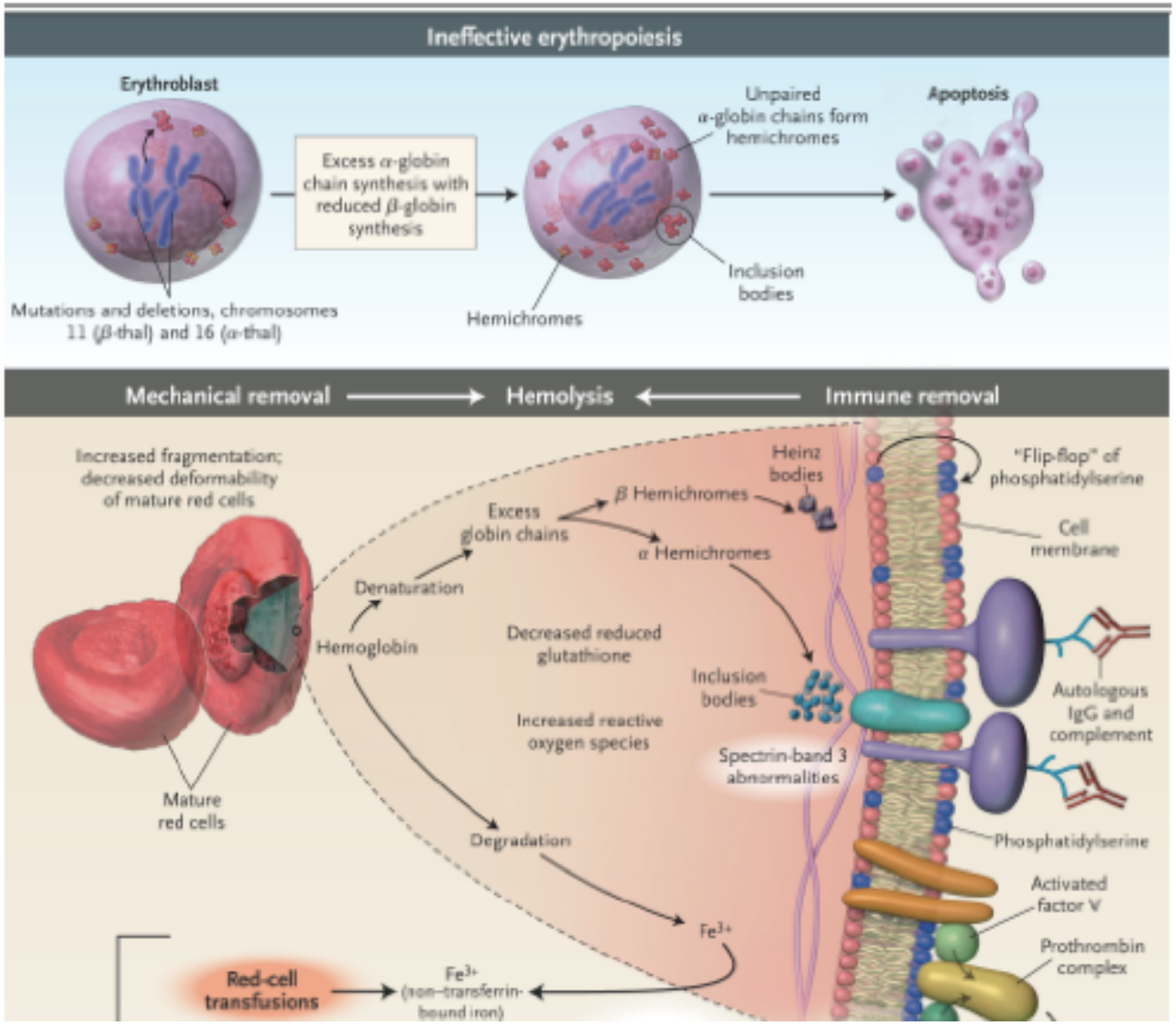
Chimerism and DLI in HSCT for non-malignant disorders

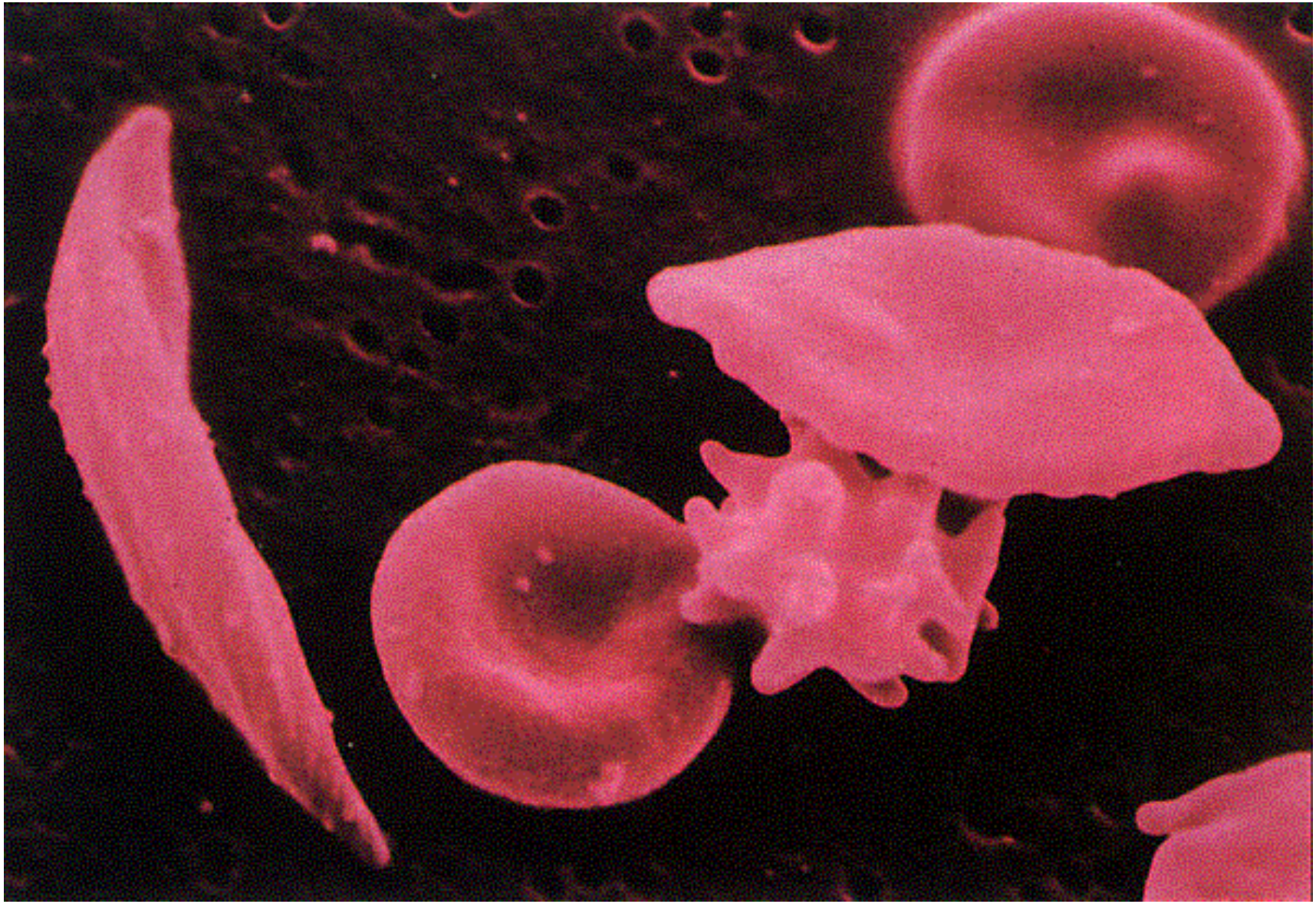
Giorgio La Nasa

Fisiopatolologia dell'eritropoiesi inefficace nella Talassemia



NEJM

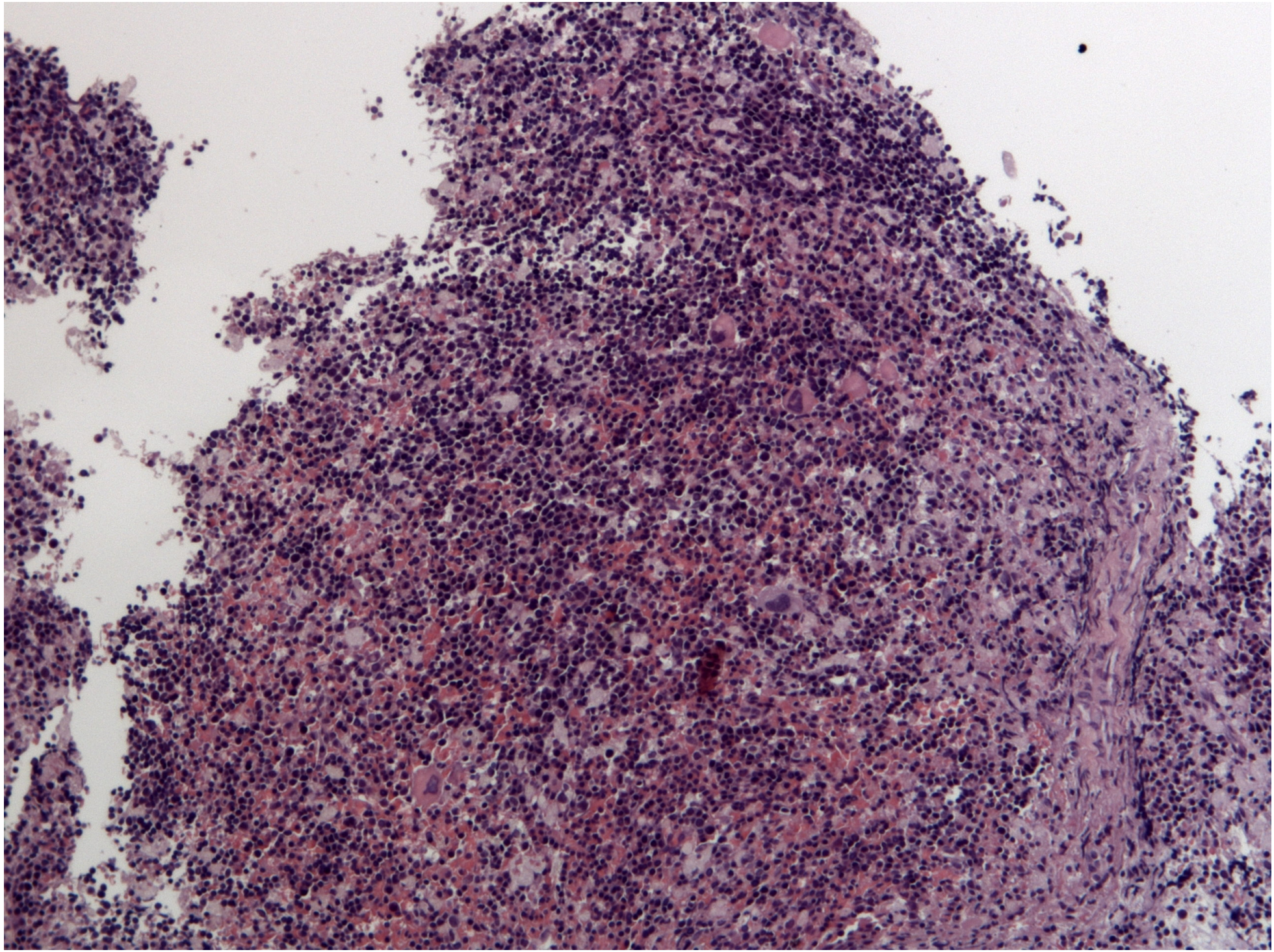
Talassemia Major



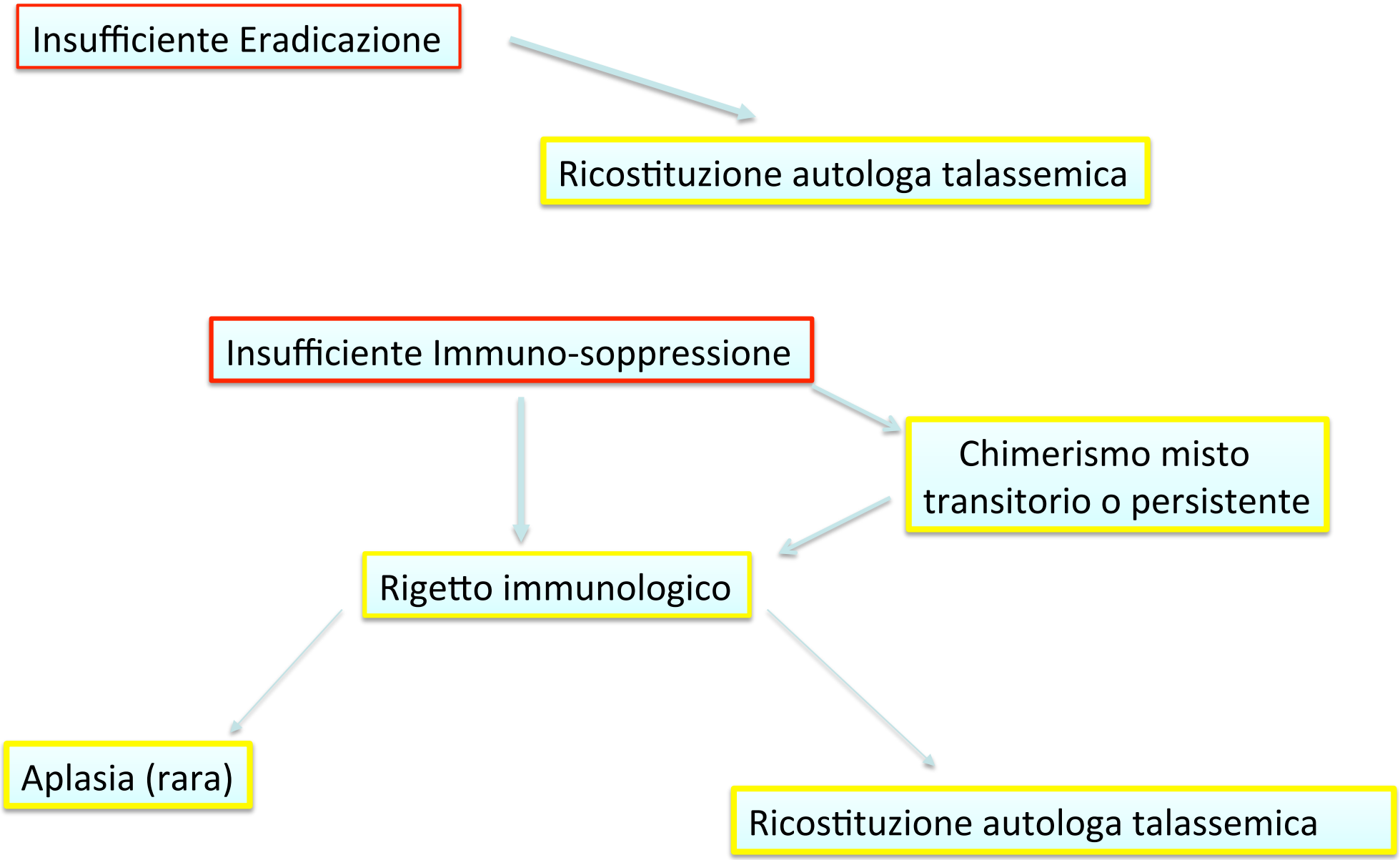
Problematiche del trapianto allogenico legate alla componente mielo-ablativa nelle emoglobinopatie

- Massa eritroide iper-espansa
  - iperplasia della serie eritroide
  - eritropoiesi inefficace
  - metaplasia eritroide
- Epatomegalia
  - Ipersiderosi
  - metaplasia eritroide
  - pazienti splenectomizzati
- Splenomegalia
  - Ipersplenismo
  - esaltata emocateresi
  - metaplasia eritroide

