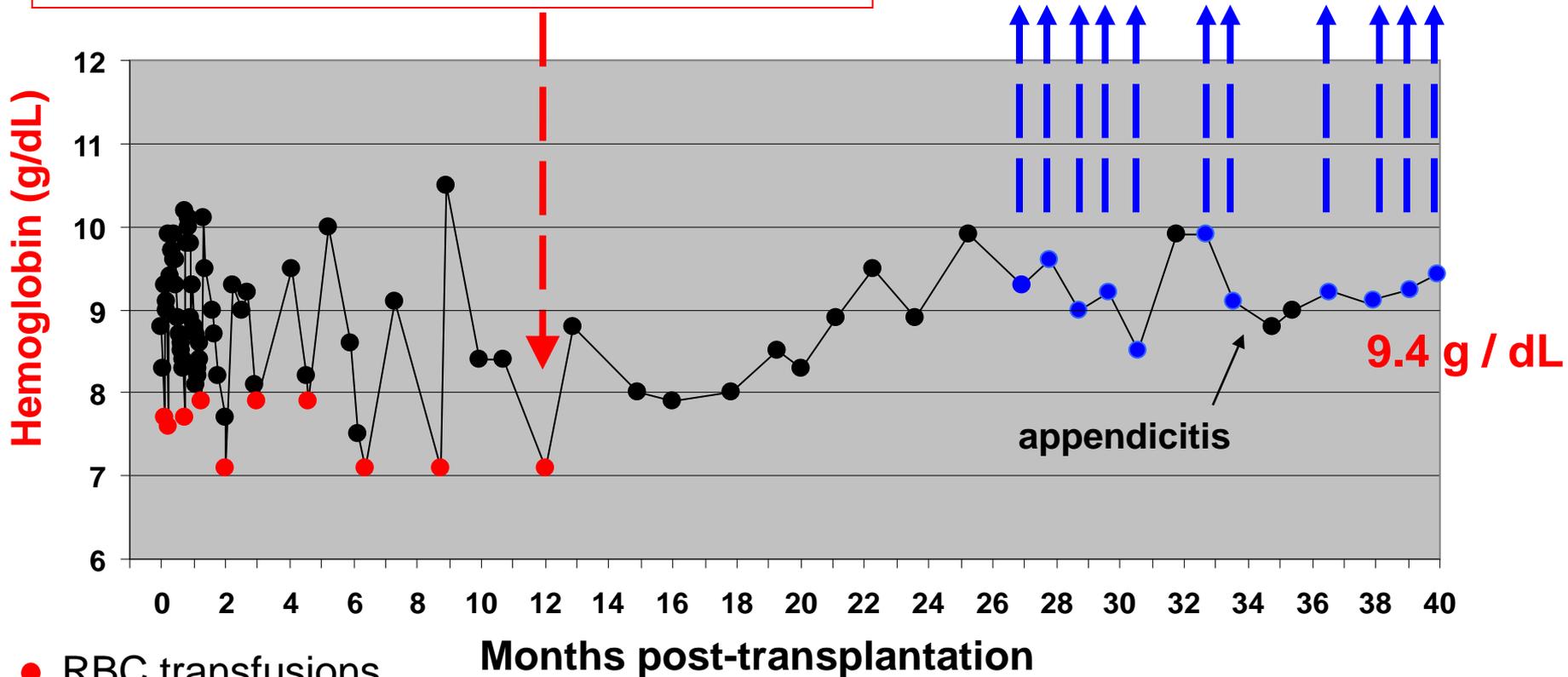
- Uneventfull transplantation at age 18 on June 7, 2007

Conversion to transfusion independence for > 2.5 years, 3.5 years post-transplant

