15° Congrès de la Société Française d'Hémaphérèse
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ECP in Italy: from the '90s to nowadays

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ASFA Guidelines 2013
JCA 2013

I	**Disorders for which apheresis is accepted as first-line therapy, either as a primary standalone treatment or in conjunction with other modes of treatment.** *Example: plasma exchange in Guillain-Barre syndrom as 1st-line standalone therapy; plasma exchange in myasthenia gravis as 1st-line in conjunction with immunosuppression and cholinesterase inhibition*
II	**Disorders for which apheresis is accepted as second-line therapy, either as a standalone treatment or in conjunction with other modes of treatment.** *Example: plasma exchange as standalone secondary treatment for acute disseminated encephalomyelitis after high-dose IV corticosteroid failure; extracorporeal photopheresis added to corticosteroids for unresponsive chronic graft-versus-host disease*
III	**Optimum role of apheresis therapy is not established. Decision making should be individualized.** *Example:extracorporeal photopheresis for nephrogenic systemic fibrosis; plasma exchange in patients with sepsis and multi-organ failure*
IV	**Disorders in which published evidence demonstrates or suggests apheresis to be ineffective or harmful. IRB approval is desirable if apheresis treatment is undertaken in these circumstances.** *Example: plasma exchange for active rheumatoid arthritis*

Recommendation	Description	Methodological quality of supporting evidence	Implications
Grade 1A	Strong recommendation, high-quality evidence	RCTs without important limitations or overwhelming evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Grade 1B	Strong recommendation, moderate quality evidence	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Strong recommendation, can apply to most patients in most circumstances without reservation
Grade 1C	Strong recommendation, low-quality or very low- quality evidence	Observational studies or case series	Strong recommendation but may change when higher quality evidence becomes available
Grade 2A	Weak recommendation, high quality evidence	RCTs without important limitations or overwhelming evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients'or societal values
Grade 2B	Weak recommendation, moderate-quality evidence	RCTs with important limitations (inconsistent results, methodological flaws, indirect, or imprecise) or exceptionally strong evidence from observational studies	Weak recommendation, best action may differ depending on circumstances or patients' or societal values
Grade 2C	Weak recommendation, low-quality or very low-quality evidence	Observational studies or case series	Very weak recommendations; other alternatives may be equally reasonable

aGvHD: (skin) II 1c

cGvHD : (skin) II 1b

a/c GvHD: (non-skin) III 2b

JACIE standard 5 th ed

B7.3 There shall be a policy addressing safe administration of extracorporeal photopheresis (ECP).

B7.3.1 There shall be a consultation with the facility that performs ECP prior to initiation of therapy.

B7.3.2 Before ECP is undertaken, there shall be a written order from a physician specifying, at a minimum, the patient's diagnosis, proposed regimen, timing of the procedure, and any other factors that may affect the safe administration of ECP.

B7.3.3 A final report of the details of ECP administered, including an assessment of the response, shall be documented in the patient's medical record.

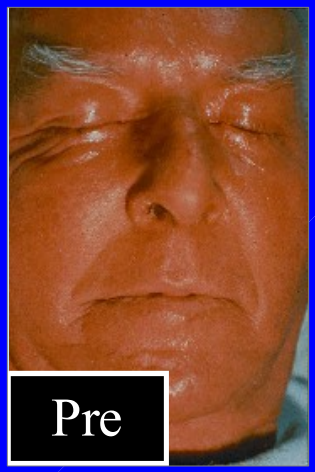
B7.3.4 The ECP procedure shall be performed according to written standard operating procedures of the facility performing the procedure appropriate for the clinical condition of the patient.

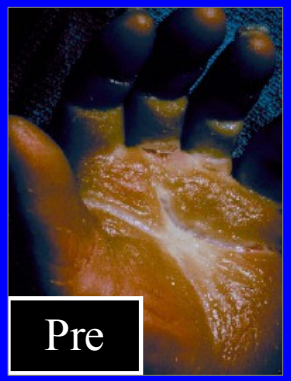
B7.3.5 Outcomes, including adverse events, related to the administration of ECP to patients within the Clinical Program shall be analyzed annually.

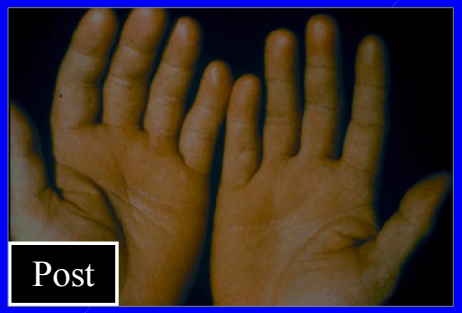
Treatment of Cutaneous T-Cell Lymphoma by Extracorporeal Photochemotherapy

Edelson R et al, NEJM 1987

9 of 37 CTCL patients: >75 response







FDA approval granted for advanced CTCL in 1988, as the first government sanctioned selective immunotherapy for any cancer.

Approximately 80% of immunocompetent CTCL patients have a >50% diminution in cutaneous involvement, while 25% experience complete responses.

Persistent (>18 month) complete responses was corroborated by loss of identifiable malignant T cell clone by TCR PCR assessment.

Toxicity is remarkably low and less than with all alternative systemic therapies.

PHOTOPHERESIS FOR THE PREVENTION OF REJECTION IN CARDIAC TRANSPLANTATION

MARK L. BARR, M.D., BRUNO M. MEISER, M.D., HOWARD J. EISEN, M.D., RANDALL F. ROBERTS, M.D., UGOLINO LIVI, M.D., ROBERTO DALL'AMICO, M.D., PH.D., RICHARD DORENT, M.D., JOSEPH G. ROGERS, M.D., BRANISLAV RADOVANČEVIĆ, M.D., DAVID O. TAYLOR, M.D., VALLUVAN JEEVANANDAM, M.D., AND CHARLES C. MARBOE, M.D., FOR THE PHOTOPHERESIS TRANSPLANTATION STUDY GROUP

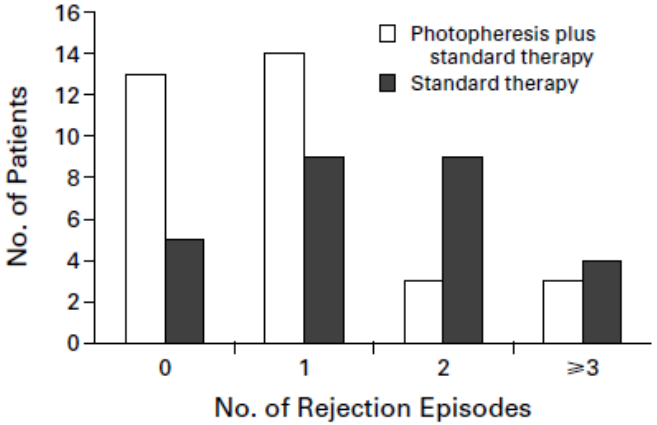


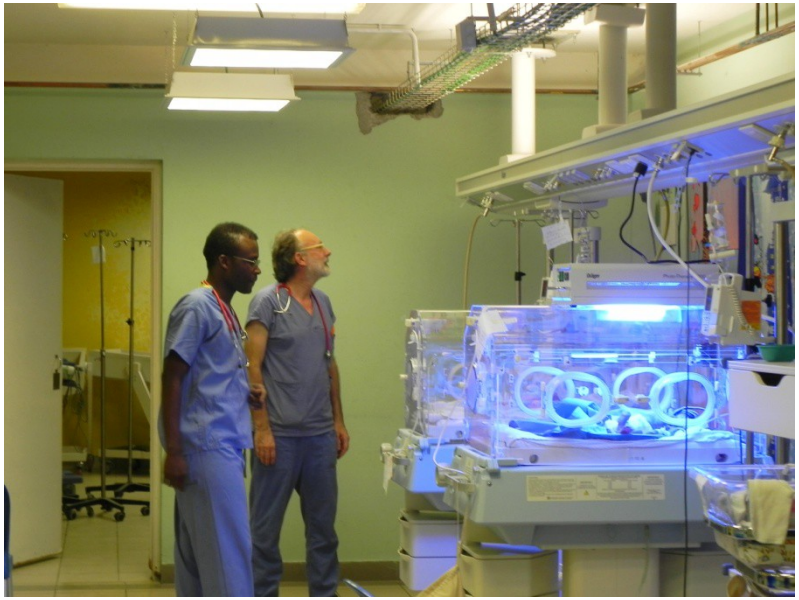
Figure 1. Number of Patients with and without Rejection Episodes during the Six-Month Follow-up.

