Mixed Chimerism and Immunological Tolerance in non malignant diseases after HSCT

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The main goal of engraftment monitoring is to determine the presence or absence of residual host cell populations after HSCT.
Engraftment Monitoring after HSCT

Donor cell

Normal Recipient cell

HSCT

Donor

Patient before HSCT

Complete Chimerism

Rejection

Mixed Chimerism

Patient after HSCT
Mixed Chimerism in Haemoglobinopathies

MC in haemoglobinopathies may be defined as

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

**Persistent** when donor and recipient cells coexist for more than 24 months after HSCT
HSCT in haemoglobinopathies, such as beta-thalassemia or sickle cell disease represents an interesting model to study mixed chimerism, since patients do not present:

- immune system alterations by underlying diseases
- malignant cells
- previous chemotherapy treatment
Haemoglobinopathies: genetic characteristics and distribution

HSCT for haemoglobinopathies - clinical results

Engraftment detection methods for HSCT in haemoglobinopathies

Early mixed chimerism (transient) and the risk of graft rejection

Persistent mixed chimerism

- thalassemic erythroid compartment evaluation

- immunological tolerance
Haemoglobinopathies: genetic characteristics and distribution

Beta Thalassemia and Sickle Cell Disease, are the most widely diffused hereditary hemoglobinopathies in the world.

Both soon became globally spread because of old and modern migration.
Beta Thalassemia
It is estimated that about **180 million** healthy carriers are present worldwide and every year there are more than **70,000** new born beta thalassemic patients.

Sickle Cell Disease
On the other side, worldwide, there are an estimated **270,000** patients affected with SCD.

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Modell B et al. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008
Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. *Blood* 2010
Freed J et al. Allogeneic cellular and autologous stem cell therapy for sickle cell disease: 'whom, when and how'. *BMT* 2012
Haemoglobinopathies: genetic characteristics and distribution

Normal asset of hemoglobin gene

Normal adult hemoglobin protein
Haemoglobinopathies: genetic characteristics and distribution

**Beta Thalassemia** is characterized by different genetic mutations that produce deficient or absent synthesis of the beta globin chains

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Type</th>
<th>β Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD39 (C-T)</td>
<td>non-sense</td>
<td>β⁰</td>
</tr>
<tr>
<td>IVS1:110 (G-A)</td>
<td>RNA processing mutant</td>
<td>β⁺</td>
</tr>
<tr>
<td>IVS1:6 (T-C)</td>
<td>RNA processing mutant</td>
<td>β⁺</td>
</tr>
<tr>
<td>IVS1:1 (G-A)</td>
<td>RNA processing mutant</td>
<td>β⁺</td>
</tr>
<tr>
<td>IVS2:745 (C-G)</td>
<td>RNA processing mutant</td>
<td>β⁺</td>
</tr>
<tr>
<td>IVS2:1 (G-A)</td>
<td>RNA processing mutant</td>
<td>β⁺</td>
</tr>
<tr>
<td>-87 (C-G)</td>
<td>transcriptional mutant</td>
<td>β⁺</td>
</tr>
<tr>
<td>CD6-A</td>
<td>frameshift</td>
<td>β⁰</td>
</tr>
</tbody>
</table>
Conventional treatment for affected individuals has improved in recent years due to advances in medical management in the prevention and treatment of complications.

Kaplan-Meier survival curves in 977 medically treated patients with thalassemia in Italy. Survival is calculated after the first decade of life, by birth cohort.


HSCT is still the only available curative option.
Haemoglobinopathies: genetic characteristics and distribution

**HSCT for haemoglobinopathies - clinical results**

Engraftment detection methods for HSCT in haemoglobinopathies

Early mixed chimerism (transient) and the risk of graft rejection

Persistent mixed chimerism

- thalassemic erythroid compartment evaluation

- immunological tolerance
In the 1980s and early 1990s, 900 consecutive unselected patients transplanted from an HLA-identical sibling donor at the transplant center in Pesaro, Italy reported a 20 years probability of thalassemia-free survival of 73%.

Figure 1. Results of hematopoietic stem cell transplantation in 900 consecutive patients, aged 1-35 years, transplanted from an HLA identical sibling in Pesaro since December 1981. Courtesy of Guido Lucarelli.

