

A Phase I/II Pilot Study of Gene Transfer for Sickle Cell Disease



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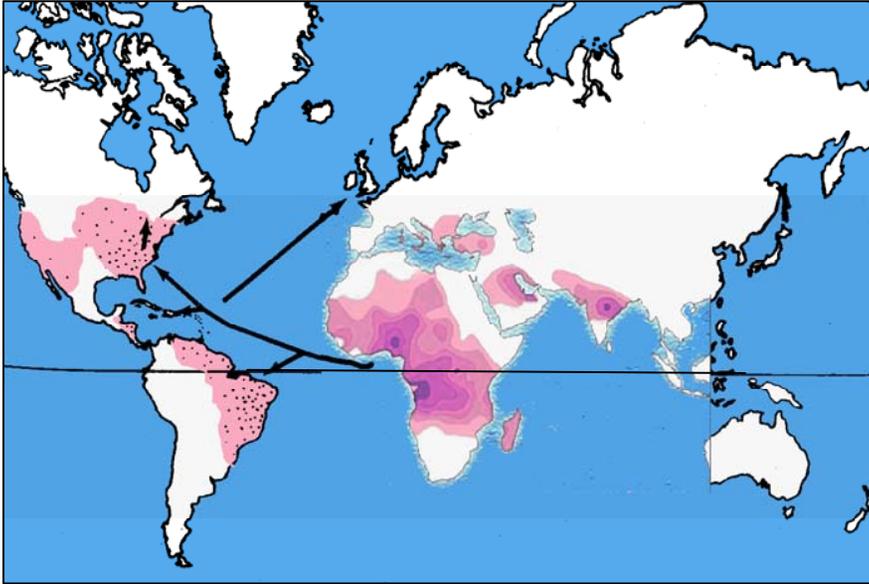
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Focus for Discussion

- *Comparative merits of hydroxyurea. It's risk: benefit ratio in children*
- *Severe disease in young children and if the same eligibility criteria will apply*
- *Eligible sickle cell patients in Cincinnati, community interest in gene transfer study*
- *Rationale for eligibility criteria*
 - *Exclusion of established pulmonary hypertension*
 - *Exclusion of adults with the most severe disease,*
 - *Patients on chronic transfusions,*
 - *Stroke*
- *Vector used to deliver γ -globin and its efficacy*
- *Thresholds for correction of sickle phenotype*
- *Expression of the transgene in human sickle marrow*
- *Lineage specificity of the lentivirus vector*
- *Safety of erythroid lineage-specific lentivirus vectors*
- *Clonal dominance seen in a thalassemia patient in France and its implications in the current trial*
- *Trial monitoring parameters: Transfusion withdrawal, distinction of transgene induced F retics and F RBC from endogenous upregulation*

Sickle Cell Disease



Graham R. Serjeant. 1985. Sickle Cell Disease (modified)

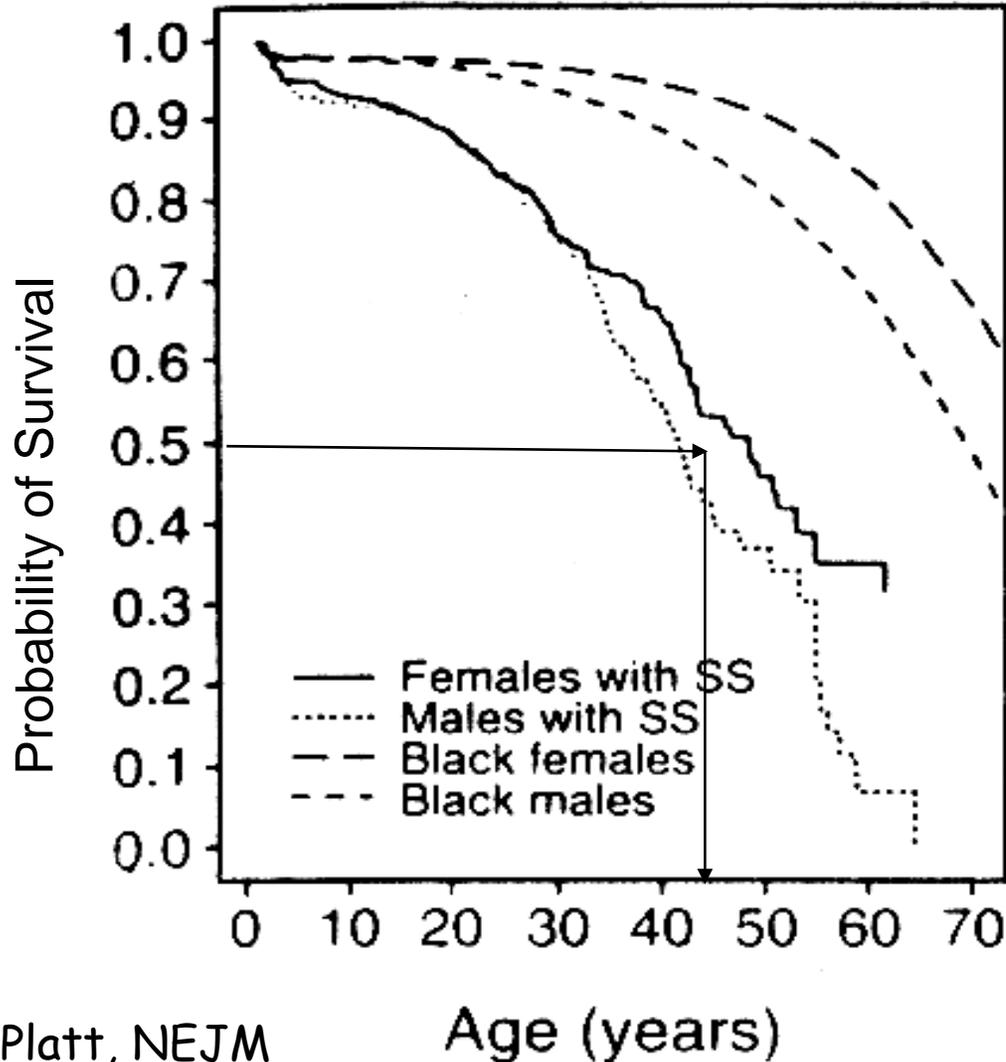
- A point mutation in β -globin gene resulting in a 'sickle hemoglobin'.
- Millions afflicted worldwide.
- 80,000 Americans with SCD.
- 2,000 affected infants born in the United States every year.
- >\$1.1 B in annual healthcare costs in the US alone.
- Better therapies are needed for this disease

- 20-25 infants born in Cincinnati per year
- 270 children with SCD followed at Cincinnati Children's Hospital Medical Center
- A total of 440 adults and children followed at CCHMC/U. of Cincinnati Hospital
- 430 adults and children with SCD followed in the Ohio Sickle Cell Alliance for Research

Differences in Clinical Manifestations of SCD

	Children	Adults
Pain	acute	acute and chronic
Infection	more	less
Splenic Sequestration	more	less
Aplastic crisis	more	less
Stroke	infarctive	hemorrhagic
Acute Chest	more	less
Orthopedic	less	more
Pulmonary hypertension	less*	more
Iron overload	begins	end stage
Renal failure	less	more
Heart failure	less	more
Reproductive problems	less	more
Medical coverage	more	less
Mortality	~ 5%	~ 35%

Life Expectancy and Causes of Death in Sickle Cell Disease



Platt, NEJM

- Chest syndrome
- Pulm hypertension
- Sudden Death
- Stroke
- Vaso-occlusion - acute multi-organ failure.
- Renal Failure
- Peri-operative
- Iron overload
- Infections

Current Therapeutic Approaches

Prevention of infections

Relief of symptoms

Hydroxyurea (increase Hb F)

Chronic Transfusions
(preventing primary and secondary stroke)

HSC Transplant (curative)

The Risk: Benefit Ratio of Hydroxyurea in Children

Benefits

Outcome	Studies, <i>n</i>	Magnitude and Consistency of Effect	Evidence Grade
HbF%	17	93%–366% increase	High
Hemoglobin	16	5%–20% increase	High
Pain crises	5	Significant reductions in 3, no difference in 1, no baseline data in 1	Moderate
Hospitalization	5	56%–87% decline in yearly rate	High
Transfusions	3	Decreased in 3 small studies	Insufficient
Neurologic events	3	Comparable stroke rates as on chronic transfusion, stable brain images	Low
Splenic function	3	Improved in 14%–45% of children by scintigraphy; no change in pitted red cells in 1 study	Low

The Risk: Benefit Ratio of Hydroxyurea in Children

Toxicities

Short Term

Neutropenia

Thrombocytopenia

Anemia

Skin/Nail changes

Headaches,

Elevations in ALT, creatinine

Long-term Risks

~15 years of experience in adults, ~12 years of experience in children shows this therapy is relatively safe

- Azoospermia and oligozoospermia, directly related to duration of therapy
- Reports of increased DNA mutagenicity (VDJ, HPRT)
- Potential teratogen in mice – no reports in humans as of now. Anecdotes of normal births, but patients on HU are on contraception.
- Hypothetical risk of cancer in patients with myeloproliferative syndromes

Hydroxyurea is the preferred approved therapy for SCD.

