XV Congresso Nazionale SIdEM
XVI Corso di aggiornamento in emaferesi

Torino, 9-12 Novembre 2011

Fotoferesi extracorporea nella GvHD e markers biologici di efficacia

Paolo Perseghin

Dipartimento di Patologia Clinica
Unità di Aferesi e nuove tecnologie trasfusionali
Ospedale San Gerardo de' Tintori
Università di Milano-Bicocca

Photopheresis or Extracorporeal Photochemotherapy (ECP)

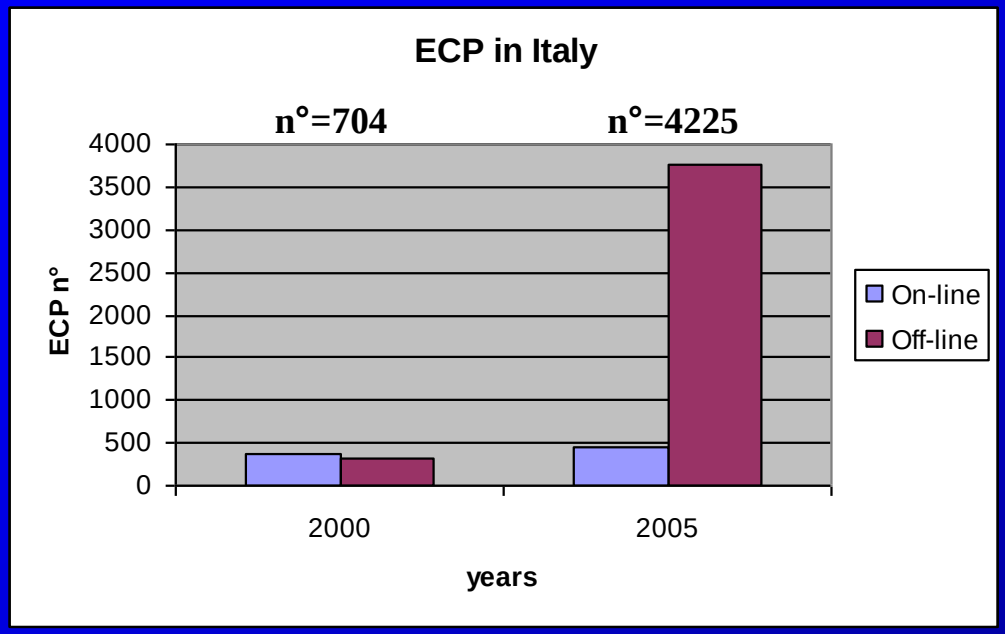
- First applied by Edelson et al. for the treatment of CTCL (1987, NEJM)
  FDA approved
- Organ rejection (kidney, heart, liver, etc…)
- SLE, Systemic Sclerosis
- Rheumatoid Arthritis, Lichen planus
- Pemphigus vulgaris
- Acute and chronic GvHD
- Post-TX Bronchilitis obliterans
- Type 1 Diabetes, Crohn disease, …
- ……

So far, published studies on ECP (mostly retrospective) differ in:

Devices and methods (Therakos vs two-step technique)

- Treatment schedule
- Patient selection criteria

Up-to-now ECP activity in Italy



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

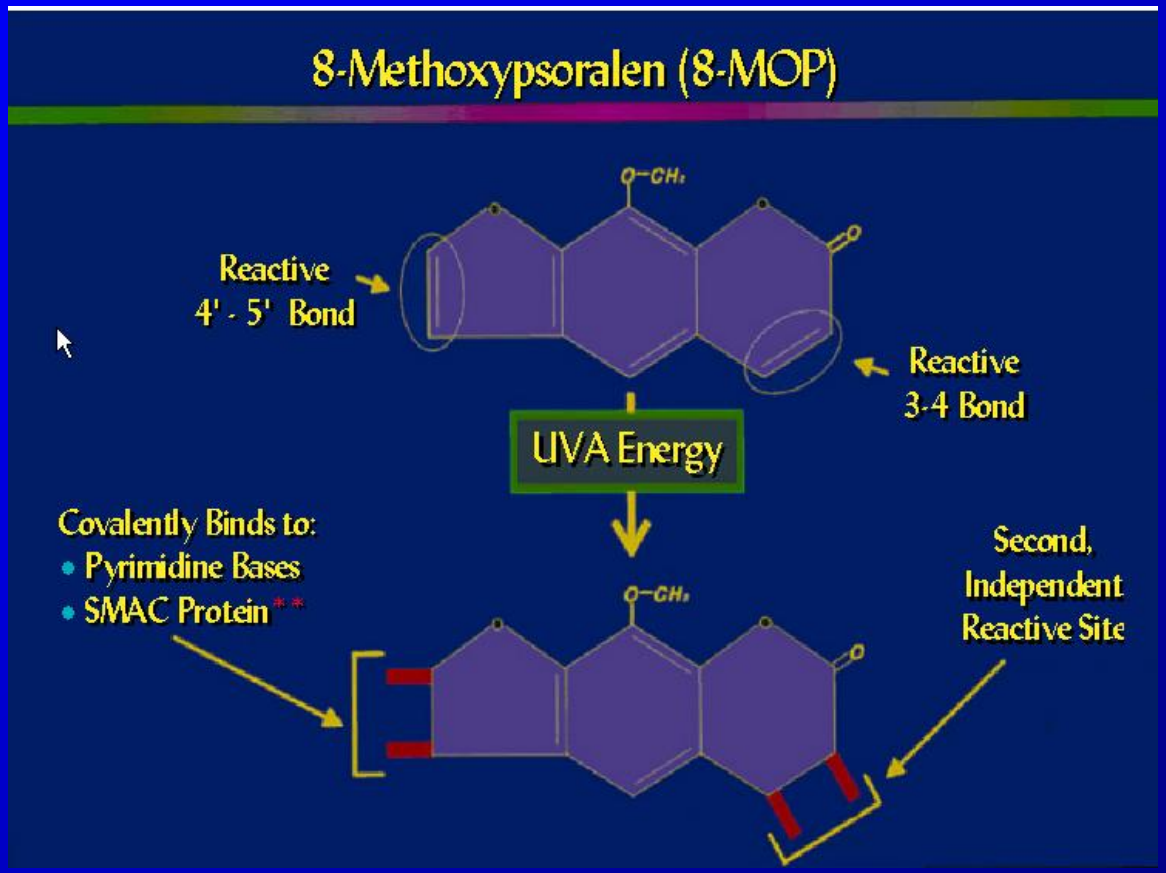
Yr 2005: preliminary data derived from 13 Apheresis centers (2)

- **On-line: 3 centers, 20 pts**
- **Off-line: 12 centers, 173 pts**

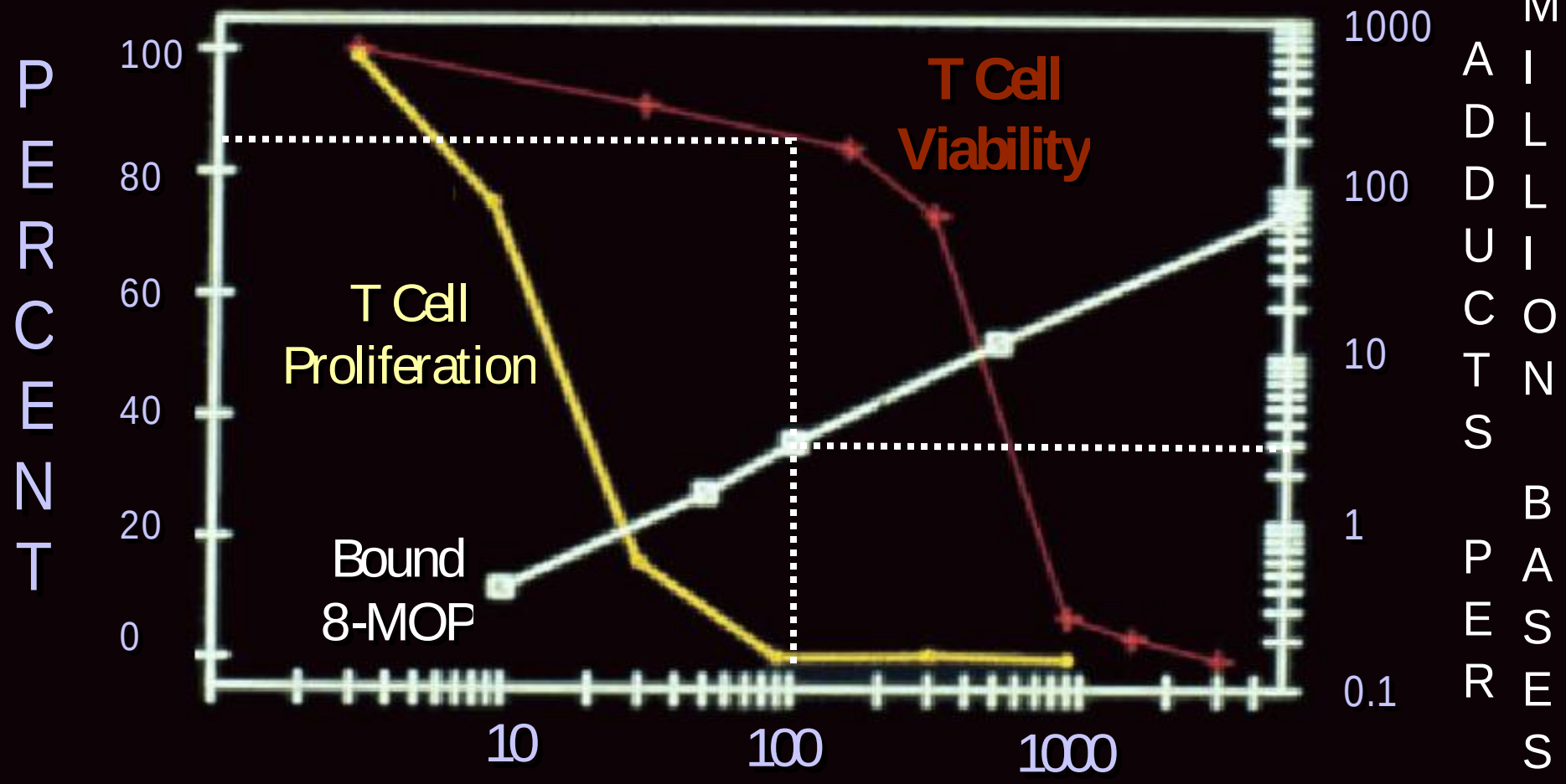
1) G. De Silvestro et al: National survey of apheresis activity in Italy. Trans Apher Sci 2004