Angelucci et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica 2014
### Table 2. Recent reports on matched sibling donor HSCT in children and adults with thalassaemia major.

<table>
<thead>
<tr>
<th>Author and Reference</th>
<th>N. of patients</th>
<th>Patient cohort/Pesaro risk category</th>
<th>Overall survival</th>
<th>Thalassemia free survival</th>
<th>Treatment related mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galambrun <em>et al.</em></td>
<td>108</td>
<td>Children all categories of risk</td>
<td>15 years 86.8%</td>
<td>15 years 69.4%</td>
<td>15 years 12%</td>
</tr>
<tr>
<td>Yesilipek <em>et al.</em></td>
<td>245</td>
<td>Children: Low: 41 Intermediate: 130 High: 63</td>
<td>1 year 85%</td>
<td>1 year 68%</td>
<td>1 year 7.75%</td>
</tr>
<tr>
<td>Li <em>et al.</em></td>
<td>82</td>
<td>Children all risk categories</td>
<td>3 years 91%</td>
<td>3 years 87%</td>
<td>3 years 8%</td>
</tr>
<tr>
<td>Choudhary <em>et al.</em></td>
<td>28</td>
<td>Children: Intermediate risk: 7 High risk: 21</td>
<td>78.5%</td>
<td>71.4%</td>
<td>21.4%</td>
</tr>
<tr>
<td>Bernardo <em>et al.</em></td>
<td>60</td>
<td>Low: 27 Intermediate: 17, High: 4 Adults: 12</td>
<td>5 years 93%</td>
<td>5 years 84%</td>
<td>7%</td>
</tr>
<tr>
<td>Sabloff <em>et al.</em></td>
<td>179</td>
<td>Low: 2% Intermediate: 42% High: 36%</td>
<td>5 years: Intermediate risk: 91% High risk: 64%</td>
<td>5 years: Intermediate risk: 88% High risk: 62%</td>
<td>Intermediate risk: 5/75 High risk 23/64</td>
</tr>
<tr>
<td>Ghavamzadeh <em>et al.</em></td>
<td>183</td>
<td>Children Low and intermediate</td>
<td>2 years PBSCs 83% BM 89%</td>
<td>2 years PBSCs 76% BM 70%</td>
<td>1 year PBSC 14% BM: 9%</td>
</tr>
<tr>
<td>Iravani <em>et al.</em></td>
<td>52</td>
<td>Children high risk: 52</td>
<td>4.1 years 80%</td>
<td>4.1 years 69%</td>
<td>4.1 years 7/52</td>
</tr>
<tr>
<td>Irfan <em>et al.</em></td>
<td>56</td>
<td>Children Low: 20 Intermediate: 20 High: 16</td>
<td>5 years BM: 73% PBSCs: 63%</td>
<td>5 years BM: 67% PBSCs: 55%</td>
<td>100 days: 10/56</td>
</tr>
<tr>
<td>Locatelli <em>et al.</em></td>
<td>259</td>
<td>Median age 8 years (range 1-24) Low: 86 Intermediate: 122 High: 51</td>
<td>6 years 95%</td>
<td>6 years 86%</td>
<td>4%</td>
</tr>
<tr>
<td>Ullah <em>et al.</em></td>
<td>48</td>
<td>Low: 31 Intermediate: 11 High: 6</td>
<td>6 years 79%</td>
<td>6 years 75%</td>
<td>20.8%</td>
</tr>
<tr>
<td>Di Bartolomeo <em>et al.</em></td>
<td>115</td>
<td>All categories</td>
<td>20 years 89.2%</td>
<td>20 years 85.7%</td>
<td>1 year 8.7%</td>
</tr>
</tbody>
</table>
Recent HSCT activity in Sickle Cell Disease
Haemoglobinopathies: genetic characteristics and distribution

**Sickle Cell Disease** is characterized by the presence of a single genetic mutation, that produce the SS hemoglobin. Loss of deformability and changes in cell morphology of the erythrocytes leads to intracellular hemoglobin polymerization.