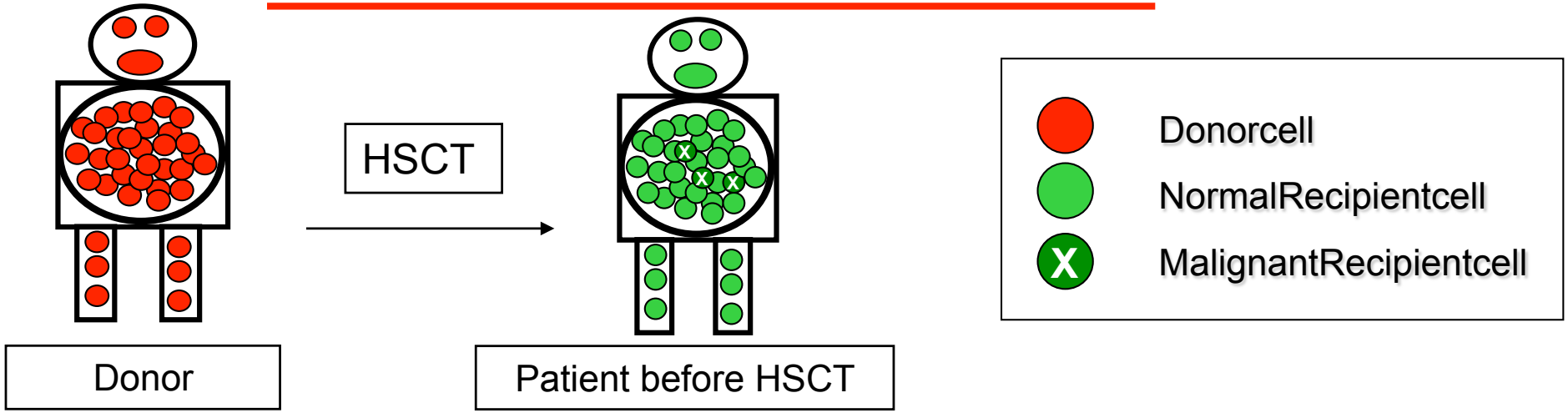
Paziente immunocompente talvolta iper-immunizzato

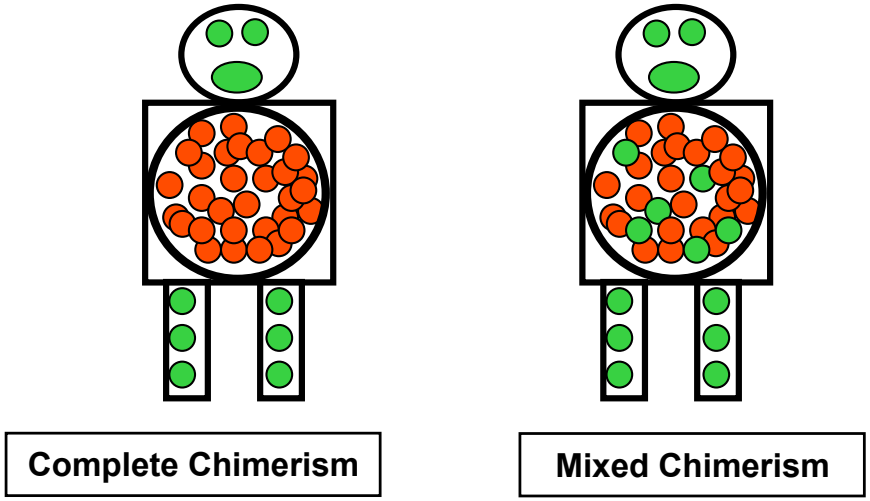


Tipi di Rigetto del Trapianto



Clinical Chimerism testing





Mixed chimerism
presence of both donor and recipient lymphohematopoiesis

Split chimerism
presence within a single compartment of different donor and recipient cells proportion

Microchimerism
presence of donor cells that are detectable only with very sensitive techniques

Patient after HSCT

Types of Chimerism

- *Mixed Chimerism*
  - presence of both donor and recipient lymphohematopoiesis
- *Split Chimerism*
  - presence within a single compartment of different donor and recipient cells proportion
- *Micro Chimerism*
  - presence of donor cells that are detectable only with very sensitive techniques
-

Mixed Chimerism in Haemoglobinopathies

MC may be defined :

Transient (TMC) *if present early after HSCT showing an evolution to graft failure or to complete chimerism*

Persistent (PMC) *when donor and recipient cells coexist for more than 24 months after HSCT*

Mixed Chimerism in Thalassemia after HSCT

The main goal of monitoring engraftment is to identify the presence or absence of residual host cells (RHC) after HSCT

The prompt determination of Mixed Chimerism (MC) my help to identify patients at high risk of relapse and rejection

Strategies for engraftment monitoring evaluation

Biological Samples:	Bone Marrow Peripheral Blood Lymphoid cell subsets
Time of investigation:	Patient Pre-transplant profile Donor profile Early Post-Transplant follow-up controls Periodic late Post-Transplant controls
Method sensitivity:	FISH, STR, Real time PCR

Engraftment detection methods after HSCT in haemoglobinopathies

in nucleated cells

- Peripheral Blood
- Lymphid Cells Subsets (CD3, CD19, CD56 etc.)
- T regulatory cells (Tr1)
- Bone Marrow
- Erythroid Precursurs (BFU-E)

Markers commonly used for mixed chimerism detection in nucleated cells

- **VNTR**
- **FISH analysis (if sex mismatched)**
- **STR (2-5%)**
- **Real Time PCR** (0.1 – 0.05%)

Increasing

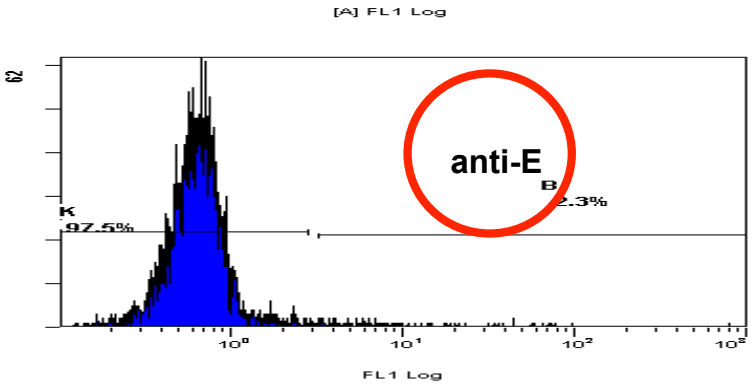
Sensitivity

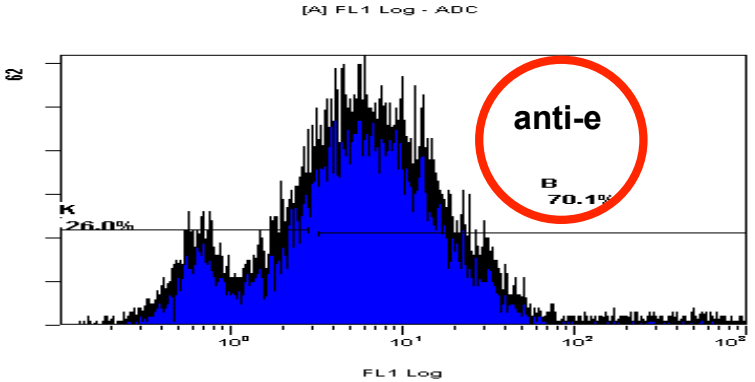
Engraftment detection methods after HSCT in haemoglobinopathies

in red blood cells

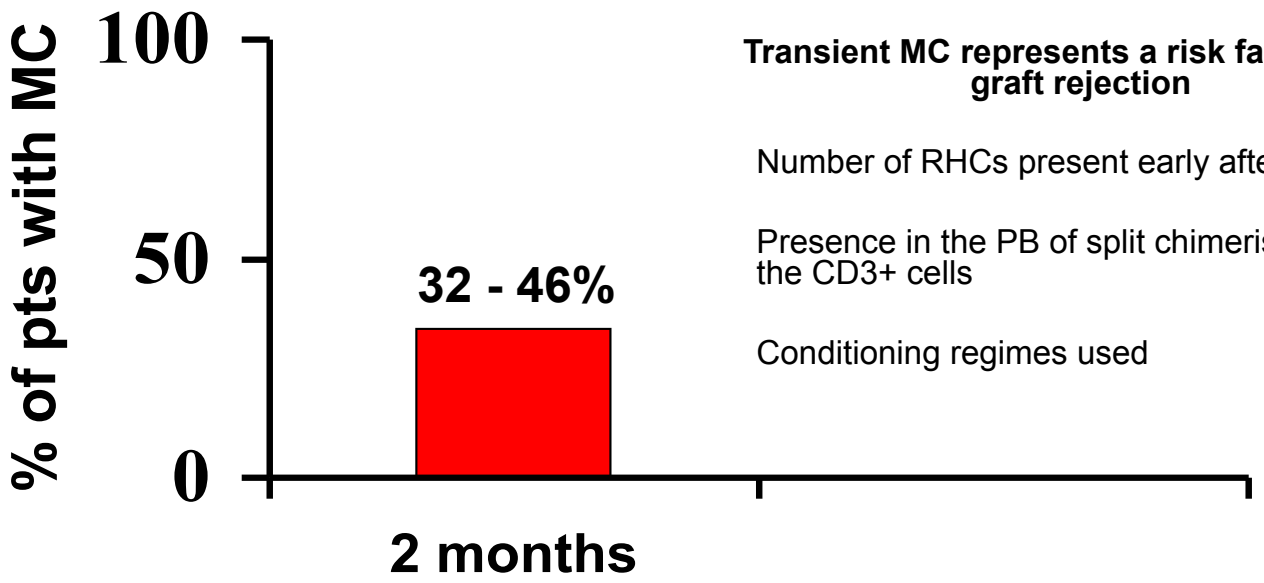
Cytofluorimetric analysis of washed RBCs, incubated with **anti-ABO** or **anti-C, c, D, E, e** monoclonal antibodies

	Patient	Donor
AB0:	**A**	**A**
Rh:	**+**	**+**
Subgroup:	**ccDEe**	**ccDee**
Useful Marker:	**E**	





Mixed Chimerism in Thalassemia after HSCT



Transient MC represents a risk factor for graft rejection

Number of RHCs present early after HSCT

Presence in the PB of split chimerism within the CD3+ cells

Conditioning regimes used

Blood Transfus. 2008 Jul;6(3):143-9.

Relationship between mixed chimerism and rejection after bone marrow transplantation in thalassaemia.

Andreani M, Testi M, Battarra M, Indigeno P, Guagnano A, Polchi P, Federici G, Lucarelli G.

Laboratorio di Immunogenetica e Biologia dei Trapianti, Fondazione IME, Roma, Italy.

Mixed Chimerism in Thalassemia after HSCT

Transient MC evolution

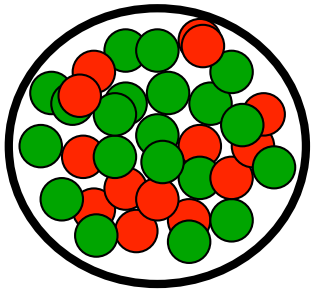
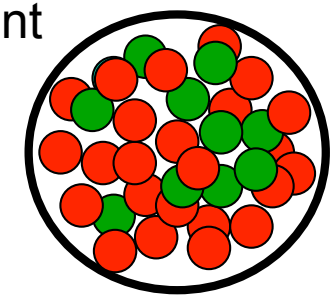
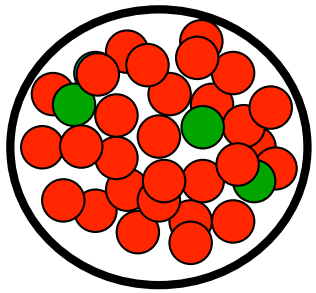
Number of RHCs present early after HSCT

Presence in the PB of split chimerism within the CD3+ cells

Conditioning regimes used

Andreani M, Testi M, Lucarelli G. Tissue Antigens. 2014

Mixed Chimerism in Thalassemia after HSCT

Chimerism status early after HSCT	Transplant outcome
 > 25%	50-60% Rejection PMC Complete Chimerism
patient  donor 10-25%	Rejection PMC Complete Chimerism
 < 10%	3-12% Rejection PMC Complete Chimerism

Patients enrolled only if a clinical control at 1 year after HSCT was performed						
	Engraftment status early after HSCT	Engraftment evolution over the time (minimum 1 year)				
Total N of Patients	**138 + 14 (152)**	**CC**	**MC level 1**	**MC level 2**	**MC level 3**	**Rejection**
MC level 1	**53**	**45**	**3**	**2**	**0**	**3 (5.6%)**
MC level 2	**5**	**4**	**0**	**0**	**0**	**1 (20%)**
MC level 3	**20**	**3**	**1**	**0**	**2**	**14 (70%)**
CC	**74**	**65**	**4**	**0**	**3**	**2 (2.7%)**

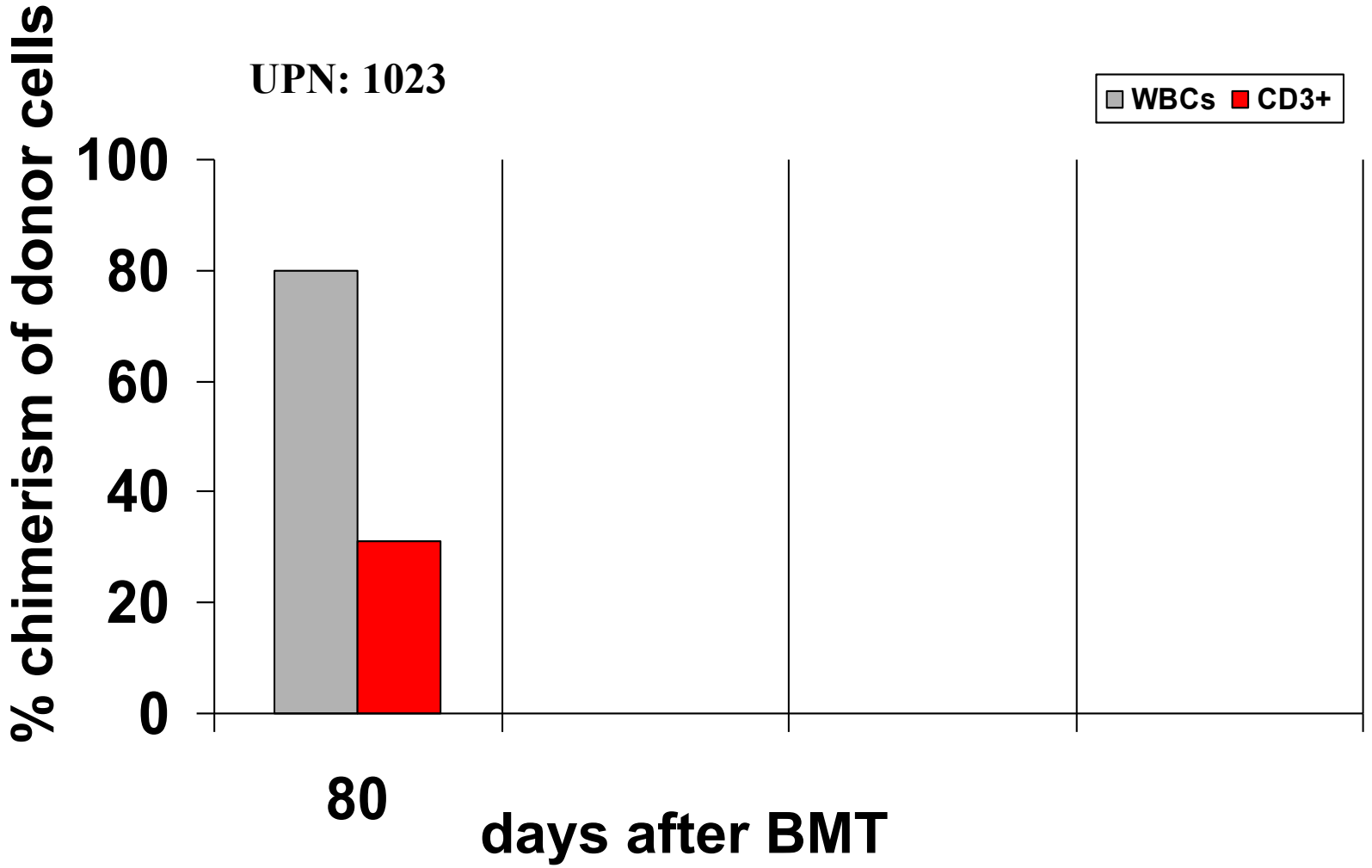
Transient Mixed Chimerism in Thalassemia after HSCT

