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

Donor cell
Normal Recipient cell
Malignant Recipient cell

Patient before HSCT

Complete Chimerism
Rejection
Relapse
Mixed Chimerism

Patient after HSCT
Engraftment Monitoring after HSCT

Mixed Chimerism

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

Definition
Natural and induced
Methods commonly used for detection
Graft rejection and relapse
Immunological tolerance
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
Engraftment Monitoring after HSCT

Mixed Chimerism

Definition

Natural and induced

Methods commonly used for detection

Graft rejection and relapse

Immunological tolerance
### Engraftment Monitoring after HSCT

<table>
<thead>
<tr>
<th>Natural</th>
<th>Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>Transfusion</td>
</tr>
<tr>
<td></td>
<td>Solid organ and HSCT transplantation</td>
</tr>
</tbody>
</table>
# Engraftment Monitoring after HSCT

<table>
<thead>
<tr>
<th>Natural</th>
<th>Induced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy</td>
<td>Transfusion</td>
</tr>
<tr>
<td></td>
<td>Solid organ and HSCT transplantation</td>
</tr>
</tbody>
</table>
Naturally acquired Mc derives primarily from maternal cells in her progeny, or cells of fetal origin in women.
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
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.
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
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 histocompatibility 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

<table>
<thead>
<tr>
<th>Natural</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Induced</strong></td>
<td>Transfusion</td>
</tr>
<tr>
<td>Solid organ and HSCT</td>
<td>transplantation</td>
</tr>
</tbody>
</table>

**Note:** HSCT stands for Hematopoietic Stem Cell 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%)
FISH for chromosome Y ed X
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.
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
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

PRE

DON

Post-BM
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
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
I4.110  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.
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).

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.
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).
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


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


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

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

Masseyac J, Coutard B.

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

Abstract

Defining the Intensity of Conditioning Regimens: Working Definitions

Andrea Bacigalupo, M.D.1, Karen Bollen, M.D.1, Doug Rizzo, M.D.1, Sergio Giralt, M.D.1, Hilario Lazouras, M.D.1, Vincent Ho, M.D.1, Jane Appleton, M.D.1, Shimon Sivan, M.D.1, Marcello Pasquini, M.D.1, Brenda M. Sandmeier, M.D.1, John Barrett, M.D.1, Didier Blaise, M.D.1, Robert Lowski, M.D.1, Mary Horowitz, M.D.1

Defining the intensity of conditioning regimens: working definitions.


San Martino Hospital, Genoa, Italy. andrea.bacigalupo@tumori.onl.it

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, Chaim Sheba Medical Center, Tel-Hashomer, Israel.

Best Practice & Research Clinical Haematology 24 (2011) 369-379
Mixed Chimerism

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

% of pts with MC

2 months

MC - 153 pts


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


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

<table>
<thead>
<tr>
<th>Chimerism status early after HSCT</th>
<th>Transplant outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 25%</td>
<td>Rejection</td>
</tr>
<tr>
<td></td>
<td>PMC</td>
</tr>
<tr>
<td></td>
<td>Complete Chimerism</td>
</tr>
<tr>
<td>10-25%</td>
<td>Rejection</td>
</tr>
<tr>
<td></td>
<td>PMC</td>
</tr>
<tr>
<td></td>
<td>Complete Chimerism</td>
</tr>
<tr>
<td>&lt; 10%</td>
<td>Rejection</td>
</tr>
<tr>
<td></td>
<td>PMC</td>
</tr>
<tr>
<td></td>
<td>Complete Chimerism</td>
</tr>
</tbody>
</table>
Patients with MC level 3 early after HSCT have a higher risk of graft rejection.

<table>
<thead>
<tr>
<th>Engraftment status at 60 days after HSCT</th>
<th>Nº Pts</th>
<th>Engraftment evolution</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC Level 3 (RHCs&gt;25%)</td>
<td>10</td>
<td>2 3 5 (50%)</td>
</tr>
<tr>
<td>CC</td>
<td>50</td>
<td>3 46 1 (2%)</td>
</tr>
</tbody>
</table>


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

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

UPN: 1023

% of donor cells

80 days after BMT

0 20 40 60 80 100

WBCs CD3+

80

UPN: 1023
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

- BU14 - CY200
- BU14 - CY160
- BU14 - CY120

Incidence of mixed chimerism:
- 2 months: 210 pts
- 24 months: 26 pts

2 months:
- BU14 - CY200: 76 pts

24 months:
- BU14 - CY160: 26 pts
- BU14 - CY120: 76 pts
Mixed Chimerism in Thalassemia after HSCT

incidence of mixed chimerism

2 months

24 months

- BU14/CY200
- BU14/CY160
- BU14/CY120

rejection

210 11/76 28 7/28

10/210
Mixed Chimerism in Thalassemia after HSCT

Incidence of mixed chimerism

- 210 pts at 2 months
- 76 pts at 24 months
- 28 pts at 24 months
- 10/155 for BU14 - CY200
- 5/56 for BU14 - CY160
- 2/26 for BU14 - CY120
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
In many cases the proportion of cells of recipient origin is extremely large.
## Persistent Mixed Chimerism