Two-tailed P=0.03 for the comparison between groups by the chi-square test for trend, with one degree of freedom.

TABLE 3. NUMBER OF PATIENTS WHO REQUIRED TREATMENT FOR INFECTION.

TYPE AND NO. OF TREATED INFECTIONS	PHOTOPHERESIS PLUS STANDARD THERAPY (N=33)	STANDARD THERAPY ALONE (N=27)	P VALUE*
Bacterial			0.76†
0	18	17	
1	11	6	
≥2	4	4	
Viral			0.76‡
0	26	20	
1	7	7	
≥2	0	0	
Fungal			0.26†
0	30	23	
1	3	2	
≥2	0	2	
Parasitic or protozoal			0.45‡
0	33	26	
1	0	1	
≥2	0	0	

*P values were calculated for each type of infection.



Up-to-now ECP activity in Italy

n°=704

n°=4225

Preliminary data 2013

Overall: 22.229 procedures

ECP (total): 5362
(2/3 off-line method

Estimated ECP/yr in Italy:
~ 7500

Yr 2000: data derived from 102 Apheresis Centers (1)

Yr 2005: preliminary data derived from 13 Apheresis centers (2)

1) G. De Silvestro et al: National survey of apheresis activity in Italy. Trans Apher Sci 2004

2) G. De Silvestro, National survey of apheresis activity in Italy Int J Art Organs 2008.

3) G. De Silvestro, personal communication, 2014

Monitoring of circulating T-cell substes: the role of T-REGs in ECP and GVHD (A.Lamioni, *Transplantation* 2005)

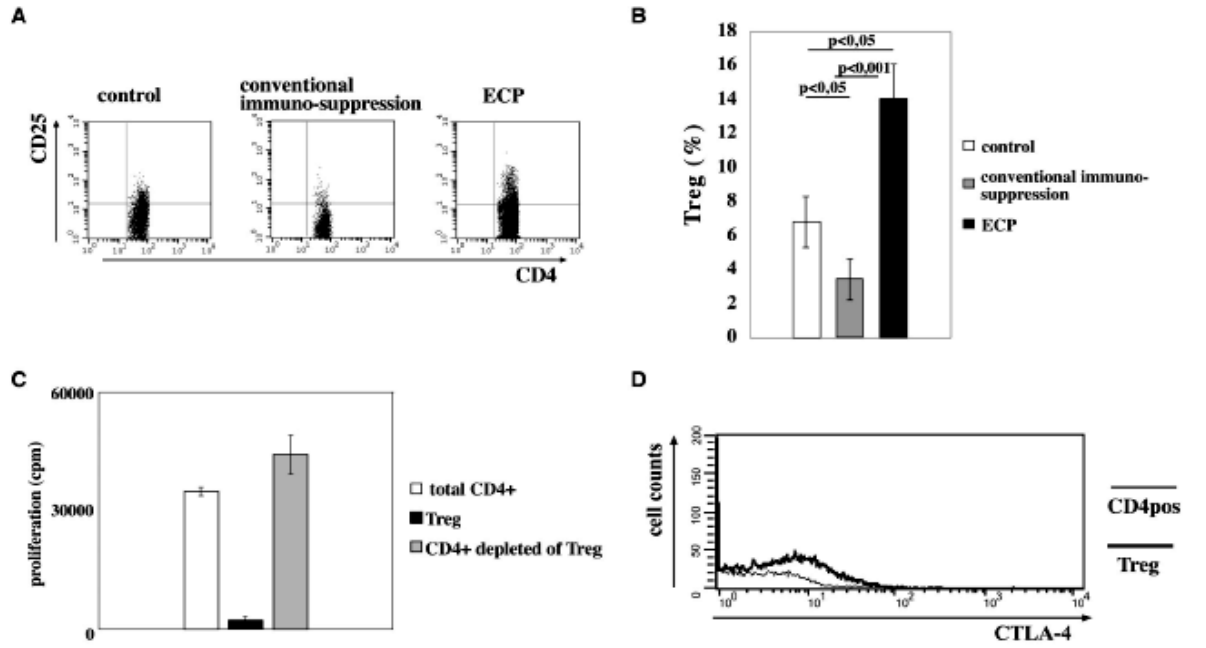


FIGURE 3. Treg cells are increased in the blood of patients treated with ECP and have a suppressive function. (A) Cells were stained with antibodies to CD4, CD3, CD69 and CD25. Dot plot shows CD25 expression of $CD4^+$ $CD3^+$ T cells in a representative control (*left*) and in patients treated either with conventional immunosuppression (CIS, *middle*) or ECP+CIS (*right*). $CD69^+$ activated cells were excluded from analysis by electronic gating. (B) The bars show the frequency of $CD4^+$ $CD25^+$ Treg cells in five normal individuals (*white bar*) and in transplanted patients treated either with CIS (six patients, *gray bar*), or with additional ECP (four patients, *black bar*). Student's *t* test was used for statistical analysis. $P < 0.05$ was considered significant. (C) Depletion of $CD4^+CD25^+$ Treg cells increases T-cell proliferation in ECP-treated patients. The white bar shows the proliferation of $CD4^+$ T cells, the grey bar the proliferation of Treg depleted $CD4^+$ cells. Treg cells do not proliferate upon stimulation (*black bar*). A representative result of three independent experiments is shown. (D) CTLA-4 surface expression of sorted $CD4^+$ T cells (*thin line*) and Treg cells (*thick line*) from an ECP-treated patient analyzed after stimulation with anti-CD3 and anti-CD28.

The Immunological Effects of Extracorporeal Photopheresis Unraveled: Induction of Tolerogenic Dendritic Cells In Vitro and Regulatory T Cells In Vivo

Andrea Lamioni,[1] Francesco Parisi,[2] Giancarlo Isacchi,[3,4] Ezio Giorda,[1] Silvia Di Cesare,[5] Attilio Landolfo,[3] Francesco Cenci,[1] Gian Franco Bottazzo,[1] and Rita Carsetti[1,6]

Effectiveness of extracorporeal photochemotherapy (ECP) in the treatment of acute and chronic GvHD, cutaneous T-cell lymphoma and immunomediated diseases: results of a three-year multicenter perspective observational study.

Perseghin P, Sciorelli G, Biagi E, Incontri A, Pagani A, Poli L, Marini M, Ferremi P, Galimberti S, Perotti C and Salvaneschi L for the Regione Lombardia ECP study group*

- 4 Institutions (Brescia, Gallarate, Monza, Pavia)
- 178 patients enrolled (2006-2009)
- 124 (70 %) with GvHD (acute= 37, chronic=87), ~2600 ECP performed
- 43 out of 124 (35%) pediatric patients

Overall response rates (in pediatric patients)

Acute GvHD

- NR= 20%
- PR+CR=80%

Chronic GvHD

- NR=12%
- PR+CR= 76%
- NV= 12% (ongoing evaluation, not yet available data)

Perseghin et al, unpublished data

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 Uderzo[3]

[1]Department of Clinical Pathology, Therapeutic Apheresis Unit, San Gerardo de' Tintori Hospital, [2]Department of Clinical Medicine, Prevention and Health Biotechnology, [3]Pediatric Clinic and [6]Division of Hematology, University of Milan-Bicocca and San Gerardo de' Tintori Hospital, Monza, [4]Division of Hematology, Riuniti Hospital, Bergamo, and [5]Division of Oncohematology, Humanitas Clinical Institute, Milan, Italy

Ther Apher Dial. 2007, 11:85-93

25 patients who underwent:

-Allogeneic related HSCT (n=18)-Allogeneic unrelated HSCT (n=3) -Haplo-identical HSCT (n=4)

who developed cGvHD refractory to conventional immunosuppressive treatment and started ECP were retrospecively analysed

Main diagnosis for HSCT: AML (8), ALL (7), CML (4),

other diseases (6)

