Ann & Robert H. Lurie
Children's Hospital of Chicago™

# Gene Therapy and Other Curative Approaches

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BEST CHILDREN'S HOSPITALS U.S.News HONOR ROLL 2012-13

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# Disclosures

| | |
|---|---|
| • Research | Novartis, GlaxoSmithKline, Amgen, Eli Lilly, Shire, bluebird bio |
| • Consultant | Novartis, ApoPharma, Baxter |
| • Speaker's Bureau | None |
| • Equity Ownership | None |
| • Royalties | None |

No discussion of off-label indications

# Objectives

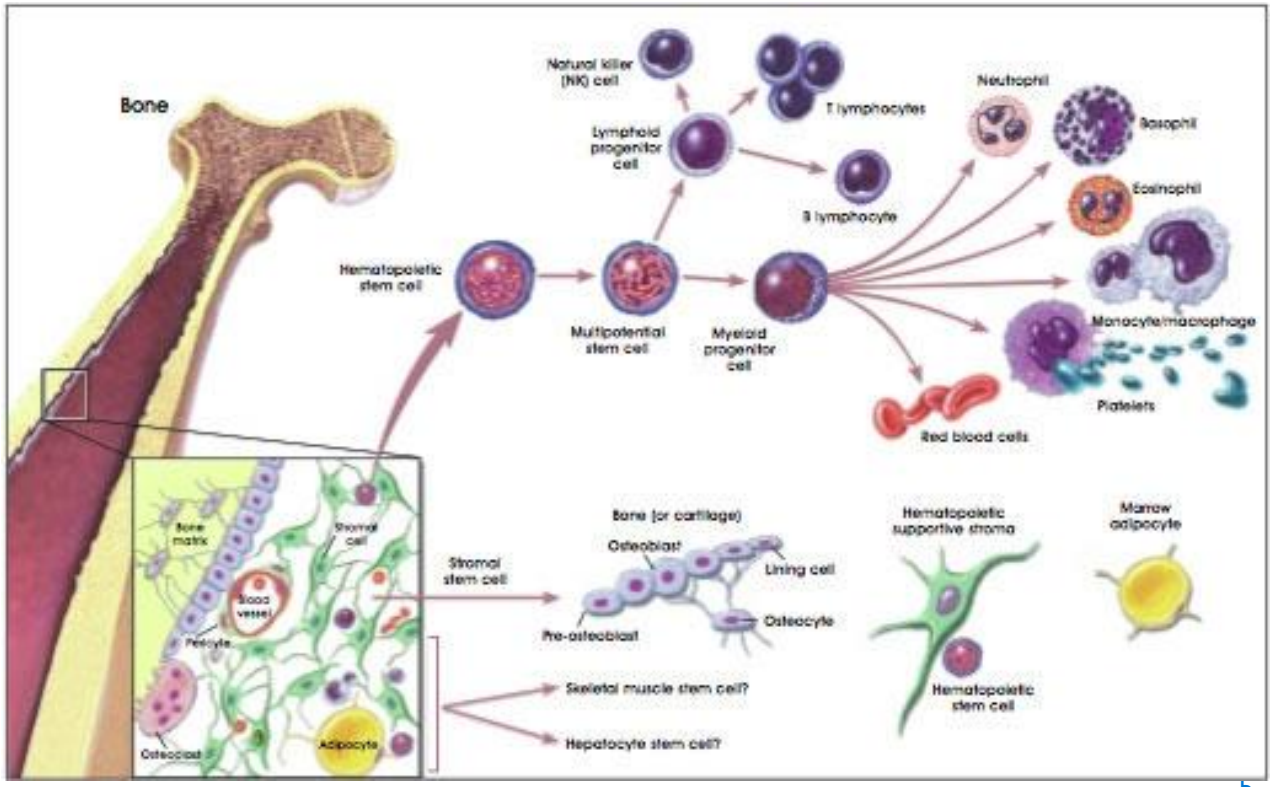
- Review rationale for stem cell transplant in thalassemia
- Describe the different types of transplant procedures
- Examine stem cell transplant outcomes
- Describe the current gene therapy strategies
- Recent results
- Future directions

# Rationale for Stem Cell Transplant

- Assist in recovery from toxic treatments
  - Cancer, leukemia
- Vehicle for delivery of a protein or gene product not necessarily related to blood production
  - Hemophilia
- Replacement of defective stem cells
  - Thalassemia

# Terminology

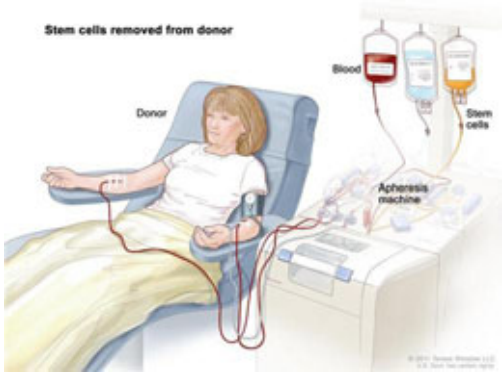
- **What Is a Hematopoietic Stem Cell?**
- A hematopoietic stem cell is a cell isolated from the blood or bone marrow that can renew itself, can differentiate to a variety of specialized cells, can mobilize out of the bone marrow into circulating blood
  - Sources
    - Bone Marrow
    - Peripheral Blood
    - Umbilical Cord



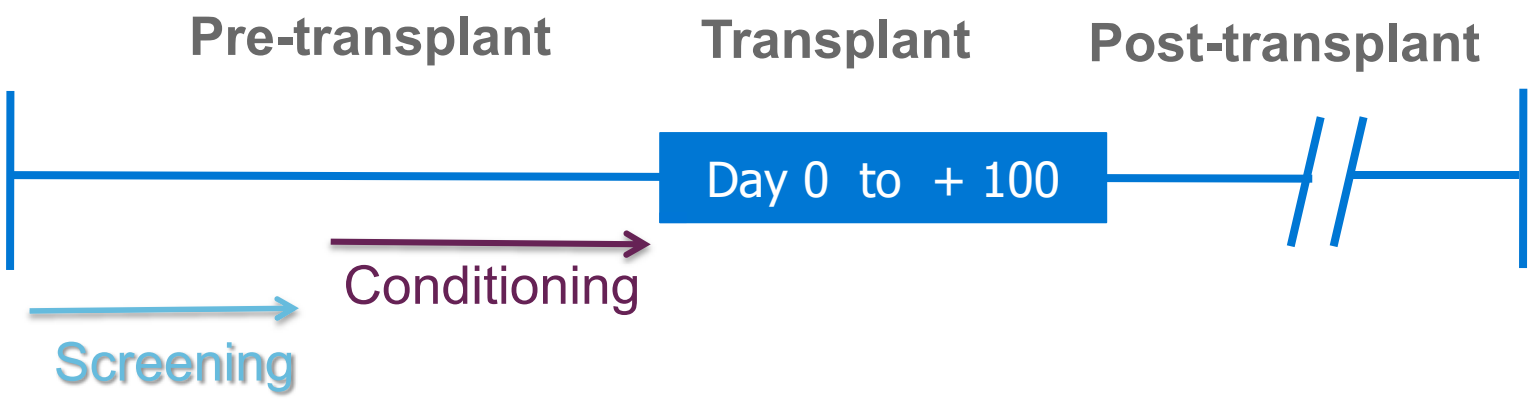

http://stemcells.nih.gov/info/scireport/pages/chapter5.aspx

# Terminology

- Types of Transplantation
  - Uses patient's own cells (Autologous)
  - Stems cells from another source (Allogeneic)
- Sources of allogeneic stem cells
  - Sibling
  - Parent
  - Unrelated donor
- Preparation (conditioning)
  - Medication (chemotherapy) or radiation that will destroy the defective cells, make room for new cells in the marrow space and/or suppress the host immune system
  - Types of Conditioning:
    - Complete marrow and immune replacement (myeloablative)
    - Reduced intensity (nonmyeloablative)

