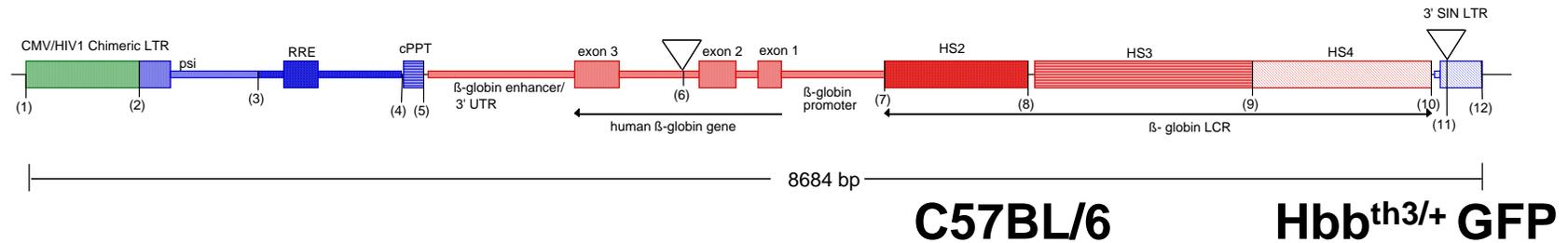


***Globin gene transfer for the treatment of  $\beta$ -thalassemia***  
***Update from MSKCC***  
**RAC, December 2, 2009**

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**Michel Sadelain, MD, PhD**  
**Director, Center for Cell Engineering**  
**Molecular Pharmacology and Chemistry Program**  
**Departments of Medicine and Pediatrics**  
**Memorial Sloan-Kettering Cancer Center**  
**New York, NY**

# Correction of anemia in $Hbb^{th3/+}$ mice



## letters to nature

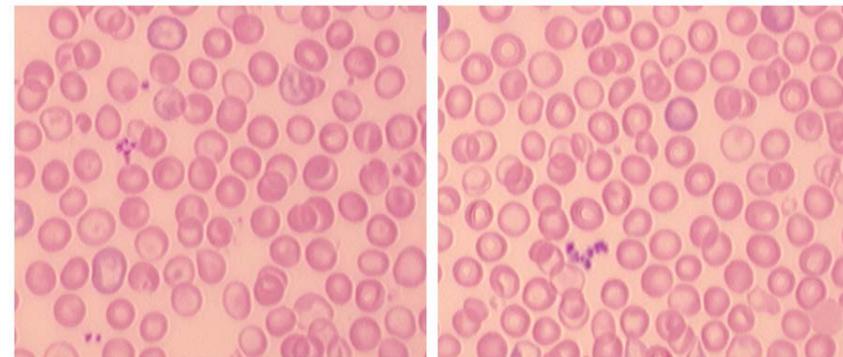
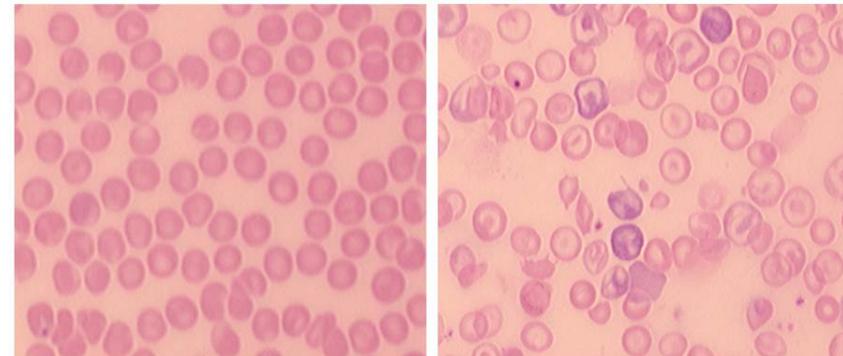
### Therapeutic haemoglobin synthesis in $\beta$ -thalassaemic mice expressing lentivirus-encoded human $\beta$ -globin

Chad May\*<sup>†‡</sup>, Stefano Rivella<sup>+</sup>, John Callegari<sup>+</sup>, Glenn Heller<sup>§</sup>,  
Karen M. L. Gaensler<sup>||</sup>, Lucio Luzzatto<sup>\*¶</sup> & Michel Sadelain<sup>\*†‡¶#</sup>

\* Department of Human Genetics, <sup>†</sup> Immunology Program, and Departments of  
<sup>§</sup> Epidemiology and Biostatistics, <sup>¶</sup> Medicine and <sup>#</sup> Pediatrics,  
Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA

<sup>‡</sup> Weill Graduate School of Medical Sciences, Cornell University, New York,  
New York 10021, USA

<sup>||</sup> Department of Medicine, University of California, San Francisco,  
California 94143, USA



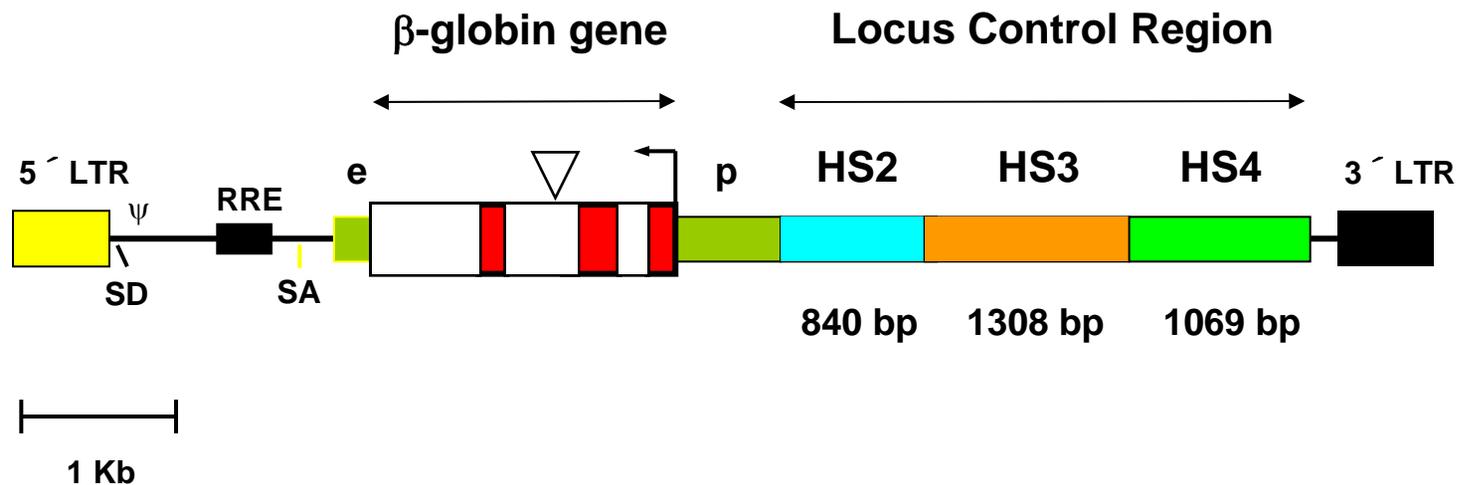
**$Hbb^{th3/+}$  TNS9**

**$Hbb^{th3/+}$  TNS9**

# EFFICACY in animal models

- correction of anemia
- prevention of secondary organ damage
- long-term expression
- peripheral selective advantage
- vector copy number-dependent expression

## Structure of the TNS9 Lentiviral Vector:

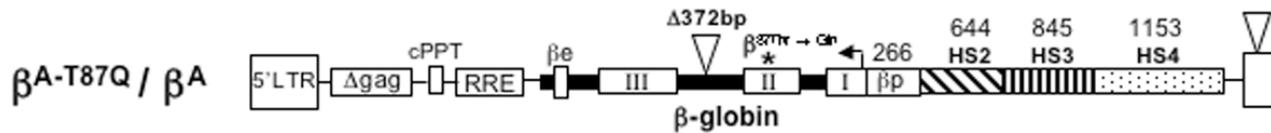


# Published $\beta$ - and $\gamma$ -globin lentiviral vectors and their expression levels



**4.0g/dL/VC**

(May *et al.*, Nature, 2000)

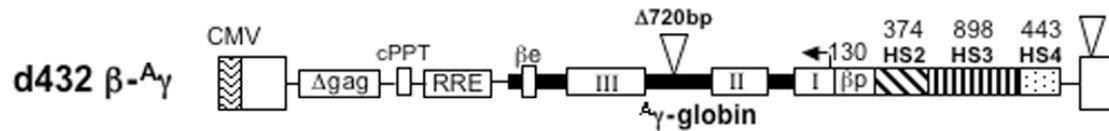


**1.9g/dL/VC**

(Pawliuk *et al.*, Science, 2001)

**1.5g/dL/VC**

(Imrem *et al.*, PNAS, 2002)