- **Median age: 17 yrs (range 6-55)**
- **Median weight: 52 Kg (range 20-81)**

- **12 patients : progressive cGvHD**
- **7 patients : "de novo" cGvHD**

at a median of 5 mths from HSCT

- **6 patients had "quiescent" cGvHD**

ECP: MECHANISMS OF ACTION

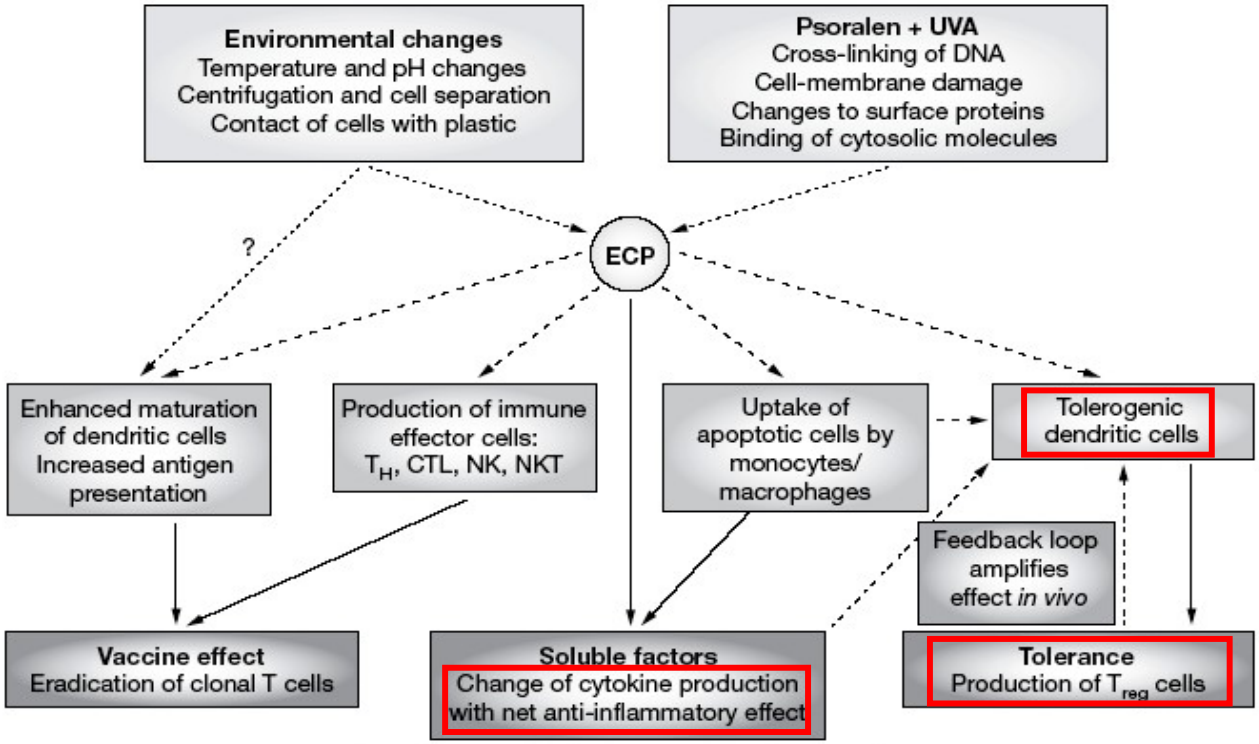


Figure 2 Proposed mechanistic pathways of extracorporeal photochemotherapy. CTL, cytotoxic T lymphocytes; DNA, deoxyribonucleic acid; ECP, extracorporeal photochemotherapy; NK, natural killer cells; NKT, natural killer T cells; T_H, T helper lymphocytes; T_{reg}, $CD4^+/CD25^+$ T-regulatory lymphocytes.

Technology Insight: ECP for the treatment of GvHD—can we offer selective immune control without generalized immunosuppression?

Scott R Marshall

NATURE CLINICAL PRACTICE ONCOLOGY JUNE 2006 VOL 3 NO 6

Monitoring of circulating T-cell substes: the role of T-REGs in allotrasplant and GVHD

Table 1

Clinical studies in allogeneic hematopoietic stem cell transplantation.

Number of patients	Identification of Treg (method)	Conclusions	References
60	$CD4^+CD25^+$ T cells (cytometry)	GvHD is associated with high frequency of $CD4^+CD25^+$ cells within the graft.	[21]
40	$CD4^+CD25^{hi}$ T cells (cytometry)	More than 100 days post-graft, patients with cGvHD have elevated numbers of $CD4^+CD25^{hi}$ T cells.	[22]
54	$CD4^+CD25^{hi}$ T cells (cytometry)	cGvHD does not correlate with the numbers of $CD4^+CD25^{hi}$ T cells in grafted patients.	[23]
34	Foxp3 (RT-qPCR)	GvHD correlates with low Foxp3 expression level in PBMCs of grafted patients.	[24]
57	$CD4^+CD25^+$ T cells (cytometry) and Foxp3 (RT-qPCR)	Patients with cGvHD have reduced frequencies of $CD4^+CD25^+$ and Foxp3-expressing T cells. These cells are functionally suppressive *in vitro*.	[25•]
47	$CD4^+CD25^{hi}$ T cells (cytometry)	The frequency of infused $CD4^+CD25^{hi}$ T cells does not correlate with the risk of GvHD in a delayed leukocyte infusion setting.	[26]
31	$CD4^+CD25^+$ T cells (cytometry) and Foxp3 (RT-qPCR)	The number of Foxp3-expressing $CD4^+CD25^+$ T cells does not correlate with GvHD in grafted patients.	[27]
49	$Foxp3^+$ cells (immunostaining)	Deficit of $Foxp3^+$ cells in the intestine of patients with GvHD.	[28••]
32	$CD4^+Foxp3^+$ T cells (cytometry)	High numbers of $CD4^+Foxp3^+$ T cells within the transplant or in the blood of grafted patients are associated with a reduced risk to develop GvHD.	[29••]

The role of $CD4^+CD25^{hi}$ regulatory T cells in the physiopathogeny of graft-versus-host disease

José L Cohen[1] and Olivier Boyer[2]

Extracorporeal Photochemotherapy Is Accompanied by Increasing Levels of Circulating cD4+CD25+GITR+Foxp3+CD62L+ Functional Regulatory T-Cells in Patients With Graft-Versus-Host Disease

Ettore Biagi,[1,4] Iolanda Di Biaso,[1] Veronica Leoni,[1] Giuseppe Gaipa,[1] Vincenzo Rossi,[1] Cristina Bugarin,[1] Giuliano Renoldi,[1] Matteo Parma,[2] Adriana Balduzzi,[1] Paolo Perseghin,[3] and Andrea Biondi[1]

