The Need for Individualized Procedures in ECP, the European Perspective!

Volker Witt, MD
St. Anna Kinderspital, Vienna, Austria
ECP = extrcorporeal photopheresis

1. Collecting Leukocytes
2. + 8 MOP
3. + UVA 1.5 J/cm²
4. Reinfusion to the patient
What is ECP?

STEPS OF THE PROCESS
• Harvesting Leukocytes
• Preparing a buffy coat
• Adding a Photosensitizer
• Transferring in a irradiation disposable
• Irradiating a “buffy coat”
• Retransfusion to the patient
What is ECP?

STEPS OF THE PROCESS

• **Harvesting Leukocytes**
• Preparing a buffy coat
• Adding a Photosensitizer
• Transferring in a irradiation disposable
• Irradiating a “buffy coat”
• Retransfusion to the patient

• **Apheresis**
  • Inline systems
  • Offline systems
• To draw peripheral blood
What is ECP?

STEPS OF THE PROCESS

- Harvesting Leukocytes
- **Preparing a buffy coat**
- Adding a Photosensitizer
- Transferring in a irradiation disposable
- Irradiating a “buffy coat”
- Retransfusion to the patient

- Directly from the Apheresis device
- Diluted by plasma
- Diluted by saline solution
- Hematocrit
What is ECP?

**STEPS OF THE PROCESS**

• Harvesting Leukocytes
• Preparing a buffy coat
• **Adding a Photosensitizer**
• Transferring in a irradiation disposable
• Irradiating a “buffy coat”
• Retransfusion to the patient

• 8-MOP
• Final concentration (?)
What is ECP?

STEPS OF THE PROCESS

• Harvesting Leukocytes
• Preparing a buffy coat
• Adding a Photosensitizer
• **Transferring in a irradiation disposable**
• Irradiating a “buffy coat”
• Retransfusion to the patient

• Chamber
• Bag
What is ECP?

STEPS OF THE PROCESS
• Harvesting Leukocytes
• Preparing a buffy coat
• Adding a Photosensitizer
• Transferring in a irradiation disposable
• **Irradiating a “buffy coat”**
• Retransfusion to the patient

• In a pivoting a bag
• By flowing through a chamber
• Constantly
• Inconstantly
• Time
• Hematocrit
• Measuring the dose
What is ECP?

**STEPS OF THE PROCESS**
- Harvesting Leukocytes
- Preparing a buffy coat
- Adding a Photosensitizer
- Transferring in a irradiation disposable
- Irradiating a “buffy coat”
- Retransfusion to the patient

- Immediately
- After incubating
As often happened in the past, ECP was invoked as a last resort in front of patients with highly unsatisfactory therapeutic proposals. In fact, that was the case of acute and chronic GvHD when ECP was even borrowed from the treatment of cutaneous T cell lymphoma.
A possible explanation of the positive impact on BOS after ECP intensification may be the higher number of cells treated over time and consequently the more intense and long lasting immunomodulation.
Moreover, differently from others, we are convinced that in this setting of patients response to ECP must be considered also stabilization or even slowing of lung function decline over time, considering that nearly all patients meet a rapid and dramatic evolution.

Extracorporeal photopheresis for bronchiolitis obliterans syndrome after allogeneic stem cell transplant: An emerging therapeutic approach?
Claudia Del Fante*, Cesare Perotti
Transfusion and Apheresis Science 56 (2017) 17–19
Extracorporeal Photochemotherapy published guidelines:

2007  French guidelines (*Kanold J.*, *Transfusion*)
acute and chronic GvHD, children

2012  Italian guidelines (*Pierielli L.*, *Transfusion*)
acute and chronic GvHD, adults and children

2014  European Academy of Dermatology and Venerology (*Knobler R.*, *JEADV*)
Guidelines on the use of ECP (aGVHD, cGVHD included)

2014  COCHRAN Review (*Weitz M.*)
acute and chronic GvHD, children

2015  British Guidelines on the clinical use of apheresis procedures ... (*Howell C, Transfusion*)

acute and chronic GvHD, adults and children
Guideline on the clinical use of apheresis procedures for the treatment of patients and collection of cellular therapy products

C. Howell,\textsuperscript{1} K. Douglas,\textsuperscript{2,3} G. Cho,\textsuperscript{4} K. El-Ghariani,\textsuperscript{5} P. Taylor,\textsuperscript{6} D. Potok,\textsuperscript{7} T. Rintala\textsuperscript{8} & S. Watkins\textsuperscript{9}