Number of RHCs present early after HSCT

Presence in the PB of split chimerism within the CD3+ cells

Conditioning regimes used

Mixed Chimerism in Thalassemia after HSCT



Mixed Chimerism in Thalassemia after HSCT

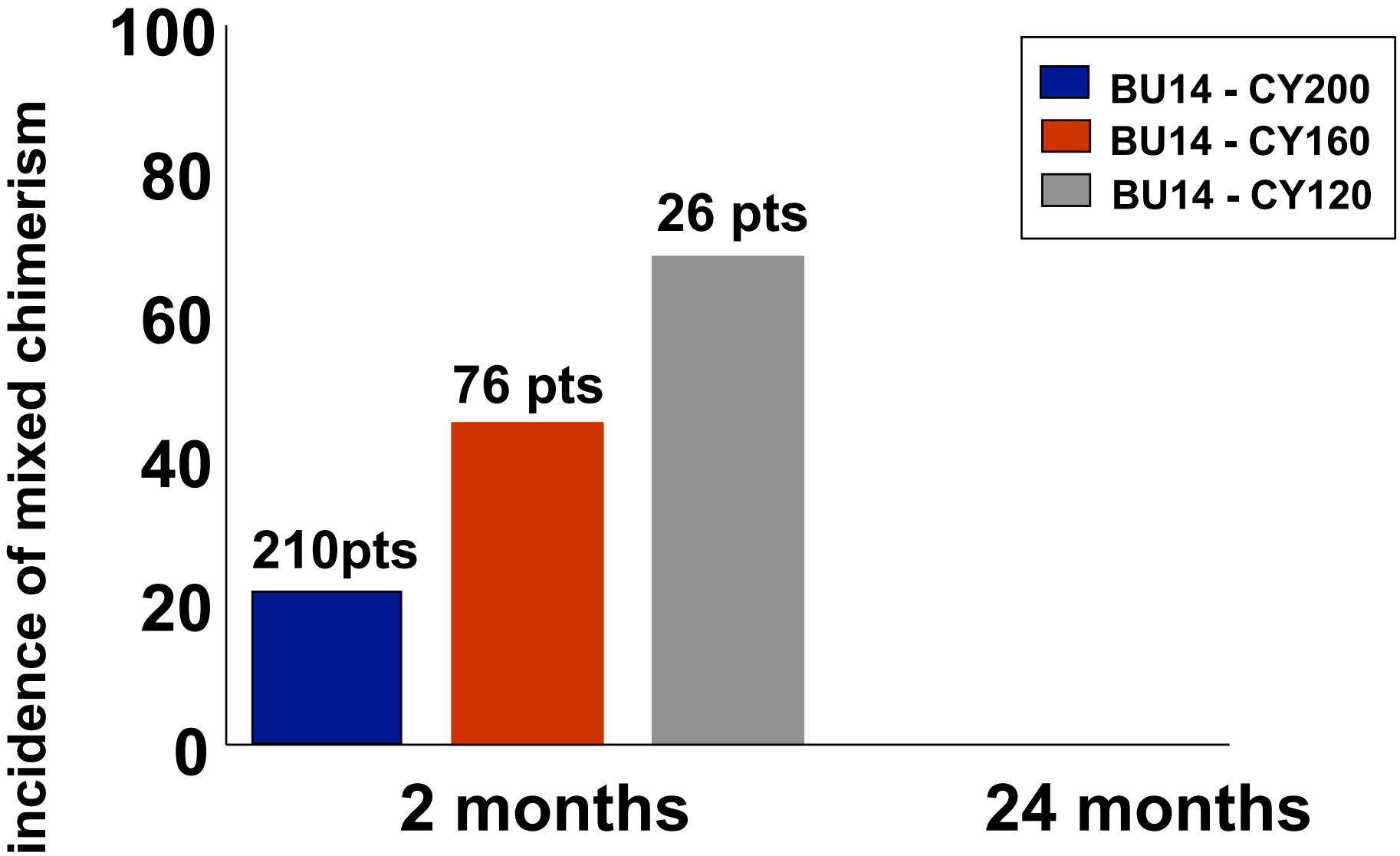
Transient MC evolution

Number of RHCs present early after HSCT

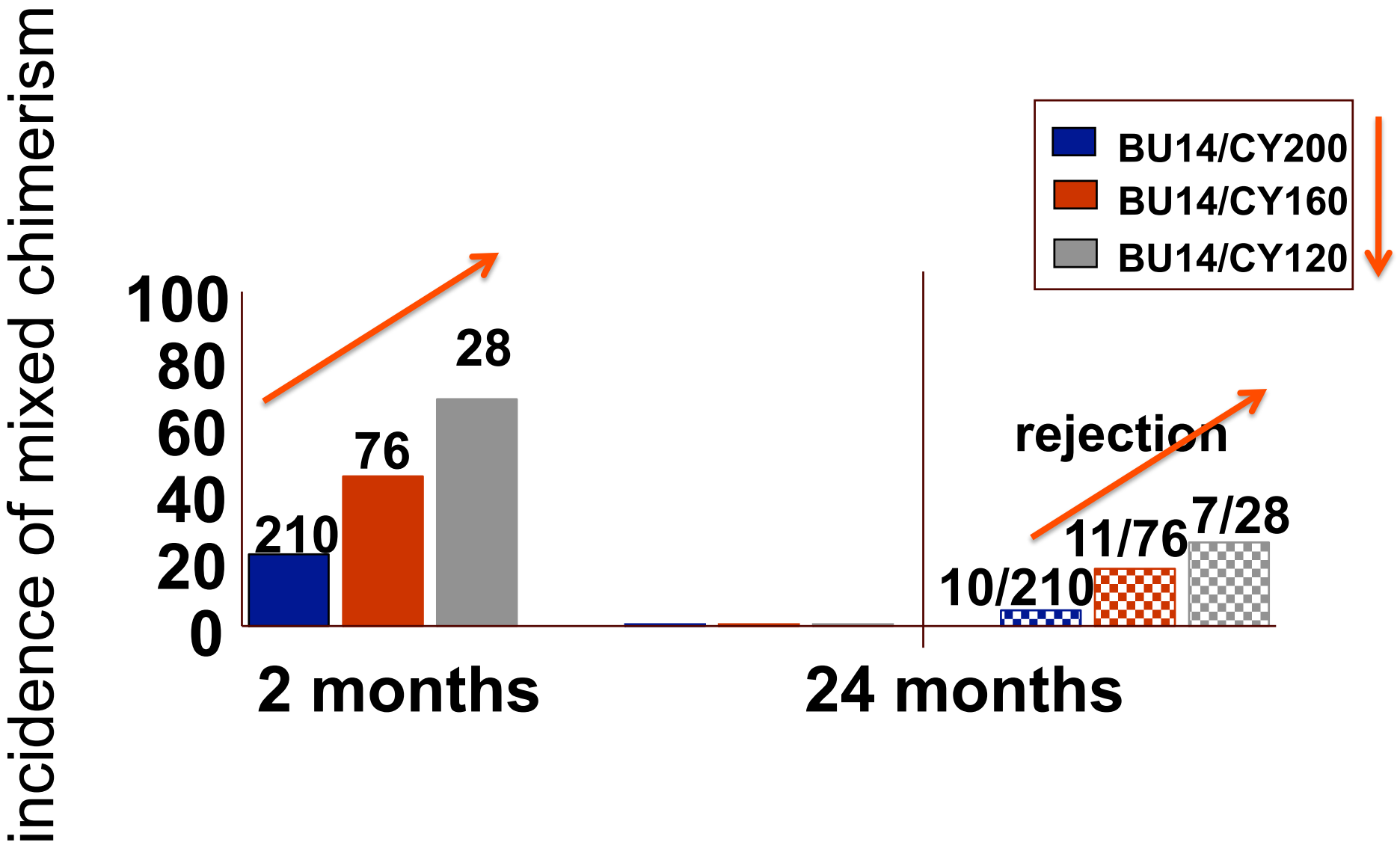
Presence in the PB of split chimerism within the CD3+ cells

Conditioning regimes used

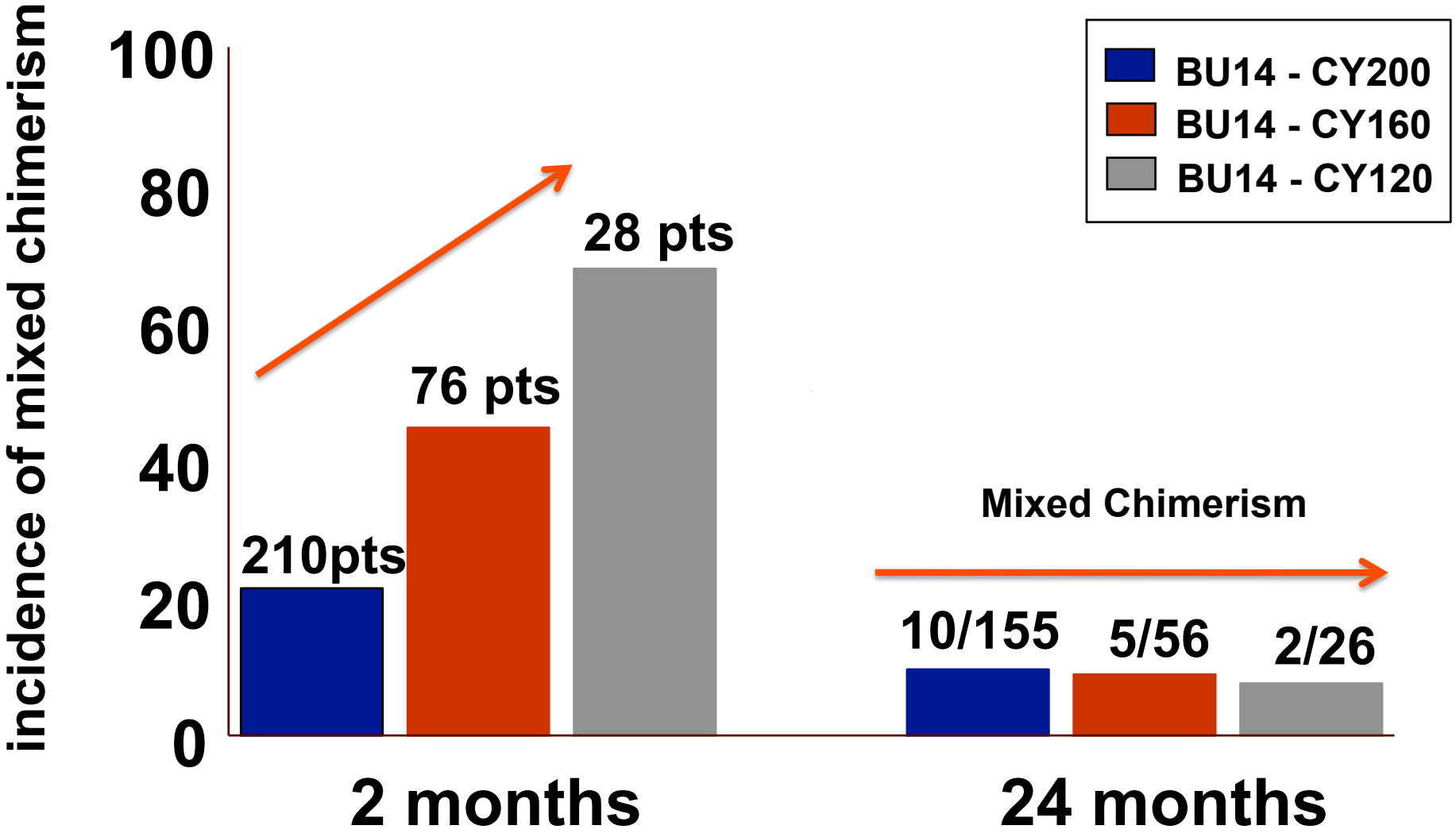
Mixed Chimerism in Thalassemia after HSCT



Mixed Chimerism in Thalassemia after HSCT



Mixed Chimerism in Thalassemia after HSCT

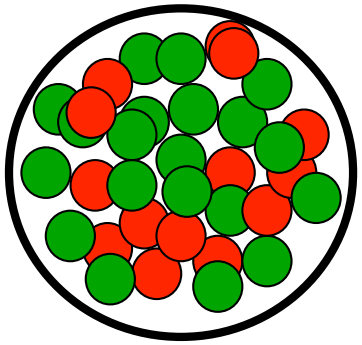


Persistent Mixed Chimerism in Thalassemia after HSCT

Clinical conditions of patients with PMC

- no red blood cells infusions
- produce high levels of hemoglobin
- no evolution to graft failure
- no evolution to complete chimerism

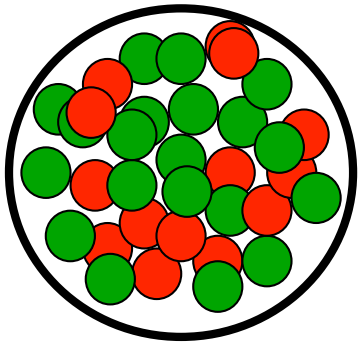
Functional graft



Status of MC characterized by the presence of large amount of RHCs

After 2 months from HSCT → **High probability of Rejection**

Andreani M, Testi M, Lucarelli G. Tissue Antigens. 2014



Status of MC characterized by the presence of large amount of RHCs

After 2 months from HSCT → **High probability of Rejection**

After 2 years from HSCT → **NO Rejection**

Time after HSCT

Andreani M, Testi M, Lucarelli G. Tissue Antigens. 2014

Persistent Mixed Chimerism

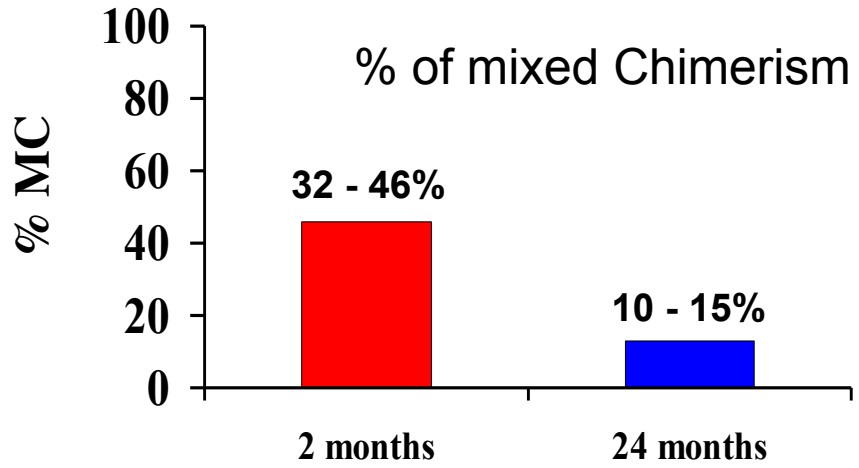
MC is defined persistent when donor and recipient cells **coexist for more than 24 months after HSCT**

no evolution to graft failure ?