OXY-STATE ↔ DEOXY-STATE
Haemoglobinopathies: genetic characteristics and distribution

Hemoglobin polymers are the cause of stroke in SCA
**Table 2. Worldwide reported outcomes of myeloablative HSCT for sickle cell disease**

<table>
<thead>
<tr>
<th></th>
<th>n = 50 (Vermylen et al. 1998)</th>
<th>n = 50 (Walters et al. 2000)</th>
<th>n = 11 (Locatelli et al. 2003)</th>
<th>n = 67 (Panevito et al. 2007)</th>
<th>n = 87 (Berneaudin et al. 2007)</th>
<th>n = 11 (Lucarelli et al. 2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OS</strong></td>
<td>93%</td>
<td>94%</td>
<td>100%</td>
<td>97%</td>
<td>92%</td>
<td>90%</td>
</tr>
<tr>
<td><strong>DFS</strong></td>
<td>85%</td>
<td>84%</td>
<td>90%</td>
<td>85%</td>
<td>86%</td>
<td>90%</td>
</tr>
<tr>
<td><strong>Rejection</strong></td>
<td>10%</td>
<td>10%</td>
<td>9%</td>
<td>13%</td>
<td>5%</td>
<td>10%</td>
</tr>
</tbody>
</table>

Abbreviations: OS, overall survival; DFS, disease-free survival.

Haemoglobinopathies: genetic characteristics and distribution

HSCT for haemoglobinopathies - clinical results

**Engraftment detection methods after HSCT in haemoglobinopathies**

Transient mixed chimerism and graft rejection

Persistent mixed chimerism
  - thalassemic erythroid compartment evaluation
  - immunological tolerance
in nucleated cells

- Peripheral Blood
- Lymphid Cells Subsets (CD3, CD19, CD56 etc.)
- T regulatory cells (Tr1)
- Bone Marrow
- Erythroid Precursors (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%)
DNA fragments analysis – STR Short tandem repeats
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.
in red blood cells

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

<table>
<thead>
<tr>
<th></th>
<th>Patient</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB0:</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Rh:</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Subgroup:</td>
<td>ccDDe</td>
<td>ccDe</td>
</tr>
<tr>
<td>Useful Marker:</td>
<td>E</td>
<td></td>
</tr>
</tbody>
</table>

- Anti-E
  - 87.5%
  - 2.3%

- Anti-e
  - 70.1%
Haemoglobinopathies: genetic characteristics and distribution
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**Transient mixed chimerism and graft rejection**

Persistent mixed chimerism
- thalassemic erythroid compartment evaluation
- immunological tolerance
Mixed Chimerism in Thalassemia after HSCT

MC - 153 pts

% of pts with MC

2 months


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


Laboratorio di Immunogenetica e Biologia dei Trapianti, Fondazione I.M.E., Roma, Italy.
Mixed Chimerism in Thalassemia after HSCT

Evolution of TMC towards:
- graft failure
- complete chimerism
- persistent mixed chimerism

Graph showing the evolution of TMC over months after HSCT, with data points indicating the percentage of donor cells over time.
Mixed Chimerism in Thalassemia after HSCT

Transient MC evolution

Number of RHCs present early after HSCT

Presence of split chimerism between PBMCs and CD3+ cells

Conditioning regimes used

Andreani M, Testi M, Lucarelli G. Tissue Antigens. 2014
Chimerism status early after HSCT

Chimerism evolution within 2 years after HSCT

RHCs < 10%

[Diagram showing cell populations labeled as 'Patient' and 'Donor', with color coding for 'Rej - Rejection', 'CC - Complete Chimerism', and 'PMC - Persistent Mixed Chimerism'].

Legend:
- Green: Patient
- Red: Donor
- Green with square pattern: Rej - Rejection
- Red: CC - Complete Chimerism
- PM: PMC – Persistent Mixed Chimerism
Chimerism status early after HSCT

Chimerism evolution within 2 years after HSCT

RHCs < 10%

Donor
Patient
Rej - Rejection
CC - Complete Chimerism
PMC – Persistent Mixed Chimerism
Chimerism status early after HSCT