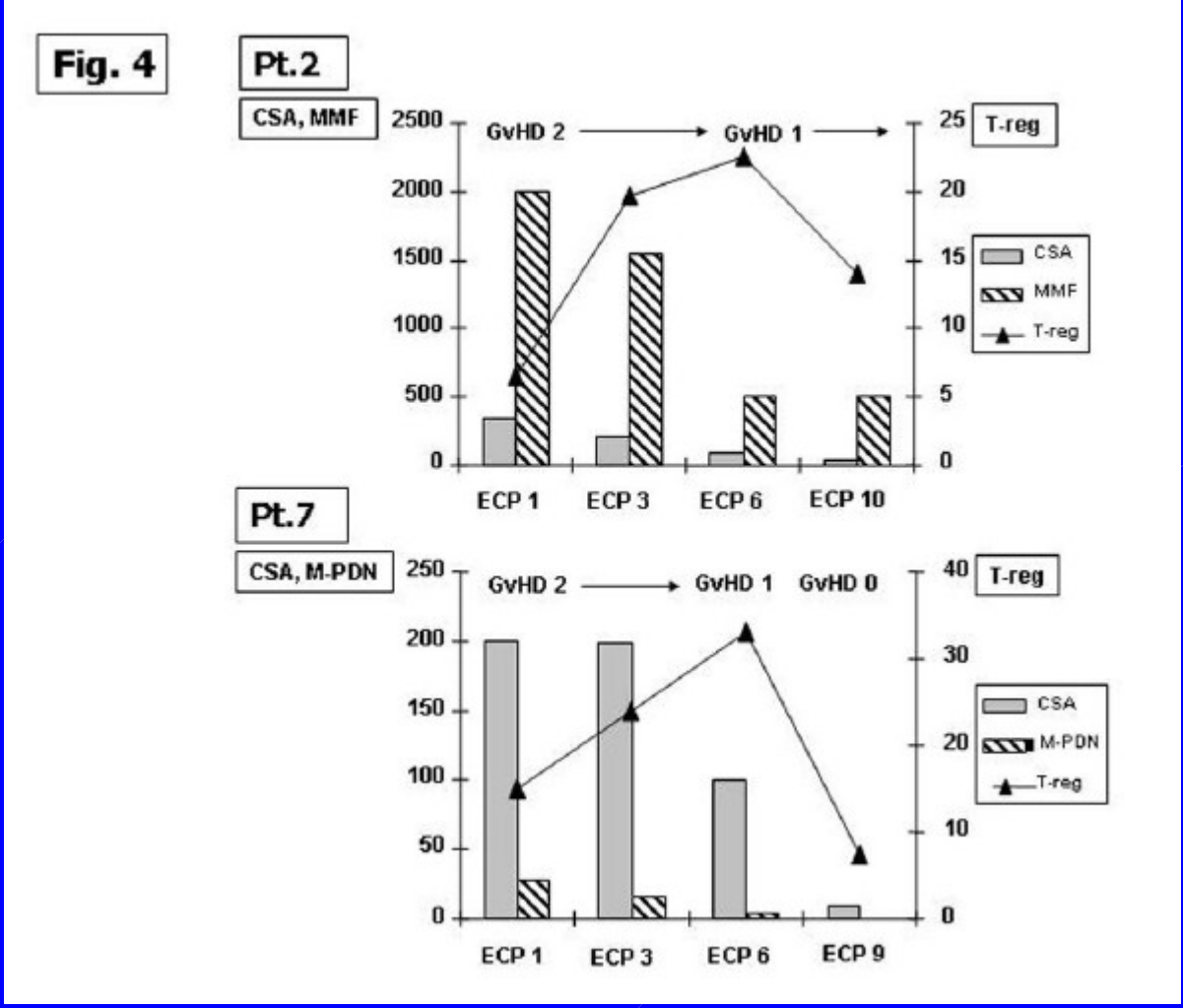
Biagi et al, Transplantation, 2007

Study design

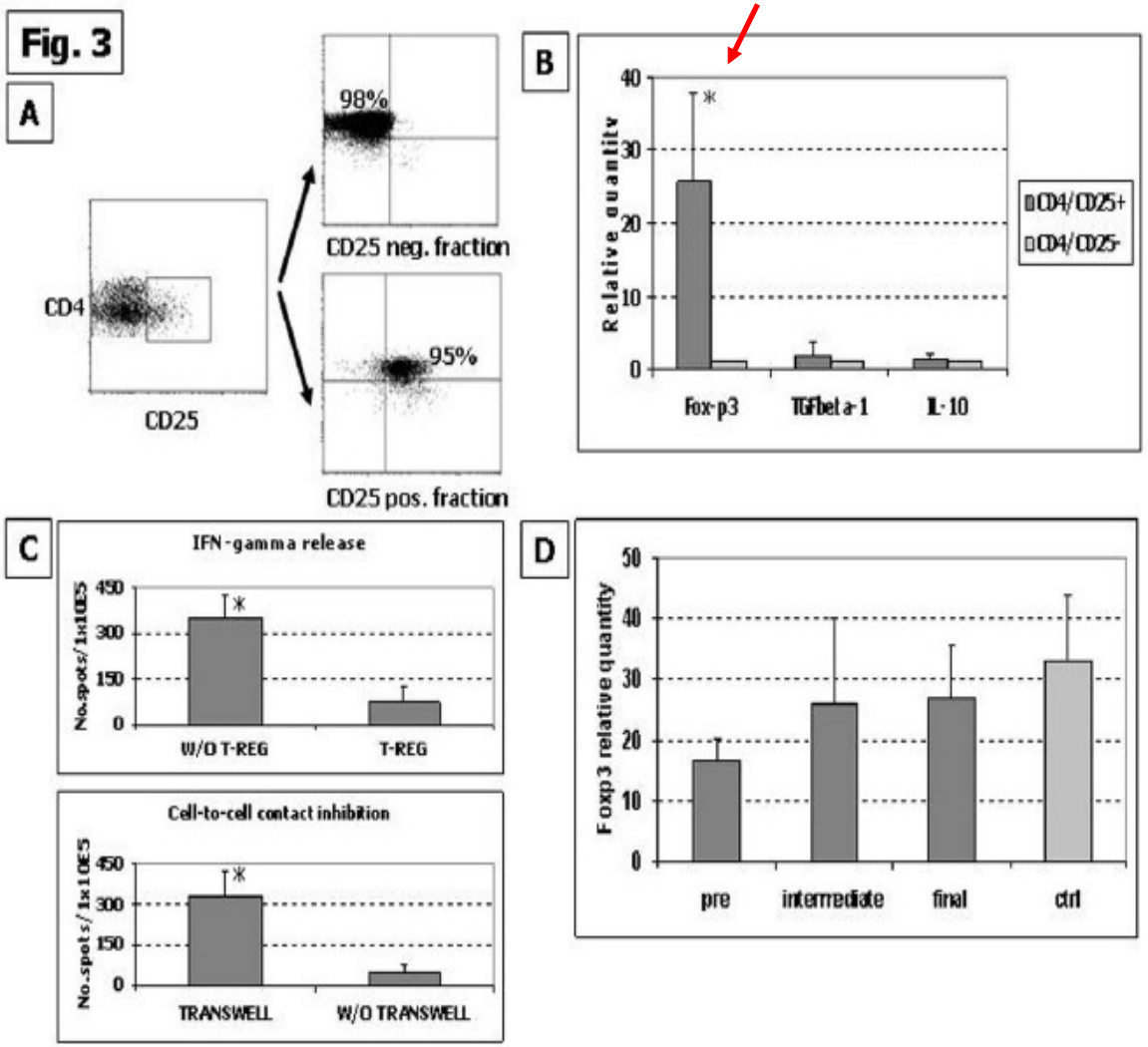
1. Immune-phenotyping of circulating T-regs
   - CD4-CD25 intermediate, bright (comparison with healthy donors and transplanted patients not receiving ECP)
   - GITR, CD45RO, CD62L and Fox-p3 (intracytoplasmic staining)

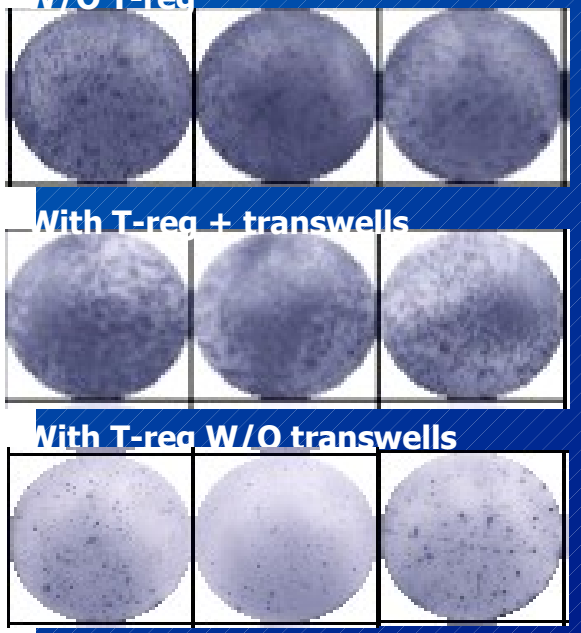
2. Functional analysis on sorted T-regs:
   - qRT-PCR for Fox-p3, IL-10, TGF-beta
   - IFN-gamma Elispot assays in allogeneic cultures
   - Trans-well experiments (cell contact inhibition)