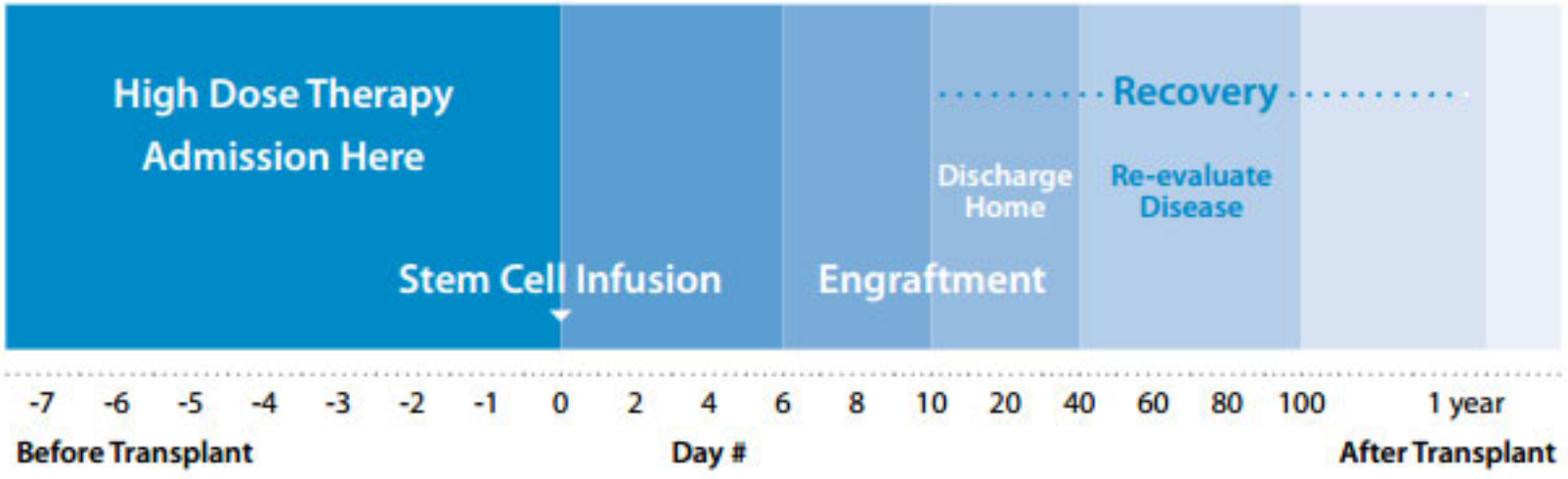


# Transplant Timeline




- Length of each phase will vary with transplant type
- Complications related to transplant type and timeline

**This diagram reflects a general overview of the transplant process:**




## Early Side Effects

- Mouth Sores
- Anemia
- Nausea Vomiting
- Hair Loss
- Loss of Appetite
- Fevers

## Late Side Effects

- Rejection
- Infection
  - Bacterial
  - Viral
  - Fungal
- GVHD
  - Skin
  - Liver
  - Gut

## Long term Effects

- Disease recurrence
- Chronic liver disease
- Malignancies
- Infertility

# The Transplant "Wish List"

- Minimal early and late side effects
- Early recovery of immune
- Low risk of transplant associated deaths, low organ toxicity
- High disease free survival with good Quality of Life
- Low graft rejection
- Low or NO Graft vs Host Disease (GVHD)
- Fertility preservation

# Thalassemia Transplant Goals

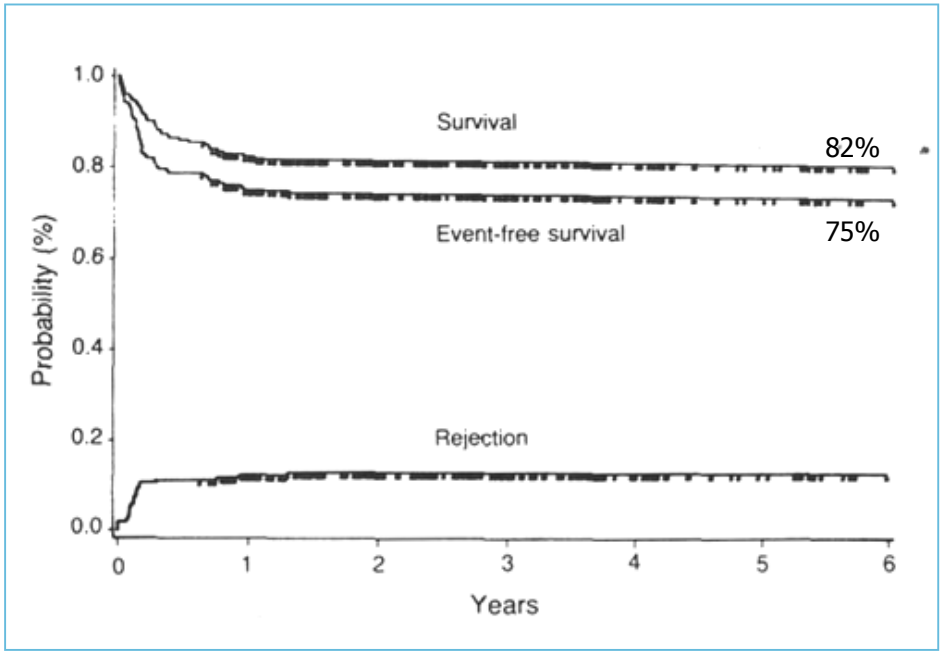
- To develop a lower toxicity regimen that can support stable engraftment in non-malignant disorders
- Limit the incidence of GVHD
- Decrease the incidence of late transplant related complications
  - Infertility
  - Chronic GVHD
  - Transplant associated malignancies

# A Balancing Act

- Transplant strategies are aimed at optimizing conditioning regimen in relation to stem cell source
- Sibling donors have superior results but most people will not have a family match
- A non-ablative approach may have fewer side effects, *but* leads to a high rate of graft rejection especially in the heavily transfused patients, patients with red cell antibodies and normal immune systems

Horan J et al. BMT 2005, Jacobsohn D et al. Lancet 2004, Iannone R et al. BBMT 2003

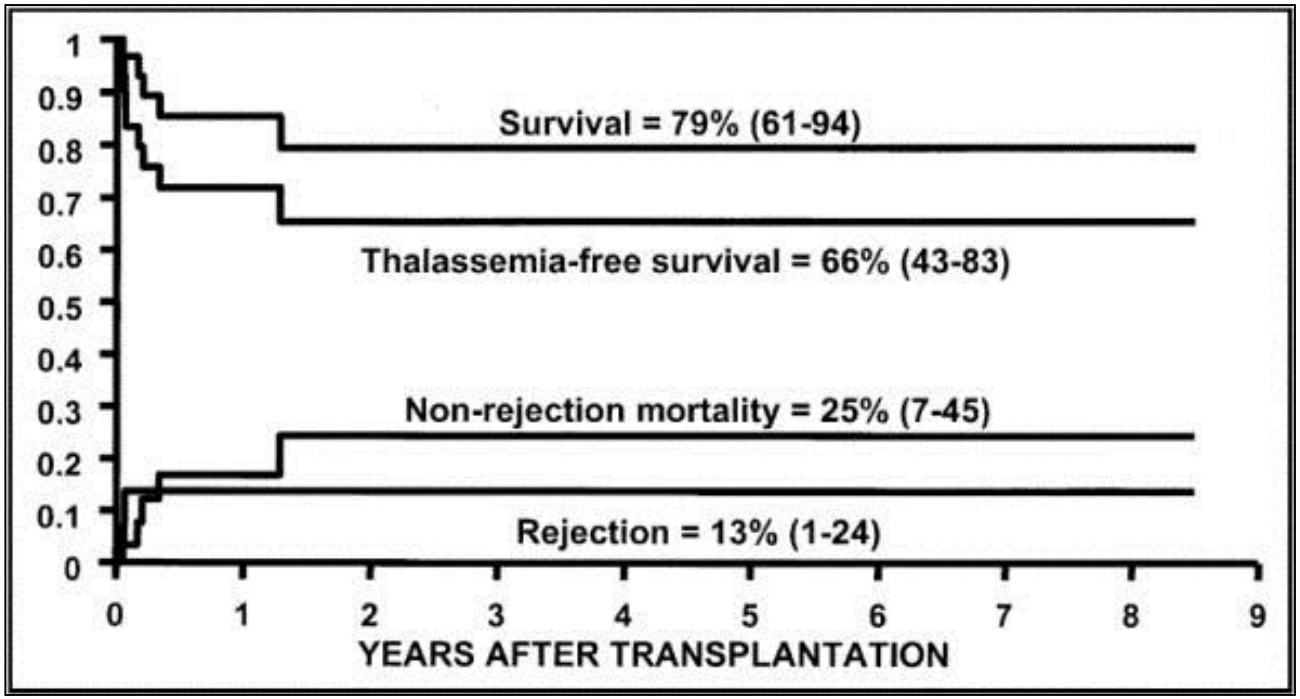
# BMT in ß Thalassemia




- Standard care SCT with HLA-matched sibling donors
  - Risk stratification based on adequacy of chelation, hepatomegaly and hepatic fibrosis
  - Myeloablative vs reduced intensity
  - Intensified conditioning for high risk patients

Lucarelli G, et al NEJM 1990; 322:417-21

# Stem Cell Source: Matched Unrelated Bone Marrow




Unrelated donor transplants for 32 patients who received transplants from HLA-matched unrelated donors (in parentheses: 95% confidence intervals at 2 years)

Current Opinion in Pediatrics. 15(1):24-31, February 2003.

