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10th International
Summer School on Immunogenetics
Stintino | Sardinia | Italy
15th - 18th September 2013

Engraftment Monitoring after HSCT

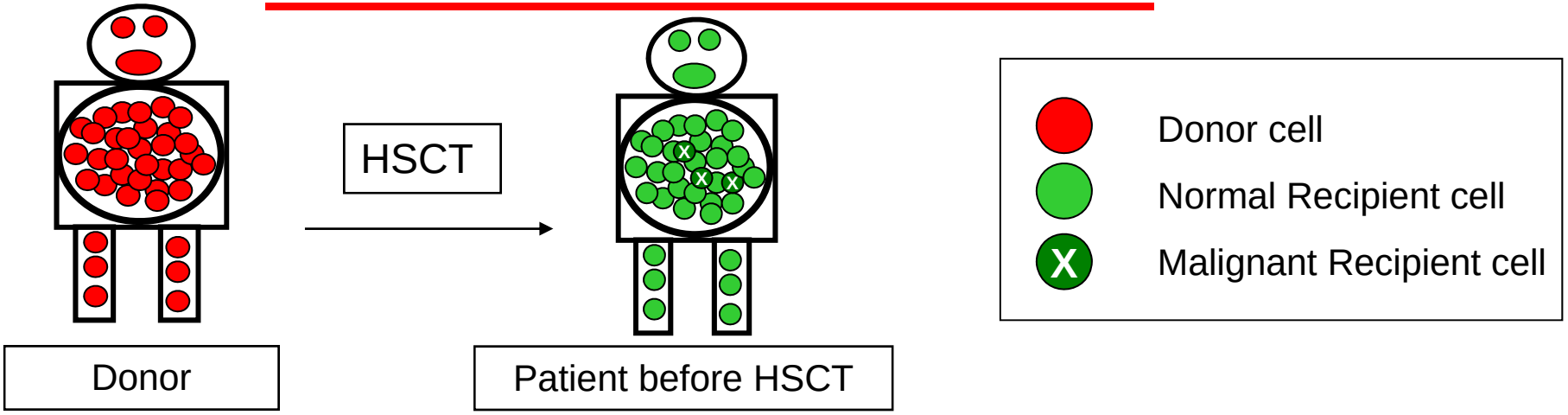
Marco Andreani
Laboratory of Immunogenetics and Transplant Biology (LIBT) - IME Foundation

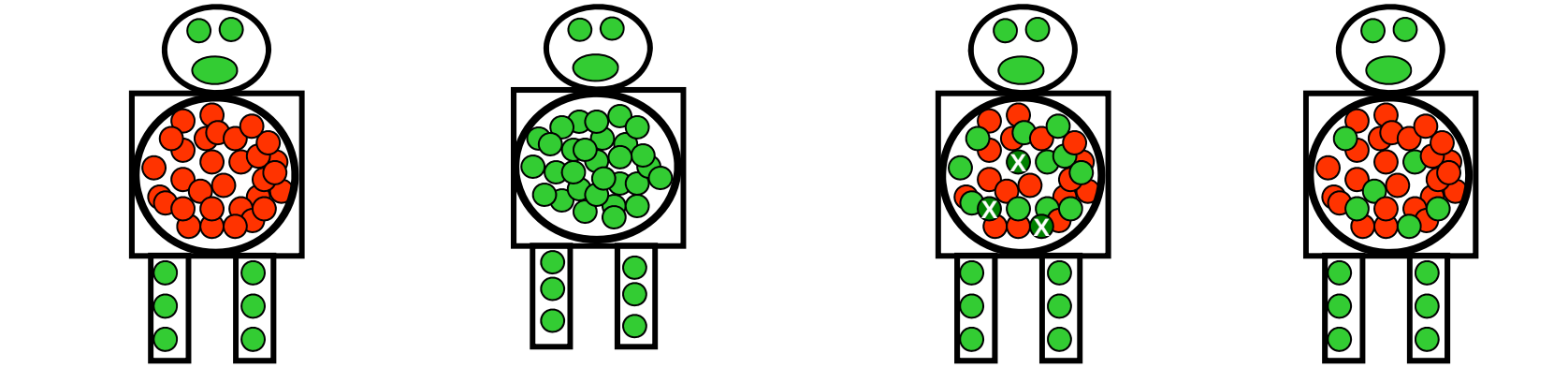
Engraftment Monitoring after HSCT

Engraftment monitoring is an important issue in HSCT

The early detection of residual host cells represents a crucial information relative to the potential outcome of the transplant

Engraftment Monitoring after HSCT





Patient after HSCT

Engraftment Monitoring after HSCT

Mixed Chimerism

- Definition
- Natural and induced
- Methods commonly used for detection
- Graft rejection and relapse
- Immunological tolerance

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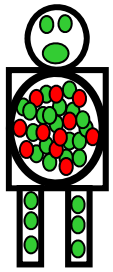

Engraftment Monitoring after HSCT

Mixed chimerism

indicates the presence of both donor and recipient lymphohematopoiesis

Split chimerism

indicates the presence within a single compartment of different donor and recipient cells proportion (example: lymphoid vs myeloid lineages)

Microchimerism

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

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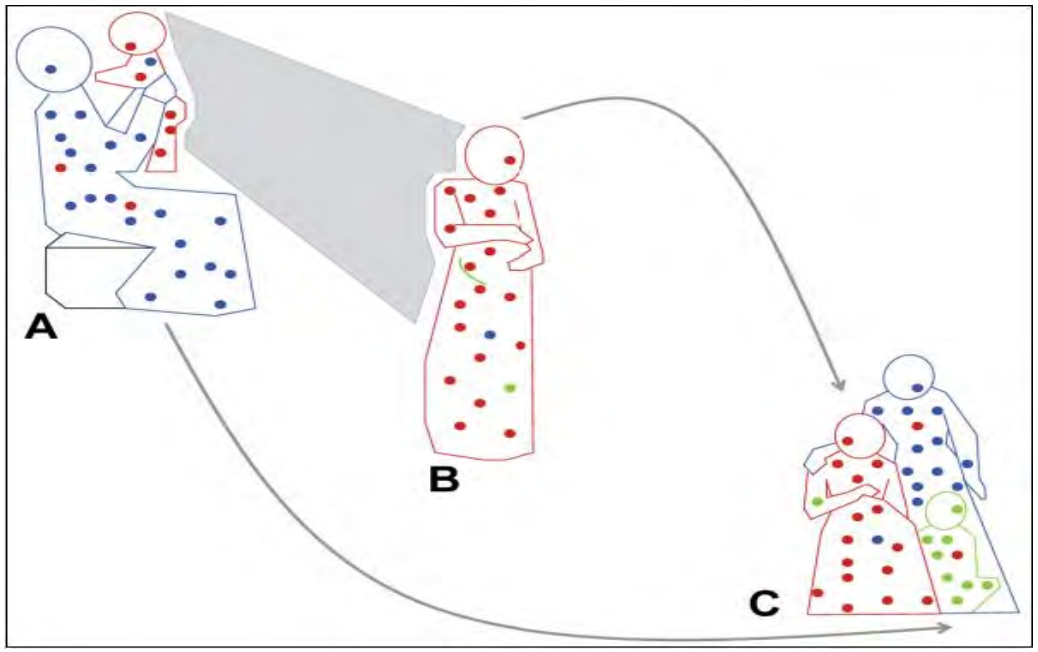
Engraftment Monitoring after HSCT

Natural	Pregnancy
Induced	Transfusion Solid organ and HSCT transplantation

Engraftment Monitoring after HSCT

Natural	Pregnancy
Induced	Transfusion Solid organ and HSCT transplantation

Naturally acquired Mc derives primarily from maternal cells in her progeny, or cells of fetal origin in women



Int J Dev Biol. Author manuscript; available in PMC 2010 June 18.
Published in final edited form as:
Int J Dev Biol. 2010; 54(2-3): 531–543.
doi: 10.1387/ijdb.082767hg

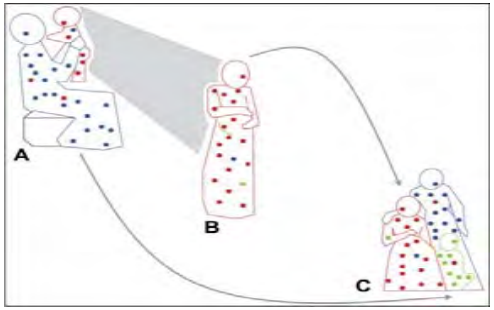
Naturally acquired microchimerism

HILARY S. GAMMILL*[,1,2] and J. LEE NELSON[1,3]

Published in final edited form as:
Trends Immunol. 2012 August ; 33(8): 421–427. doi:10.1016/j.it.2012.03.002.

The Otherness of Self: Microchimerism in Heath and Disease

J. Lee Nelson, MD
Immunogenetics, Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. Department of Medicine, University of Washington, Seattle, Washington, USA

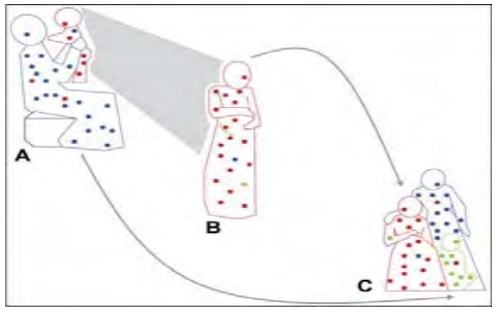


Both maternal and fetal Mc are detected in hematopoietic cells including T and B cells, monocyte/macrophages, NK cells and granulocytes

Mc appears also to generate cells such as myocytes, hepatocytes, islet cells and neurons

Pregnancy, Microchimerism, and the Maternal Grandmother

Hilary S. Gammill[1,2*], Kristina M. Adams Waldorf[1,2], Tessa M. Aydelotte[1], Joëlle Lucas[1], Wendy M. Leisenring[1], Nathalie C. Lambert[3], J. Lee Nelson[1,4]



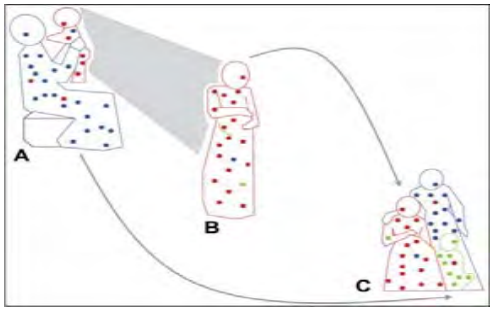
PLoS ONE | www.plosone.org 1 August 2011 | Volume 6 | Issue 8 | e24101

Interactions between a pregnant woman and her acquired microchimerism cell populations may have the potential to influence normal reproduction and be a positive favorable prognostic factor for preeclampsia

Science. 2008 December 5; 322(5907): 1562–1565. doi:10.1126/science.1164511.

Maternal Alloantigens Promote the Development of Tolerogenic Fetal Regulatory T Cells in Utero

Jeff E. Mold[1,2], Jakob Michaëlsson[3], Trevor D. Burt[1,4], Marcus O. Muench[5], Karen P. Beckerman[6,*], Michael P. Busch[5], Tzong-Hae Lee[5], Douglas F. Nixon[1], and Joseph M. McCune[1,†]



Mold showed that the human fetal immune system takes advantage of an additional mechanism: the generation of regulatory T cells (Tregs) that suppress fetal immune responses

Natural microchimerism

NIPA e NIMA

HLA-G

Indirect evidence that maternal microchimerism in cord blood mediates a graft-versus-leukemia effect in cord blood transplantation

Jon J. van Rood[a,1], Andromachi Scaradavou[b], and Cladd E. Stevens[b,2]

[a]Department of Immunohematology and Blood Transfusion, Leiden University Medical Center, 2333 ZA, Leiden, The Netherlands; and [b]National Cord Blood Program, New York Blood Center, New York, NY 11101

PNAS | February 14, 2012 | vol. 109 | no. 7 |

During pregnancy women can develop B- and T-cell immunity against the inherited paternal antigens (IPAs) of the fetus, such as HLA, peptides of minor histocompatibilty antigens, and possibly onco-fetal antigens. The biological and pathological role of these pregnancy-induced immunological events is only understood in part. **However, anti-IPA immunity in the mother persists for many decades after delivery and may reduce relapse in offspring with leukemia after HLA-haploidentical transplantation of maternal hematopoietic stem cells (HSC).** We hypothesized that maternal anti-IPA immune elements cross the placenta and might confer a potent graft-versus-leukemia effect when cord blood (CB) is used in unrelated HSC transplantation. In a retrospective study of single-unit CB recipients with all grafts provided by the New York Blood Center, we show that patients with acute myeloid or lymphoblastic leukemia (n = 845) who shared one or more HLA-A, -B, or -DRB1 antigens with their CB donor's IPAs had a significant decrease in leukemic relapse posttransplantation [hazard ratio (HR) = 0.38, $P < 0.001$] compared with those that did not. Remarkably, relapse reduction in patients receiving CB with one HLA mismatch (HR = 0.15, $P < 0.001$) was not associated with an increased risk of severe acute graft-versus-host disease (HR = 1.43, $P = 0.730$). Our findings may explain the unexpected low relapse rate after CB transplantation, open new avenues in the study of leukemic relapse after HSC transplantation (possibly of malignancies in general), and have practical implications for CB unit selection.