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Recommendations

1. ECP is recommended as first line therapy for erythrodermic CTCL (1A).
2. ECP may be considered as second line treatment in skin, mucosal and liver cGvHD (1B).
3. ECP schedule should be paired treatments on consecutive days, repeated fortnightly for a minimum assessment period of 3 months (1C).
4. Patients managed by ECP for cGvHD should have their disease monitored by staff trained in the application of the National Institutes of Health (NIH) assessment standards (Pavletic \textit{et al.}, 2006) (1C).
5. ECP is suggested as one of a number of second line agents for steroid-refractory acute GvHD (2C).
6. ECP may be considered for prophylaxis or treatment of graft rejection in heart or lung transplantation (2C).
7. Application of ECP in alternative settings should be considered in the context of appropriate clinical trials (1C).
The current methods for delivery of ECP use ‘closed’ or ‘open’ systems. Open ECP necessitates collection of a buffy coat on a cell separator, separation of the buffy coat from the system and subsequent UVA irradiation in a separate device, before reinfusion to the patient. By contrast the closed system incorporates an internal UVA source in the apheresis device with no separation of the buffy coat from the device, before return to the patient.

While the open system has the potential advantages of increased cell dose and the ability to manipulate the cells further, there is an increased risk of microbial contamination with this technology.

The closed system devices have integrated mononuclear cell collection and 8-methoxypsoralen/UVA irradiation, and regulatory approval for treating patients (Wong, 2012).
Guidelines on the Use of Therapeutic Apheresis in Clinical Practice—Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Seventh Special Issue

Joseph Schwartz,¹ Anand Padmanabhan,² Nicole Aqui,³ Rasheed A. Balogun,⁴ Laura Connelly-Smith,⁵ Meghan Delaney,⁶ Nancy M. Dunbar,⁷ Volker Witt,⁸ Yanyun Wu,⁹ and Beth H. Shaz¹,¹⁰,¹¹*
<table>
<thead>
<tr>
<th>Condition</th>
<th>Procedure</th>
<th>Rejection</th>
<th>Prophylaxis</th>
<th>Desensitization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic (neuro-) dermatitis (atopic eczema), recalcitrant</td>
<td>ECP IA TPE</td>
<td>III</td>
<td>III</td>
<td>2C</td>
</tr>
<tr>
<td>Cardiac transplantation</td>
<td>ECP ECP TPE TPE</td>
<td>Cellular/recurrent rejection</td>
<td>Rejection prophylaxis Desensitization</td>
<td>Antibody mediated rejection</td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma; mycosis fungoides; Sezary syndrome</td>
<td>ECP ECP</td>
<td>Erythrodermic</td>
<td>Non-erythrodermic</td>
<td>I</td>
</tr>
<tr>
<td>Dermatomyositis/polymyositis</td>
<td>TPE ECP</td>
<td></td>
<td></td>
<td>IV</td>
</tr>
<tr>
<td>Graft-versus-host disease</td>
<td>ECP ECP ECP ECP</td>
<td>Skin (chronic)</td>
<td>Non-skin (chronic)</td>
<td>Skin (acute)</td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Adsorptive cytapheresis Adsorptive cytapheresis ECP</td>
<td>Ulcerative colitis</td>
<td>Crohn’s Disease</td>
<td>Crohn’s Disease</td>
</tr>
<tr>
<td>Condition</td>
<td>ECP</td>
<td>TPE</td>
<td>TPE</td>
<td>Bronchiolitis obliterans syndrome</td>
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<td>-----------------------------------------------------</td>
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<tr>
<td>Lung transplantation</td>
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<tr>
<td>Nephrogenic systemic fibrosis</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Cutaneous T-cell lymphoma; mycosis fungoides; Sezary syndrome</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Pemphigus vulgaris</td>
<td>TPE</td>
<td>ECP</td>
<td>IA</td>
<td></td>
</tr>
<tr>
<td>Psoriasis</td>
<td>ECP</td>
<td>Ads.cytaph. Lymphocyt</td>
<td>TPE</td>
<td>Disseminated pustular</td>
</tr>
<tr>
<td>Scleroderma (systemic sclerosis)</td>
<td>TPE</td>
<td>ECP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Why we use ECP in GVHD?
main complications of immunosuppressive therapies

- infections
- endocrine dysfunction
- avascular necrosis
- multiorgan failure
- secondary malignancies
- quality of life?
Corticoid and IS therapy sparing effect needed(!?)
Immunological benefits

• Maeda A et al. (2005) Intravenous infusion of syngeneic apoptotic cells by photopheresis induces antigen-specific regulatory T cells.
  • *J Immunol* 174: 5968–5976
• Patients receiving ECP respond normally to new immune challenges and do not lose their immunity against infections, gained either from previous exposure to the pathogen or through vaccination.
  • Suchin KR et al. (1999)
• Extracorporeal photochemotherapy does not suppress T- or B-cell responses to novel or recall antigens.
  • *J Am Acad Dermatol* 41: 980–986
ECP in children

• Advantages:
  • Selective downregulation of GVHD without increasing infection or malignancy relapse
  • Possibility to reduce general immunosuppression
  • Minimal acute side effects
  • No reported long-term side effects

• Problems:
  • Time consuming
  • Venous access
  • Circulating extracorporeal blood volume
  • Priming with blood
  • Unvalidated procedures
  • Few randomized trials
  • Experienced staff necessary
Why ECP in paediatric practice merits attention?