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

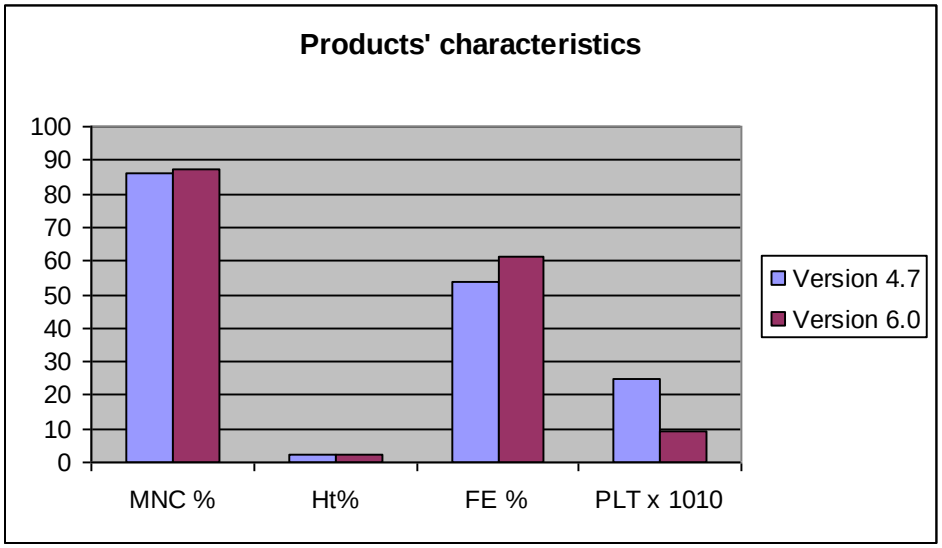
8-Methoxypsoralen (8-MOP)



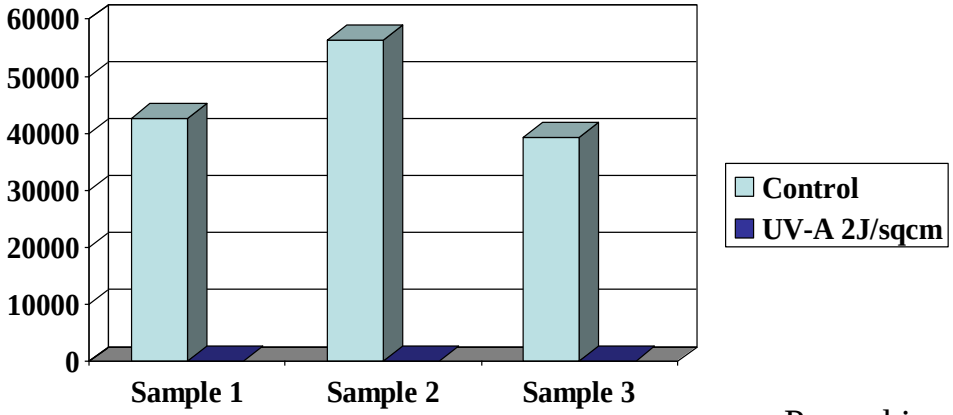
EFFECTS of 8-MOP and UVA on T CELL RESPONSE to MITOGEN



Procedure validation



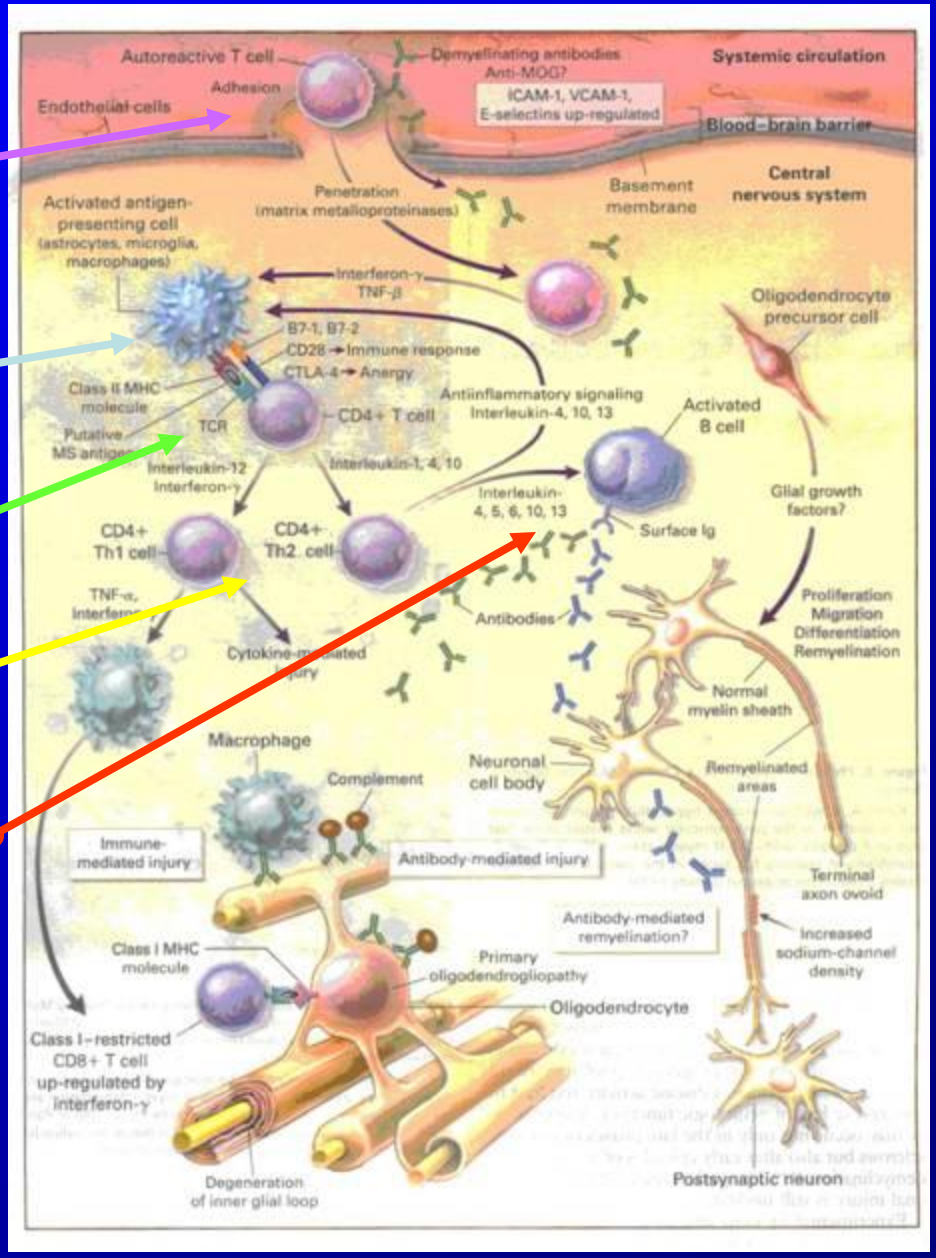
MNC response to PHA stimulation (cpm)



Perseghin et al, J Clin Apher 2001

Mechanisms of ECP action

- modification of endothelial adhesion molecules with reduced T-lymphocyte migration
- modification of the expression of MHC molecules on the plasmamembrane
- alteration of the TCR of the activated cells
- shift in Th1/Th2 balance
- enhancement in the regulatory action of CD8+ T-cells activated by the antigen
- 8-MOP cross-linking with DNA leading to apoptotic death of the activated cells within 10-15 days
- generation of DCs from monocytes
- generation of clone-specific suppressor T-cells



Extracorporeal photopheresis induces apoptosis in the lymphocytes of cutaneous T-cell lymphoma and graft-versus-host disease patients

J. Bladon and P. C. Taylor *Department of Haematology, Rotherham General Hospital, South Yorkshire*

British Journal of Haematology, 1999, 107, 707–711

Lymphocytes treated by extracorporeal photopheresis can down-regulate cytokine production in untreated monocytes

John Bladon, Peter C. Taylor

Department of Haematology, Rotherham General Hospital, South Yorkshire, UK

Photodermatol Photoimmunol Photomed 2005; 21: 293–302

ECP-treated lymphocytes of chronic graft-versus-host disease patients undergo apoptosis which involves both the Fas/FasL system and the Bcl-2 protein family

Arch Dermatol Res (2003) 295 : 175–182

M. Di Renzo · P. Rubegni · P. Sbano · A. Cuccia
C. Castagnini · G. Pompella · A. L. Pasqui · P. L. Capecchi
A. Auteri · F. Laghi Pasini · M. Fimiani