Engraftment Monitoring after HSCT

Natural	Pregnancy
Induced	Transfusion
	Solid organ and HSCT transplantation

Induced mixed chimerism

Microchimerism and trasfusion after polytrauma

Engraftment Monitoring after HSCT

Natural	Pregnancy
Induced	Transfusion
	Solid organ and HSCT transplantation

Engraftment Monitoring after HSCT

Mixed Chimerism

- Definition
- Natural and induced
- **Methods commonly used for detection**
- Graft rejection and relapse
- Immunological tolerance

What is needed for clinically relevant analysis

Panel of genetic loci sufficient to differentiate donors from recipients

Quick turn-around-time

Sensitivity – ability to detect low numbers of cells

Small sample size

Markers commonly used for mixed chimerism detection

- Red blood cell phenotyping
- Karyotyping
- HLA typing

- FISH analysis

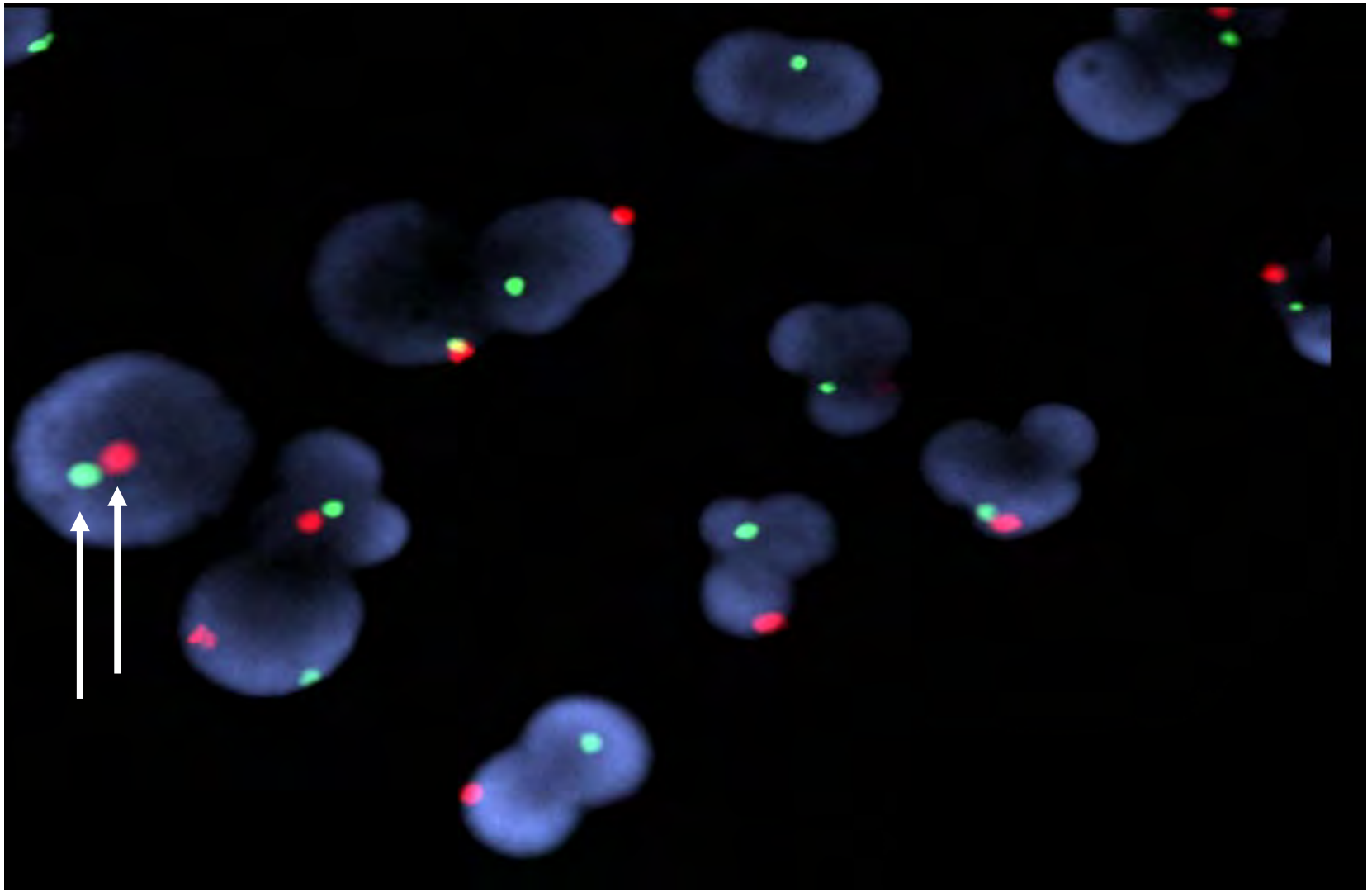
- VNTR

- STR (2-5%)

- Real Time PCR (0.1 – 0.05%)

Increasing Sensitivity

FISH for chromosome Y ed X



DNA fragments analysis – STR

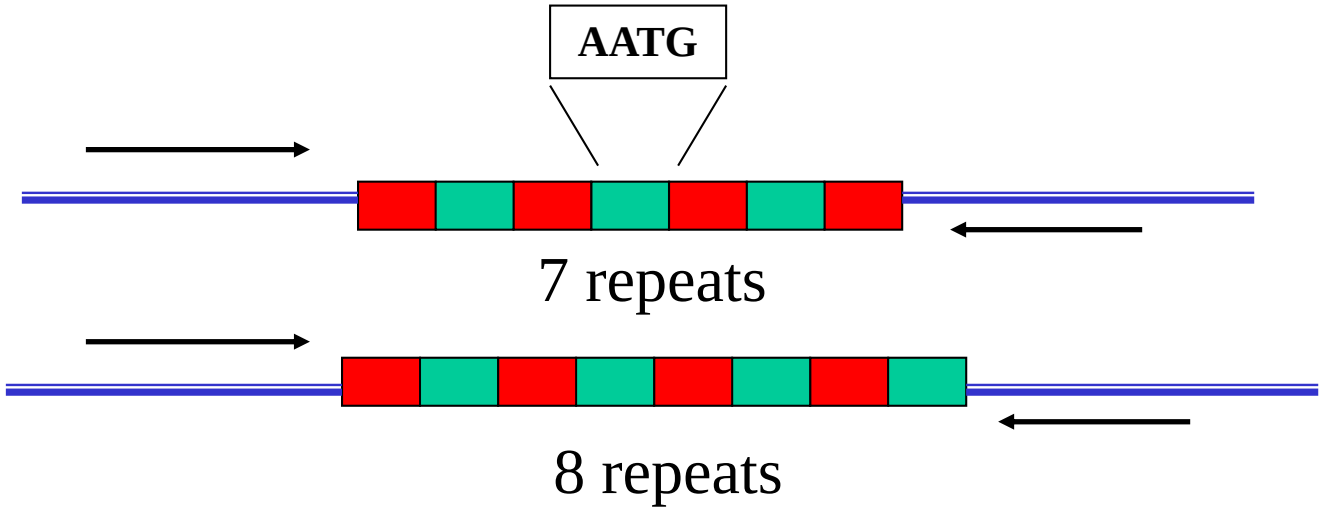
Short tandem repeats

Short tandem repeat (STR) are polymorphic DNA loci that contain a repeat nucleotide sequence

The STR repeat unit can be from 2 to 7 nucleotides in length

DNA fragments analysis – STR

Short tandem repeats

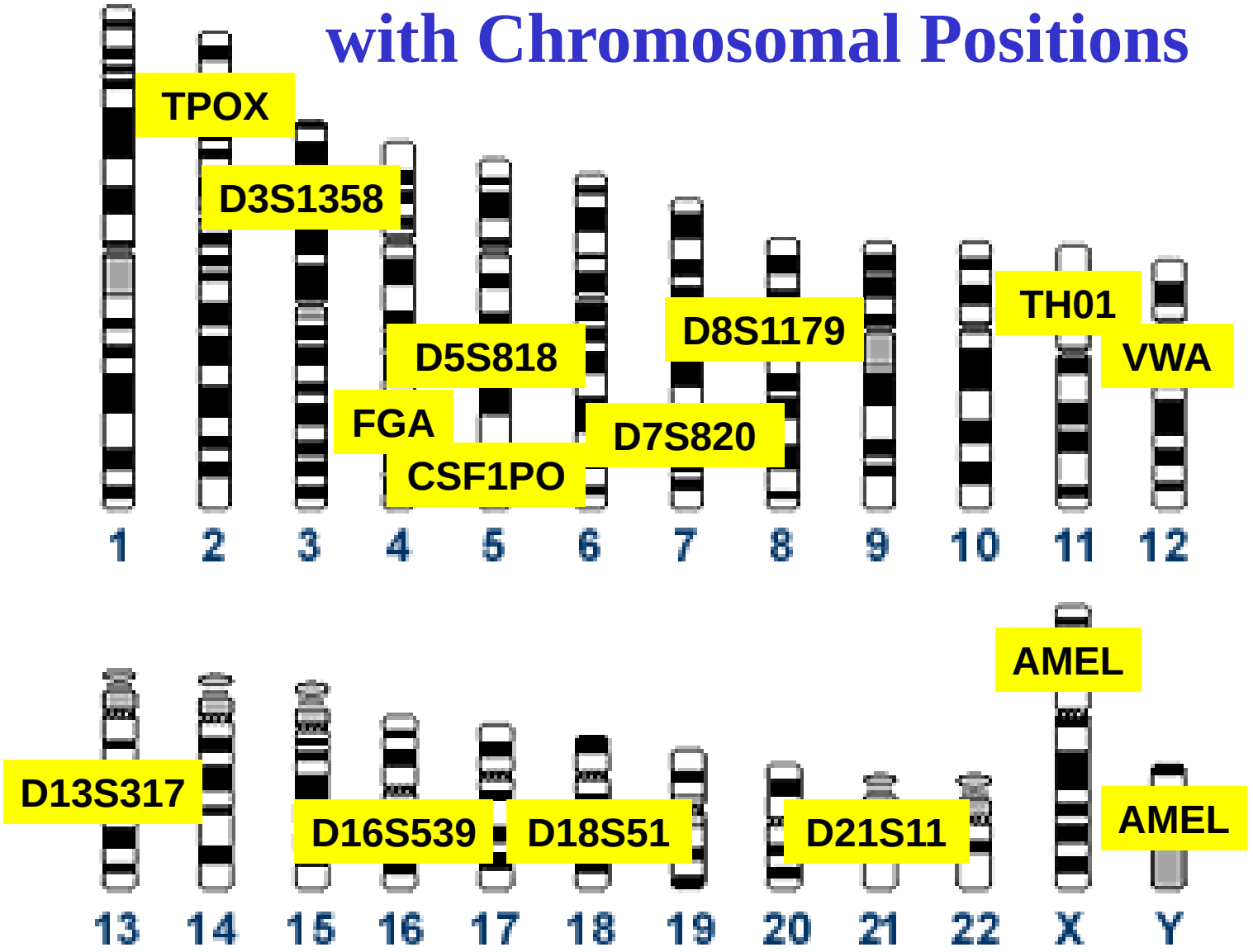


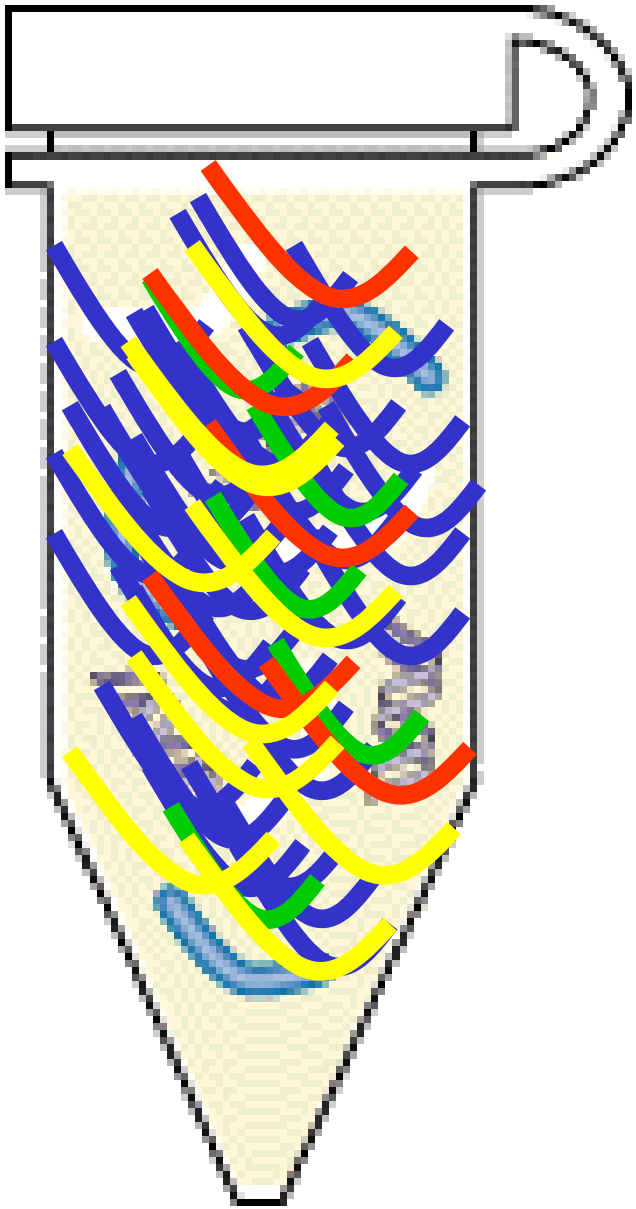
the repeat region is variable between samples while the flanking regions where PCR primers bind are constant