# Haplo-identical Stem Cell Transplant

- Parent serves as donor for child
- Unmanipulated marrow versus T-cell depleted or CD34+ selected
- Myeloablative conditioning in most cases
  - Modified intensity?
- Requires extended immuosuppression
- Early results mixed
  - 31 children with thalassemia
  - 19 full engraftment, 3 partial but txn independent
  - 2 deaths, 7 graft rejection

(Sodani, et al 2011)

# Unrelated Donor Stem Cell Transplant in Thalassemia (URTH Study)

- Goal: To estimate the efficacy and toxicity of unrelated donor transplant in thalassemia
- Conditioning: Reduced Intensity

| | |
|---|---|
| Hydroxyurea (30mg/kg) | day -50 to -21 |
| Alemtuzumab (48 mg total) | day -21 to -19 |
| Fludarabine (150 mg/m$^2$ total) | day -8 to -4 |
| Thiotepa (4mg/kg IV q 12 hrs) | day -4 |
| Melphalan (140 mg/m$^2$) | day -3 |

- Stem cell source: Full HLA matched unrelated bone marrow or umbilical cord blood with high cell dose, minor mismatch
- Results: 23 patients enrolled
  - 14 marrow, 9 UCB
  - Overall survival 82%; Disease-free survival 78% at 1 year

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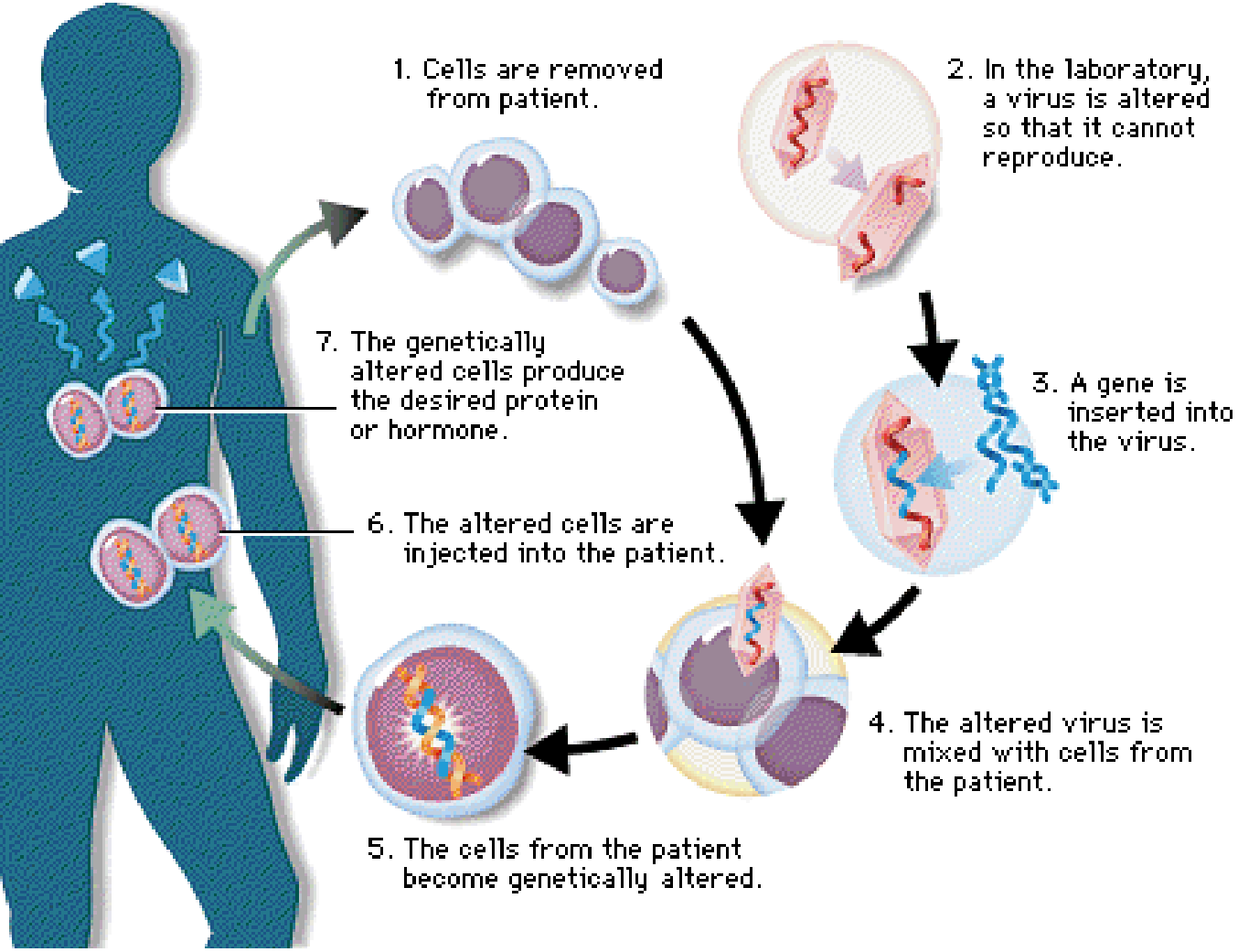
# Gene Therapy for Thalassemia