no evolution to complete chimerism

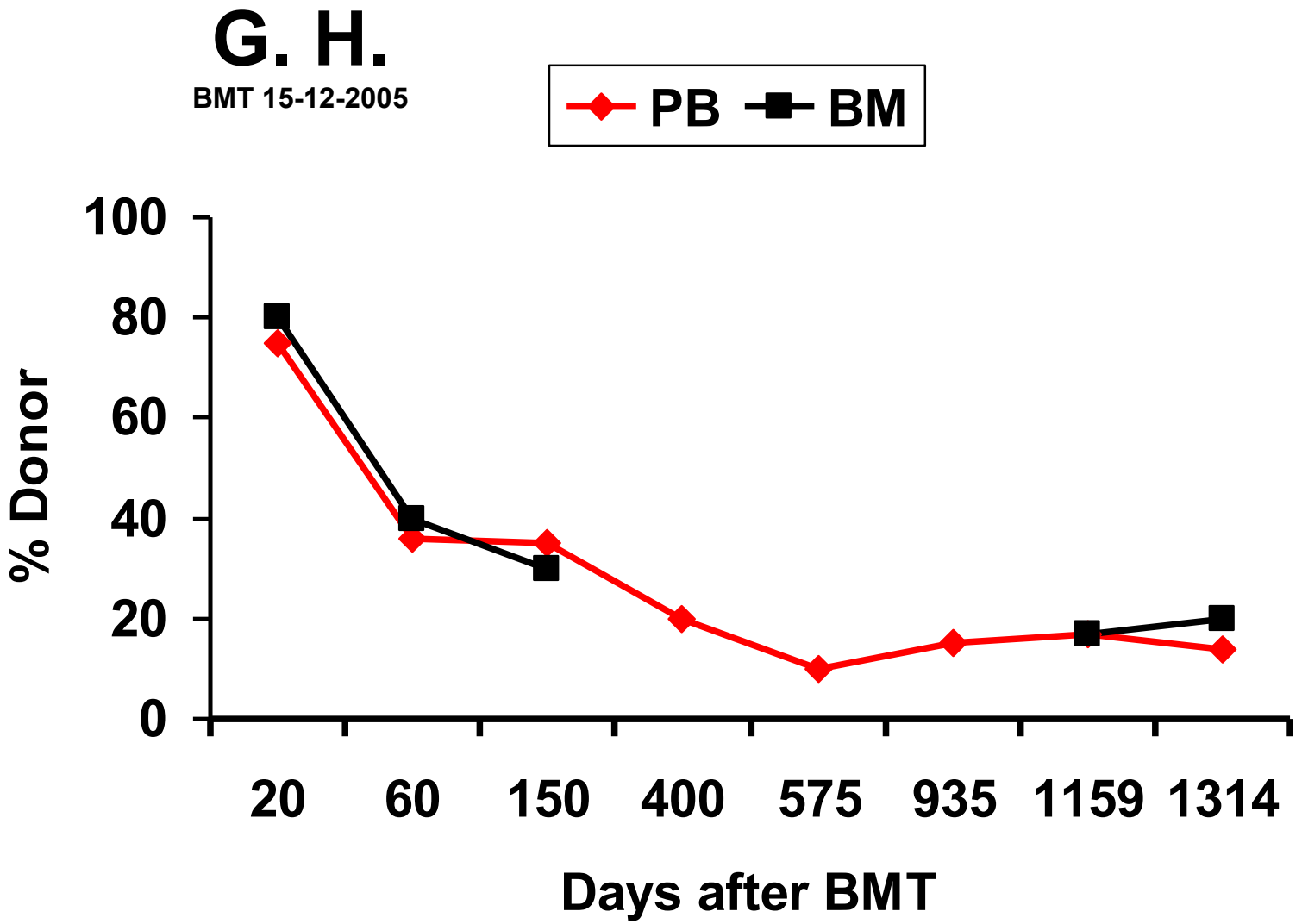
Persistent Mixed Chimerism

Functional graft



In many cases the proportion of cells of recipient origin is extremely large

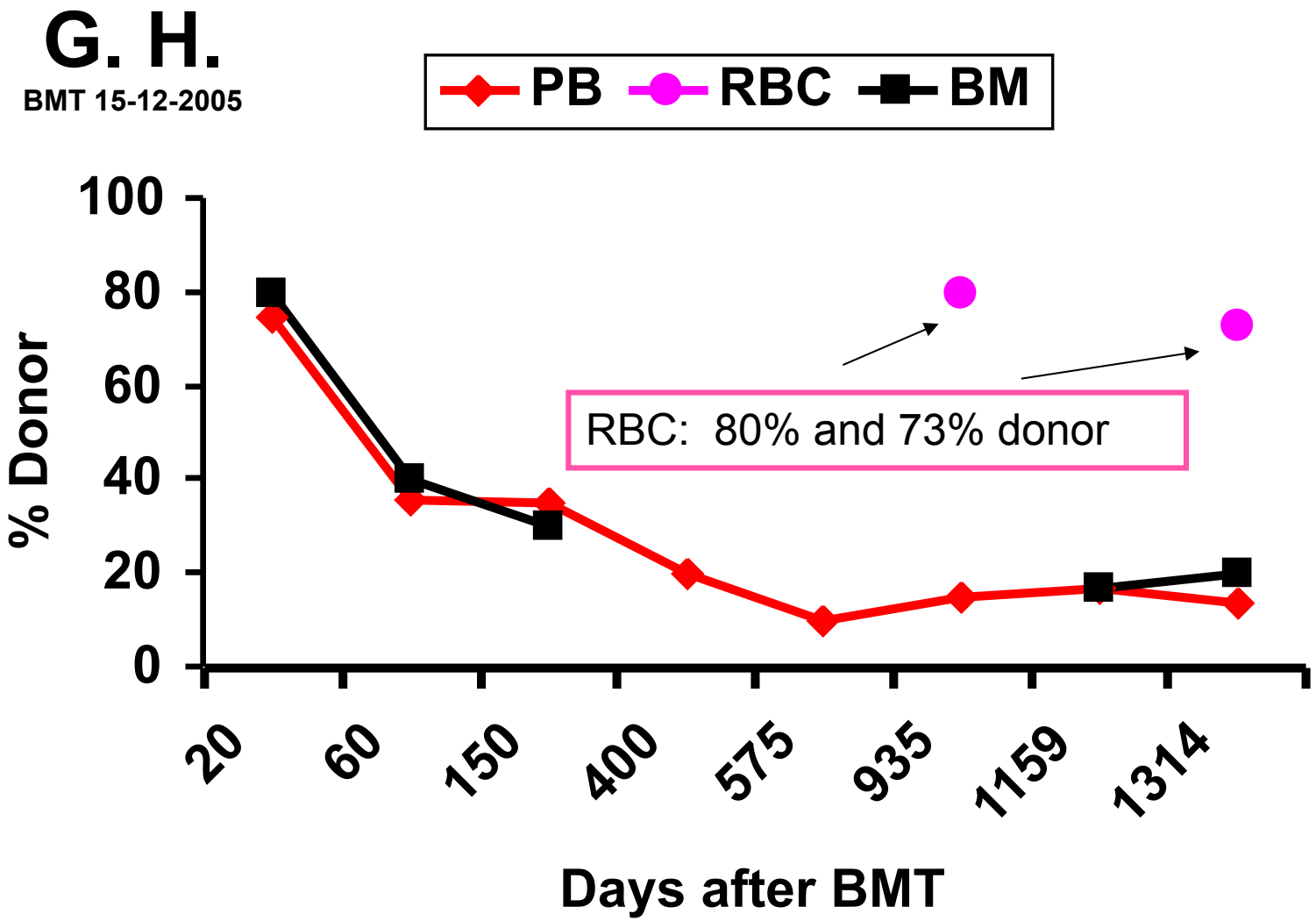
Mixed Chimerism in Thalassemia after HSCT



Persistent Mixed Chimerism

Split chimerism between PBMCs and RBCs

Mixed Chimerism in Thalassemia after HSCT



Clinical Chimerism testing

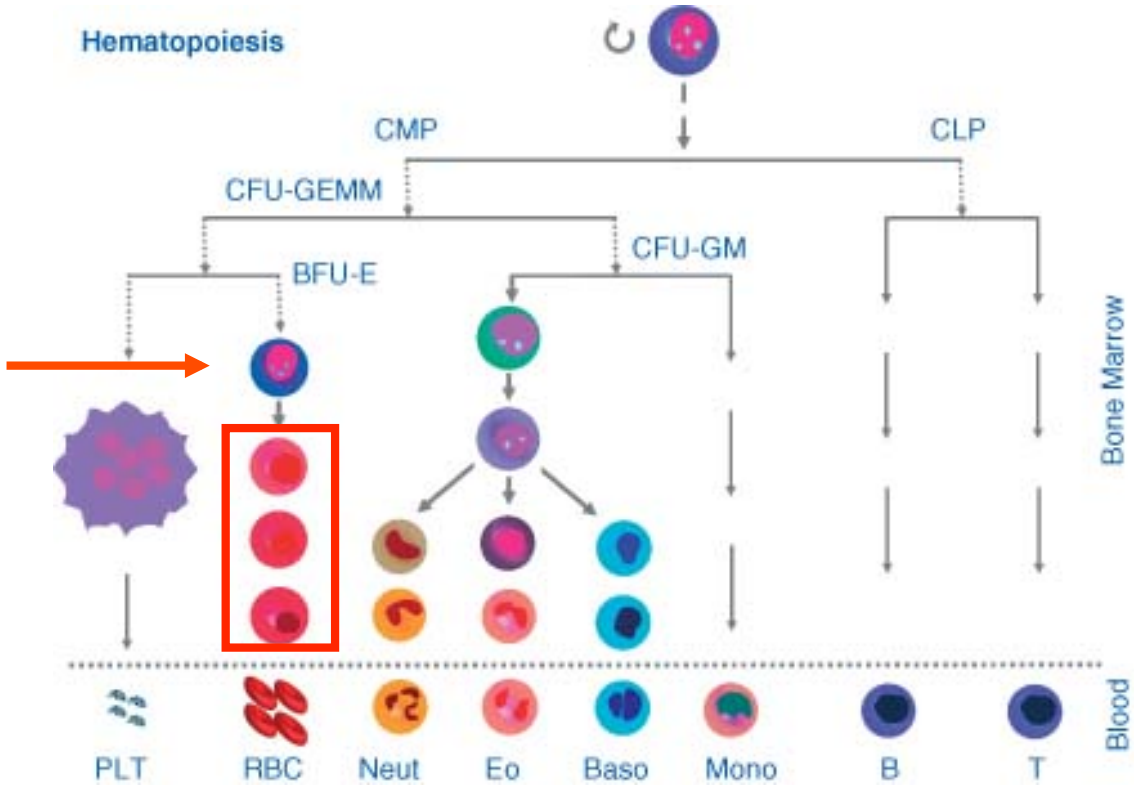
Persistent Mixed Chimerism

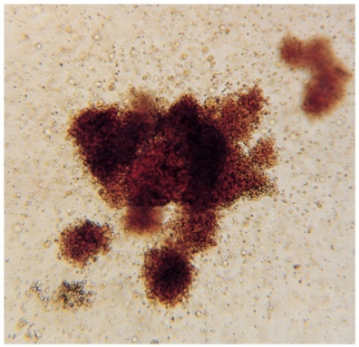
- Control of the erythroid expansion
- Immunological Tolerance