Homozygote = both alleles are the same length

Heterozygote = alleles differ and can be resolved from one another

13 CODIS Core STR Loci with Chromosomal Positions



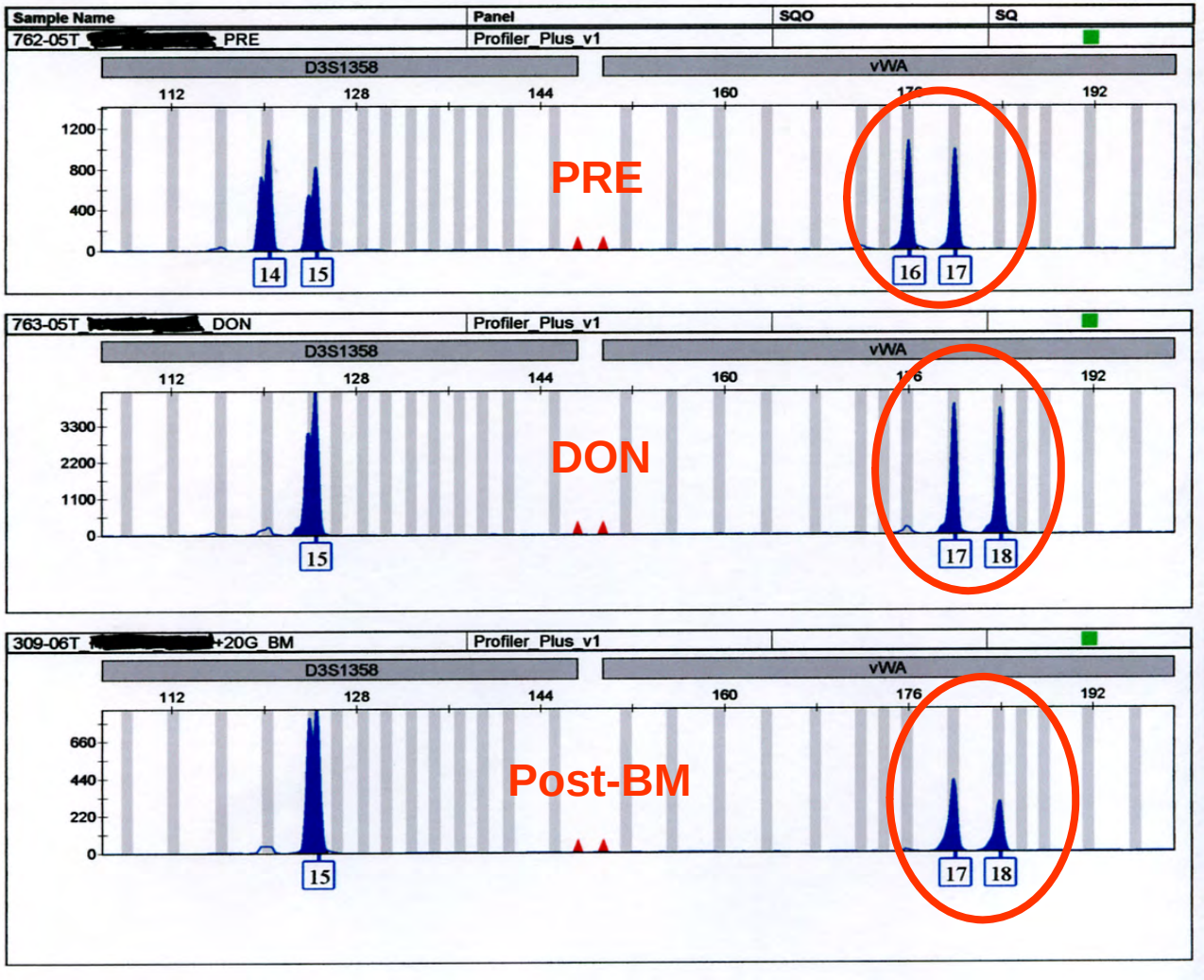


Multiplex PCR

- Over 10 Markers Can Be Copied at Once
- Sensitivities to levels less than 1 ng of DNA
- Ability to Handle Mixtures and Degraded Samples
- Different Fluorescent Dyes Used to Distinguish STR Alleles with Overlapping Size Ranges

DNA fragments analysis – STR

Short tandem repeats



Markers commonly used for mixed chimerism detection

- Red blood cell phenotyping
- Karyotyping
- HLA typing

- FISH analysis

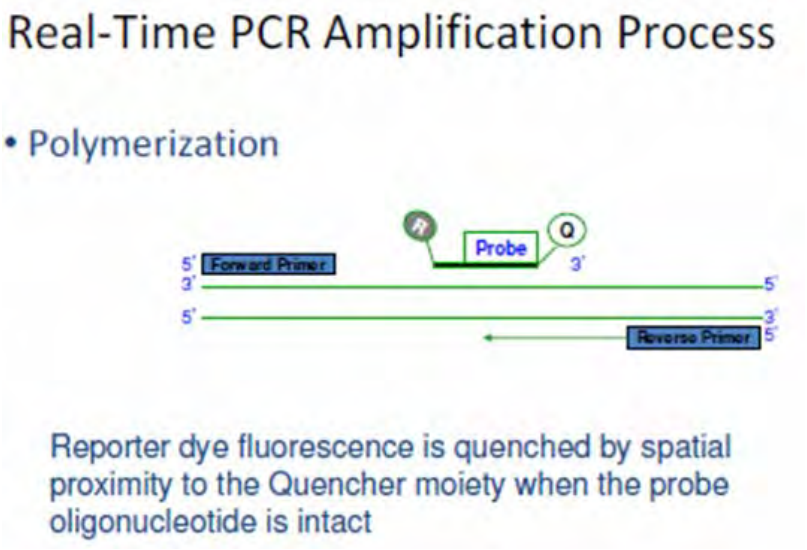
- VNTR

- STR (2-5%)

- Real Time PCR

Real-Time PCR Chimerism

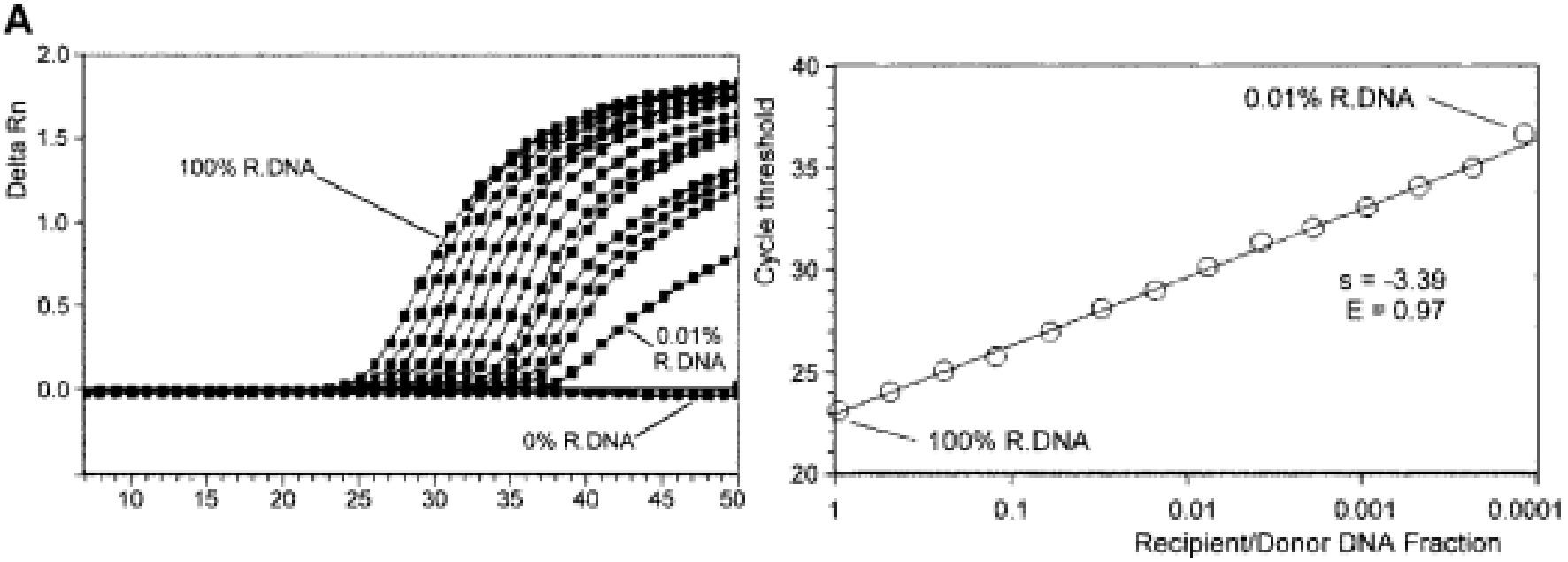
Sensitivity: Detection of 0.05%of minor component in a mixed DNA sample when starting with 250ng of DNA



Real-Time PCR Chimerism

Quantitative assessment of hematopoietic chimerism after bone marrow transplantation by real-time quantitative polymerase chain reaction

Mehdi Alizadeh, Marc Bernard, Bruno Danic, Charly Dauriac, Brigitte Birebent, Christine Lapart, Thierry Lamy, Pierre-Yves Le Prisé, Alain Beauplet, Dominique Bories, Gilbert Semana, and Erwann Quelvennec



BLOOD, 15 JUNE 2002 • VOLUME 99, NUMBER 12

Leukemia. 2012 Aug;26(8):1821-8. doi: 10.1038/leu.2012.66. Epub 2012 Mar 7.

The EuroChimerism concept for a standardized approach to chimerism analysis after allogeneic stem cell transplantation.

Lion T, Watzinger F, Preuner S, Kreyenberg H, Tilanus M, de Weger R, van Loon J, de Vries L, Cavé H, Acquaviva C, Lawler M, Crampe M, Serra A, Saglio B, Colnaghi F, Biondi A, van Dongen JJ, van der Burg M, Gonzalez M, Alcoceba M, Barbany G, Hermanson M, Roosnek E, Steward C, Harvey J, Frommlet F, Bader P.

Wide use of the European-harmonized protocol for chimerism analysis presented will provide a basis for optimal diagnostic support and timely treatment decisions.

I4.000 Haemopoietic Chimaerism and Engraftment (HCE) Monitoring

I4.100 Standards in L1.0000, L1.3400, L2.1100, L2.1200, L2.2000, L2.3100 and L2.3300 also apply.

I4.110 The polymorphic gene system(s) used for HCE monitoring must be identified and documented with regards to allelic variability

I4.120 Where locally developed PCR primers/probes are used, their sequence and specificity must be documented.

I4.130 Donor and patient specific alleles must be determined using appropriate reference material and documented.

I4.140 Optimal ranges of DNA quantity and purity must be defined and documented. If a sample falls outside these optimal ranges, a statement must be included on the report.

I4.150 Criteria for assignment of HCE results, on a qualitative or quantitative basis, must be defined.

I4.160 The sensitivity of the HCE assay must be validated.

I4.170 When multiple PCR primers are used in the same tube (multiplex PCR), results must take into account possible amplification bias.

I4.180 Results must be validated using DNA mixtures from two individuals at defined ratios/concentrations, before implementation into clinical use.

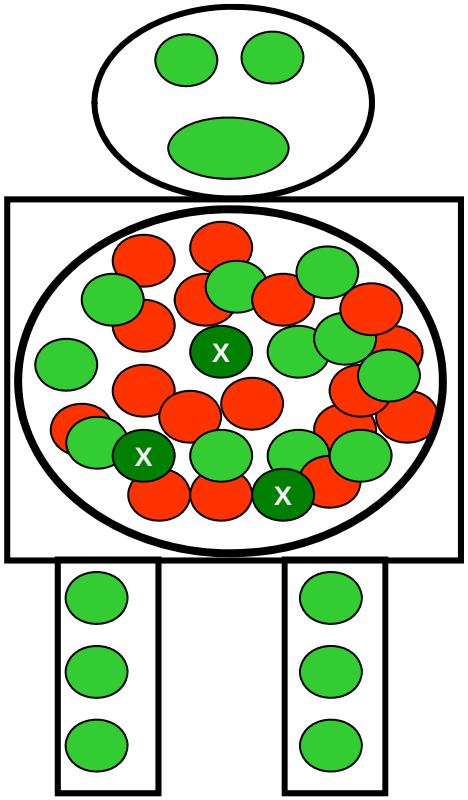
I4.190 When HCE testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population must be documented and taken into account in the analysis of the results. If this is not possible it must be clearly stated in the report.

Engraftment Monitoring after HSCT

Mixed Chimerism

- Definition
- Natural and induced
- Methods commonly used for detection
- Graft rejection and **relapse**
- Immunological tolerance

Relapse of the disease



The chimeric status of the patients post allo-HSCT has assumed increased importance in addition to the determination of MRD analysis with specific tumor markers

Methods to predict disease relapse after chemotherapy or HSCT allow early intervention may improve the probability of long-term disease free survival (DFS)

Am J Pharmacogenomics. 2004;4(3):177-87.

Diagnostic chimerism analysis after allogeneic stem cell transplantation: new methods and markers.

Thiede C.

Medical Department, University Hospital Carl Gustav Carus, Technical University Dresden, Dresden, Germany.

Ann Hematol. 2013 Aug 2. [Epub ahead of print]

A fast and simple approach for the simultaneous detection of hematopoietic chimerism, NPM1, and FLT3-ITD mutations after allogeneic stem cell transplantation.

Waterhouse M, Bertz H, Finke J.

Department of Hematology and Oncology, University Medical Center Freiburg, Hugstetter Str. 55, 79106, Freiburg, Germany, miguel.waterhouse@uniklinik-freiburg.de.

They developed a multiplex PCR for use in the **simultaneous detection of hematopoietic chimerism and mutations** in nucleophosmin (NPM1) and fms-like tyrosine kinase-3 internal tandem duplication (FLT3-ITD)

Bone Marrow Transplant. 2013 Aug;48(8):1070-6. doi: 10.1038/bmt.2013.2. Epub 2013 Feb 4.

CD34(+) lineage specific donor cell chimerism for the diagnosis and treatment of impending relapse of AML or myelodysplastic syndrome after allo-SCT.

Rosenow F, Berkemeier A, Krug U, Müller-Tidow C, Gerss J, Silling G, Groth C, Wieacker P, Bogdanova N, Mesters R, Büchner T, Kienast J, Berdel WE, Stelljes M.

Department of Medicine A/Hematology and Oncology, University of Muenster, Muenster, Germany.

Unsorted and lineage-specific donor engraftment were measured in 134 patients: 43 patients had an incomplete CD34(+) chimerism with no other evidence of relapse

Relapse-free survival at 3 years of the 91 patients with stable donor chimerism was 74% while in the 43 patients with incomplete donor chimerism was 40%

Relapse of the disease

After HSCT with reduced intensity conditioning regimens, high probability to observe mixed chimerism

Therapeutic intervention after HSCT, including DLI

Different Conditioning regimens for HSCT and DLI

Bone Marrow Transplant. 2013 Feb;48(2):238-42. doi: 10.1038/bmt.2012.131. Epub 2012 Jul 9.

The impact of center experience on results of reduced intensity: allogeneic hematopoietic SCT for AML. An analysis from the Acute Leukemia Working Party of the EBMT.

Giebel S, Labopin M, Mohty M, Mufti GJ, Niederwieser D, Cornelissen JJ, Janssen JJ, Milpied N, Vindelov L, Petersen E, Arnold R, Bacigalupo A, Blaise D, Craddock C, Nagler A, Frassoni F, Sadus-Wojciechowska M, Rocha V.

Curr Oncol Rep. 2013 Aug 21. [Epub ahead of print]

Hematopoietic Stem Cell Transplantation for Acute Myeloid Leukemia: To Whom, When, and How.

Magenau J, Couriel DR.

Division of Hematology/Oncology, University of Michigan Comprehensive Cancer Center, 1500 E. Medical Center Dr., Med C409, Ann Arbor, MI, 48109-5948, USA, johnmage@umich.edu.