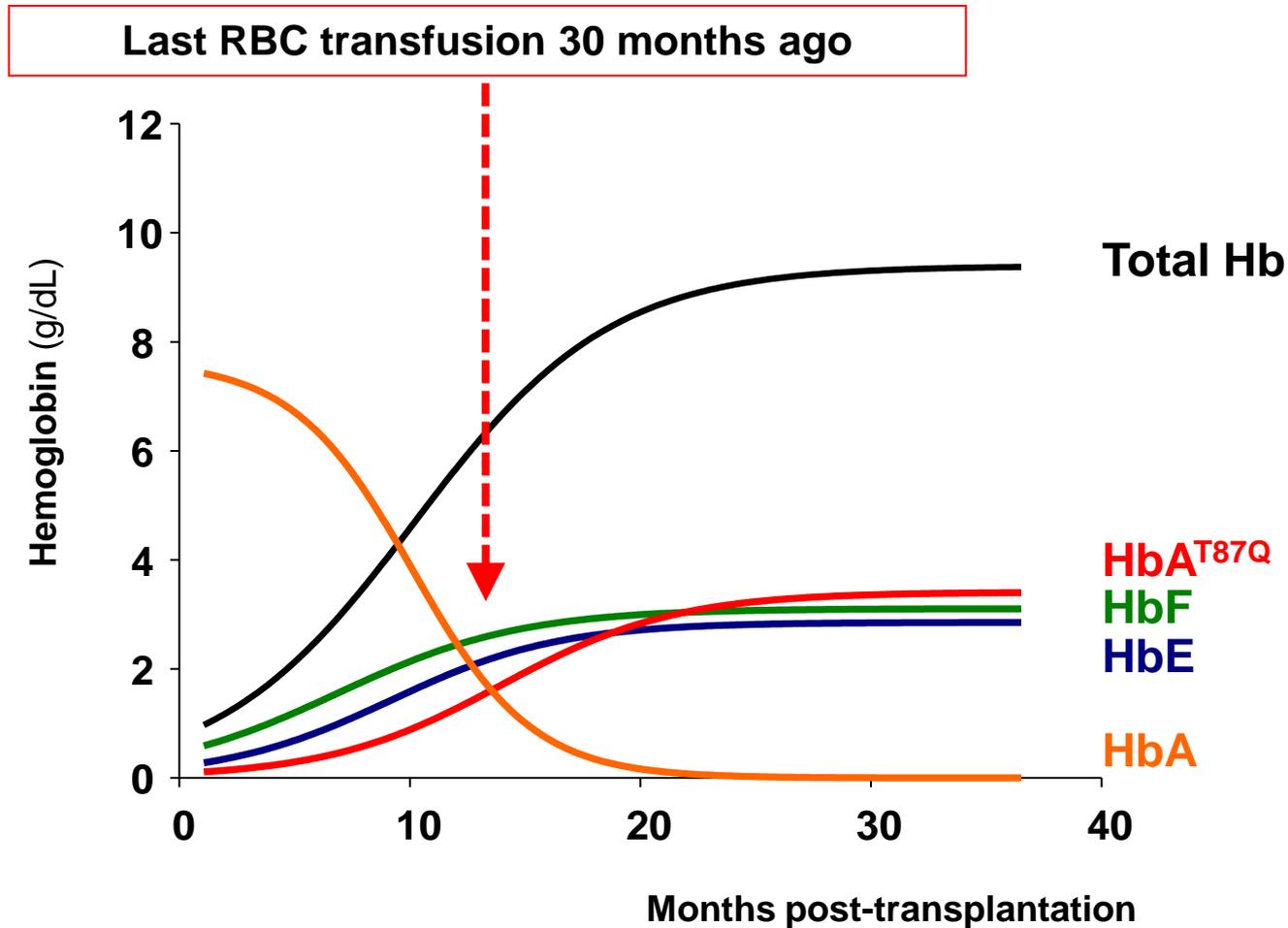
Transplantation on June 7, 2007

Last RBC transfusion on June 6, 2008

Phlebotomies (200 ml each)



Differential contributions of Hb species



≈ 3.5 g/dL vector-derived β -globin chain

Sustained amelioration of the thalassemic phenotype

- Complete MCH correction (28.4 pg)
- Calculated expression $\beta A^{T87Q} > 70\%$ endogenous βA -globin on a per gene basis
- Calculated increased RBC lifespan > 8.5 -fold
- Decreased circulating erythroblasts > 3 -fold (> 9 -fold for vector⁺ cells)
- Continuous decrease in plasma ferritin levels
- Excellent clinical status (full time job)

Percentages of vector bearing cells in blood and bone marrow (qPCR – 3 years post-transplant)

BONE MARROW (36 months)

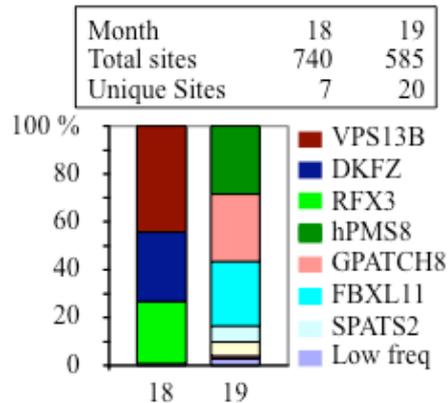
All nucleated cells	21.0 %	 ≈ 20%
CD34+	25.0 %	
CD45+	19.4 %	
Erythroblasts (glycoA+ CD71+)	34.6 %	

BLOOD (35 months)

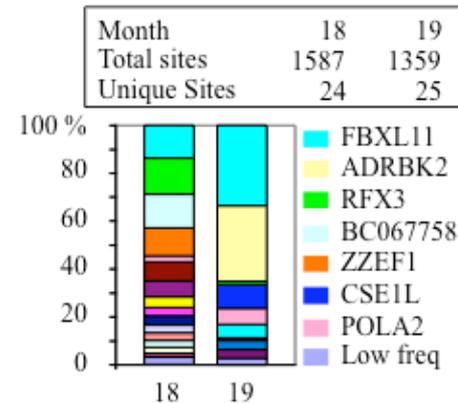
All nucleated cells	8.9 %	← Correction dyserythropoiesis	
Granulo-Monocytes (CD15+)	18.6 %		
B Lymphocytes (CD19+)	9.4 %		
T Lymphocytes (CD3+)	2.3 %		← Memory T cells not exposed
Erythroblasts (CD45- CD71+)	1.1 %		

Integration site (IS) analysis by DNA pyrosequencing (1.5 year post-BMT)