**1.1g/dL/VC**

(Persons *et al.*, Blood, 2003)



**2.0g/dL/VC**

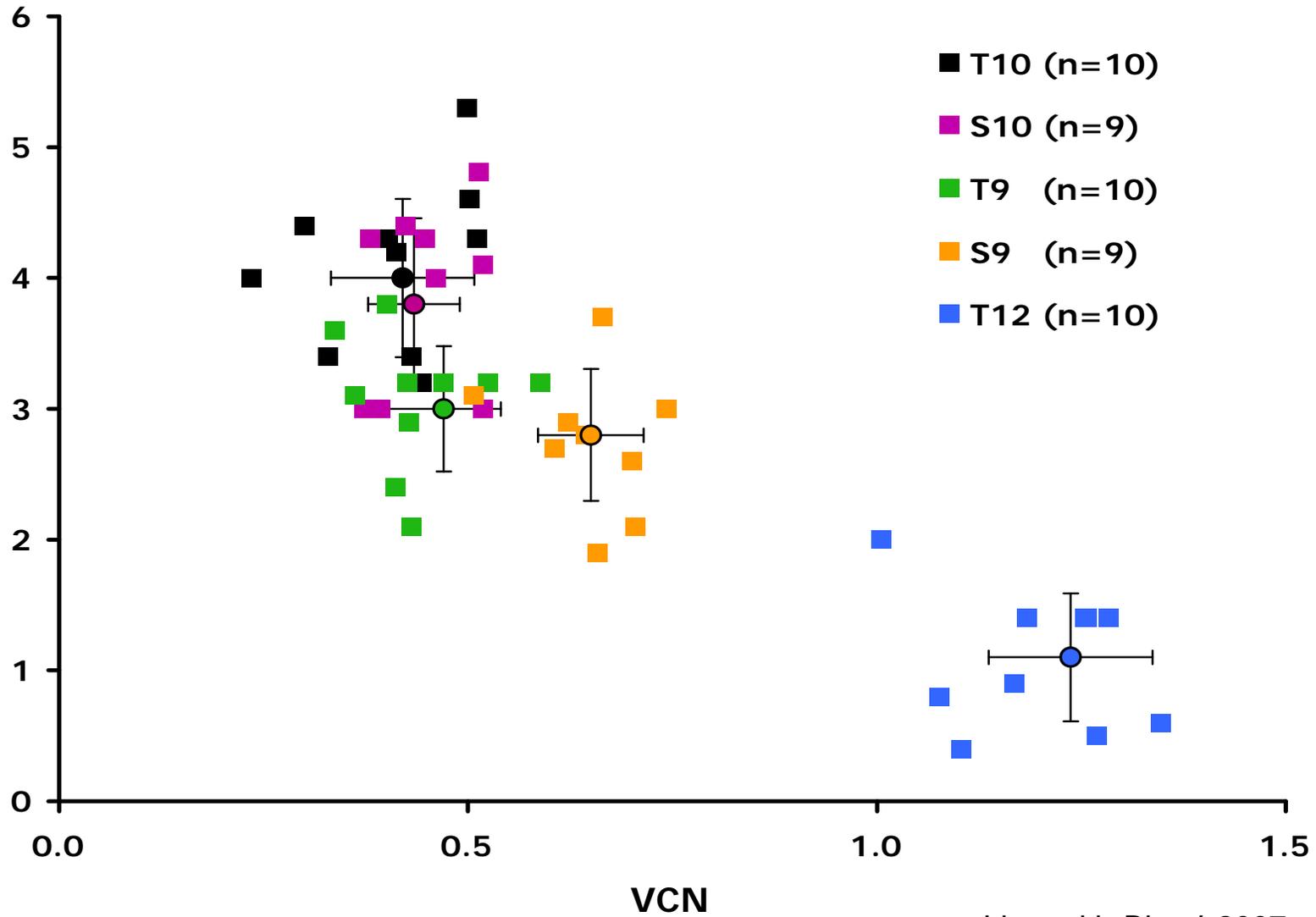
(Hanawa *et al.*, Blood, 2004)



**2.1g/dL/VC**

(Levasseur *et al.*, Blood, 2003)

# Comparative evaluation of different globin lentiviral vectors in $Hbb^{th3/+}$ thalassemic mice



Lisowski, *Blood*, 2007

# MSKCC trial to investigate globin gene transfer

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A phase I open-label clinical trial for the treatment of  $\beta$ -thalassemia major with autologous CD34+ hematopoietic progenitor cells transduced with a lentiviral vector encoding the normal human  $\beta$ -globin gene. PI: Dr. Farid Boulad, Memorial Sloan-Kettering Cancer Center, New York, NY.

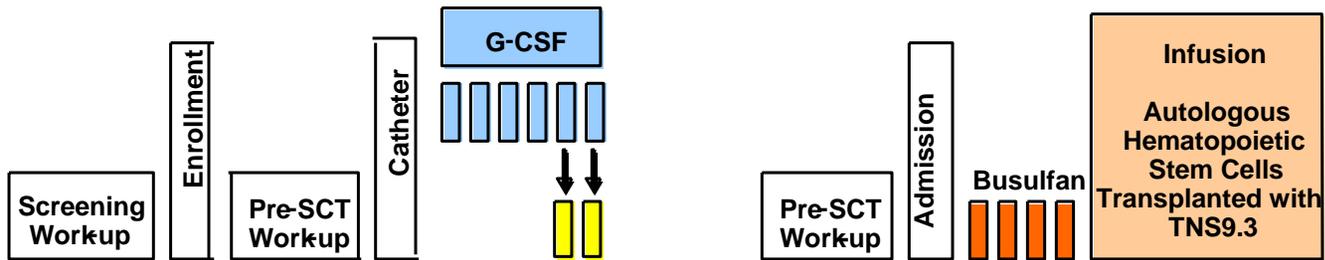
We will transduce **the normal human  $\beta$ -globin gene**, using the **TNS9.3.55 vector**, in **G-CSF-mobilized CD34+ cells**, from patients with  $\beta$ -thalassemia major **age 15 and above**, who **lack a matched donor** (extended HLA typing), following **reduced intensity conditioning (8 mg/kg Bu)**.

To open first at **MSKCC** (PI: Farid Boulad), then **NHLBI** (PI: John Tisdale) and **U.Washington/G. Papanicolaou Hospital** (PI: Lila Yannaki).

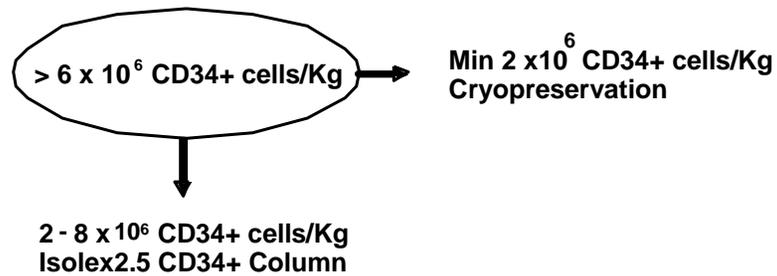
RAC review June 20, 2007; IND submission pending.