T-regs increase in ECP-responders allows immunosuppressive drug tapering



Biagi et al. Transplantation, 2007

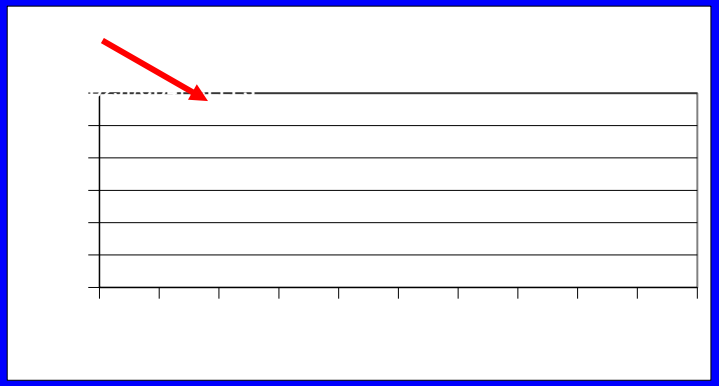


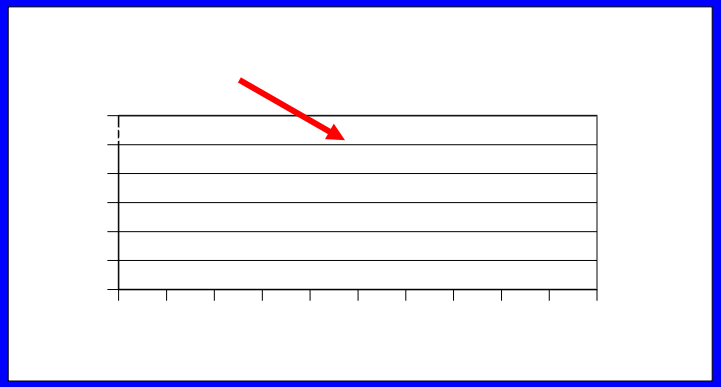


T-regs show inhibitory capacity towards allo-reactivity

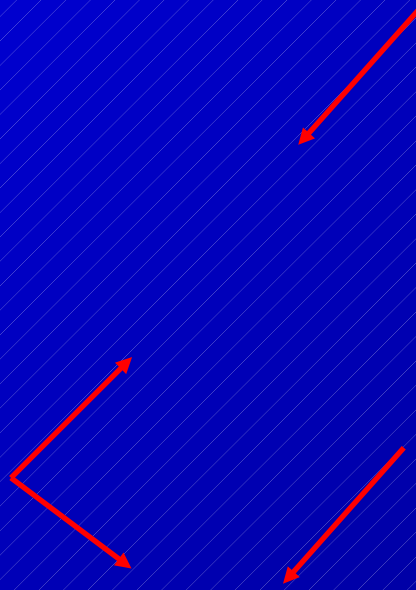
Biagi et al. Transplantation, 2007

T-regs variations in ECP-responders with acute (left) and chronic (right) GvHD



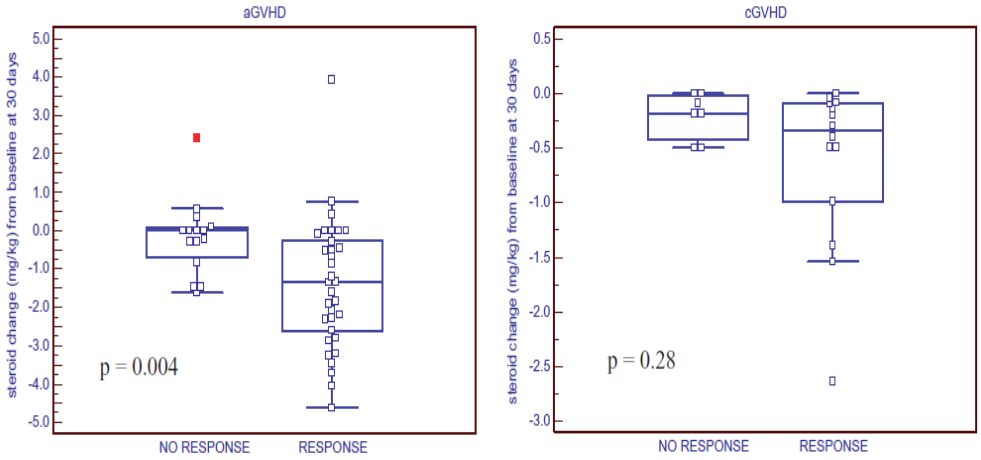


Absence of response to ECP is correlated to more than 1-log higher secretion of IL-17 by Th17 cells, particularly evident at 3 cycles



Extracorporeal photochemotherapy in graft-versus-host disease: a longitudinal study on factors influencing the response and survival in pediatric patients

Cesare Perotti, Claudia Del Fante, Carmine Tinelli, Gianluca Viarengo, Luigia Scudeller, Marco Zecca, Franco Locatelli, and Laura Salvaneschi



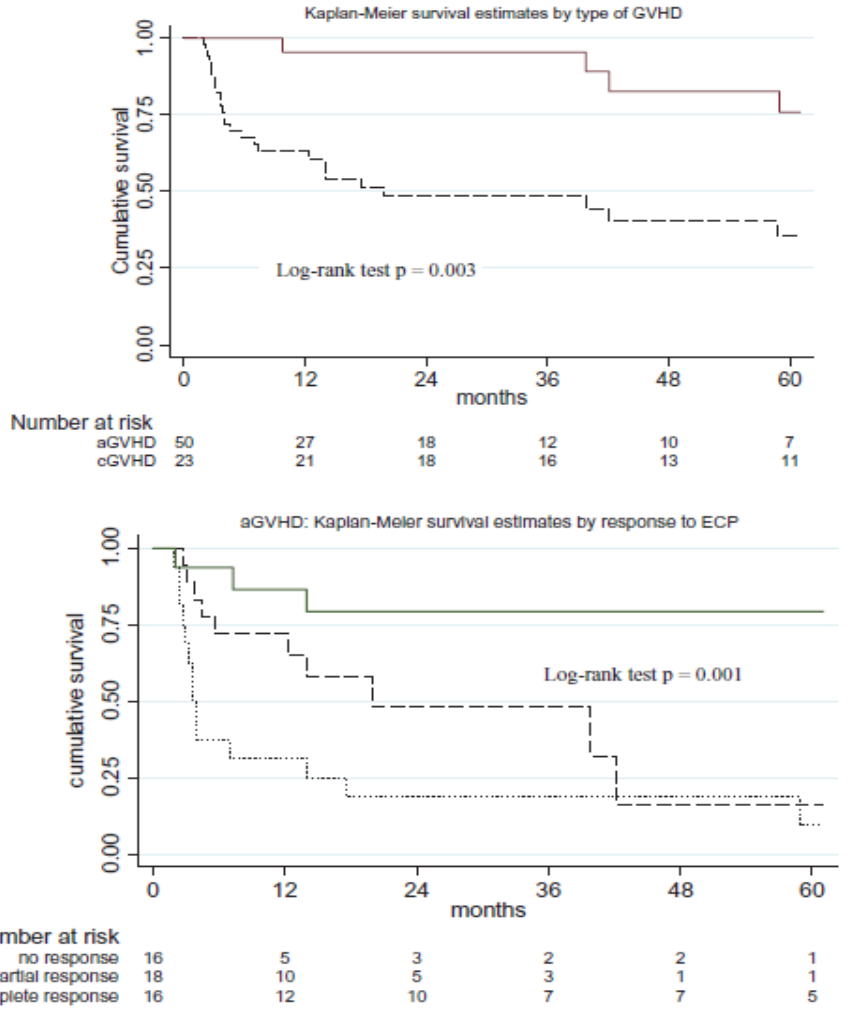


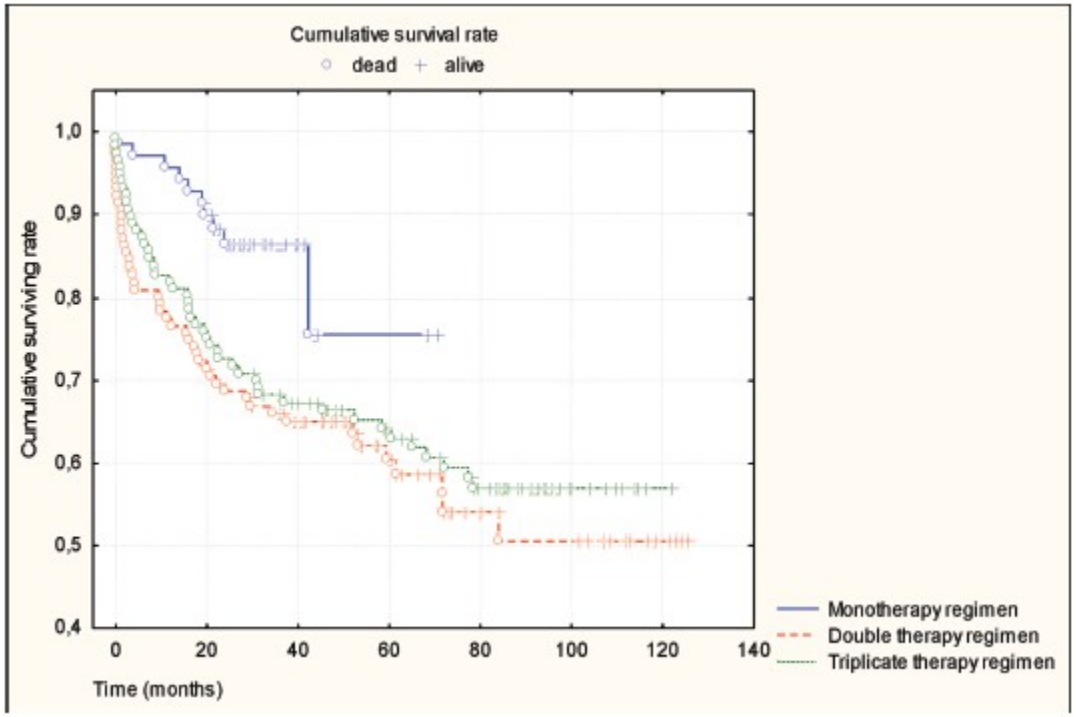
Fig. 2. Survival Kaplan-Meier estimates in 50 aGVHD (– – –) and 23 cGVHD (—) pediatric patients (months from transplantation). Bottom figure: ··· = NR; – – – = PR; — = CR.