RHCs < 10%

RHCs > 25%

Chimerism evolution within 2 years after HSCT

Rej - Rejection
CC - Complete Chimerism
PMC – Persistent Mixed Chimerism

Chimerism status early after HSCT

RHCs < 10%

RHCs > 25%

Chimerism evolution within 2 years after HSCT

Rej - Rejection
CC - Complete Chimerism
PMC – Persistent Mixed Chimerism

Donor
Patient

Rej
CC
PMC
Chimerism status early after HSCT

RHCs < 10%

RHCs > 25%

Chimerism evolution within 2 years after HSCT

Rej - Rejection

CC - Complete Chimerism

PMC – Persistent Mixed Chimerism
Mixed Chimerism in Thalassemia after HSCT

Transient MC evolution

Number of RHCs present early after HSCT

Presence of split chimerism between PBMCs and CD3+ cells

Conditioning regimes used

Andreani M, Testi M, Lucarelli G. Tissue Antigens. 2014
Mixed Chimerism in Thalassemia after HSCT

UPN: 1023

% of donor cells

80 days after BMT

80

WBCs CD3+

80
Mixed Chimerism in Thalassemia after HSCT

Transient MC evolution

Number of RHCs present early after HSCT

Presence of split chimerism between PBMCs and CD3+ cells

Conditioning regimes used

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

- **2 months**: 210 pts
- **24 months**: 76 pts, 26 pts

**Legend**:
- BU14 - CY200
- BU14 - CY160
- BU14 - CY120

**Incidence of mixed chimerism**
Mixed Chimerism in Thalassemia after HSCT

Incidence of mixed chimerism over 2 months and 24 months.

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

2 months:
- BU14/CY200: 210
- BU14/CY160: 76
- BU14/CY120: 28

24 months:
- Rejection: 10/210, 11/76, 7/28
Mixed Chimerism in Thalassemia after HSCT

incidence of mixed chimerism

2 months

- BU14 - CY200: 210 pts
- BU14 - CY160: 76 pts
- BU14 - CY120: 28 pts

24 months

- BU14 - CY200: 10/155
- BU14 - CY160: 5/56
- BU14 - CY120: 2/26
Haemoglobinopathies: genetic characteristics and distribution
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Engraftment detection methods after HSCT in haemoglobinopathies
Transient mixed chimerism and graft rejection

**Persistent mixed chimerism**
- thalassemic erythroid compartment evaluation
- immunological tolerance
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

Patients are cured from beta-thalassemia although they present a functional graft
Mixed Chimerism in Thalassemia after HSCT

G. H.
BMT 15-12-2005

% Donor

Days after BMT

20 60 150 400 575 935 1159 1314

PB
BM

0 20 40 60 80 100
<table>
<thead>
<tr>
<th>Patients</th>
<th>Follow-up</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>
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Transient mixed chimerism and graft rejection

**Persistent mixed chimerism**
- thalassemic erythroid compartment evaluation
- immunological tolerance
What do you find if you monitor the engraftment status in the red blood cells?

Split Chimerism
between nucleated and red blood cells
Mixed Chimerism in Thalassemia after HSCT

G. H.
BMT 15-12-2005

RBC: 80% and 73% donor

Days after BMT

% Donor
Markers commonly used for mixed chimerism detection in nucleated cells

- Peripheral Blood
- Lymphid Cells Subsets (CD3, CD19, CD56 etc.)
- T regulatory cells (Tr1)
- Bone Marrow
- Erythroid Precursors (BFU-E)
Mixed Chimerism in Thalassemia after HSCT

Control of the erythroid compartment expansion

BFU-E: mostly are of recipient origin

Singularity pick up and extract DNA

STR analysis
Mixed Chimerism in Thalassemia after HSCT

G. H.
BMT 15-12-2005

Days after BMT

% Donor

RBC: 80% and 73% donor

BFU-E in BM: 23% donor

PB
RBC
BM
BFU-E
Mixed Chimerism in Thalassemia after HSCT

Control of the erythroid compartment expansion
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

Erythroid expansion
Bone marrow of a thalassemic patient before HSCT transplant

100% RHCs

Normal erythropoiesis

Erythroid expansion

Bone marrow of a thalassemic patient with PMC after HSCT transplant

70% RHCs

< 30% Donor
Bone marrow of a thalassemic patient before HSCT transplant

100% RHCs

Bone marrow of a thalassemic patient with PMC after HSCT transplant

70% RHCs

< 30% Donor

Normal erythropoiesis

Erythroid expansion
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**Persistent mixed chimerism**
- thalassemic erythroid compartment evaluation
- immunological tolerance
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
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.