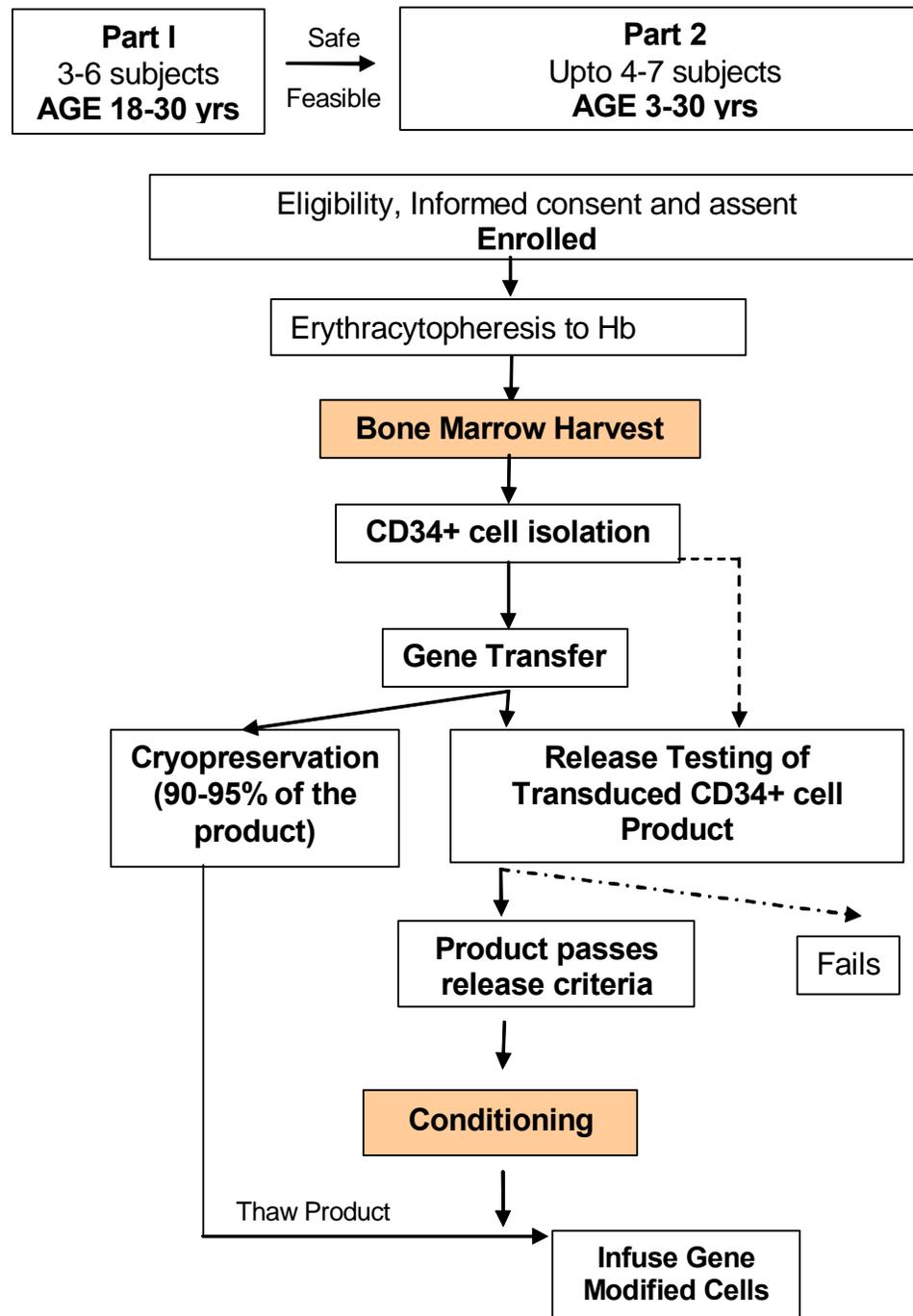
We do not plan to enroll those individuals who are currently tolerating hydroxyurea and have stable disease.

The Cincinnati Sickle Cell Center invests significant effort into ensuring compliance in our pediatric population.

We would offer gene transfer only to those:
that have been treated with hydroxyurea and have not tolerated it
those who have not responded to hydroxyurea
those that, as an adult, have chosen not to take hydroxyurea.

Patients enrolling into this study will be informed of the importance of compliance in following through with all safety assessments in the gene therapy study

Study Design



Eligible Subjects

The Ohio Sickle Cell Alliance for Research (OSCAR)

Consists of 4 high quality comprehensive sickle cell programs located in close proximity of each other with the following currently followed patients with SCD

	SS	SC	S-β thalassemia	OTHER	TOTAL
Cincinnati Children's	154	73	32	13	272
U.Cincinnati Hospital	109	36	15	8	168
Ohio State University	117	45	14	4	180
Columbus Children's	140	72	20	13	245
TOTAL	520	226	81	38	865

We anticipate approximately **175- 200** patients to be eligible for this study in OSCAR

We have an outreach to Dayton Children's Hospital and University of Louisville Sickle Cell Centers that follow approximately 150 patients each.

Therefore, we do not anticipate problems with having adequate # of eligible patients

Eligible Patients in the Cincinnati Area

99 of 310 patients with Hb SS and S β -thalassemia are eligible from Cincinnati Children's and University of Cincinnati Hospital

41 adults, 18-30 years of age without history of stroke or abnormal TCD and not on hydroxyurea (refusal, unable to tolerate or non-responders), but with frequent vaso-occlusive crises, and/or recurrent acute chest syndrome are potentially eligible

- 21 adult patients followed at Cincinnati Children's

- 20 adult patients followed at University Hospital in Cincinnati

58 children, 3-17 years of age meet the eligibility criteria (causes are recurrent severe acute chest syndromes, multiple admissions for acute vaso-occlusions, failure to thrive/perform routine activities from severe disease symptoms/symptomatic anemia that are not on hydroxyurea due to reasons listed above or on chronic transfusions for stroke)

CINCINNATI CHILDREN'S HOSPITAL PATIENTS ON CHRONIC TRANSFUSIONS

A total of 37 patients followed at the Cincinnati Children's Hospital are on chronic transfusion therapy.

- 16 for secondary stroke prevention (history of CVA)
- 10 for primary stroke prevention (patients with abnormal TCD)
- **11** for other reasons including frequent pain episodes, recurrent ACS (several with ICU admissions for ACS), FTT, and other reasons.
 - Three of the patients in this group failed hydroxyurea therapy
 - Three patients are younger than 6 years of age.

Patient Interest in Gene Transfer

An Initial Assessment Performed at the Cincinnati Sickle Cell Center
Annual Sickle Cell Research Day, Aug 2009



An event attended by more than 500 patients and families of patients

A talk on gene transfer research followed by a survey questionnaire:

- The majority of patients or parents stated an interest.

- Ones interested

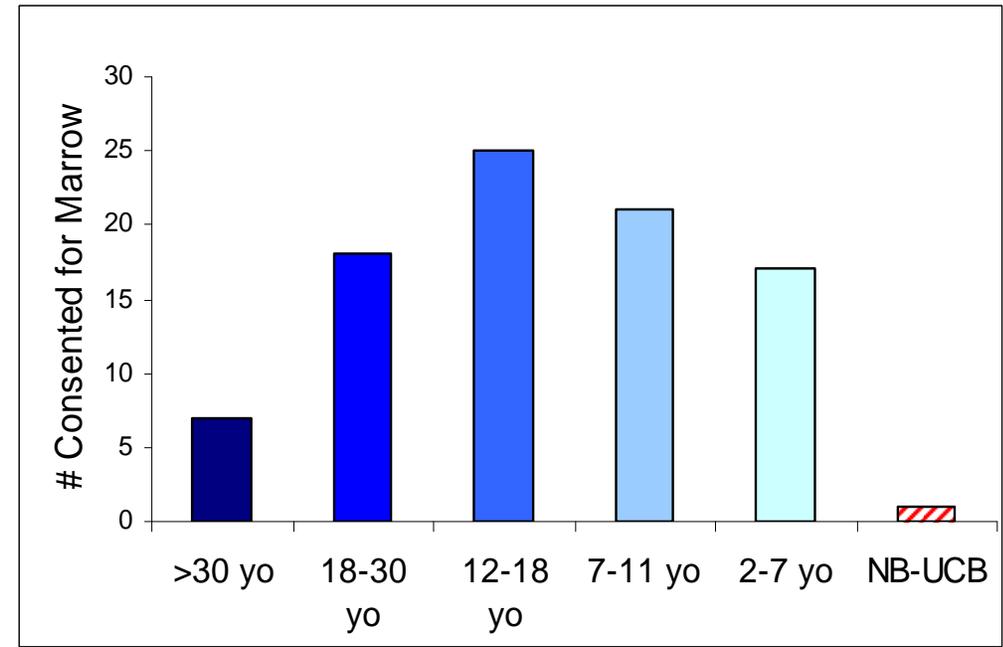
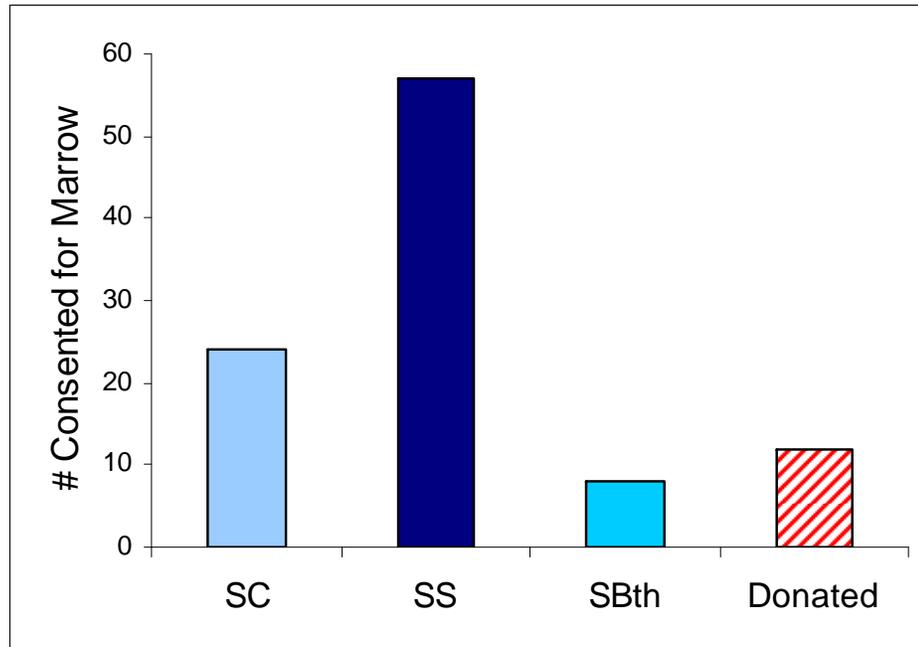
 - wanted to know more about gene transfer therapy

 - volunteered for focus groups

 - signed up to donate bone marrow or blood

- We have plans to have focus groups and education in the near future

89 Patients With SCD at CCHMC+UC have Consented To Donate Bone Marrow For *Gene Transfer Research*



- Adults consent to a bone marrow aspirate donation for research only
- Parents consent to bone marrow aspirate donation when their child undergoes a medically indicated surgical procedure
- One parent with a 2 yo with severe SCD disease had prenatal diagnosis when pregnant, and asked us to collect and store the umbilical cord blood in a GMP-compliant manner for future gene therapy.

Overall Rationale for Initial Inclusion and Exclusion Criteria

- We recognize that this experimental therapy should first be tested in adults with the most severe disease and plan to enroll adults initially.
- We propose establishing the safety and feasibility in carefully chosen adults with severe enough disease to justify an experimental protocol, but not place them at risk of study related mortality.
- The initial three patients will be adults as they can give fully informed consent.
- Bone marrow harvest has not been performed in patients with SCD.
- Our inclusion and exclusion criteria are adapted from a multicenter study on reduced intensity transplants in patients with SCD and discussions with the study PI, Dr. Shenoy. A local pilot was performed by her in 13 patients and based upon the safety and feasibility, the BMT protocol was implemented.

Rationale for Extending the Study to Children

The study will be performed on three adults for safety and feasibility.

Enrollments of adults will continue but eligibility will be extended to children once safety and feasibility are established. Children are the ultimate target of this therapy.