# OVERALL TREATMENT PLAN

## Patient Clinical Care



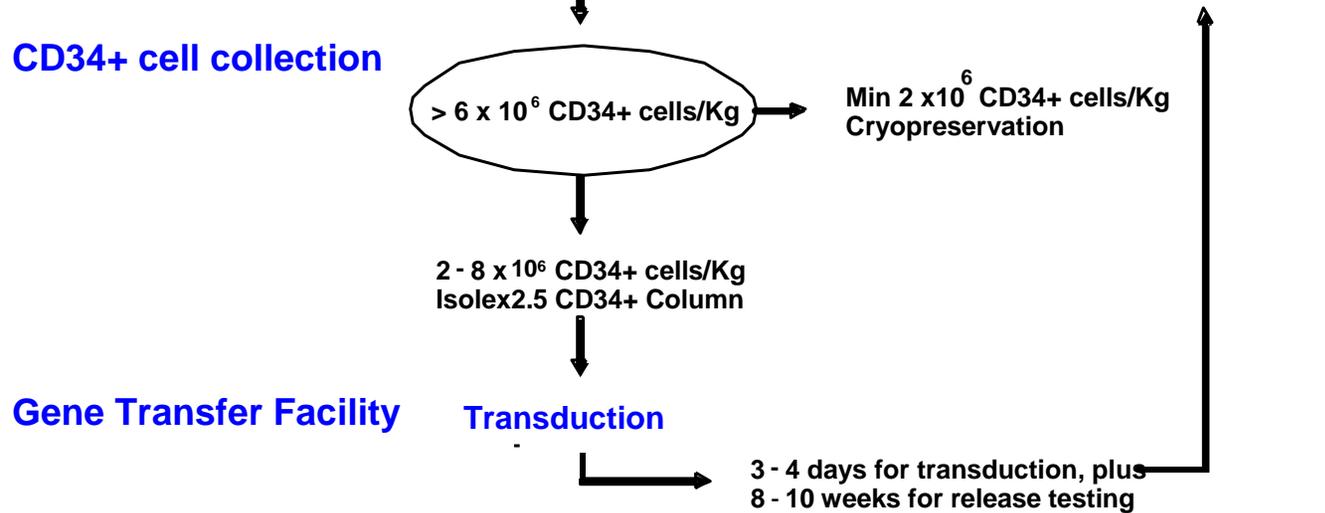
## CD34+ cell collection



## Gene Transfer Facility

### Transduction

3 - 4 days for transduction, plus  
8 - 10 weeks for release testing



## Safe and effective G-CSF mobilization in adults with $\beta$ -thalassemia major

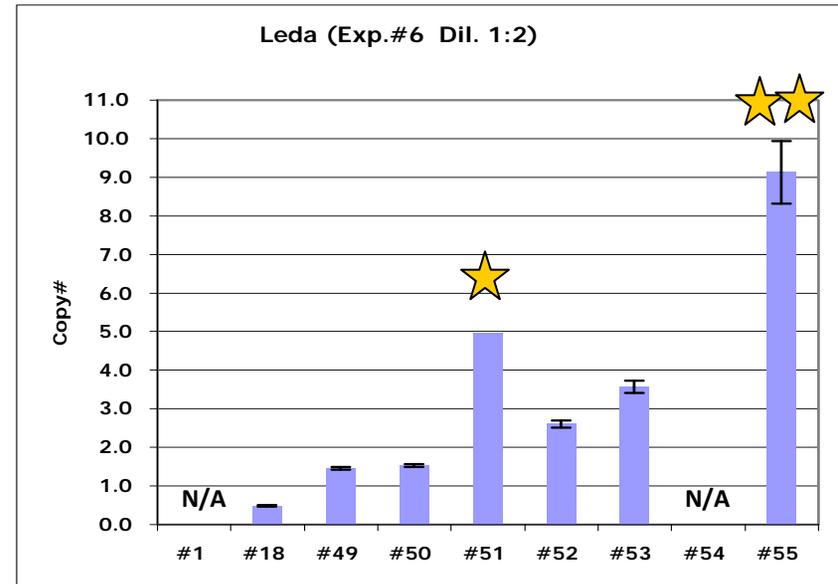
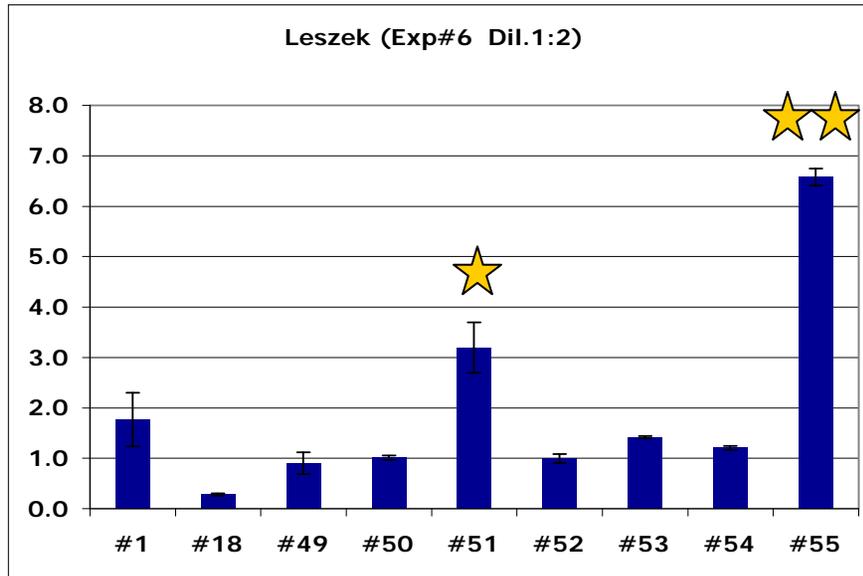
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		+1	+2	+3	+4	+5	+6
Pt #1	WBC	9.6	42.8	56.9	NA	61.4	65.2
	ANC	6.9	38.7	52.3	NA	52.7	55.4
	Total CD34					3.03 x 10 <sup>6</sup>	4.95 x 10 <sup>6</sup>
	HGB	10.3	9.5	9.9	NA	9.8	9.2
Pt #2	WBC	3.5	18.8	19.7	24.9	44.6	46.5
	ANC	1.8	13.7	17.2	20.9	40.1	43.3
	%CD34					0.2%	0.1%
	Total CD34					6.4 x 10 <sup>6</sup>	5.6 x 10 <sup>6</sup>
	HGB	11.3	11.6	11.0	11.1	10.8	10.3
Pt #3 splectomized	WBC	10.1	60.1	69.4	66.0	74.6	73.8
	ANC	5.9	53.5	65.0	57.8	59.8	56.7
	Total CD34					4 x 10 <sup>6</sup>	4.5 x 10 <sup>6</sup>
	HGB	11.9	11.3	11.1	11.0	11.3	10.6

QuickTime™ and a  
Photo - JPEG decompressor  
are needed to see this picture.

GMP vector production at MSKCC, New York, NY

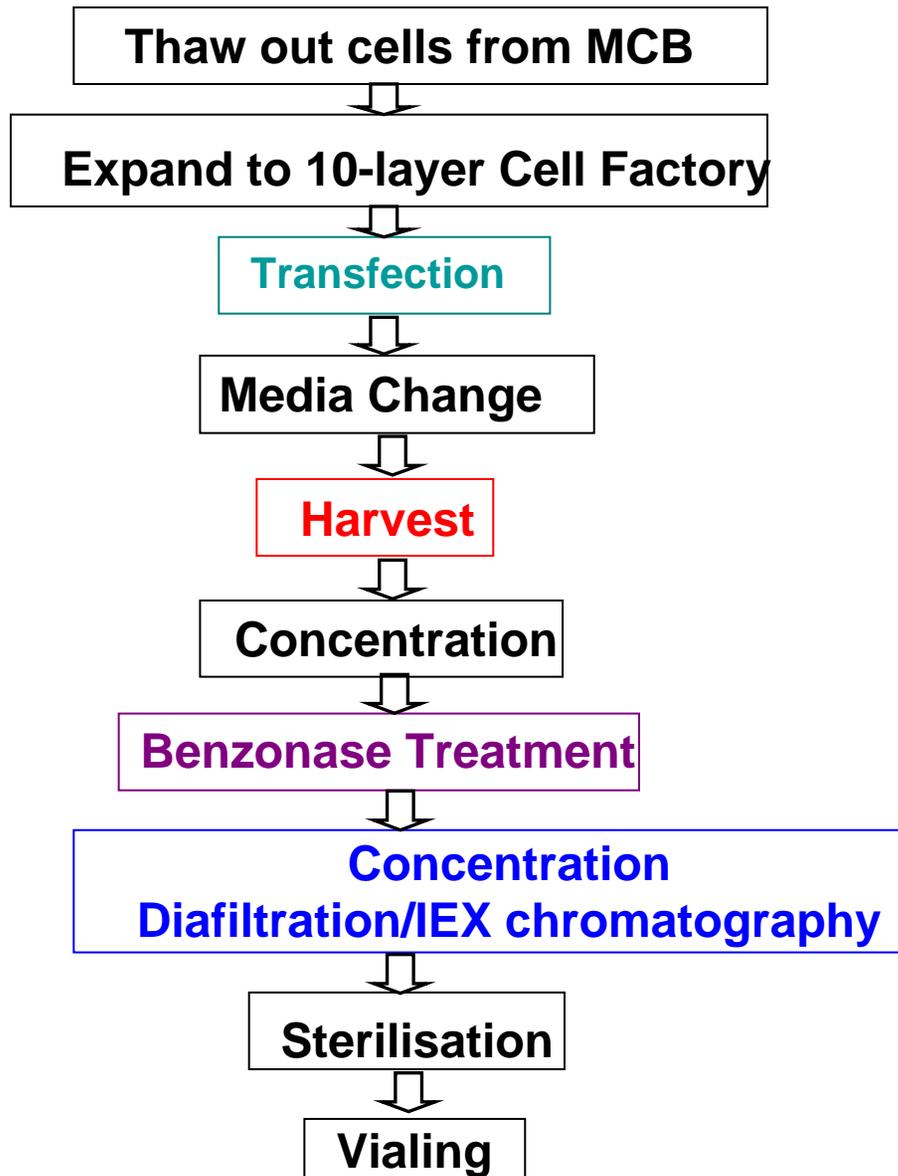
# From TNS9.3 to TNS9.3.55



Construct	copy#	fold increase vs #18
#1	1.766	6.2
#18	0.283	1.0
#49	0.903	3.2
#50	1.012	3.6
#51	3.192	11.3
#52	0.993	3.5
#53	1.420	5.0
#54	1.205	4.3
#55	6.579	23.2

