T Cell subset recovery after Hematopoietic Stem Cell Transplantation and chronic Graft versus Host Disease development

Maria Soares, PhD

João Lacerda Lab
Allogeneic Hematopoietic Stem Transplantation

Graft Donor types
- Related, unrelated, matched or haploidentical

Graft Sources
- Bone Marrow
- Mobilized PBSC
- Umbilical Cord Blood

Passweg, J et al Bone Marrow Transpl 2018 (53)
T cell reconstitution in Hematopoietic Stem Transplantation

- Donor Graft
- Recipient
- HLA-matched Donor

Donor mature T cells

Donor HSCs

Engraftment

Recipient Bone Marrow

T cell precursors

Recipient Thymus

de novo Naïve T cell production

Recipient Peripheral T cell Pool

Homeostatic T cell proliferation

Recipient Peripheral T cell Pool
Hematopoietic Stem Transplantation

- Graft source
- Conditioning regimen
- Age of patient
- GVHD prophylaxis
- GVHD
Graft versus Host Disease (GVHD)

**Acute**
- Skin, Liver and GI tract.
- Stages of severity for each organ.
- Grades I to IV.

**Chronic**
- Skin, Mouth, Eyes, GI, Liver, Lung, Muscle-Joint, Genital.
- Grades 0 to 3 per organ.
- Mild, Moderate, Severe.

Ferrara et al. Clinical Hematology, Elsevier 2006: Graft versus host disease, 1235-1251
Pathophysiology of cGVHD

MacDonald et al. Blood 2017;129:13-21
• Effect on survival depends on severity:
  - mild cGVHD associated with improved survival
  - severe cGVHD associated with increased mortality

• Associated with *loss of immunological tolerance*

Relies on the balance between effector & Treg cells

- **T cell recovery**
  - Effector T cell subsets
    - CD4\(^+\) T cells
    - CD8\(^+\) T cells
  - Regulatory T cells
    - CD4\(^+\) Treg cells
Regulatory T Cells (Treg)

- Constitute 1-3% of peripheral CD4+ cells and are mostly suppressive.
- Impaired Treg function associated with autoimmune diseases.
- CD3+CD4+ Foxp3+CD25^{high} CD127^{low}
Loss of Immune Tolerance → GVHD

CD4+ Tcon cells
CD8+ T cells

CD4+ Treg cells

Analyzing T cell reconstitution after HSCT and cGHVD development
February 2013

17 patients excluded (early disease relapse or death from either infection or aGVHD in the first 9 months post-transplant)

No cGVHD
n=22
(Median Follow-up 483 days)

December 2016
patients initiating follow-up
n=72

cGVHD
n=18
(Median Follow-up 682 days)
- Patients underwent allo-HSCT from unrelated donors after a RIC regimen with fludarabine 125mg/m², melphalan 140mg/m² and thymoglobulin (4–6mg/Kg depending on HLA compatibility).
- Chronic GVHD diagnosis and staging performed according to the 2014 NIH criteria.
- GVHD prophylaxis: cyclosporine and mycophenolate mofetil.
Study Protocol:

- Phenotypic analysis of patients undergoing unrelated HSCT after transplant for up to 2 years.
- Flow cytometry was performed on whole blood at fixed time points.
- PBMC were stored for future additional analysis.

Time points for sample collection:

- cGVHD onset: Median 230 days after HSCT.
**Phenotypic panel (Flow cytometry)**

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Treg and Tcon identification

TREG Gating

TCON Gating
• From months 9 to 18: decreased Treg in cGVHD vs No cGVHD patients.