Eligibility criteria will be re-reviewed after the adults are enrolled and before the protocol becomes open to children by the DSMB, FDA, and IRB.

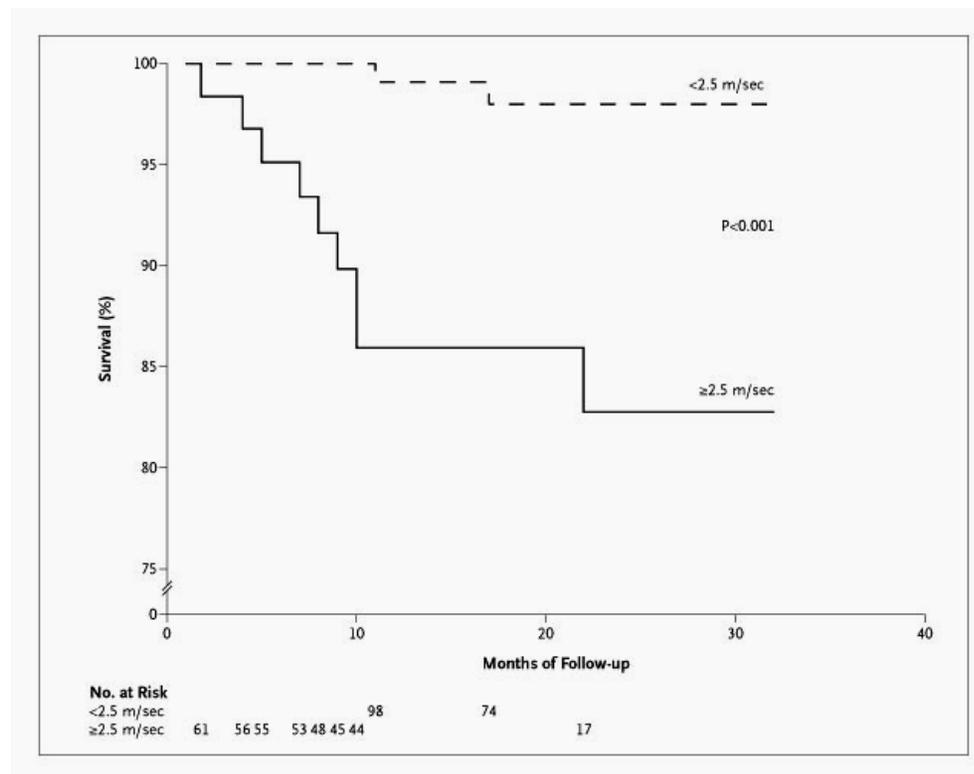
If the safety profile in adults does not provide sufficient evidence for safety and feasibility, we would expect to continue to enroll only adult patients until there is more compelling evidence to allow us to reduce the age for inclusion.

Children would most benefit when organ damage is still reversible. Eligibility criteria may change, based upon the safety and feasibility data in adults

Rationale of inclusion of adults with TR jet velocity >2.5 m/s

TR jet velocity of >2.5 m/s is associated with pulmonary hypertension in adults, and a ten times higher mortality. Mortality is seen largely in patients with TR jet velocities >3 m/s.

TR jet velocity >3 or established pulmonary hypertension would impose significant risk to adults undergoing study procedures and not allow adequate follow up for assessing the safety and feasibility of the study.



(Gladwin, NEJM 2004)

TR jet velocity >2.5 m/s in children

Similar results from TR jet velocity >2.5 m/s have not been seen in children

A recent study showed that TR jet velocity >2.6 along with indices of excessive hemolysis may be more predictive of pulmonary hypertension in children.

(Miniti, Hematologica, 2009)

Another recent assessment in children shows that elevated TR jet velocity >2.5 m/s but <2.7 m/s is transient. Only TR jet velocity >2.7 shows consistent elevation and may be associated with pulmonary hypertension.

(Pashankar, Pediatrics, 2008)

Overall, recent evidence suggests that increases in peak pulmonary pressures are transient and reversible in children, and likely become irreversible in adults.

Therefore, we recognize that TR jet velocity criteria would need to be revisited when the study is extended to children.

Rationale for Exclusion of Patients with Stroke

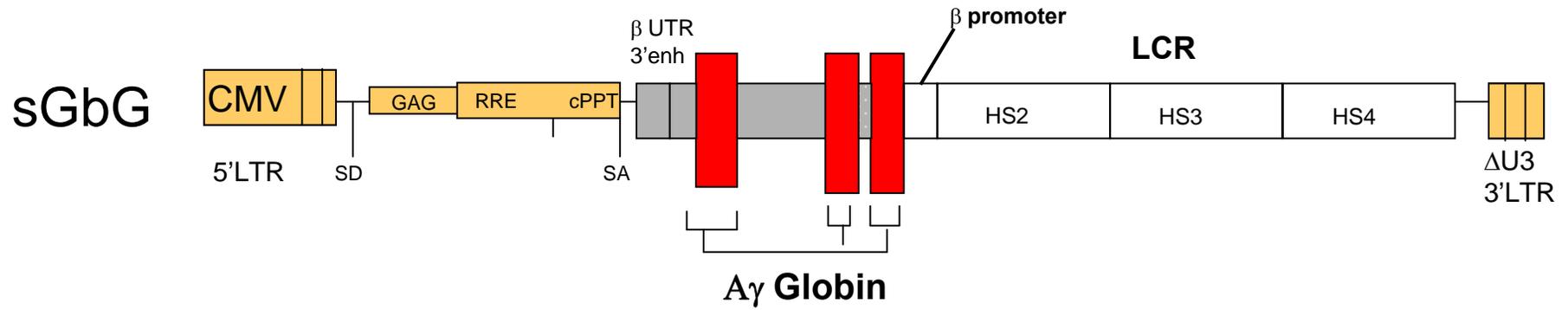
We have not established that gene transfer would result in a high fetal hemoglobin concentration

Hydroxyurea, an agent that increases fetal hemoglobin, is currently under investigation for stroke prevention

If the results of the SWITCH and TWITCH studies show that increases in fetal hemoglobin are protective against secondary and primary strokes, respectively, AND gene transfer shows sustained increased fetal hemoglobin in subjects, the inclusion criteria will be revisited.

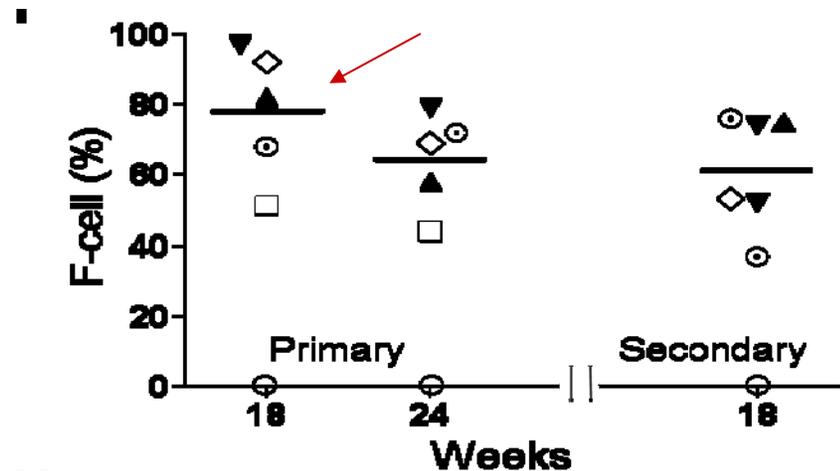
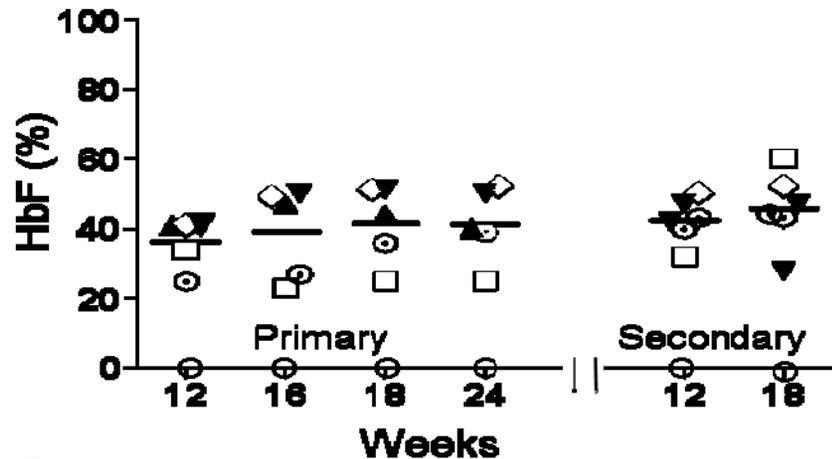
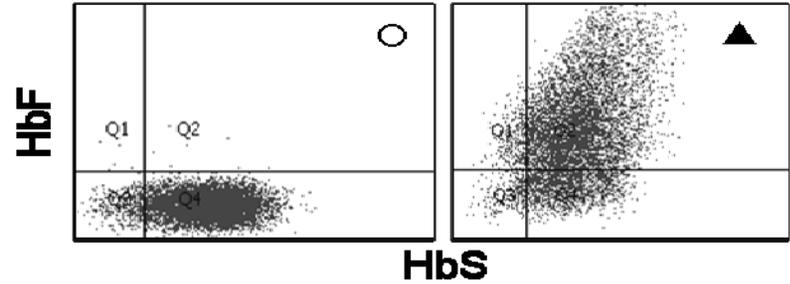
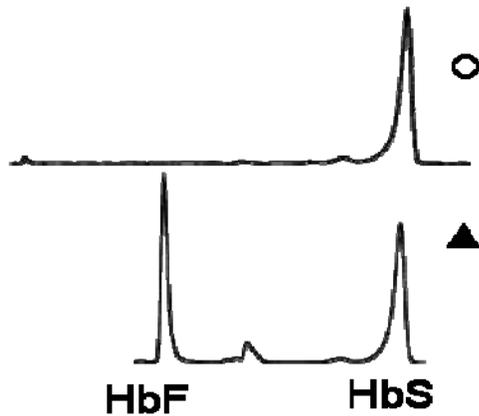
Gene Transfer Preclinical Studies

