



bluebirdbio™

Transforming the Treatment  
OF SEVERE GENETIC DISORDERS

## PROTOCOL HGB-204

**A PHASE 1/2, OPEN LABEL STUDY EVALUATING THE SAFETY AND EFFICACY OF GENE THERAPY IN SUBJECTS WITH B-THALASSEMIA MAJOR BY TRANSPLANTATION OF AUTOLOGOUS CD34+ STEM CELLS TRANSDUCED EX VIVO WITH A LENTIVIRAL  $\beta^A T87Q$ -GLOBIN VECTOR (LENTIGLOBIN® BB305 DRUG PRODUCT)**

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JUNE 19, 2012  
RAC Meeting

Presentation by Dr. Davidson, Chief Medical Officer, and Dr. Finer, Chief Scientific Officer, bluebird bio

# PRESENTATION OVERVIEW

## Outline

1

*Background on  $\beta$ -Thalassemia Major*

2

*Overview of supporting preclinical and clinical data*

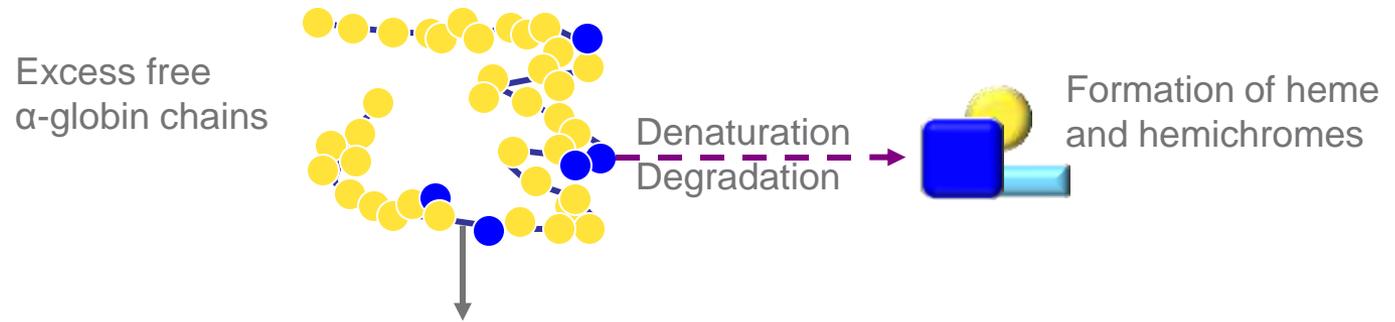
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*Outline of clinical protocol*

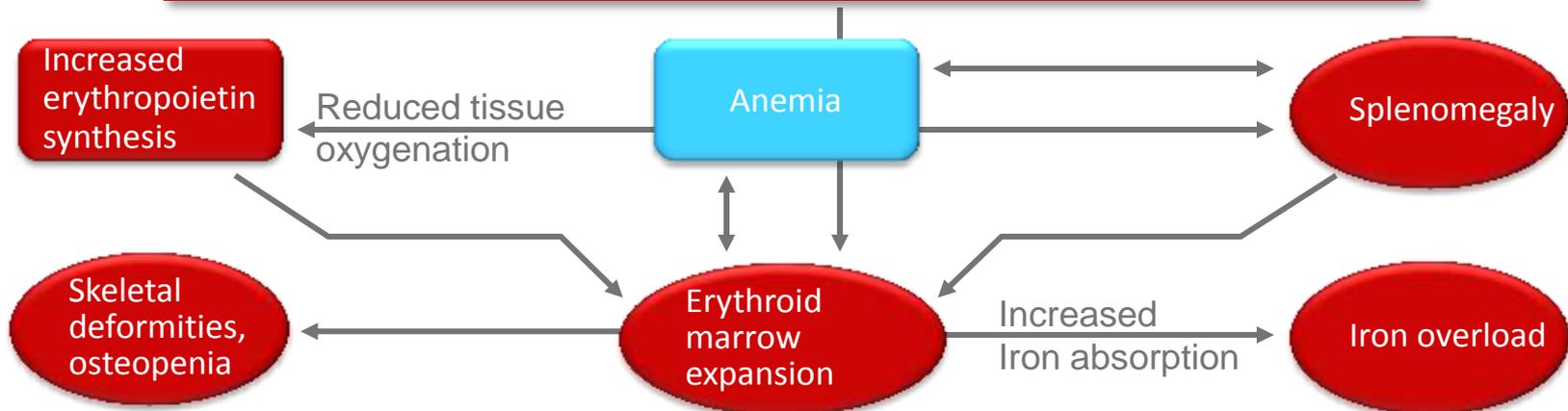
4

*Responses to specific reviewer questions*

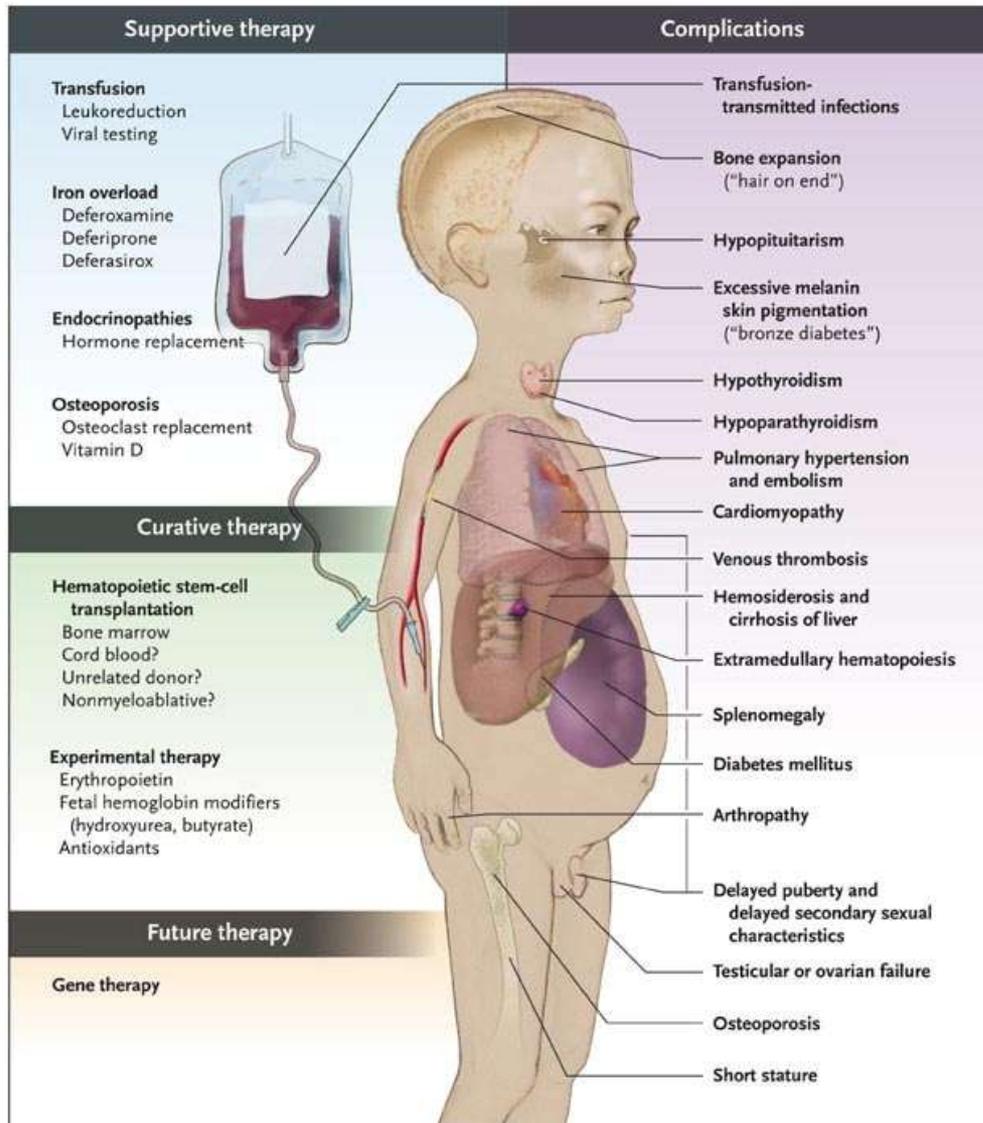
# $\beta$ -Thalassemia Pathophysiology Summary



- Ineffective erythropoiesis
- Chronic anemia and hemolysis
- Iron overload



# Clinical Overview of $\beta$ -Thalassemia



Regular blood transfusion and iron chelation have considerably improved survival resulting in a larger proportion of adult thalassemia patients.

However, disease- and treatment-related complications progress in most over time, causing severe morbidity and shortened life expectancy.

