

# Gene therapy for the Haemoglobinopathies: meeting the global need

Thalassaemia and the Haemoglobinopathies  
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# Haemoglobinopathies Worldwide

- ~7% of world's adult population are carriers (~475 million).  
71% of 229 countries, and these 71% of countries include 89% of all births worldwide
- 6.5% of world's children are carriers; equally divided between thalassaemia and sickle cell disease.
- >330,000-affected births/year worldwide (83% SCD; 17% Thal).  
3.4% of global child deaths under 5yrs  
60% of the total and 70% of the pathology are in Africa.

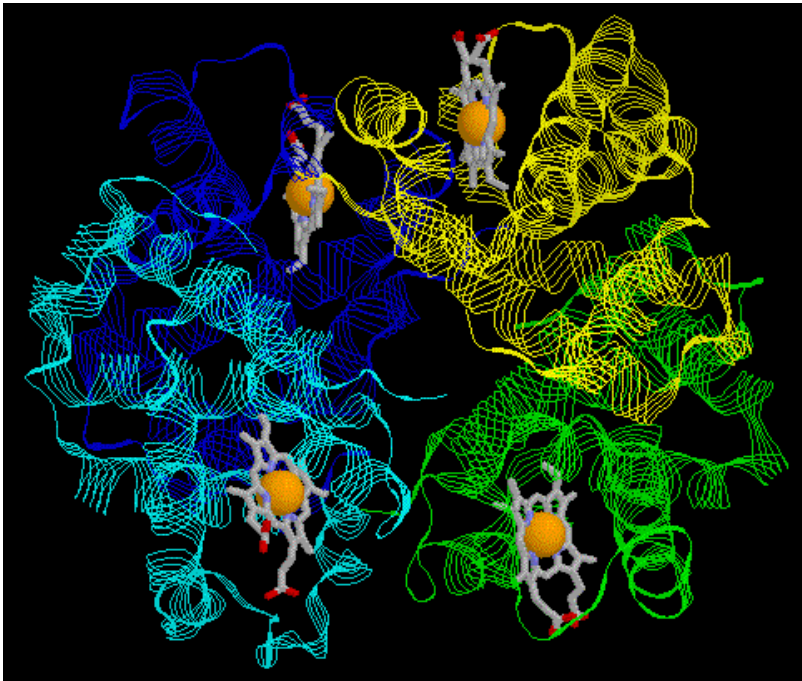
Modell B & Darlison M (2008) Bulletin of the World Health Organization **86**: 480-487

# Adult Haemoglobin (HbA)

2  $\alpha$ -globin chains

2  $\beta$ -globin chain

4 haem groups



# Gene Therapy for the thalassaemia

Requires stable introduction of a normal functioning  $\beta$ -globin gene unit into the bone marrow stem cells of the patient. A minimum of 50% of normal levels of  $\beta$ -globin required for curative therapeutic effect.

What we need:

$\beta$ -globin gene control elements to provide required high level production to balance  $\alpha:\beta$  chain ratio

Therapy gene delivery system for efficient stable integration into DNA of patient bone marrow stem cells

- $\beta$ -globin gene “LCR” master regulator
- Lentiviral gene delivery vector
- Ex-vivo protocol

# Gene therapy for thalassaemia

## - *ex-vivo* protocol -

Isolate blood stem cells from patient bone marrow and grow under laboratory conditions