Construct	copy#	fold increase vs #18
#1	n/a	n/a
#18	0.485	1.0
#49	1.460	3.0
#50	1.524	3.1
#51	4.965	10.2
#52	2.606	5.4
#53	3.569	7.4
#54	0.000	0.0
#55	9.129	18.8

# Large Scale Lentiviral Production Flow Chart



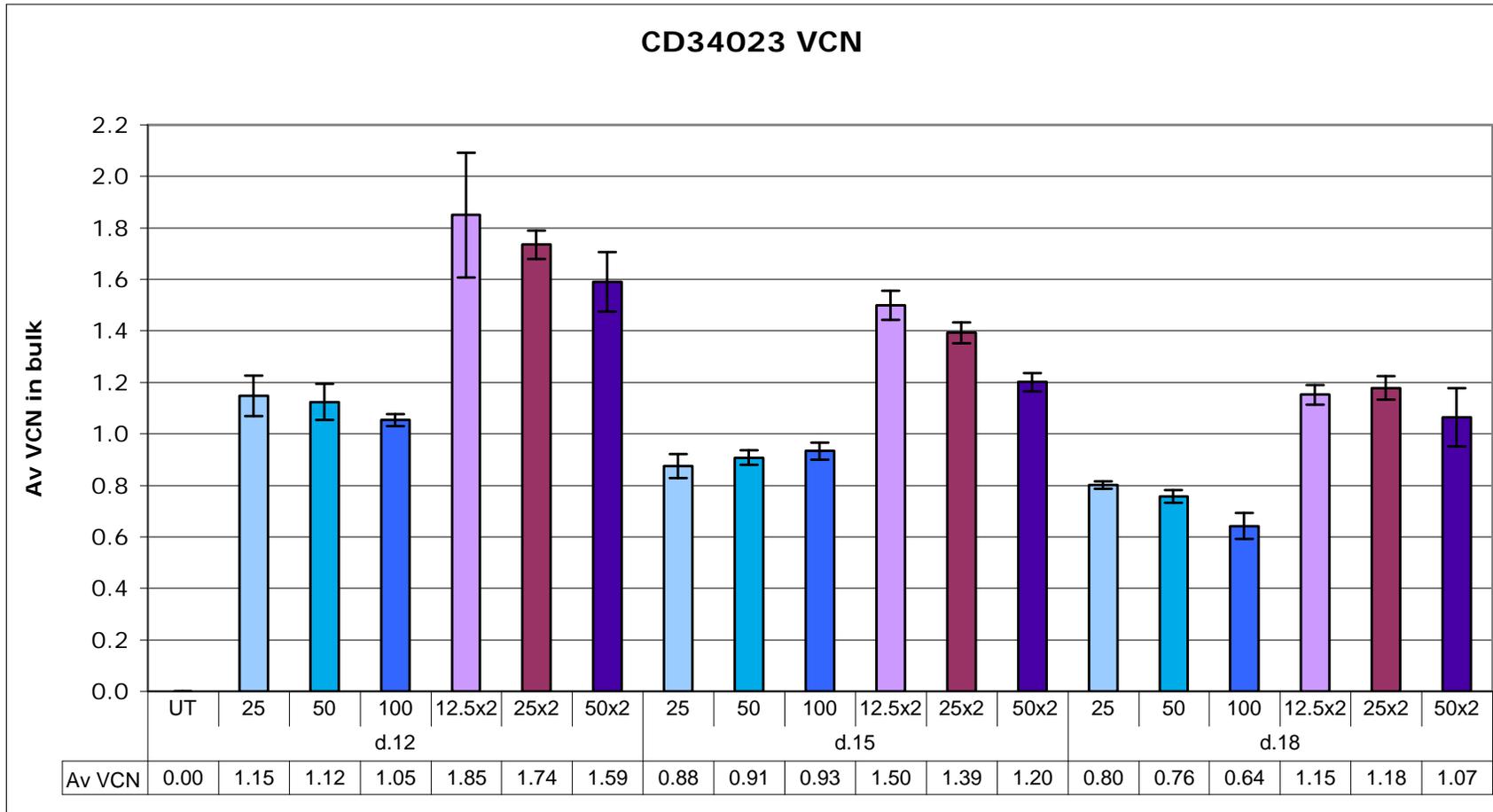
10 to 25 liter

Increasing Purity

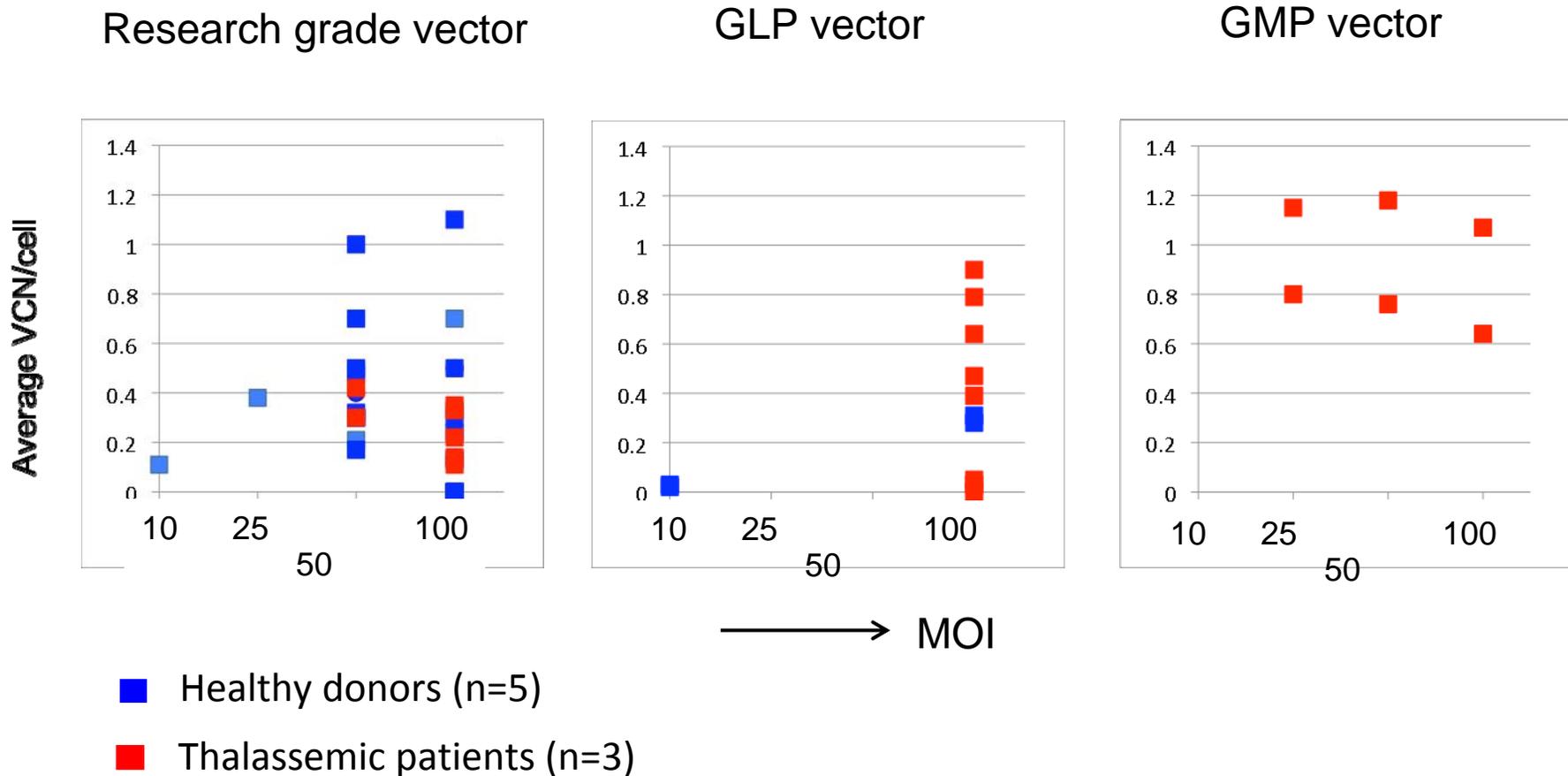


50-150 ml