# $\beta$ -Thalassemia – Standard of care

## Significant unmet medical need

### Poor Quality of Life

#### Chronic transfusions

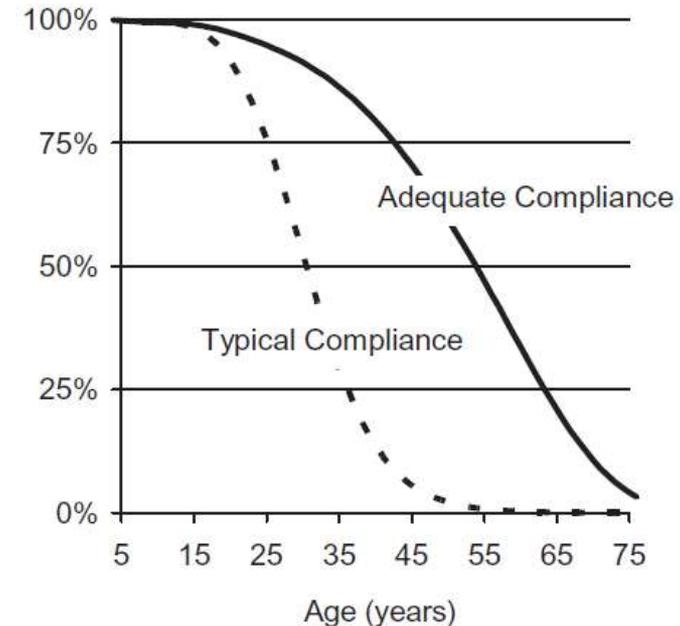


- Every 3-6 weeks

#### Iron chelation therapy

- Chelation improves survival, but is frequently inadequate due to problems with compliance, efficacy, tolerability

### Survival



Estimates based on DFO compliance  
Delea et al. *Transfusion* 2007 (47):1919-29

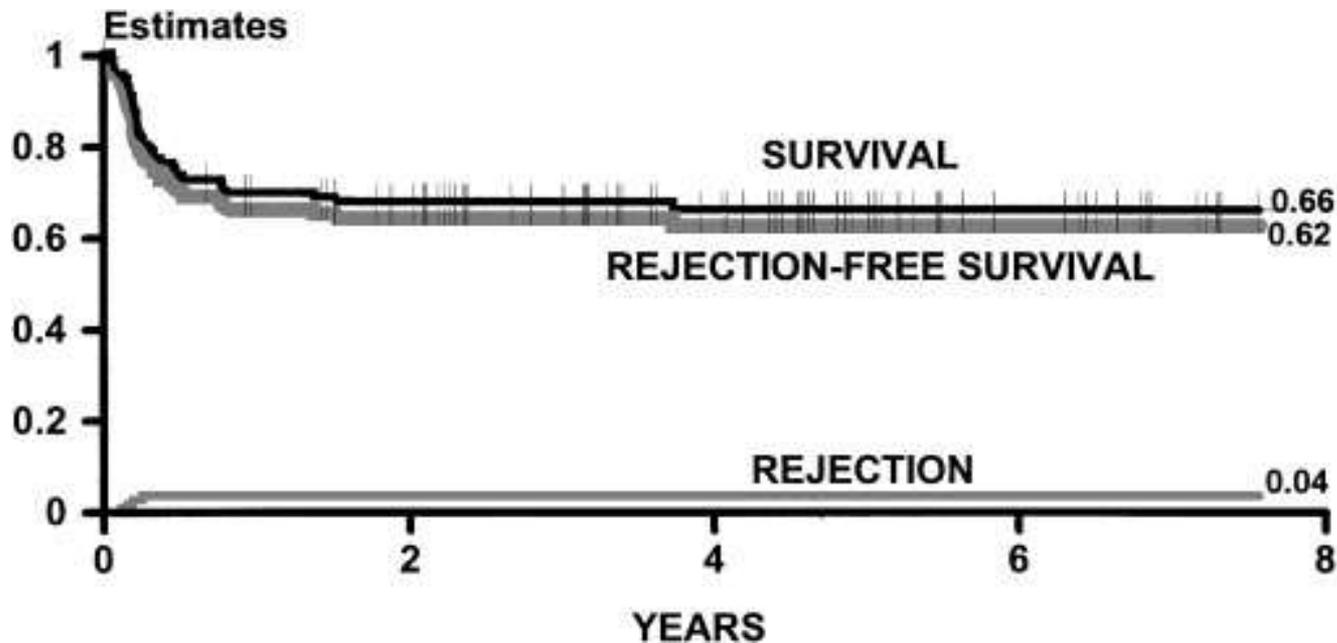
- Iron Overload - Tissue damage occurs early in life and most patients ultimately die from iron overload induced cardiac toxicity
- Allo BMT may be curative; but availability of sibling matched donor <25% and transplant complicated by GVHD, engraftment failure, chronic immunosuppression

# Rationale for Gene Therapy in Adults w/ $\beta$ -Thalassemia Major

HSCT currently offers the only hope of cure, with as low as 10-15% mixed donor chimerism (Vora, BMT, 2011)

- Only <25% thalassemia patients have a non-affected HLA matched sibling donor
- To minimize risk of graft rejection/GVHD MUD graft should optimally match by high resolution typing for HLA-A, -B, -C, -DRB, -DQB1 antigens for 10/10
  - -DPB1 disparities may also contribute to increased graft rejection and GVHD [Fleischhauer Blood 2006]
- Low number of progenitor cells in UCB limits use in adults
  - No adequate studies assess UCBT in adults with thalassemia
- In children, the balance of risks and benefits favors allogeneic HSCT over medical therapy for those who have an HLA-matched sibling
- Transplant in adult patients is characterized by higher transplant-related toxicity due to more advanced disease
  - Adults undergoing HLA-matched HSCT might expect a transplant-related mortality ~30% and thalassemia-free survival of ~70% [Gaziev Ann N Y Acad Sci. 2005; La Nasa BMT 2005]
  - Thus, the risk/benefit balance for allogeneic HSCT in adults is uncertain
- Lentiviral gene therapy with autologous HSCT is expected to be associated with lower transplant related mortality and morbidity versus allogeneic HSCT
  - Procedure related mortality for autologous HSCT  $\leq$  1%
  - Conditioning with only busulfan; no cyclophosphamide or other immunosuppressants required
  - Avoid risks of graft rejection, GVHD, chronic immunosuppression

# Allogeneic HSCT Outcomes in Adults with $\beta$ -Thalassemia



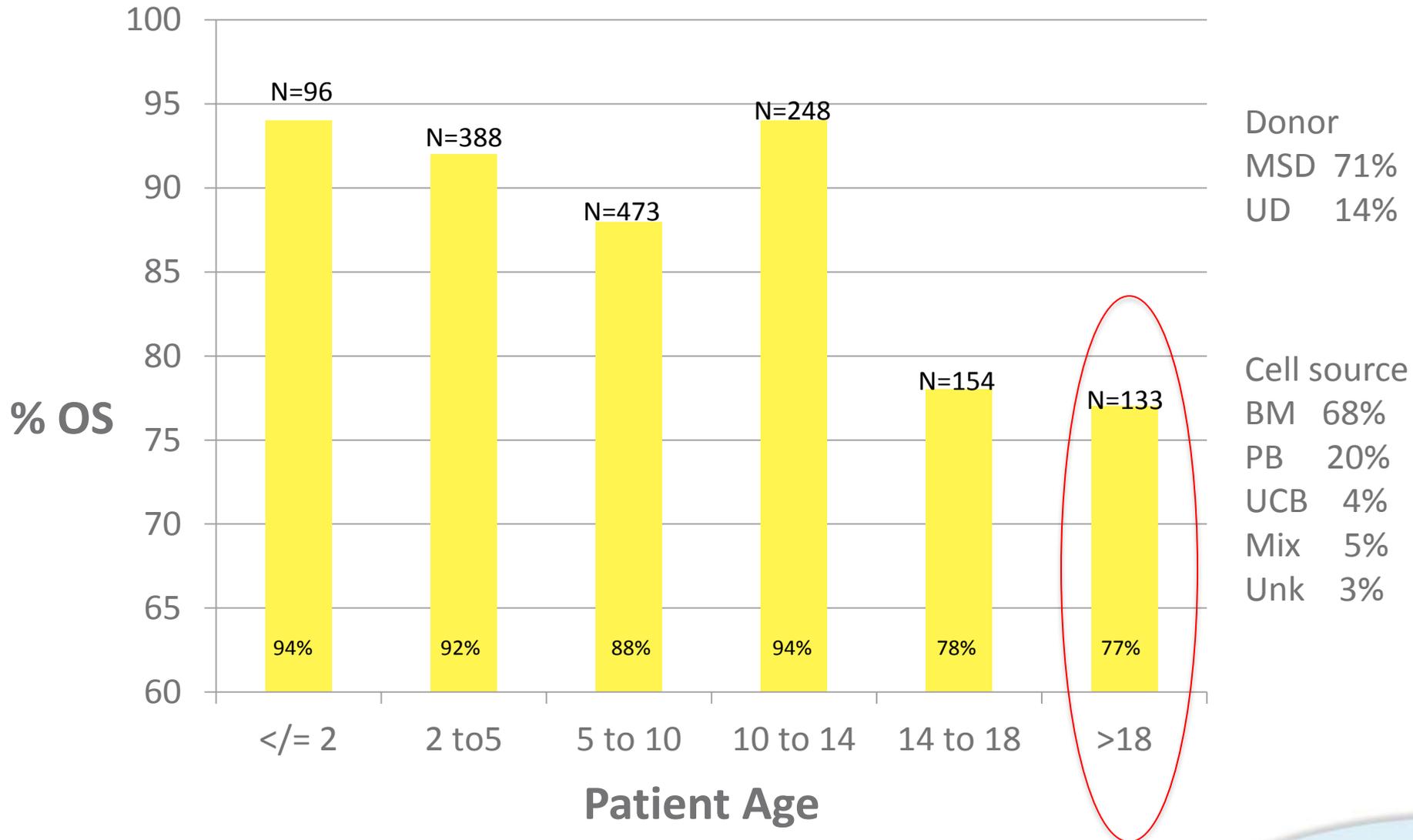
**Kaplan-Meier estimates of survival and thalassemia-free survival and cumulative incidence estimates of rejection for 107 adult**