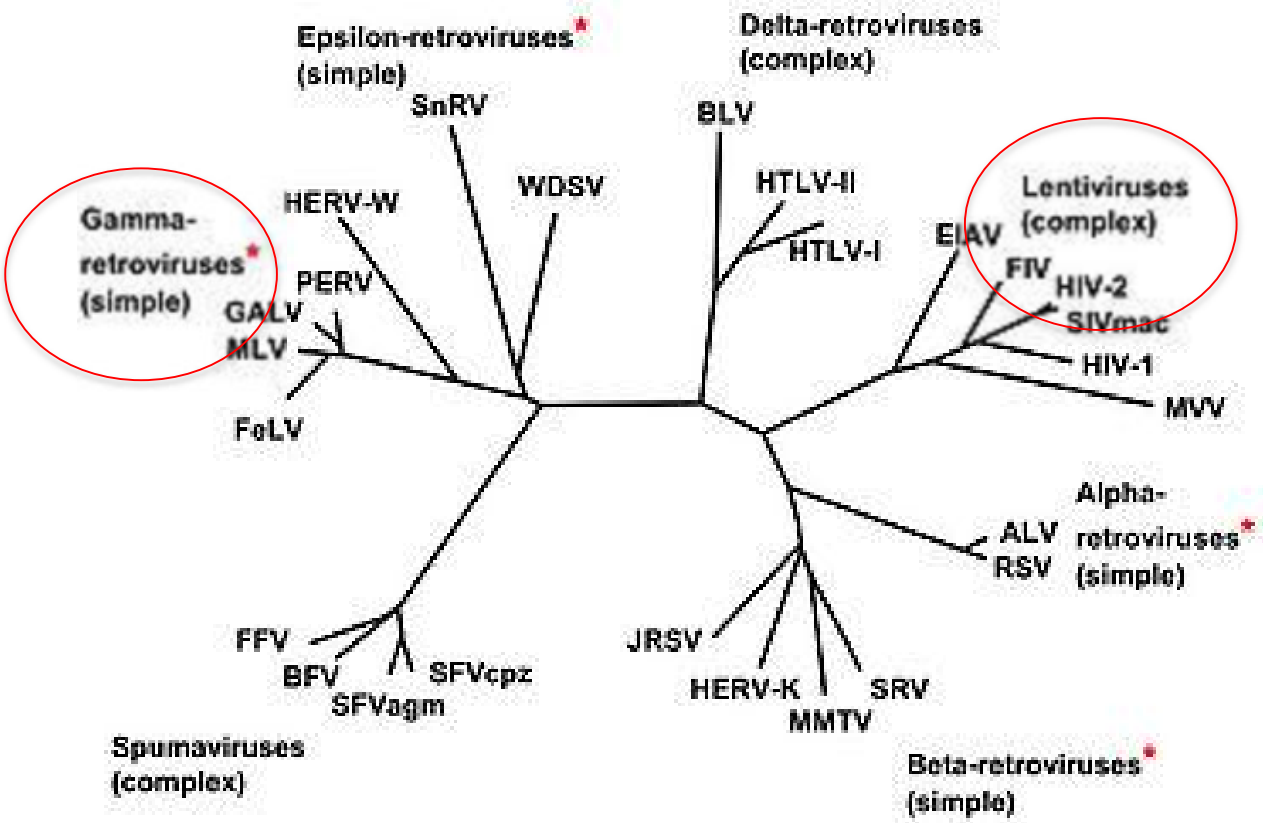


# Thalassemia Gene Therapy Trials

- St. Jude Children's Research Hospital
  - Not a treatment study
  - Retroviral vector mediated globin gene transfer
  - Looking for patients with thalassemia or sickle cell to donate marrow for use in mouse models of the conditions
- Memorial Sloan-Kettering Cancer Center
  - TNS9.3.55 Lentiviral vector for gene transfer into autologous stem cells
  - Adults >age 18 years, beta thalassemia major, LIC <15
  - Enrollment open
- bluebird bio
  - LentiGlobin lentiviral vector for Ex vivo globin gene transfer
  - Adults age 18-35 years, HbE-beta thal or beta thal major
  - 4 U.S. sites, 2 international
  - First gene therapy in U.S. performed in March 2014

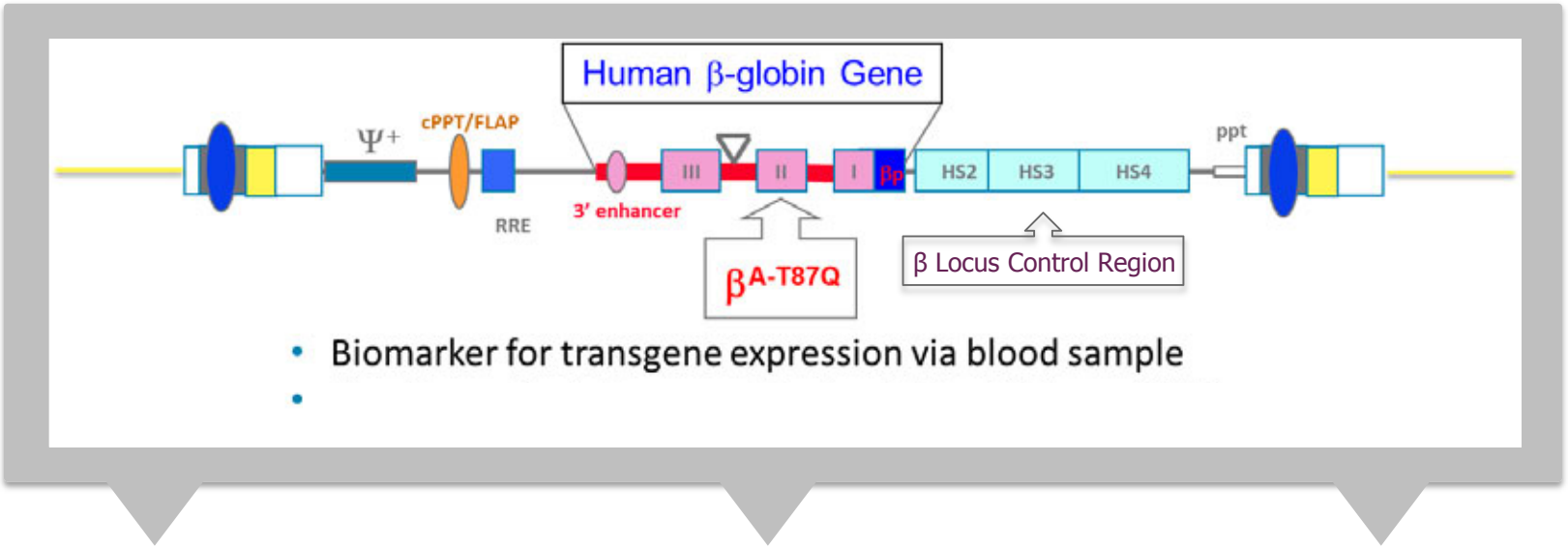
https://clinicaltrials.gov

# The retrovirus family



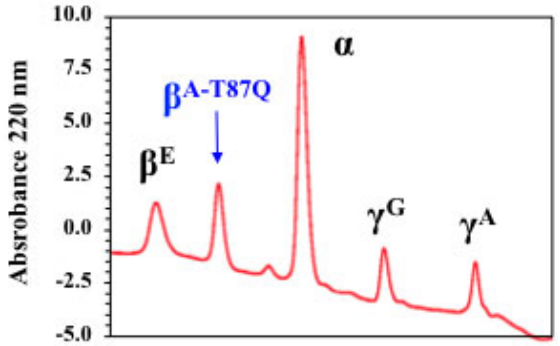

- A family of enveloped RNA viruses that replicates in the host via reverse transcription followed by integration into host DNA.
- Often used in gene therapy when the goal is to have stable genomic information passed from one cell generation to the next.

# β-Thalassemia: LentiGlobin Design




*Lineage Specific Expression*

*(Erythrocytes)*




*Philippe Leboulch*

# Gene Therapy Phases and Timeline

- Screening (4-6 weeks)
  - MRI (heart and Liver)
  - Other imaging: Chest X-ray, abdominal ultrasound
  - Liver Biopsy
  - Lung function tests
  - Heart function tests (ECG, echocardiogram)
  - Blood tests
  - Consultation with fertility specialist
- Mobilization (4-6 days)
  - GCSF, daily
  - Plerixafor, 1-2 days
  - Daily lab tests