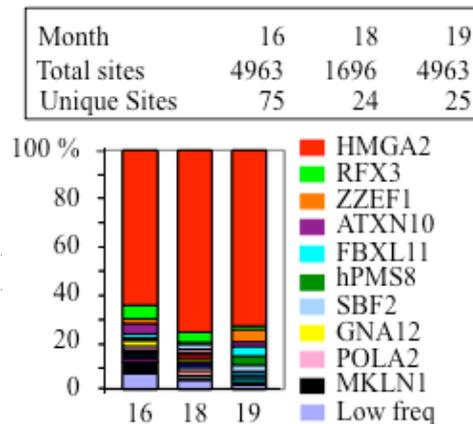
**T lymphocytes
(CD3+)**



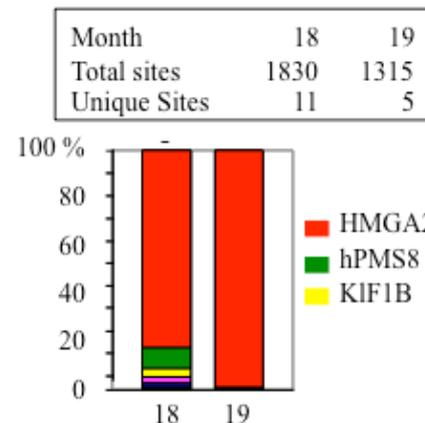
**B lymphocytes
(CD19+)**



**Granulocytes –
Monocytes (CD15+)**

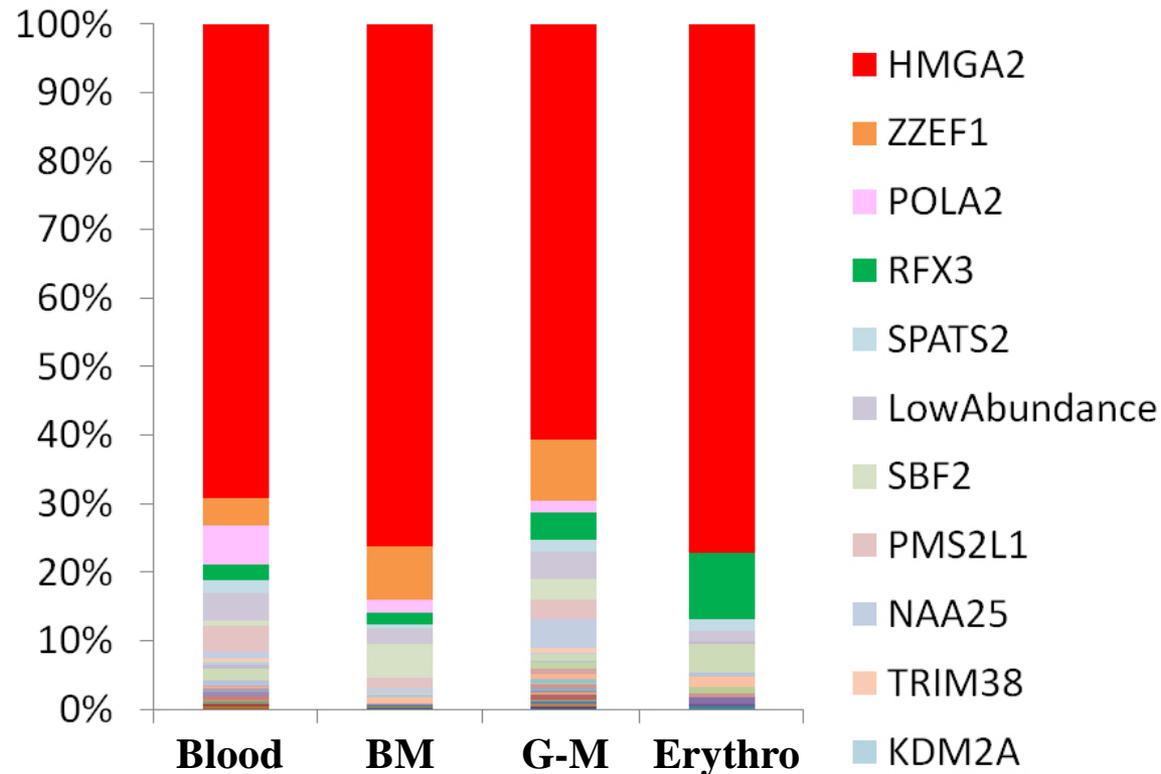


**Erythroblasts
(CD45-/CD71+)**



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

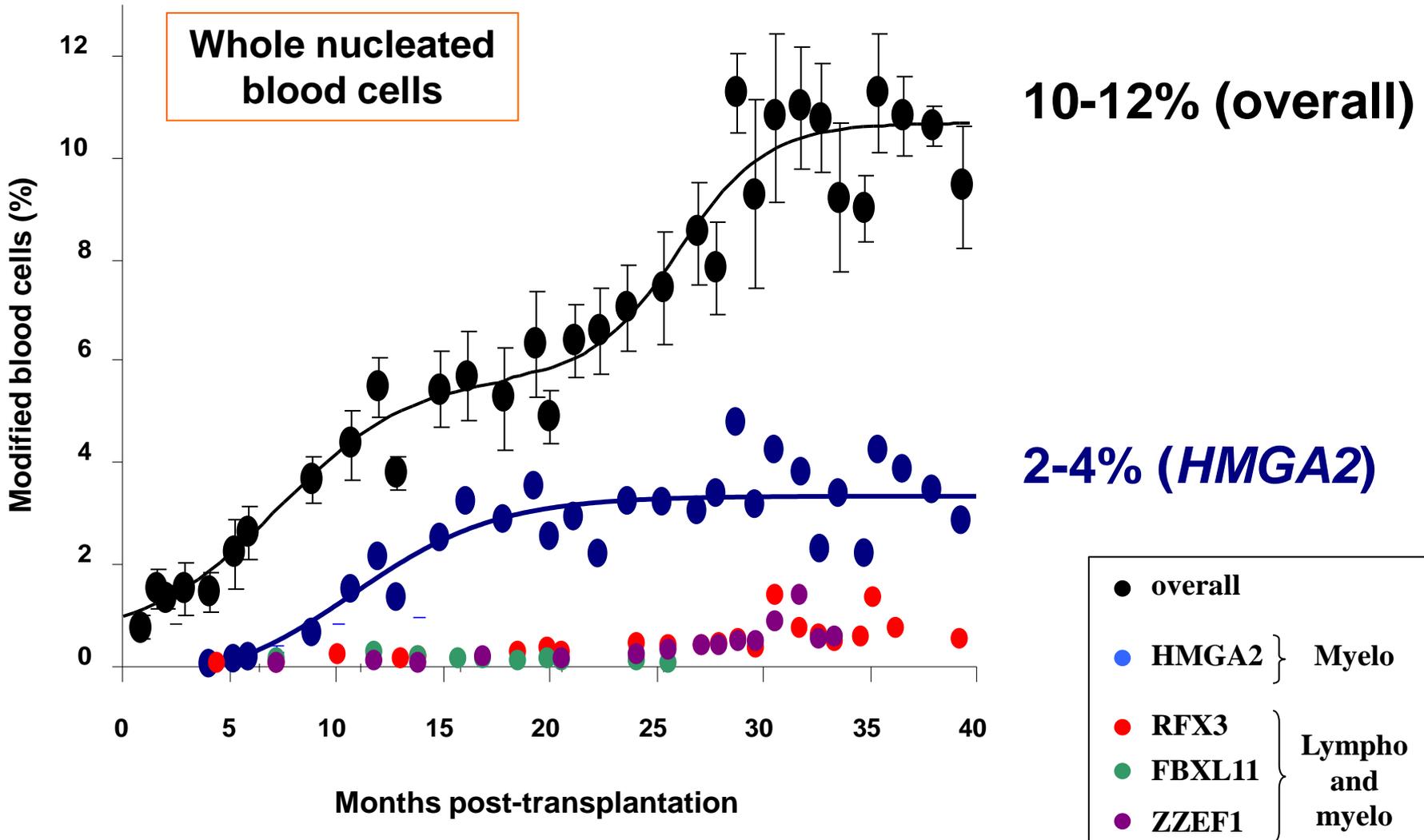
Integration site (IS) analysis by DNA pyrosequencing (3 years post-BMT)