An HIV-1 Based Self-inactivating Lentivirus Vector

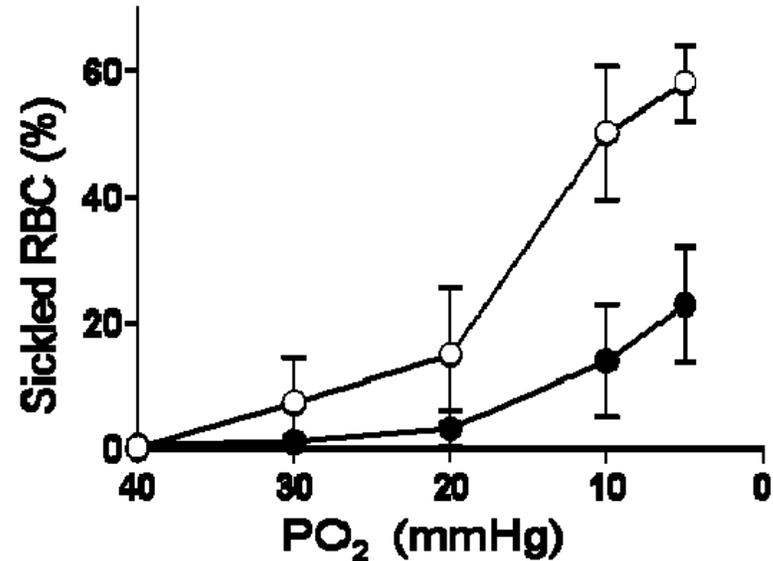
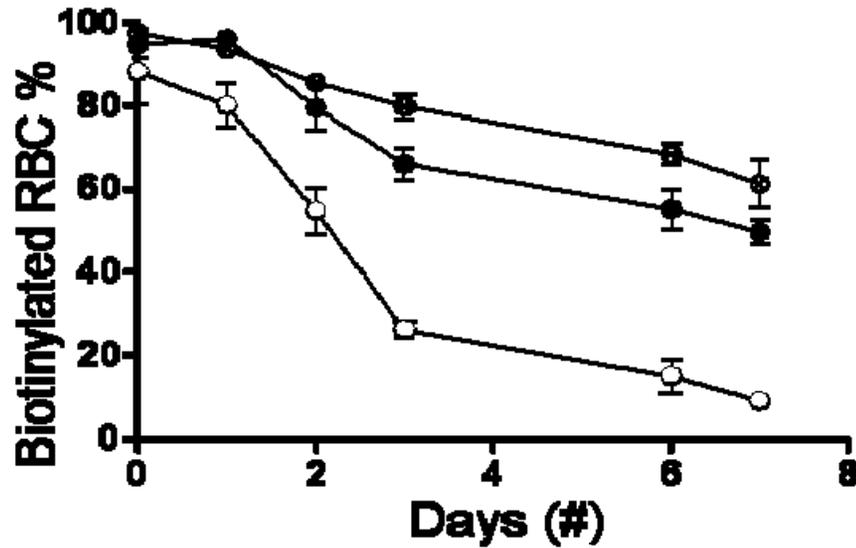
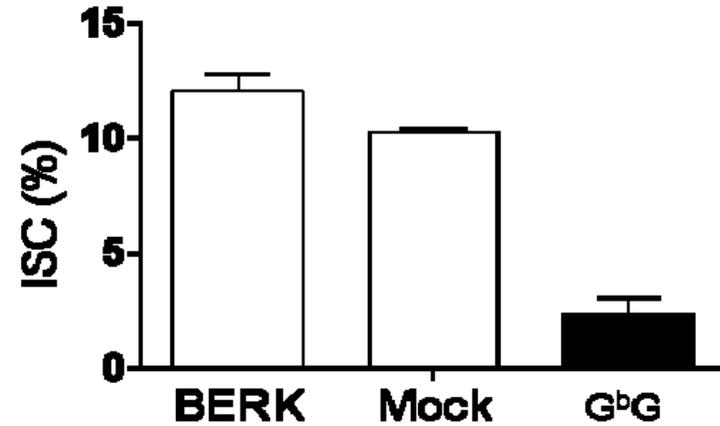
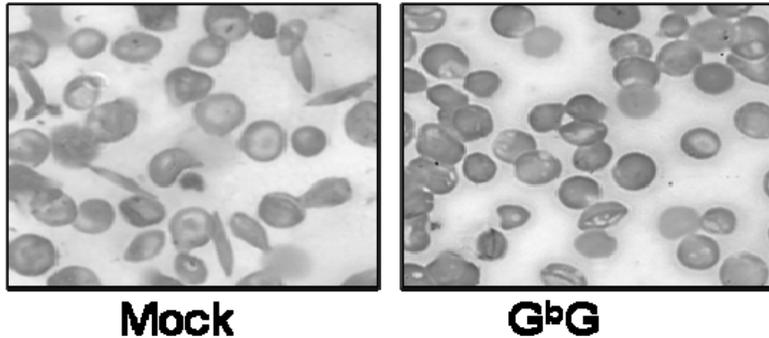


Myeloablative Transplant Model

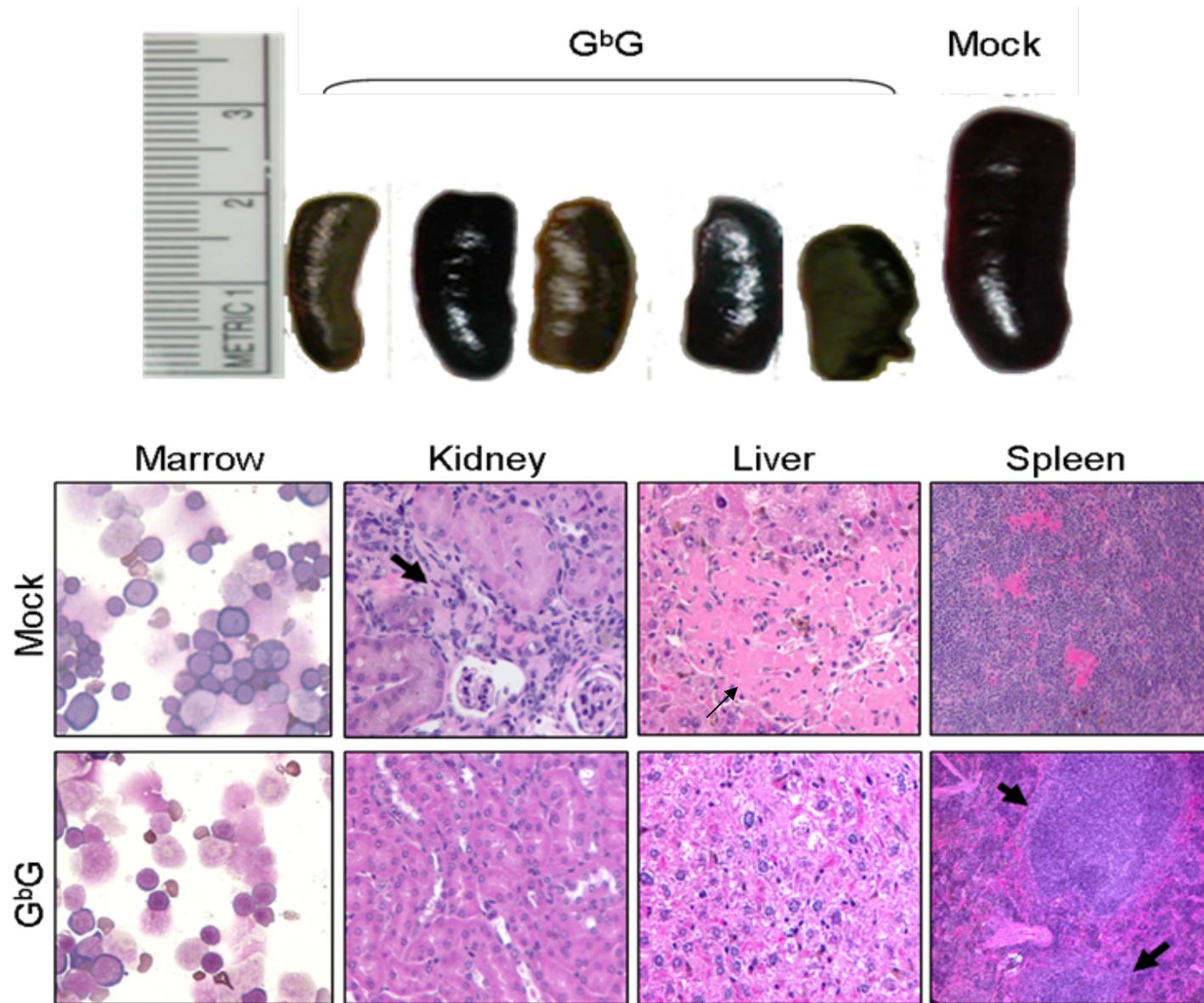
Berkeley → C57Bl/6J mice following myeloablative conditioning
Primary transplants – 6 months, secondary transplants 6-8 months