Mixed Chimerism in Thalassemia after HSCT

Control of the erythroid compartment expansion

BFU-E: donor – recipient origin

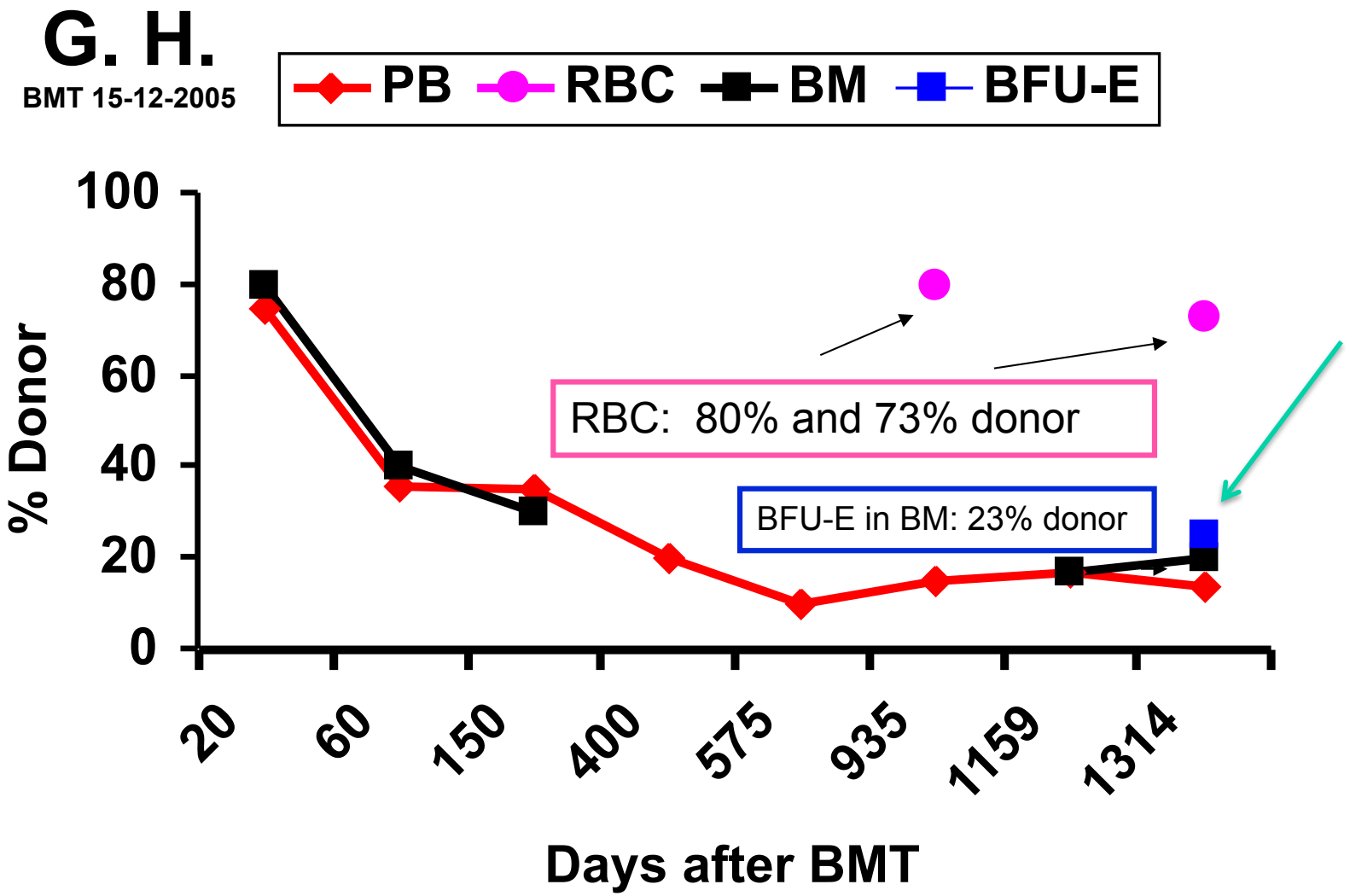




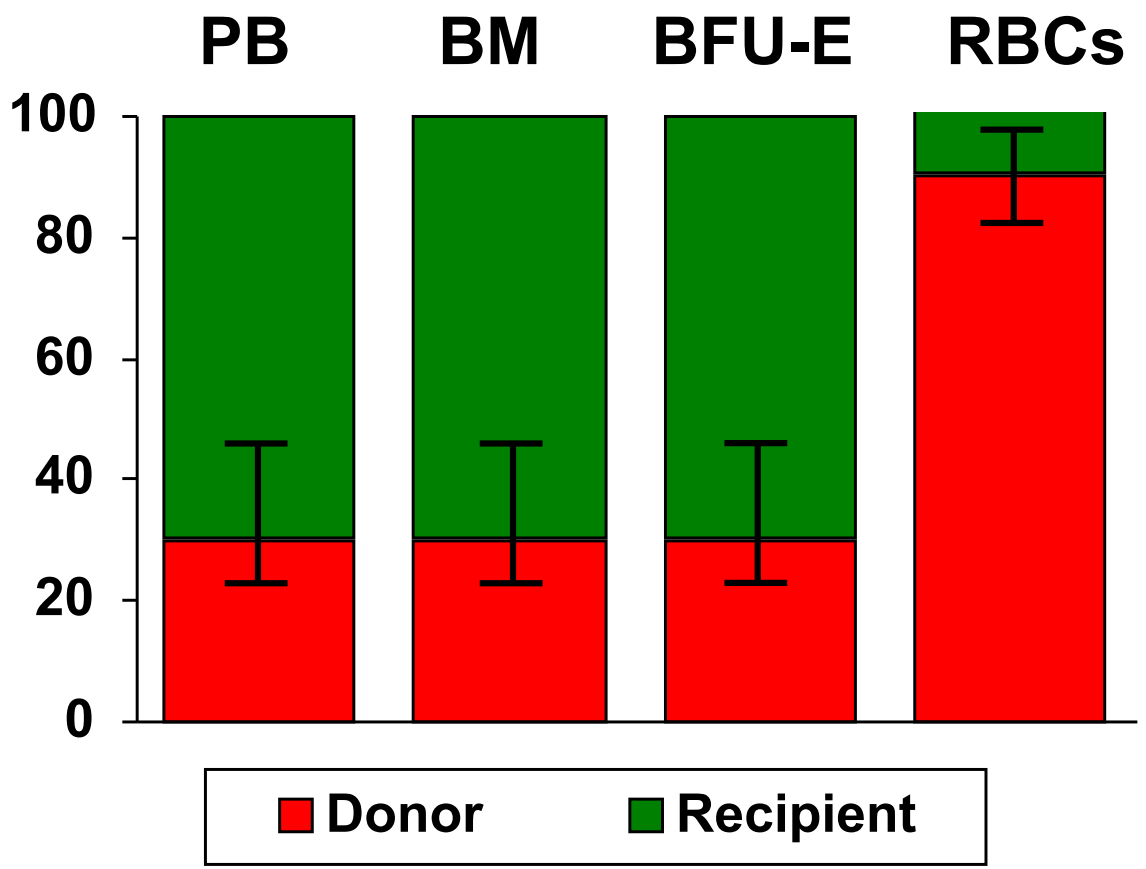
DNA extraction

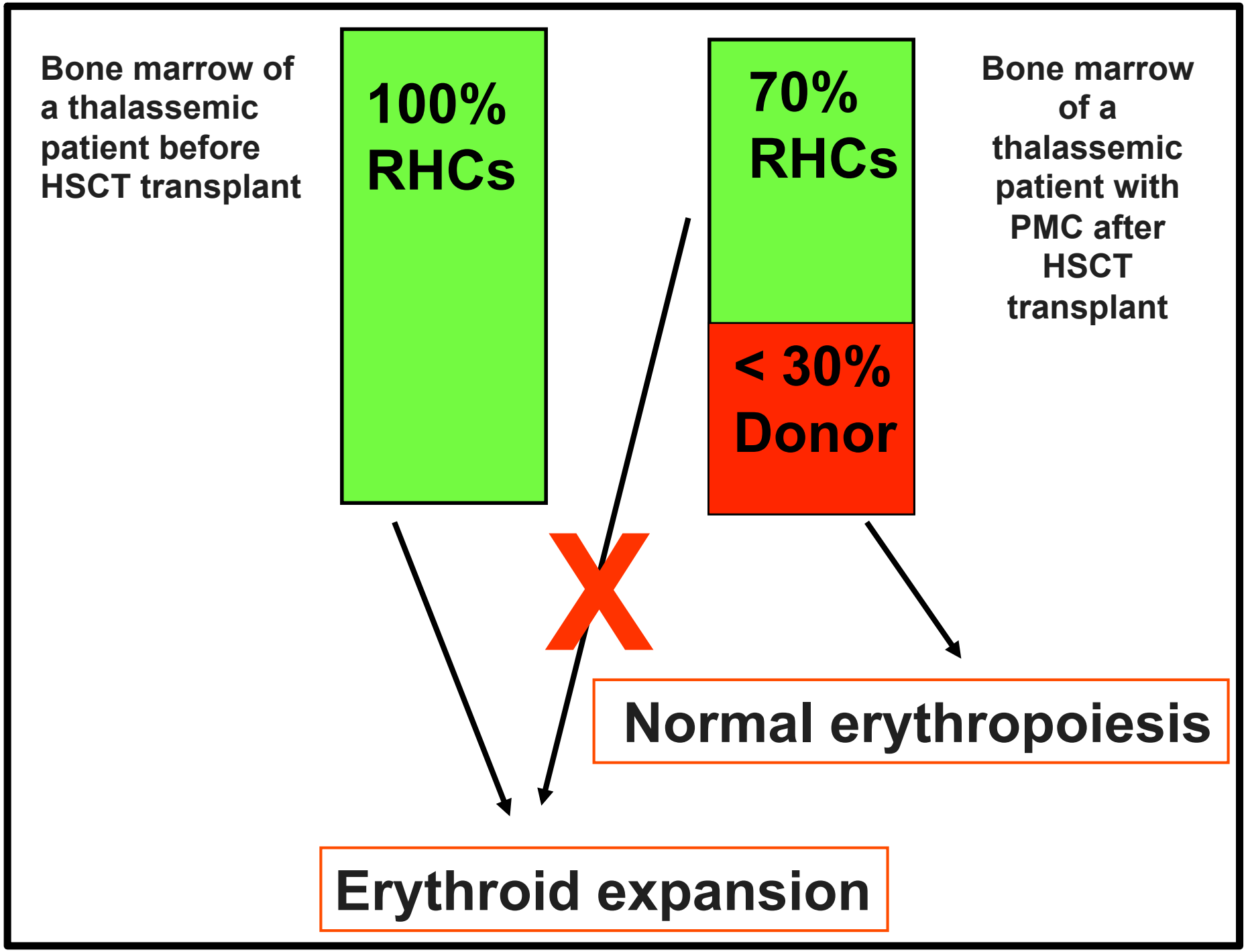
STR analysis

Mixed Chimerism in Thalassemia after HSCT



Split chimerism between RBCs and peripheral blood (PB) bone marrow (BM) and erythroid precursors (BFU-E) in a patient with PMC at 60 months after HSCT




Bone marrow of a thalassemic patient before HSCT transplant
100% RHCs
70% RHCs
< 30% Donor
Bone marrow of a thalassemic patient with PMC after HSCT transplant
X
Normal erythropoiesis
Erythroid expansion

Mixed Chimerism in Thalassemia after HSCT

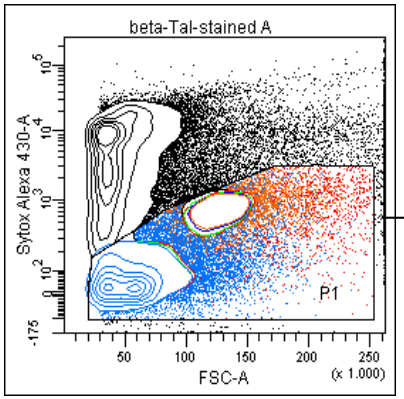
Control of the erythroid compartment expansion

Primary BFU-E: donor – recipient origin

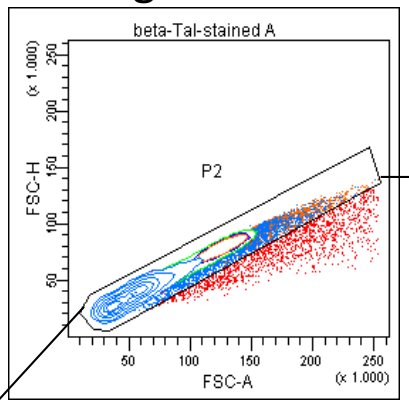
Secondary BFU-E: Self renewal capability of donor and recipient BFU-E

Erythroblasts subpopulations sorting

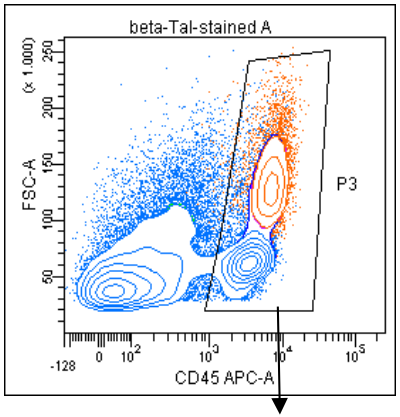
Exp 1-Day 0



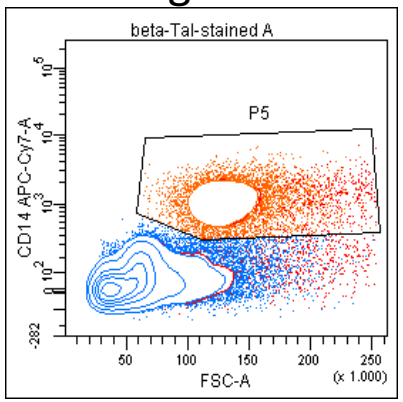
P1-gated cells



P2-gated cells

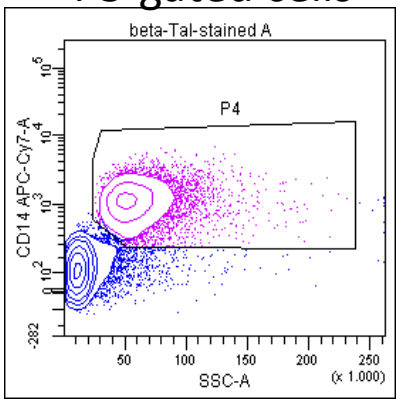


P2-gated cells

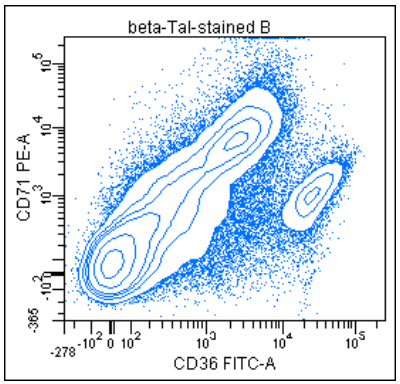


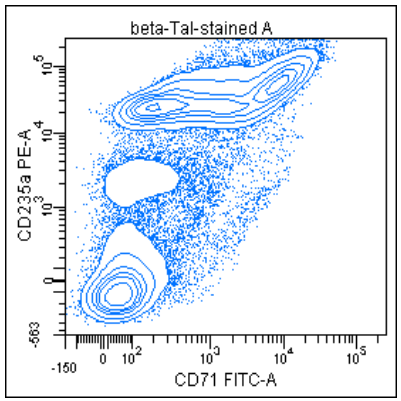
Tube: stained B			
Population	#Events	%Parent	%Total
All Events	152.063		100,0
P1	65.057	42,8	42,8
P2	62.785	96,5	41,3
P3	21.599	34,4	14,2
P4	7.561	35,0	5,0
NOT(P4)	14.038	65,0	9,2
P5	7.392	11,8	4,9
NOT(P5)	55.393	88,2	36,4

P3-gated cells

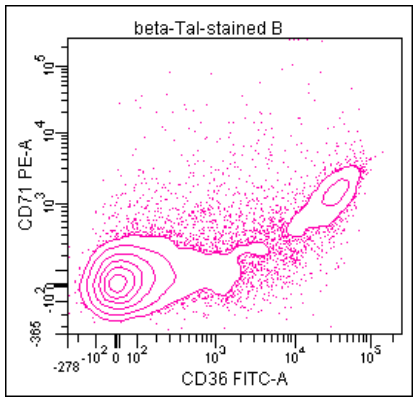


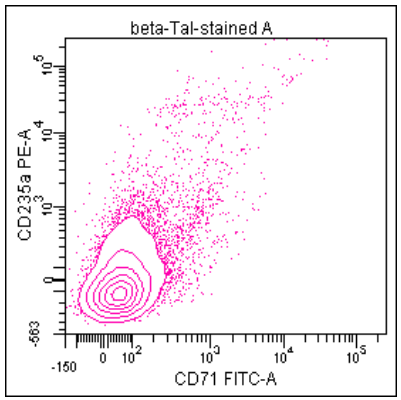
Not P5-gated cells





Not P4-gated cells





Mixed Chimerism in Thalassemia after HSCT

Preliminary results for Teyab: +3001 days after HSCT

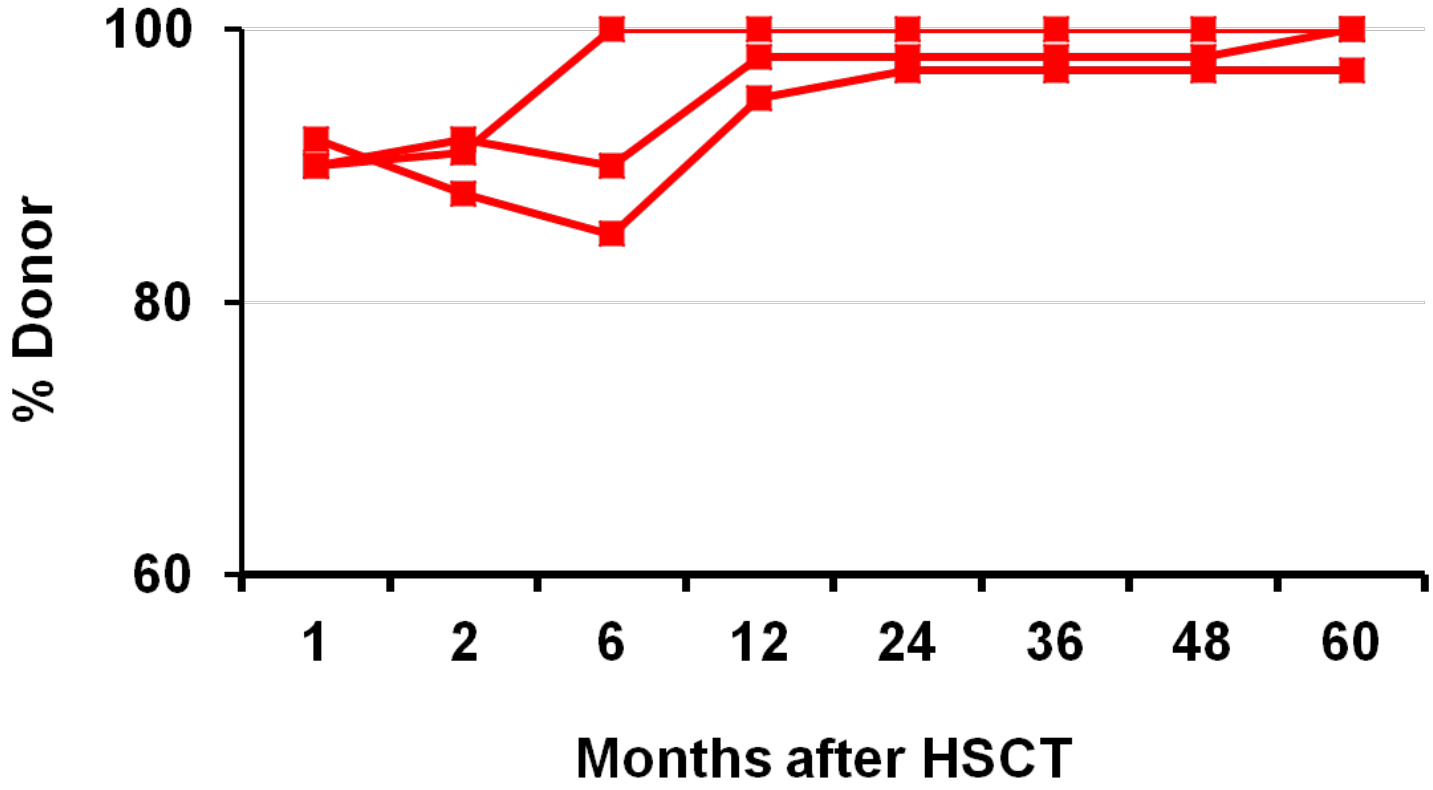
Chimerismo Misto in BM - Teyab								
Days	BM % DON	Sorting precursosri eritroidi						RBC %DON
		Popol. Control	CD34+	Proer.	Er. Bas	Er. Poly	Er. Ort	
3001	44	51	32	50	40	60	NV	100