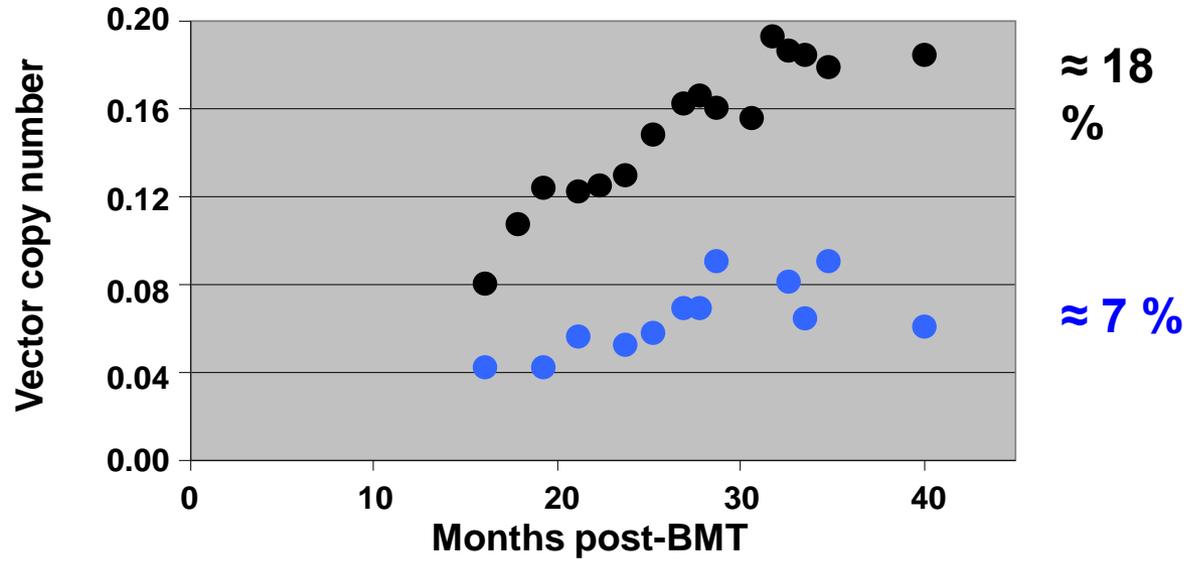
Stability of *HMGA2* relative clonal dominance

Other clones are highly stable but at lower levels

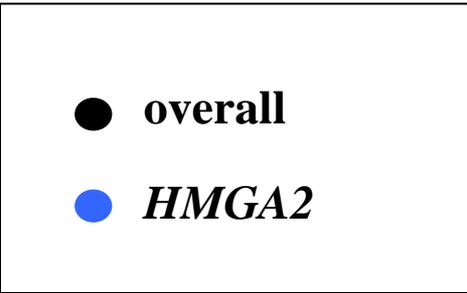
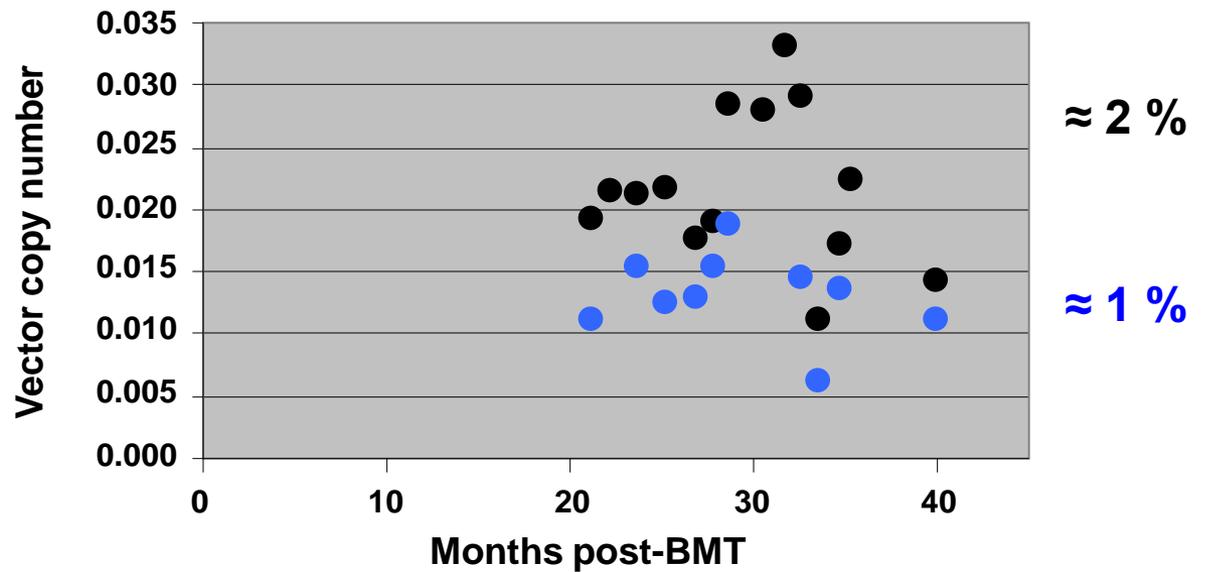
Quantification by qPCR of vector copy numbers in blood cells (overall, *HMGA2*, *RFX3*, *ZZEF1*, *FBXL11* IS)