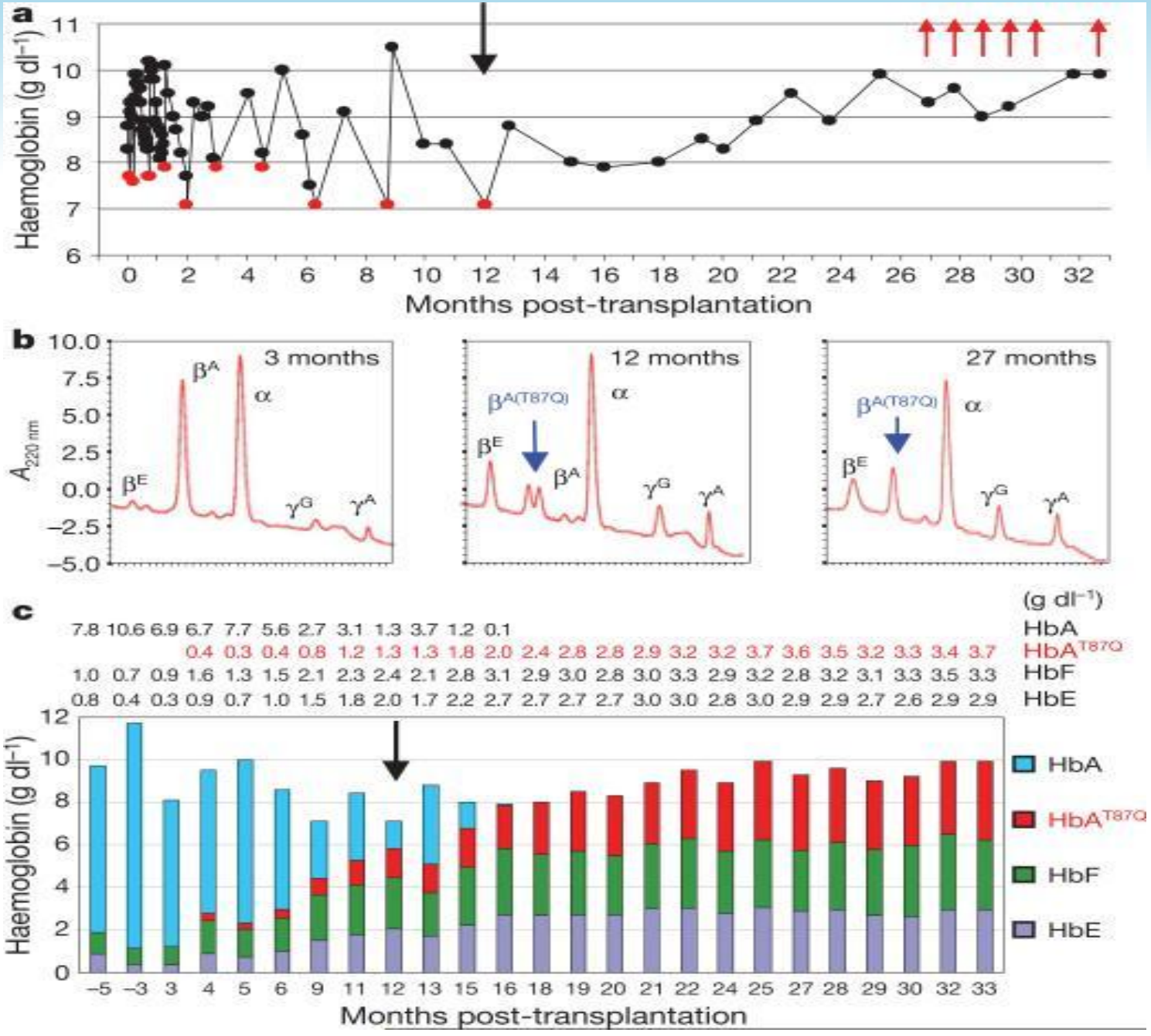
# Gene Therapy Phases and Timeline

- Stem Cell Harvest (2-3 days)
  - IV access?
  - Peripheral Blood CD34 Stem Cell Collection
  - Back up stem cell collection (PB or marrow)

*LentiGlobin Preparation (4-8 weeks)*

- Conditioning
  - IV Bulsufan x 4 days, rest for 3 days
- Infusion of Modified Stem Cells (Day 0)
- Recovery
  - Supportive care, pending engraftment
- Follow up

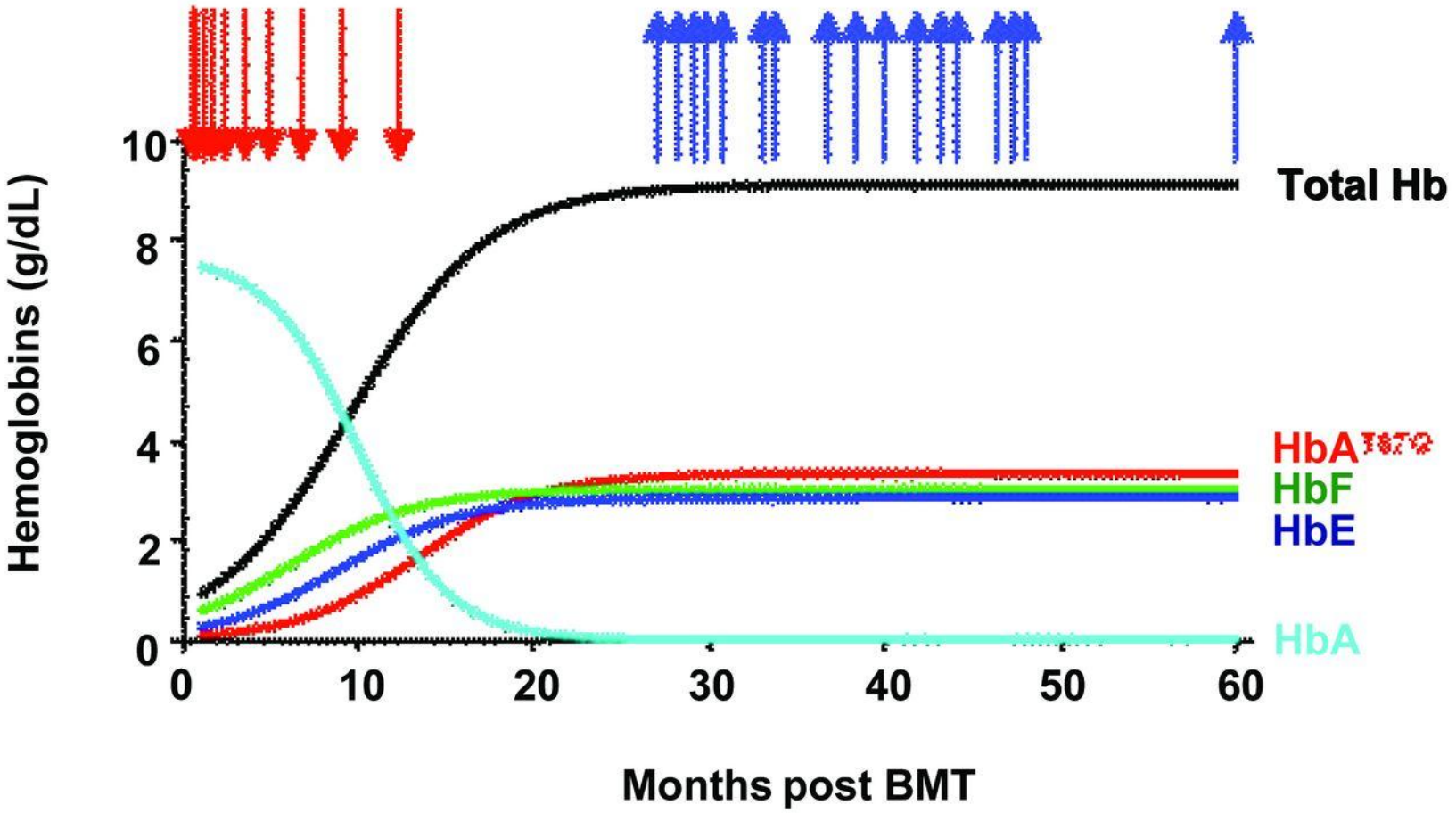
3-4 weeks






Nature. 2010 September 16; 467(7313): 318–322.

# Concentration of hemoglobins in blood.




Payen E , and Leboulch P Hematology 2012;2012:276-283

# Risks of Gene Therapy

- Conditioning toxicity
  - Mouth sores (mucositis)
  - Hair Loss
  - Liver problems (rare, highly unlikely)
  - Lung scarring (rare, highly unlikely)
- Graft Failure
  - Depends on number of cells given
  - Conditioning important in heavily transfused patients
- Cancer (insertional oncogenesis)
- Viral infection
- Sterility

# What is "insertional oncogenesis"?

- Insertion of the viral genomic material into the host DNA is semi-random
- Insertional oncogenesis occurs when this insertion occurs within or near genes that are responsible for cell proliferation or survival and deregulates the expression of these genes
- This propensity to cause insertional oncogenesis is a well known property of the γ-retroviruses
  - Hence, their names – murine leukemia virus, feline leukemia virus

# Modifications to decrease risk of insertional oncogenesis

- Three critical differences:

1) Vectors (BB305) are self-inactivating (SIN):
   - These vectors have a deletion in the long terminal repeat (LTR) that abolishes the promoter activity of the LTR and therefore minimize the potential for activation of cellular oncogenes
2) bluebird bio vectors (BB305) are lentiviral, not γ-retroviral
   - Unlike γ-retrovirus, lentivirus does not preferentially integrate upstream of genes being actively expressed (in close proximity to the gene control elements)
3) BB305 incorporates the red cell-specific human β -globin promoter and β -globin Locus Control Region elements
   - Transcription only occurs in erythroid lineage

*Montini et al, JCI 2009*

Abstract # S742

Oral presentation at the European Hematology Association (EHA), June 14, 2014:

# Improving Gene Therapy For β-Thalassemia Major: Initial Results From Study Hgb-205

M. Cavazzana, JA Ribeil*, E. Payen*, F. Suarez, O. Negre, Y. Beuzard, F. Touzot, R. Cavallesco, F. Lefrere, S. Chretien, P. Bourget, F. Monpoux, C. Pondarre, B. Neven, F. Bushman, M. Schmidt, C. von Kalle, L. Sandler, S. Soni, B. Ryu, R. Kutner, G. Veres, M. Finer, S. Blanche, O. Hermine, S. Hacein-Bey-Abina, P. Leboulch