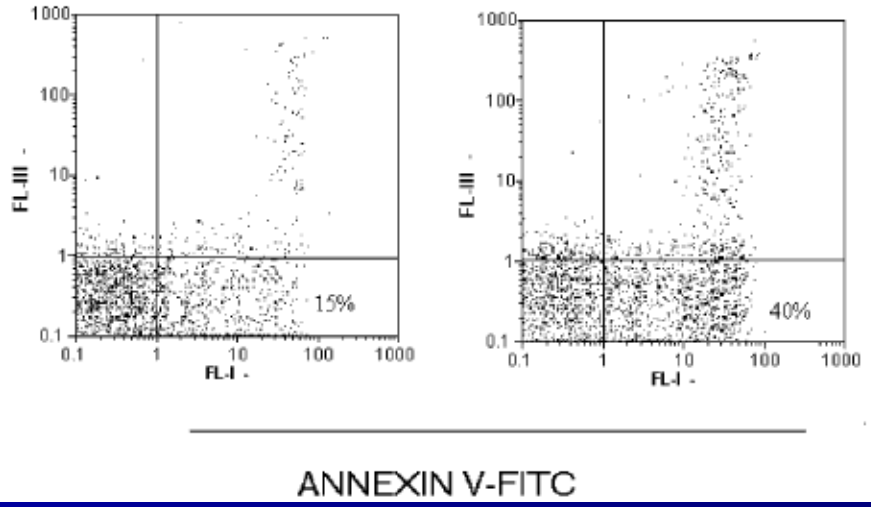


Fig. 1A, B Percentage of annexin V^+/PI^- lymphocytes. PBMC were isolated from peripheral blood before ECP (sample 1, A) and from ECP-treated buffy coat (sample 3, B) and cultured for 48 h. The percentage of annexin V^+/PI^- lymphocytes was determined by flow cytometry after setting the gate on lymphocytes. Shown is one experiment out of ten performed

ECP: Putative mechanism/s of action

Klassen J, Curr Oncol 2010

- **Clearance of apoptotic cells by antigen-presenting cells results in differentiation of those cells into a more tolerogenic phenotype leading to decreased stimulation of effector T cells or their deletion.**
- **Production of anti-inflammatory cytokines, especially interleukin 10, is increased .**
- **Production of pro-inflammatory cytokines, especially interleukin 12 and tnfα, is decreased .**
- **Generation of CD4+, CD25+, GITR+, Foxp3+, CD62L+ T-regulatory cells occurs .**

It is of considerable interest that the T- and B-cell responses to novel and recall antigens remain intact in patients treated with **ECP**. Thus, there appears to be a reduced risk of infections with the use of **ECP** as compared with the use of other immunosuppressive agents

Extracorporeal photo-apheresis for the treatment of steroid-resistant graft versus host disease ☆,☆☆

Kristin Baird *, Alan S. Wayne

Pediatric Oncology Branch, Center for Cancer Research (CCR), National Cancer Institute (NCI), National Institutes of Health (NIH), Bethesda, MD, USA

GvHD: an unresolved issue

- 15-20.000 allo-HSCT/yr
- La ridotta mortalità peri-HSCT ha incrementato il n° dei long-term survivor e quindi il rischio di sviluppare GvHD
- aGvHD:
  - -30-50 % matched-related HSCT
  - -50-80 % matched-URD HSCT
  - - grade III: 25% survivor a 5 anni
  - - grade IV: 5% survivor a 5 anni

Terapia standard: PDN ± CSA: risposta 40-60 %... Altre terapie (MSC)

- cGvHD:
  - - 30-60 % (PBSC > BM)
  - - Poor prognosis : 40 % survivor a 5 anni
  - - Good prognosis: 70% survivor a 5 anni
  - - Terapia: PDN, CSA, Tacrolimus, MMF, etarnecpt, sirolimus, Rituximab, ECP

Transfusion and Apheresis Science 41 (2009) 209–216

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)				

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

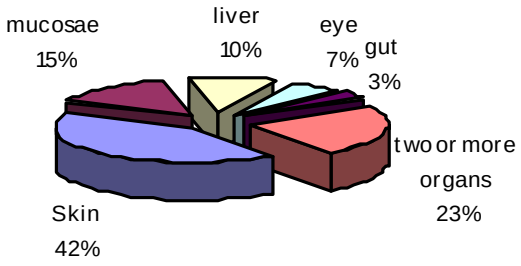
25 patients who underwent:

-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

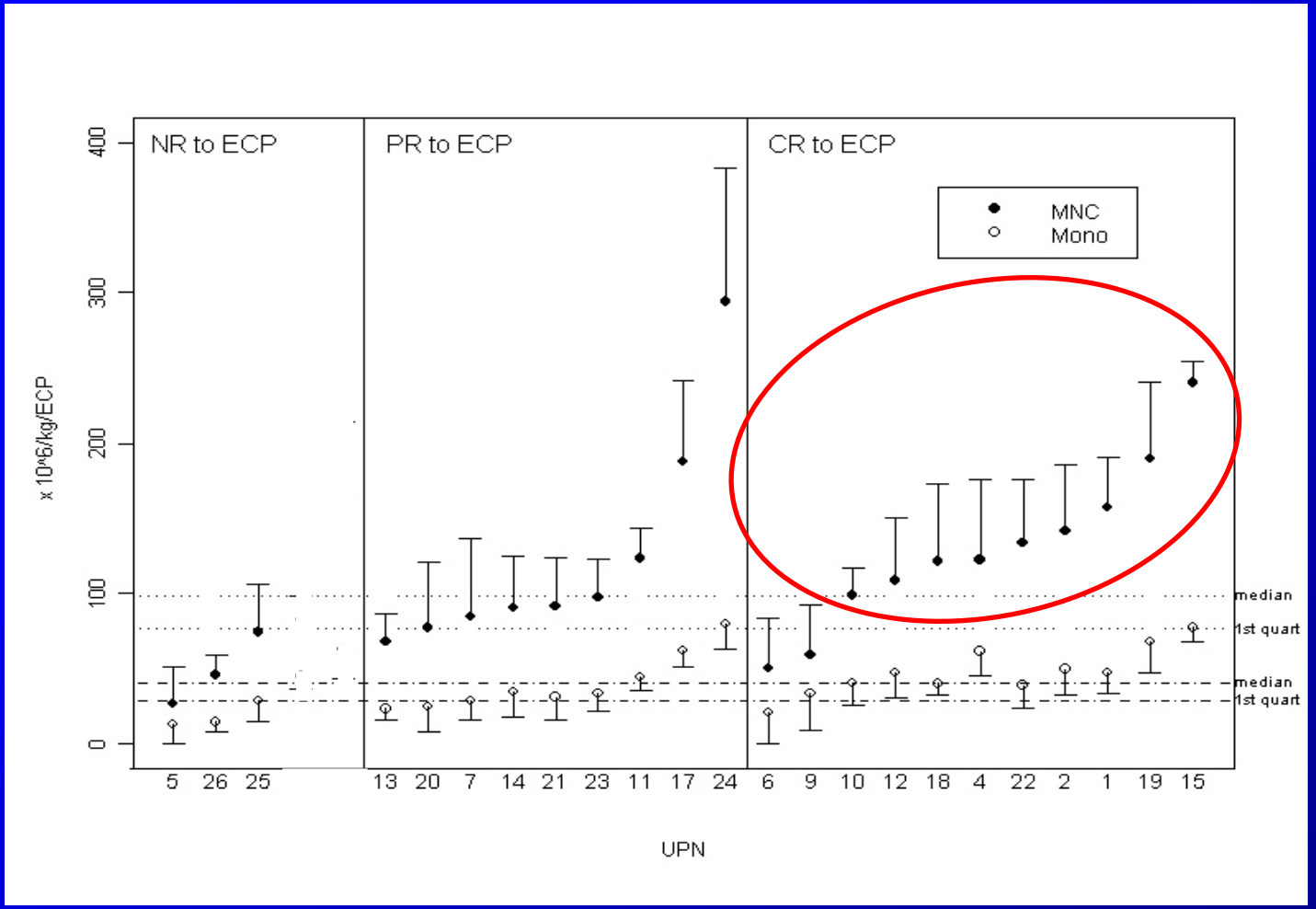
Main diagnosis for HSCT: AML (8), ALL (7), CML (4), other diseases (6)

- **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**



A cell dose/ECP of at least 75 x10^6 MNC/Kg identified 85 % of responsive patients (CR+PR)

100x 10^6/MNC/Kg identified 82 % of CR.

Perseghin et al. Ther Apher Dial, *2007*

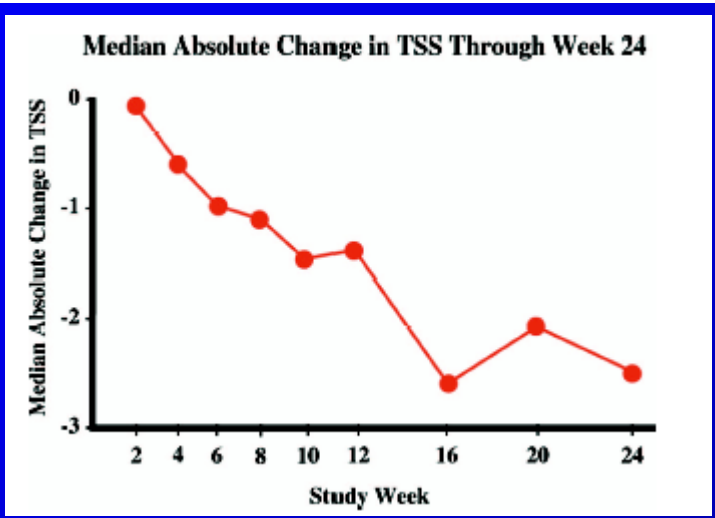
A multicenter prospective phase 2 randomized study of extracorporeal photopheresis for treatment of chronic graft-versus-host disease

*Mary E. D. Flowers,[1] Jane F. Apperley,[2] Koen van Besien,[3] Ahmet Elmaagacli,[4] Andrew Grigg,[5] Vijay Reddy,[6] Andrea Bacigalupo,[7] Hans-Jochem Kolb,[8] Luis Bouzas,[9] Maurioette Michallet,[10] H. Miles Prince,[11] Robert Knobler,[12] Dennis Parenti,[13] Jose Gallo,[13] and *Hildegard T. Greinix[14]

BLOOD, 1 OCTOBER 2008 • VOLUME 112, NUMBER 7

Table 3. Total Skin Score (TSS) and corticosteroid response to ECP treatment

Parameter	Week 12			Week 24	
	ECP, n = 48	Control, n = 47	*P*	ECP, n = 48	Control, n = 47*
Median percent change from baseline in TSS	−14.5	−8.5	.48	−31.4	N/A
> 50% reduction in corticosteroid dose, n (%)†	12 (25)	6 (12.8)	.13	19 (39.6)	N/A
> 50% reduction in corticosteroid dose and > 25% improvement in TSS, n (%)	4 (8.3)	0 (0.0)	.04	11 (22.9)	N/A
> 50% reduction in corticosteroid dose and final corticosteroid dose of < 10 mg/day, n (%)†	10 (20.8)	3 (6.4)	.04	17 (35.4)	N/A



