« Immunothérapie adoptive anti-virale dans le contexte de greffe de cellules souches hématopoïétiques »
« Antiviral T–cell therapy in the setting of hematopoietic stem cell transplantation »

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Infections:
main causes of death after HSCT

- SCID treated patients:
  Infections: 56%
  GvHD: 25%
  B cell lymphoproliferative disorders: 5%

- Non SCID treated patients:
  Infections: 70%
  GvHD: 9%
  Conditioning regimen toxicity: 9%
  B cell lymphoproliferative disorders: 5%
  Rejection: 3%

(Antoine C et al., The Lancet, 2003)
Viral infections:
ADV important cause of morbidity and mortality after HSCT

- Incidence of adenovirus (ADV)
  
  In adults: 3-13% (Chakrabarti S et al., Blood, 2002; Walls T et al., Lancet Infect Dis, 2003 and Baldwin A et al., Bone Marrow Transplantation, 2000)

  In pediatric: 20-30% (Flomenberg P et al., Journal of Infect Dis, 1994; Hale GA et al., Bone Marrow Transplantation, 1999; Howard DS et al., Clin Infect Dis, 1999 and Lion T et al., Blood, 2003)

- Overall mortality of ADV infection: 18-26% (Bruno B et al., Blood, 1997; Howard DS et al., Clin Infect Dis, 1999; La Rosa AM et al., Clin Infect Dis, 2001)

- Disseminated ADV infection mortality: 60% (Lion T et al., Blood, 2003)

- Limited action of antiviral drugs (Matthes-Martin S et al., Transplant Infectious Disease, 2012)
Viral infections:
CMV important cause of morbidity and mortality after HSCT

- Reactivation of cytomegalovirus (CMV) 60 to 70% of sero-positive patients after SCT (Miller W et al., Blood, 1986; Hiwarkar P et al., Bone Marrow transplantation, 2012)

- Limited action and toxicity of antiviral drugs: treatment failure in 45 to 55% of patients. A real resistance of virus has been demonstrated in only < to 5% of cases (Van Der Beek MT et al., Antivir Ther, 2012)
GvHD:
main cause of complications and death after HSCT

- GvHD is the main obstacle to allogeneic HSCT with an incidence and a severity significantly associated with mismatched and unrelated donors (Flowers ME et al., Blood, 2011)

- The global incidence of GvHD varies from 30 to 60% after allogeneic HSCT with 50% of mortality (Barton-Burke M et al., Oncology, 2008)
Adoptive immune therapy

Two main indications:

- Non-manipulated Donor Lymphocyte Infusion (DLI)

- Manipulated DLI: anti-viral T cell lymphocytes used in refractory viral infections, after allogeneic HSCT, before immune reconstitution
Adoptive immune therapy
Non-manipulated DLI

Indications:

- To promote anti-tumoral effect (GvL effect)

- To improve engraftment (chimerism shift)
Adoptive immune therapy
Non-manipulated DLI

- DLI prevent relapses of hematologic malignancies after allogeneic HSCT

  • PBMC collected by apheresis on a separator of blood cells (Cobe)

  • In the first studies the number of injected cells was very variable (0.1 - 15 x 10^8 cells/kg)
Adoptive immune therapy
Non-manipulated DLI and GvL

Adoptive immune therapy
Non-manipulated DLI and engraftment

Nonmyeloablative Stem Cell Transplants (NST)
(“reduced intensity conditioning transplants” or “mini-transplants”)
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Adoptive immune therapy
Manipulated DLI-Anti-viral T cells
HSCT

Donor cells
- HSC
- T-cell precursors
- Memory T cells

Reconstitution of T-cell compartment
- New naive T-cells
  - Complete repertory
  - Effective response to infections
- Memory T-cells
  - Disturbed T-cell repertory
  - Incomplete response to infections

Peripheral expansion
Thymic maturation
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Restoration of Viral Immunity in Immunodeficient Humans by the Adoptive Transfer of T Cell Clones

Stanley R. Riddell,* Kathe S. Watanabe, James M. Goodrich, Cheng R. Li, Mounzer E. Agha, Philip D. Greenberg

Methodology
- 3 couples D+/R+ (genoidentical BM SCT)
- CD3+/CD8+/CD4- clones amplified in vitro during 5 to 12 weeks:
  *PBMC of the Donor + CMV-infected autologous fibroblasts
  *CD8+ T cells were cloned from the cultures by the limiting-dilution method after 7 to 14 days
  *Clones CD8+ cytotoxic T lymphocytes specific for CMV (depleted of CD4+ T cells) + irradiated autologous EBV-tranformed B lymphoblasts as feeder cells + IL-2 + anti-CD3 monoclonal antibody or autologous CMV-infected fibroblasts to stimulate the T cells
  *Amplification by cyclic stimulation at 10 to 12 days intervals with the stimulation
- Weekly intravenous administration from D30 after SCT and during 4 weeks (5.10^7 à 1.10^9 CTL/m²)
- Maintained immunosupression (Cyclosporine/Prednisone)