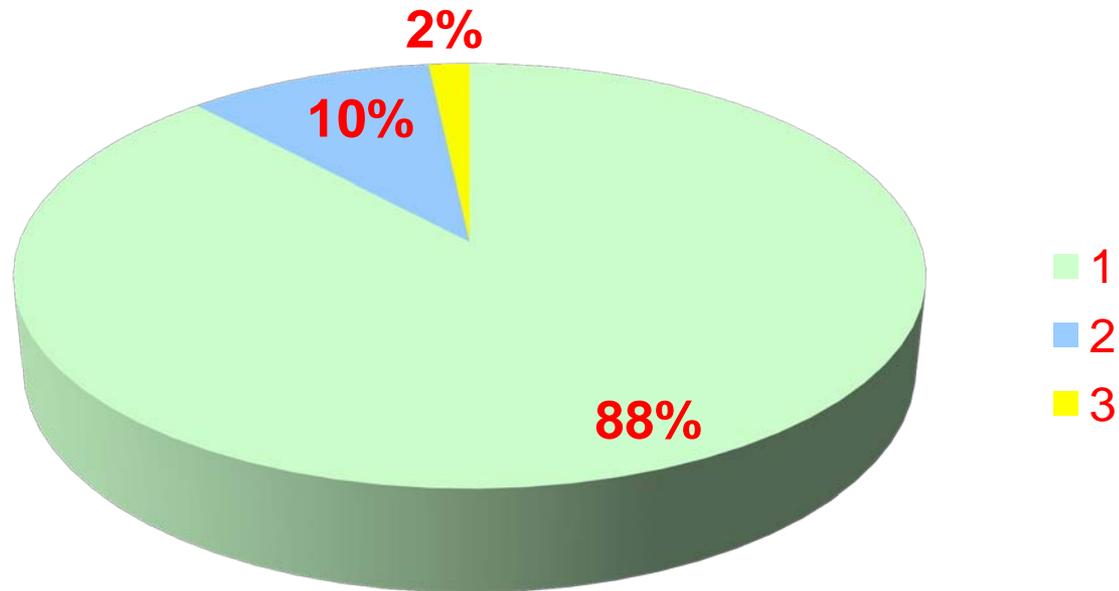
**Blood CD15+
cells (GM)**



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

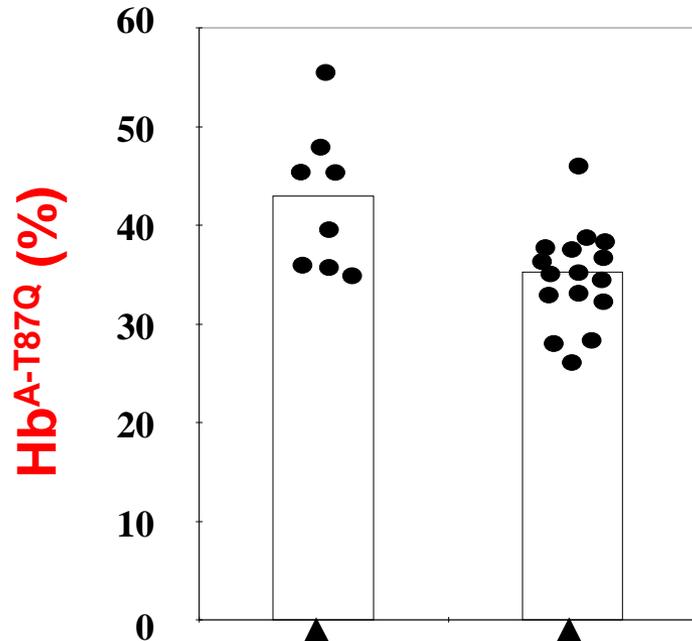


Proportion of vector bearing BFU-Es 40 months post-transplant



1. Non modified BFU-Es
2. BFU-Es genetically modified at *HMGA2* IS
3. BFU-Es genetically modified at other sites

Absence of significant position effect for βA^{T87Q} -globin expression regardless of integration site (IS) dominance