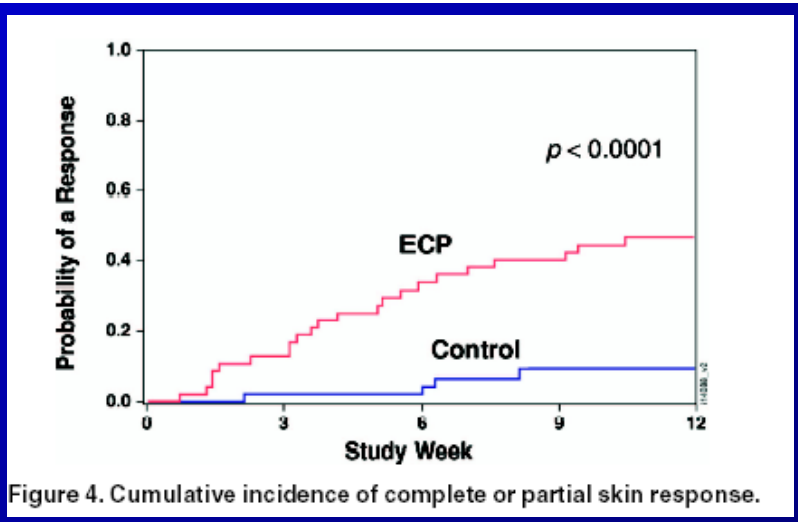


Figure 4. Cumulative incidence of complete or partial skin response.

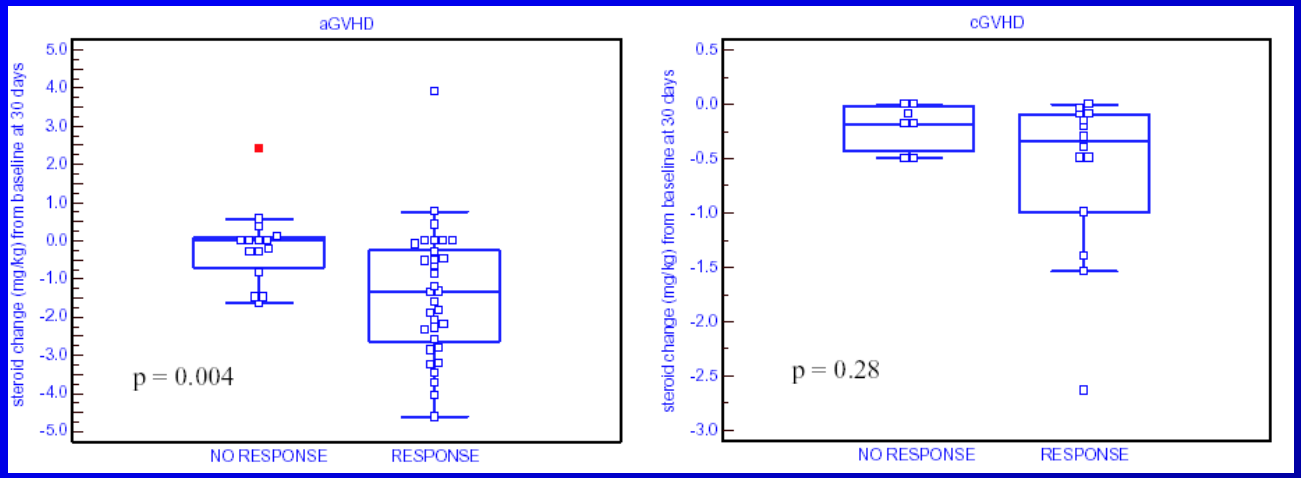
95 pazienti

The results of this study suggest that ECP may have a steroid-sparing effect in the treatment of chronic GVHD, as evidenced by reduction in corticosteroids concomitant with improvement in skin disease assessed by a blinded observer.

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

Perotti et al, Transfusion 2010

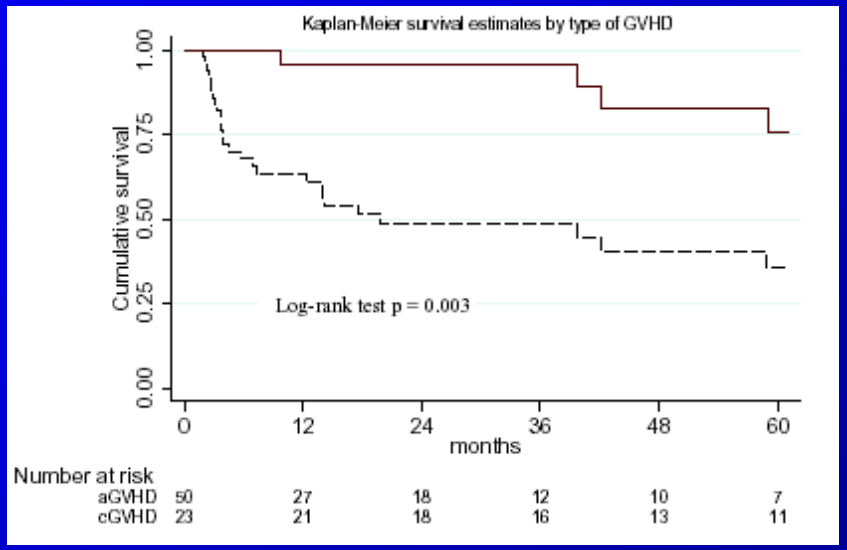
Dose PDN

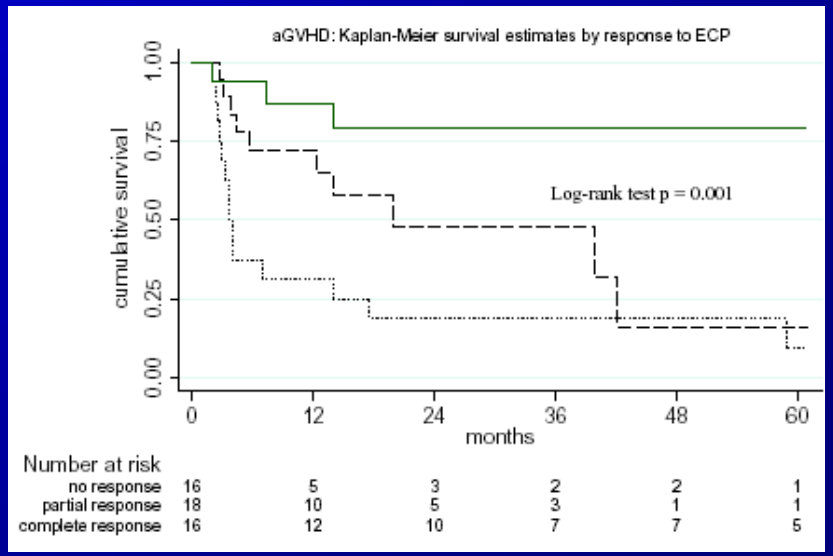


A gg+ 30 dall'inizio

50 p. aGvHD

23 p. cGvHD





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

Table 1

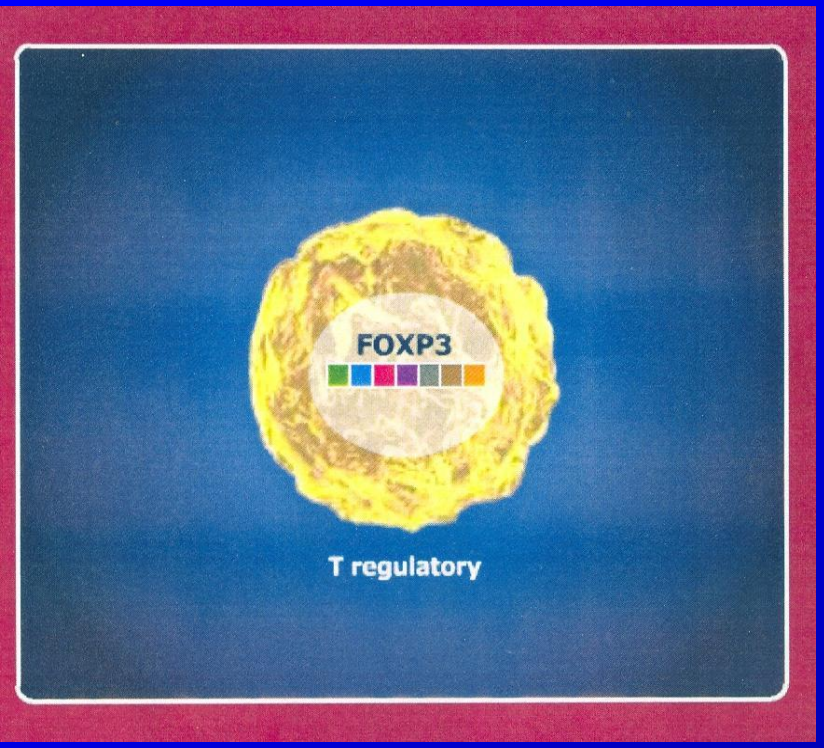
Clinical studies in allogeneic hematopoietic stem cell transplantation.