# Vector copy number (VCN) in bulk CD34+ cells

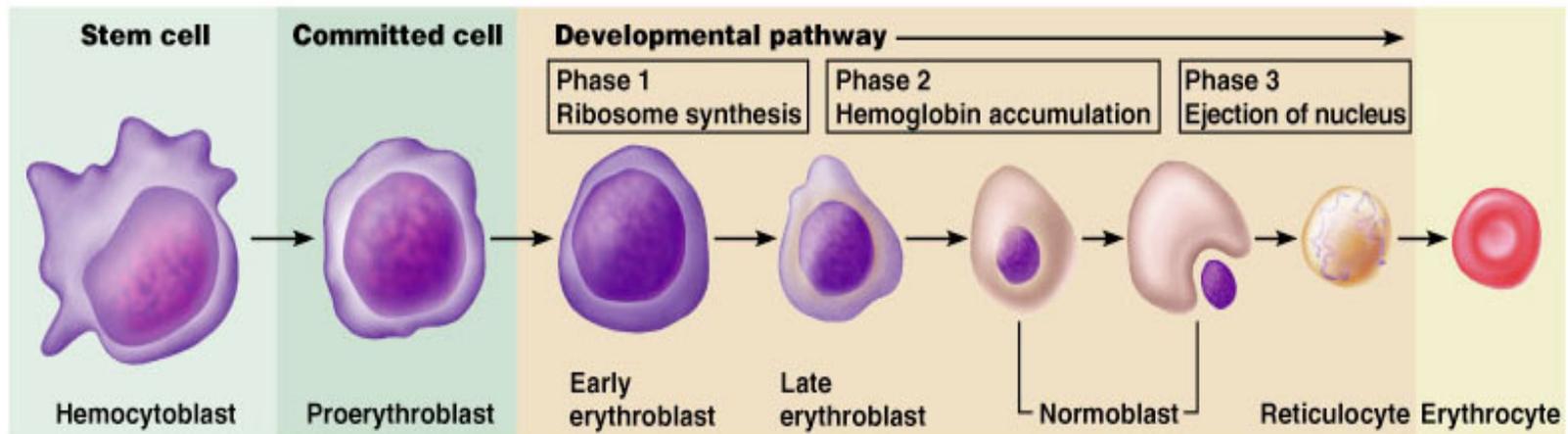


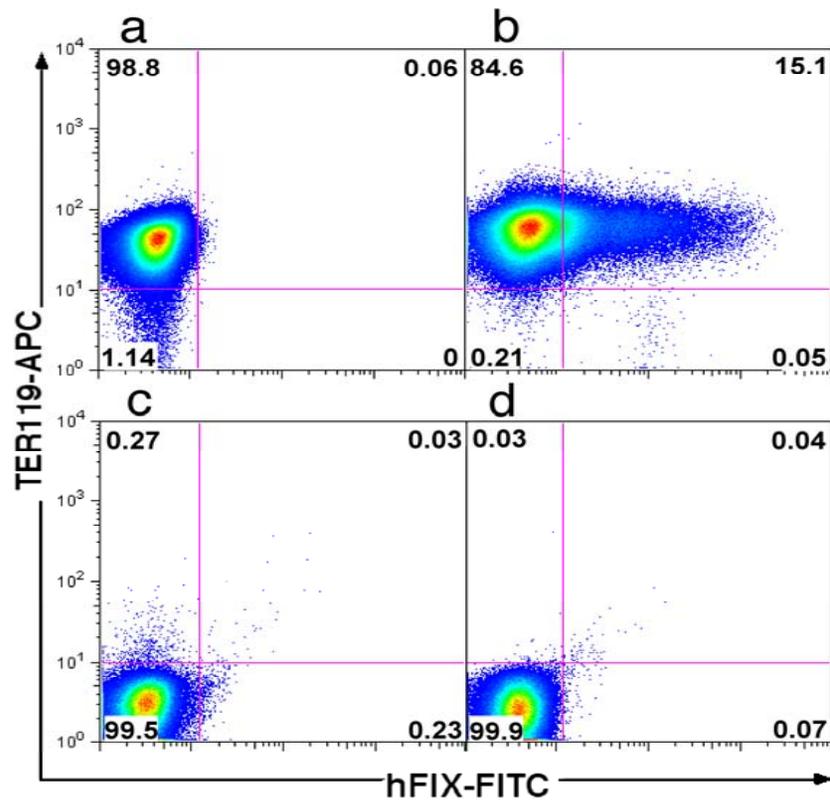
# Effective gene transfer in thalassemic patient CD34+ cells



# Targeting gene expression to late-stage erythropoiesis

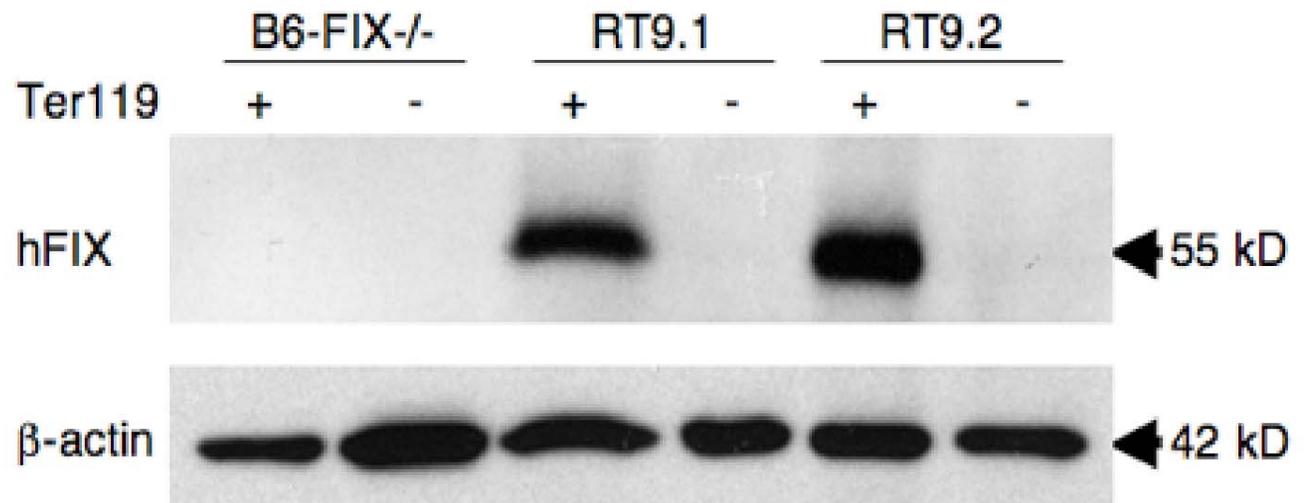
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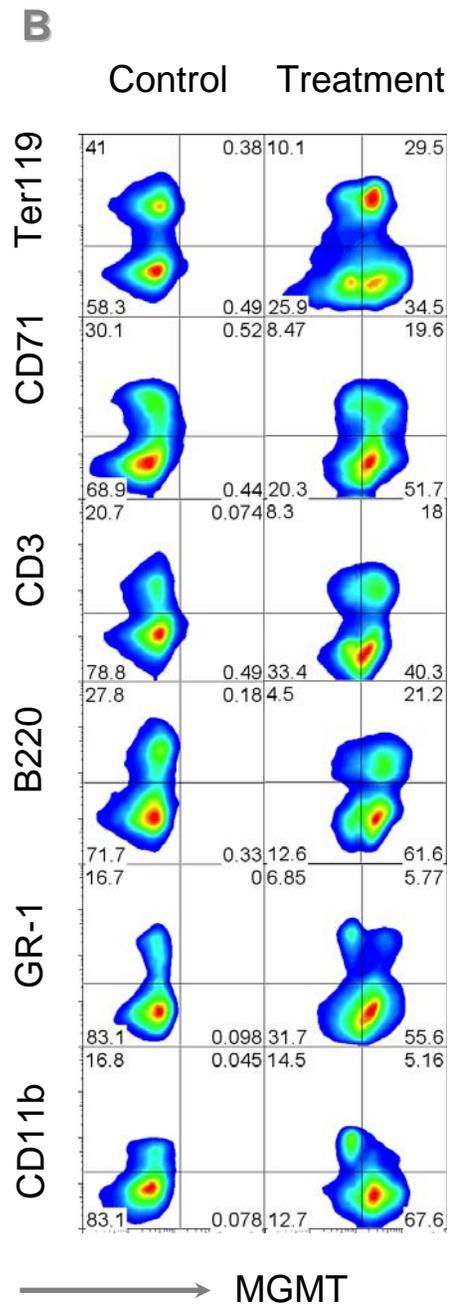
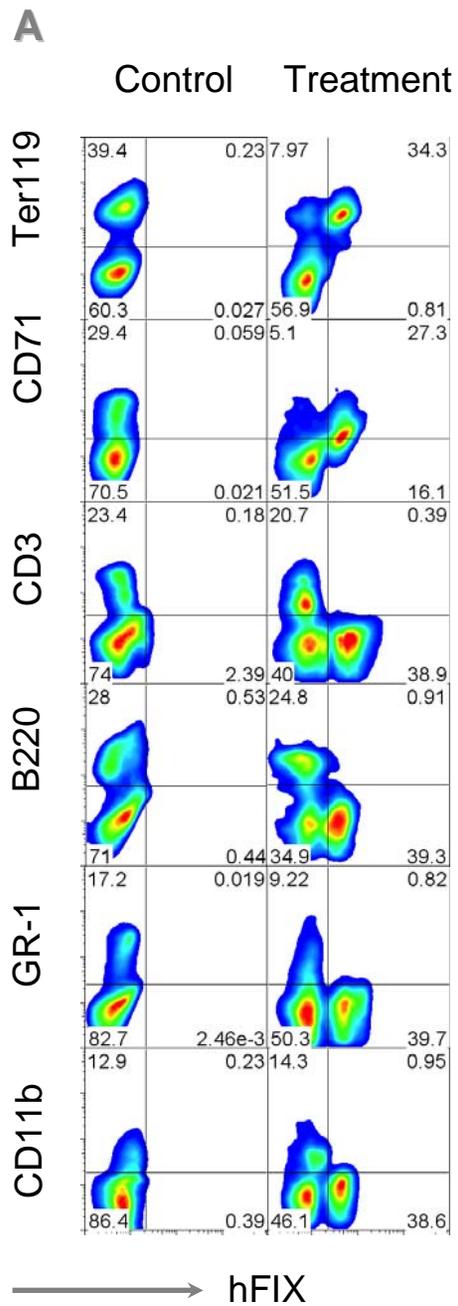