**pts transplanted between Nov 1988 and Sept 1996. Statistics updated as of Dec 1997**

# EBMT Hemoglobinopathy Registry

## Overall Survival in 1492 Consecutive HSCT Transplants for Thalassemia Major after Jan 1, 2000

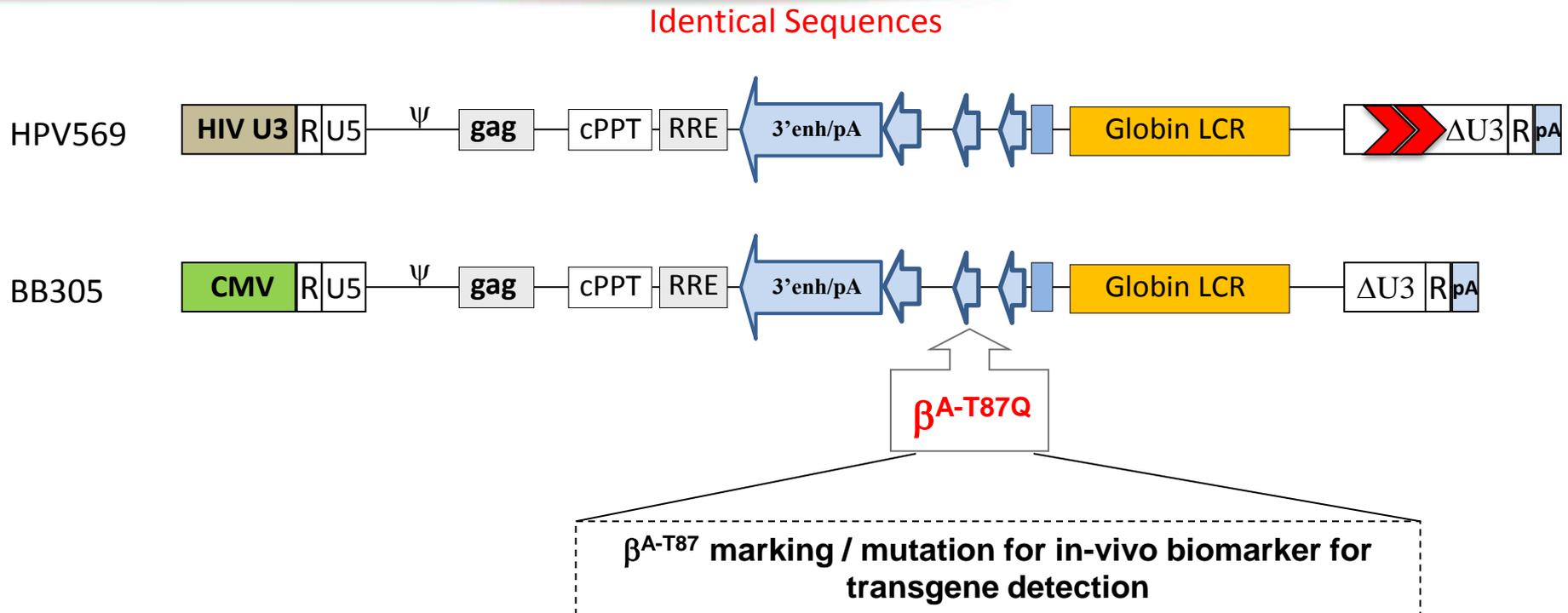
Baronciani et al. ASH 2011 abstract # 905



# Clarifications on LentiGlobin lentiviral vectors

- **LentiGlobin HPV569** was used in on-going clinical study LG001 in France, based on founding work conducted by Prof. P. Leboulch (University of Paris and Harvard Medical School) (*Science* 2001, *PNAS* 2002).
- Three patients have been treated in this study:
  - Subject 1002 and 1003 were treated in 2006 and 2007
  - Subject 1004 was treated in November 2011
  - bluebird bio is not planning to treat any additional patient with LentiGlobin HPV569 Drug Product
- In the proposed protocol, HGB-204, bluebird bio will use **LentiGlobin BB305**

# LentiGlobin BB305 Lentiviral Vector



## **BB305 versus HPV569:**

- insulators were removed
- Internal vector sequences are identical
- globin gene and control sequences unchanged
- 5' HIV U3 promoter/enhancer replaced with a 5' CMV promoter/enhancer
- tat no longer necessary in lentiviral vector manufacturing

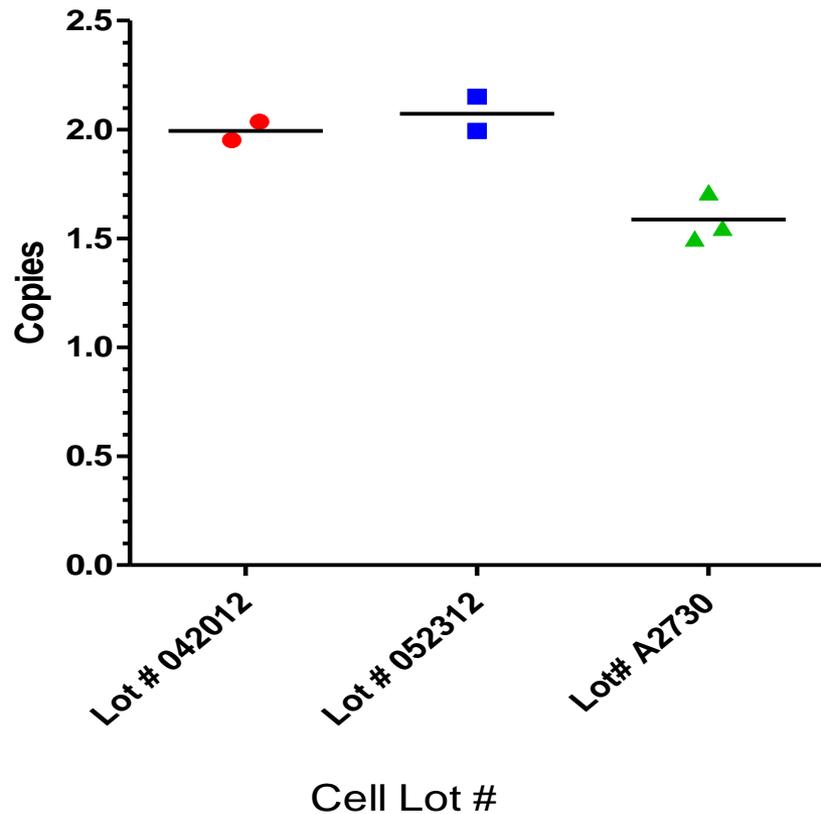
# Supporting Preclinical Data

- LentiGlobin HPV569 key safety studies:
  - Ronen *et al.*, 2011 – HMGA2 insertion site not identified
  - Study Report VI – Spi-1 mice – no preference for induction of leukemic clones
- LentiGlobin BB305:
  - Hb expression and transductions efficiency in CD34+ Sickle Cell Anemia cells
  - Hb expression, transduction efficiency and long-term stem cell potential in CD34+ SCA cells (includes comparison to HPV569)
  - IVIM assays completed (*integration site analysis on-going*)
  - Single dose toxicology study in  $\beta$ -thalassemic (Hbb th1/th1) mice - *on-going*

# LentiGlobin BB305 High Transduction Efficiency

Vector copy numbers of 1.5 – 2 can be achieved in normal hCD34+ cells

VCN of LentiGlobin Transduced mPB CD34+ Cells



mPB: mobilized peripheral blood

# SCA CD34+ in vitro Pharmacology (Efficacy) Study

## BB305 vs HPV569

### Experimental Plan

Frozen BM SCA CD34+ cells ( $\beta^E/\beta^S$ )



Pre-stimulation



Transduction



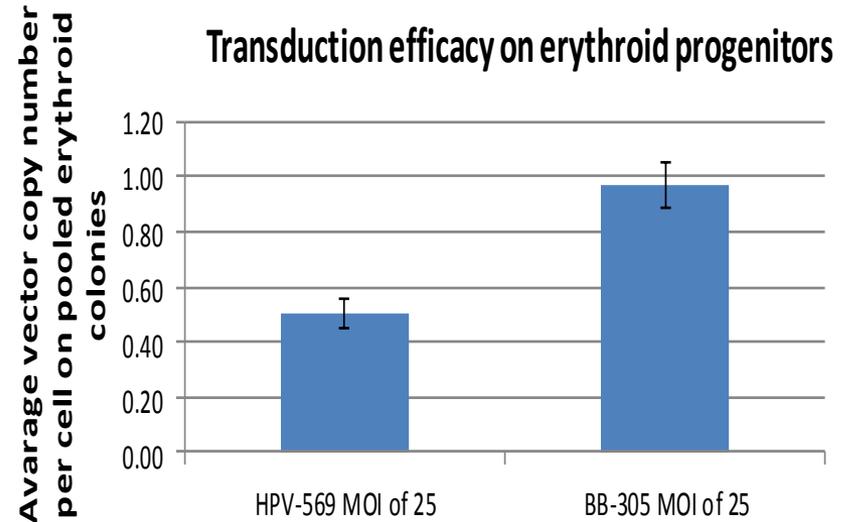
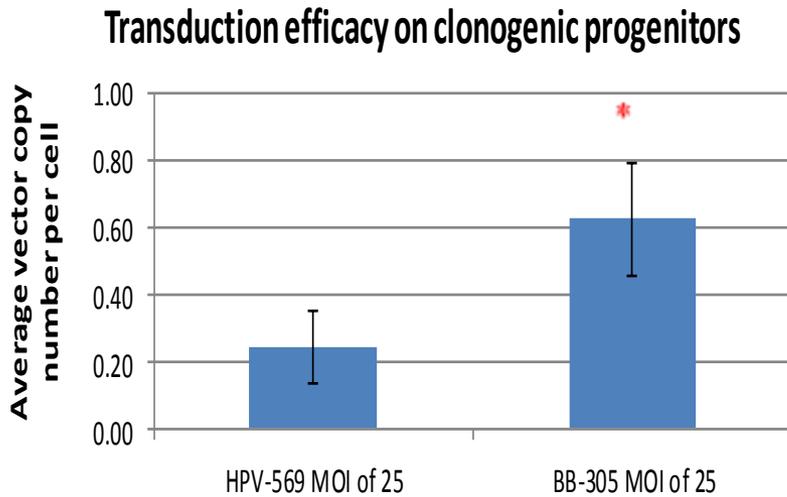
Clonogenic Assays



- Average vector copy number per cell on pooled BFU-E colonies (qPCR)
- Globin chain analysis on pooled BFU-E colonies (RP-HPLC)
- Proportion of genetically modified BFU-E colonies (PCR on individual BFUs)
- Hemoglobin expression in individual BFU-E colonies (IE-HPLC)
- Average vector copy number per cell on pooled LTC-CFC (qPCR)