Abstract

BRIEF ARTICLES

ASBMT American Society for Blood and Marrow Transplantation

Defining the Intensity of Conditioning Regimens: Working Definitions

Andrea Bacigalupo, M.D.,[1] Karen Ballen, M.D.,[2] Doug Rizzo, M.D.,[3] Sergio Giralt, M.D.,[4] Hillard Lazarus, M.D.,[5] Vincent Ho, M.D.,[6] Jane Apperley, M.D.,[7] Shimon Slavin, M.D.,[8] Marcelo Pasquini, M.D.,[3] Brenda M. Sandmaier, M.D.,[9] John Barrett, M.D.,[10] Didier Blaise, M.D.,[11] Robert Lowski, M.D.,[12] Mary Horowitz, M.D.[3]

Biol Blood Marrow Transplant. 2009 Dec;15(12):1628-33. doi: 10.1016/j.bbmt.2009.07.004. Epub 2009 Sep 1.

Defining the intensity of conditioning regimens: working definitions.

Bacigalupo A, Ballen K, Rizzo D, Giralt S, Lazarus H, Ho V, Apperley J, Slavin S, Pasquini M, Sandmaier BM, Barrett J, Blaise D, Lowski R, Horowitz M.

San Martino Hospital, Genoa, Italy. andrea.bacigalupo@hsanmartino.it

Biol Blood Marrow Transplant. 2013 Jul;19(7):1094-101. doi: 10.1016/j.bbmt.2013.04.021. Epub 2013 Apr 28.

Pilot study of prophylactic ex vivo costimulated donor leukocyte infusion after reduced-intensity conditioned allogeneic stem cell transplantation.

Kumar AJ, Hexner EO, Frey NV, Luger SM, Loren AW, Reshef R, Boyer J, Smith J, Stadtmauer EA, Levine BL, June CH DL, Goldstein SC.

Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA, USA. Anita.kumar@uphs.upenn.edu

Optimizing the conditioning regimen for allogeneic stem-cell transplantation in acute myeloid leukemia; dose intensity is still in need

Avichai Shimoni, MD, Director, Department of Bone Marrow Transplantation *, Arnon Nagler, MD, Professor, Director Division of Hematology and Bone Marrow Transplantation

Division of Hematology and Bone Marrow Transplantation, Chaim Sheba Medical Center, Tel-Hashomer, Israel

Best Practice & Research Clinical Haematology 24 (2011) 369–379

Engraftment Monitoring after HSCT

Mixed Chimerism

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Mixed Chimerism in Thalassemia after HSCT

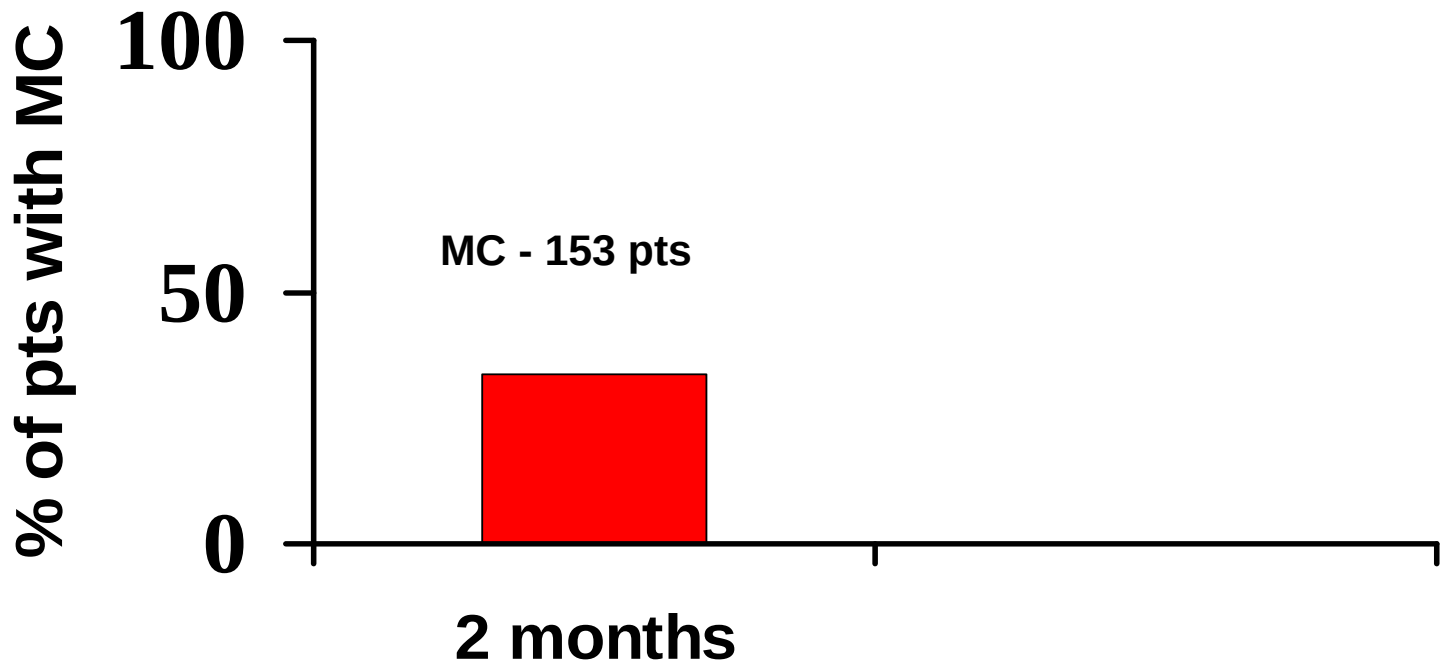
Thalassemia represent an unique model in order to study the coexistence of donor and recipient cells after HSCTs due to the

absence of residual malignant host cells

Mixed Chimerism in Thalassemia after HSCT

Transient Mixed Chimerism

Mixed Chimerism in Thalassemia after HSCT



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

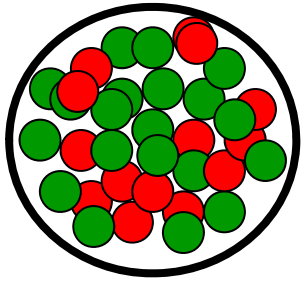
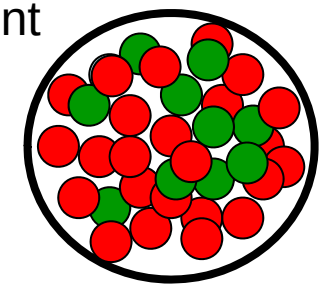
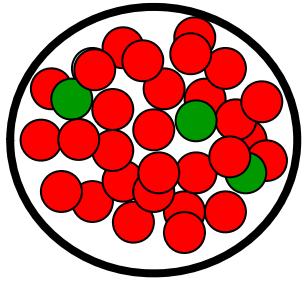
Transient MC represents a risk factor for graft rejection

Number of RHCs present early after HSCT

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

Conditioning regimes used

Mixed Chimerism in Thalassemia after HSCT

Chimerism status early after HSCT	Transplant outcome
 > 25%	Rejection PMC Complete Chimerism
patient  donor	Rejection PMC Complete Chimerism
 < 10%	Rejection PMC Complete Chimerism

Mixed Chimerism in Thalassemia after HSCT

Patients with MC level 3 early after HSCT have a higher risk of graft rejection

Engraftment status at 60 days after HSCT **Patients analyzed: 105**	**N° Pts**	**Engraftment evolution**		
		MC	**CC**	**Rej**
MC Level 3 **(RHCs>25%)**	10	2	3	**5 (50%)**
CC	50	3	46	**1 (2%)**

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

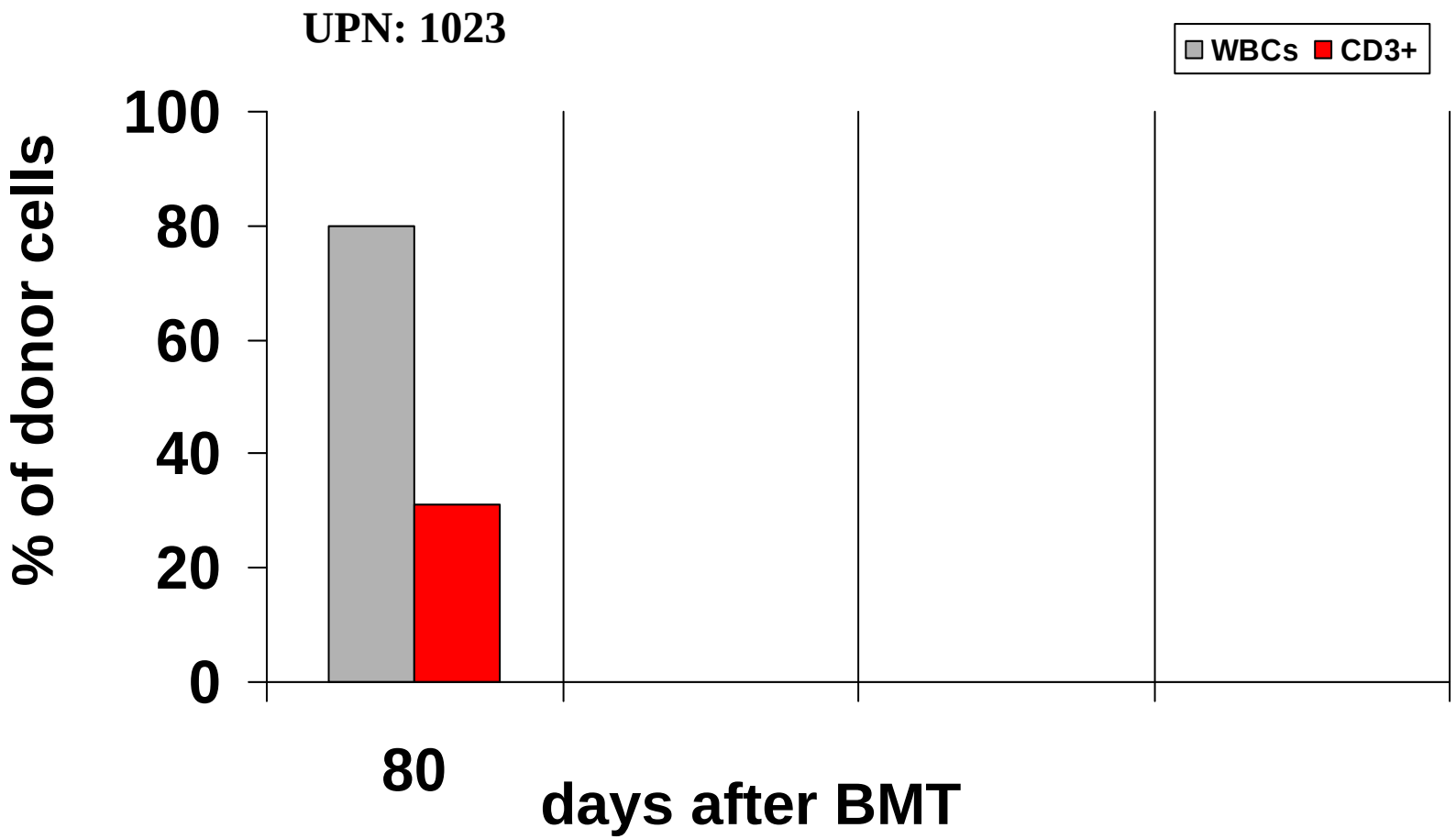
Transient MC represents a risk factor for graft rejection

Number of RHCs present early after HSCT

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

Conditioning regimes used

Mixed Chimerism in Thalassemia after HSCT



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 regimens used

Mixed Chimerism in Thalassemia after HSCT

Conditioning Regimens

Eradication:

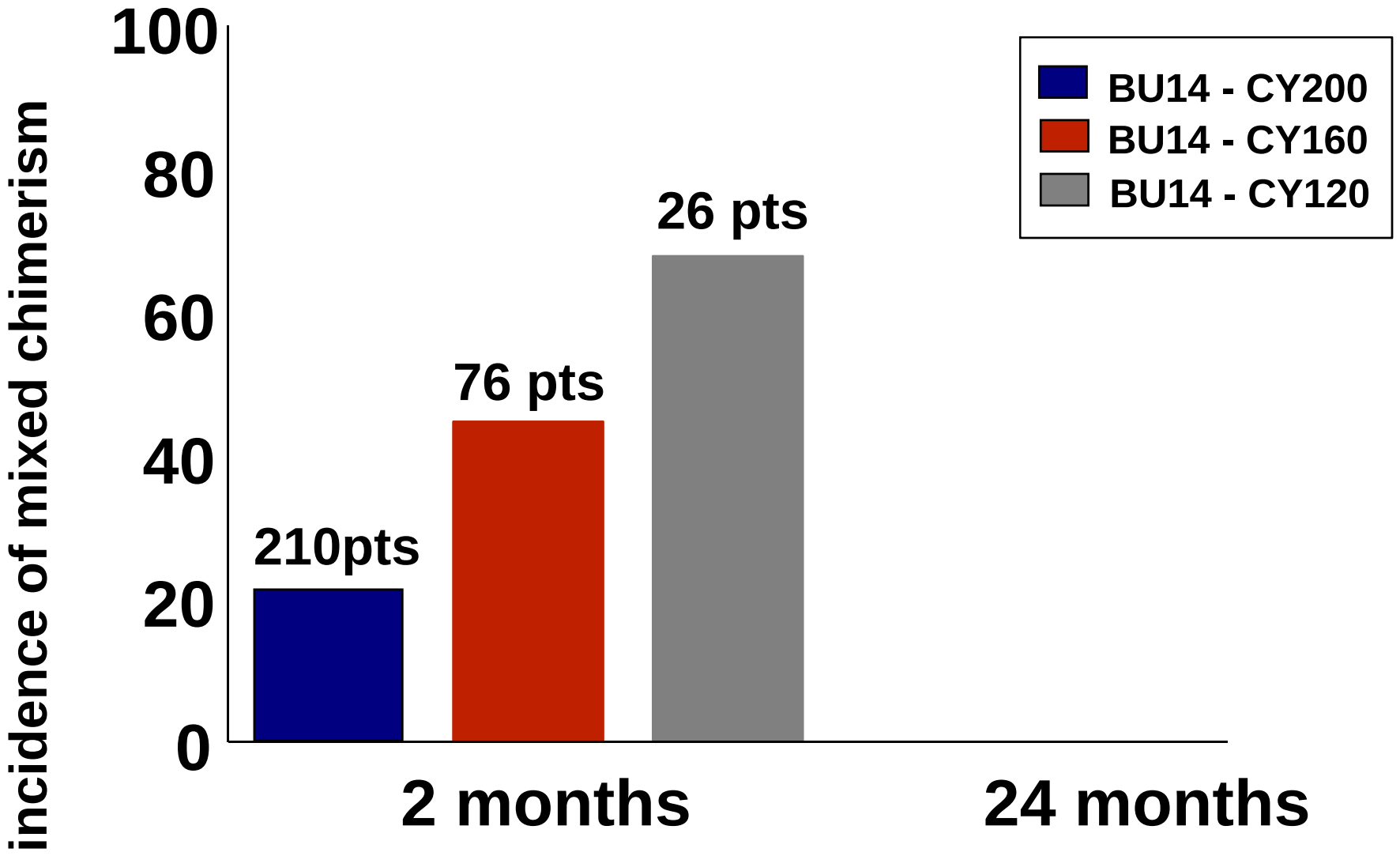
- BU 14 mg/Kg

Immunosuppression

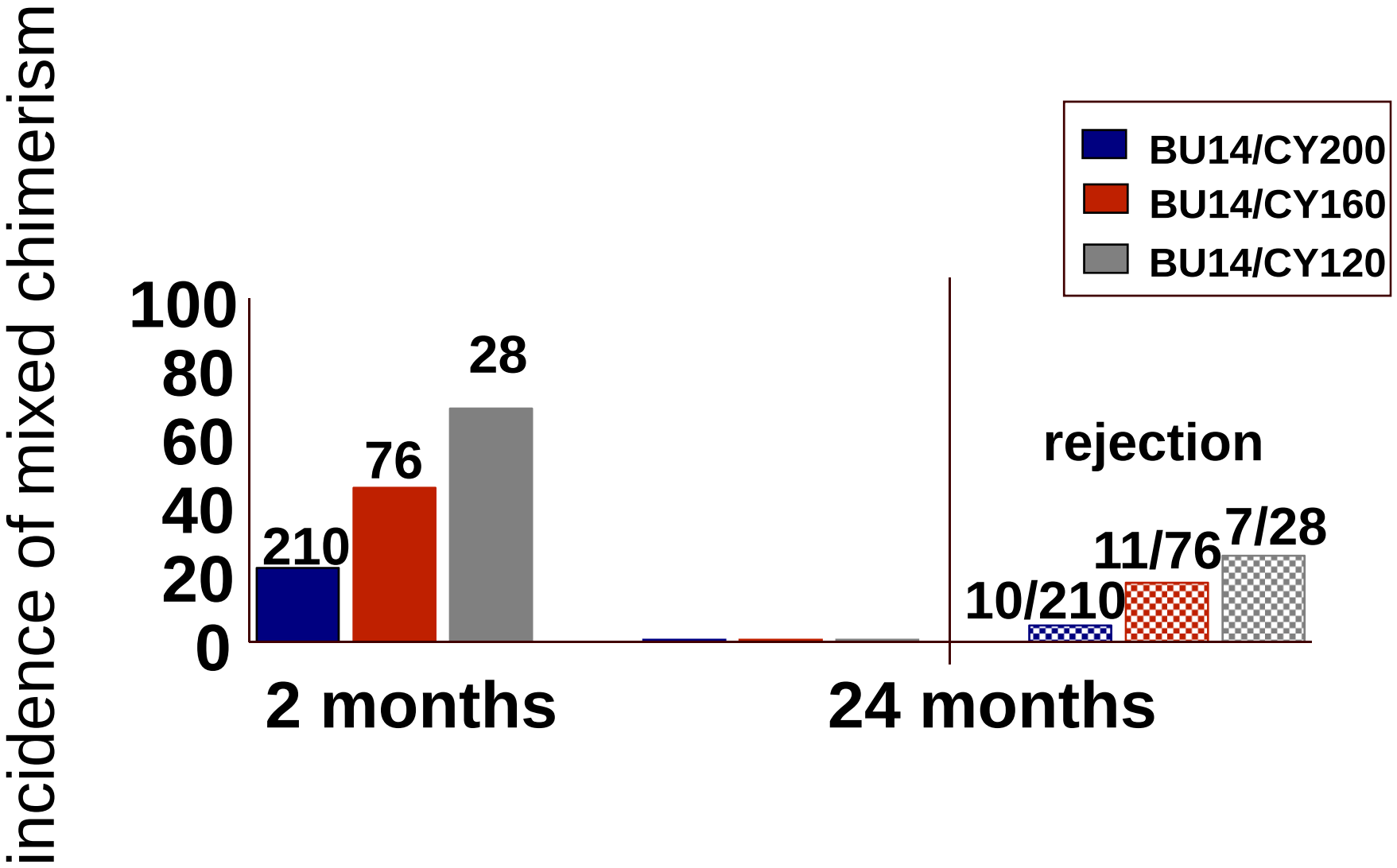
Three different CY doses:

- 200mg/Kg
- 160mg/Kg
- 120mg/Kg

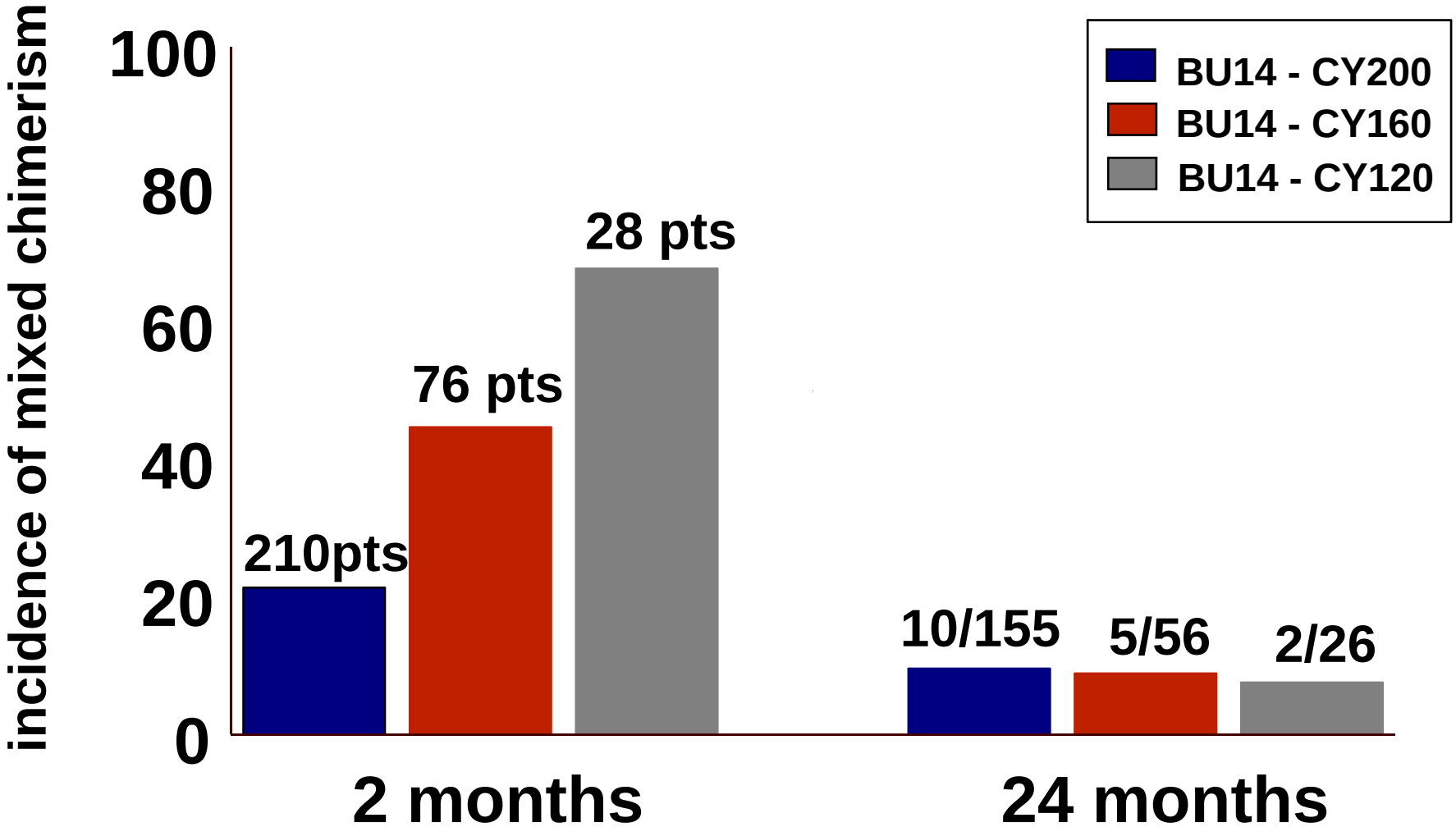
Mixed Chimerism in Thalassemia after HSCT



Mixed Chimerism in Thalassemia after HSCT



Mixed Chimerism in Thalassemia after HSCT



Persistent Mixed Chimerism

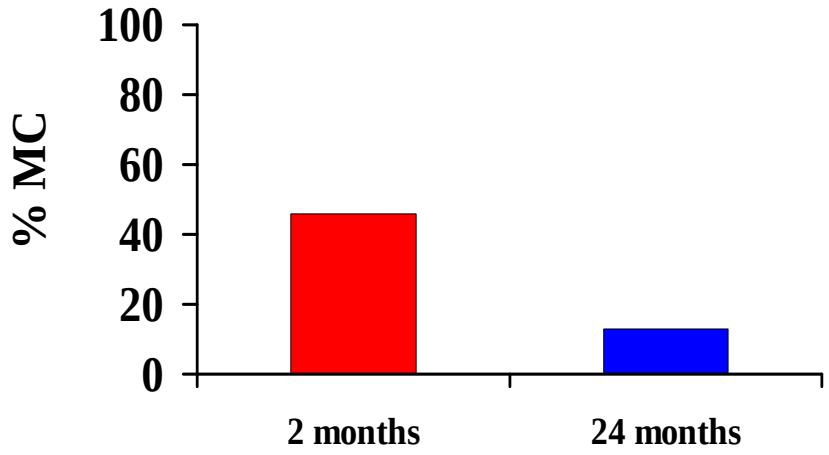
MC is defined persistent when donor and recipient cells **coexist for more than 24 months after HSCT**