Mixed Chimerism in Thalassemia after HSCT

Establishment of immune tolerance with development of T regulatory cells

% Donor

Months after HSCT

1 2 6 12 24 36 48 60
Mixed Chimerism in Thalassemia after HSCT

Establishment of immune tolerance with development of T regulatory cells

Maintenance of a status of immune tolerance due to the presence of T regulatory cells
Markers commonly used for mixed chimerism detection in nucleated cells

- Peripheral Blood
- Lymphid Cells Subsets (CD3, CD19, CD56 etc.)
- T regulatory cells
- Bone Marrow
- Erythroid Precursors (BFU-E)
INDUCTION OF PERIPHERAL TOLERANCE
BY REGULATORY T CELLS

CD4\(^{-}\) 8\(^{-}\) \(\gamma\delta^+\) CD4\(^+\) NK-T CD8\(^+\)

- Tr1
- nTr (CD4\(^+\)CD25\(^+\)FOXP3\(^+\))
Identification and selection of Tr1 cells in PBMC of patients with PMC after HSCT for beta-thalassemia

T cell cloning

Identification of IL-10 producing T cells by intracellular cytokine staining (ICS) in PBMCs
Identification and selection of Tr1 cells in PBMC

T cell cloning

PBMC

Positive selection of CD4+ T cells

limiting dilution

0.3 cells/well

Tr1 cell clones

T cell clone characterization:
- Cytokine profile IL-10/IL-4 ratio >8
- Proliferative capacity
- Suppressive activity

Courtesy of Dr Silvia Gregori
Tr1 cell clones: cytokine production profile

Tr1 clones = ratio IL10/IL4 > 8

<table>
<thead>
<tr>
<th>clone</th>
<th>IL10</th>
<th>IL2</th>
<th>IL4</th>
<th>IFNg</th>
<th>IL17</th>
<th>IL10/IL4</th>
</tr>
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<tbody>
<tr>
<td>#246</td>
<td>4700</td>
<td>0</td>
<td>124</td>
<td>770</td>
<td>128</td>
<td>37,9</td>
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<tr>
<td>#93</td>
<td>2756</td>
<td>29</td>
<td>30</td>
<td>1020</td>
<td>112</td>
<td>91,9</td>
</tr>
<tr>
<td>#282</td>
<td>12350</td>
<td>20</td>
<td>266</td>
<td>630</td>
<td>0</td>
<td>46,4</td>
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<tr>
<td>#46</td>
<td>6510</td>
<td>174</td>
<td>246</td>
<td>1825</td>
<td>64</td>
<td>26,5</td>
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<tr>
<td>#143</td>
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<td>176</td>
<td>18070</td>
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<td>50,6</td>
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<tr>
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<td>1005</td>
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<td>285,0</td>
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<td>4245</td>
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<td>67,5</td>
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<tr>
<td>#103</td>
<td>1620</td>
<td>20</td>
<td>20</td>
<td>425</td>
<td>0</td>
<td>81,0</td>
</tr>
</tbody>
</table>

 Courtesy of Dr Silvia Gregori
Number of T cell clones

Are Tr 1 cell clones present in large number in patients with PMC after HSCT for haemoglobinopathies?
Mixed Chimerism in Thalassemia after HSCT

Host/Donor T cell clones origin
STR analysis

Do Tr1 cell clones originate both from host and donor in patients with PMC after HSCT for haemoglobinopathies?
Host/Donor T cell clones origin

STR analysis

<table>
<thead>
<tr>
<th>ORIGIN</th>
<th>1(^{\text{st}}) T cell cloning</th>
<th>2(^{\text{nd}}) T cell cloning</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOST</td>
<td>\n° clones</td>
<td>n° Tr1</td>
</tr>
<tr>
<td></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>