# Mean VCN in Pooled BFU-E Colonies derived from SCA CD34+ cells

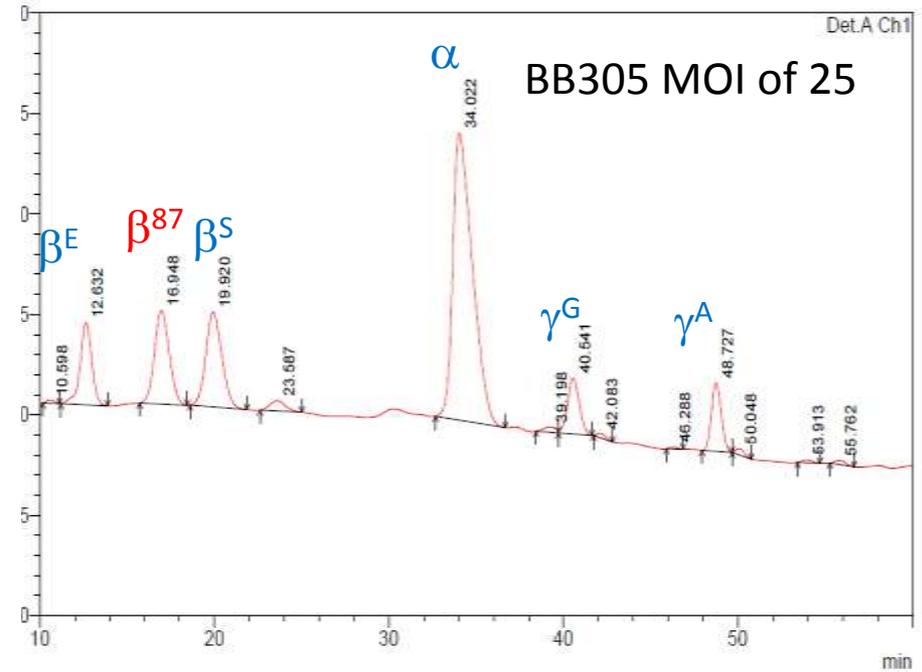
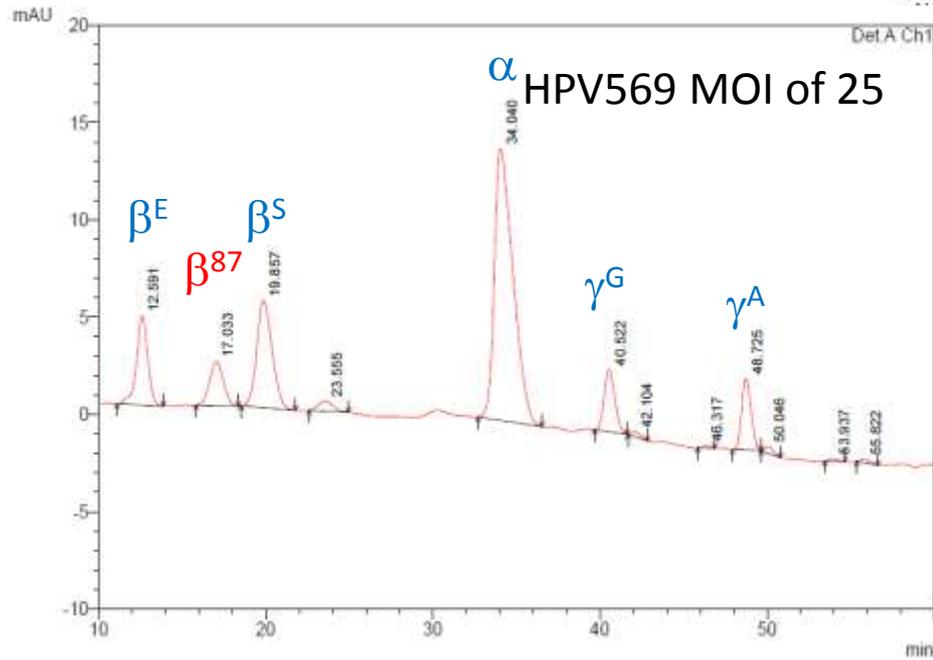
BB305 yields improved transduction than HPV569



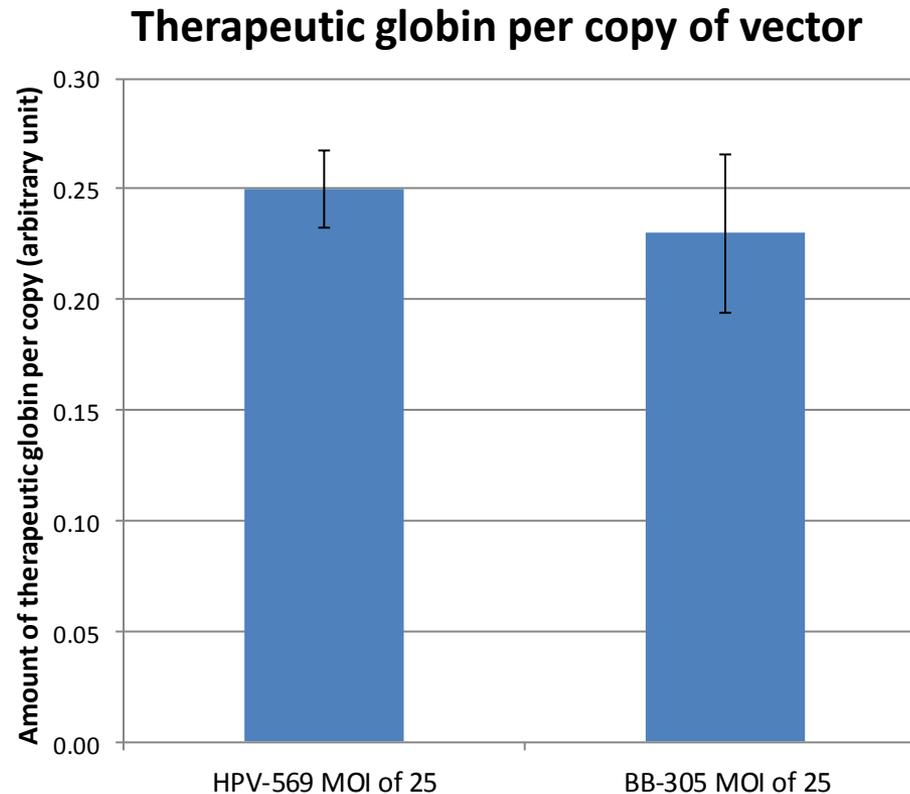
BB305 provides 2x transduction of BFU-E compared to HPV569

# HPLC Analysis of Globin Chains in Pooled BFU-E Colonies

## BB305 yields identical expression with greater transduction vs. HPV569



# $\beta^{A-T87Q}$ -globin expression per copy of vector



The amounts of  $\beta^{A-T87Q}$ -globin produced per copy of vector are not statistically different between the two vectors.

# *IVIM genotoxicity assay for globin vectors*

## **Objectives:**

- Assess genotoxicity of HPV569 and BB305 vectors in murine primary hematopoietic cells in vitro
- Compare genotoxicity of test vectors with established positive and negative controls

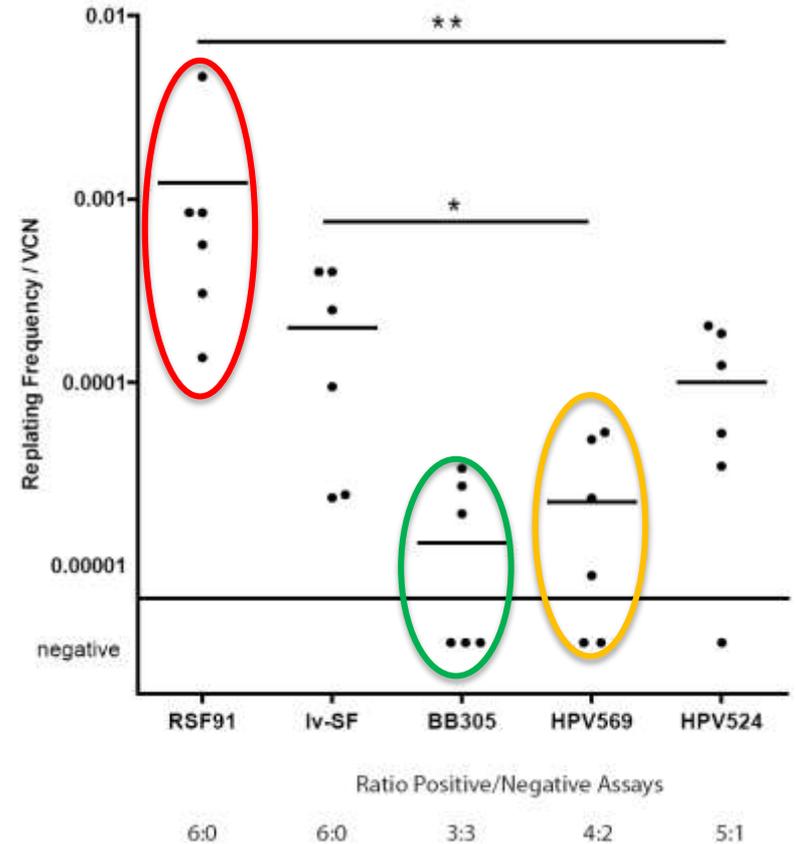
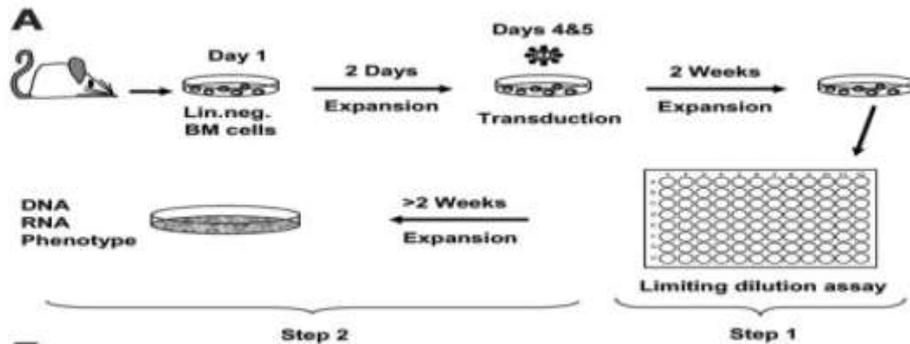
## **Experimental set up:**

- Negative control: Mock transduced cells receiving no viral vector
- Positive control#1: RSF91.GFPgPRE ( $\gamma$ -retroviral vector – referred to as **RSF91**)
- Positive control#2: RRL.PPT.SF.eGFP.pre (lentiviral vector – referred to as **lv-SF**)
- Test Vectors: Lenti-Globins:BB305, HPV569