no evolution to graft failure

no evolution to complete chimerism

Persistent Mixed Chimerism

Functional graft

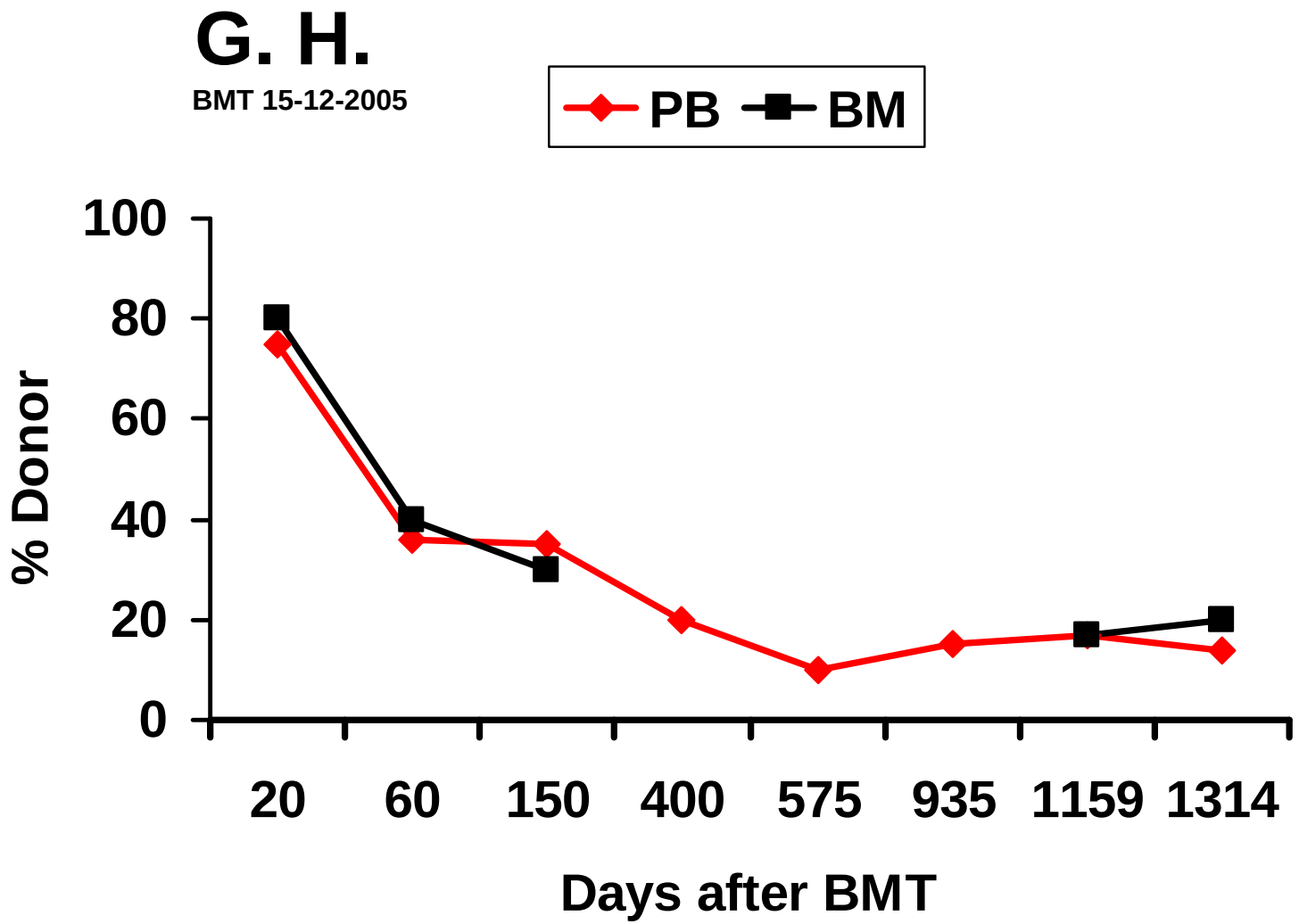


In many cases the proportion of cells of recipient origin is extremely large

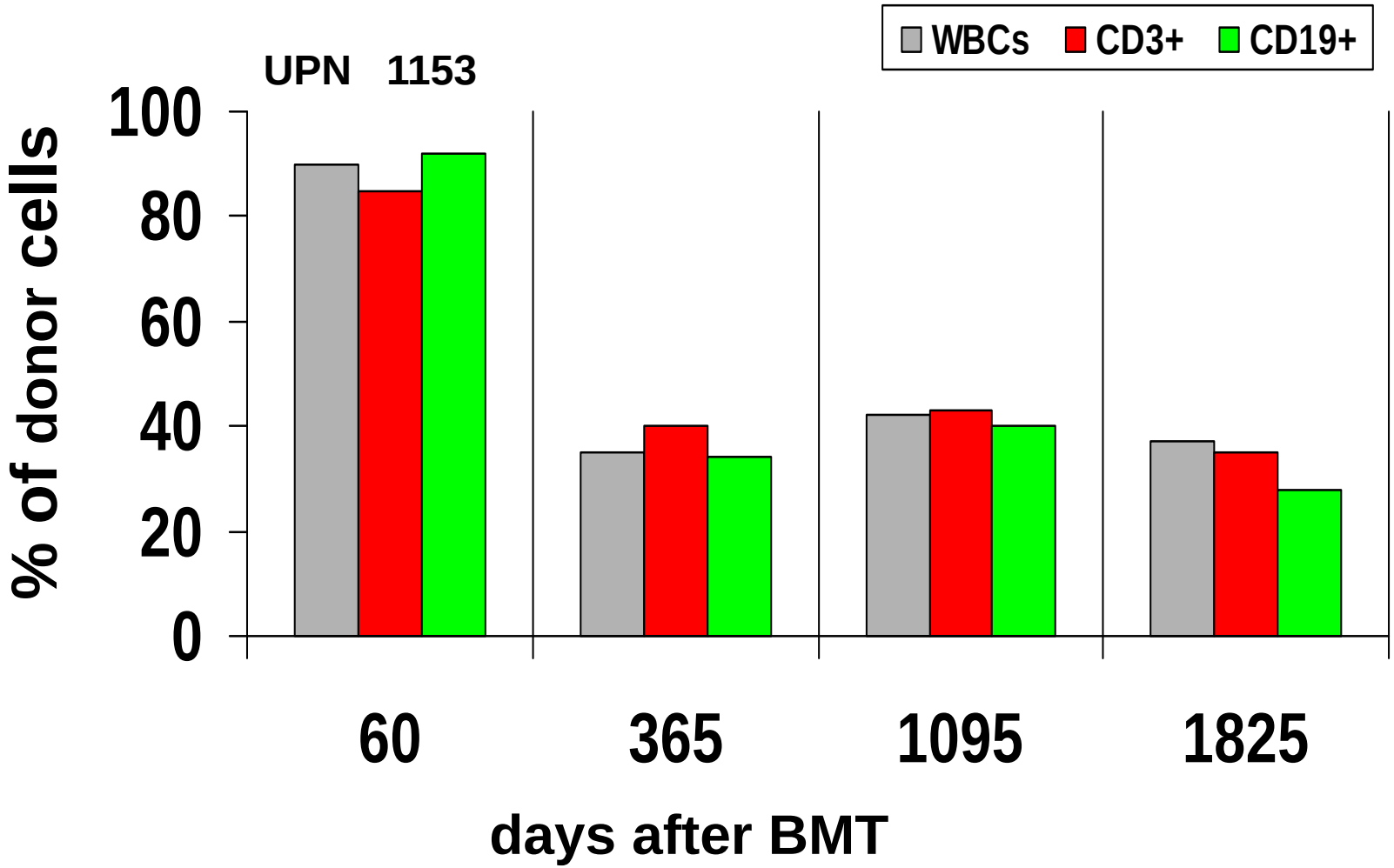
Persistent Mixed Chimerism

Patients	Follow-up (years)	Proportion of donor nucleated cells
Sal A	7	80
Tey M	7	46
Sat F	14	25
You M	5	29
Ven M	17	70
Tri A	17	84
Kyr A	9	18
Fre G	14	40
Cel S	10	59
Car F	17	20
Gan H	7	15

Persistent Mixed Chimerism



Persistent Mixed Chimerism



Persistent Mixed Chimerism

Mostly of the studies reported in literature showed the presence of mixed chimerism in the **nucleated cells**….

….rather than in the mature **erythrocytes**, **cells functionally crucial** for the patients affected by **haemoglobinopathies**.

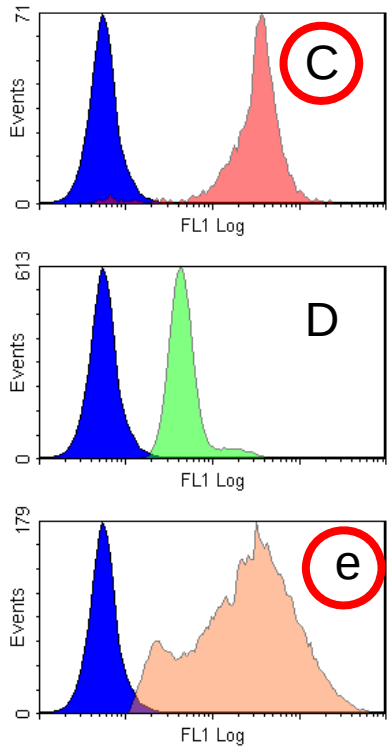
Persistent Mixed Chimerism

Donor engraftment in RBCs

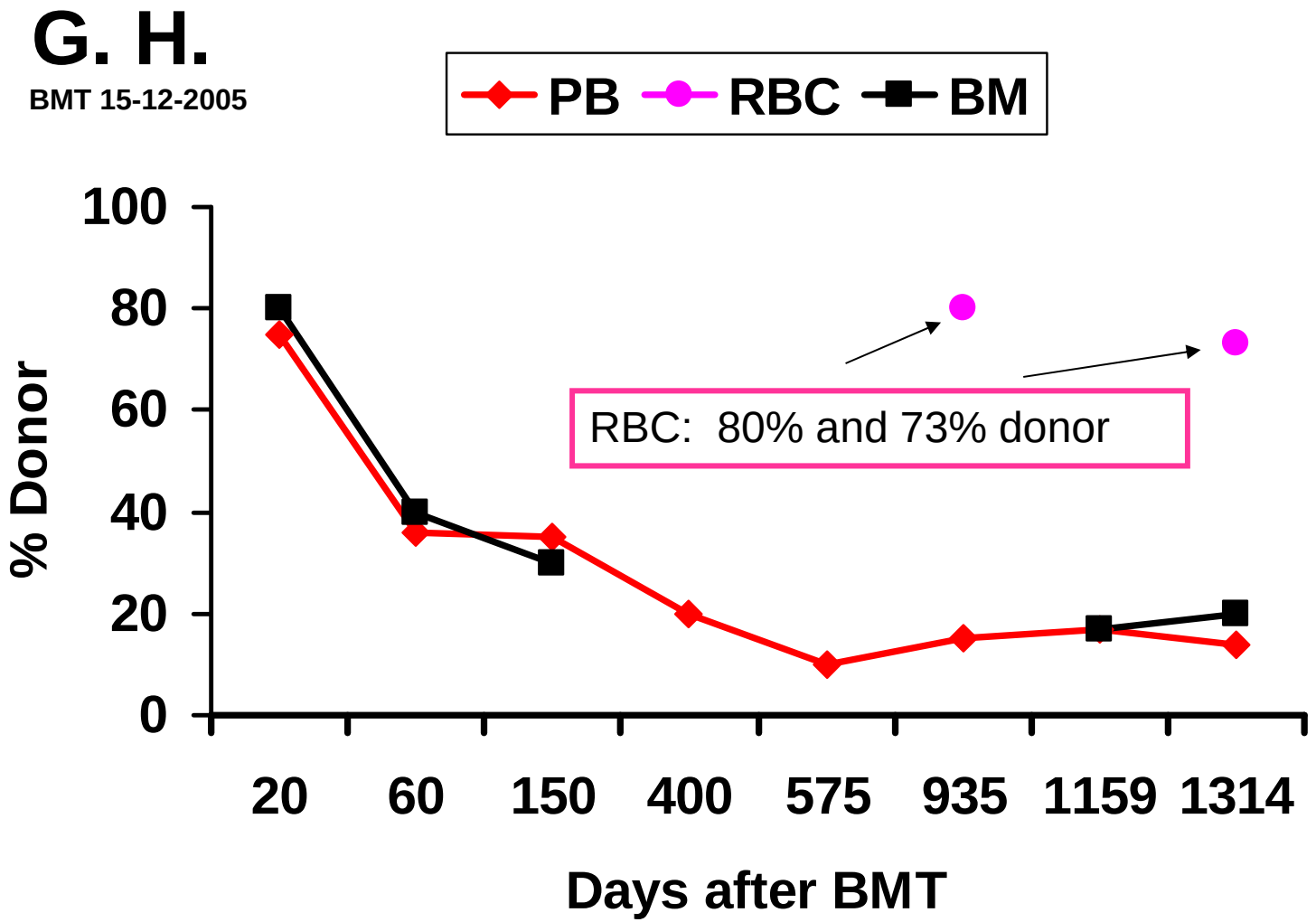
We investigated the presence of MC in the red blood cells by citofluorimetry analysis to detect chimerism in RBC using ABO or Rh differences

Mixed Chimerism in Thalassemia after HSCT

Donor: “C” and “e” pos



Mixed Chimerism in Thalassemia after HSCT



PERSISTEN MIXED CHIMERISM

Other thalassemic patients with PMC

Mixed Chimerism in Thalassemia after HSCT

Patients	Follow-up (years)	% donor nucleated cells	% donor RBCs
SA	3	72	**100**
AB	4	59	**99**
TM	7	50	**100**
SF	10	25	**80**
GH	3	15	**80**

Andreani M, Haematologica. 2011 Jan;96(1):128-33.

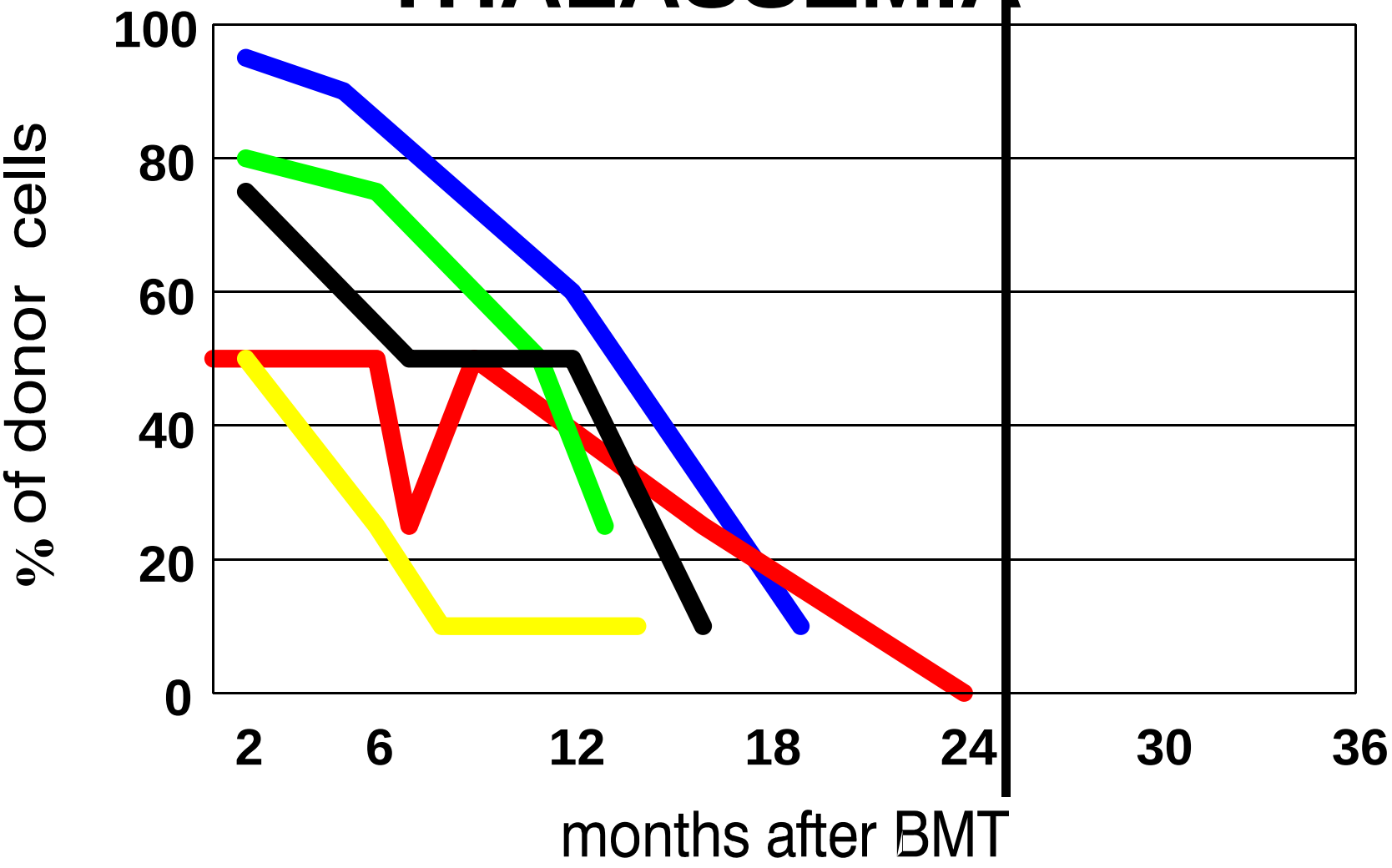
PMC in pazienti talassemici dopo TMO

Patients	Follow-up (years)	% donor nucleated cells	% donor RBCs	% donor BFU-E
SA	3	72	**100**	75
AB	4	59	**99**	50
TM	7	50	**100**	55
SF	10	25	**80**	28
GH	3	15	**80**	30