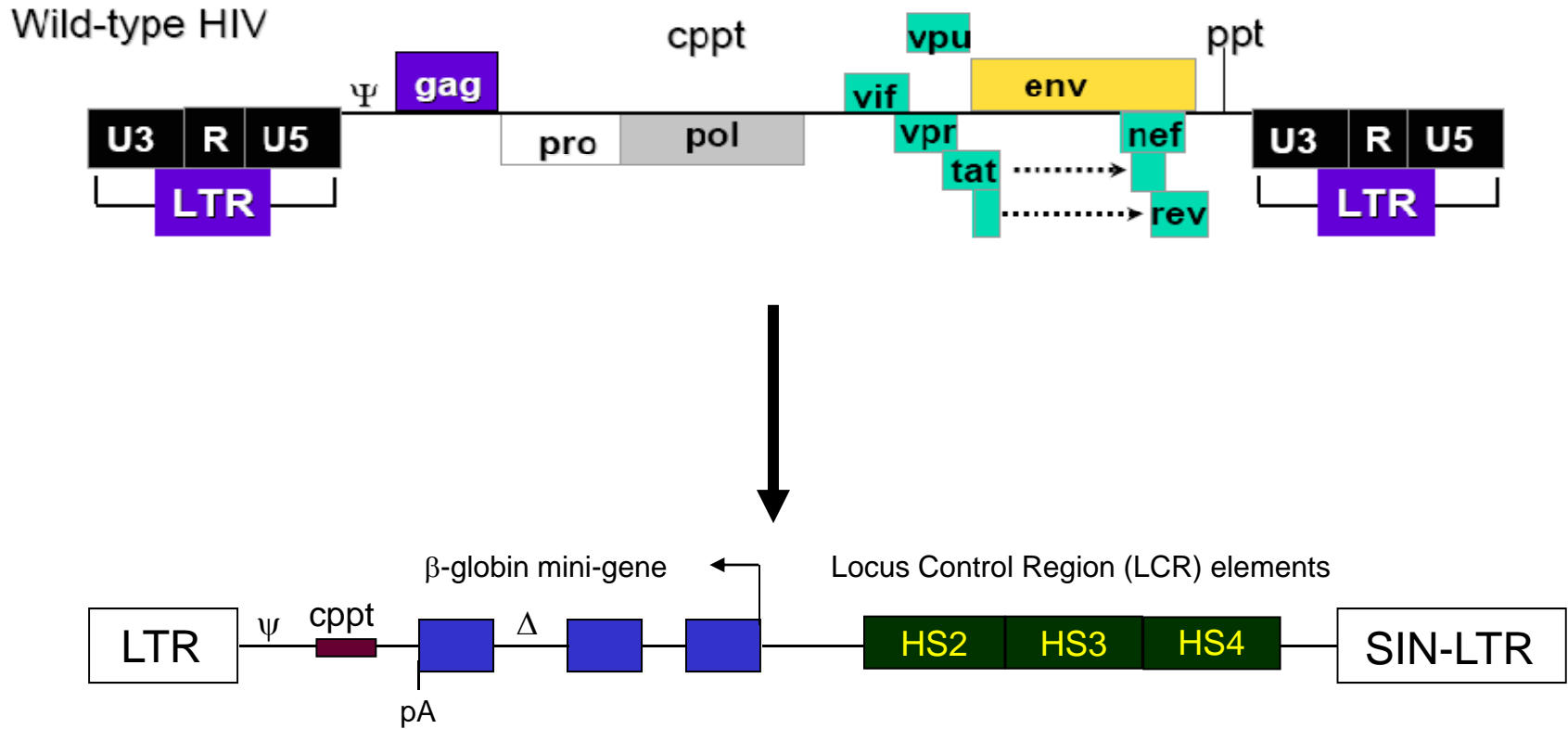
Infect bone marrow stem cells with globin lentiviral therapy gene medicine



Return (infuse) genetically corrected bone marrow stem cells to “myeloablated” (chemotherapy treated) patient (as in bone marrow transplant)

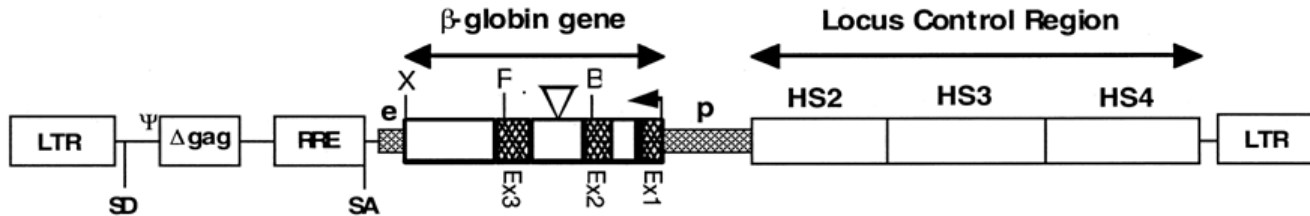
**Note: treatment of the patients own bone marrow stem cells thereby avoiding the need for a donor and complications of rejection/graft verses host disease.**