Clinical Chimerism testing

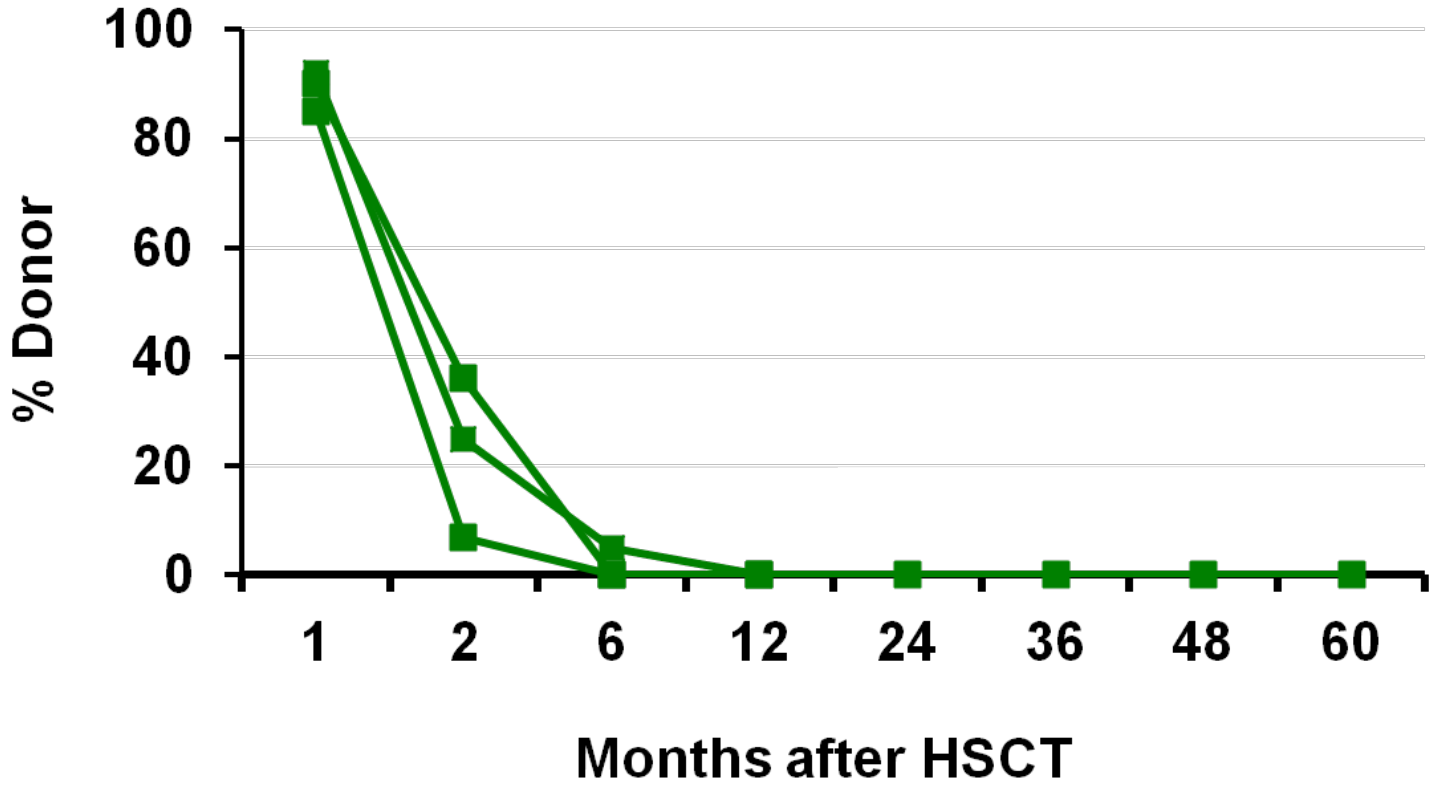
Persistent Mixed Chimerism

- Control of the erythroid expansion
- Immunological Tolerance

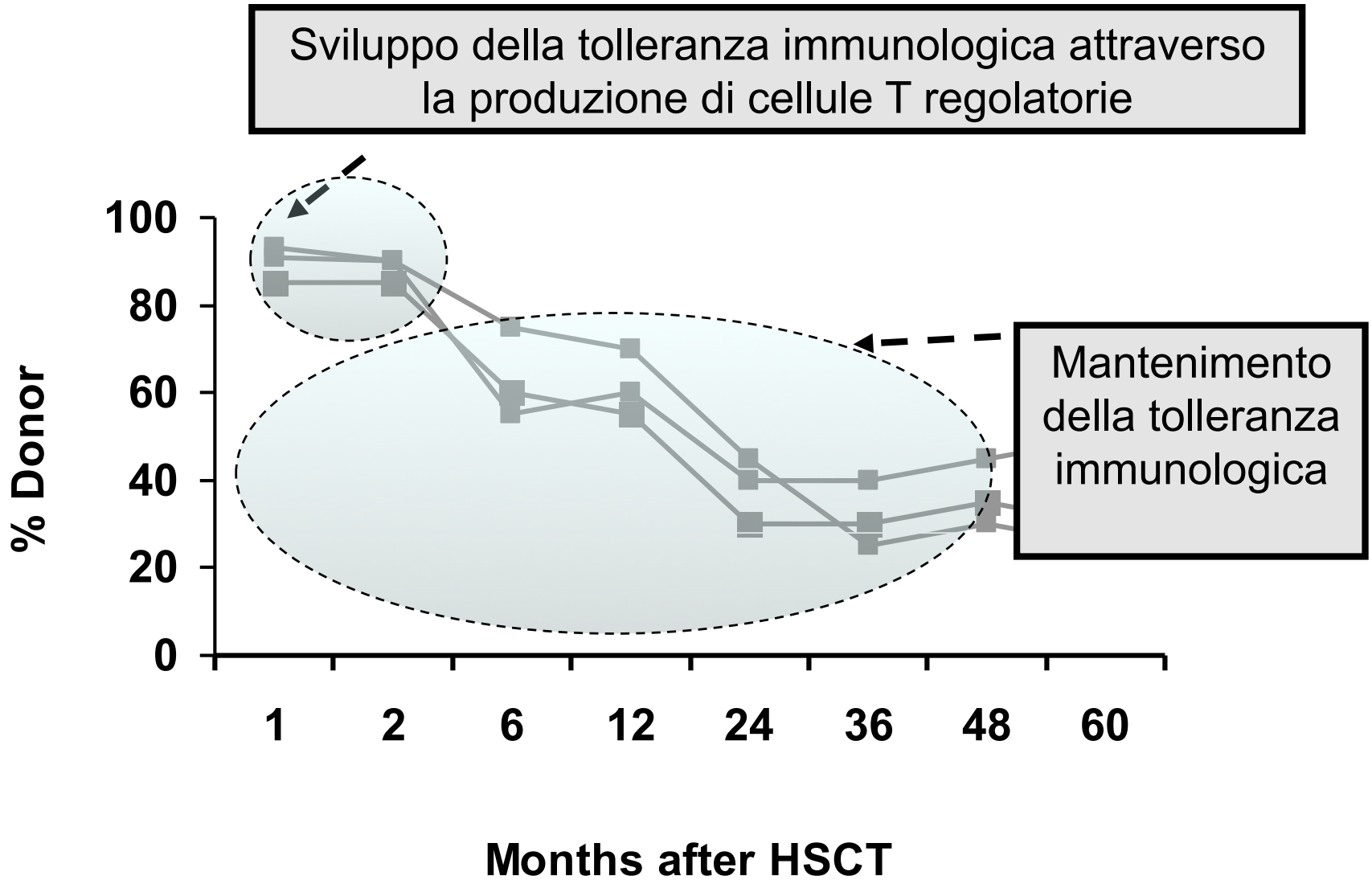
Tolleranza immunologica dopo trapianto di CSE nelle emoglobinopatie



Tolleranza immunologica dopo trapianto di CSE nelle emoglobinopatie



Tolleranza immunologica dopo trapianto di CSE nelle emoglobinopatie



Induzione della tolleranza periferica tramite cellule T regolatorie

INDUCTION OF PERIPHERAL TOLERANCE BY REGULATORY T CELLS

CD4^{-} 8^{-} | $\gamma\delta^{+}$ | **CD4^{+}** | NK-T | CD8^{+}

- Tr1
- nTr (CD4^{+}CD25^{+}FOXP3^{+})

COMMON MECHANISMS:

- Suppression of proliferation/cytokine production *in vitro*
- Suppressive cytokines and/or inhibitory receptors

MORE RECENT ACHIEVEMENTS

Definition of new tolerogenic markers for Tr1 cells:

Granzyme B

CD49b and LAG-3

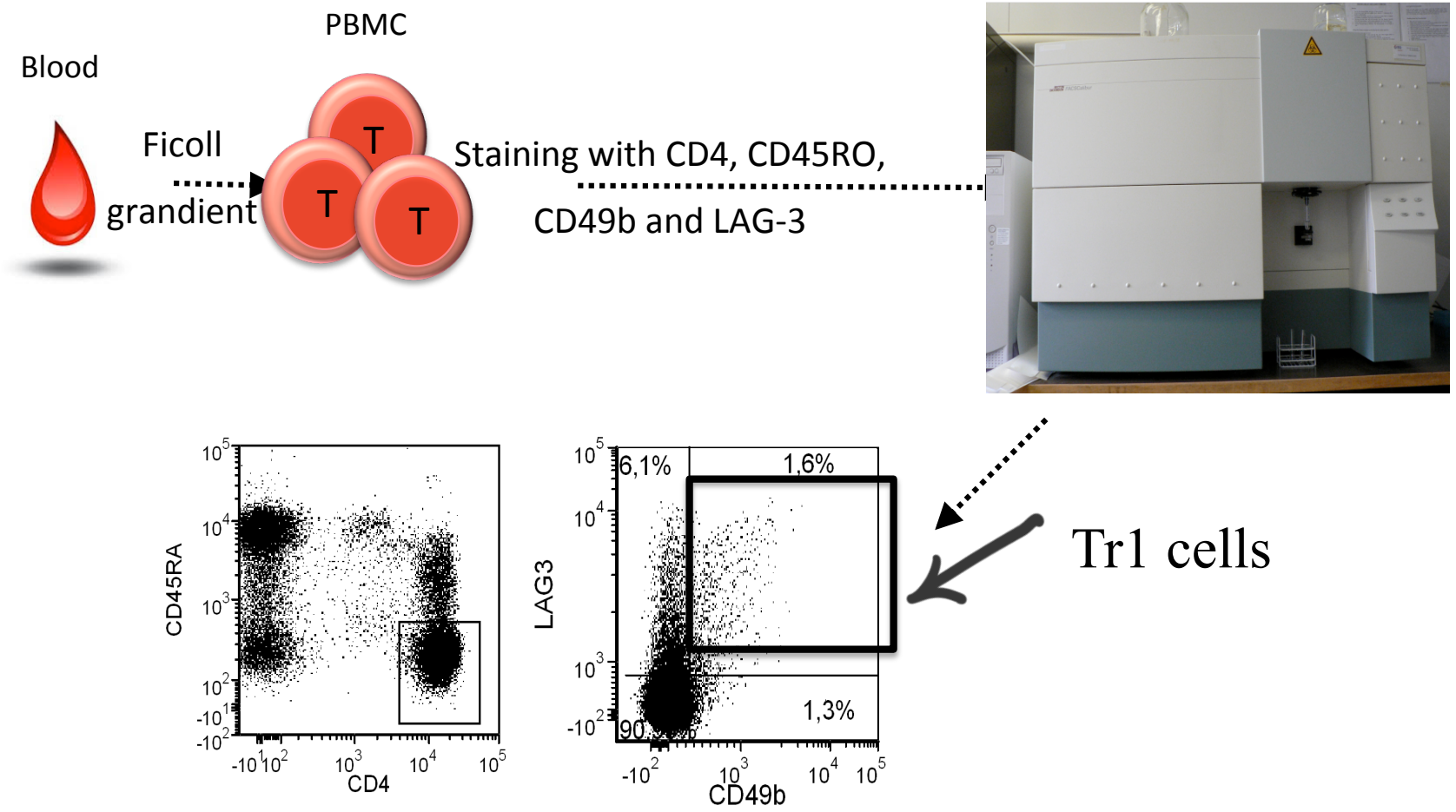
MORE RECENT ACHIEVEMENTS

Definition of new tolerogenic markers for Tr1 cells:

GranzymeB

CD49b and LAG-3

Identificazione di cellule Tr1 umane



Identificazione e selezione delle cellule Tr1 dopo trapianto di CSE nella talassemia

origine sia del donatore che del ricevente

	Clonaggio in vitro	
Origine	n° cloni	**n° Tr1**
Ricevente	**12**	**5**
Donatore	**16**	**7**

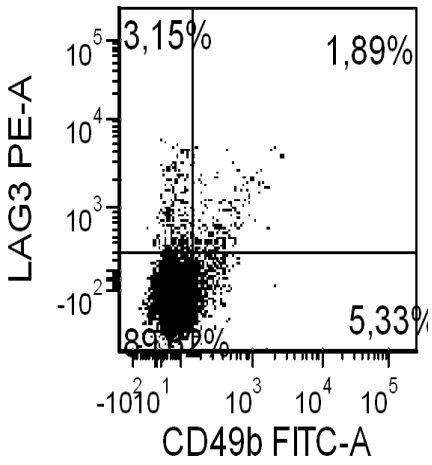
producono alti livelli di IL-10 se confrontati con cloni Tr1 di individui normali

clone	IL10	IL2	IL4	IFNg	IL17	IL10/IL4	origin
#246	4700	0	124	770	128	37,9	D
#93	2756	29	30	1020	112	91,9	D
#282	12350	20	266	630	0	46,4	H
#46	6510	174	246	1825	64	26,5	D
#143	8910	151	176	18070	0	50,6	H
#17	5700	46	20	1005	0	285,0	H
#131	1350	114	0	4245	0	67,5	D
#103	1620	20	20	425	0	81,0	D

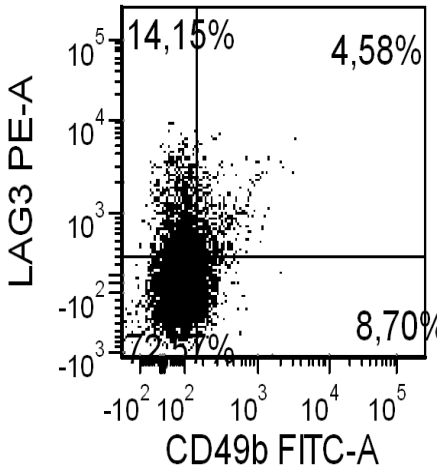
Serafini G, Andreani M, Testi M et al. *Haematologica. 2009*