Mixed Chimerism in Thalassemia after HSCT

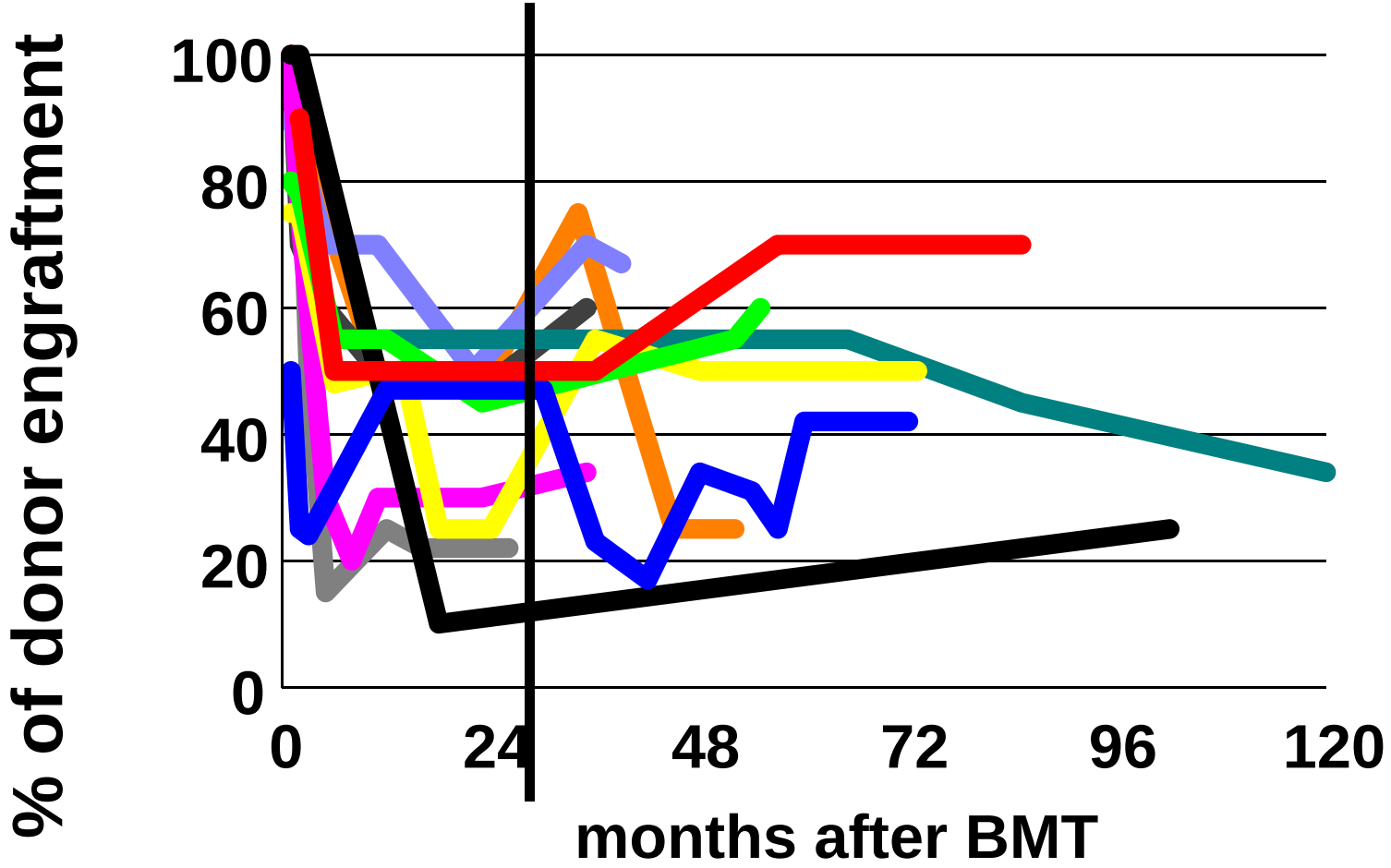
- Detection of mixed chimerism in the nucleated cells
- Difference between transient and persistent mixed chimerism
- The detection of mixed chimerism in red blood cells
- Control of the erythroid compartment expansion
- Associated between PMC and specific immune tolerance

LATE REJECTION AFTER BMT IN THALASSEMIA



MIXED CHIMERISM AFTER BMT IN THALASSEMIA

evolution of M.C. in 11 patients with RHCs > 25%



Mixed Chimerism in Thalassemia after HSCT

Associated between PMC and specific immune tolerance

- Presence of T regulatory cells
- Donor – Recipient Origin
- Specific suppressor activity

Mixed Chimerism in Thalassemia after HSCT

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- Specific suppressor activity

Mixed Chimerism in Thalassemia after HSCT

In the recent years many studies have shown that mixed chimerism is strongly associated with a state of immunotolerance, particularly in the clinical experience of solid organ transplantation.

Sachs DH, Sykes M, Kawai T, Cosimi AB. **Immuno-intervention for the induction of ransplantation tolerance through mixed chimerism.** Semin Immunol. 2011 Jun;23(3):165-73.

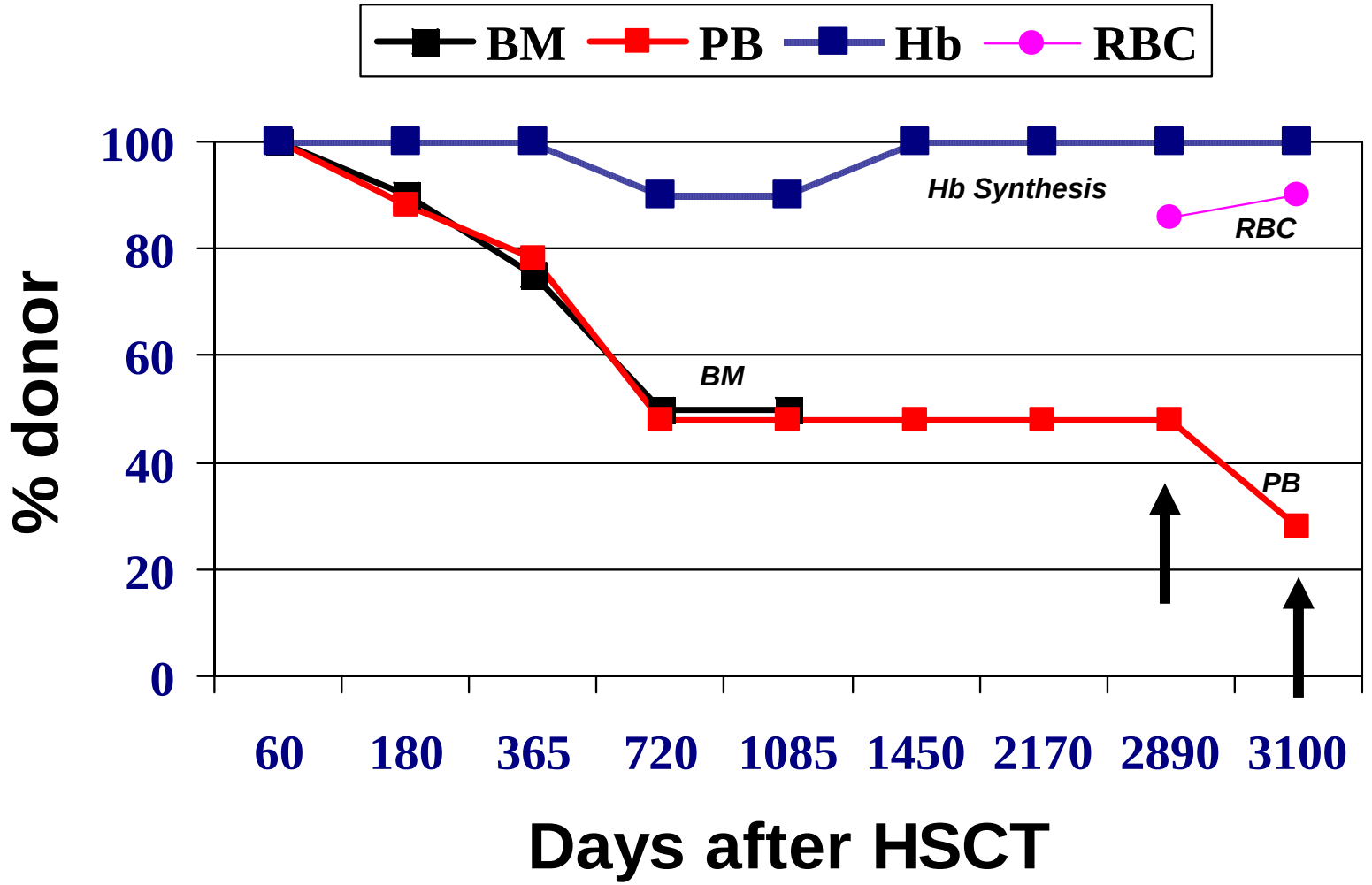
Mathes DW, Hwang B, Graves SS, Edwards J, Chang J, Storer BE, Butts-Miwongtum T, Sale GE, Nash RA, Storb R. Tolerance to vascularized composite allografts in canine mixed hematopoietic chimeras. Transplantation. 2011 Dec 27;92(12):1301-8.

Sykes M, Immune tolerance: mechanisms and application in clinical transplantation, Journal of internal medicine, 2007 Blackwell Publishing.

Mixed chimerism as an approach to transplantation tolerance

The ability to achieve transplantation tolerance with HCT has been well documented in patients who first received HCT with conventional myeloablative conditioning to treat a haematological malignancy, and later accepted an organ transplant from the same donor without chronic immunosuppressive therapy.

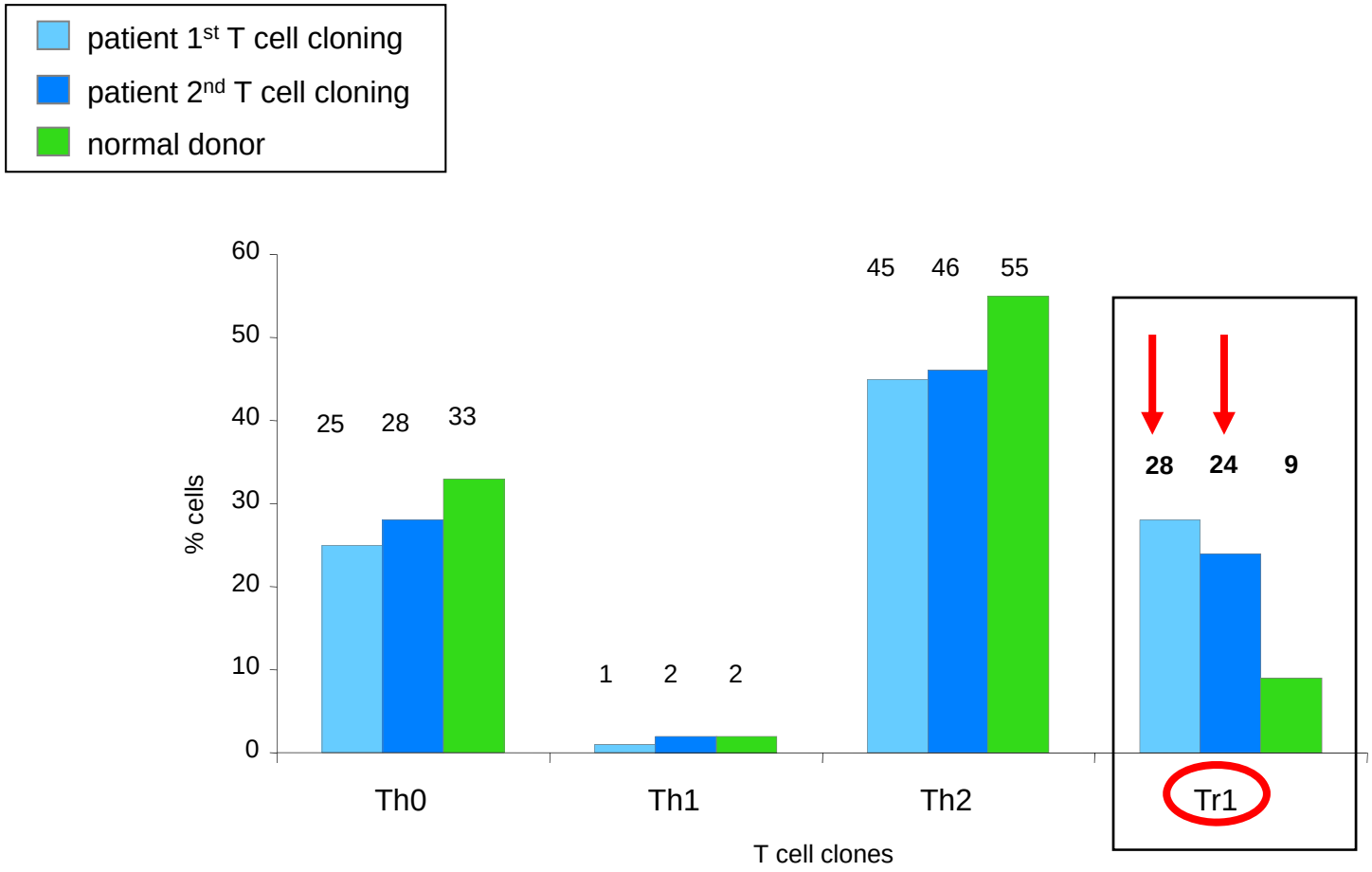
Mixed Chimerism in Thalassemia after HSCT



Mixed Chimerism in Thalassemia after HSCT

Two different cloning of CD4+ cells from a patient with PMC

Mixed Chimerism in Thalassemia after HSCT



Serafini G, Andreani M, Testi M et al. *Haematologica. 2009 Oct;94(10):1415-26*

Mixed Chimerism in Thalassemia after HSCT

- Presence of T regulatory cells
- Donor – Recipient Origin
- Specific suppressor activity

Host/Donor T cell clones origin (STR analysis)

*Number of Tr1 cell clones from the host/donor T cell clones characterized

	1st T cell cloning	
ORIGIN	n° clones	**n° Tr1**
HOST	12	**5***
DONOR	16	**6***
TOTAL	28	**11**

Tr1 cell clones of both origins

Both host and donor Tr1 cell clones are present *in vivo* in the patient with PMC.

Host/Donor T cell clones origin (STR analysis)

*Number of Tr1 cell clones from the host/donor T cell clones characterized

	1st T cell cloning		2nd T cell cloning	
ORIGIN	n° clones	**n° Tr1**	n° clones	**n° Tr1**
HOST	12	**5***	26	**7***
DONOR	16	**6***	20	**6***
TOTAL	28	**11**	46	**13**

Tr1 cell clones of both origins

Both host and donor Tr1 cell clones are present *in vivo* in the patient with PMC.