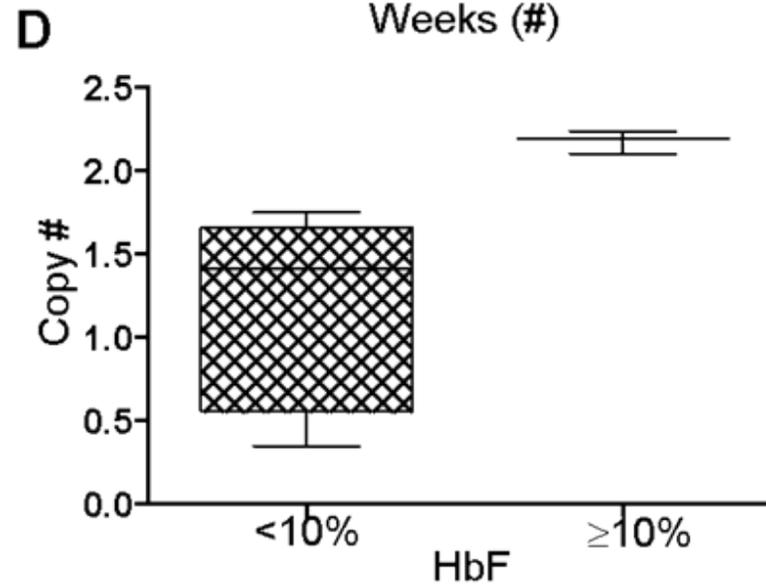
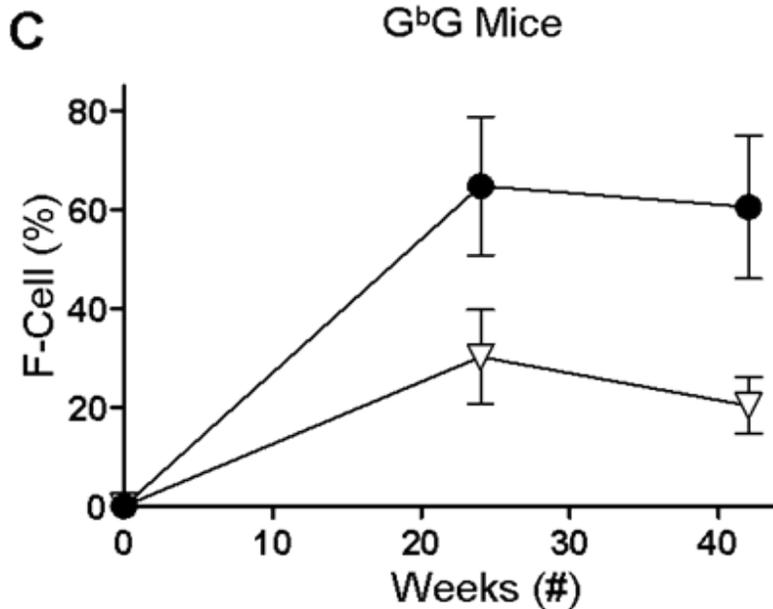
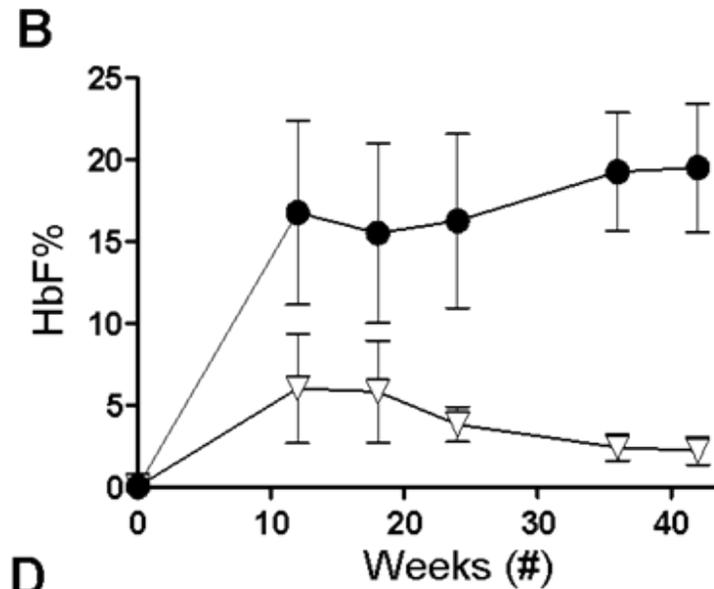
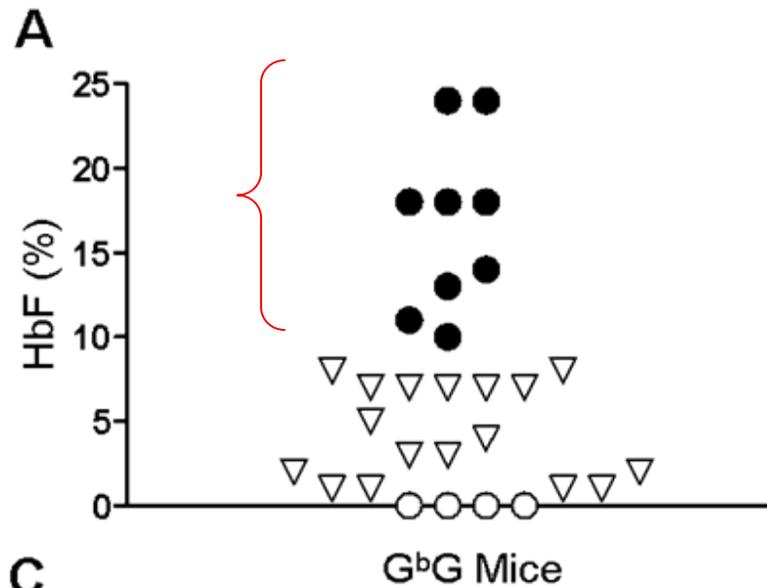
Functional Correction of RBC Parameters



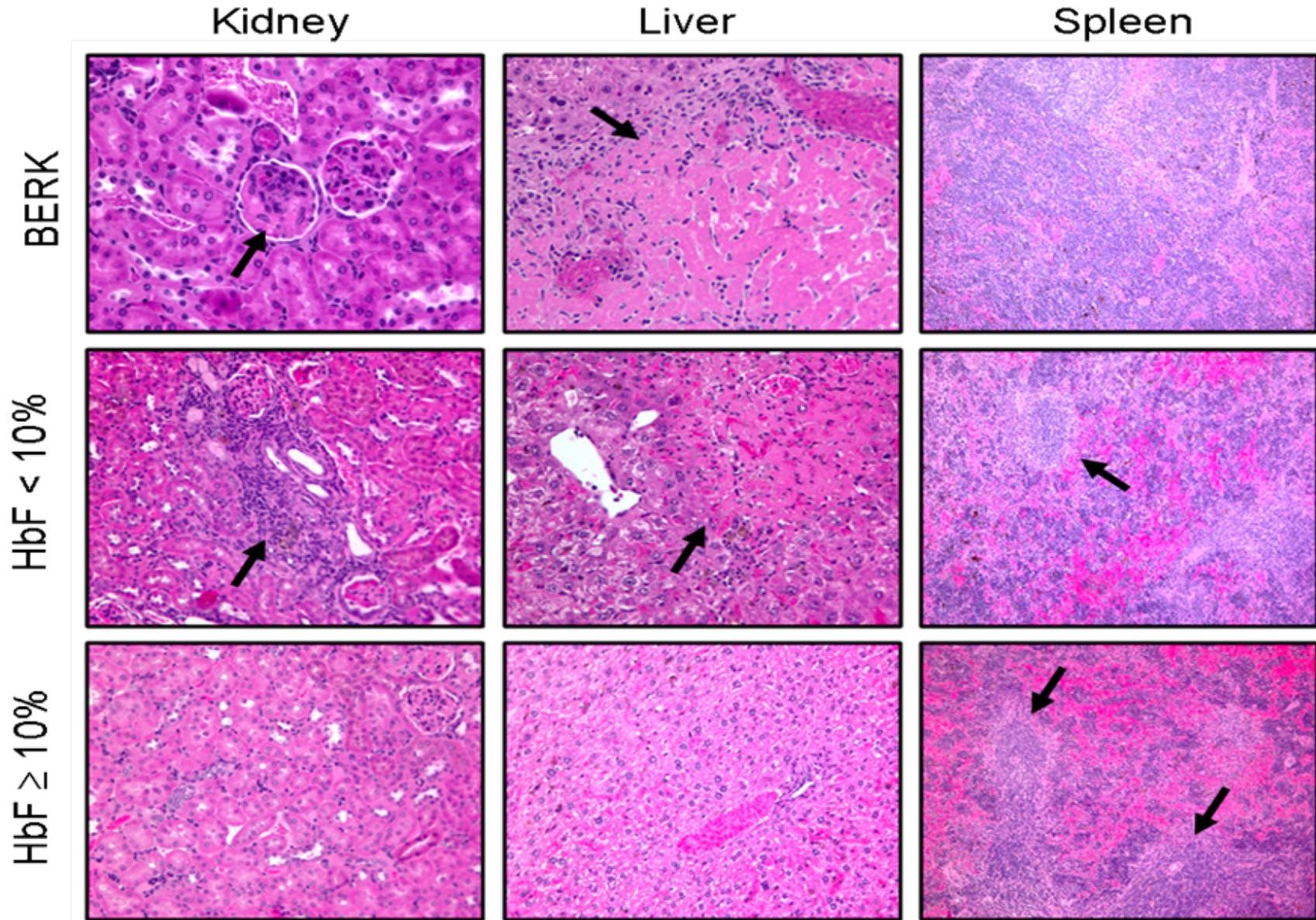
Correction of Organ Pathology



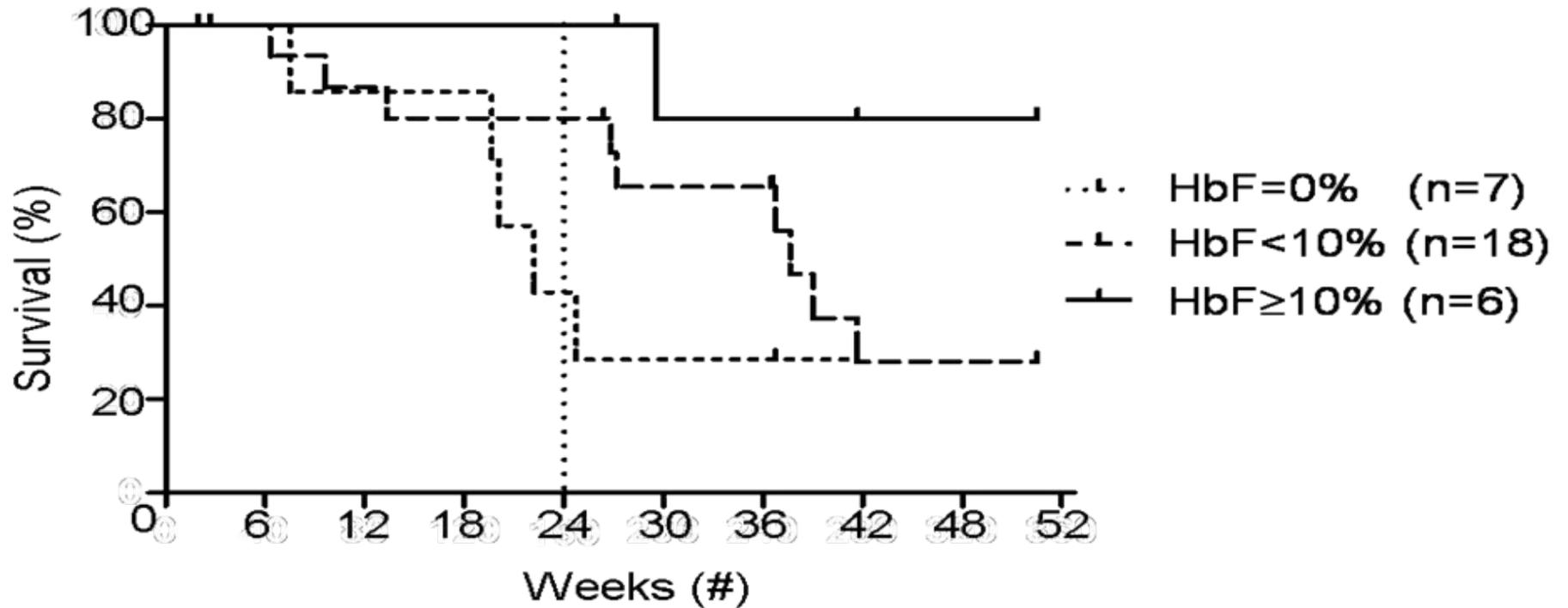
Reduced Intensity Transplant Model Berkeley → Berkeley