# Globin Lentiviral Gene Therapy Vectors

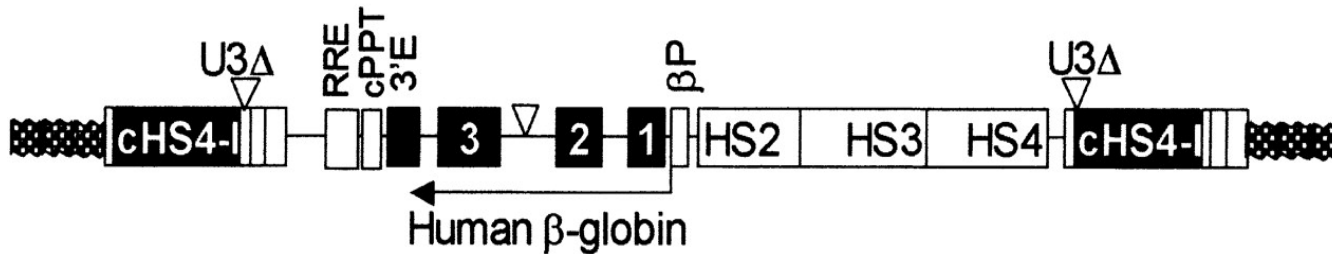


# Globin Lentiviral Vectors : $\beta$ -thalassaemia

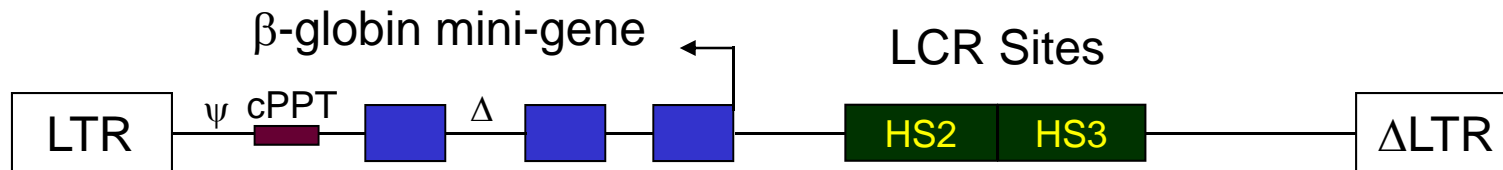
Michele Sadelain Lab (2000)



Punam Malik Lab. (2004)



Giuliana Ferrari and Michael Antoniou Labs (2008)



Correction of  $\beta$  - thalassaemia in mouse models using mouse and human bone marrow haematopoietic stem cells:

# Clinical Trials

Michele Sadelain (USA): approved

- George Stamatoyannopoulos (USA/Greece): approved

Puman Malik (USA): approved

Giuliana Ferrari (Italy): pending

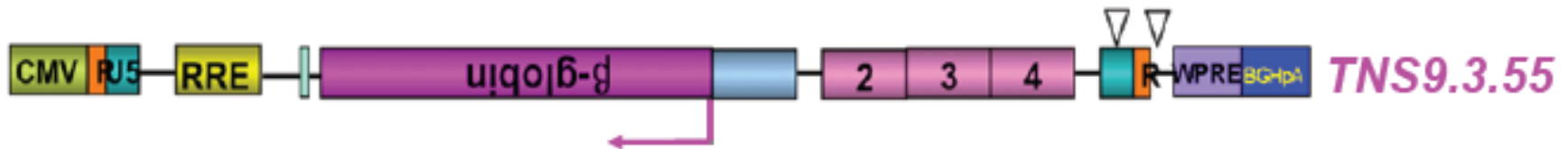
Philip Leboulche (France): started 2006; recommencing

**All will use ex vivo procedure**

# Clinical Trials

Michele Sadelain with Errant Gene Therapeutics, LLC™  
(EGT), USA

*Ex vivo* with partial myeloablation (low dose Busulfan)  
Approved by USA RAC and FDA. Recruiting patients.



See TIF website for proposed trial details – “Damascus Meeting Report”.