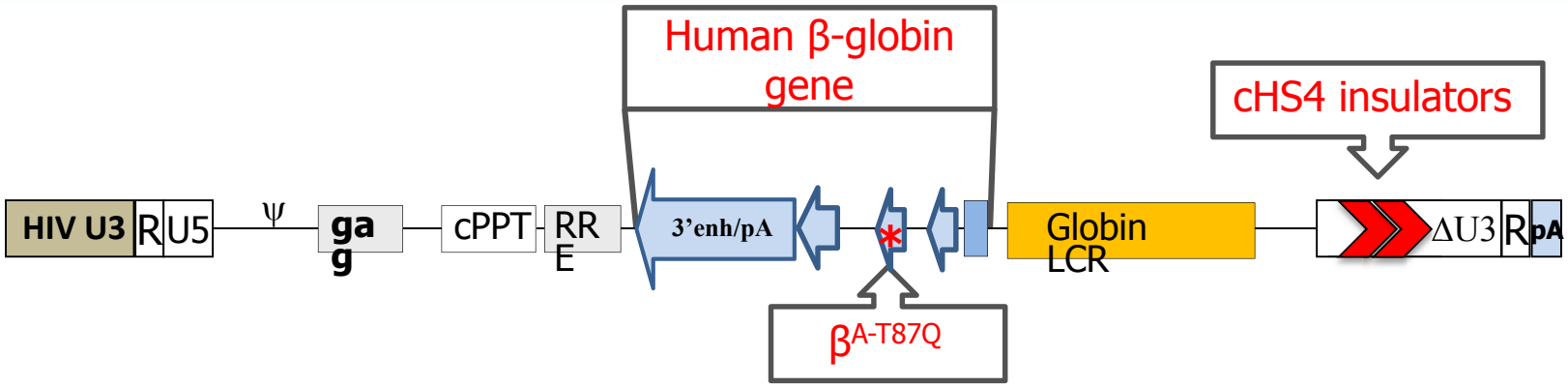
**these authors contributed equally*

# EHA presentation on Gene Therapy Studies for Thalassemia

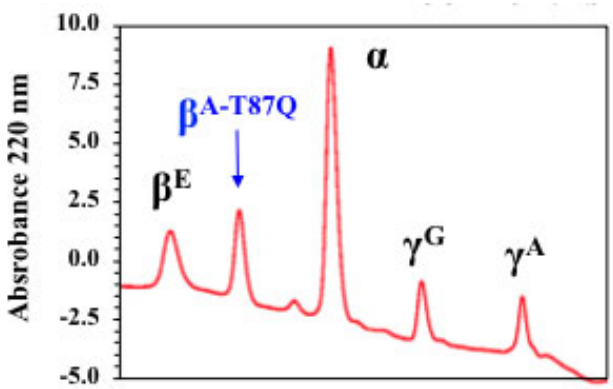
- Long term follow up from early bluebird bio study and initial report of subjects on second study (HGB-205) that mirrors US trial (HGB-204)
- Procedures: Stem cell collection, gene transfer in the lab, conditioning with IV busulfan, infusion of modified stem cells containing LentiGlobin sequences

| Study | Lentiviral vector | Current status |
|---|---|---|
| 1 (LG001) | HPV569 | Study closed, update presented |
| 2 (HGB-205) | BB305 | Enrolling, initial results on first 2 subjects presented |

# Study 1:HPV569 lentiviral vector




- Globin production is under transcriptional control of an erythroid-specific promoter and enhancer




- $\beta^{A\text{-}T87Q}$-globin allows for monitoring of protein levels produced using HPLC

# Study 1 – LG001

| Subject | Outcome |
|---|---|
| **1** | Not treated |
| **2** | Low number of stem cells infused, no engraftment, received rescue cells |
| **3** | Engrafted, 6 years follow-up |
| **4** | Engrafted, 2 years follow-up |

| | Subject 3 | Subject 4 |
|---|---|---|
| Age | 18 | 22 |
| Genotype | $\beta^0/\beta^E$ | $\beta^0/\beta^E$ |
| $CD34^+$ VCN | 0.6 | 0.3 |
| $CD34^+$ cell dose (x $10^6$/kg) | 4.9* | 4.3 |

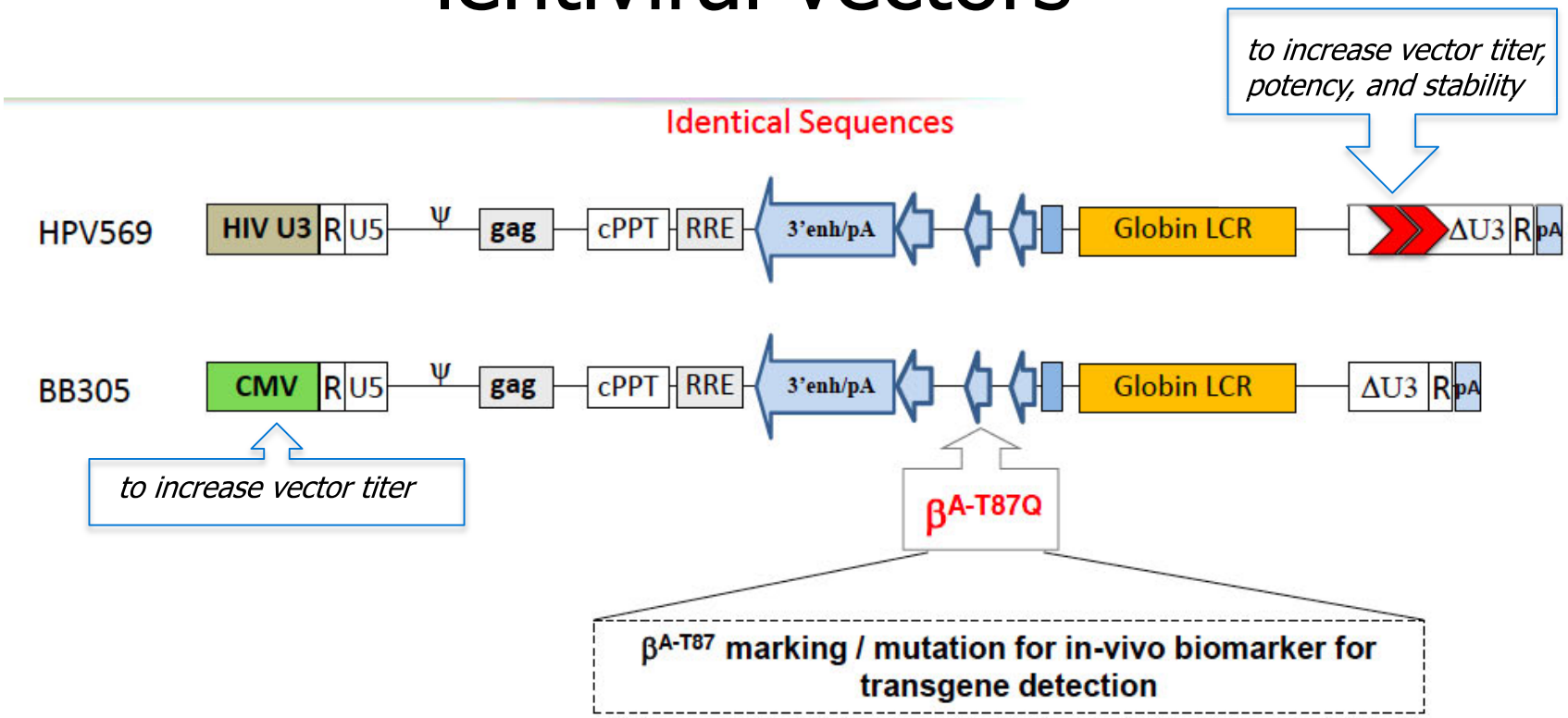
**Subject 3 source of CD34+ cells was bone marrow*

# Study 1: safety

| | Subject 3 | Subject 4 |
|---|---|---|
| Follow-up period | 6 years | 2 years |
| Day of neutrophil engraftment<br>*ANC > 500/µL* | Day 27 | Day 19 |
| Day of platelet engraftment<br>*Unsupported platelet count > 20,000/µL* | Day 40 | Day 130 |
| Non-laboratory ≥Grade 3 AEs | None | Mucositis, metrorrhagia, epistaxis, mouth bleeding |
| SAEs occurring ≥ Day 0 | None | Thrombocytopenia |
| Insertion site analysis | Multiple clones (25-50 detected at each timepoint) | Polyclonal (90-200 clones detected at each timepoint) |