A substantial number of children undergo HSCT for non-malignant disorders and will not benefit from the GvHD.

The toxicities of systemic treatment in a growing child should be considered.

Therefore, addition of an effective pharmacologic-immunosuppression-sparing agent is of crucial importance for long-term outcome of these patients.

Excellent safety profile of ECP
Studies of Photopheresis for Treatment of Acute Graft-vs-Host Disease in Children and Adult

TABLE 1. aGVHD: ECP case series*

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Number</th>
<th>Age (year)</th>
<th>Skin N</th>
<th>Skin R</th>
<th>Liver N</th>
<th>Liver R</th>
<th>Gastrointestinal N</th>
<th>Overall response</th>
<th>Survival (%)</th>
<th>Steroid Reduction or stop</th>
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</thead>
<tbody>
<tr>
<td>Greinix, 2003</td>
<td>21</td>
<td>27-55</td>
<td>21</td>
<td>17</td>
<td>12</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>8</td>
<td>57</td>
</tr>
<tr>
<td>Messina, 2005</td>
<td>33</td>
<td>0-20</td>
<td>33</td>
<td>27</td>
<td>15</td>
<td>9</td>
<td>20</td>
<td>25</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>Kanold, 2005</td>
<td>41</td>
<td>&lt;18</td>
<td>40</td>
<td>40</td>
<td>22</td>
<td>&gt;13</td>
<td>25</td>
<td>25</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Garban, 2005</td>
<td>12</td>
<td>23-63</td>
<td>12</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td>Greinix, 2006</td>
<td>59</td>
<td>21-60</td>
<td>&gt;47</td>
<td>23</td>
<td>&gt;14</td>
<td>15</td>
<td>4</td>
<td>9 (CR+PR)</td>
<td>47</td>
<td>47</td>
</tr>
<tr>
<td>Berger, 2007</td>
<td>15</td>
<td>6-18</td>
<td>14</td>
<td>11</td>
<td>7</td>
<td>5</td>
<td>10</td>
<td>6</td>
<td>66</td>
<td>Stopped in all responders</td>
</tr>
<tr>
<td>Kanold, 2007</td>
<td>12</td>
<td>4-18</td>
<td>10</td>
<td>10</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>66</td>
<td>Stopped after 17-294 days</td>
</tr>
<tr>
<td>Perrotti, 2008</td>
<td>23</td>
<td>18-66</td>
<td>&gt;15</td>
<td>4</td>
<td>&gt;4</td>
<td>10</td>
<td>&gt;4</td>
<td>5</td>
<td>48</td>
<td>5 tapered</td>
</tr>
<tr>
<td>Calore, 2008</td>
<td>15</td>
<td>1-18</td>
<td>13</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>14</td>
<td>14</td>
<td>48</td>
<td>5 tapered</td>
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<tr>
<td>Merlin, 2010</td>
<td>12</td>
<td>2-18</td>
<td>11</td>
<td>4</td>
<td>4</td>
<td>9</td>
<td>6</td>
<td>5</td>
<td>83</td>
<td>6 stopped; 3 tapered</td>
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<tr>
<td>Perrotti, 2010</td>
<td>50</td>
<td>&lt;18</td>
<td>47</td>
<td>39</td>
<td>24</td>
<td>24</td>
<td>11</td>
<td>8</td>
<td>44</td>
<td>8 stopped; 25 tapered</td>
</tr>
</tbody>
</table>

* Empty cells: no data reported in the paper.
† Including patients with skin and other organ GVHD.
CR = complete response; N = number of assessed patients; NR = no response; PR = partial response; R = number of responders.
Fig 1. Acute GVHD: survival according to the response to ECP. The 5-year overall survival of patients responding to ECP was significantly better than that of non-responders, being 69.5% (95% CI 50.2–80.9) and 12.5% (95% CI 0–34.5) respectively (P = 0.001).
Update on extracorporeal photochemotherapy for graft-versus-host disease treatment

J Kanold\(^1\), C Messina\(^2\), P Halle\(^1\), F Locatelli\(^3\), E Lanino\(^4\), S Cesaro\(^2\) and F Deméocq\(^1\), on behalf of the Paediatric Diseases Working Party of the European Group for Blood and Marrow Transplantation (EBMT)