# Clinical Trials

## Philip Leboulche, Paris, France

First Phase I/II gene therapy clinical trial; started 2006

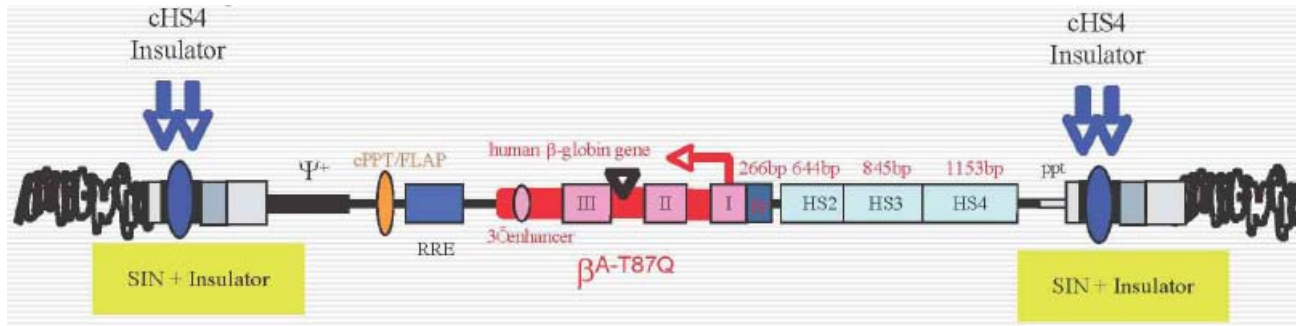
5  $\beta$ -thalassaemia; 5 sickle cell disease patients; 5-35 years of age

**Ex vivo approach; full chemotherapy-conditioning programme (Busulfex)**

By end of 2006, two patients with  $\beta$ -thalassaemia treated.

**Trial controversies:** use of a high-risk full chemotherapy-conditioning programme as part of a protocol whose success is still far from being certain let alone matching what is currently achievable with a sibling-donor bone marrow transplant.

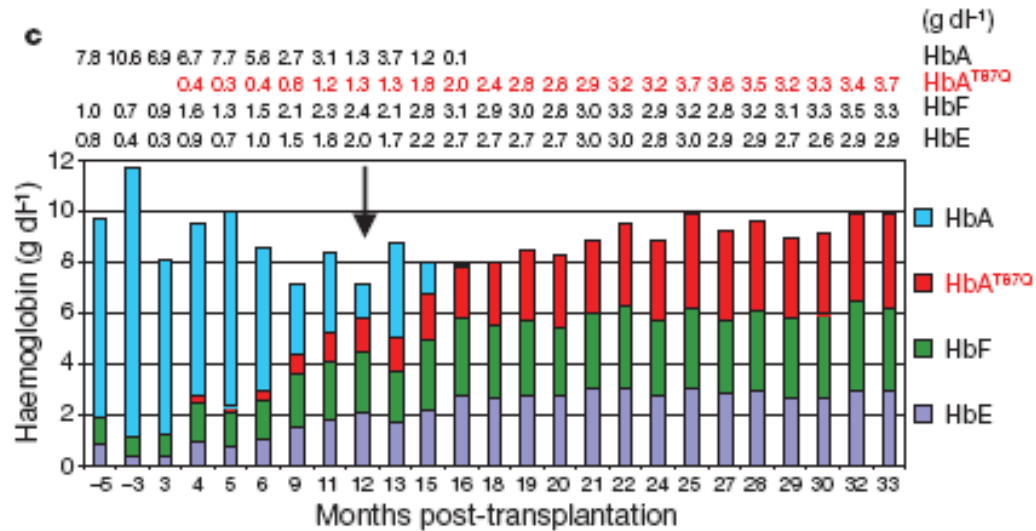
# Thalassaemia Clinical Trial : Paris 2006



- 18 years old
- $\beta^E/\beta^0$
- Transfusion dependent – one per month
- Treated Sept 2007

Paul Louis Beauchesne

# Thalassaemia Clinical Trial : Paris 2006



Marina Cavazzana-Calvo M et al. (2010) *Nature*, **467**: 318-323

# Thalassaemia Gene Therapy Trial: Paris 2006

## A very special case

### □ To date:

- Slow gradual increase in therapy vector  $\beta$ -globin
- Last transfusion June 08
- Stabilised at 9-10g/dl Hb

### □ Equal contribution from:

- $\beta E$
- HbF
- Therapy vector  $\beta$ -globin  
50% of vector  $\beta$ -globin from only a single, expanded parent stem cell caused by  
**host gene disruption event**