- No AEs related to drug product, including no RCL nor malignancy

# HPV569 (Study 1) and BB305 (Study 2) lentiviral vectors



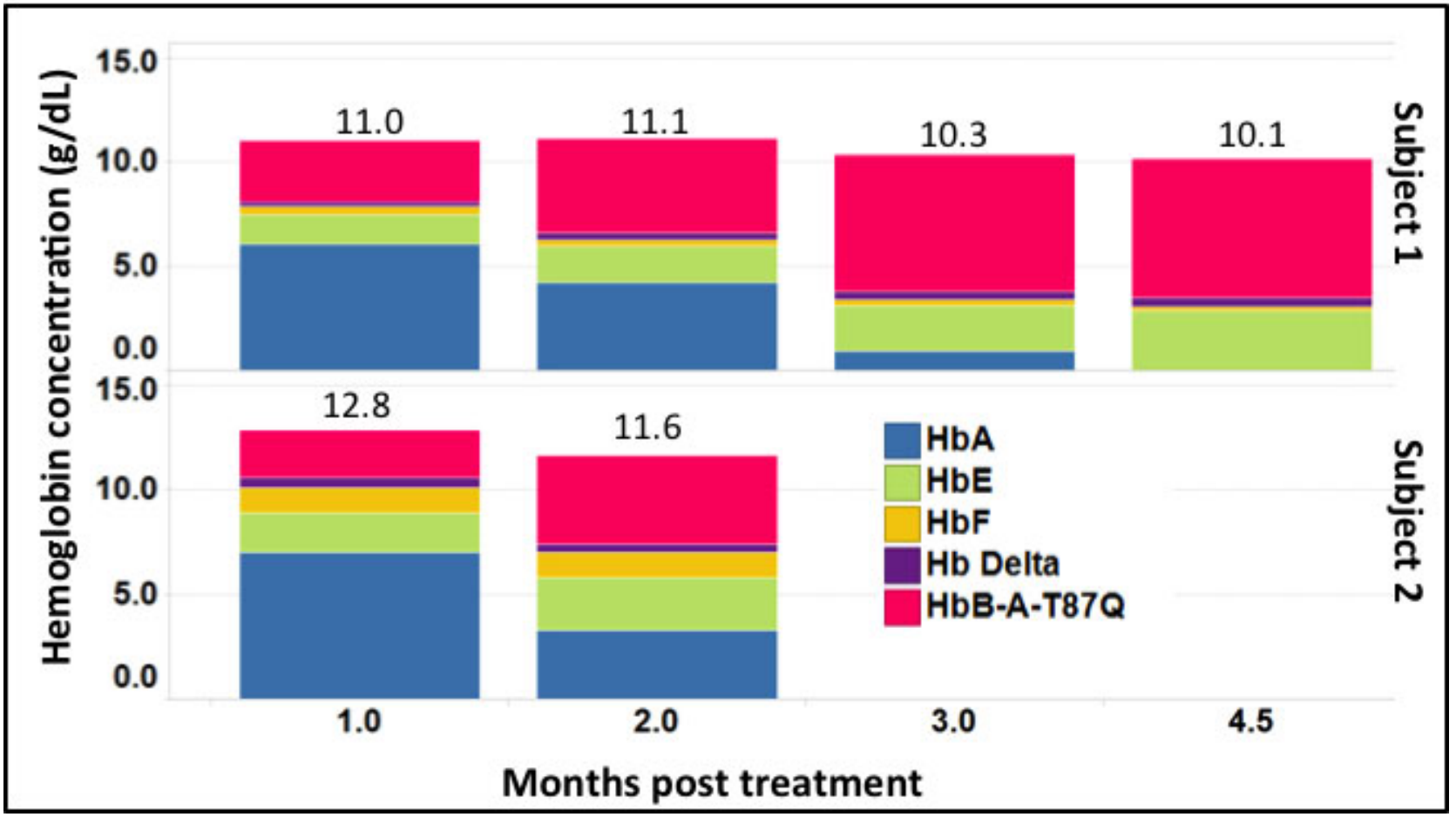

**BB305 versus HPV569:**

- 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

# Study 2: subject characteristics

| | Subject 1 | Subject 2 |
|---|---|---|
| Age at Enrollment | 18 | 16 |
| Genotype | $\beta^0/\beta^E$ | $\beta^0/\beta^E$ |
| $CD34^+$ VCN | 1.5 | 2.1 |
| $CD34^+$ cell dose (x $10^6$/kg) | 8.9 | 13.6 |

# Study 2: Early, high production of $\beta^{A\text{-}T87Q}$ globin



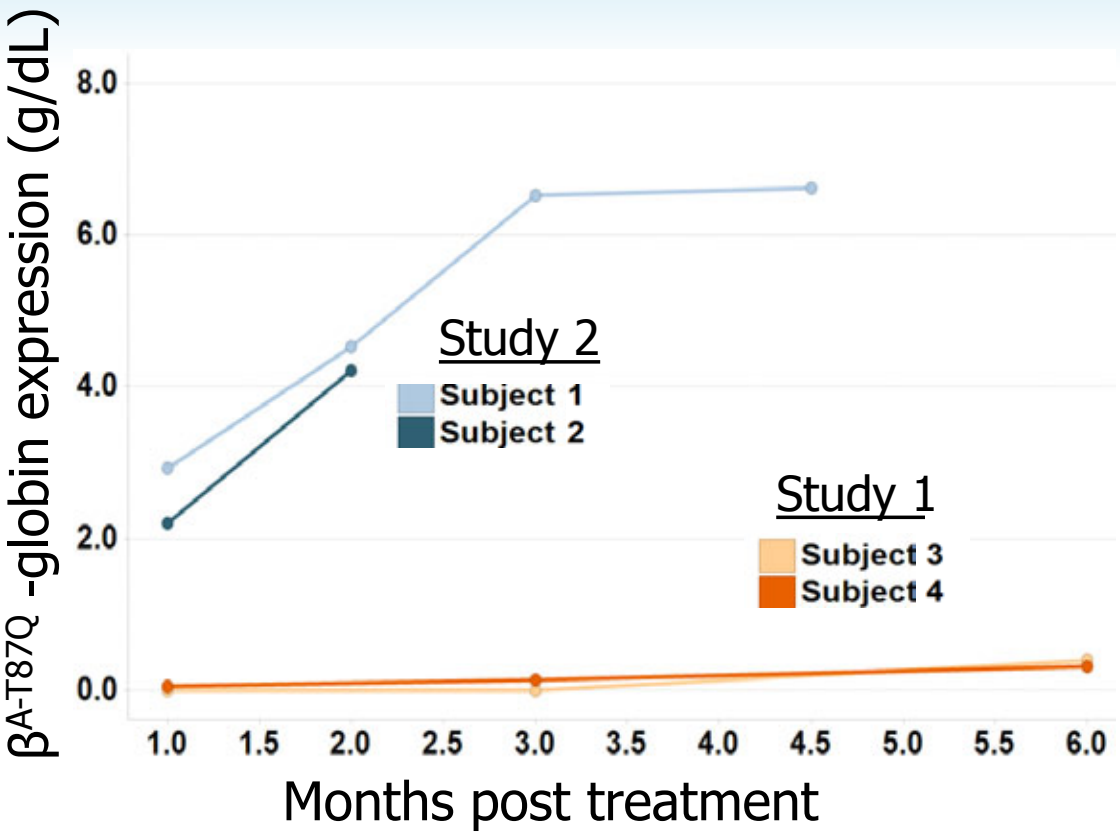

- Subject 1: producing 6.6 g/dL of $\beta^{A\text{-}T87Q}$ -globin at 4.5 months
- Subject 2: producing 4.2 g/dL of $\beta^{A\text{-}T87Q}$ -globin at 2 months

# Study 2: Safety

| | Subject 1 | Subject 2 |
|---|---|---|
| Follow up period | 4.5 months | 2 months |
| Day of neutrophil engraftment<br>*ANC > 500/µL* | Day 13 | Day 15 |
| Day of platelet engraftment<br>*Unsupported platelet count > 20,000/µL* | Day 17 | Day 24 |
| Non-laboratory ≥Grade 3 AEs | Mucositis[1] | Mucositis |
| SAEs occurring ≥ Day 0 | None | None |
| Insertion site analysis | At 3 Months: highly polyclonal (>1000), no clonal dominance | Not yet available |

- No AEs related to drug product, including no RCL nor malignancy

# Kinetics of $\beta^{A\text{-}T87Q}$ expression




- Gradual increase in production from the LentiGlobin modified stem cellsIn Study 2, rapid production of therapeutic globin (weeks as opposed to one year)
- Both subjects in Study 2 have near-normal hemoglobin levels without transfusion support (neither subject has required a transfusion post-engraftment)

# LentiGlobin expression and transfusion independence

| RBC transfusion independence | | | |
|---|---|---|---|
| | Subject 3 | Subject 1 | Subject 2 |
| Study | 1 | 2 | 2 |
| Vector | HPV569 | **BB305** | **BB305** |
| Day of last transfusion | Month 12 | Day 10 | Day 12 |
| Duration since last transfusion | >5 years | >125 days | >48 days |