\(^1\)Unité Biolclinique de Thérapie Cellulaire, C.H.U. Clermont-Ferrand, France; \(^2\)Pediatric hematology and oncologic Unity, University of Padua, Italy; and \(^3\)IRCCS Policlinico S Matteo, Pavia, Italy; \(^4\)IRCCSG, Gaslini, Genova, Italy

<table>
<thead>
<tr>
<th>Author</th>
<th>Patients</th>
<th>Response</th>
<th>No response</th>
<th>Skin</th>
<th>Liver</th>
<th>Gut</th>
<th>Lung</th>
<th>Joint</th>
<th>Muco</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Resolved</td>
<td>Improved</td>
<td>Stable</td>
<td>Worsen</td>
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<td></td>
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</tr>
<tr>
<td>Acute GvHD</td>
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<tr>
<td>Padova, Pavia, Genova, Monza (Italy) 1992–2003</td>
<td>33</td>
<td>18</td>
<td>7</td>
<td>27/33</td>
<td>9/15</td>
<td>15/20</td>
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<tr>
<td>Jena (Germany) 1997</td>
<td>1</td>
<td>1</td>
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<td>—</td>
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<tr>
<td>Nantes (France) 1997</td>
<td>2</td>
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<tr>
<td>Clermont Ferrand (France) 1996–2003</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>4/4</td>
<td>4/5</td>
<td>1/3</td>
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<tr>
<td>Overall acute GvHD</td>
<td>41</td>
<td>73%</td>
<td>27%</td>
<td>80%</td>
<td>59%</td>
<td>64%</td>
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<tr>
<td>Chronic GvHD</td>
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<tr>
<td>Padova, Pavia, Genova, Monza (Italy) 1992–2003</td>
<td>44</td>
<td>15</td>
<td>10</td>
<td>19</td>
<td>20/36 ESS</td>
<td>12/20</td>
<td>10/21</td>
<td>6/14</td>
<td>8/14</td>
</tr>
<tr>
<td>Augsburg (Germany) 1996</td>
<td>1</td>
<td>1</td>
<td>—</td>
<td>—</td>
<td>1/1</td>
<td>1/1</td>
<td></td>
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<tr>
<td>Nancy (France) 1996</td>
<td>1</td>
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<td>Nantes (France) 1997</td>
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<td>7</td>
<td>8/12</td>
<td>11/12</td>
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<td>2/2</td>
<td>6/7</td>
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<tr>
<td>Overall chronic GvHD</td>
<td>63</td>
<td>63%</td>
<td>37%</td>
<td>60%</td>
<td>73%</td>
<td>59%</td>
<td>47%</td>
<td>62%</td>
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</table>
Extracorporeal Photopheresis versus Anticytokine Therapy as a Second-Line Treatment for Steroid-Refractory Acute GVHD: A Multicenter Comparative Analysis

Madan Jagasia 1,*,1, Hildegard Greinix 2,*, Marie Robin 3, Emma Das-Gupta 4,*, Ryan Jacobs 1, Bipin N. Savani 1, Brian G. Engelhardt 1, Adetola Kassim 1, Nina Worel 2, Robert Knobler 2, Nigel Russell 4, Gerard Socie 3,1

1 Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee
2 Department of Internal Medicine I, Medical University of Vienna, Vienna, Austria
3 Service d’Hématologie Greffe de Moelle, Saint Louis Hospital, Paris, France
4 University of Nottingham, Division of Epidemiology and Public Health, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom

Figure 2. Overall survival (OS) stratified by treatment group. Survival is measured from onset of ECP or non-ECP intervention.
The National Institutes of Health global score predicts the risk of non relapse mortality and disease-free survival in children with chronic graft-versus-host disease.

Children with severe chronic graft-versus-host disease had significantly higher non relapse mortality and lower disease-free survival.

Probability of continuing chronic graft-versus-host disease 8 years after onset of severe chronic graft-versus-host disease was 36%.

Among survivors of more than 5 years, 22% had a performance score below 70%.

Efforts to lower the risk of severe chronic graft-versus-host disease are necessary in pediatric hematopoietic stem cell transplantation.
When could we use it in GVHD?
Figure 6  Cumulative incidence of grades II–IV acute GVHD.

Figure 7  Adjusted probability of disease-free survival.

Figure 8  Adjusted probability of survival.