Tr1 cell clones of both origins
### Tr1 cell clones: cytokine production profile

**Tr1 clones** = ratio IL10/IL4 > 8

<table>
<thead>
<tr>
<th>clone</th>
<th>IL10</th>
<th>IL2</th>
<th>IL4</th>
<th>IFNγ</th>
<th>IL17</th>
<th>IL10/IL4</th>
<th>origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>#246</td>
<td>4700</td>
<td>0</td>
<td>124</td>
<td>770</td>
<td>128</td>
<td>37,9</td>
<td>D</td>
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<tr>
<td>#93</td>
<td>2756</td>
<td>29</td>
<td>30</td>
<td>1020</td>
<td>112</td>
<td>91,9</td>
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<tr>
<td>#282</td>
<td>12350</td>
<td>20</td>
<td>266</td>
<td>630</td>
<td>0</td>
<td>46,4</td>
<td>H</td>
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<tr>
<td>#46</td>
<td>6510</td>
<td>174</td>
<td>246</td>
<td>1825</td>
<td>64</td>
<td>26,5</td>
<td>D</td>
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<tr>
<td>#143</td>
<td>8910</td>
<td>151</td>
<td>176</td>
<td>18070</td>
<td>0</td>
<td>50,6</td>
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<td>0</td>
<td>285,0</td>
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<tr>
<td>#131</td>
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<td>0</td>
<td>4245</td>
<td>0</td>
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<td>D</td>
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<tr>
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<td>20</td>
<td>20</td>
<td>425</td>
<td>0</td>
<td>81,0</td>
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</tr>
</tbody>
</table>

Courtesy of Dr Silvia Gregori
Suppressive activity

Do **Tr1 cell clones have specific suppressive activity** in patients with PMC after HSCT for haemoglobinopathies?
Suppressive activity

Suppression?

Adding of Tr1 PMC recipient or donor clones

Recipient

Donor

Alloreactivity

+++

IFN-gamma production

read – out
Suppressive activity of host and donor alloreactive Tr1 cell clones

Suppressive activity

Yes, both host and donor Tr1 cell clones have specific suppressive activity
Identification and selection of Tr1 cells in PBMC

Alternatively identification of IL-10 producing T cells by intracellular cytokine staining (ICS) in PBMCs

\[
\text{p=ns} \quad \text{p<0.01} \quad \text{p<0.01}
\]

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{chart.png}
\end{figure}

*Serafini et al. Hematologica 2009*

Courtesy of Dr Silvia Gregori
Identification and selection of Tr1 cells in PBMC

T cell cloning

PBMC

Positive selection of CD4+ T cells

limiting dilution

0.3 cells/well

Tr1 cell clones

LIMITATION:

not applicable for routine screening of patients

Alternatively identification of IL-10 producing T cells by intracellular cytokine staining (ICS) in PBMCs

LIMITATION:

it requires in vitro re-stimulation;
not only Tr1 cells produce IL-10. Serafini et al. Hematologica 2009

% of CD4+ IL-10 producing T cells

N D PMC pts CC pts

p=ns

p<0.01

p<0.01

Courtesy of Dr Silvia Gregori
Definition of a new tolerogenic marker by gene expression profiling of human Tr1 cell clones:

Co-expression in Tr1 cells of:

CD49b (Integrin, alpha 2 subunit of VLA-2 receptor)
LAG-3 (lymphocyte activation gene 3)

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

Oral presentation (O 40) at EFI Stockholm meeting by Silvia Gregori
Identification of human Tr1 cells

FACS analysis

Staining with CD4, CD45RO, CD49b and LAG-3

Blood → Ficoll gradient → PBMC

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

 Courtesy of Dr Silvia Gregori
CD45RA⁻CD49b⁺LAG-3⁺ Tr1 cells are highly represented in PMC

Tolerant patients after BMT

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

Courtesy of Dr Silvia Gregori
Early transient MC represents a risk factor for graft rejection after HSCT in haemoglobinopathies

If MC is present after HSCT for a period longer than two years it becomes persistent

Large proportion of Tr1 cells are present in patients with PMC after HSCT for haemoglobinopathies

Tr1 cells originate both from donor and host

Tr1 cells suppress both host and donor-specific T-cell mediated responses

The co-expression of CD49b and LAG-3 enables the isolation of highly suppressive human Tr1 cells
Acknowledgments

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

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

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

Centro Trapianti Fondazione
IME - Roma
Guido Lucarelli
Javid Gaziev
Pietro Sodani