A Novel Immunosuppressive Strategy Combined with Preemptive Antiviral Therapy Improves the Eighteen-Month Mortality in HCV Recipients Transplanted with Aged Livers

Lucio Urbani,[1,6] Alessandro Mazzoni,[2] Piero Colombatto,[3] Lucia Bindi,[4] Gianni Biancofiore,[4] Carlo Tascini,[5] Francesco Menichetti,[5] Maurizia Rossana Brunetto,[3] Fabrizio Scatena,[2] and Franco Filipponi[1]

Transplantation • Volume 86, Number 12, December 27, 2008

Monotherapy: 69 patients (CyA in 61 patients, FK in 5, and MMF in 3) associated with ECP (64 patients) and induction with Ab-CD25 (67 patients).
Double-drug regimen: 116 patients (steroids+CyA in 75 patients, steroids+FK in 19, steroids+MMF in 8, and CyA+MMF in 14) associated with ECP (9 patients) and induction with Ab-CD25 (59 patients).
Triple-drug regimen: 117 patients (steroids+CyA+AZA in 69 patients, steroids+FK+AZA in 7, steroids+CyA+MMF in 34, and steroids+FK+MMF in 7) associated with ECP (5 patients) and induction with Ab-CD25 (26 patients).



Extracorporeal photopheresis for the treatment of acute and chronic graft-versus-host disease in adults and children: best practice recommendations from an Italian Society of Hemapheresis and Cell Manipulation (SIdEM) and Italian Group for Bone Marrow Transplantation (GITMO) consensus process

Luca Pierelli, Paolo Perseghin, Monia Marchetti, Chiara Messina, Cesare Perotti, Alessandro Mazzoni, Andrea Bacigalupo, Franco Locatelli, Paolo Carlier, and Alberto Bosi for Società Italiana di Emaferesi and Manipolazione Cellulare (SIdEM) and Gruppo Italiano Trapianto Midollo Osseo (GITMO)

TRANSFUSION 2013;53:2340-2352.

The panel first met on February 7, 2011, and agreed on addressing the following issues:

1. Indications of ECP in aGVHD.
2. Indications of ECP in cGVHD.
3. Indications of ECP in GVHD prophylaxis.
4. Patient evaluation and contraindications to ECP.
5. ECP procedure.

The whole group discussed the proposed recommendations according to the Nominal Group Technique[23] by which participants were first asked to comment in round-robin fashion on their preliminary votes and then to propose a new vote, after this phase of discussion. If an 80% consensus on the statement was not achieved, the choices were further discussed, and a second vote was taken. If an 80% consensus was still not attained, the issue was declared unresolved, and no further attempt was made. The final statements were then agreed upon during three meetings held in Bologna, Italy, between March and July 2011. The conclusions of the project were endorsed by the GITMO and SIdEM. MM and PP were charged with preparing a preliminary draft of recommendations, which was, in turn, reviewed and approved by the whole panel.

TABLE 1. aGVHD: ECP case series*

Author, year	Number	Age (year)	Skin		Liver		Gastrointestinal		Overall response	Survival (%)	Steroid Reduction or stop
			N	R	N	R	N	R			
Greinix, 2000[24]	21	27-55	21	17	12	4	4	0	8	57	
Messina, 2003[25]	33	0-20	33	27	15	9	20	15			
Kanold, 2005[26]	41	<18	40	40	22	>13	25	25	30		
Garban, 2005[27]	12	23-63	12	10	2		5	3	9 (CR+PR)		Stopped in all responders
Greinix, 2006[28]	59	21-60	57†	>47	23	>14	15	>9		47	Stopped after 17-284 days
Berger, 2007[29]	15	6-18	14	11	7	5	10	6	CR 9; NR 6	66	
Kanold, 2007[30]	12	4-18	10	10	9	6	6	5	CR 7; PR 3; NR 2;	80	6 stopped; 3 tapered
Perfetti, 2008[31]	23	18-66	22	>15	4		10	>4	12	48	5 tapered
Calore, 2008[32]	15	1-18	13	12	1	1	14	14	CR 11; PR 4	85	10/15 stopped
Merlin, 2010[33]	12	2-18	11		4		9		CR 6; PR 4	57	
Perotti, 2010[34]	50	<18	47	39	24	24	11	8	CR 16; PR 18	44	8 stopped, 25 tapered

* Empty cells: no data reported in the paper.

† Including patients with both skin and other organ GVHD.

CR = complete response; N = number of assessed patients; NR = no response; PR = partial response; R = number of responders.

Question 2: Is ECP a therapeutic option for sparing immunosuppressive therapy in patients with viral reactivation?

Recommendations. ECP represents a promising approach for treatment of aGVHD in patients for whom further immunosuppression is contraindicated due to viral reactivation or other infectious complications.

Background. Steroids and other immune-suppressive drugs used in the treatment of GVHD are associated with a high risk of infection-related mortality.[26,35] ECP has not been associated with the same frequency of viral reactivation and infectious complications as other more immune-suppressive therapies.[26] ECP has been most successfully applied in hepatitis C–positive patients receiving solid organ transplantation.[33] Therefore, in view of this consideration and of the immune-modulatory effect rather than the panimmunosuppressive one of ECP, the Expert Panel emphasized the high appropriateness of ECP in this setting.

Question 13: Which is the appropriate technology for performing ECP?

Recommendations. ECP may be performed with two methods that differ on the basis of the procedure of MNC collection and UV-A irradiation:

1. In-line procedure: ECP is performed using a single device that provides the MNC collection with a discontinuous or continuous flow method and then the irradiation with UVA. So far, only Uvar XTS and Cellex Therakos systems (Therakos, Inc., Raritan, NJ) allow for this specific ECP treatment.
2. Off-line procedure: ECP is performed by employing two devices:
   i. MNC collection is performed with either a continuous-flow cell separator such as the COBE Spectra (CaridianBCT, Denver, CO), COM.TEC (Fresenius SE & Co., Bad Homburg, Germany), or Amicus (Fenwal, Inc., Lake Zurich, IL) or a discontinuous-flow device: Haemonetics MCS plus (Haemonetics Corp., Braintree, MA);
   ii. the UVA irradiation step can be performed by using the PIT System (Med Tech Solutions, Modena, Italy) or Theraflex-ECP (MacoPharma, Mouvaux, France), both equipped with appropriate UVA irradiation bag.

Question 16: How should ECP quality be monitored?

Recommendations. According to European Guidelines for minimal cell manipulation (Directive 2006/86/EC; Regulation 1394/2007/EC), off-line procedure should be performed in a Class A laminar-air-flow cabinet located in a Class D laboratory. During off-line procedures, cultures of the product for aerobic and anaerobic bacteria and fungi should be done immediately before reinfusion into the patient. Sterility controls before the introduction of 8-MOP are encouraged at least in two nonconsecutive off-line procedures of each therapeutic course.

….don’t forget the patients…

ECP: yet unanswered questions

Clinical and biological issues

1) Indications

2) Patient selection and accrual

3) Treatment schedule(s)

4) Clinical response evaluation

5) Long-term treatment ?