<table>
<thead>
<tr>
<th>Patients</th>
<th>Follow-up (years)</th>
<th>Proportion of donor nucleated cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sal A</td>
<td>7</td>
<td>80</td>
</tr>
<tr>
<td>Tey M</td>
<td>7</td>
<td>46</td>
</tr>
<tr>
<td>Sat F</td>
<td>14</td>
<td>25</td>
</tr>
<tr>
<td>You M</td>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>Ven M</td>
<td>17</td>
<td>70</td>
</tr>
<tr>
<td>Tri A</td>
<td>17</td>
<td>84</td>
</tr>
<tr>
<td>Kyr A</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Fre G</td>
<td>14</td>
<td>40</td>
</tr>
<tr>
<td>Cel S</td>
<td>10</td>
<td>59</td>
</tr>
<tr>
<td>Car F</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>Gan H</td>
<td>7</td>
<td>15</td>
</tr>
</tbody>
</table>
Persistent Mixed Chimerism

G. H.
BMT 15-12-2005

% Donor

Days after BMT

PB  BM
Persistent Mixed Chimerism

UPN 1153

% of donor cells

0 20 40 60 80 100

60 365 1095 1825

days after BMT

WBCs  CD3+  CD19+

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.
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

[Graphs showing mixed chimerism]
Mixed Chimerism in Thalassemia after HSCT

G. H.
BMT 15-12-2005

Days after BMT

% Donor

PB
RBC
BM

RBC: 80% and 73% donor
PERSISTENT MIXED CHIMERISM

Other thalassemic patients with PMC
## Mixed Chimerism in Thalassemia after HSCT

<table>
<thead>
<tr>
<th>Patients</th>
<th>Follow-up (years)</th>
<th>% donor nucleated cells</th>
<th>% donor RBCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>3</td>
<td>72</td>
<td>100</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
<td>59</td>
<td>99</td>
</tr>
<tr>
<td>TM</td>
<td>7</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>SF</td>
<td>10</td>
<td>25</td>
<td>80</td>
</tr>
<tr>
<td>GH</td>
<td>3</td>
<td>15</td>
<td>80</td>
</tr>
</tbody>
</table>

### PMC in pazienti talassemici dopo TMO

<table>
<thead>
<tr>
<th>Patients</th>
<th>Follow-up (years)</th>
<th>% donor nucleated cells</th>
<th>% donor RBCs</th>
<th>% donor BFU-E</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>3</td>
<td>72</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>AB</td>
<td>4</td>
<td>59</td>
<td>99</td>
<td>50</td>
</tr>
<tr>
<td>TM</td>
<td>7</td>
<td>50</td>
<td>100</td>
<td>55</td>
</tr>
<tr>
<td>SF</td>
<td>10</td>
<td>25</td>
<td>80</td>
<td>28</td>
</tr>
<tr>
<td>GH</td>
<td>3</td>
<td>15</td>
<td>80</td>
<td>30</td>
</tr>
</tbody>
</table>
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 THALASSEMA
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

Associated between PMC and specific immune tolerance

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


**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

Days after HSCT

% donor

BM
PB
Hb
RBC

Hb Synthesis

RBC

BM
PB
Mixed Chimerism in Thalassemia after HSCT

Two different cloning of CD4+ cells from a patient with PMC
Mixed Chimerism in Thalassemia after HSCT

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

<table>
<thead>
<tr>
<th>ORIGIN</th>
<th>n° clones</th>
<th>n° Tr1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOST</td>
<td>12</td>
<td>5*</td>
</tr>
<tr>
<td>DONOR</td>
<td>16</td>
<td>6*</td>
</tr>
<tr>
<td>TOTAL</td>
<td>28</td>
<td>11</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>ORIGIN</th>
<th>1st T cell cloning n° clones</th>
<th>1st T cell cloning n° Tr1</th>
<th>2nd T cell cloning n° clones</th>
<th>2nd T cell cloning n° Tr1</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOST</td>
<td>12</td>
<td>5*</td>
<td>26</td>
<td>7*</td>
</tr>
<tr>
<td>DONOR</td>
<td>16</td>
<td>6*</td>
<td>20</td>
<td>6*</td>
</tr>
<tr>
<td>TOTAL</td>
<td>28</td>
<td>11</td>
<td>46</td>
<td>13</td>
</tr>
</tbody>
</table>

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

Recipient

Donor

Alloreactivity

+++

IFN-gamma production

Adding of Tr1 PMC recipient or donor clones

read – out

Suppression ?
Suppressive activity of host and donor alloreactive Tr1 cell clones
Suppressive activity of host and donor alloreactive Tr1 cell clones

Graphs showing IFN-γ production in response to stimulation with αCD3/αCD28 mAb and F28, F17, F1, E10, and F29. Host clone 18 stimulation results in 75% and 23% IFN-γ production. Donor clone 30 stimulation results in 68% and 27% IFN-γ production.
Suppressive activity of host and donor alloreactive Tr1 cell clones

Patient PBMC + αCD3/αCD28 mAb +F28 +F17 +F1 +E10 +F29

IFN-γ production

HOST

Donor PBMC + αCD3/αCD28 mAb +F28 +F17 +F1 +E10 +F29

IFN-γ production

HOST

Host clone 18 + αCD3/αCD28 mAb +F17 +E18 +F29 +F13 +R40

IFN-γ production

HOST

Donor clone 30 + αCD3/αCD28 mAb +F17 +F29 +F14 +F24 +F27

IFN-γ production

DON
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

MORE RECENT ACHIEVEMENTS

Definition of other tolerogenic markers:

Role of Granzyme B

New markers for Tr1 cells
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.
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 regimen, 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