**Note:** without any one of above 3 compounding components patient will still need transfusions; that is, gene therapy would be ineffective

# Future Advances

Future advances to make gene therapy a widely applicable technology include:

- Therapy gene units to provide reproducible, stable expression; not prone to silencing
- **Need *in vivo* delivery approaches**

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# Pre-natal Gene Therapy

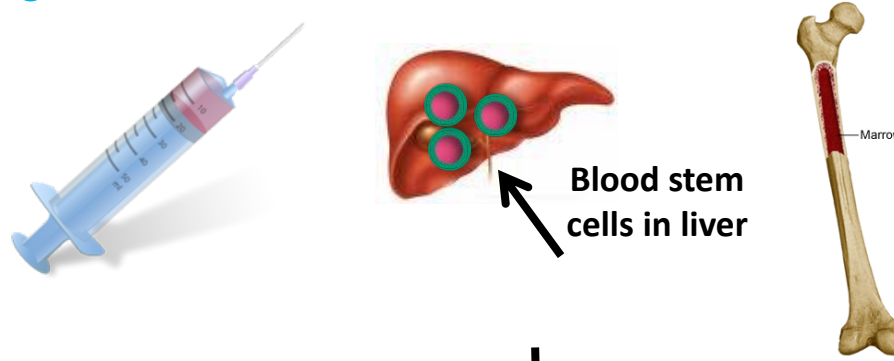
## Why Pre-natal foetal gene therapy?

- Lower therapy gene vector dose required; cheaper & safer
- Prevents disease onset; baby born cured
- Exploits period of rapid expansion and migration of blood stem cells
- Applicable to  $\alpha$ -thalassaemia
- Avoids/overcomes ethical and moral problems associated with pregnancy termination
  
- More universally applicable; direct *in vivo* procedure
- Ease of application using standard foetal surgery methods; out-patient basis
- Can have a significant global impact

# Pregnancy period



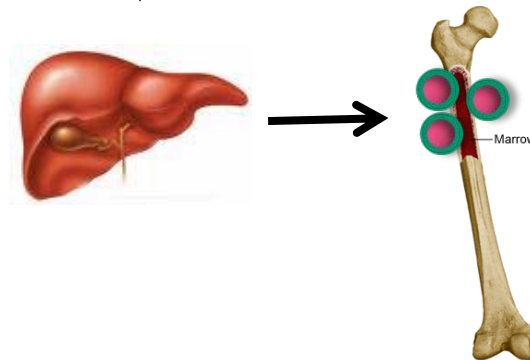
**A**



**Pre-natally**

↓ **Migration**

**B**



**Post-natally**

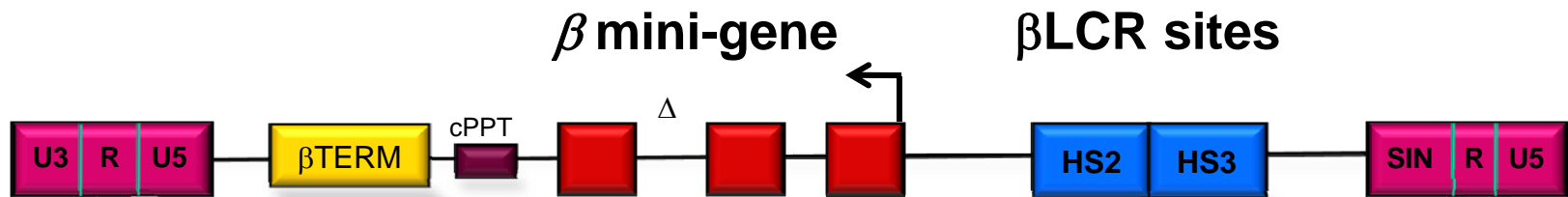
## Development of *pre-natal* gene therapy for thalassaemia

Concept:

A. Globin lentiviral therapy gene medicine correction of blood stem cells **pre-natally in foetal liver**

B. Migration of corrected blood stem cells to **bone marrow post-natally**

# GLOBE-2 Lentiviral Vector

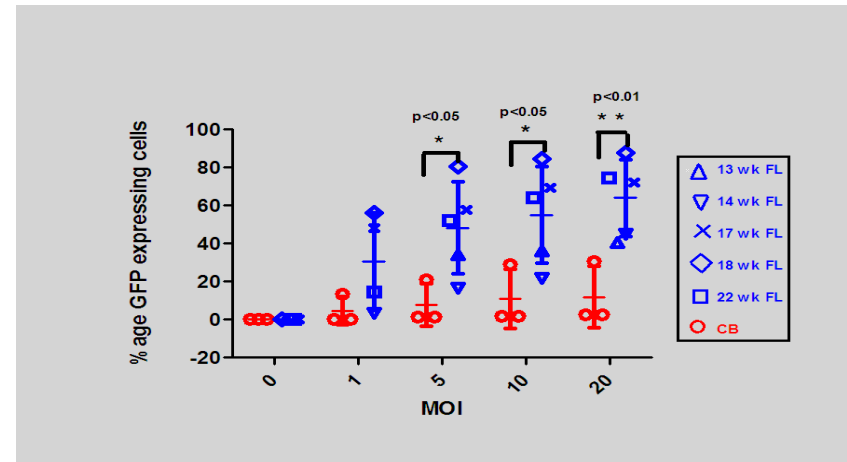
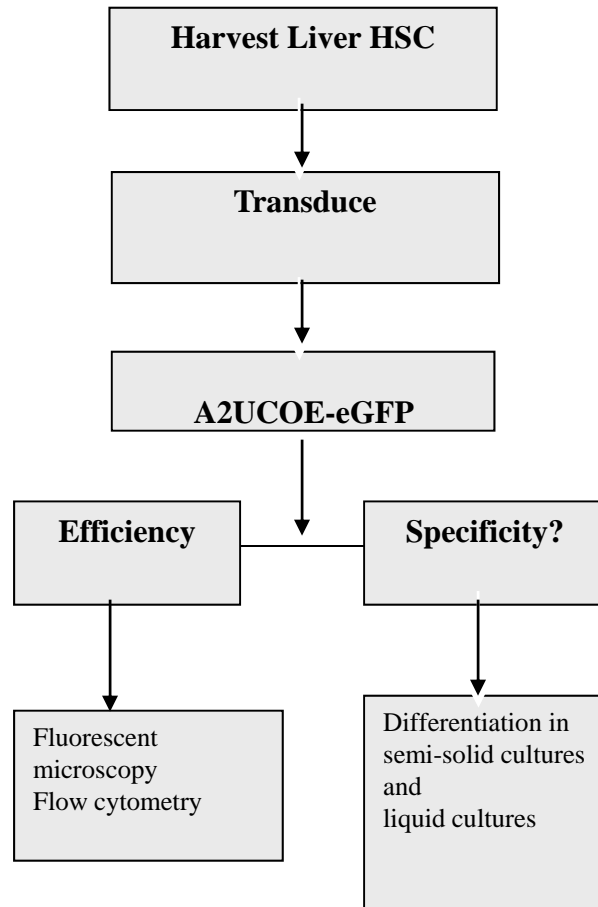


# Successful post-natal erythroid expression of **GLOBE-2** following *in utero* targeting of HSC

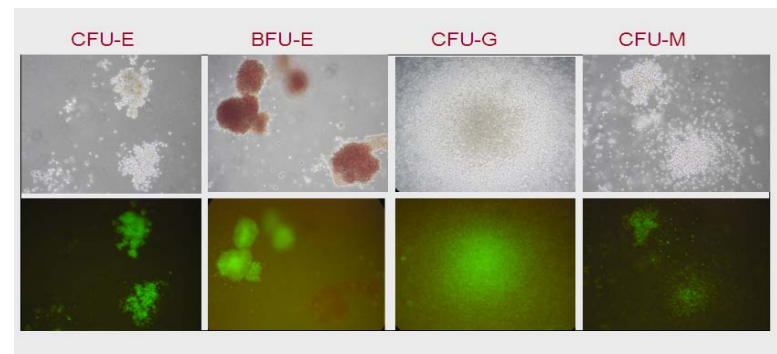
no	gender		cage	copy number	Hemoglobin levels (Hemoglobin ELISA) ng/ml
1	male		CG14377	1.4055	0.153
2	male	control 1	CG14377	0.1089	0.256
3	male		CG14379	0.4327	0.155
4	male		CG14379	0.3762	0.079
5	male		CG14379	1.6138	0.182
6	male	control2	CG14379	-0.5879	0.082
7	female		CG14378	2.9104	0.174
8	female	control3	CG14378	-2.8124	0.09