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]

FOXP3

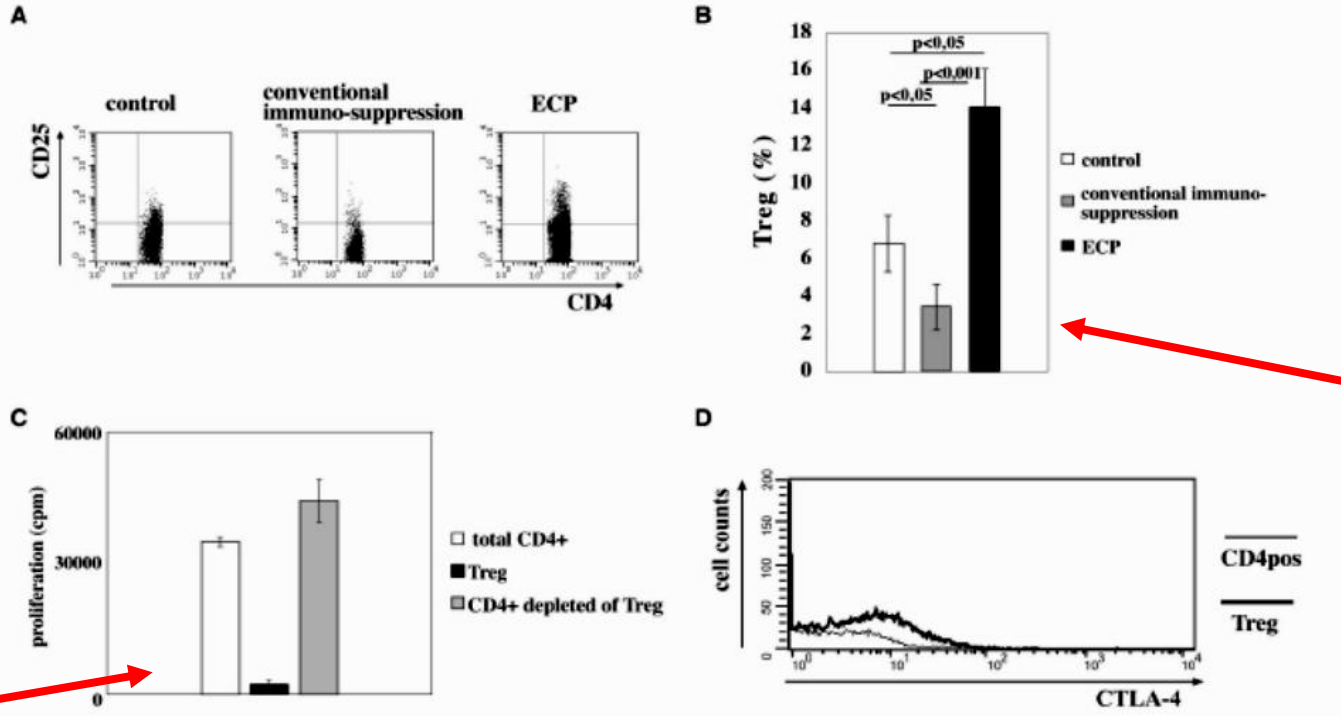


1. Member of the forkhead transcription factor family
2. FOXP3 gene maps to chromosome Xp11.23
3. Expressed, exclusively, within the nuclei of CD4+CD25+ regulatory T cells
4. Selective marker for regulatory T cells
5. Involved in the activation, differentiation and homeostasis of T-reg

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]

Transplantation, 2005



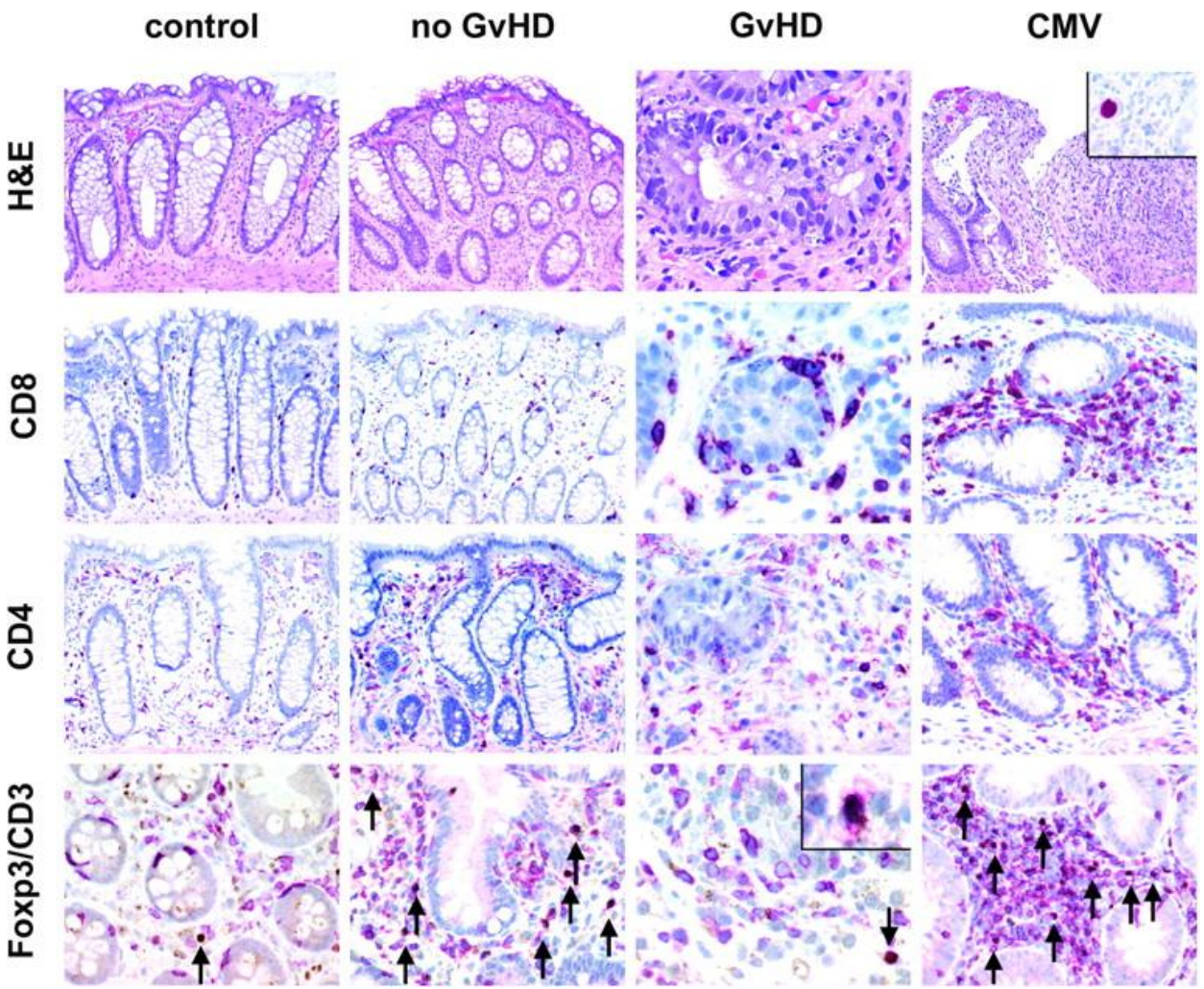
% T reg

Inibizione proliferazione

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.

blood
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY

Figure 2. Histology and immunohistochemistry for CD4+, CD8+, and CD3+FOXP3+ T cells of representative colonic biopsies from healthy controls and patients with no GvHD, with GvHD after bone marrow transplantation, and with CMV infection



Rieger, K. et al. Blood 2006;107:1717-1723

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]

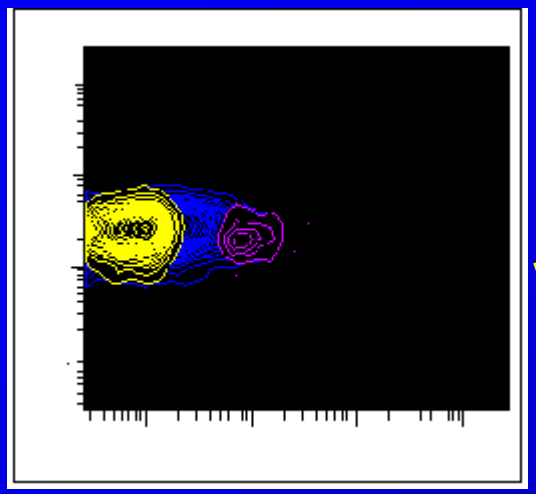
Biagi et al, Transplantation, 2007

Study design

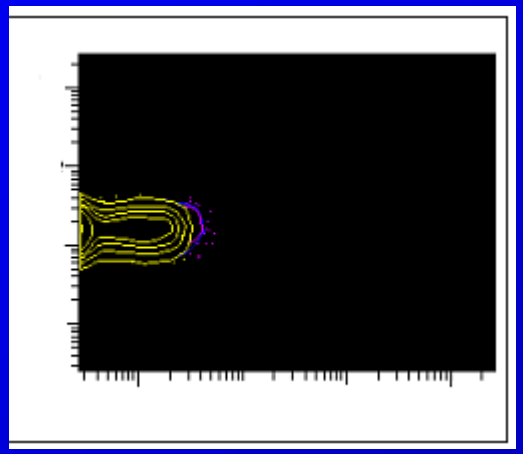
1. Immune-phenotyping of circulating T-regs
   - CD4-CD25 intermediate, bright (comparison with healthy donors and transplanted patients not receiving ECP)
   - GITR, CD45RO, CD62L and Fox-p3 (intracytoplasmic staining)

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)