Extracorporeal photopheresis for the prevention of acute GVHD in patients undergoing standard myeloablative conditioning and allogeneic hematopoietic stem cell transplantation

PJ Shaughnessy1, BI Bolwell2, K van Besien3, M Mistek4, A Gring5, A Doslab6, HM Prince7, S Durrah8, O Ilan9, D Pirelli6, J Gallo6, F Foss10, J Apperley11, M-J. Zhang12, MM Horowitz13 and S Adibyakhat14

1Texas Transplant Institute, San Antonio, TX, USA; 2Cleveland Clinic Foundation, Cleveland, OH, USA; 3University of Chicago, Chicago, IL, USA; 4Department of Hematology and Transplantation, University Hospital Bratislava, Bratislava, Slovakia; 5Royal Melbourne Hospital, Melbourne, Australia; 6St Vincent’s Hospital, Sydney, Australia; 7Peter MacCallum Cancer Institute and University of Melbourne, Melbourne, Australia; 8Royal Brisbane Hospital, Brisbane, Australia; 9Akita University Medical School, Akita, Japan; 10Therakos, Exton, PA, USA; 11 Mayo Clinic Center, Hennepin, CT, USA; 12Rammanan Hospital Imperial College, London, UK; 13Roswell Park Cancer Institute, Buffalo, NY, USA and 14University of Kansas Medical Center, Kansas City, KS, USA
These preliminary data may indicate a potential survival advantage with ECP for transplant recipients undergoing standard myeloablative hematopoietic cell transplantation.
How to use ECP in GVHD?
Indications for ECP in the paediatric setting: guidelines

**acute and chronic GvHD**

1. not responding to steroid
2. responding with intolerable side effects
   (ECP combined with 2nd line treatment)

**chronic GvHD**

3. unsuccessfully treatment with > 3 lines of therapy (resistant, dependent, late-stage disease)
4. limited, regardless of other therapies

**acute GVHD**

5. grade IV with 1st and 2nd line therapies
Algorithm for aGVHD treatment used in our center.

**Diagnosis of aGVHD**

- Optimize CsA therapy
- Methyprednisolone 2 mg/kg
- Program ECP

**Contraindications to steroid therapy**

**Critic situation, not immediately available ECP, no response to steroid**

- ECP

**Second line treatment:**

- Mycophenolate mofetil, FK, Rapamycin

**Second line treatment:**

- No responders to steroid and/or ECP
- ECP available

- Second line treatment: Mycophenolate mofetil, FK, Rapamycin

**Third line treatment:**

- Mesenchymal Stem Cells, Methotrexate
Which system?

• „However the machine currently approved is designed for patients over 40 kg of body weight. Significant fluid shift and venous access are major concerns when ECP is performed in children. Various modifications of the ECP procedure have been tried to manage patients with los body weight. Experience with ECP in children is limited but preliminary data also showed favorable response in children with resistant GVHD.“

Chan KW J Clin Apher.2006;21(1):60-4
Extracorporeal volume of apheresis systems

![Bar chart showing extracorporeal volume for different systems.](chart.png)
UVAR system
ECP: on-line and off-line methods

On-line (Therakos)

Off-line: I° step = MNC collection

Off-line: II° step = 8-MOP and UV-A irradiation
Acute mechanical hemolysis as a complication of extracorporeal photopheresis in a low-weight child

Robert A. DeSimone¹ | Sandeep N. Wontakal¹ | Alexander K. Lyashchenko¹ | Joseph Schwartz¹,²
ECP program from 2005 until today

• Patients non responsive to SIT and need for 2\textsuperscript{nd} line treatment
  • aGVHD
  • cGVHD

• System
  • > 40 kg UVAR XTS, > 30 kg Cellex (no blood priming)
  • < 40 kg AMICUS, Fenwal CS, AMICUS, OPTIA plus COMBI light plus and later on MACOGENIC
    • (1x Apherese, 2x Reinfusion)
  • in special cases „MINI“ ECP
UVAR Cellex

• Double needle or single needle method applicable
• Validation and qualification is done at our department
ECP program „offline method 1 apheresis 2 reinfusions“

- Day 1: Divided in 2
- Day 2: Stored overnight at +4°C
This procedure can only be performed in a GMP laboratory
Monday 9h-13h

Wednesday 9h-13h

Friday 9h-13h

Monday 9h-12h

• **Cryopreservation** of mononuclear cells

• **Sequential reinfusion** of thawed and ECP treated cells

Monday 12h-13h

Wednesday 12h-13h

Friday 12h-13h

**Reduction of the number of aphereses**
Cryopreservation induces lymphocyte apoptosis while retaining the stimulatory function of dendritic cells.
Methods

• Healthy donors

• Isolation of PBMC by centrifugation on Ficoll-Hypaque gradients (density, 1.077)

• Cryopreservation
  - DMSO : 3,5 %
  - Albumin : 10 %
  - HES : 92,3 %
  - -80°C without controlled-rate freezing

• Thawing in 40°C water until completely melted, then RPMI is added, supplemented with 10% human AB serum. Washed twice to remove DMSO.