**In vivo hFIX expression is erythroid-specific**

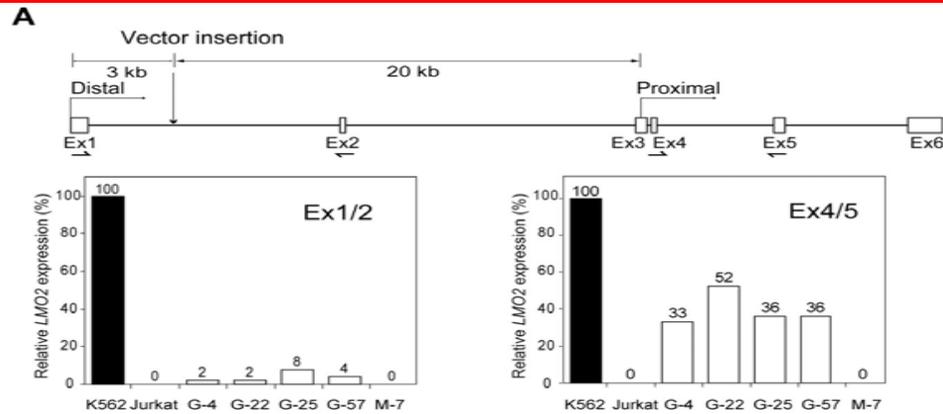
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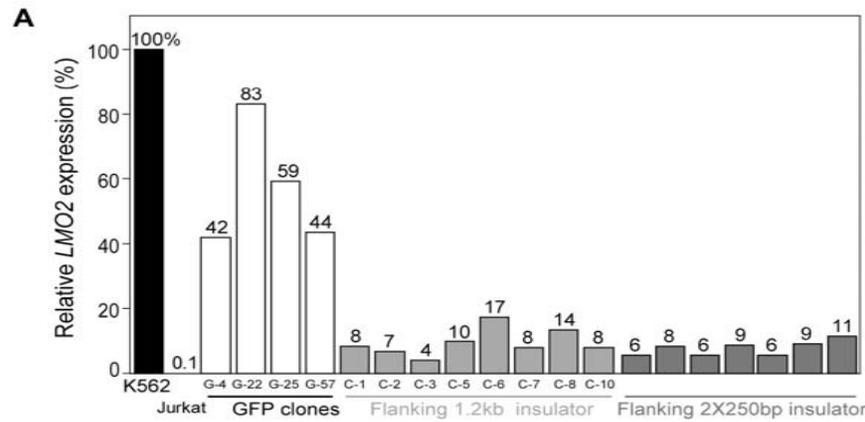
Chang et al, *Nat Biotechnol*, 2006



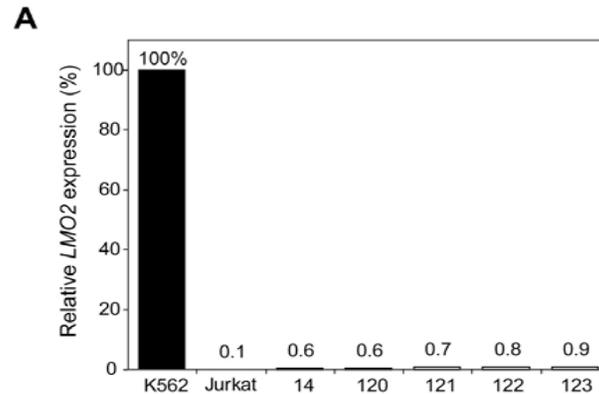
# Ryu, et al., An Experimental System for the Evaluation of Retroviral Vector Design to Diminish the Risk for Proto-oncogene Activation. *Blood*, 2008



**Figure 3. Activation of the distal and proximal *LMO2* promoters by the LTR.** (A) The origin of the 2 transcripts from human *LMO2*, both of which encode the same protein, is indicated along with the relative distances of the retroviral insertion site from the 2 transcriptional start sites. The relative *LMO2* expression from the 2 promoters was established in several clones using TaqMan Gene Expression Assays. The location of each primer pair is indicated on the diagram. Results are expressed as the percentage of the K562 cell control. (B) Northern blot analysis of RNA from 2 clones having an LTR-GFP insertion (G-22 and G-25) and positive control (K562) and negative control (Jurkat) cells was performed. The transcript from the proximal promoter was more abundant than that from the distal promoter in both clones, consistent with the results obtained with the qRT-PCR analysis in panel A.



**Figure 5. Attenuation of *LMO2* activation by a chromatin insulator (cHS4).** (A) The R-6 clone was transduced with scAAV-Cre and transfected with a plasmid containing the LTR-GFP cassette flanked by 1.2-kb cHS4 or 2 × 250-bp core insulator fragments, and single cell clones were identified in which cassette exchange had occurred. The relative *LMO2* expression in those clones was compared by qRT-PCR. Both flanking insulators reduced *LMO2* expression 3- to 15-fold over that observed in clones having the noninsulated LTR-GFP cassette. (B) Western blot confirmed reduction of *LMO2* protein expression by flanking insulator fragments in 6 clones in which the 1.2-kb cHS4 insulator flanked the LTR-GFP cassette relative to the G-25 and R-6 clones. Jurkat cells served as a negative control. (C) The relative GFP expression in clones with or without insulator elements flanking the LTR-GFP cassette was compared by FACS each week for 4 weeks. The average MFI of GFP expression in each clone set is represented by a horizontal bar. LTR-GFP cassette (○), LTR-GFP cassette flanked by a 1.2-kb insulator fragment (●), LTR-GFP cassette flanked by 2 × 250-bp core insulator fragments (■).



**Figure 6. *LMO2* is not activated by globin regulatory elements.** A cassette containing the GFP coding sequences under the control of  $\beta$ -globin gene promoter along with the human  $\beta$ -globin LCR was used in the cassette exchange reaction in R-6 cells. Five clones were confirmed as having undergone the predicted exchange. Activation of the *LMO2* gene in all 5 clones was minimal, if any, as determined by qRT-PCR (A) or Western blot analysis (B).