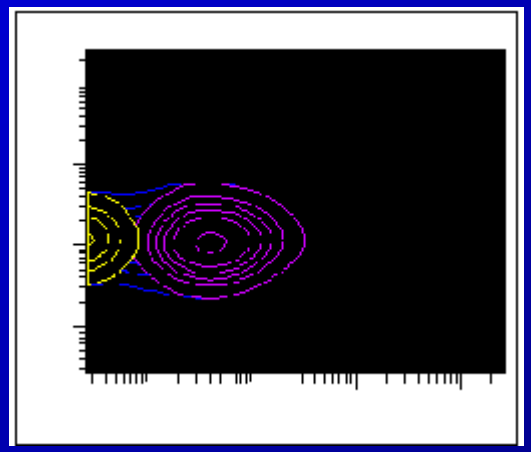
T-reg functional analysis: FACS sorting



CD25

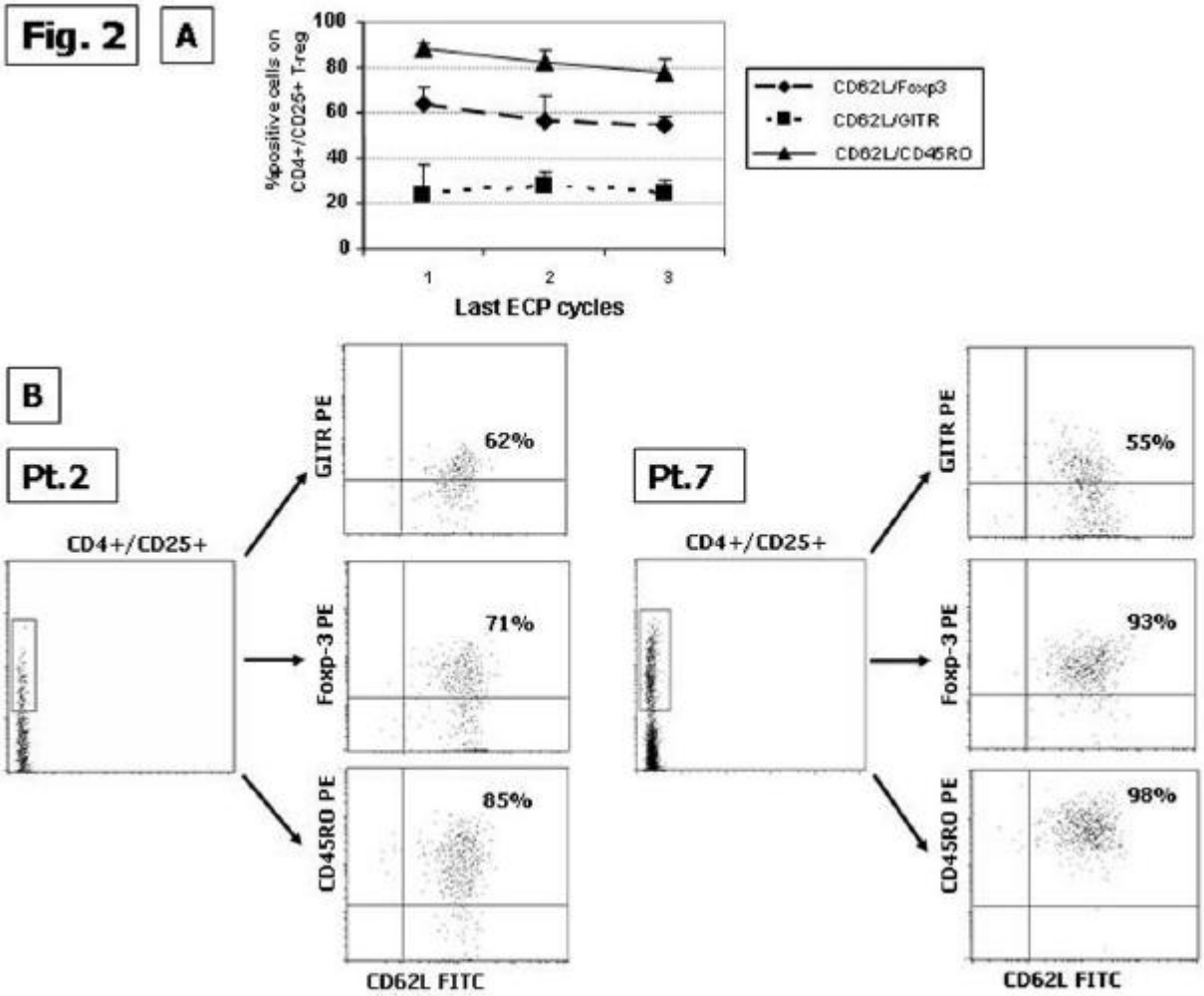


CD25 neg. fraction



CD25 pos. fraction

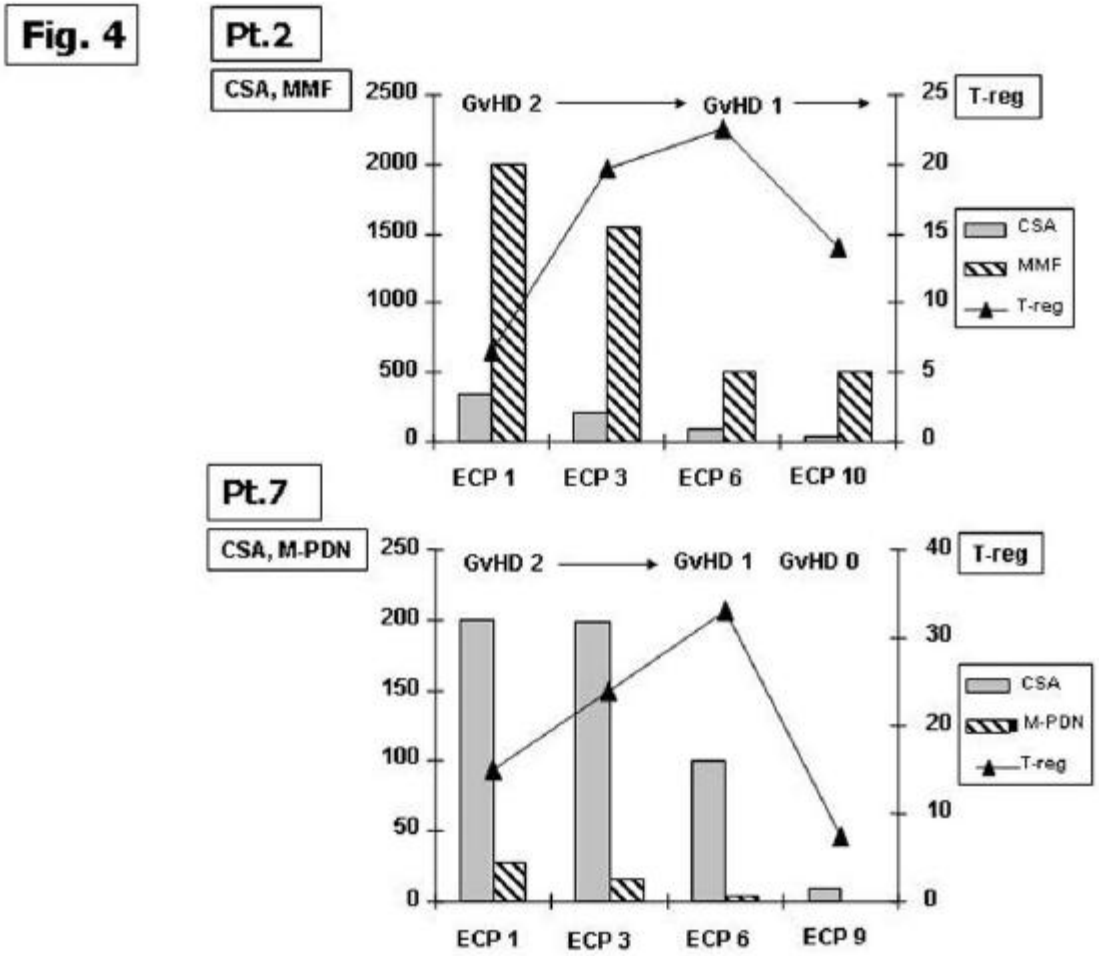
- qRT-PCR for Foxp-3, TGF-beta, IL-10
- IFN-gamma Elispot for alloreactivity inhibition



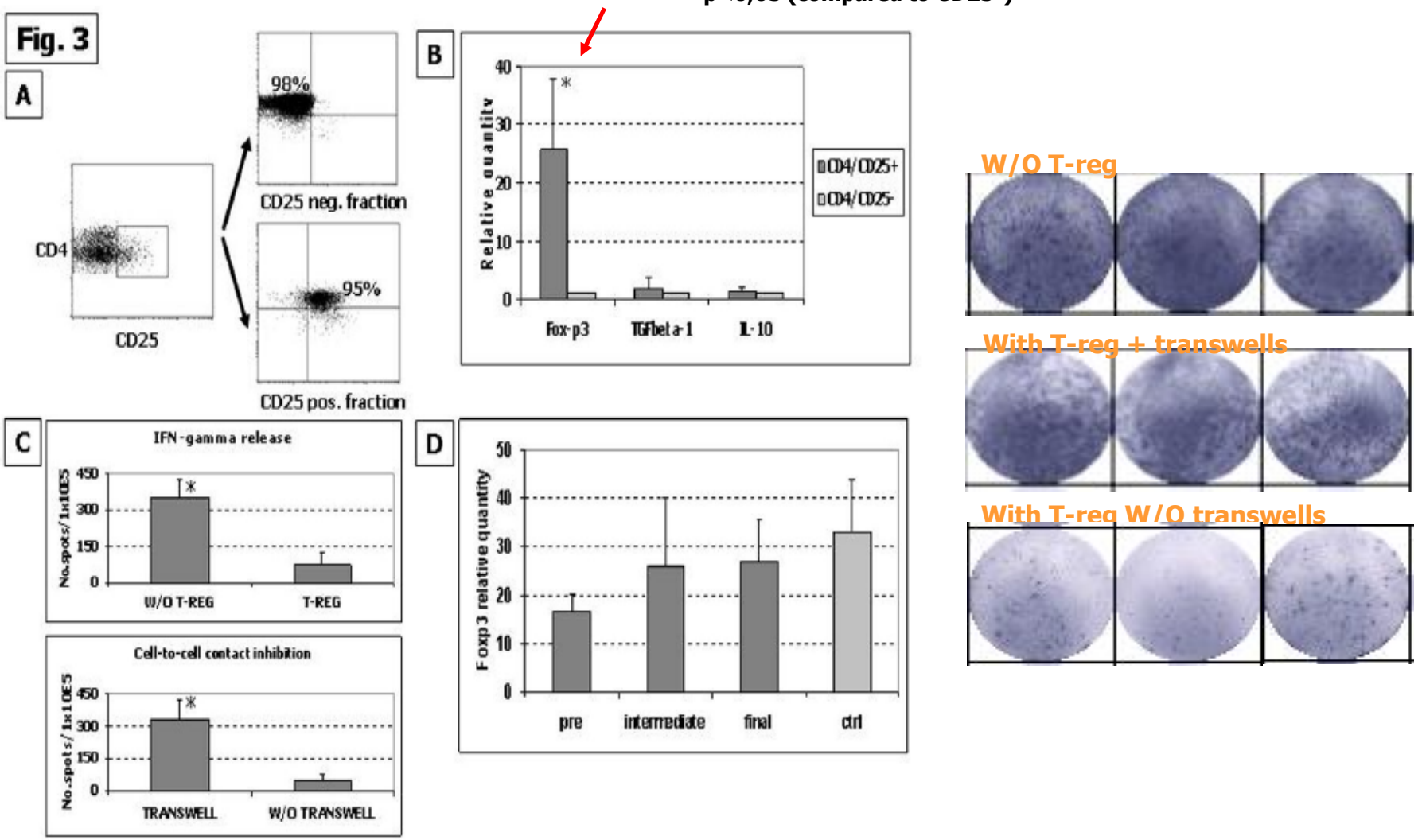
CD62L mediates homing into peripheral lymph-nodes, whereT-regs exert their inhibitory action towards allo-reactive T cells in organs affected by GvHD (GI tract)