• ECP: Vilber-Lourmat irradiator in UVA transparent bag (Macopharma)
Cryopreservation for ECP – in vitro assessment

ECP treated cells - Viability (Trypan Blue Exclusion)

- Fresh cells
- Cryopreserved cells

Viable cells (%)

<table>
<thead>
<tr>
<th></th>
<th>Before ECP</th>
<th>h1</th>
<th>h24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable cells (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryopreserved cells</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
ECP treated cells – Apoptosis
(Annexin V – IP)

![Graph showing Annexin + cells (%) before and after ECP treatment]

- Fresh cells
- Cryopreserved cells

Before ECP

H1 after ECP
Functional assessment - MLR

Judgment criterion: proliferation in MLR

Measured with CFSE dye dilution

Stimulator cells: $A^* = \text{mother} - 30 \text{ Gys}$
Reactive cells: $B = \text{daughter} – \text{labelled with cfse}$
Tested cells: $\text{ECP treated B cells}$

7 days culture in RPMI-1640 supplemented with human AB serum, Hepes, antibiotics and glutamine
A^*+B_{CFSE} \ 2:1

A^*+B_{CFSE}+B_{ECP} \ 2:1:1

A^*+B_{CFSE}+B_{ECP} \ {CRYO} \ 2:1:1
Cryopreservation for ECP – in vitro assessment

Cryopreservation for ECP

- in vitro assessment

Slides from Merlin E. ©
Cryopreserved-cell ECP for GVH in children – Clermont-Fd experience

Freezing
- DMSO : 3,5 %
- Albumin : 10 %
- HES : 92,3 %
- -80°C without controlled-rate freezing

Thawing
Washing twice
Cryopreserved-cell ECP for GVH in children – Clermont-Fd experience

Days of ECP therapy

<table>
<thead>
<tr>
<th></th>
<th>ECP center</th>
<th>Referring hospital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell infusion (ECP)</td>
<td>⬤ ⬤ ⬤ ⬤ ⬤ ⬤</td>
<td>⬤ ⬤ ⬤ ⬤ ⬤</td>
</tr>
<tr>
<td>Apheresis</td>
<td>⬤ ⬤ ⬤ ⬤ ⬤ ⬤ ⬤</td>
<td>⬤ ⬤ ⬤ ⬤ ⬤</td>
</tr>
</tbody>
</table>

0 10 20 30 40

- ECP: Ex Vivo Cellular Therapy
- GVH: Graft-versus-host Disease
- Clermont-Fd: Clermont-Ferrand, France
Immunosuppression
Salt-free diet
Hypertension
Colitis
Troubles de l'humeur
Neutropenia

Fed up
Venous access
Transfusions
Logistical issues

Short term
Shortterm

Immunosuppression
Salt-free diet
Hypertension
Colitis
Troubles de l’humeur
Neutropenia

Fed up
Venous access
Transfusions
Logistical issues
MINI ECP → simple method

1. Drawing blood from peripheral vascular access
2. Fluid substitution with cristalloid solutions if needed
3. Centrifugation of the product
4. Squeezing of the buffy coat
5. Reinfusion of the residual erythrocyte fraction
6. Dilution of the buffy coat to hematocrit < 5%
7. Plus 8-Methoxypsoralen
8. Irradiation with UVA 2,0 - 2,5 J/cm²
9. Reinfusion of the treated buffy coat
Time needed

3. + 4. + 6. + 7.  8.

1.  2.  5.  9.

180 min

15.01.2015
Facility Resources

GMP facility

3. + 4. + 6. + 7.

Laboratory

8.

1. 2. 5. 9.

Day care center/ ward

15.01.2015
MINI ECP in practise

• SCID (Omenn-Syndrom, RAG1 Mutation), 3y alt, multiple premorbidity pre SCT (viral infections, BCG infection, surgery Ileum resection ,...)
• SCT MUD (BM) 17.12.2013
• Fulminatn engraftment (d+13)
• Severe acut GvHD skin/liver/gut:
  - exanthema
  - Bilirubin increase
  - bloody diarrhea (transfusion dependend)

• Nonresponse to IST with steroid, high dose CSA/FK506 and MMF (MMF ex d +28)

  ➔ start MINI ECP d +20 (05.01.2014): 2x/week
Mini ECP in very low bodyweight patients (< 10kg BW)

Cave circulatory!
Monitoring: HF, RR

Hickman ZVK single lumen
Hickman CVC single lumen

Bag with ACD-A

Syringe to take blood

pRBC (isovolemic Hemodilution))

HA 5%
steps:
1. Drawing 10ml blood from CVC
2. Direct to sample bag
3. Reinfuse pRBC 1:1 with HA 5%
skin: remission
liver: remission
gut: partial response

Outcome

- Reduction of the liver size
- Decrease of skin rush
- Reduction of steroids

15.01.2015
Extracorporeal Photochemotherapy for the Treatment of Chronic Graft-Versus-Host Disease: Trend for a Possible Cell Dose-Related Effect?