6) Mechanisms of action

Technical issues

1) Suitable devices (pediatric patients)

2) Venous accesses

3) Certification (FDA, CE, etc)

4) Procedure validation

5) 8-MOP

ECP schedule (1)

Author	Year/ journal	Pts (n°)	Diagnosis	Method	Schedule	Cell dose
Greinix	**2000 Blood**	21	aGvHD (II-IV)	On-line	2/w until improvement then 2/2-4 w	**No**
		Response: 100%, 67 % and 12 % (gr. II, III and IV) max. after a median of 4 courses (8 ECP or 2 mts)				
Kanold (review)	**2003 Transf. Aph. Sci**	73	19: aGvHD 54:cGvHD	Both	2w x3w, then 2/2w, 2/4w 3w x 3w then tapering 2w/2w until response 2w/3w x 6 mts	**Most no**
		Response: aGvHD= 63 %, cGvHD=75%				
Foss	**2005 BMT**	25	cGvHD	On-line	2w/2w in 17 pts 1w/until response in 8 pts	**No**
		Response: 66-70%				
Couriel	**2006 Blood**	71	cGvHD	On-line	2-4/w then tapering (1/w) to 2/2w	**No**
		Response: 61 % (overall)				

ECP schedule (2)

Author	Year/ journal	Pts n°	Diagnosis	Method	Schedule	Cell dose
Garban	**2005 Haematol**	27	12:aGvHD 15:cGvHD	Off-line	2w x 3w, then according to response (1w)	**Yes**
		Response: aGvHD=75%, cGvHD= 87 %				
Greinix	**2006 Haematol**	59 (21 p.r)	aGvHD	On-line	2/1-2 w, then 2/ 2-4 w	**No**
		Response: 82% skin, 61 % gut and liver, lower when combined				
Perseghin	***2007*** **Ther Apher Dial**	25	cGvHD	Off-line	2w x 3w, then 2w/2w and 2w/4w	**Yes**
		Response : 80% (maintained > 30 mts in 90% pts)				

Take home message

- …..ECP is a powerful tool and that we are still learning the best way to use it.
- It is time to set up a strong and fruitful cooperation between those centers which currently perform ECP, designing multicenter trials, which aim to ascertain the real effectiveness of ECP and the best way to apply it, asking for financial support from central national and /or European institutions to avoid any company interference.

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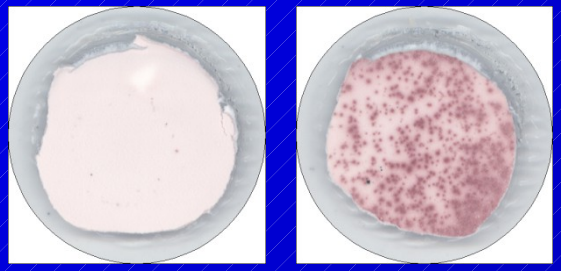
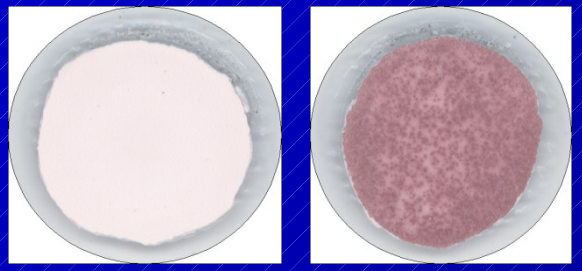
Thank you!

Th17-secreting cells (by Elispot), the principal responsible for GvHD, inversely correlate with % of circulating T cells

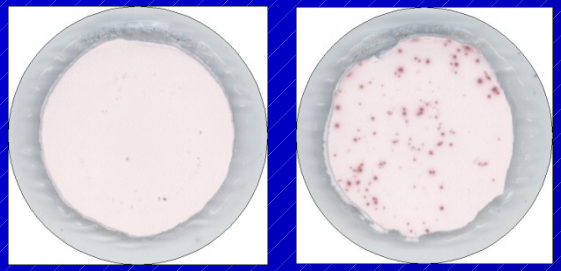
Unstimol PMA/Ionomycin



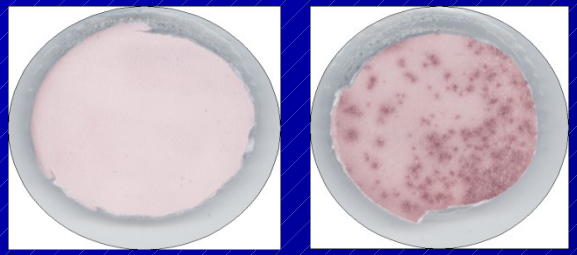
I° CYCLE ECP
%Foxp3/CD4+CD25+ cells = 0%



III° CYCLE ECP
%Foxp3/CD4+CD25+ cells = 0%



VII° CYCLE ECP
%Foxp3/CD4+CD25+ cells = 20%



X° CYCLE ECP
%Foxp3/CD4+CD25+ cells = 0%

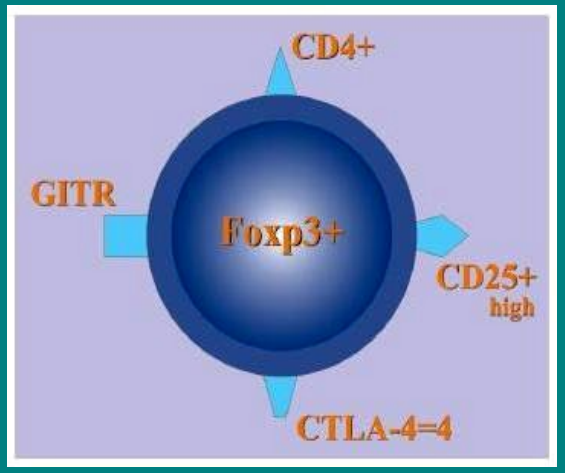
Patients clinical details

TABLE 1. Patient characteristics

Patient	Age (years)	Diagnosis	HSCT	GVHD (type/organs)	Primary treatment	ECPs (n)	Response	Status
1	10	CML	Haplo	Acute/skin	M-PDN/CSA	20	Partial	Deceased, MOF
2	18	Rel NHL	MUD	Acute/skin, gut, liver	M-PDN/CSA/MMF	22	Partial	Drugs tapered
3	12	Fanconi	MUD	Acute/skin	M-PDN/CSA	22	Complete	Drugs discontinued
4	8	MDS	MUD	Acute/skin	M-PDN/CSA	20	Complete	Drugs discontinued
5	6	Rel ALL	Related	Chronic/skin, gut, liver	M-PDN/CSA/MMF	24	Complete	Drugs discontinued
6	16	ALL	MUD	Chronic/skin	M-PDN/CSA	22	Partial	Drugs tapered
7	32	ALL	MUD	Chronic/skin	M-PDN/CSA/MMF	18	Complete	Drugs tapered
8	62	AML	Related	Chronic/skin, eyes	M-PDN/CSA/MMF	20	Partial	Drugs tapered
9	64	AML	MUD	Chronic/skin, oral mucosa, eyes, liver	M-PDN/MMF	22	Complete	Drugs discontinued
10	36	NHL	Related	Chronic/skin, oral mucosa, eyes, liver	M-PDN/CSA	24	Partial	Drugs tapered

Regulatory T cells (T-reg)

- T-cell subset with immunosuppressive properties, useful for self-tolerance maintenance
- Determinant role in inducing and maintaining the tolerance towards the transplanted graft



Various types of T-regs have been described:
- Natural occurring T-reg (CD4, CD25, CD62L, CTLA-4, R, CD45RO, Foxp3)
- Inducible T-reg

Clinically relevant after BMT: increased circulating levels of natural occurring T-regs are related with decreased incidence and severity of GvHD (*Zorn E., Blood 2005)*