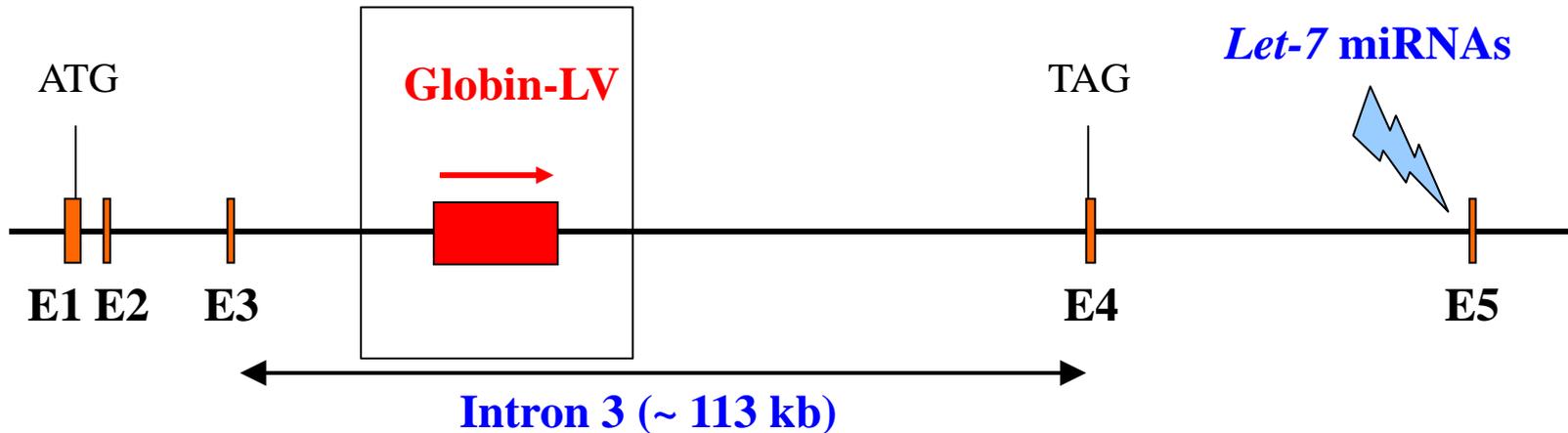
Globin HPLC analysis of individual vector-bearing (PCR+) BFU-Es

Homogenous γ -globin expression in single BFU-E colonies regardless of vector integration sites (HMGA2 or not)

12 – 20 months post-transplantation (during HMGA2 IS dominance)

0 – 6 months post-transplantation (before HMGA2 IS dominance)

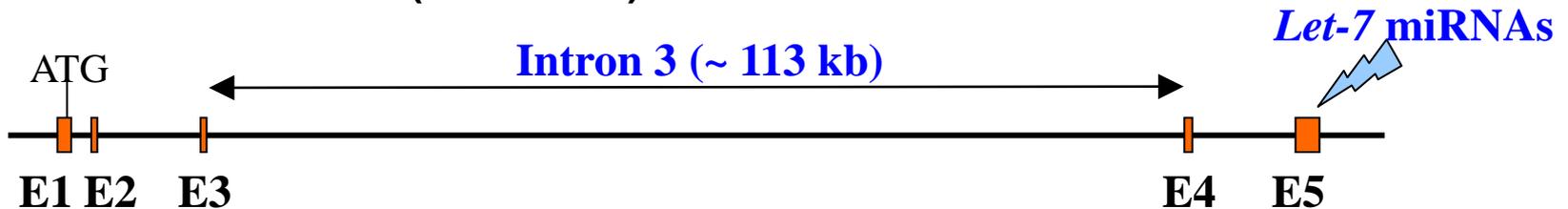
Physiological regulation of HMGA2 expression



- Normal degradation of RNA by *Let-7* miRNAs (multiple targets in E5)
- Expression largely restricted to embryonic tissues and adult stem cells
- Decreased expression in HSCs correlated with aging (Morrison and coll.)
- Increased expression in fetal liver vs. adult HSCs (Eaves and coll.)

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)

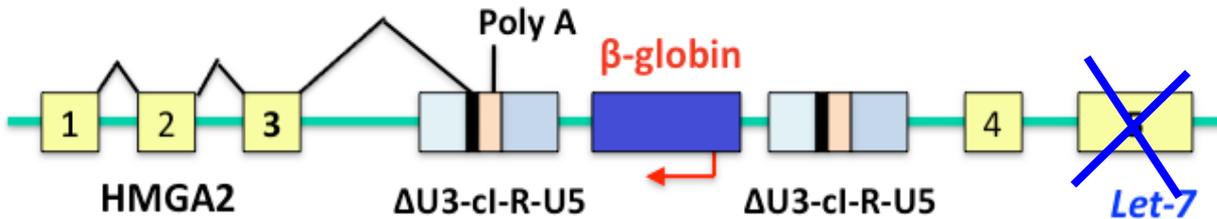
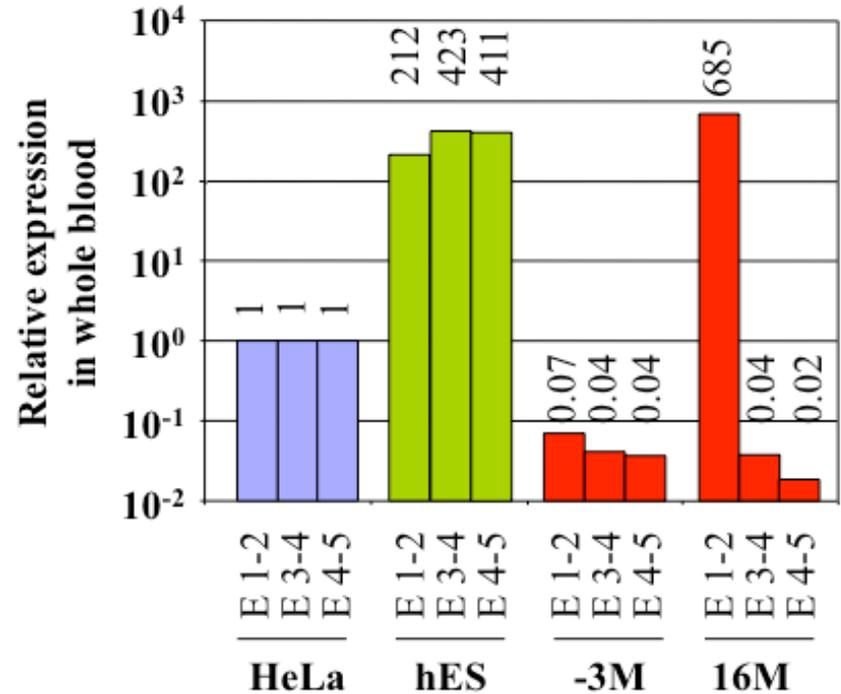


Main mechanism of activation of HMGA2 correlated with malignancy

- Expression of full-length 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 a genome-wide mouse oncogene screen (Copeland and coll.)