Paolo Perseghin,1 Stefania Galimberti,2 Adriana Balduzzi,3 Sonia Bonanomi,3 Valentina Baldini,1 Attilio Rovelli,3 Maria Dassi,1 Alessandro Rambaldi,4 Luca Castagna,5 Paola Corti,3 Enrico M Pogliani,6 and Cornelio Uderzo3

1Department of Clinical Pathology, Therapeutic Apheresis Unit, San Gerardo de’ Tintori Hospital, 2Department of Clinical Medicine, Prevention and Health Biotechnology, 3Pediatric Clinic and 4Division of Hematology, University of Milan-Bicocca and San Gerardo de’ Tintori Hospital, Monza, 4Division of Hematology, Rianiti Hospital, Bergamo, and 5Division of Oncohematology, Humanitas Clinical Institute, Milan, Italy
TABLE 2. Results of the univariate logistic analysis on no response to ECP treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>No of patients</th>
<th>No of NR</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous aGVHD</td>
<td>Yes</td>
<td>18</td>
<td>3</td>
<td>0.50</td>
<td>0.06–3.91</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>7</td>
<td>2</td>
<td>0.81</td>
<td>0.43–15.52</td>
</tr>
<tr>
<td>Months from HSCT to cGVHD</td>
<td>= 3.3 (100 days)</td>
<td>13</td>
<td>3</td>
<td>0.85</td>
<td>0.69–1.06</td>
</tr>
<tr>
<td></td>
<td>3.3–6</td>
<td>9</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;6</td>
<td>3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months from cGVHD to ECP</td>
<td>0–6</td>
<td>12</td>
<td>4</td>
<td>1.42</td>
<td>0.12–17.46</td>
</tr>
<tr>
<td></td>
<td>6–12</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;12</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cGVHD grading</td>
<td>Limited</td>
<td>4</td>
<td>1</td>
<td>0.99</td>
<td>0.98–1.01</td>
</tr>
<tr>
<td></td>
<td>Extensive</td>
<td>21</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. organ involved</td>
<td>1</td>
<td>11</td>
<td>1</td>
<td>3.99</td>
<td>0.29–55.46</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>7</td>
<td>2</td>
<td>3.99</td>
<td>0.29–55.46</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>3.99</td>
<td>0.29–55.46</td>
</tr>
<tr>
<td>Platelet count at first ECP (×10³/μL)</td>
<td>≤100</td>
<td>8</td>
<td>3</td>
<td>0.98</td>
<td>0.97–1.01</td>
</tr>
<tr>
<td></td>
<td>&gt;100</td>
<td>17</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean dose of MNC cells (&gt;10%/kg.b.w. ECP)</td>
<td>0–75</td>
<td>6</td>
<td>3</td>
<td>0.96</td>
<td>0.95–1.01</td>
</tr>
<tr>
<td></td>
<td>75–100</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;100</td>
<td>12</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean dose of Mono cells (&gt;10%/kg.b.w. ECP)</td>
<td>0–25</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25–35</td>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;35</td>
<td>14</td>
<td>2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CI, confidence interval; ECP, extracorporeal photochemotherapy; HSCT, hematopoietic stem cell transplantation; MNC, mononuclear cell; Mono, monocyte; NR, no response.

FIG. 1. Mean cell doses/kg b.w. of mononuclear cells and monocytes infused per procedure throughout the entire ECP course. Each bar represents the standard deviation of the individual profiles, while the horizontal lines are the median and the first quartile of the MNC (dotted line) and Mono (dashed lines) mean values, respectively. CR, complete response; ECP, extracorporeal photochemotherapy; MNC, mononuclear cells; Mono, monocyte; NR, no response; PR, partial response.
## Celldose

[cells x 10^6/kg bw]

<table>
<thead>
<tr>
<th></th>
<th>Lymphocyte</th>
<th>Monocyte</th>
<th>Granulocyte</th>
</tr>
</thead>
<tbody>
<tr>
<td>MINI (n=65)</td>
<td>5.37</td>
<td>2.44</td>
<td>3.99</td>
</tr>
<tr>
<td>OFFLINE (n=136)</td>
<td>46.7</td>
<td>20.3</td>
<td>14.9</td>
</tr>
<tr>
<td>INLINE*) (n=134)</td>
<td>25.4</td>
<td>6.2</td>
<td>21.8</td>
</tr>
</tbody>
</table>