FONDAZIONE TETTAMANTI
Per lo studio delle leucemie ed emopatie infantili

Patients characteristics

1. Prospectic analysis on **10 patients**
   - Acute GVHD (4 pts)
   - Chronic GvHD (6 pts)

2. ECP numbers
   - Range: 14-24
   - Median: 20

3. Median Follow-up (from 1st ECP)
   - Range: 5-12 months
   - Median: 9 months

TABLE 2. cGVHD: ECP case series (>10 patients)*

Author, year	Number	Age (years)	Interval (days) since cGVHD (since SCT)	Skin		Liver		Lung		Oral mucosa		Gastrointestinal	
				N	R	N	R	N	R	N	R	N	R
Smith, 1998[38]	18	5-53	2-47	10	4	13	12	3	0	7	2		
Greinix, 1998[39]	15	25-55	4-23	15	15	10	9			11	11		
Zic, 1999[40]	11			8	6	5	2			10	2	3	3
Child, 1999[41]	11	18-47		10	10	6	6	5	2	4	3		
Salvaneschi, 2001[42]	14	6-20	1-110	12	10	9	6	1	1	12	8		
Apisarnthanarax, 2003[43]	32	>70	10-1515 (109-1906)	32	18	17	0			11	0	11	0
Messina, 2003[25]	44	0-20	12-3300 (10-280)	36	20	20	16	14	6	26	0	21	10
Seaton, 2003[44]	28	18-51	12-5000 (305-5098)	21	10	25	0	9	0	14	3		
Rubegni, 2005[45]	32	18-60	(30-1700)	27	22	22	10	5	2	25	23		
Kanold, 2005[26]	63			51	31	33	24	15	7	33	0	27	16
Foss, 2005[46]	25	18-59	(242-2928)	25	20	6	0	2	2	13	6	2	2
Garban, 2005[28]	15	14-62	<365 days in 11 of 15	12	12	3	1	3	3			9	7
Bisaccia, 2006[47]	14	25-57	30-793 (5-96)	14	7	5	5	3	1	7	3		
Couriel, 2006[48]	71	>70		56	33	21	15	11	6	9	7	3	2
Perseghin, 2007[49]	25	6-55	(100-726)	25	20	6	4			9	7	2	2
Kanold, 2007[30]	15	5-18	37-1399	12	9	11	6	1	0	7	6	6	5
Flowers, 2008†[19]	48	16-67	85-2743 (69-637)	48	17	14	3	9	1	30	16	2	1
Perotti, 2010[34]	23	<18		22	22	4	4	3	2	8	4	5	6
Dignan, 2011[50]	82	14-69	(>184)	75	57					39	29		
Greinix, 2011†[20]	29	20-67	120-2400	29	9								
Del Fante, 2012[51]	102	33-54	102-287 (87-883)	91	NA	44	NA	13	NA	66	NA	30	NA

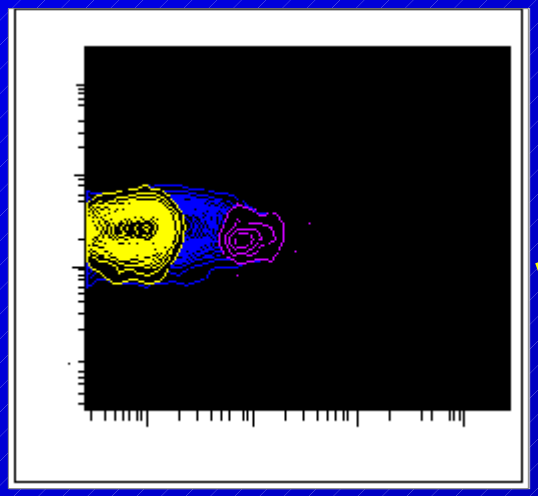
* Empty cells: no data reported in the paper.
† Randomized clinical trials, NA = not available (not detailed in the paper).
N = number of assessed patients; R = number of responders.

TABLE 3. cGVHD: ECP case series (>10 patients)

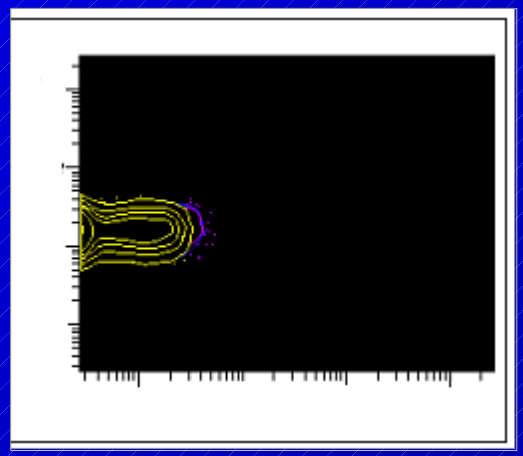
Author, year	Number	Overall response	Complete response	Number of surviving patients*
Smith, 1998[39]	18	6	3	7
Greinix, 1998[39]	15	15	6	14
Zic, 1999[40]	11			8
Child, 1999[41]	11			9
Salvaneschi, 2001[42]	14	9	4	38
Apisarnthanarax, 2003[43]	32	18	7	
Messina, 2003[25]	44	25†	15†	13‡
Seaton, 2003[44]	28			24
Rubegni, 2005[45]	32	25		
Kanold, 2005[26]	63	40		50
Foss, 2005[46]	25	16		15
Bisaccia, 2006[47]	14			11
Couriel, 2006[48]	71	43	14	29§
Perseghin, 2007[49]	25	18	11	19
Kanold, 2007[30]	15	11	4	10
Flowers, 2008[19]	48			47
Jagasia, 2009[52]	31	20	3	16‖
Akhtari, 2010[53]	25	14		16¶
Perotti, 2010[34]	23	11		18
Greinix, 2011[20]	29	9		
Del Fante, 2012[51]	102	82	16	80††

* Overall survival reported at 5-year follow-up.
† Patients assessed for response were 34.
‡ Overall survival in responders 96% versus 58% of nonresponders.
§ Overall survival at 5 years in responders 63% versus 10% in nonresponders.
‖ Overall survival at 3 years was significantly higher in responders.
¶ Overall survival at 2 years 88% in responders versus 18% in nonresponders.
†† At a median of 3.6 years. Empty cells: no data reported in the paper.
[Correction added after online publication 10-Jan-2013: Note ** has been removed.]

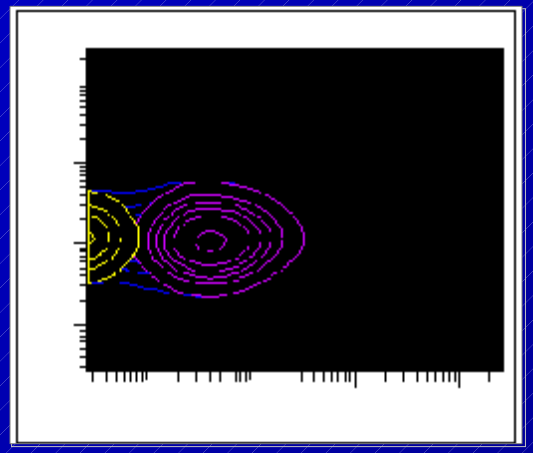
T-reg functional analysis: FACS sorting



CD25



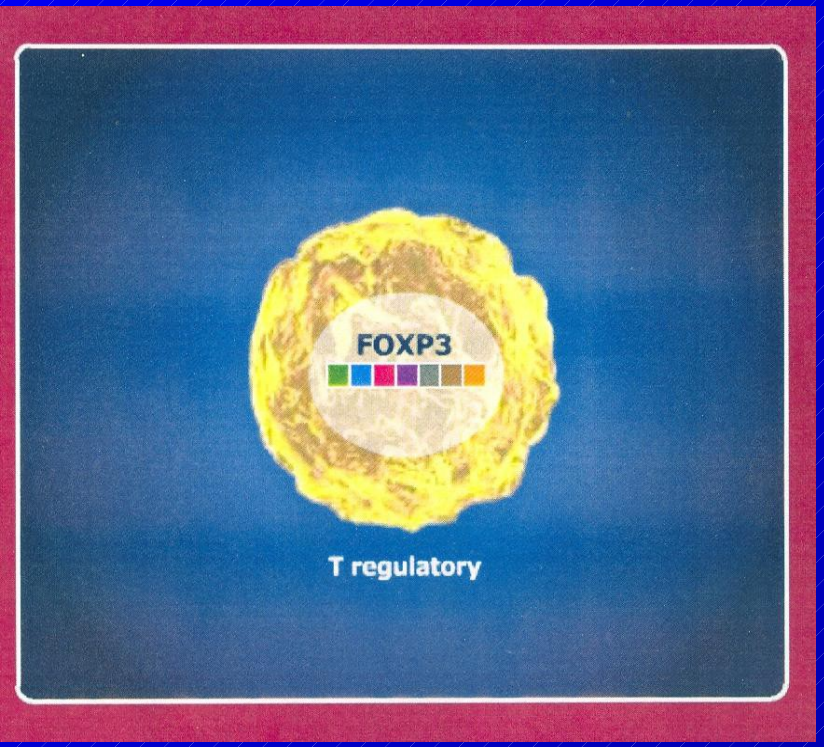
CD25 neg. fraction



CD25 pos. fraction

- qRT-PCR for Foxp-3, TGF-beta, IL-10
- IFN-gamma Elispot for alloreactivity inhibition

FOXP3



1. Member of the forkhead transcription factor family
2. FOXP3 gene maps to chromosome Xp11.23
3. Expressed, exclusively, within the nuclei of CD4+CD25+ regulatory T cells
4. Selective marker for regulatory T cells
5. Involved in the activation, differentiation and homeostasis of T-reg

Patients responding to ECP present a marked increase of T-reg, which is not observed in ECP non-responder patients. T-reg are, as expected, Foxp3-positive