CD45RA^{-}CD49b^{+}LAG-3^{+} Tr1 cells are highly represented in PMC

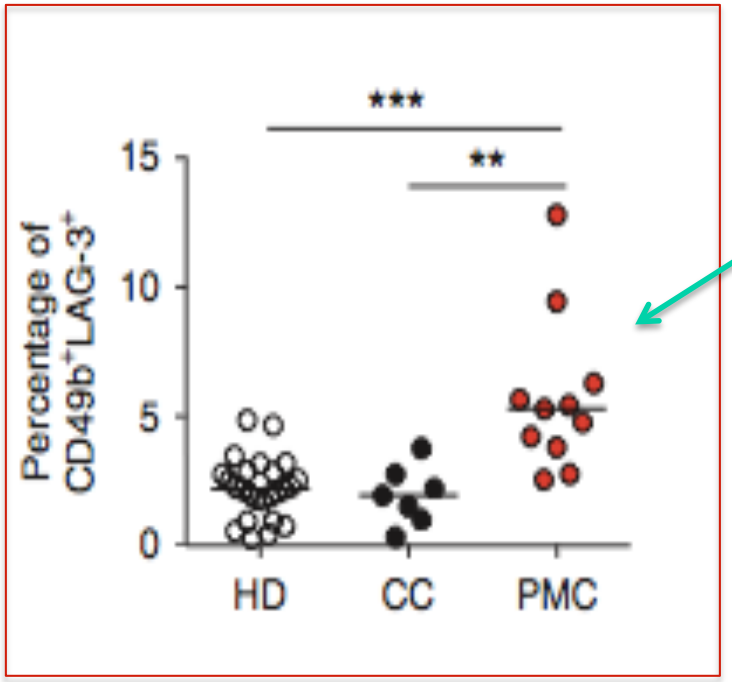
Control Pt
CC



Tolerant Pt
PMC

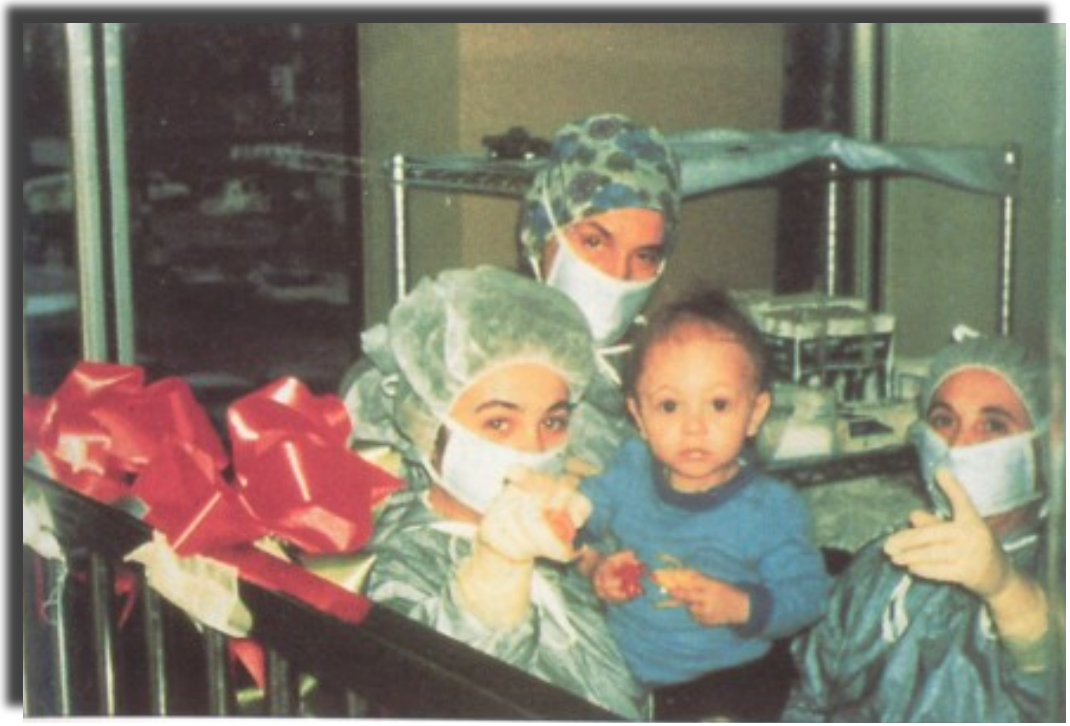


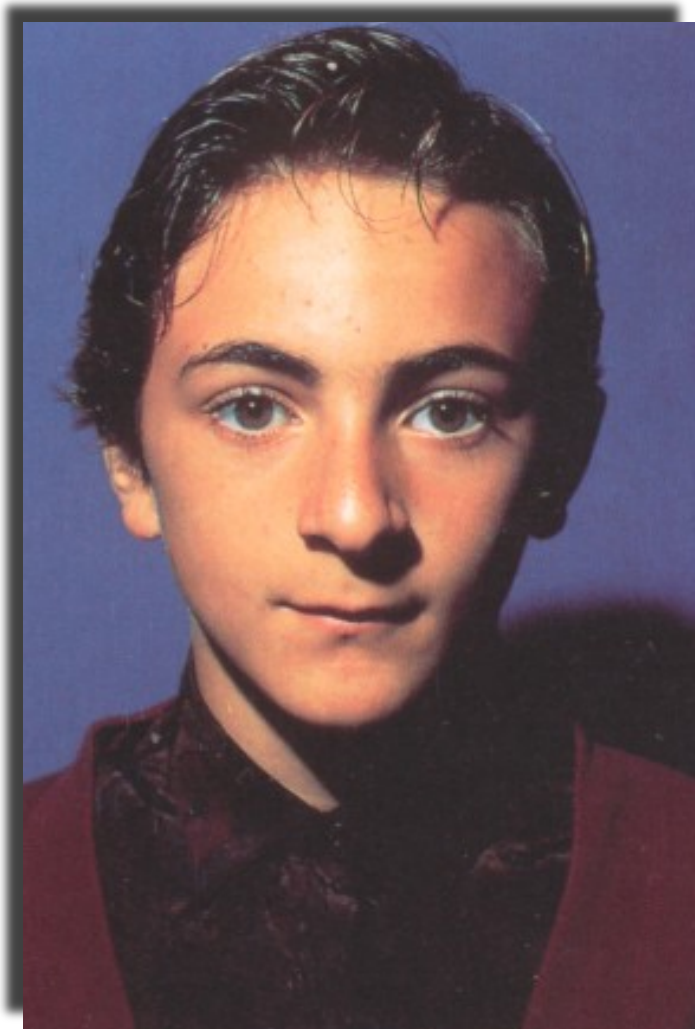
Tolerant patients after BMT



Gagliani N., Magnani C., and Huber S:, Nat Med 2013

Primo paziente talassemico sottoposto a trapianto di midollo osseo (Seattle 1981)





Primo paziente talassemico sottoposto a trapianto di midollo del 1981

Evoluzione (BMT 1981)

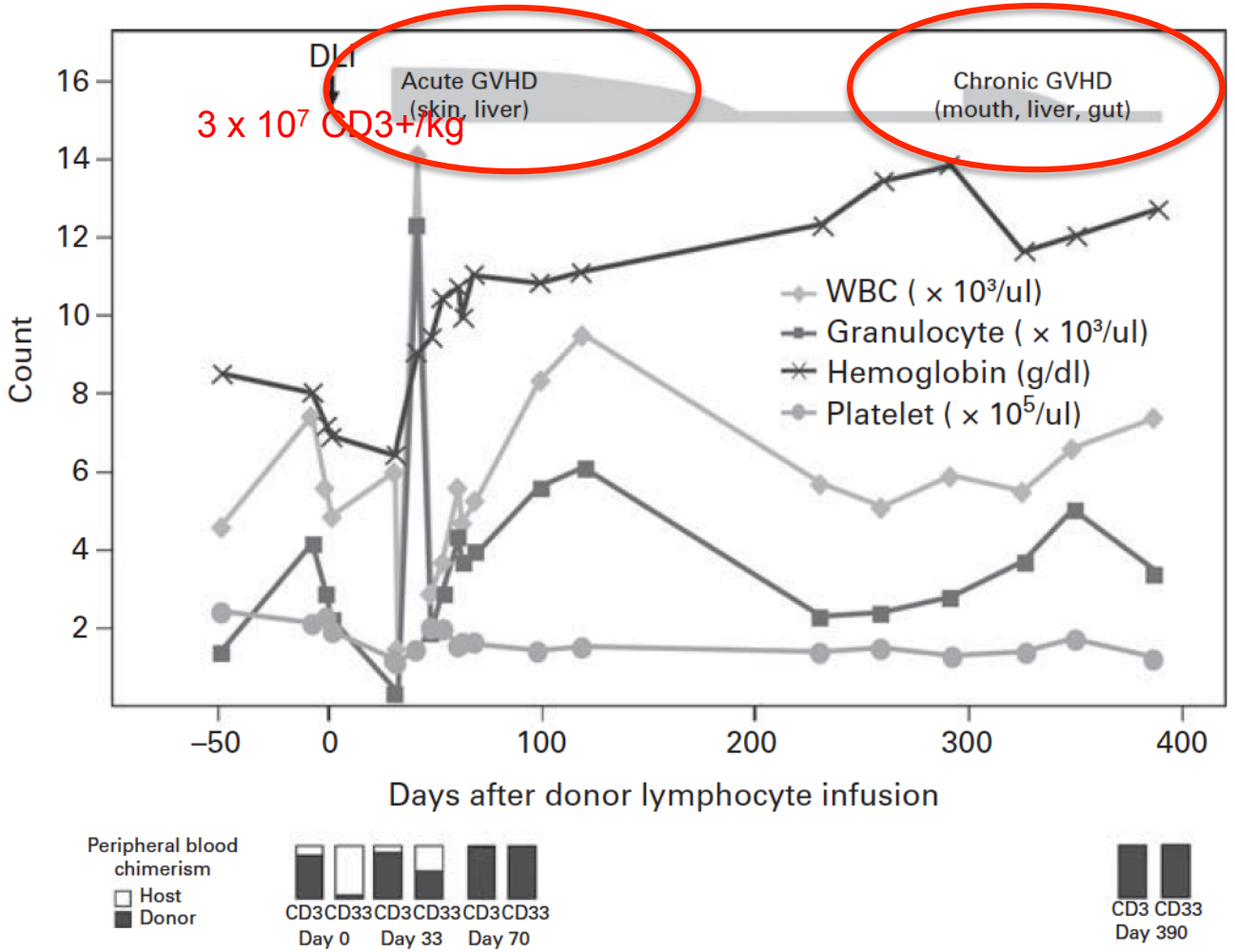
- Rigetto del trapianto documentato nel 2013
- Ripresa terapia di supporto trasfusionale
- Ripresa terapia ferrochelante

Bone Marrow Transplantation (2011) 46, 1037–1038

www.nature.com/bmt

LETTER TO THE EDITOR

Recurrence of β-thalassemia major more than 20 years after HLA-identical sibling BMT treated successfully with donor lymphocyte infusion



Bone Marrow Transplantation (2010) 45, 1047–1051

www.nature.com/bmt

ORIGINAL ARTICLE

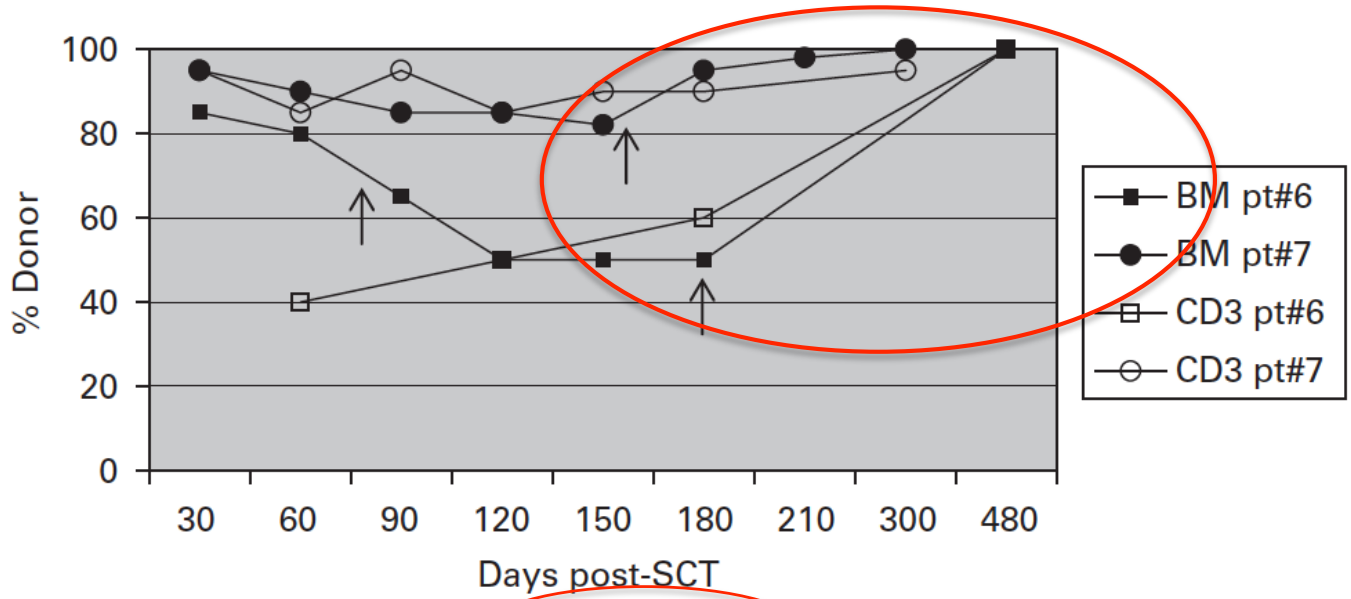
Escalating doses of donor lymphocytes for incipient graft rejection following SCT for thalassemia

I Frugnoli[1], B Cappelli[1,2], R Chiesa[1,2], E Biral[1,2], A Noè[1], C Evangelio[1], M Fossati[1], S Napolitano[1], F Ciceri[3], MG Roncarolo[1,2,4] and S Marktel[1,2]

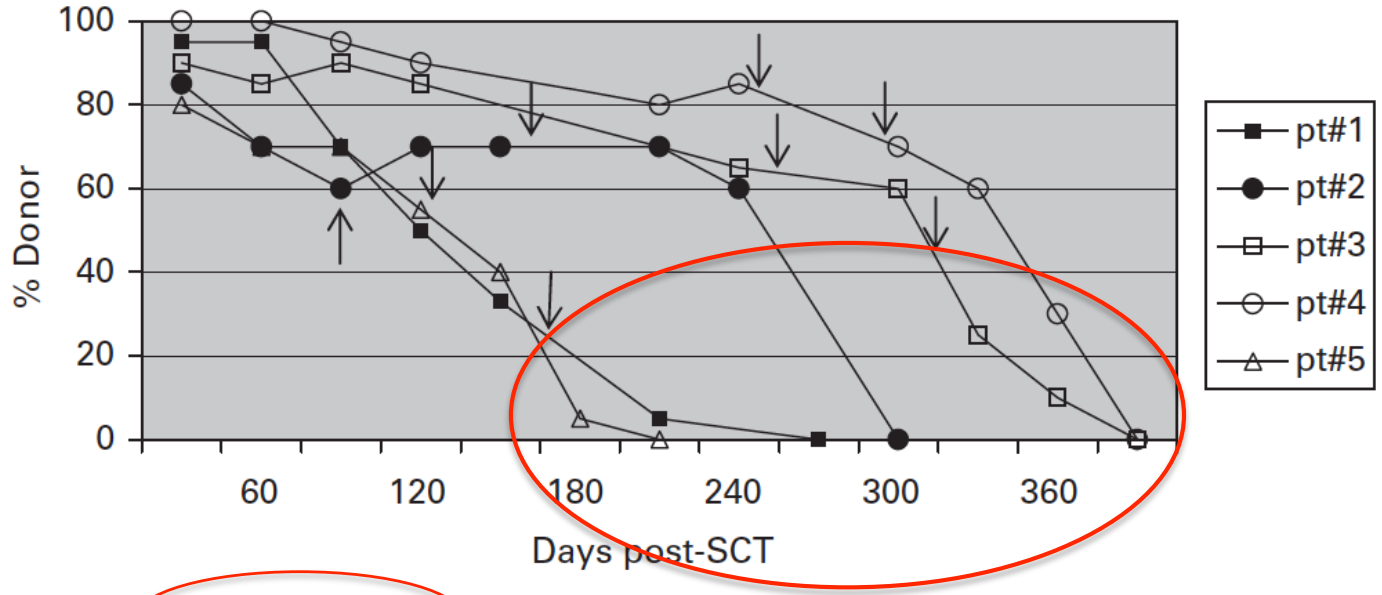
1 x 10^7 CD3+/kg 3 x 10^7 CD3+/kg

Table 1 Characteristics of recipients, donors, SCT and post transplantation chimerism

Patient (gender, age)	*Pesaro class*	*Donor type (gender, age)*	*CD34 ($\times 10^6$/kg)*	*CD3 ($\times 10^6$/kg)*	*% Donor on day 60 (BM/PB/CD3)*	*SCT- 1st DLI (days)*	*% Donor at 1st DLI (BM/PB/CD3)*	*GVHD prophylaxis at 1stDLI*	*SCT- 2nd DLI (days)*	*% Donor at 2nd DLI (BM/PB/CD3)*	*Outcome*
#1 (M, 5)	III	HLA ID SIB (F, 12)	13.4	84	95/NA/NA	167	35/35/NA	None			Rejection
#2 (F, 10)	III	HLA ID SIB (F, 14)	5.7	77	70/65/55	106	60/70/NA	CyA (9 mg/kg)	175	70/65/35	Rejection
#3 (M, 13)	III	HLA ID SIB (M, 15)	6.0	72	85/85/80	264	65/75/60	CyA (2.8 mg/kg)	329	25/20/NA	Rejection
#4 (M, 8)	III	HLA ID SIB (M, 16)	6.1	89	100/95/95	267	85/85/70	CyA (2.6 mg/kg)	306	70/70/75	Rejection
#5 (M, 5)	II	PHENO-ID (F^a, 27)	3.3	47	70/60/40	135	55/55/25	CyA (7.7 mg/kg)			Rejection
#6 (F, 8)	II	HLA ID SIB (M, 25)	6.0	114	80/70/40	90	65/65/40	FK506 (0.10 mg/kg)	176	50/50/60	Full donor
#7 (F, 4)	II	HLA ID SIB (F, 17)	5.4	66	95/95/85	174	82/82/90	FK506 (0.12 mg/kg)			Full donor



Donor chimerism on BM and PB CD3 positive cells in class II patients treated with DLI. Arrows indicate DLI infusions.



Donor chimerism on BM in class III patients treated with DLI. Arrows indicate DLI infusions.