Arumugan, et al., Genotoxic Potential of Lineage-specific Lentivirus Vectors Carrying the  $\beta$ -Globin Locus Control Region. *Molecular Therapy*, 2009

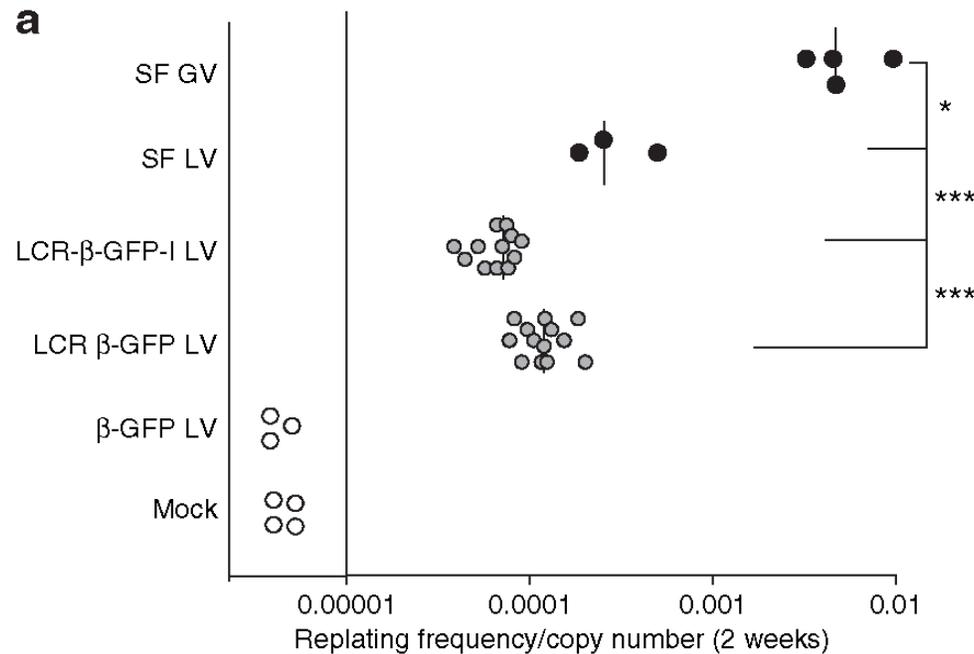
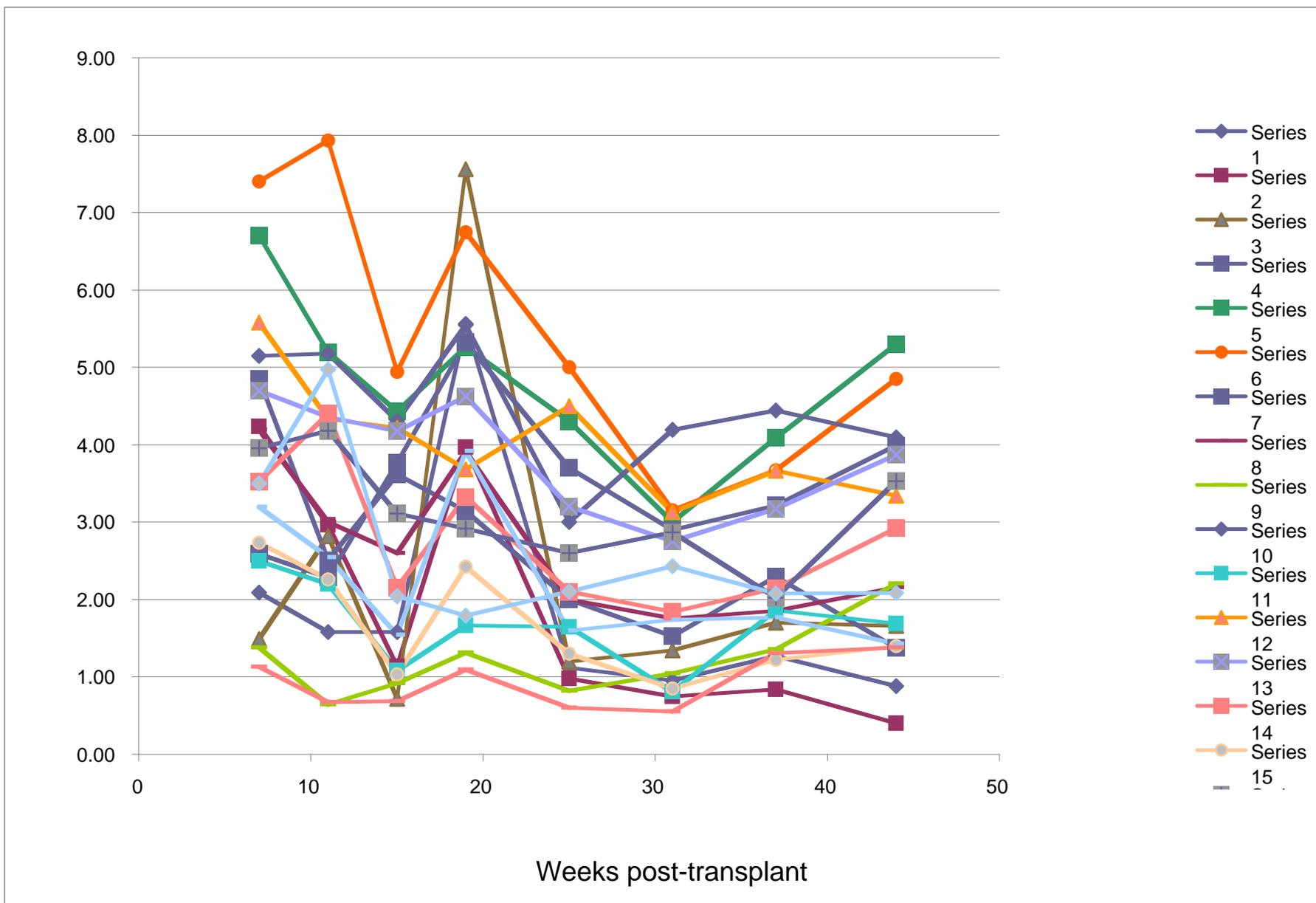


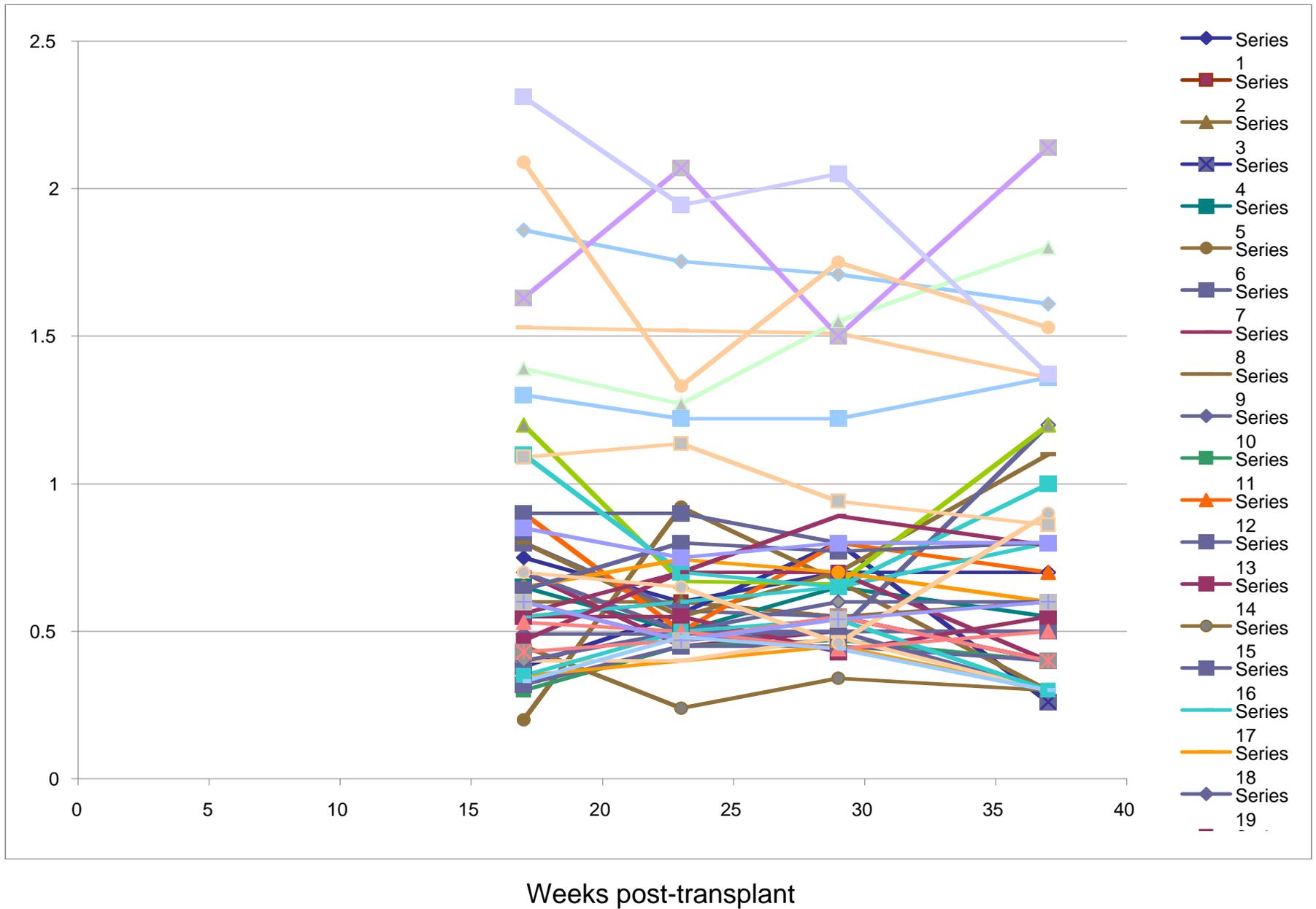
Figure 1 Frequency of generation of IVIM clones from lineage-negative primary hematopoietic cells scored at 2 weeks and at 5 weeks (actual replating frequency). (a) Two-week replating frequency (x axis) per vector copy. The y axis shows a schematic representation of the vectors. The in vitro immortalization (IVIM) clonal frequency of SF GV, a vector driven by the spleen focus forming virus (SFFV) LTR, and SF LV, carrying an internal SFFV promoter/enhancer was compared to LV carrying the lineage specific  $\beta$ -globin promoter and LCR enhancer (LCR- $\beta$ -GFP). The replating frequency of three LCR- $\beta$ -GFP insulated LV carrying different cHS4 insulator fragments in the U3 deletion are combined and plotted. An enhancer-less vector, carrying only the  $\beta$ -promoter and mock transductions were negative controls. All vectors were compared with the SF GV (SF91.eGFP.pre) for their genotoxic potential using Student's "t"-test (unpaired and two tailed). Open circles represent the replating frequency/copy number from independent transductions. Open circles below the horizontal line indicate independent transductions, which did not give rise to any replating clones. Replating frequency was normalized for the mean copy number in the pooled cell population prior to replating. Median is indicated by the black line. P-values are \*P < 0.05, \*\*\*P < 0.001 indicated in the graph.