Soares, M. et al. Frontiers in Immunology, March 2019
T cell reconstitution

- Homeostatic T cell proliferation
- *de novo* Thymic T cell production

Peripheral T cell Pool
### Prospective analysis of immune reconstitution after allo-HSCT

#### Phenotypic panel (Flow cytometry)

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Reduced Ki-67 in patients developing cGVHD

Reduced Treg numbers in cGVH may be partly due to reduced proliferation.
No significant differences in Bcl-2 or CD95 levels between patient groups

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Naïve /Memory T Cell Subset Identification

- **Central Memory (CM): RA-CD62L+**
- **Effector Memory (EM): RA-CD62L-**
- **Effector Memory RA (EMRA): RA+CD62L-**
- **Stem Cell Memory (SCM) RA+CD62L+CD95+ vs Naïve: RA+CD62L+CD95-**

- **SCM:** Play a role in the maintenance of long term immunological memory. Increased SCM have been associated to autoimmune conditions.

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**Apoptosis**

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Distinct subset reconstitution in CD4 and CD8 in cGVHD
17 patients excluded (early disease relapse or death from either infection or aGVHD in the first 9 months post-transplant)

- cGVHD, n=18
  - Median Follow-up 682 days
- No cGVHD, n=22
  - Median Follow-up 483 days

Patient population

- Acute GVHD only, n=9
- No GVHD, n=13
- No acute GVHD, n=1

February 2013

December 2016

patients initiating follow-up, n=72
The effects of acute GVHD on SCM Tcon and CD8

- Adding aGVHD as a covariate in the Linear Mixed Effects analysis:
  - during the first 6 months after HSCT, SCM Tcon and CD8 percentages and counts are significantly affected by aGVHD (p<0.01).

- GVHD development is associated to early increase in SCM
- sustained increase in SCM CD8 subset in cGVHD
T cell reconstitution/Maintenance

(1) T-cell Receptor Excision Circles (TREC) Content analysis
- Reduction in TREC content
- Loss of CD31 Expression

(2) Quantification of CD31+ Naïve CD4+ T cells

Homeostatic T cell proliferation

denovo Thymic T cell production
Reduced TREC content in cGVHD

Dário Ligeiro
Centro de Histocompatibilidade do Sul
cGVHD negatively impacts thymic function

Reduced RTE (CD31+Naïve) Treg and Tcon in

Significantly different slopes for RTE Tcon absolute counts:
- RTE Tcon increase in the absence of cGVHD but not in cGVHD.

RTE Tcon Linear Mixed Effects analysis of the first 6 months

The effect of acute GVHD on RTE Tcon

- HC
- No GVHD
- Ac GVHD
- Ac & Ch GVHD
Does reduced RTE in cGVHD impact on TCR Diversity?
cGVHD and TCR Diversity

CD4

HC

No cGVHD

cGVHD

% within VB Family

Early

Late

VB Family Number

CD8

HC

No cGVHD

cGVHD

% within VB Family

Early

Late

VB Family Number

Dário Ligeiro, Centro de Histocompatibilidade do Sul, Lisboa
cGVHD negatively impacts TCR Diversity in CD4+ T cells
cGVHD development is associated with reduced Treg recovery after allo-HSCT, particularly of Naive and SCM subsets.

- Significantly reduced RTE counts in Tcon and Treg
- Significantly reduced TREC content and TCR VB diversity in Tcon

Impaired Treg recovery likely due to a combination of reduced proliferation and thymic production within the CD4 T cell compartment.

Initial increases in RTE Tcon in Acute GVHD and in Acute and Chronic GVHD patients.

Potential deleterious role for RTE Tcon early after HSCT, while RTE Tcon present at later time-points in the absence of cGVHD likely result from adequate thymic selection processes.

Summary

Soares, M. et al. Frontiers in Immunology, March 2019
Summary

- cGVHD development is associated to increases in Naive, SCM and EMRA CD8 T cells. -apparent early after HSCT and persists throughout follow-up,

Potential involvement of these cells in cGVHD.

- significantly reduced CD8 sjTREC content in cGVHD high prevalence of skewed and oligoclonal TCR repertoires.