Pediatr Blood Cancer 2012;58:453–458

The Value of Donor Lymphocyte Infusions in Thalassemia Patients at Imminent Risk of Graft Rejection Following Stem Cell Transplantation

Gulsun Tezcan Karasu, MD,[1] M. Akif Yesilipek, MD,[1]* Sibel Berker Karauzum, PhD,[2] Vedat Uygun, MD,[1]
Esra Manguoglu, PhD,[2] Alphan Kupesiz, MD,[1] and Volkan Hazar, MD[1]

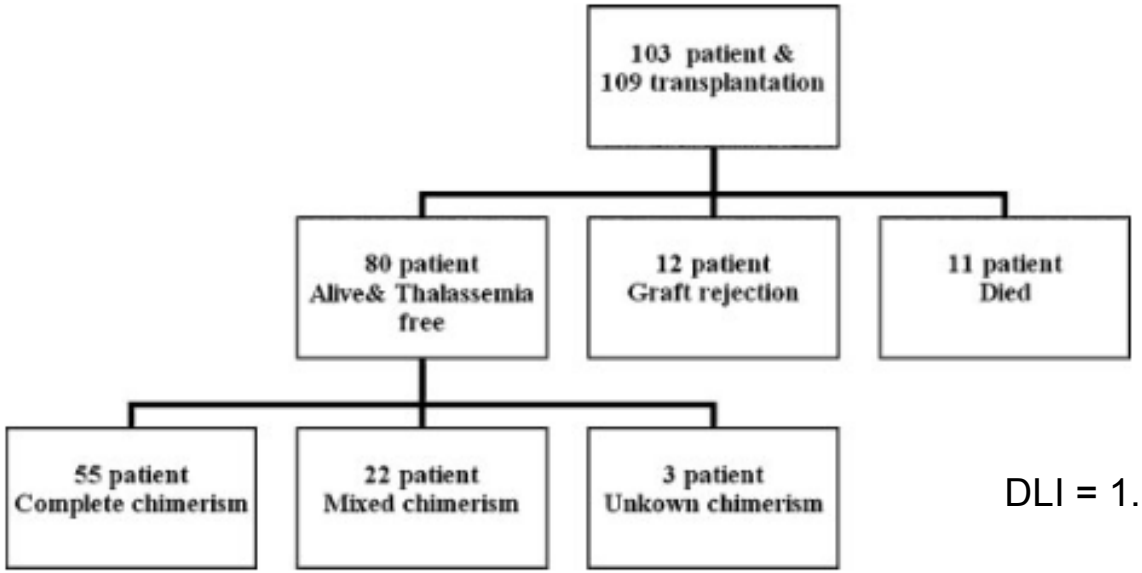


Fig. 1. Transplant outcomes of 103 patients.

DLI = 1.5 x 10^7 CD3+/kg median n° 2 (1-6)

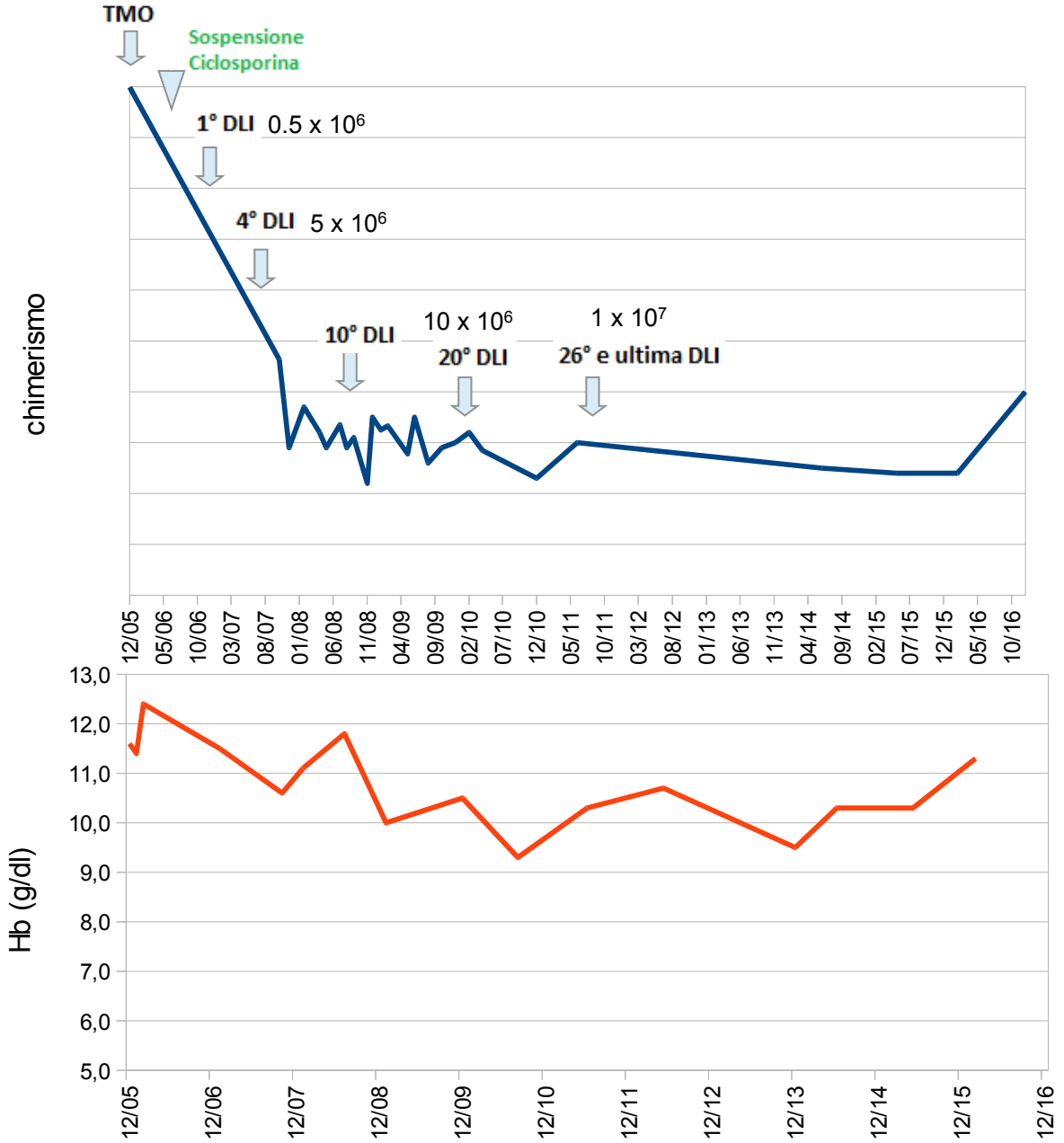
7 *early-* DLI group (group 1)
4 *defferred*-DLI group (group 2)
10 *late*- DLI group (group 3)

Results :
CC = 3 (16%)
MC= 9 (47%)
GR= 7 (37%)

Group 1: GR = 57%
Group 2: GR = 75%
Group 3: GR = 20%

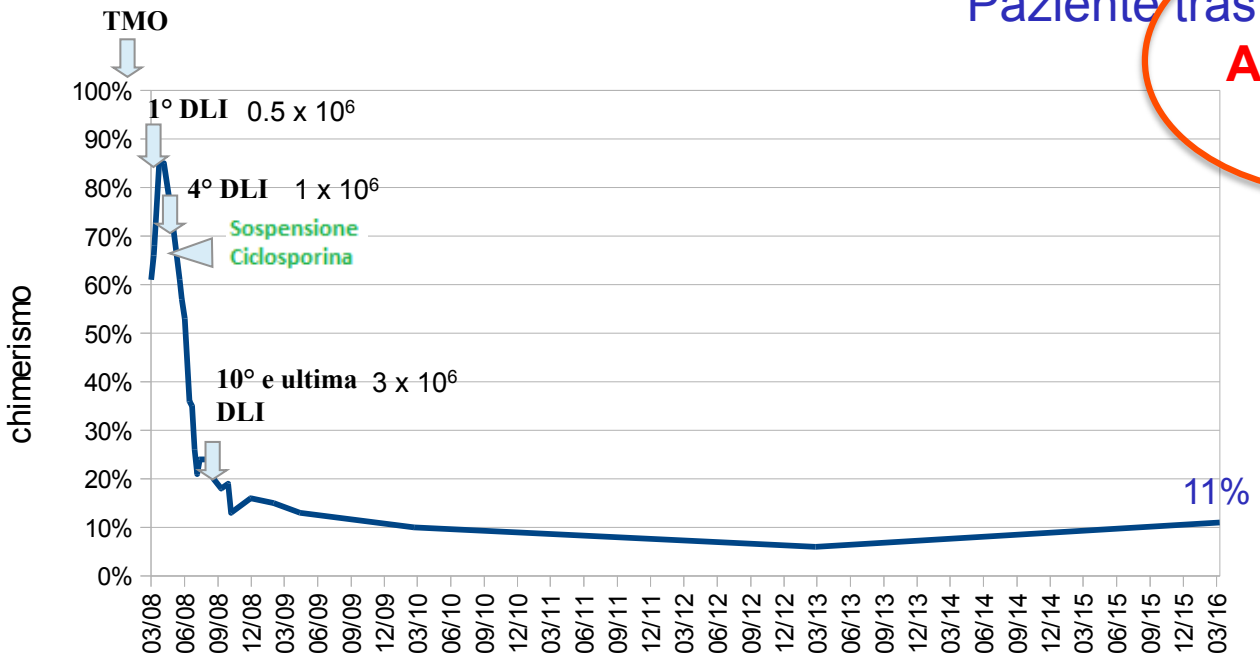
G.D. TMO 28/12/2005

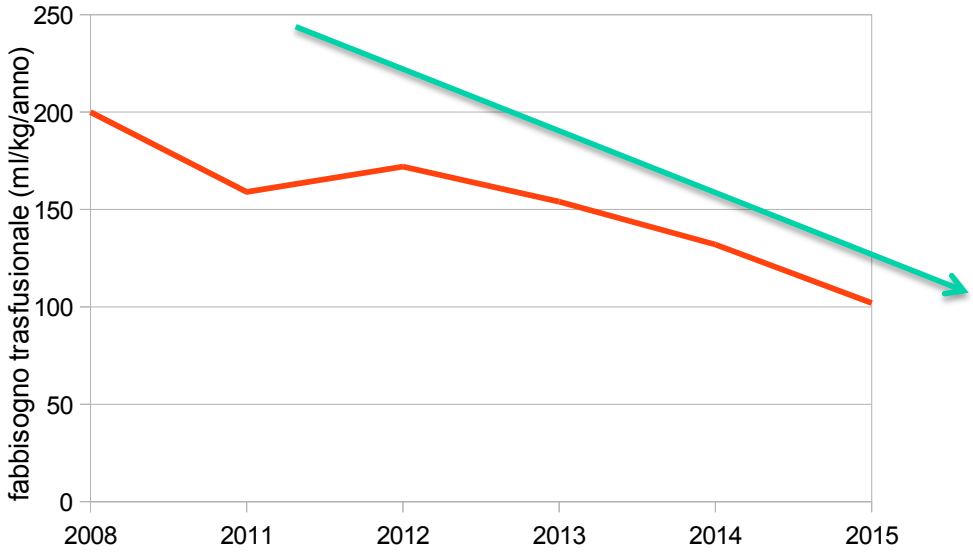
Outcome : PMC



R.M. 12/02/2008

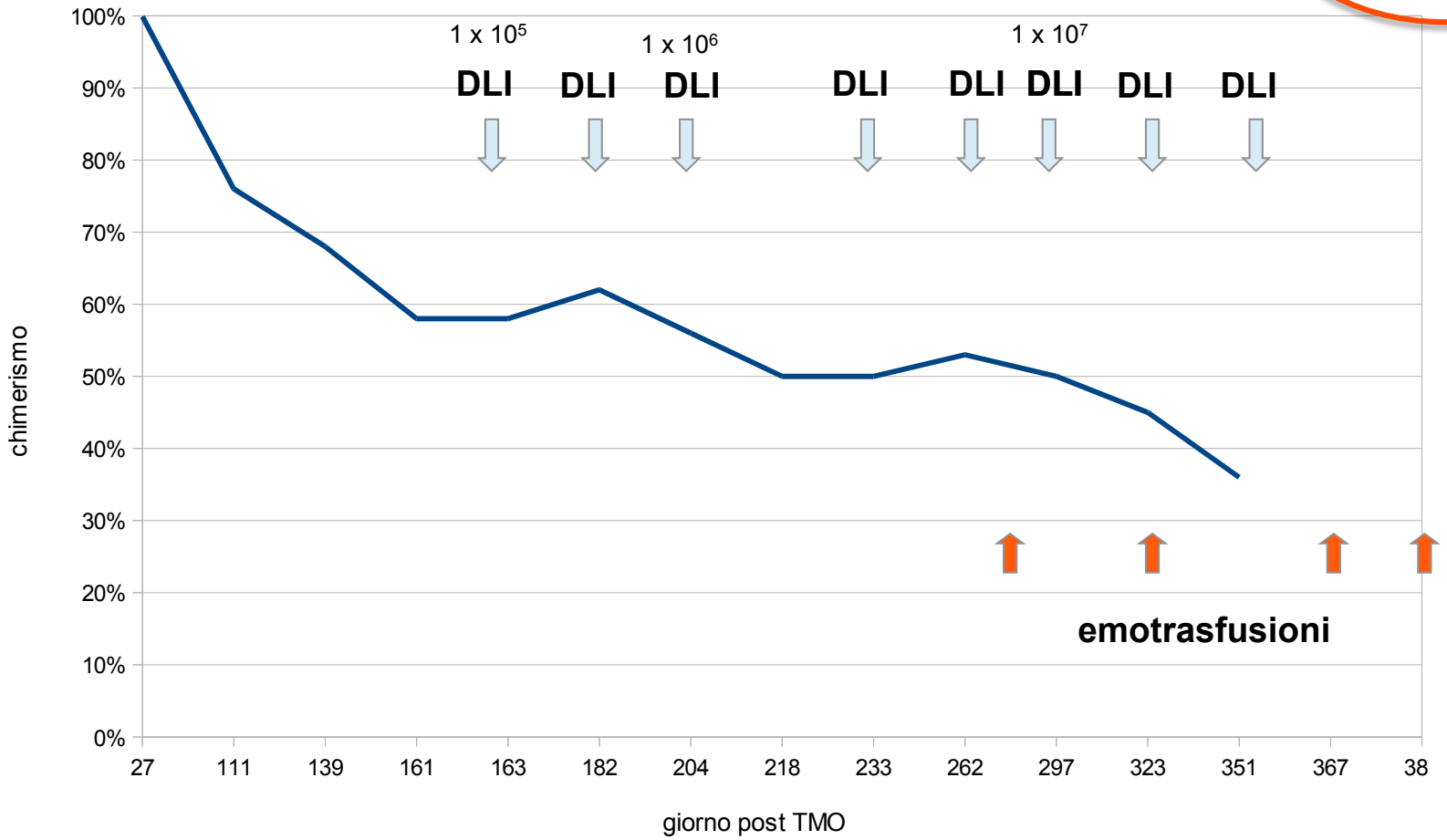
GF
Paziente trasfusione dipendente
A+ in 0+





C.M. 22/12/2015

PCM
Tx dipendente
A+ in 0+



All rules have their exception

An index case

Male, 18 y.o.

Transfusion dependent Thalassemia Major

July 2013: HSCT from UD; full engraftment, no major complication

+6m Mixed chimerism (15%) >>> Stop Cyclosporine

+9m Mixed chimerism 30%: DLI program

+11m Starts DLI, with monthly escalating doses

$3 \times 10^5 \rightarrow 1 \times 10^6 \rightarrow 3 \times 10^6 \rightarrow 3 \times 10^6$

+16m After 4 DLIs, progressive increase of recipient cells (50%)

$\rightarrow 1 \times 10^7$/Kg

+17m Severe post-DLI aplasia: ANC<200, Plt<10, RBC transfusions

No allogeneic recovery (>50 dd in aplasia); chimerism 30%

Hospitalized for >50 dd

Rescued with high-dose steroids and cyclosporine

ANC response only (plt<10)

+20m Still severe thrombocytopenia, symptomatic; stable mixed chimerism 50%

+21m Infectious complication (viral), again severe pancytopenia

All rules have their exception

An index case

+22m Rescue with autologus HSC cryopreserved before allogeneic HSCT

With or without conditioning?

Conditioning as for immune-mdeiated aplastic anemia:

Flu	*30 mg/mq*	*x4*
Cyclophosphamide	*300 mg/mq*	*x4*
Anti-thymocyte globuline (rabbit)	*3,75 mg/kg*	*x2*

Comorbidities: KPC colonization (+ non-KPC Klebsiella)

Complications:

Serum sickness (liver involvement)

Sepsis by the non-KPC Klebsiella, with rabdomiolysis

Low HSCT dose (around 1.5 x 10^6/kg)

Finally engrafted at +25 (ANC) and +40 (plt)

Alive with his major thalassemia

Grazie per l'attenzione

Adriana Vacca
Eugenia Piras

MariaGrazia Orofino
Antonio Piroddi