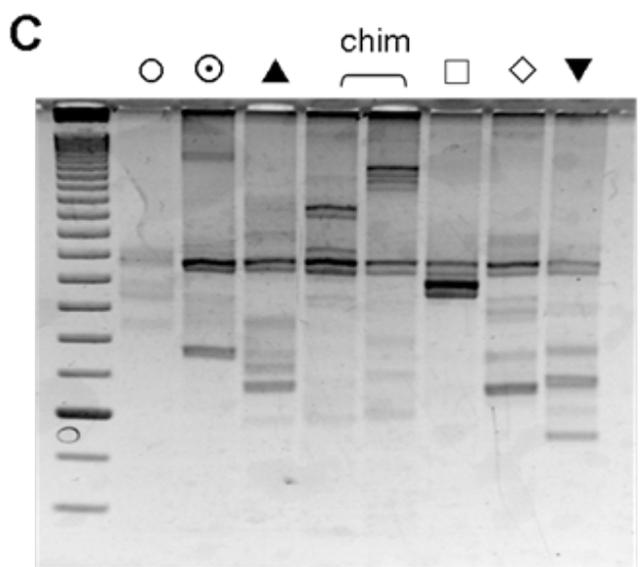
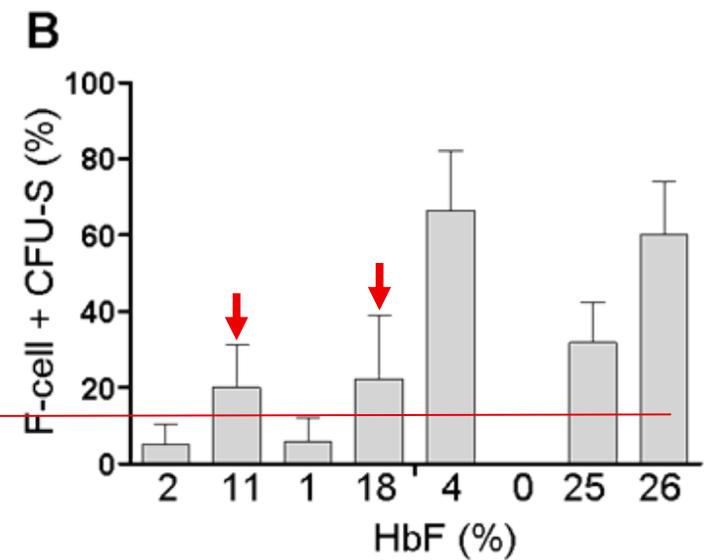
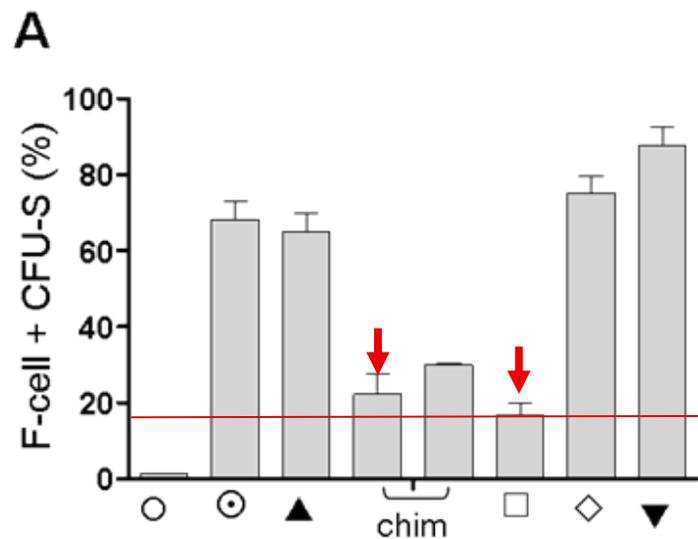
Correction of Organ Pathology if HbF $\geq 10\%$



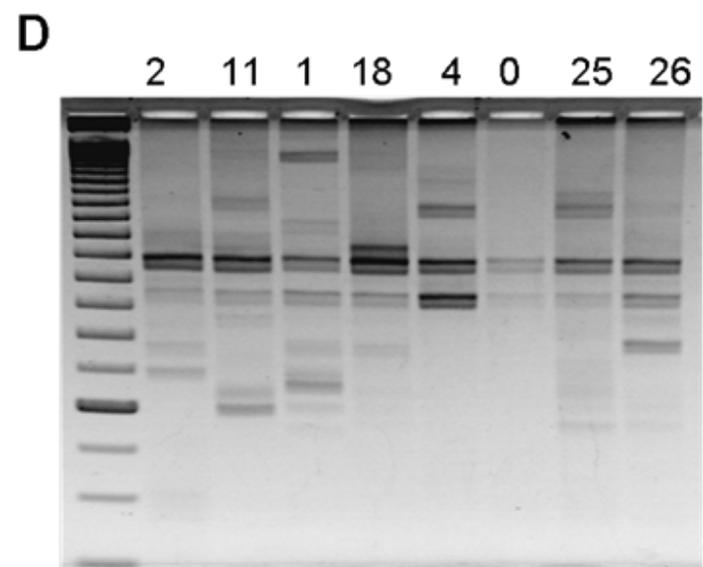
Correction of SCA in $G^bG > 10$ Mice Improves Survival



Proportion of Transduced HSC Required for Correction



Myeloablative Transplant
(Berkeley → C57BL/J6)



Reduced-Intensity Transplant
(Berkeley → Berkeley)