Haemoglobin Human sample control: 0.107

# Stable **A2UCOE-eGFP** marker gene lentiviral vector expression in human fetal HSC and their progeny

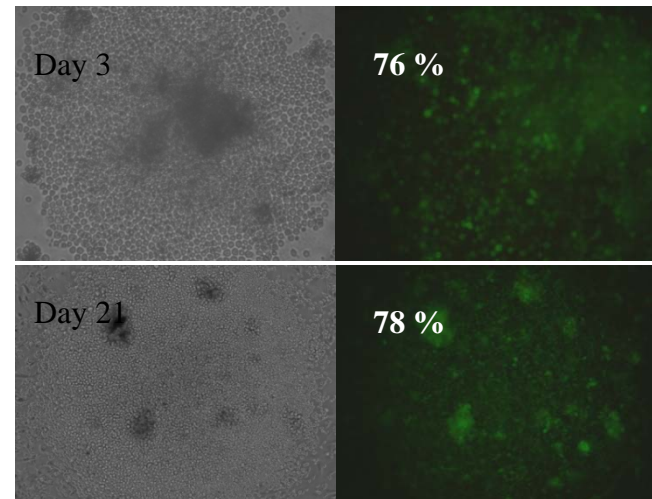
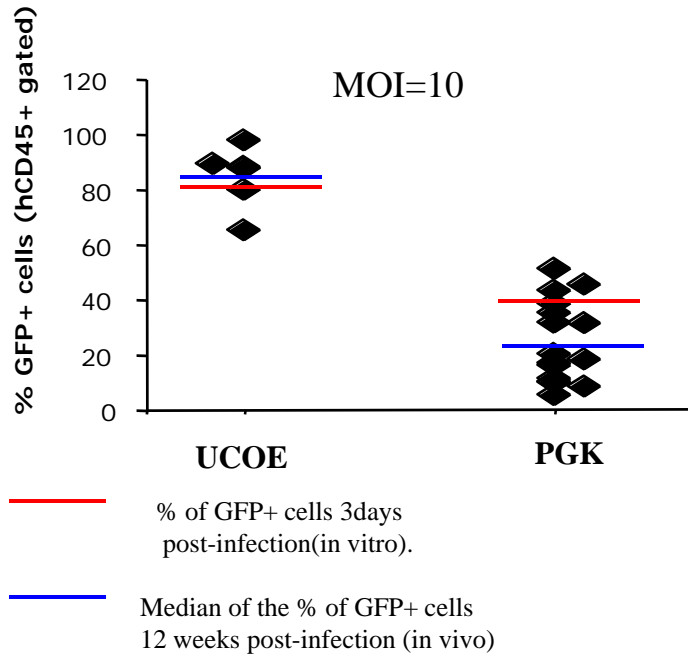


Increase in transduction efficiency with increasing gestation.



Transduced cells retain multilineage differentiation capacity.

# Stable **A2UCOE-eGFP** marker gene lentiviral vector expression in human fetal HSC and their progeny



**Sustained transgene expression of UCOE-GFP after transplantation**

**Continued expression of UCOE-GFP in culture**

# Summary and Future

Lentiviral vectors effectively mark (transduce) blood stem cells in the foetal liver

Long-term marking in peripheral blood including red blood cells

**GLOBE-2** new generation  $\beta$ -thalassaemia lentiviral vector can produce therapeutic levels of  $\beta$ -globin after pre-natal delivery

# Gene therapy for thalassaemia: are we there yet ...?

## Clear way forward:

Lentiviral vectors with LCR- $\beta$ -globin transcription units look very promising

**Are current vectors and protocols good enough to cure thal major?**

## Future advances:

Therapy gene designs that provide effective therapy at single copy per cell

## Other unanswered questions:

Partial or full myeloablation?

Genotoxicity potential of vectors still needs to be further assessed

## Novel approaches:

Pre-natal treatment of developing foetus with vectors for global impact

**At long last the future looks good!**

# Acknowledgements

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**GLOBE Vector**

**Giuliana Ferrari, Telethon Institute  
for Gene Therapy, Milan**