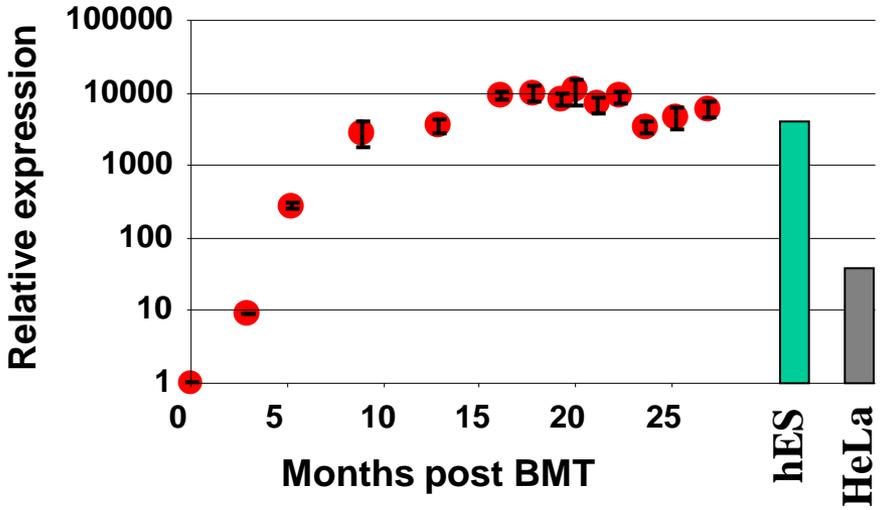
Is partial clonal dominance linked to HMGA2 activation ?

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

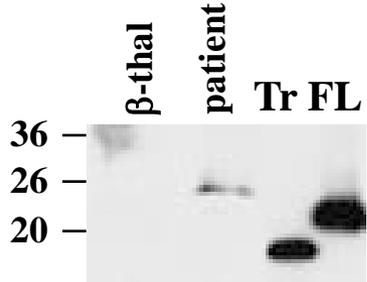


Sequencing of the main HMGA2 transcript:
Aberrant splicing within the vector insulator + polyadenylation

Evidence of high dysregulated HMGA2 mRNA and protein expression in erythroid cells



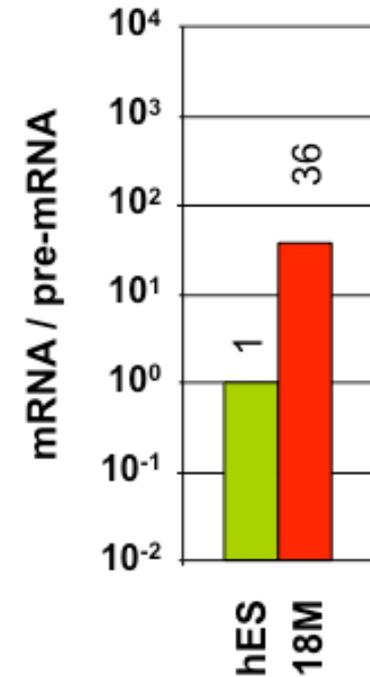
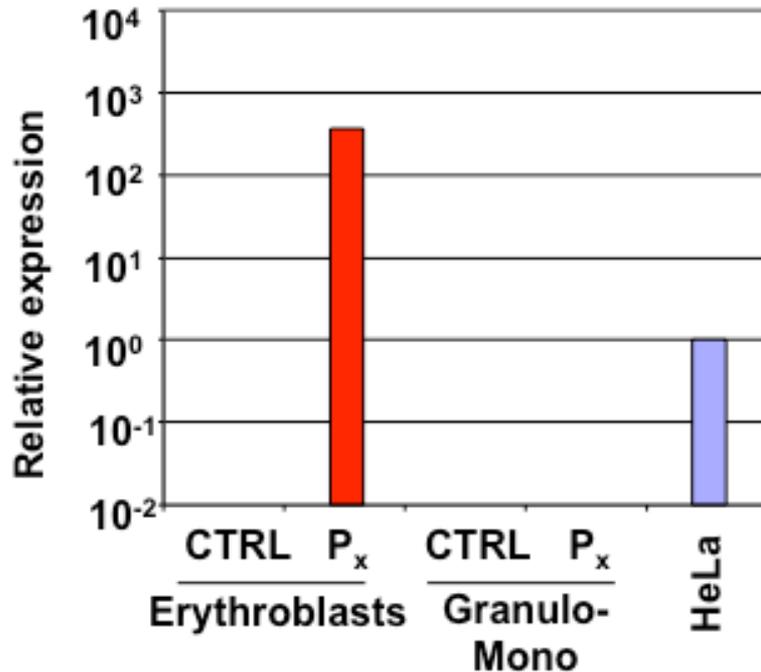
High expression level: x10,000 (whole blood cells) by RT-qPCR