# Peripheral blood VCN - Cohort 3 (n = 15)

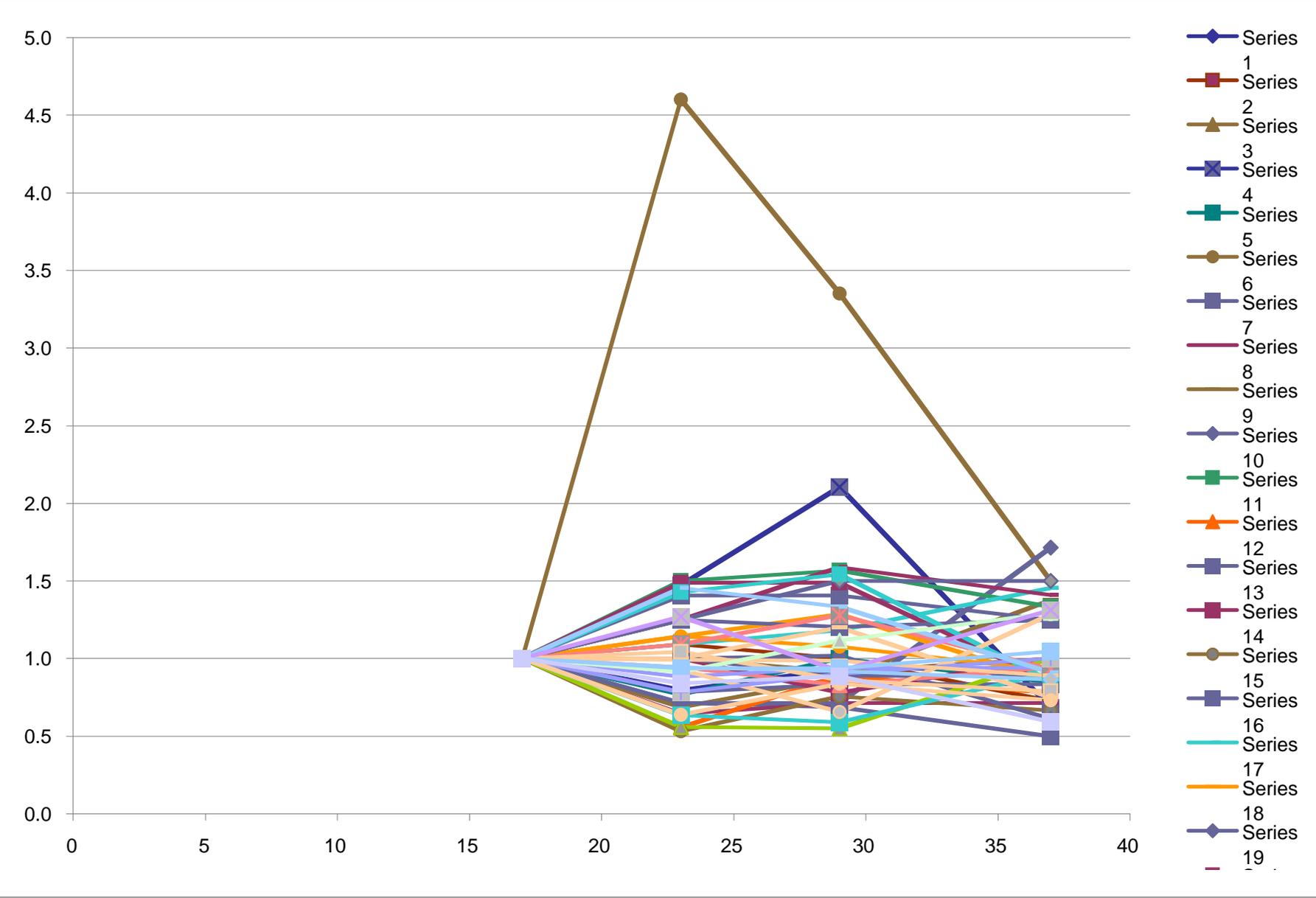


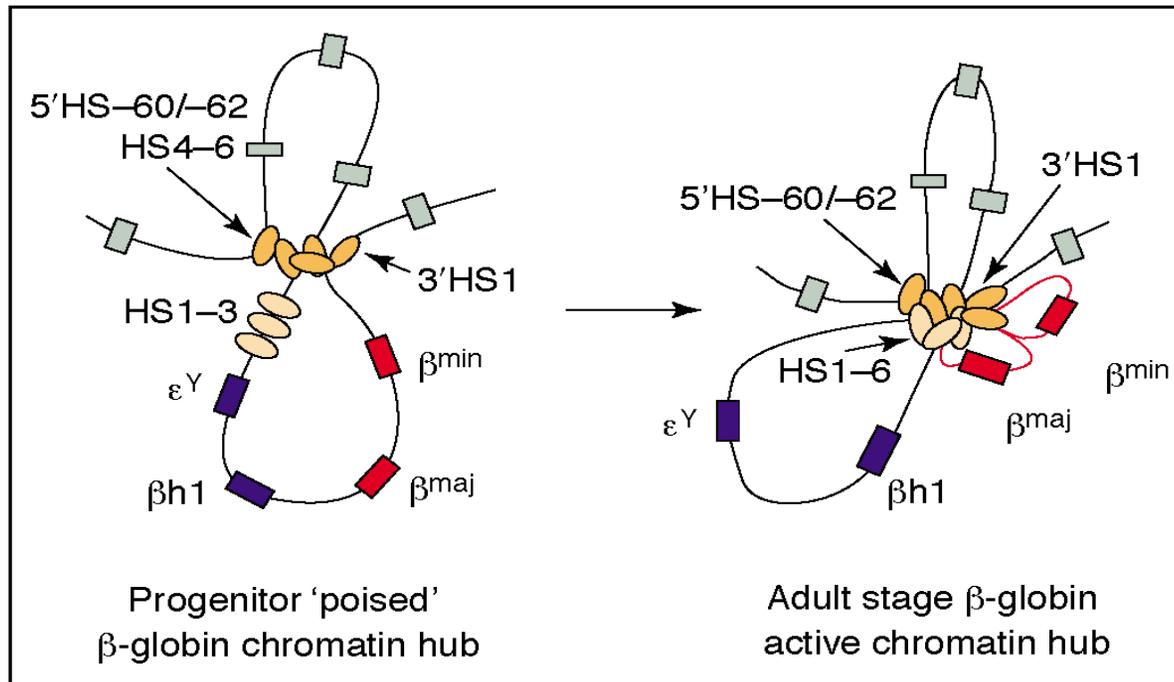


# Peripheral blood VCN - Cohort 4 (n=19)



Peripheral blood VCN / normalized - Cohort 4 (n=19)





**Figure 3.** Intra-chromosomal looping in the mouse  $\beta$ -globin locus. In erythroid progenitors, a poised chromatin hub forms consisting of the locus flanking  $3'HS1$ ,  $5'HS-60/-62$  and LCR  $HS4-6$  (dark orange ovals). An active chromatin hub (ACH) at the mouse  $\beta$ -globin locus in fetal liver cells, which have an adult pattern of globin gene expression, incorporates the remaining LCR  $HS1-3$  (light orange ovals) into the active chromatin hub (ACH). The globin genes join the ACH when they are being actively transcribed, leaving the inactive genes looped away. Adapted, with permission, from Ref. [27].

Ann Dean, On A Chromosome far, far away: LCRs and Gene Expression. *Trends in Genetics*, 2006.