Biagi et al. Transplantation, 2007

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



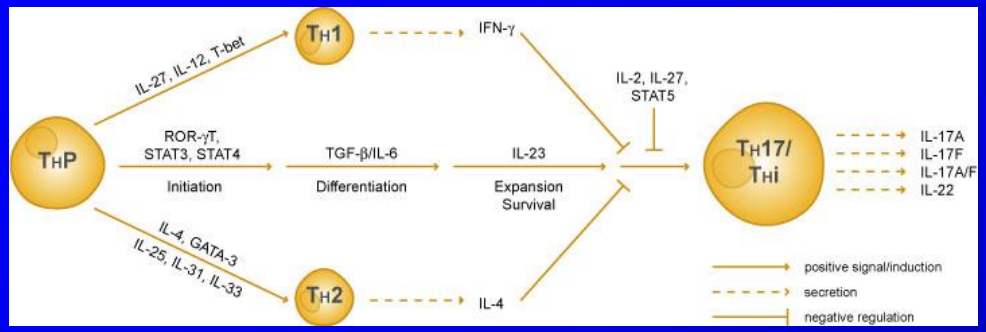
Biagi et al. Transplantation, 2007

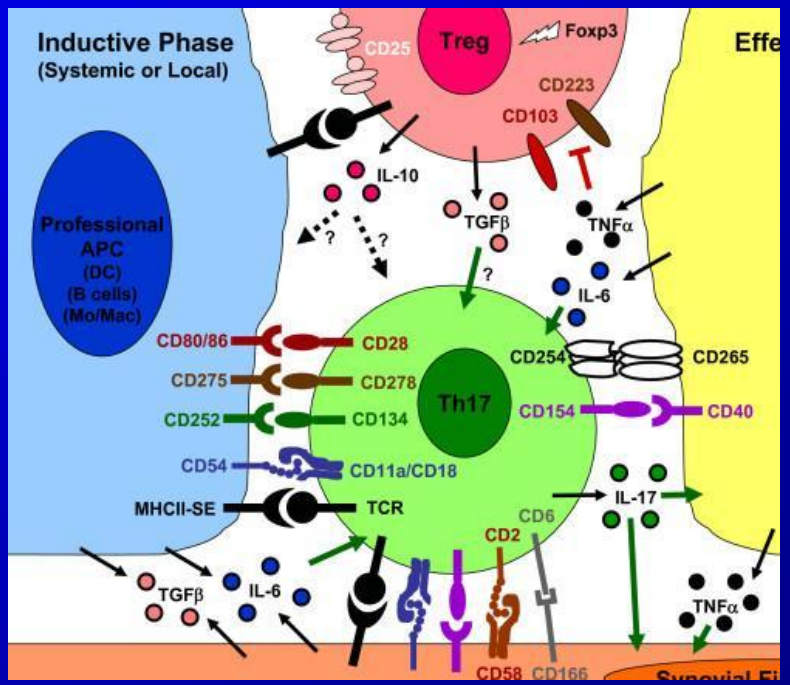


T-regs show inhibitory capacity towards allo-reactivity

Biagi et al. Transplantation, 2007

Next step: Trigger role of Th17 Lymphocytes (IL 17 producers) in GvHD





IL-17 plays a central role in the induction of autoimmune tissue injuries and inflammation, in allograft rejection and in hypersensitivities.

Regulatory T Cells and Extracorporeal Photochemotherapy: Correlation With Clinical Response and Decreased Frequency of Proinflammatory T Cells

Iolanda Di Biaso,[1] Lucia Di Maio,[1] Cristina Bugarin,[1] Giuseppe Gaipa,[1] Erica Dander,[1] Adriana Balduzzi,[1] Matteo Parma,[2] Giovanna D'Amico,[1] Paolo Perseghin,[3] Andrea Biondi,[1] and Ettore Biagi[1,4]

Transplantation • Volume 87, Number 9, May 15, 2009

Median age: 22 years (4-64)

Sex (M/F): 21/6

Transplantation type :

- **HAPLO = 10**
- **MUD = 13**
- **RELATED= 4**

GvHD:

- **acute= 9**
- **chronic= 18**

Initial therapy:

- **M-PDN / CSA = 11**
- **M-PDN / CSA / MMF = 5**
- **M-PDN / CSA / MMF/ETANER/MPDN = 4**
- **M-PDN / CSA / MMF/ GLIVEC = 3**
- **M-PDN / MMF = 2**
- **M-PDN / CSA / MMF/GLIVEC/RITUX = 1**
- **M-PDN/CSA/MMF/GLIVEC/RITUX/ETANER=1**

Number of ECP:

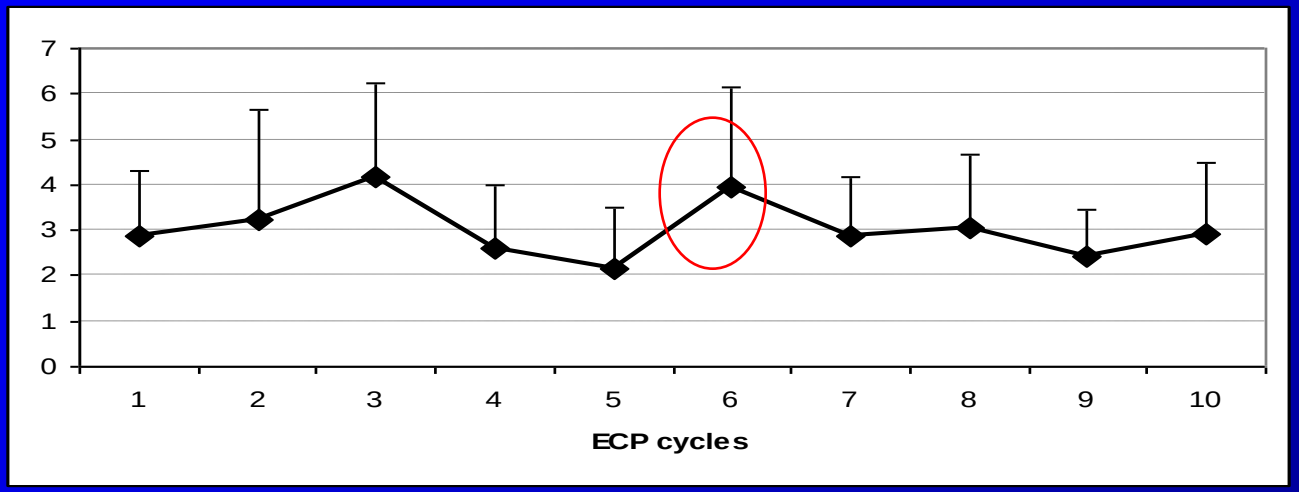
- **mean 20**
- **range 14-29**

Follow-up (from the first ECP):

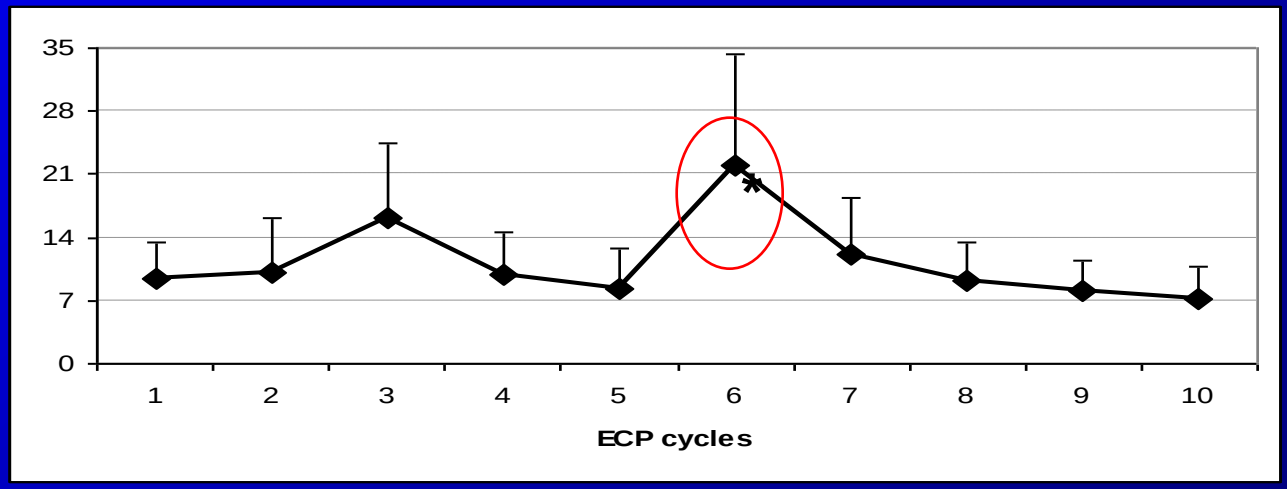
- **median 22 months**
- **range 18-26 months**