Detection of the protein in erythroid cells derived from BFU-Es *in vitro*

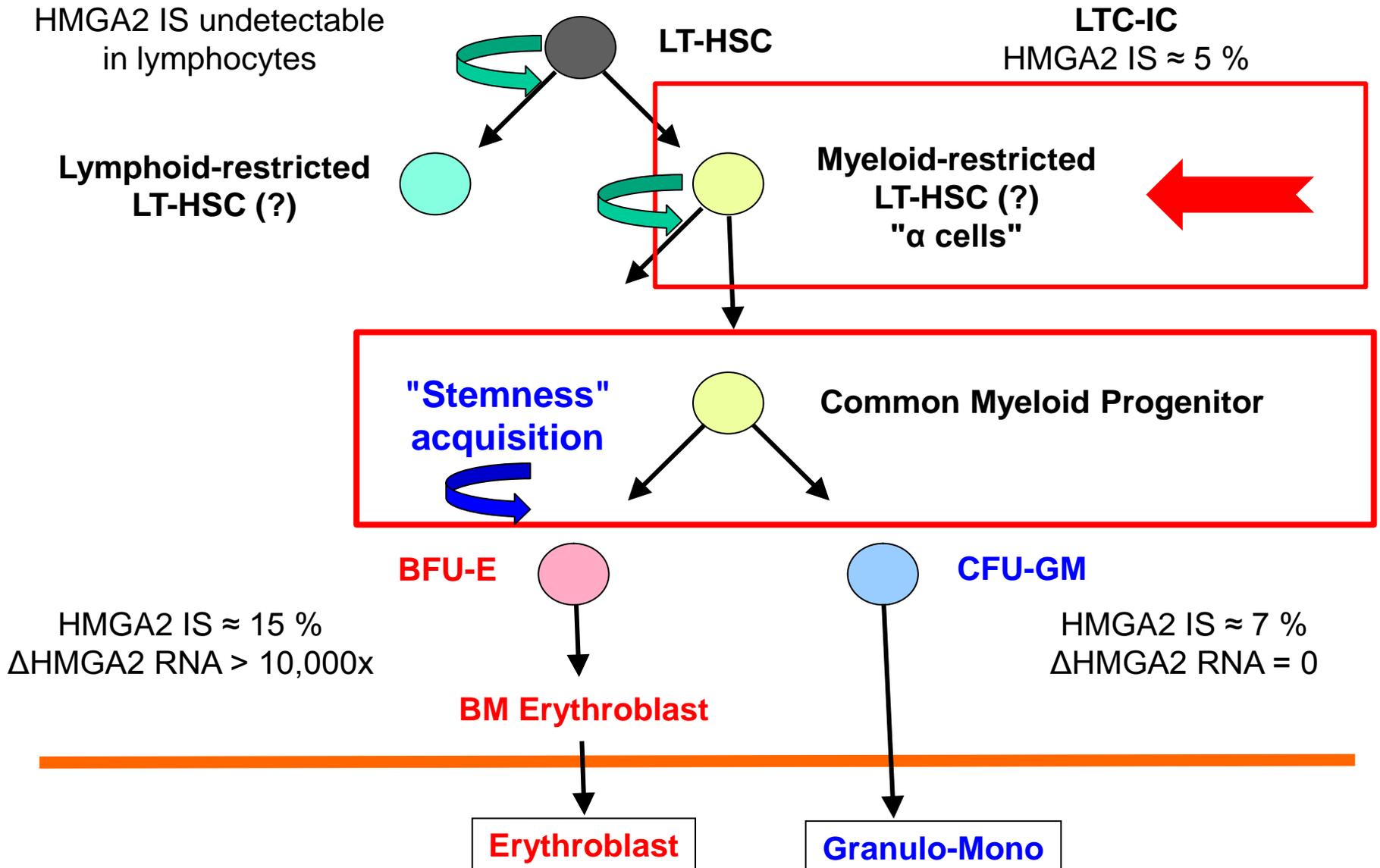
LCR - mediated effect *vs.* loss of Let-7 miRNA control

RT-qPCR



- 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

Proposed target of Globin LV-HMGA2 IS clone formation



Is hematopoietic homeostasis maintained ?

- **Asymptotic stabilization of the clone relative dominance**
- **Normal blood and bone marrow cytology – Normal cytoflurometry analysis**
- **Normal karyotype and high resolution CGH-array chromosomal analysis – No Trisomy 8 with specific probe**
- **Lack of mutation detected in *MPL*, *JAK2* or *TET2***
- **Normal LTC-IC counts**
- **Lack of cytokine-independence in CFU-C assays**
- **Absence of HMGA2-driven amplification of the patient's myeloid cells in transplanted NSG mice (*coll. with C. Eaves*)**
- **Physiologic HMGA2 overexpression in fetal liver hematopoiesis (C. Eaves)**
- **Benign evolution over 17 years of quasi-complete clonal dominance for truncated *HMGA2* in a patient with PNH (*T. Kinoshita*)**

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 “apparent” polyclonal patterns of IS (ALD, SCID)

True lack of genetic dysregulation resulting in growth advantage OR peaceful co-existence of cells with minor genetic dysregulation?

Importance of the HSC dose:

- In **deterministic** mathematical models, increasing dose of transduced HSCs results in paradoxical increase in appearance of polyclonality though with increased underlying risk ("MORE cannot be LESS")
- In certain **stochastic** mathematical models, increasing dose of transduced HSCs results in balanced co-suppression

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**
- **Absence of clinical adverse event**
- **No known vector improvements likely possible: splicing unavoidable – insulators without guaranty of improved safety – newly discovered "non-coding long transcripts"**
- **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*)**
- **Necessity to further evaluate Benefit / Risk ratio in larger cohort**
- **Appropriately updated informed consent forms**

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