Safety Features of the Vector

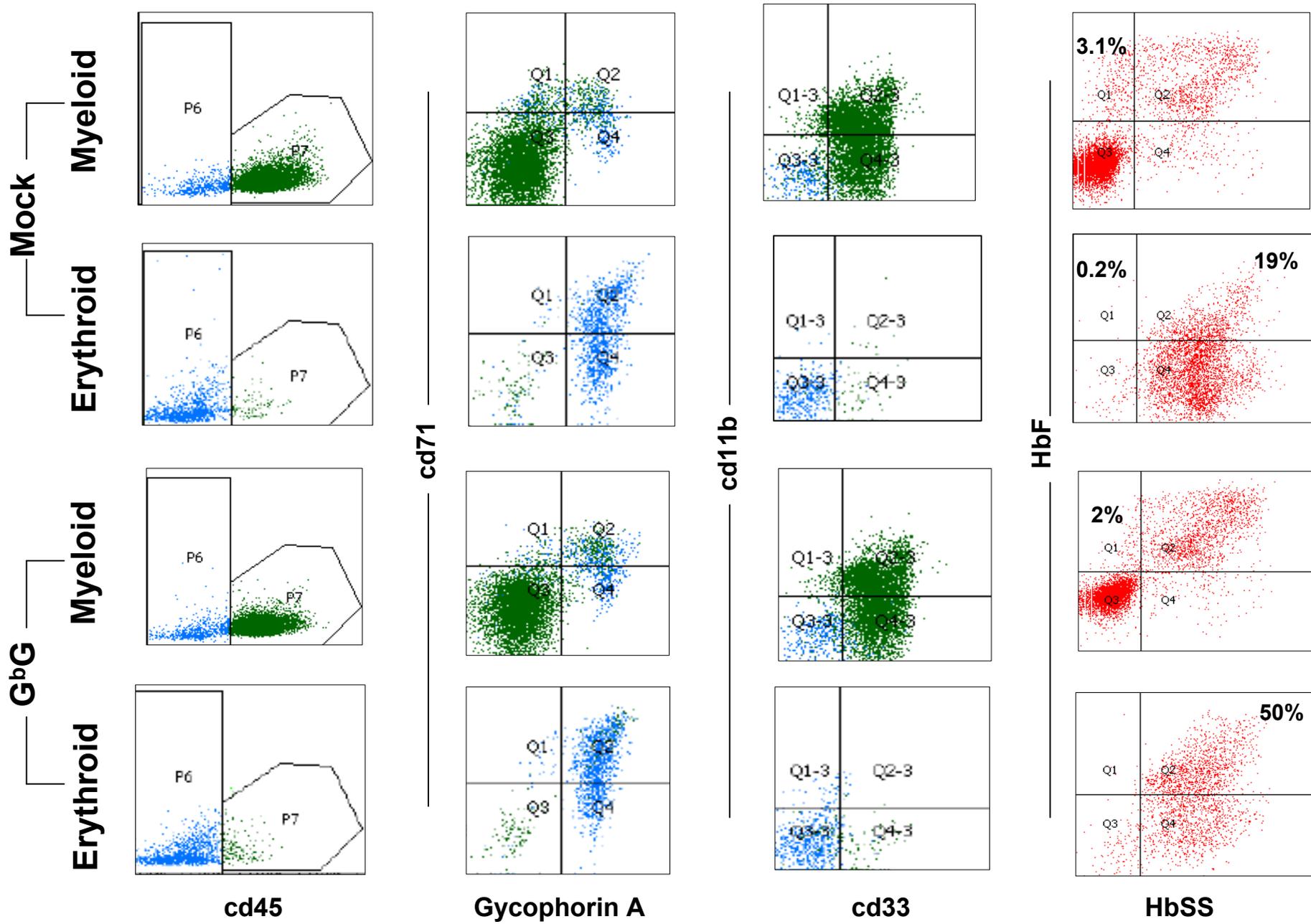
Self-inactivating design

Expression of γ -globin from this vector is restricted to the erythroid lineage

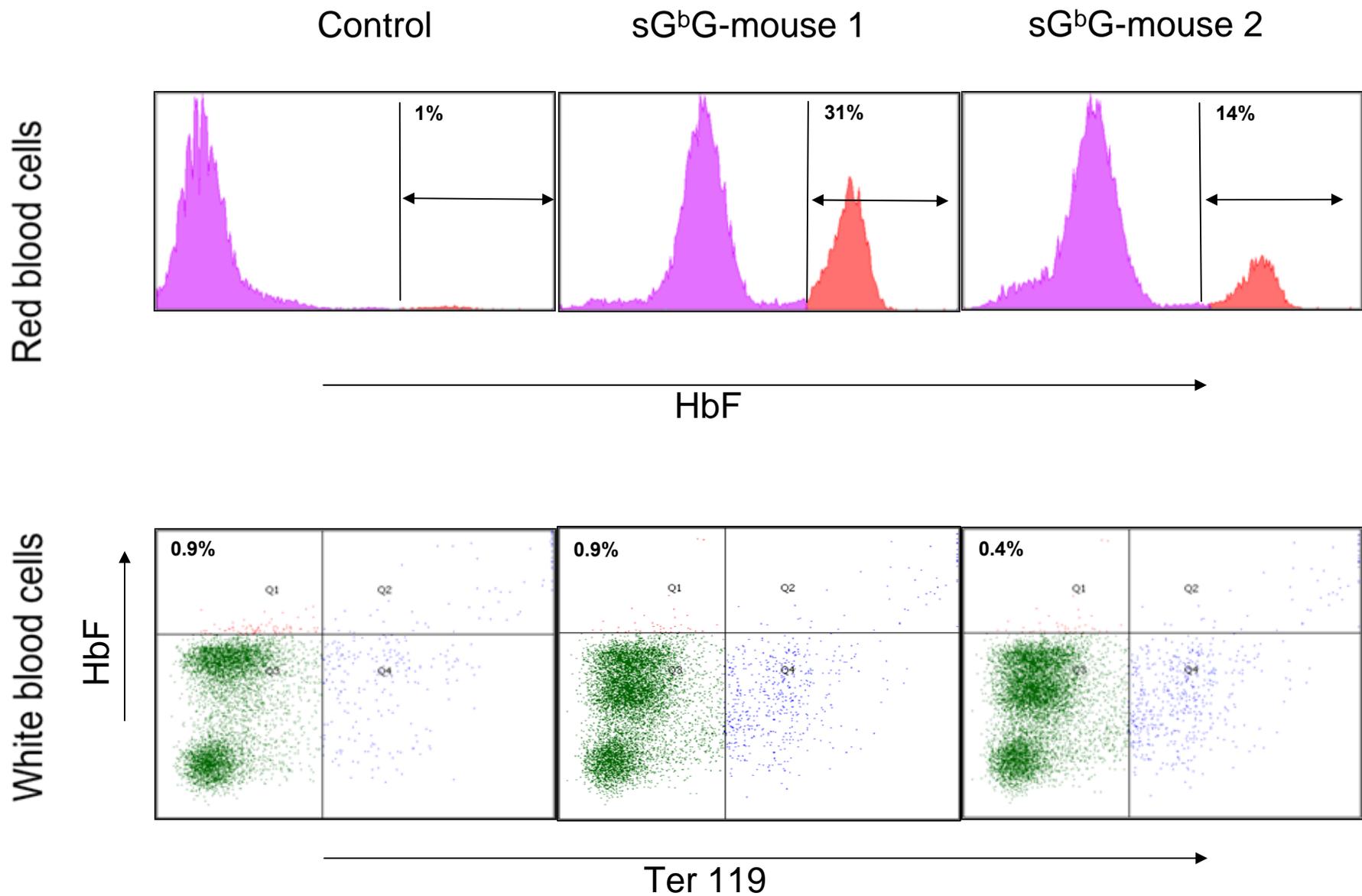
Erythroid transcriptional machinery is lost during terminal differentiation

Safety studies on primary murine progenitors show nearly 200 fold lower genotoxicity as compared to the conventional retroviral vectors

Human Sickle CD34+ Cells show Expression Restricted to Erythroid Cells



Mice transplanted with sGbG modified HSC show erythroid-restricted expression



Frequency of IVIM assay mutants following replating at 2 and 5 weeks

Vector	Average IVIM wells (2 wk)	Replating Frequency (2 wk)	Fold Reduction in IVIM (2wk)	Vector Copy number	Actual Replating Frequency (5wk) *	Immortalized Mutant Clones at 5 weeks	Actual Replating Freq/Copy (5wk) **	Fold Reduction in IVIM mutants
SF GV	83	1 in 50		4.2	0.0200000		0.0048	
	96	1 in 22		4.6	0.0450000		0.0098	
	79	1 in 58		5.2	0.0172000		0.0033	
	86	1 in 44		4.8	0.0227000		0.0047	
	344/384	0.02600	1	4.7	0.0260000	100%	0.00560	1
SF LV	8	1 in 1229		4.3	0.0008000		0.00018	
	20	1 in 440		4.4	0.0023000		0.00052	
	13	1 in 687		5.7	0.0014500		0.00026	
	41/288	0.00148	18	4.8	0.0015000	100%	0.00032	18
LCR β -GFP LV	2	1 in 4750		1.8	0.0001000		0.00006	
	3	1 in 3150		2.6	0.0001000		0.00004	
	4	1 in 2350		2.1	0.0002100		0.00010	
	4	1 in 2350		2.3	0.0001000		0.00004	
	3	1 in 3150		2.5	0.0000520		0.00002	
	4	1 in 2350		2.8	0.0001000		0.00004	
	3	1 in 3150		1.7	0.0000000		0.00000	
	3	1 in 3150		3	0.0000520		0.00002	
	3	1 in 3150		2.9	0.0000520		0.00002	
	2	1 in 4750		2.7	0.0000000		0.00000	
	3	1 in 3150		2.7	0.0000520		0.00002	
	2	1 in 4750		1.9	0.0000000		0.00000	
		36/1152	0.00030	87	2.4	0.0000681	28%	0.00003
β -GFP LV	0	< 1 in 9550		2.5	0			
	0	< 1 in 9550		2.4	0			
	0	< 1 in 9550		7.1	0			
	0	∞		4	NA	0	0	\square
Mock	0	0		0	0			
	0	0		0	0			
	0	0		0	0			
	0	0		0	0			
	0	∞		0	NA	0	0	∞

*Assuming a direct correlation between in vitro immortalisation potential and copy number

**With LCR containing vectors, one third of clones appearing at 2 weeks could be expanded by 5 wks. All clones picked for expansion with the SFFV driven GV or LV vectors at 2 weeks were expanded robustly at 5 weeks. Expansion of SF driven vectors was 4.5 ± 1.2 higher.

An Experimental System for the Evaluation of Retroviral Vector Design to Diminish the Risk for Proto-oncogene Activation

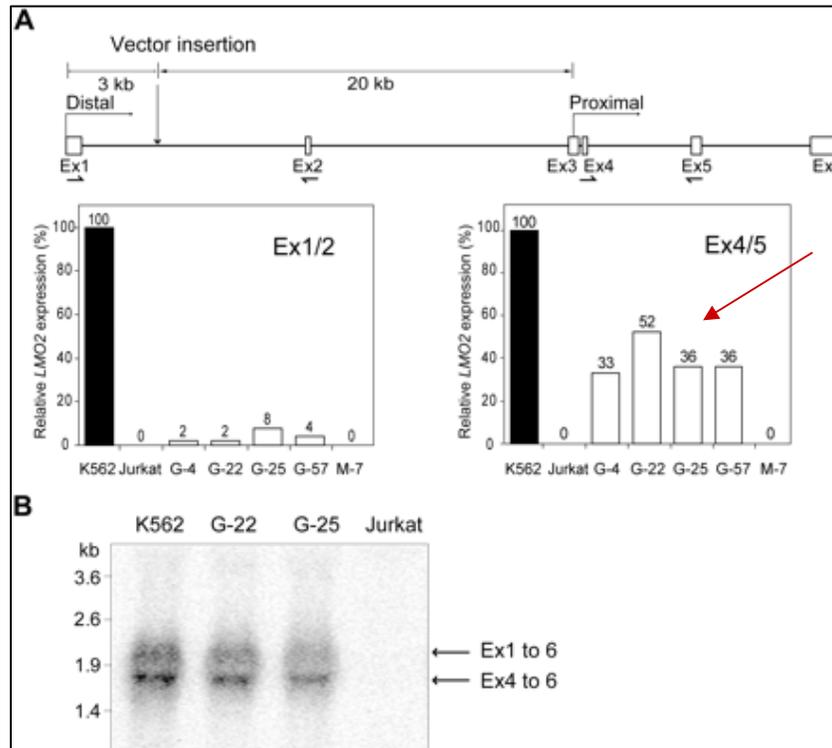


Figure 3. Activation of the distal and proximal LMO2 promoters by the LTR. (A) The origin of the 2 transcripts from human LMO2, both of which encode the same protein, is indicated along with the relative distances of the retroviral insertion site from the 2 transcriptional start sites. The relative LMO2 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.

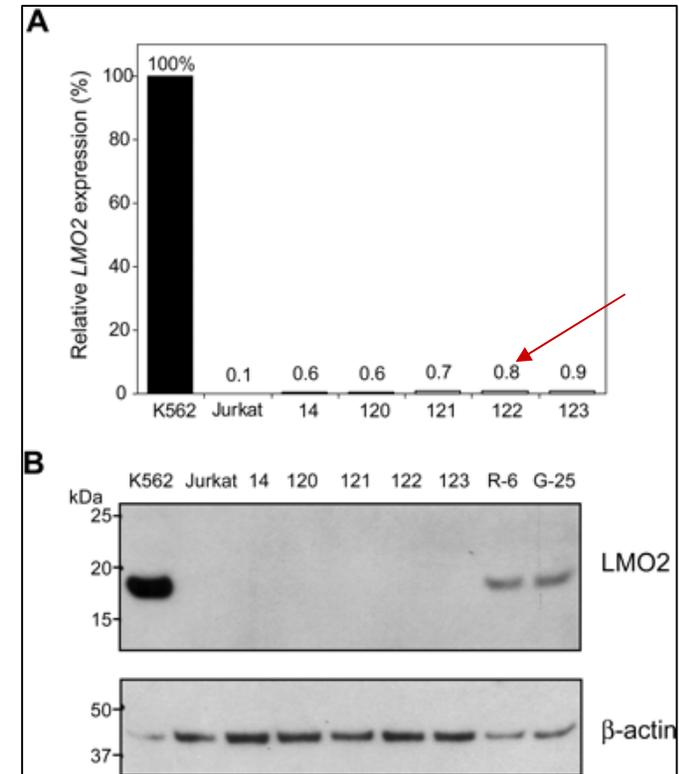


Figure 6. LMO2 is not activated by globin regulatory elements. A cassette containing the GFP coding sequences under the control of γ -globin gene promoter along with the human γ -globin LCR was used in the cassette exchange reaction in R-6 cells. Five clones were confirmed as having undergone the predicted 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).