# Improved Safety of Lentivirus Vectors

## LentiGlobin BB305 vector demonstrates strong safety profile

LentiGlobin BB305 demonstrates lowest IVIM activity, nearly 100x < positive control and equivalent to LentiGlobin HPV569 lentiviral vector



# Nonclinical Conclusions

- The LentiGlobin vector has been redesigned to create LentiGlobin BB305:
  - Improved vector titer
  - Significantly higher transduction efficiency in CD34+ cells
  - Equivalent globin expression per vector copy
  - In vitro safety has been demonstrated in IVIM assays
  - On-going in vivo single dose toxicology study in thalassemic mice
    - No excessive mortality in the “in life” part of the study
    - No gross findings at necropsy
- Data supports proceeding with HGB-204 in  $\beta$ -Thalassemia major patients

# $\beta$ -Thalassemia Clinical Development Plan Summary

- ***Our goal is to reduce/eliminate the need for blood transfusions by introducing the  $\beta^{A-T87Q}$ -globin gene into a patient's own stem cells***
- ***Autologous transplantation avoids GVHD and rejection***
- ***Initial Subject data published in Nature (Cavazzana-Calvo et al., 2010)***
- ***Improved vector and transduction process for HGB-204***

**nature**

September 2010

## **Transfusion independence and *HMGA2* activation after gene therapy of human $\beta$ -thalassaemia**

Marina Cavazzana-Calvo<sup>1,2\*</sup>, Emmanuel Payen<sup>3,4,5\*</sup>, Olivier Negre<sup>3,4,5,6</sup>, Gary Wang<sup>7</sup>, Kathleen Hehir<sup>8</sup>, Floriane Fusil<sup>3,4,5</sup>, Julian Down<sup>8</sup>, Maria Denaro<sup>8</sup>, Troy Brady<sup>7</sup>, Karen Westerman<sup>8,9</sup>, Resy Cavallesco<sup>9</sup>, Beatrix Gillet-Legrand<sup>6</sup>, Laure Caccavelli<sup>1,2</sup>, Riccardo Sgarra<sup>10</sup>, Leila Maouche-Chrétien<sup>3,4</sup>, Françoise Bernaudin<sup>11</sup>, Robert Girot<sup>12</sup>, Ronald Dorazio<sup>8</sup>, Geert-Jan Mulder<sup>8</sup>, Axel Polack<sup>8</sup>, Arthur Bank<sup>13</sup>, Jean Soulier<sup>5</sup>, Jérôme Larghero<sup>5</sup>, Nabil Kabbara<sup>5</sup>, Bruno Dalle<sup>5</sup>, Bernard Gourmel<sup>5</sup>, Gérard Socie<sup>5</sup>, Stany Chrétien<sup>3,4,9</sup>, Nathalie Cartier<sup>14</sup>, Patrick Aubourg<sup>14</sup>, Alain Fischer<sup>1,2</sup>, Kenneth Cornetta<sup>15</sup>, Frédéric Galacteros<sup>16</sup>, Yves Beuzard<sup>3,4,5</sup>, Eliane Gluckman<sup>5</sup>, Frederick Bushman<sup>7</sup>, Salima Hacein-Bey-Abina<sup>1,2\*</sup> & Philippe Leboulch<sup>3,4,9\*</sup>

# Pre-transplant clinical history

## Subject 1003 with $\beta^E/\beta^0$ -thalassemia major (LG001)

- Then 18 year old male with severe  $\beta^E/\beta^0$ -thalassemia and no HPFH or  $\alpha$  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  $\mu\text{mol/g}$ ).
- No related, genotypical HLA-matched donor. Match strict inclusion and exclusion criteria.



Transplantation at age 19 on June 7, 2007 using LentiGlobin HPV569

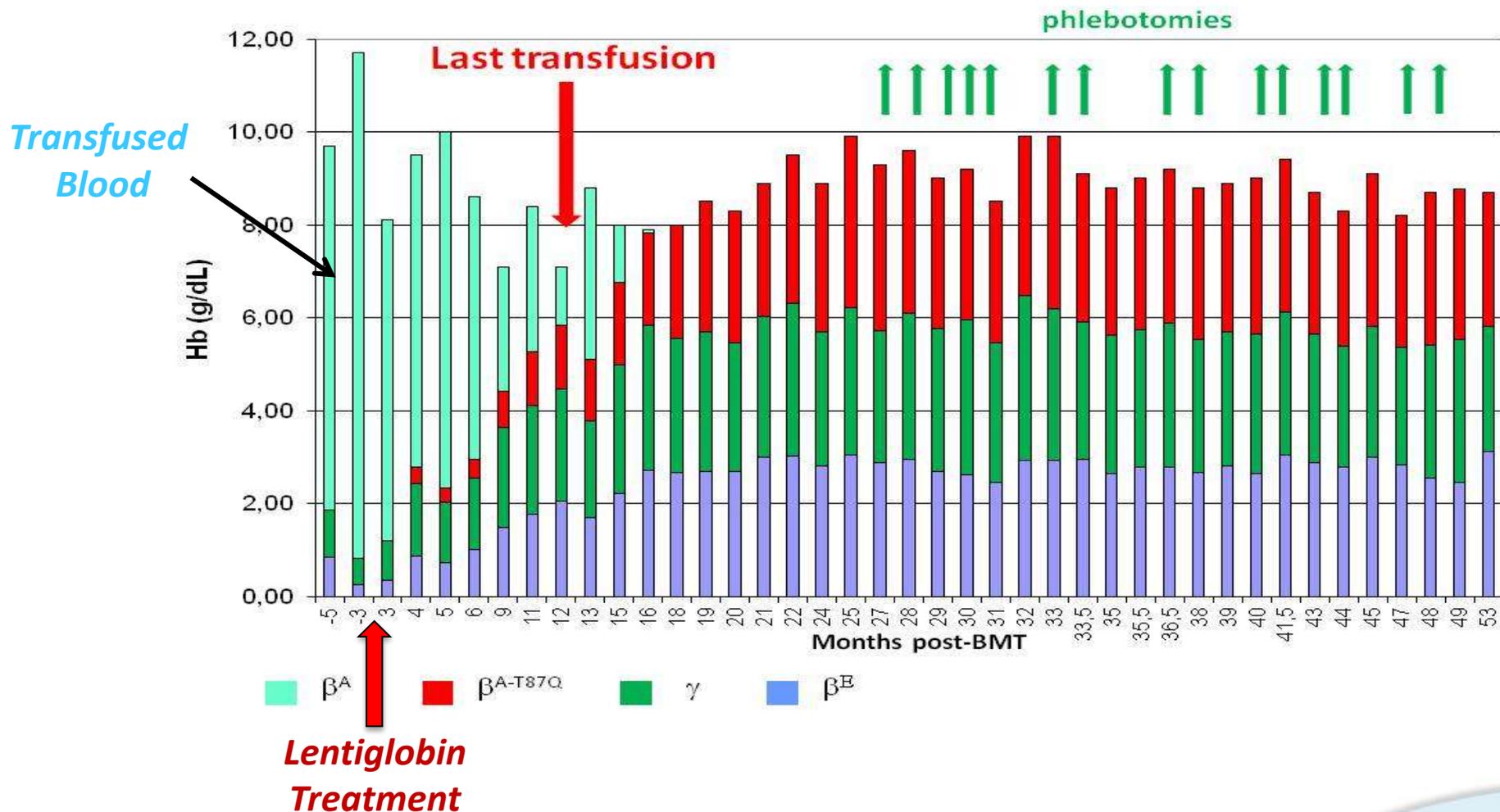
# Promising Efficacy in $\beta$ -Thalassemia

1<sup>st</sup> patient transfusion independent for 4 years (Subject 1003 – LG001)