Mixed Chimerism in Thalassemia after HSCT

- Presence of T regulatory cells
- Donor – Recipient Origin
- Specific suppressive activity

Suppressive activity

read – out

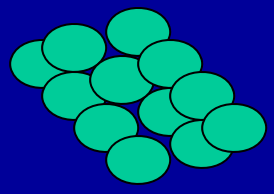
Alloreactivity

Recipient

Donor

+++++

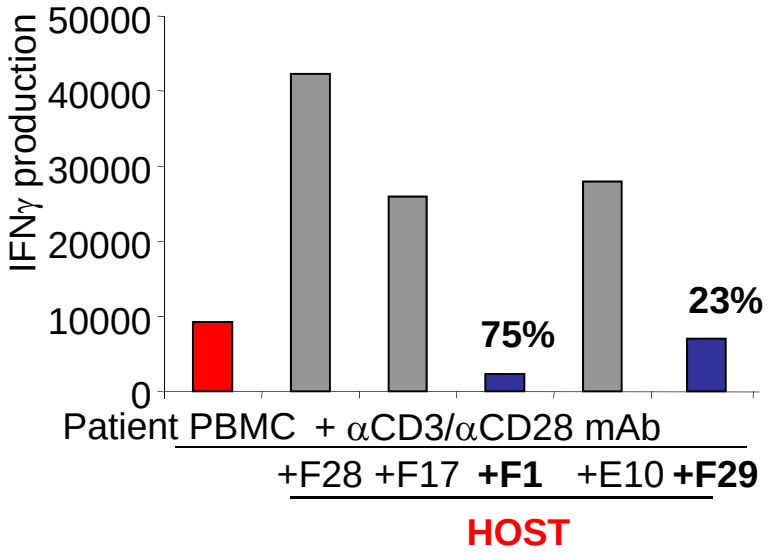
IFN-gamma production



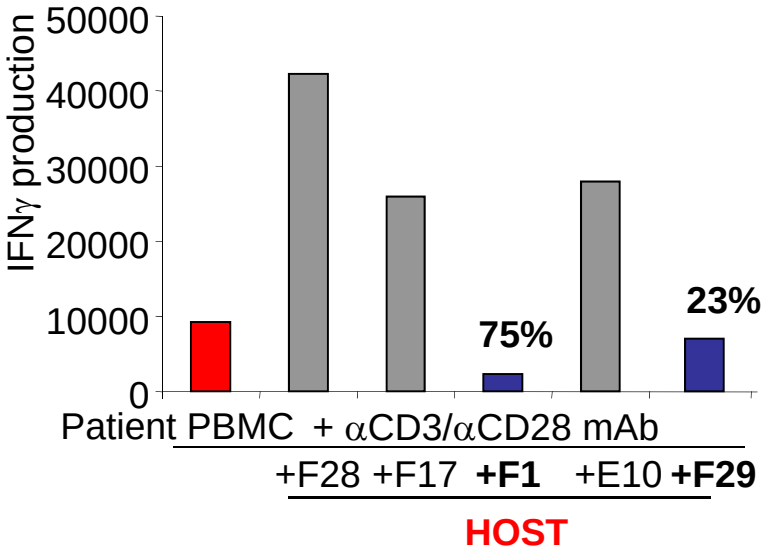
Suppression ?

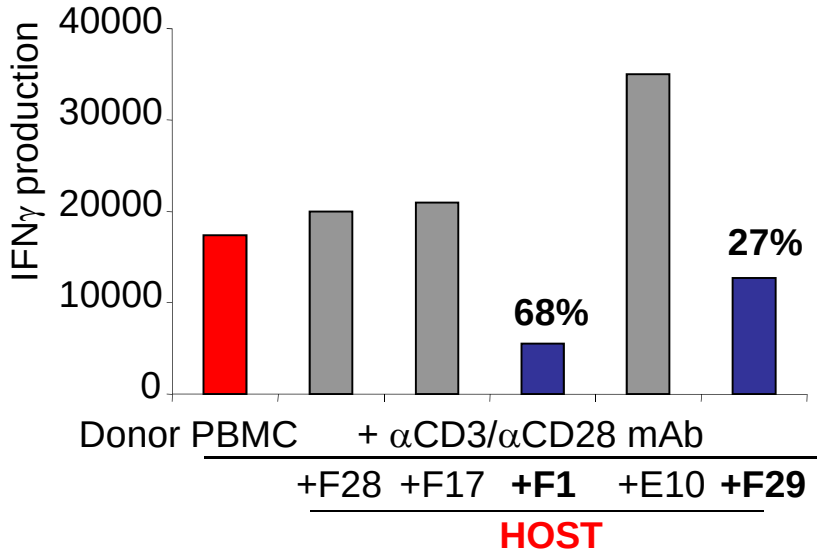
Adding of Tr1 PMC recipient or donor clones

Suppressive activity of host and donor alloreactive Tr1 cell clones

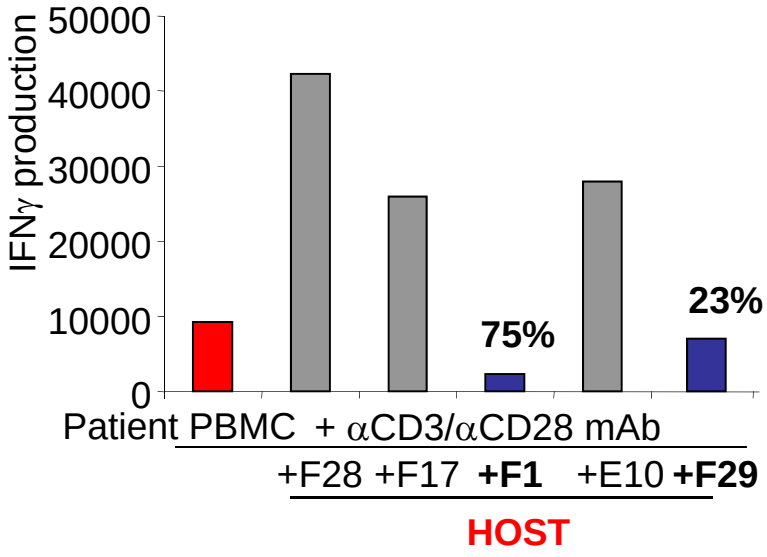


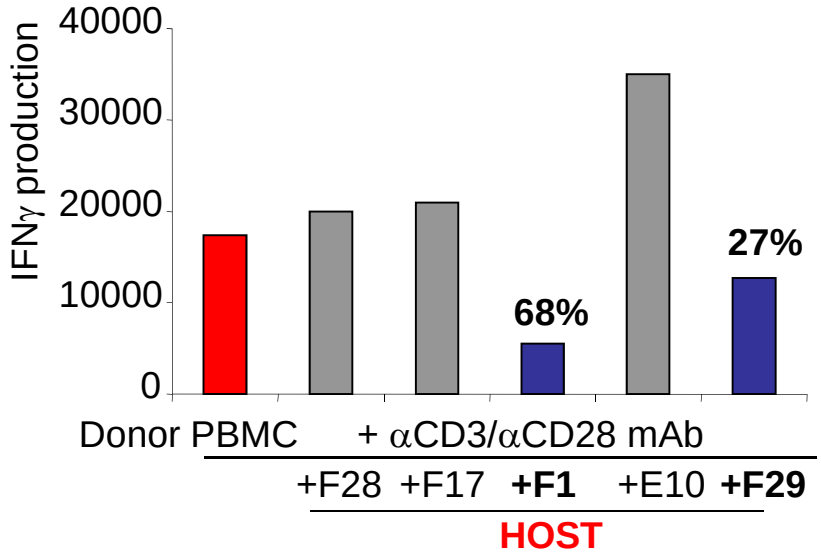
Suppressive activity of host and donor alloreactive Tr1 cell clones

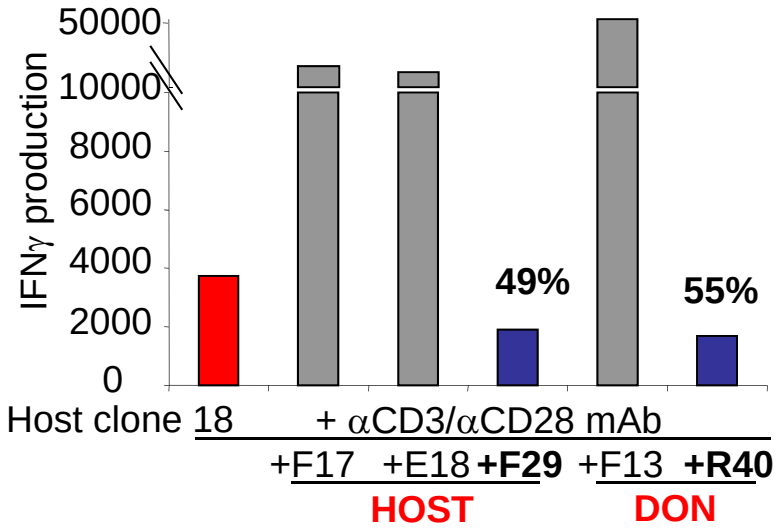


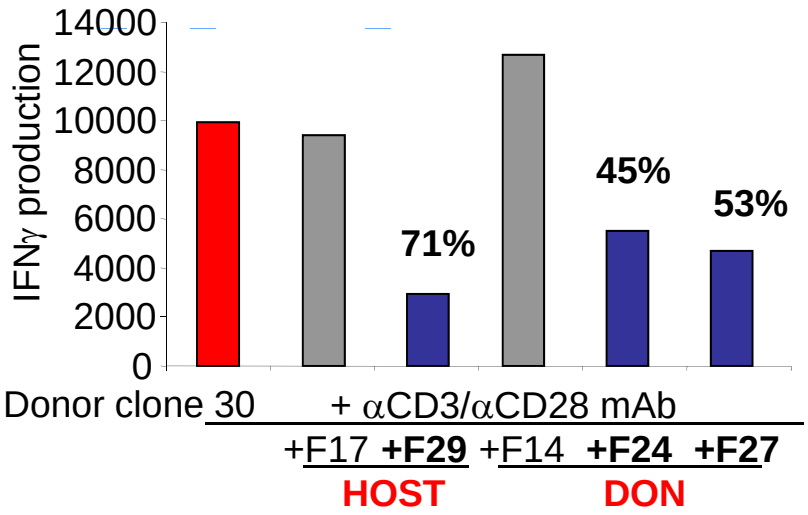


Suppressive activity of host and donor alloreactive Tr1 cell clones









THALASSEMIA PATIENTS AFTER HAPLOIDENTICAL BM HSC THAT DEVELOPED PMC

Higher frequency of IL-10-producing T cell clones in the patient with PMC compared to normal donor:

- are of both donor and host origin

- secrete high amounts of IL-10 compared to Tr1 clones from normal donor

- suppress both host and donor- specific T-cell mediated responses

Serafini G, Andreani M, Testi M et al. *Haematologica. 2009 Oct;94(10):1415-26*

MORE RECENT ACHIEVEMENTS

Definition of other tolerogenic markers:

Role of Granzyme B

New markers for Tr1 cells

Eur J Immunol. 2011 Jun;41(6):1652-62. doi: 10.1002/eji.201041120. Epub 2011 May 13.

Killing of myeloid APCs via HLA class I, CD2 and CD226 defines a novel mechanism of suppression by human Tr1 cells.

Magnani CF, Alberigo G, Bacchetta R, Serafini G, Andreani M, Roncarolo MG, Gregori S.

San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Scientific Institute, Milan, Italy.

Tr1 cells specifically lyse myeloid APC through a granzyme B (GZB)- and perforin (PRF)- dependent mechanism that requires HLA class I recognition, CD54/Lymphocyte Function-associated Antigen (LFA)-1 adhesion and activation via CD2.

High frequency of GZB expressing CD4+ T cells is detected in tolerant patients and correlates with elevated occurrence of IL-10-producing CD4+ T cells.

The modulatory activities of Tr1 cells are not only due to suppressive cytokines but also to specific cell-to-cell interactions which lead to selective killing of target cells and possibly bystander suppression

Nat Med. 2013 Jun;19(6):739-46. doi: 10.1038/nm.3179. Epub 2013 Apr 28.

Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells.

Gagliani N, Magnani CF, Huber S, Gianolini ME, Pala M, Licona-Limon P, Guo B, Herbert DR, Bulfone A, Trentini F, Di Serio C, Bacchetta R, Andreani M, Brockmann L, Gregori S, Flavell RA, Roncarolo MG.

San Raffaele Telethon Institute for Gene Therapy (HSR-TIGET), Division of Regenerative Medicine, Stem Cells and Gene Therapy, San Raffaele Scientific Institute, Milan, Italy.

The coexpression of CD49b and LAG-3 enables the isolation of highly suppressive human Tr1 cells from in vitro anergized cultures and allows the tracking of Tr1 cells in the peripheral blood of subjects who developed tolerance after allogeneic hematopoietic stem cell transplantation.

The use of these markers makes it feasible to track Tr1 cells in vivo and purify Tr1 cells for cell therapy to induce or restore tolerance in subjects with immune-mediated diseases.

CONCLUSIONS

Tr1 cells are critically involved in induction and maintenance of PMC after HSCT in B-Thal pts; however, the mechanisms underlying PMC induction are still elusive.

The establishment of active tolerance in the lymphoid compartment might contribute to the high engraftment of the donor's over recipient's erythroid cells.

Definition of the mechanisms involved in tolerance associated with Tr1 cells and PMC will provide tools for designing new protocols for in vivo induction of tolerance via PMC, and information for reducing pretransplant conditioning regiment, besides contributing to the design gene therapy strategy.

Acknowledgments

Centro Trapianti Fondazione IME - Roma
Guido Lucarelli
Javid Gaziev
Pietro Sodani
Katia Paciaroni
Fabio Torelli
Gioia De Angelis
Cecilia Alfieri
Cristiano Gallucci
Domenica Simone
Andrea Roveda
Luisa Cardarelli
Marco Marziali
Antonella Isgrò

HSR TIGET – Milano
Silvia Gregori
Giorgia Serafini
Chiara Magnani
Rosa Bacchetta
Katharina Fleischhauer
Maria Grazia Roncarolo

Servizio Trasfusionale S.Orsola Bologna
Andrea Bontadini
Pierluigi Tazzari
Francesca Ricci

ISS - Roma
Massimo Sanchez
Valentina Tirelli

Policlinico di Tor Vergata - Roma
Lidia De Felice
Francesca Agostini
Daniela Fraboni

Acknowledgments

LIBT Fondazione IME
Manuela Testi
Maria Troiano
Mariarosa Battarra
Tiziana Galluccio
Rossella Condello
Annalisa Guagnano
Giuseppe Testa
Chiara Stellitano
Renata Rosati
Andrea Di Luzio
Simona De Petris
Eleonora Palladini
Martina Mangione