- In Study 2, rapid production of therapeutic globin (weeks as opposed to one year)
- Both subjects in Study 2 have near-normal hemoglobin levels without transfusion support (neither subject has required a transfusion post-engraftment)

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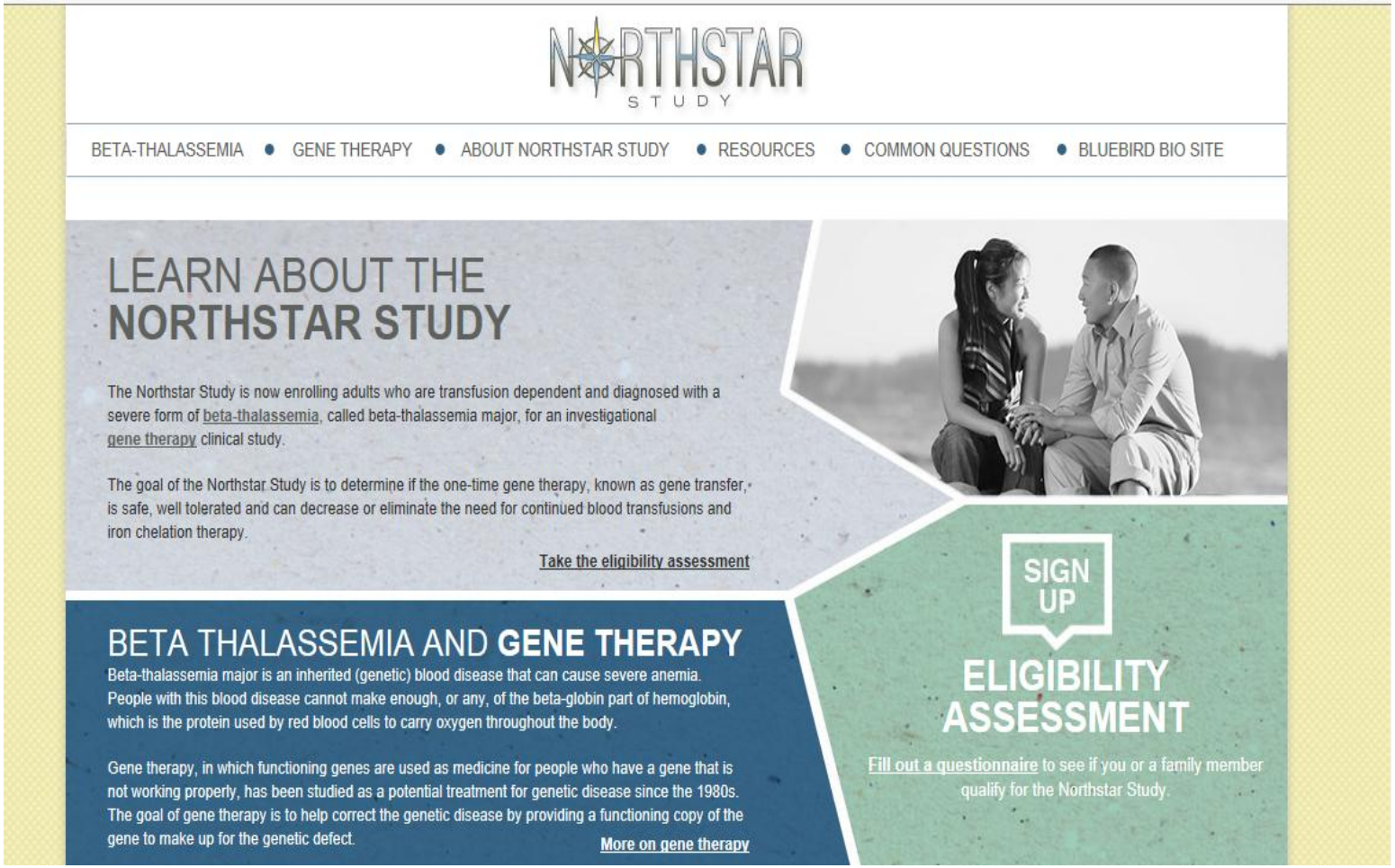
# North Star Study




Northstar website: www.northstarstudy.com

# North Star study

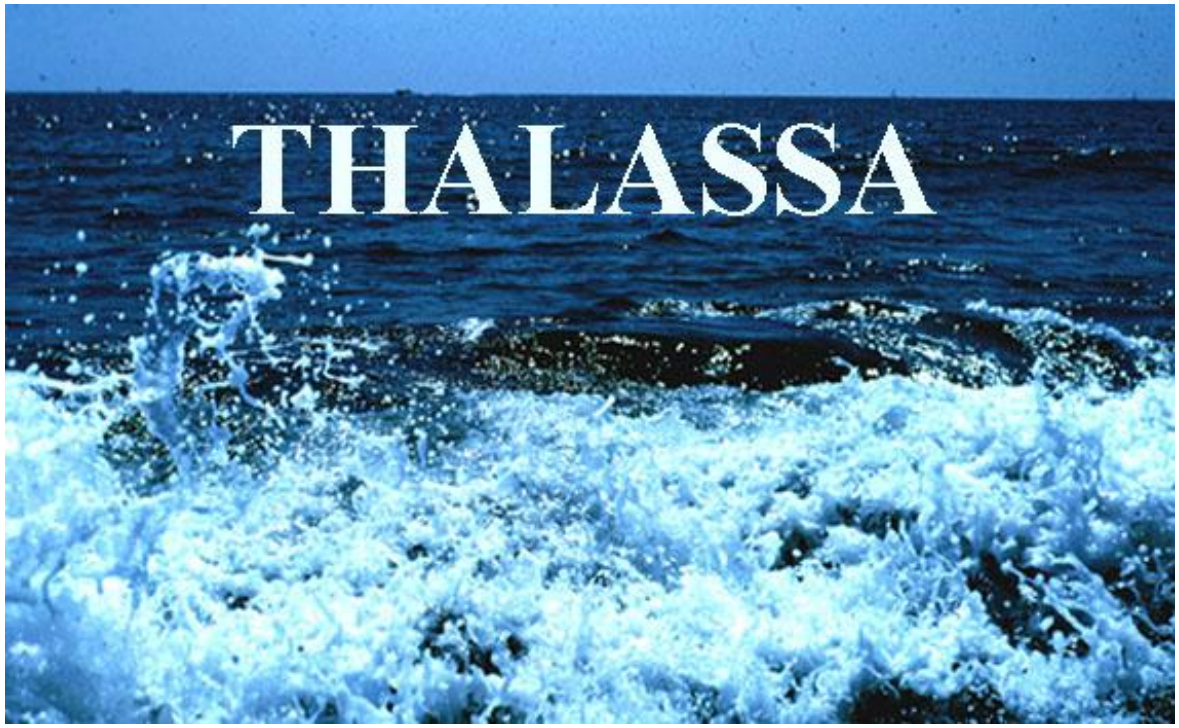
- Clinical study of Gene Therapy for Thalassemia is open and enrolling at 4 sites in the U.S.:
  - Beta thalassemia major or HbE/Beta thalassemia
  - Adults age 18-35 years
  - Some exclusions
- More info for interested patients:
  - Visit the active site listed on http://clinicaltrials.gov; Identifier: NCT01745120.

# Accepted and Experimental Approaches to Transplantation in Thalassemia

| Stem cell source | Conditioning | |
|---|---|---|
| HLA Identical sibling, marrow | Myeloablative<br>Reduced Intensity | Accepted |
| HLA well matched unrelated | Myeloablative | Accepted |
| HLA identical sibling umbilical cord | Reduced intensity | Accepted |
| HLA matched unrelated umbilical cord | Reduced Intensity | *Experimental* |
| Haploidentical parent | Immunomyeloablative | *Experimental* |
| Autologous (gene therapy) | Myeloablative<br>Reduced Intensity | *Stay tuned!* |

# Summary

- Transplant efforts should be aimed at optimizing conditioning in relation to the stem cell source
- Reducing intensity of conditioning is feasible for thalassemia and is likely to preserve fertility
- Supportive care details need significant attention
- Decision to offer transplant should balance risks involved with the approach and the severity of disease
- Early gene therapy results are promising, may provide cures and should be investigated further in a research setting




***EFKHARISTO* (THANKS!)**

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# Thank You