nature

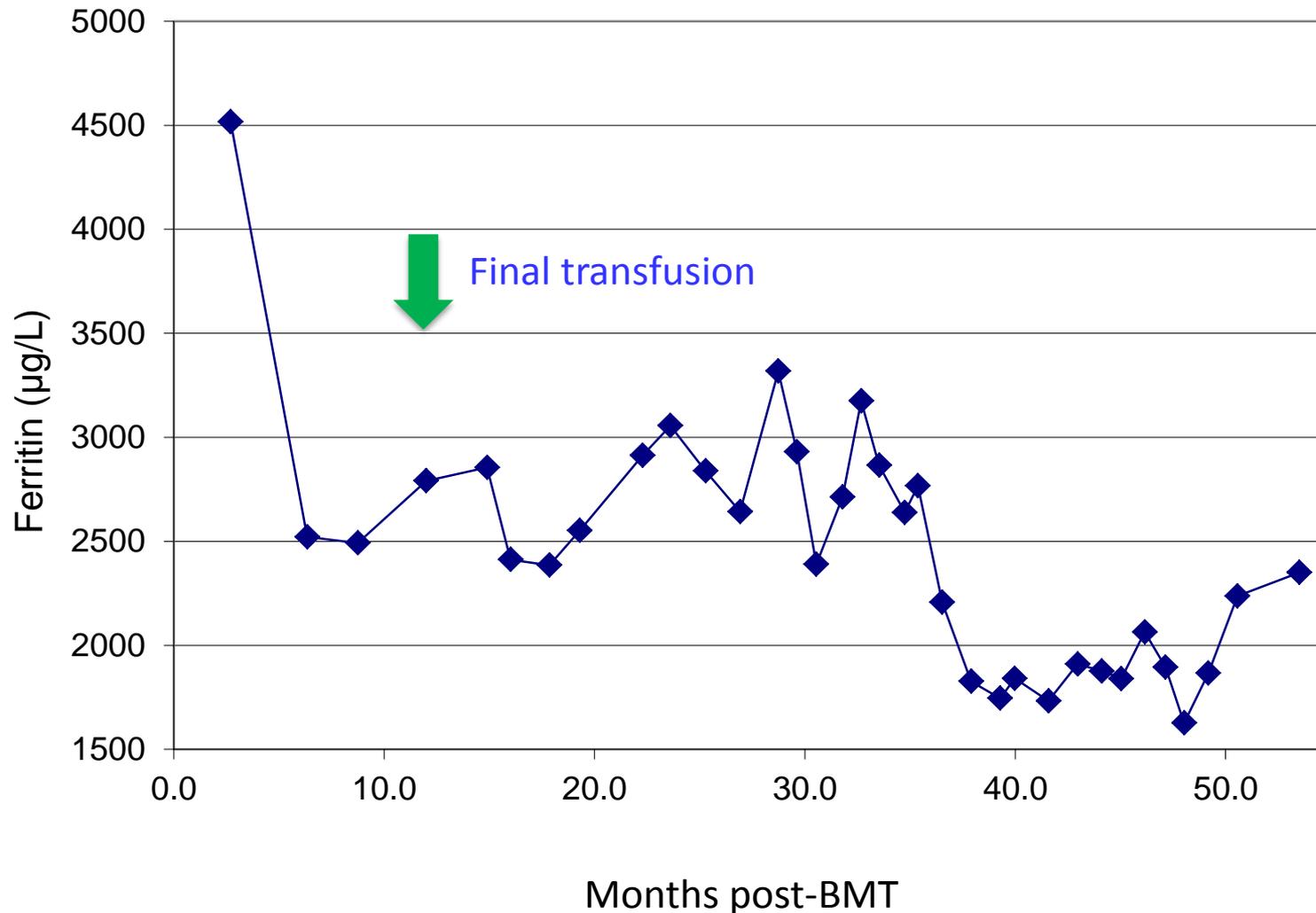
Published  
Sept 2010

Hemoglobin concentrations in blood



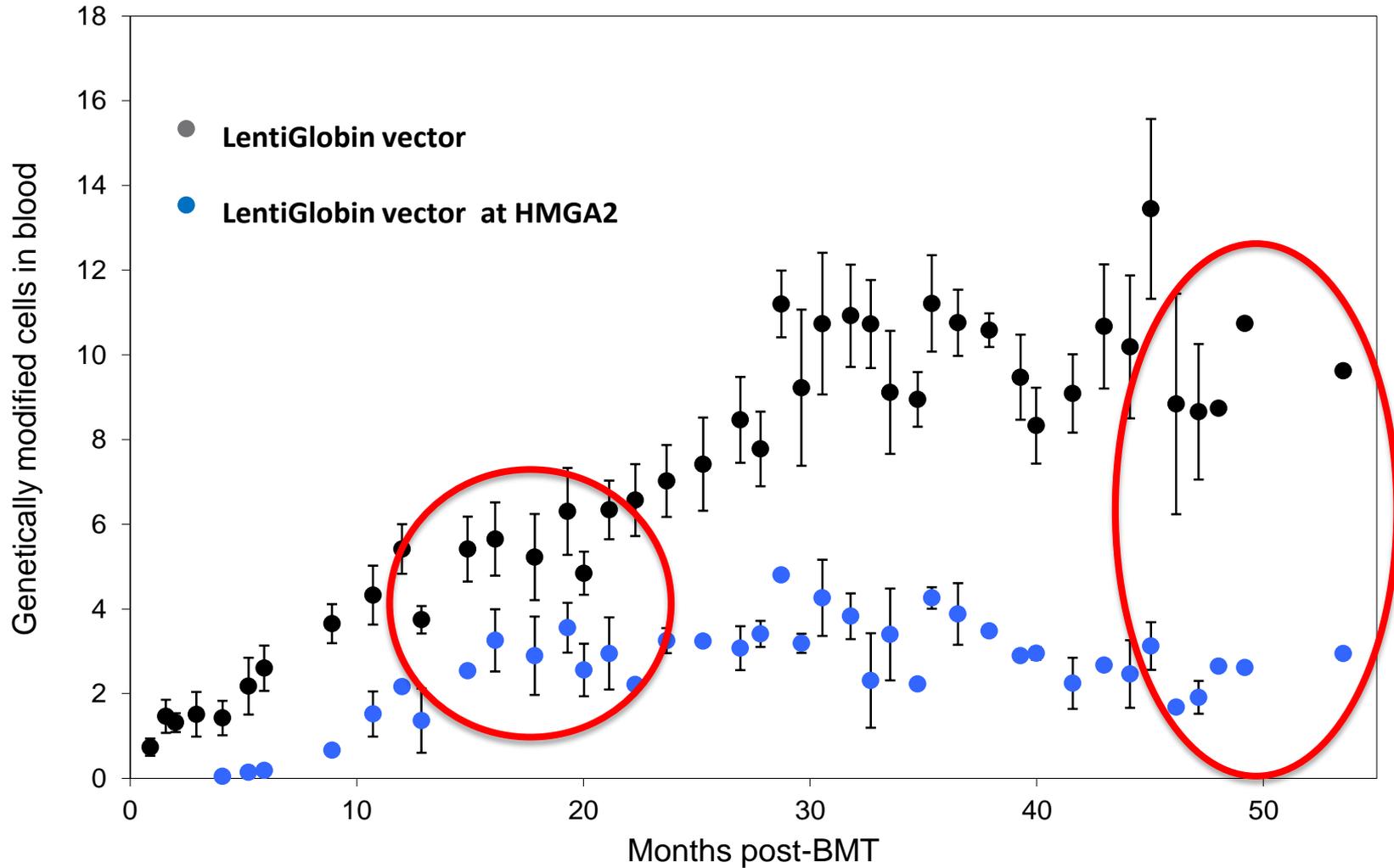
# Decreasing Ferritin concentration in patients blood

*Stopped phlebotomy at Month 48*



# Proportion of genetically modified cells in blood

*Percentage of HMGA2 expanded clone is decreasing over time*





# HMGA2 IS is frequently retrieved by DNA pyrosequencing in vivo after retroviral and lentiviral human CD34+ gene transfer

## HMGA2 in X-SCID trial ( $\gamma$ -RV vector)

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

- several in HMGA2 Intron 3
- several with tendency to increase with time and then stabilize
- 2 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

## Wiskott Aldrich trial ( $\gamma$ -RV vector)

## MGMT glioblastoma trial ( $\gamma$ -RV vector)

# $\beta$ -Thalassemia Subject 1004 Treated Nov 2011 in LG001 (LentiGlobin HPV569 Lentiviral Vector)

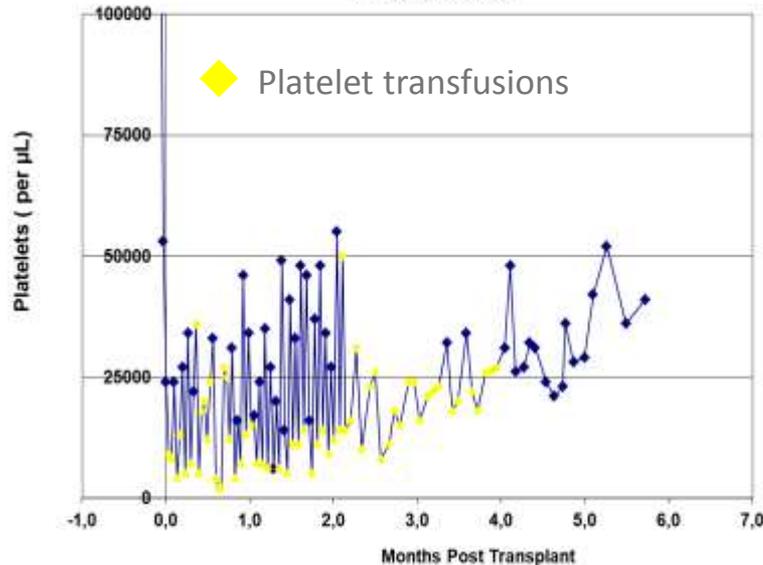
- 23 yr old woman,  $\beta^E/\beta^0$ -Thal Major
- Transfusion dependent since her 2<sup>nd</sup> month of life. No HLA matched sibling donor
- Transplantation was uneventful. Engrafted neutrophils by day 22 and had delayed platelet reconstitution (no related complications)
- Has returned to full time work
- Red blood cell transfusions are now being tapered down to stimulate erythropoiesis and increase  $\beta^{A-T87Q}$ -globin production

Prof. Marina Cavazzana-Calvo, Principal Investigator

Prof. Philippe Leboulch, Study Director

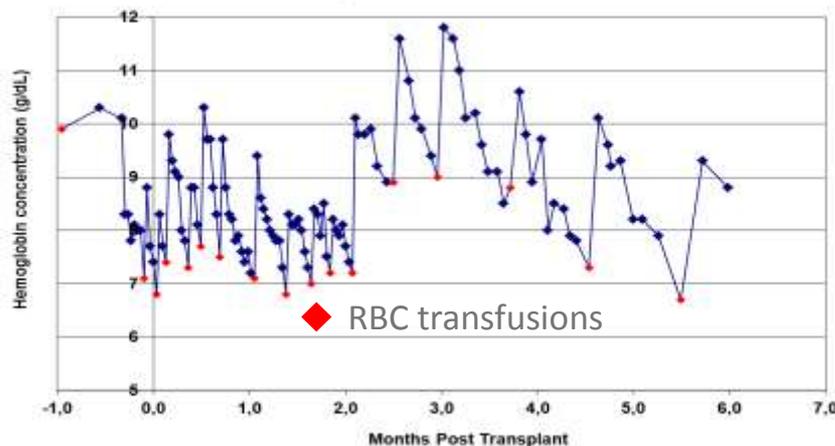
# Subject 1004 is clinically stable with improving hematologic parameters

Patient #1004  
Platelet Count

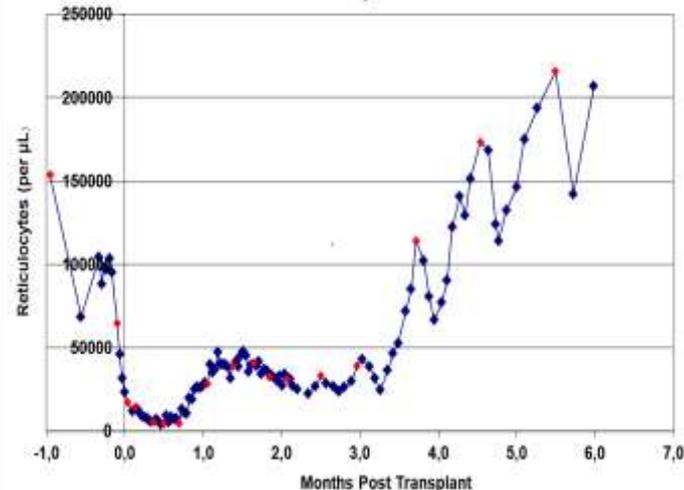


- Platelet count recovering
- Frequency of RBC transfusions decreased to stimulate hematopoiesis
  - Associated rise in reticulocyte count