Conclusion
- Reconstitution of CD8+ CMV-specific CTL responses/No CMV infection
- Long technique with high risk of contamination
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Methodology
- 14 couples D+/R+ : genoidentical BM SCT
- CD3+/CD8+/CD4- clones amplified in vitro during 5 to 12 weeks on CMV-infected autologous fibroblasts + irradiated autologous EBV-tranformed B lymphoblasts as feeder cells + IL-2 + cyclic stimulations
- Weekly intravenous administration between D30 and D40 after SCT and during 4 weeks (5.107 à 1.109 CTL/m2)
- Maintained immunosupression

Conclusion
- No toxicity
- Reconstitution of CD8+ CMV-specific CTL responses in the 14 R
- No CMV infection (no positive CMV viremia or disease)
- Long technique with high risk of contamination
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Methodology
- 15 Donors CMV+
- CD3+/CD8+ and CD3+/CD4+ clones amplified *in vitro* during 14 to 21 days on donor autologous CMV-infected monocyte-derived dendritic cells (IL-4 and GMCSF during 7 days) + IL-2

Conclusion
- Interesting technique allowing to have anti-viral T CD4+ and T CD8+ cells (T CD4+ and T CD8+ cooperation and longer anti-viral effect?)
- Applicable to all CMV+ donors
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Isolation and expansion of human adenovirus–specific CD4⁺ and CD8⁺ T cells according to IFN-γ secretion for adjuvant immunotherapy

Tobias Feuchtintera, Peter Langa, Klaus Hamprechtc, Michael Schumm, Johann Greila, Gerhard Jahn, Dietrich Niethammer, and Hermann Einsele

aUniversity Children’s Hospital, bMedizinische Klinik II, and cDepartment of Virology, Eberhard-Karls University, Tübingen, Germany
(Received 30 April 2003; revised 16 December 2003; accepted 23 December 2003)

Methodology
- New fast technique (24h)
- Generation of CD3+/CD8+ and CD3+/CD4+ anti-ADV lymphocytes using cell enrichment by the IFN-γ secretion assay and selection with magnetic beads including short periods of in vitro expansion

Conclusion
New fast and simple technique allowing the generation of anti-ADV T cells
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Adoptive transfer of cytomegalovirus-specific CTL to stem cell transplant patients after selection by HLA-peptide tetramers

Mark Cobbold,1 Naeem Khan,1 Batoul Pourgeysari,1 Sudhir Tauro,2 Dorothy McDonald,4 Husam Osman,3 Mario Assenmacher,3
Lucinda Billingham,1 Colin Steward,6 Charles Crawley,7 Eduardo Olavarria,1 John Goldman,7 Ronjon Chakraverty,1
Premini Mahendra,2 Charles Craddock,1,2 and Paul A.H. Moss1,2

(Cobbold M et al., J Exp Med, 2005)

Methodology
- 9 couples D+/R+ : 6 genoidentical SCT and 3 MUD
- Fast in vitro selection of CMV specific CD3+/CD8+ lymphocytes by HLA-peptide tetramers followed by selection with magnetic beads and direct injection without preliminary cells’ manipulation to R (<4h)

Conclusion
- Effective technique with 8/9 patients achieving undetectable viral load
- No toxicity
- Only T CD8+ cells were injected
- Tetramer-based isolation depends on the donor’s HLA haplotype
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Safe adoptive transfer of virus-specific T-cell immunity for the
treatment of systemic adenovirus infection after allogeneic
stem cell transplantation
(Feuchtinger T et al., BJH, 2006)

Methodology
- 9 couples D+/R+ : 3 MUD, 4 MMUD and 2 Haplo SCT
- Generation of CD3+/CD8+ and CD3+/CD4+ anti-ADV lymphocytes using
cell enrichment by the IFN-γ secretion assay with magnetic beads (24h)
- 1.2-50x10³/kg T cells were infused for adoptive transfer

Conclusion
- No toxicity except in 1/9 patients (GvHD II)
- Adoptive transfer of ADV-specific immunity was successful in 5 of 6
evaluable patients
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Adoptive transfer of pp65-specific T cells for the treatment of chemorefractory cytomegalovirus disease or reactivation after haploidentical and matched unrelated stem cell transplantation

(Feuchtinger T et al., Transplantation, 2010)

Methodology
- 18 couples D+/R+ : 3 MUD, 3 MMUD and 12 Haplo SCT
- Generation of CD3+/CD8+ and CD3+/CD4+ anti-CMV lymphocytes using cell enrichment by the IFN-γ secretion assay with magnetic beads (24h)
- 21x10³/kg T cells were infused for adoptive transfer