Clonal dominance Induced by Aberrant Transcript Termination

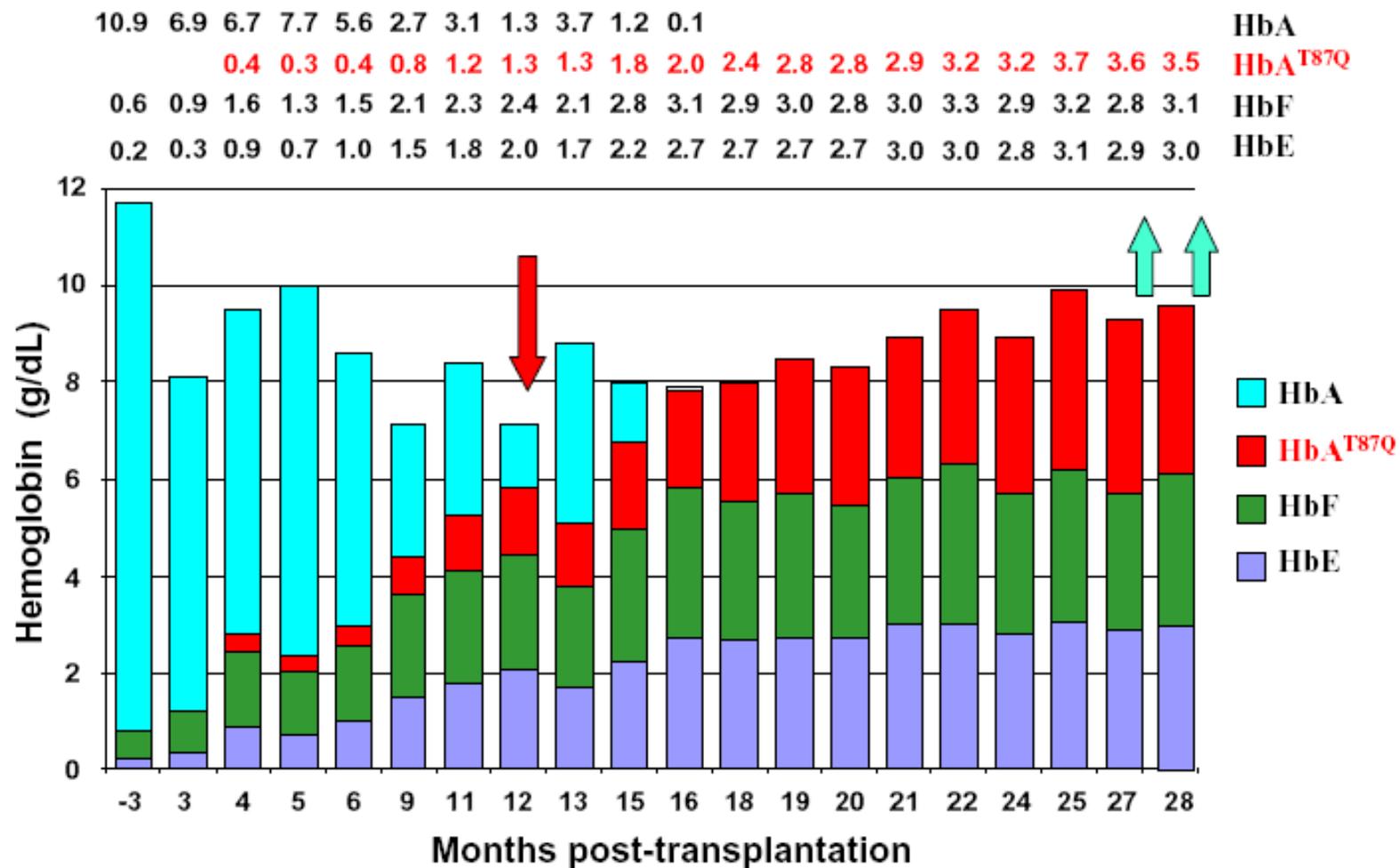
Pre-transplant clinical history of Patient "PLB"

- **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

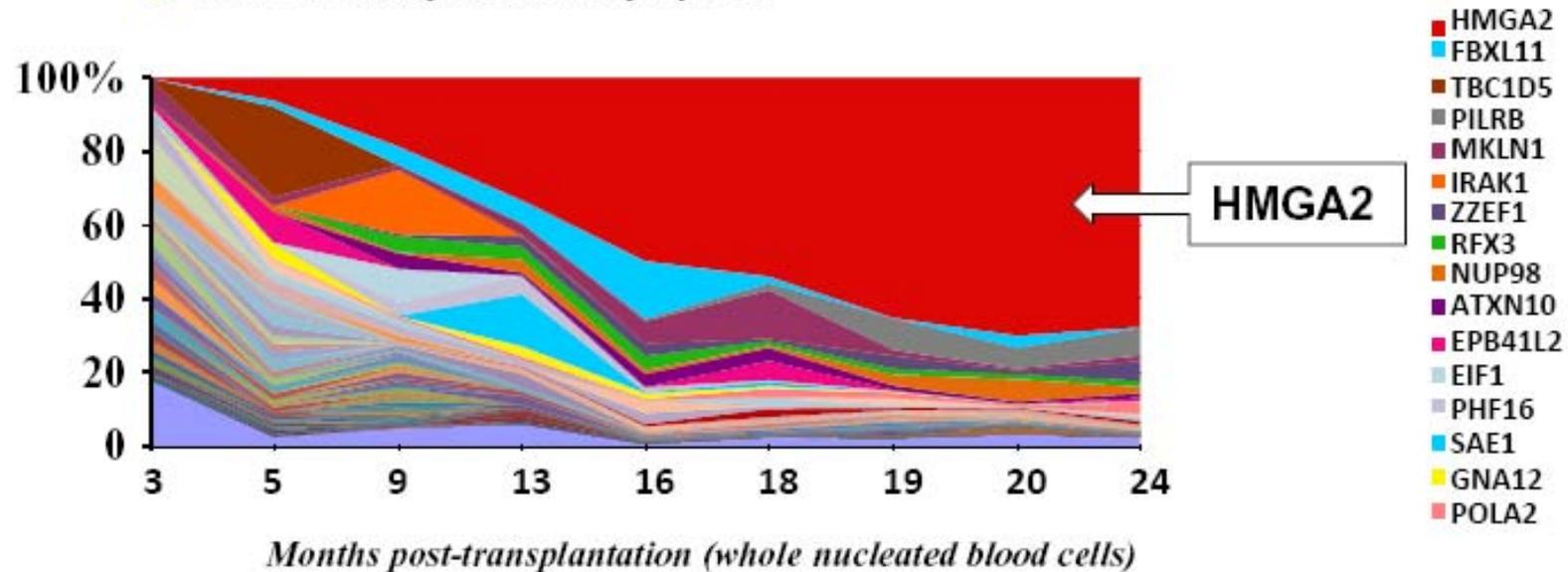
Conversion to transfusion independence (II)



This information is taken from P.Leboulch, as presented to the RAC on December 2, 2009 (slide #13).

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)

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

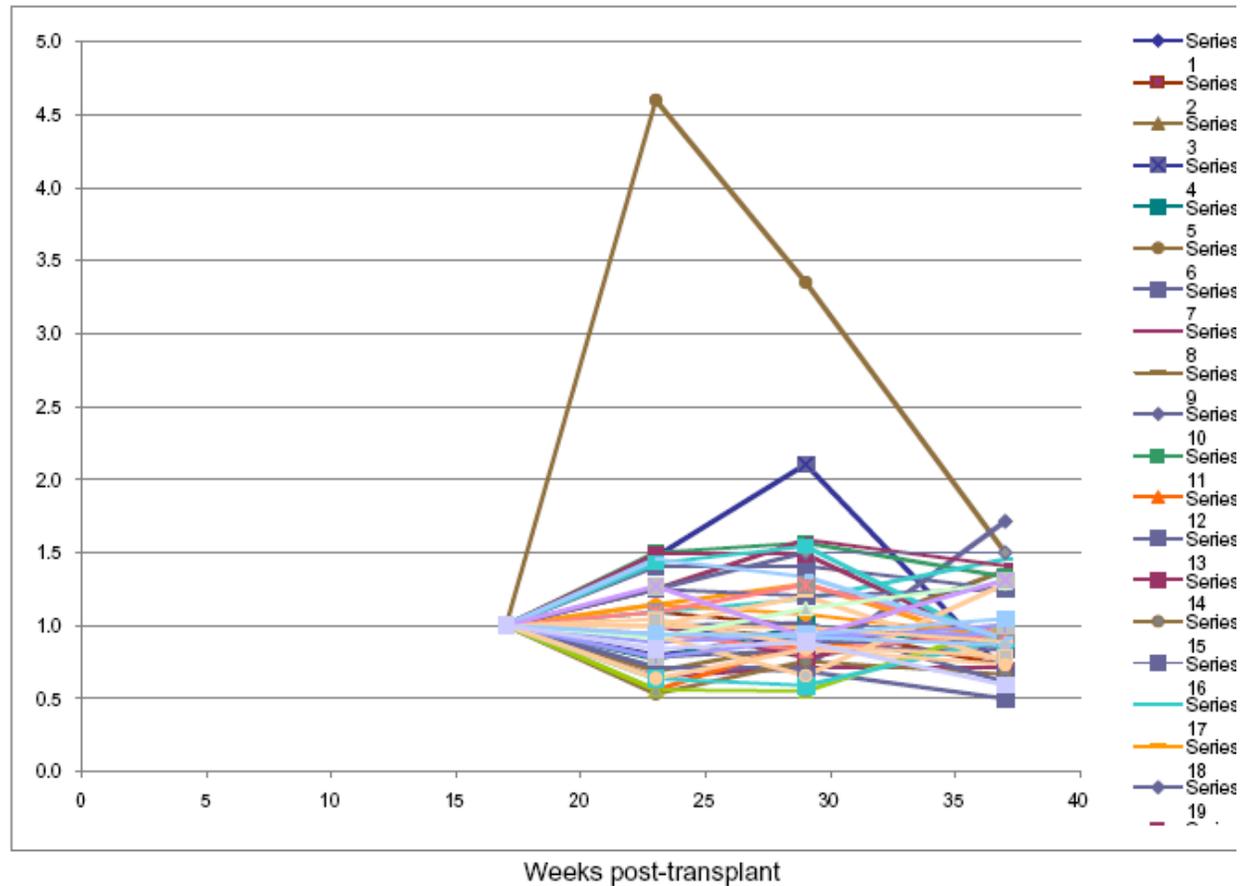
"Peaceful co-existence" of cells with minor genetic dysregulation

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**

This information is taken from M.Sadelain, as presented to the RAC on December 2, 2009 (slide #22).

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



In summary,

1. Clonal Dominance from insertion into HMGA2 intron and dysregulation of HMGA2 occurred in one patient with β -thalassemia treated with a lentivirus vector
2. This has resulted in no clinical adverse event thus far (has been beneficial)
3. Similar insertions have been seen in other gene therapy trials (X-SCID trial with the use of retroviral vectors and ALD trial with the use of LTR promoter driven lentivirus vector) with either no clonal expansion, or temporary expansion
4. This insertion has been shown with other benign proliferations
5. Some degree of transient clonal fluctuation has been observed in mice by Dr. Sadelain using a β -globin lentivirus vector.

We realize that the risk associated with a monoclonal dominance can be grave and result in malignancy.

If clonal dominance is detected in one patient enrollment into the trial will be placed on hold. The RAC, FDA, DSMB and IRB will be informed and we will seek their guidance to continue.

If two patients develop a monoclonal dominance, enrollment to the trial will be suspended and subjects followed until the clinical importance of the event can be determined by the DSMB, FDA and RAC.

The knowledge gained from the follow up of the one patient with thalassemia in the French trial will also provide insight.

Trial Monitoring Queries

Transfusion withdrawal: We plan a gradual withdrawal of transfusions starting at 3 months, and dependent upon a rise in Hb F as outlined in the clinical protocol (pg 28-29)

Hb F monitoring upon transfusion withdrawal:
We realize that our transgene is identical to the endogenous $A\gamma$ globin. Hence transient rises in Hb F will need to be corroborated with increased vector marked BFUe in peripheral blood or bone marrow to distinguish from endogenous upregulation.