Patient #1004  
Hemoglobin concentration

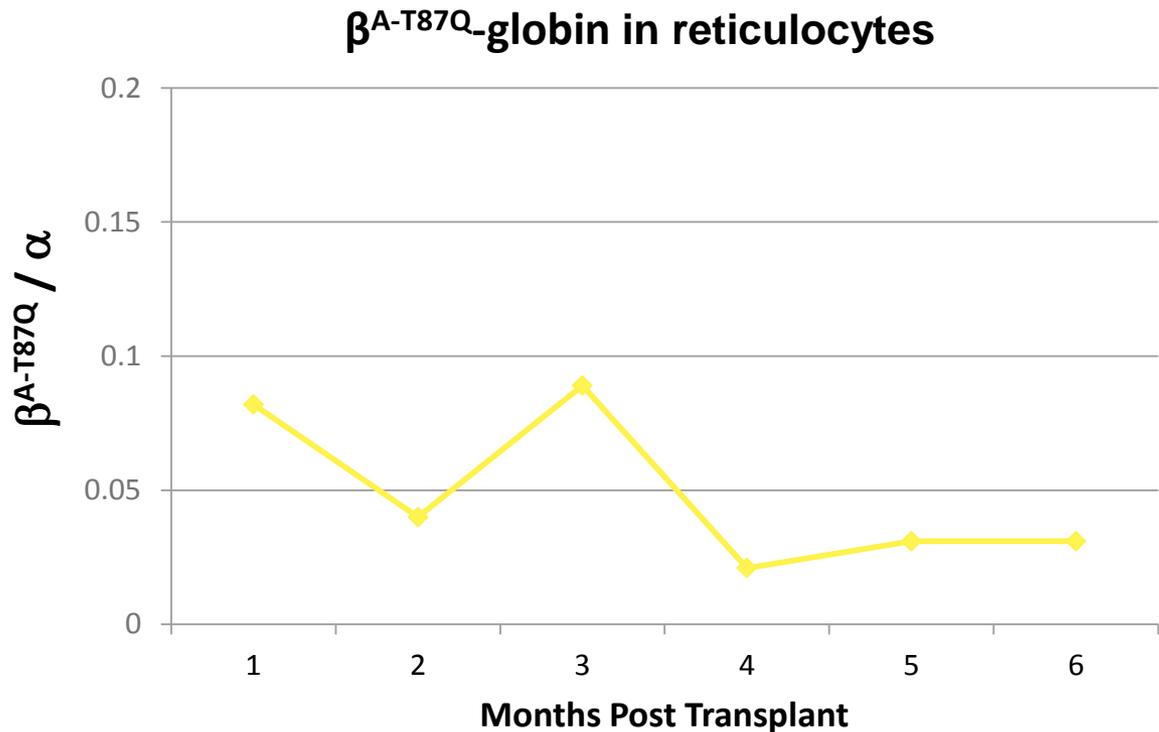


Patient #1004  
Reticulocyte count



# Subject 1004 $\beta^{A-T87Q}$ globin levels are stable

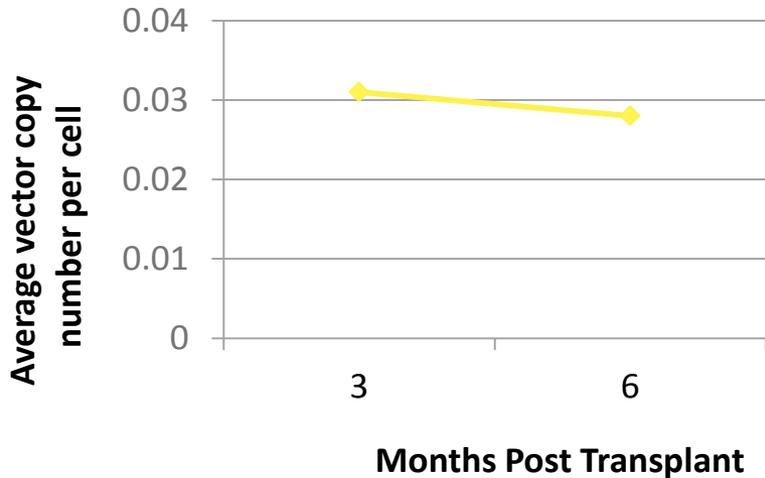
## First detection in whole blood



- Stable levels of  $\beta^{A-T87Q}$ -globin in reticulocytes
- Trace of  $\beta^{A-T87Q}$  detectable by HPLC in whole blood at Month 6
- Levels similar to those detected in Subject 1003 at same time points

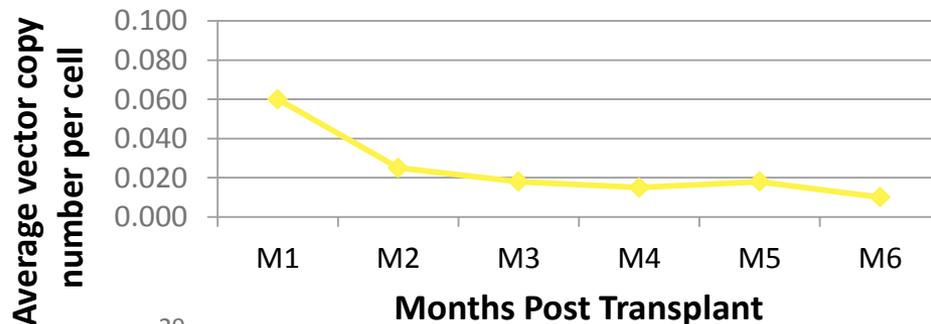
# Stable Vector Copy Number in Subject 1004

## Average Vector Copy Number in Neutrophils

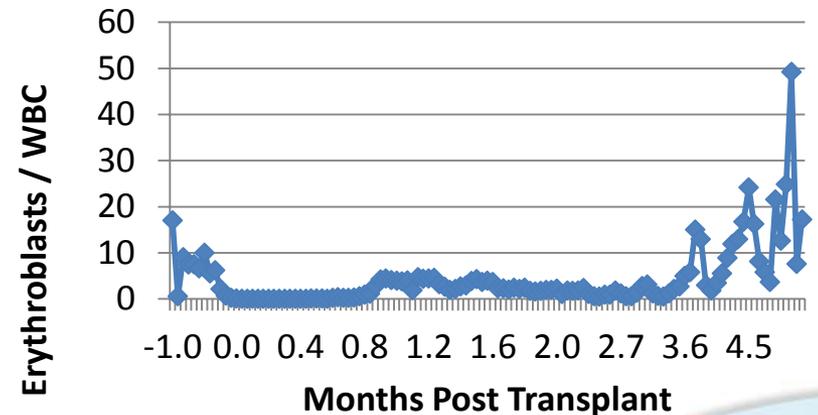


- Stable VCN in neutrophils
- Month 6 dip in whole blood VCN due to dramatic rise in peripheral erythroblasts
- Erythroblasts undergoing normal differentiation remain in bone marrow, while those circulating are largely “uncorrected” and dysfunctional

## Average Vector Copy Number in Whole Blood



## Erythroblasts/WBC



# Clinical Conclusions

- Long term safety and efficacy observed in Subject 1003 treated with LentiGlobin HPV569 lentiviral vector in clinical study LG001
- Subject 1004 has been treated and successfully engrafted
- Early gene marking in Subject 1004 is similar to previously treated subject

# Overview of HGB-204 Clinical Protocol

A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy in Subjects with  $\beta$ -Thalassemia Major by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral  $\beta^{A-T87Q}$ -Globin Vector (LentiGlobin<sup>®</sup> BB305 Drug Product)

## Eligibility Criteria

- **Adults ages 18-35 (n=15)**
- **Diagnosis of  $\beta$ -thalassemia requiring  $\geq 100$  mL/kg/year of packed RBCs or  $\geq 8$  transfusions of pRBCs per year for the prior 2 years**
- **Eligible for allogeneic bone marrow transplant**

### Exclusions

- Availability of a willing 10 / 10 HLA matched HSCT donor
- Presence of Pesaro Class III (poor compliance of iron chelation therapy, hepatomegaly and liver fibrosis)
- Severe iron overload, which in opinion of physician is ground for exclusion

### Efficacy Endpoints

- Maintenance of total Hb  $\geq 8.5$  g/dL for 6 months (between M18 and M24)
- RBC transfusion requirements
- Assessment of dyserythropoiesis by analysis of reticulocytes and erythroblasts
- Vector marking and  $\beta^{A-T87Q}$ -globin expression in erythroid progenitors and red blood cells

### Safety Endpoints

- Success of engraftment, transplant related events, overall survival
- Integration site analyses and monitoring for clonal skewing and leukemia

# ACKNOWLEDGEMENTS

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Yves Beuzard

Christopher Baum

Michael Rothe

Rick Bushman



# Additional Responses to Reviewers Comments

## Overview

### Outline

1

*Clinical*

2

*Preclinical*

3

*Consent*

# PATIENT POPULATION FOR CLINICAL TRIAL HGB-204

“With overall and disease free survival rates: 91% and 88%, respectively, for patients with Pesaro risk class II with available HLA-matched sibling donors, why include these patients at this time?”

“Since conventional treatment is effective for many patients, are there ways to limit subject selection to those most likely to be at risk with conventional therapy? Poor adherence to chelation therapy is apparently a major reason for early death, but it is also an exclusion criterion for the study. Does this mean that subjects are selected from among those who might do well with conventional treatments?”

- Patients with  $\beta$ -thalassemia major requiring  $\geq 8$  transfusions per year
- Patients with available 10/10 HLA-matched hematopoietic stem cell donor will be excluded
- Patients with poor compliance will also be excluded because they are not likely to be compliant with intensive monitoring required in HGB-204 and because iron overload leads to diffuse tissue damage predicting poor outcome from transplantation.