Conclusion
- No toxicity
- Adoptive transfer of CMV-specific immunity was successful in 83% of evaluable patients
Adoptive immune therapy
Manipulated DLI-Anti-viral T cells

Specific T cells for the treatment of CMV and/or ADV in the context of HSCT

Results of our clinical protocol

Principal coordinator of the study : Pr M Cavazzana
Development, follow-up and interpretation : Liliane Dal Cortivo and Rita Creidy
Hôpital Necker (Paris)
Project

- Therapeutic, non comparative
- Prospective, open multicenter phase I/II clinical trial
- 30 subjects: 25 CMV-infected patients and 5 ADV-infected patients
- Follow-up period: 6 months after the last infusion
Objectives

- **Primary objective**: CMV viral load at D21 after injection

- **Secondary objectives**:
  - Immediate safety
  - Immunological reconstitution (*in vivo* expansion)
  - Safety and efficacy for the ADV infection (viral load at D21 after injection)
Inclusion criteria

- Adult and pediatric patients from 7 national hospitals
- CMV and/or ADV infection resistant or intolerant to conventional antiviral treatment (blood PCR +)
- Or CMV or ADV organ disease without systemic replication
- Free of GvHD at inclusion or with pre-existing GvHD controlled by steroids < 1mg/kg/day
- Donor eligibility criteria
Procedure (1) :

Clinimacs cytokine secretion (CCS) assay IFN-γ

IFN-γ secretion
CliniMACS IFN-γ enrichment reagent (beads)
Immunomagnetic selection of T lymphocytes IFN-γ+

antigen-specific restimulation
labeling with Catch Reagent
secretion period
fluorescent and magnetic labeling

Activated T lymphocytes
Cytokine secreting cell
Activated T lymphocytes
Cytokine secreting cell
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Cytokine secreting cell
Donor leukapheresis

1 x 10^9 cells

PepTivator CMV pp65 or AdV5 Hexon stimulation at 37°C

CliniMACS IFN-γ

Catchmatrix reagent + IFN-γ enrichment reagent

Column selection

Specific (CMV or ADV) T cells

Infusion and 6 months follow-up

In vitro expansion and MLR

Procedure (2) :
CCS assay IFN-γ and infusion

Before column

After column
Selection of memory anti-CMV lymphocytes

<table>
<thead>
<tr>
<th>Donor</th>
<th>After stimulation</th>
<th>After immuno selection</th>
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<tbody>
<tr>
<td>CD4</td>
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<tr>
<td>72%</td>
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<td>CD8</td>
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<td>86%</td>
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IFN-γ
*In vivo* expansion of CMV specific T CD4+ and CD8+ cells at D14 and D28 after the infusion (D0)
Results (1)

- Clinical trial: Sept 2010-Oct 2013
- 16 inclusions, 15 treated patients
- 8 children (6m-24m) and 7 adults (24y-63y)
- Very heterogeneous pathologies: immune deficiencies and various hematologic malignancies
- Donor/recipient HLA:
  - Genoidentical 4
  - Haploidentical 5 (TCD by CD34+ immunoselection)
  - MUD 4
  - MMUD 2 (TCD by CD34+ immunoselection)
- 1/15: partial donor chimerism
- 4/10 presented moderate GvHD: 3 grade II and 2 GvHD I
Results (2)

- 10 patients were treated for CMV infection, 8 for ADV infection (3 patients had both infections)

- The average of injected CMV-specific T cells was 3540/Kg

- The average of injected ADV-specific T cells was 3739/Kg

- The isolated cells displayed low alloreactivity *in vitro*

- Immediate infusion was well tolerated

- 2 patients died within weeks of infusion, due to the underlying viral disease

- By day 21, adoptive transfer resulted in :

  * *in vivo* expansion 6 out of 8 evaluable patients treated with CMV-specific T cells and a complete virologic and clinical response in 2 of these

  * *in vivo* expansion 2 out of 3 evaluable patients treated with ADV-specific T cells and a complete virologic and clinical response in all 3
A progressive decrease in blood viral load was concomitant with CD4 and CD8 IFN-γ+ T cell reconstitution after 2 injections of anti-CMV T cells.
A progressive decrease in the viral load in the CSF just prior to CD4 and CD8 IFN-γ+ T cell reconstitution after 2 injections of anti-CMV T cells.
Conclusions

- Three key advantages of the present T-cell-based approach are: simplicity, rapidity and safety (GMP) of production.

- This technique does not depend on the donor’s HLA haplotype (in contrast to tetramer or multimer-based isolation).

- Infused CTL are CD4+ and CD8+ T cells.

- The number of injected CTL can be discussed and increased in genoidentical CST without a real risk of GvHD.

- The timing of the treatment is important: preemptive treatment just after HSCT might be more potent.
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Study monitoring: URC Paris Centre, E. Henri, V. Jolaine
Sponsor: AP-HP
Funding: National PHRC 2010