*INLINE = UVAR XTS
ECP FOR ACUTE GVHD WITH DIFFERENT METHODS (MINI; OFFLINE; INLINE) IN PEDIATRIC PATIENTS

Volker Witt, Herbert Pichler, Christina Peters, Susanne Matthes, Anita Lawitschka, Wolfgang Holter

St. Anna Kinderspital, UKKJ Medical University Vienna, Austria, Kinderspitalgasse 6, 1090 Vienna, Austria, volker.witt@stanna.at

Poster EBMT 2014, Milano
<table>
<thead>
<tr>
<th>n patients</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>age</td>
<td>6.86 a (0.82 – 17.9)</td>
</tr>
<tr>
<td>bw</td>
<td>22 kg (7 – 60)</td>
</tr>
<tr>
<td>female</td>
<td>11</td>
</tr>
<tr>
<td>male</td>
<td>12</td>
</tr>
<tr>
<td>Interval Tx =&gt; aGVHD</td>
<td>19 d (9 – 87)</td>
</tr>
<tr>
<td>Indication for ECP</td>
<td>23 x refractory to SIT</td>
</tr>
<tr>
<td>Interval Tx =&gt; first ECP</td>
<td>36 d (16 – 90)</td>
</tr>
<tr>
<td>Interval aGCHD =&gt; first ECP</td>
<td>13 d (3 – 29)</td>
</tr>
<tr>
<td>Interval first ECP =&gt; last ECP</td>
<td>51 d (9 – 372)</td>
</tr>
<tr>
<td>n ECP per patient</td>
<td>10 reinfusion (3 – 55)</td>
</tr>
<tr>
<td>GVHD</td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>23 (21/23 CR)</td>
</tr>
<tr>
<td>Gut</td>
<td>9 (8/9 CR)</td>
</tr>
<tr>
<td>Liver</td>
<td>10 (8/10 CR)</td>
</tr>
<tr>
<td>Lung</td>
<td>0</td>
</tr>
<tr>
<td>response</td>
<td>21/23 (91%)</td>
</tr>
</tbody>
</table>
aGHVD ECP method

![Bar chart showing the comparison between MINI, offline, and inline methods with respective values of 6, 13, and 4.](image-url)
n ECP / patient
aGVHD method of ECP

![Graph showing overall survival after transplantation with different methods]

<table>
<thead>
<tr>
<th>Chi-Quadrat</th>
<th>Degree of freedom</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.047293083</td>
<td>2</td>
<td>0.976630847</td>
</tr>
</tbody>
</table>
aGVHD response at 3 months to ECP
Conclusion aGVHD

- ECP is in our hands an effective second line therapy in acute GVHD. Neither the method used nor the individualized schedule applied seems to influence the outcome. Due to the small patient number, this report could be only a step forward to prospective randomized trials, bringing hopefully an answer to these questions. Interestingly we could show in our collective, that the response to ECP treatment for aGVHD is a predictor of overall survival.
Conclusion MINI ECP

• Interesting method
• No international agreement or acceptance whether blood, tissue or ATMP regulations should be applied
• Low technical efforts, low economic expenses => ? Low budget ECP?
• Data registry needed
Outcome

**Acute GVHD**
- 14/14 improved after in median after 11 treatments
  - Organ involvement
    - 14 skin (14 improved)
    - 6 liver (5 improved)
    - 4 GI (4 improved)
- 4 died due to infection and MOF not due to the apheresis procedure (Mini ECP) and improvement could not be evaluated.

**Chronic GVHD**
- 17 improved
  - 17 skin
  - 3 gut
  - 3 liver
  - 6 mucosa
- 7 did not improved
  - 4 skin (but in 2 skin improved)
  - 3 mucosa
  - 1 liver
  - 1 gut
duration of immunosuppressiv treatment in cGVHD
Outcome from all patients with acute, overlap and chronic GVHD from 1999 until today
Survival and Improvement in patients with cGVHD

timepoint:
1.ECP + 1 month

End point +3 months
Conclusions ECP in children:

• ECP is an approach to GvHD therapy that appears to offer control of GvHD without generalized immunosuppression and its associated complication
• Outcome is associated with response to ECP
• Despite different harvest and photochemotherapy methods, response to treatment seems to be independent
• High response rates in cutaneous and extracutaneous GVHD manifestations
• Steroid-sparing effect
• Improved quality of life and OS
• No negative effect on GvL
• Well tolerated, minimal side-effects during therapy and no reported long term side effects
Thank you for your attention