# UMBILICAL CORD BLOOD TRANSPLANT (UCBT)

Since unrelated umbilical cord blood has had some success, what makes the proposed intervention a potentially better treatment than cord blood? Is there a way to recruit subjects who are unlikely to benefit from cord blood transplants?

HGB-204 is restricted to adults with  $\beta$ -thalassemia major

Main factor limiting use of unrelated UCBT in adults is UCB low number of progenitor cells

Because of low number of HSCs in UCBT, patients may experience increased risk of early fatal complications due to lower engraftment rate of donor hematopoiesis, delayed kinetics of neutrophil recovery, and lack of adoptive transfer of pathogen-specific memory T-cells since UCB lymphocytes are naïve cells

Increasing cell dose with dual UCBT may improve engraftment, especially in adults, though at a cost of increased GVHD. However, little published evidence is currently available on the long-term immune reconstitution and clinical benefit of dual UCBT in non-malignant diseases in patients without an HLA-matched sibling donor (Sideri et al.2011).

Consequently, at this time it is not possible to define a population of adult patients with  $\beta$ -thalassemia major who should be excluded from the trial to receive UCBT.

# DETERMINING RELATIVE ABUNDANCE OF ABERRANT TRANSCRIPTS IN PATIENT SAMPLES

How will this study remain vigilant for aberrant events? ... DNA-based analysis will not necessarily detect potential transcriptional abnormalities. ... Using LAM PCR on reverse transcribed RNA could be considered.

If clonal analysis of peripheral blood with gene marking in  $> 1\%$  of the white blood cells reveals that greater than 1/5th ( $>20\%$ ) of the gene modified population is derived from a single clone, LAM PCR and 454 pyrosequencing will be carried out to assess insertion site sequence of the clone and the location of the site in relation to known gene loci.

If the target gene is known to control differentiation, proliferation, or identified within the Retrovirus Tagged Cancer Gene Database (RTCGD), we will initiate an analysis of whether insertion leads to aberrant transcripts of these targeted genes.

# IMPACT OF POTENTIAL CLONAL SKEWING/DOMINANCE ON CONTINUATION OF HGB-204

Are there any specific criteria such as static percentage or changes over time that would lead to stopping the trial? Or will this be evaluated on a case-by-case basis with respect to the specific cell type experiencing clonal outgrowth? Does the insertional event in the HMGA2 gene in the French trial constitute an “insertional mutagenesis” even that would stop this study?

Decisions on suspending or stopping enrollment for clonal skewing in the absence of overt malignancy will be made on a case-by-case basis in collaboration with the Principal Investigators at the clinical sites, the study Data Monitoring Committee (DMC), the regulatory authorities and the Sponsor.

The clonal skewing associated with the HMGA2 integration event in Subject 1003 stabilized at a relatively low percentage of peripheral blood nucleated cells (2-3%) and has not been associated with any adverse events over five years of follow-up. As such, this has not been considered a stopping event, and a similar perspective would be applied to HGB-204.

# POTENTIAL ENROLLMENT SUSPENSION CRITERIA RELATED TO POTENTIAL INSERTIONAL ONCOGENESIS

“The protocol states that detection of leukemia/lymphoma due to vector-mediated insertional oncogenesis will be one criterion for temporary suspension of the protocol. The investigators should also consider clonality in any patient as a criterion for suspension/evaluation. Although there is no evidence of progression to any disease in the 1 French patient with clonal expansion likely due to integration in the HMGA2 gene, given that the current vector has not yet been tested in human populations there is still potential for integration site effects at other loci that could be oncogenic.”

Clonal skewing in the absence of overt malignancy will be carefully assessed, and decisions to suspend or stop enrollment made in collaboration with the Principal Investigators at the clinical sites, the study Data Monitoring Committee (DMC), regulatory authorities and the Sponsor.

# SUBSEQUENT ALLOGENEIC HSCT

Is there reason why inclusion in this study would preclude a subject from receiving subsequent allogeneic hematopoietic stem cell transplantation from an appropriately matched bone marrow donor? For example, perhaps a once unwilling donor sibling may become a willing donor after witnessing an unsuccessful attempt at gene therapy.

While it would be possible for a patient to receive a subsequent allogeneic hematopoietic stem cell transplant following participation in this trial, the repeated exposure to myeloablative chemotherapy could increase the risk of adverse events. This procedure would fall outside of the HGB-204 study, and the treating physician will be responsible for the decision whether to proceed with allogeneic transplant.

# POTENTIAL CHALLENGES WITH MOBILIZATION

Is it known if mobilized HSCs (or bone marrow HSCs) from a person with beta-thalassemia major are particularly more difficult to harvest or to transplant? If so, perhaps this information should be included in the informed consent document.

Mobilization of sufficient CD34+ hematopoietic stem cells from adult patients with  $\beta$ -thalassemia major using G-CSF or plerixafor is achievable.

In a recent study (Yannaki et al. 2012) assessing mobilization of peripheral CD34+ hematopoietic stem cells with G-CSF or plerixafor in patients with severe  $\beta$ -thalassemia, it was shown that in splenectomized patients G-CSF could result in hyperleukocytosis requiring a reduction in G-CSF dose and consequently prolonging the time required to collect sufficient CD34+ cells.

This information will be added to the Informed Consent.

# KEY ELEMENTS IN CRITERIA USED FOR RELEASE

I would appreciate knowing the (non-proprietary) key elements in the criteria used to release the lentiviral vector and drug product (transduced) cells that are in Tables 3 and 4 of Appendix M.

## Key elements for lentiviral vector release:

- Infectious titer
- Vector Expression
- Absence of Replication Competent Lentivirus (RCL)

## Key elements for of Drug Product (transduced cells) release :

- Transduction efficiency in CD34+ cells
- $\beta^{A-t87Q}$ -globin protein expression in patient bulk erythroid cell progeny
- Cell count and viability

## TRANSDUCTION EFFICIENCY (1 of 2)

“...It was [not] clear what the criteria were for determining whether sufficient numbers of HSC were transduced prior to engraftment. What is the cutoff (both minimum and maximum [...]) for determining whether sufficient numbers of cells have been transduced prior to engraftment? How has this cutoff been determined? [...]

Studies in allogeneic transplant suggest that as little as 10-15% mixed donor chimerism results in transfusion independence (Alfred & Vora, 2011; Andreani *et al.*, 2011). As the cells in an allogeneic transplant contain two gene copies, one would anticipate a vector copy number of 0.3 should be sufficient for transfusion independence; however, as the vector level of expression is only 70% (Cavazzana-Calvo *et al.*, 2010) of the endogeneous gene per copy, we have increased the minimum vector copy number for release of the transduced cells (drug product) to 0.5.

The maximum vector copy number for release of transduced cells (drug product) has been set at a conventionally adopted upper target of 3, which is thought to minimize the risks of insertional oncogenesis.

# TRANSDUCTION EFFICIENCY (2 of 2)

Will they attempt to correlate initial transduction efficiency with final Hb levels [...]? Is there any way to measure the actual transduction frequency [...] between the different patient samples[...]? Copy number/cell could have an impact on integration site effects.”

In study LG001, transduced cells from Subject 1003 had a mean vector copy number (VCN) of 0.6 at release (qPCR Day 7) and most recently (Month 55 post-transplant), the mean vector copy number from his peripheral blood was 0.105. This Subject has shown clinical benefit five years after transplant with no gene therapy associated serious adverse events.

Correlates of biomarkers and clinical outcome, including correlation of initial transduction efficiency with Hb levels, will be assessed. HGB-204 is an open label study and on-going analysis of data will occur and could influence conduct of the trial.

We will measure integrated vector copy number (VCN) in CD34+ cells pre-infusion for drug product release by assessment of mean vector copy number by qPCR in Day 7 bulk cultures.

During conduct of the clinical study we will determine mean VCN in peripheral blood nucleated cells by qPCR. Through the use of appropriate reference standards and assay controls, we will assess relationship between VCN at infusion and long-term Hb expression.

We can compare mean VCN by pPCR in peripheral blood between different subjects who will be enrolled in the clinical trial.

# DRUG PRODUCT MANUFACTURING SITE

The study will be performed at 3 different sites. Will the drug product (HSC vector transductions and analyses) be performed at the different sites or at a single site? If the former, is there an SOP in place for use at the different sites?

The LentiGlobin BB305 Drug Product manufacturing process (transduction of autologous hematopoietic stem cells with lentiviral vector LentiGlobin BB305) will be conducted at a single manufacturing site.

Manufacturing process will be conducted under cGMP conditions at the contract manufacturing organization in the USA.

All analysis associated with in-process testing for the Drug Product manufacturing process will also be conducted this facility.

# INTEGRATION SITE ANALYSIS IN IVIM ASSAYS

I understand the data presented in Figure 15 of Appendix M, in the in vitro immortalization assay, there was increased replating frequency in HPV-569 transduced cells. Has any integration site or transcript analysis been performed on these cells to look for clonal dominance or read-through transcription?

The slight increase in re-plating frequency with LentiGlobin HPV569 lentiviral vector observed in the IVIM assay was well within the assay variance and was not statistically significant. However the integration sites of the expanded clones for both vectors are being analyzed and will be carefully compared and evaluated.

# $\beta^E$ -GLOBIN EXPRESSION

“On page 58 of Appendix M, the investigators state the expression of the E globin transgene is exclusively in the erythroid lineage. Has this actually been tested with HPV-569?”

The  $\beta^E$ -globin gene expressed in the patient is the patient's endogenous  $\beta^E$ -globin gene and it is not expressed from the LentiGlobin lentiviral vector. Both LentiGlobin HPV569 and BB305 lentiviral vectors are expressing the  $\beta^{A-T87Q}$ -globin variant protein.

$\beta^E$ -globin is by definition expressed only in erythroid cells: this is the patient's endogenous globin chain.

# COMMENTS REGARDING CONSENT FORM

- Changes requested with regards to the wording of the Informed Consent will be implemented.
- Clarifications requested will be made. The corresponding sections of the Informed Consent will be revised accordingly.

***End of Presentation***