- Naïve CD8 in GVHD may originate from defective negative selection mechanisms resulting from thymic tissue damage leading to the output of self-reactive CD8 clones that may further differentiate and mediate disease.
So... what next?...
Repair of tissue and organ damage in refractory chronic GVHD after hematopoietic stem cell transplantation by the infusion of purified allogeneic donor regulatory T lymphocytes

1. João F. Lacerda, Hospital Santa Maria / Faculdade de Medicina da Universidade de Lisboa / Instituto de Medicina Molecular (IMM), Lisboa, Portugal - Coordinator
2. Matthias Edinger, Universitätsklinik, Regensburg
3. Frédéric Baron, Laboratory of Cell and Gene Therapy, CHU Sart-Tilman, Liège, Belgium
4. Mario Arpinati, Department of Hematology, University Hospital S.Orsola-Malpighi, Bologna, Italy
5. Marie-Laure Yaspo, Max Planck Society, Max Planck Institute for Molecular Genetics, Berlin, Germany
6. Hans Lehrach, Alacris Theranostics GmbH, Berlin, Germany
7. Marta García-Fiñana, Department of Biostatistics, Faculty of Health & Life Sciences, University of Liverpool, Liverpool, UK
8. Jose Luis Perez-Simon, Instituto de Biomedicina Sevilla, IBIS, Seville, Spain

TREGeneration has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 643776.
Clinical Trial Designs

Series of **5 parallel independent Phase I/II clinical trials** exploring the effect of donor Treg in:
- acute GVHD (1 trial)
- chronic GVHD (4 + 1 trials)

**Primary endpoint:**

- **Safety** – safety and maximum tolerated dose (MTD) of donor Treg infusion in steroid-refractory cGvHD patients within a period of 12 weeks post-infusion

**Secondary endpoints:**

- **Feasibility of Treg infusion** – feasibility of achieving a successful Treg-enriched product
- **Clinical response** – assess clinical response up to 1 year after Treg infusion
**Treg Purification – CliniMACS**

**Standard CliniMACS** CD8/CD19 co-depletion followed by CD25 positive selection (*Lis, Bos, Lie, Bol*)

**CliniMACS** followed by cell sorting naïve Treg and expansion (Reg)

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**CD8 / CD19 depletion CD25 isolation**

(CE and GMP reagents)
# Phase I Treg Doses

## Lisbon
- A - 0.5 x 10^6 cells/kg (n=5) - COMPLETED
- B - 1 x 10^6 cells/kg (n=5) - COMPLETED
- C - 2-3 x 10^6 cells/kg (n=5) - COMPLETED

## Bologna
- A - 0.16 x 10^6 cells/kg/month x 3 months (n=5)
- B - 0.33 x 10^6 cells/kg/month x 3 months (n=5)
- C - 0.66 x 10^6 cells/kg/month x 3 months (n=5)

## Boston
- A - 0.1 x 10^6 cells/kg + IL-2 1 x 10^6 U/m^2/day SC x 8 weeks (n=5)
- B - 0.5 x 10^6 cells/kg + IL-2 1 x 10^6 U/m^2/day SC x 8 weeks (n=5)
- C - 1 x 10^6 cells/kg + IL-2 1 x 10^6 U/m^2/day SC x 8 weeks (n=5)

## Liège
- Up to 2-3 x 10^6 cells/kg + IL-2 1 x 10^6 U/m^2/day SC x 8 weeks (n=10)

## Regensburg
- A - 1 x 10^6 cells/kg (n=3)
- B - 5 x 10^6 cells/kg (n=3)
- C - 10 x 10^6 cells/kg (n=3)
Immune monitoring by flow cytometry

- Comprehensive panel comprising **T**, **B**, **NK** and **DC** subsets, as well as markers of thymic generation, proliferation and susceptibility to apoptosis

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In vivo tracking of infused cells by NGS

- Compare the clonotypes present in the patient (pre- and post-Treg infusion) and in the infused Treg product within:
  - Treg
  - Conventional CD4 T cells (Tcon)
  - CD8 T cells
- Are infused Treg clonotypes persisting after infusion?
- Are Treg converting into an effector phenotype post-infusion?
Repair of tissue and organ damage in refractory chronic GVHD after hematopoietic stem cell transplantation by the infusion of purified allogeneic donor Treg...to be continued...
All the patients involved