%CD4+CD25+ T-reg increases during ECP administration, particularly after 6 cycles (overall analysis)

%CD4+CD25+/ CD3+ cells

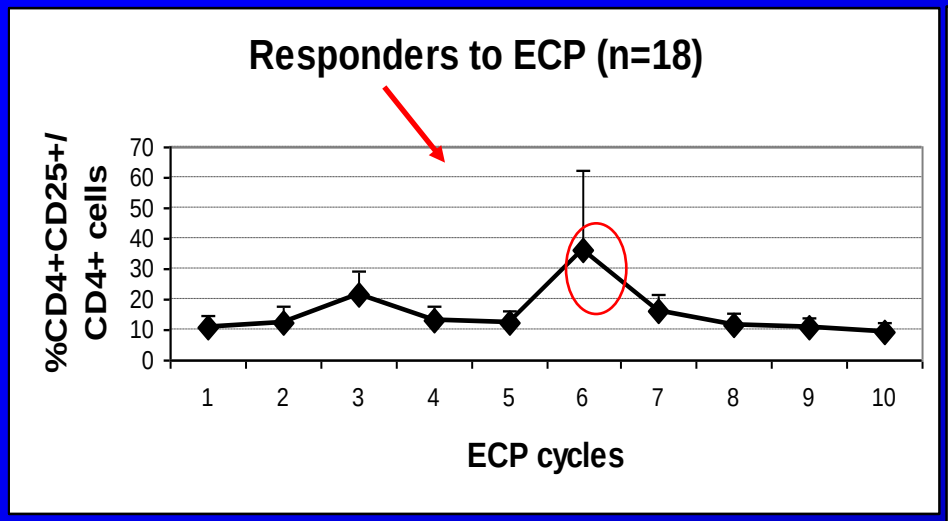


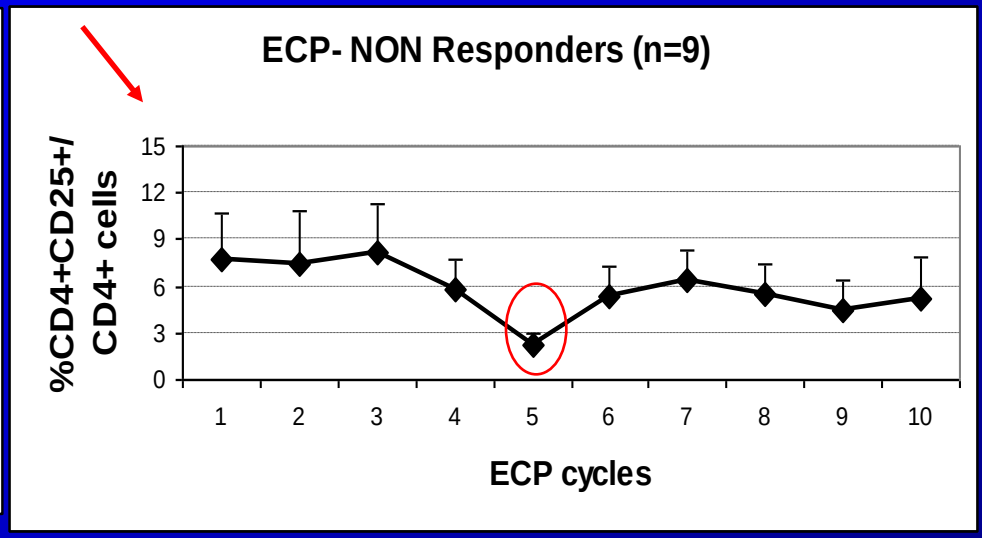
%CD4+/ CD4+CD25+cells

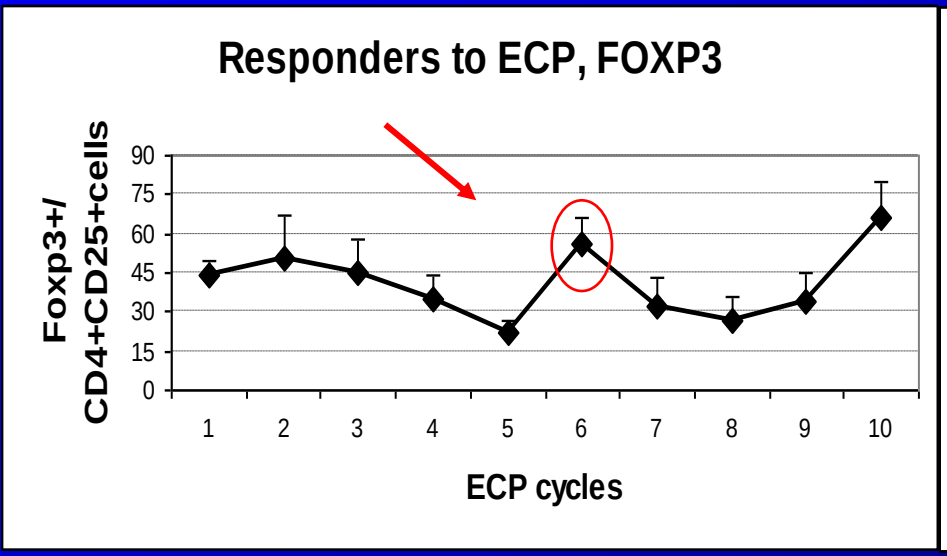


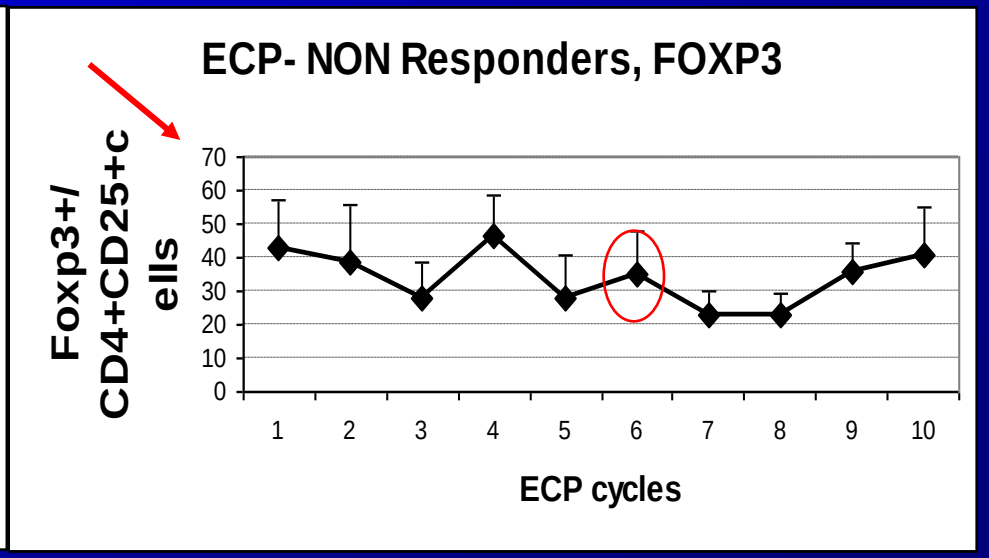
* Increase after 6 cycles was statistically significant when compared to time 0 and to control populations represented by healthy donors and GvHD-affected ECP-untreated patients

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

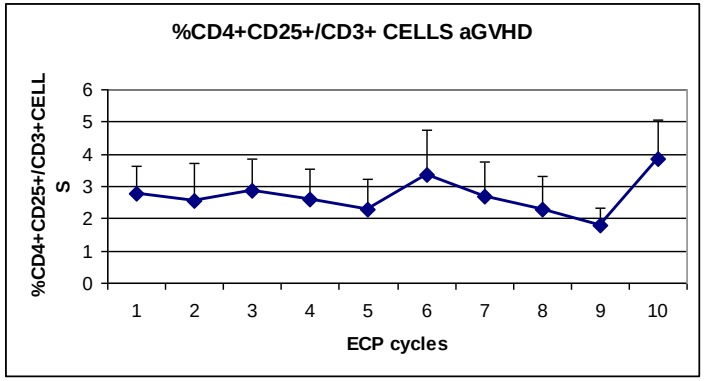


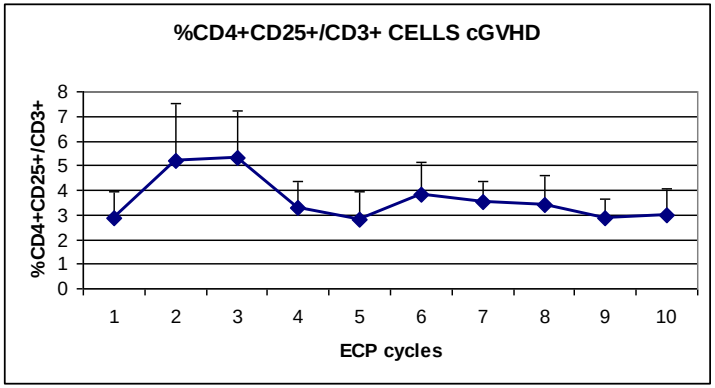


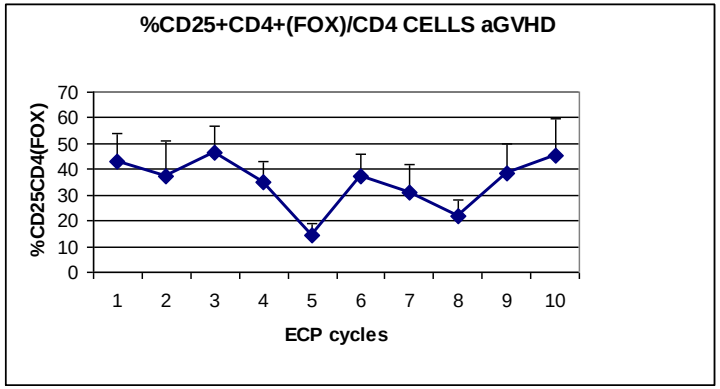


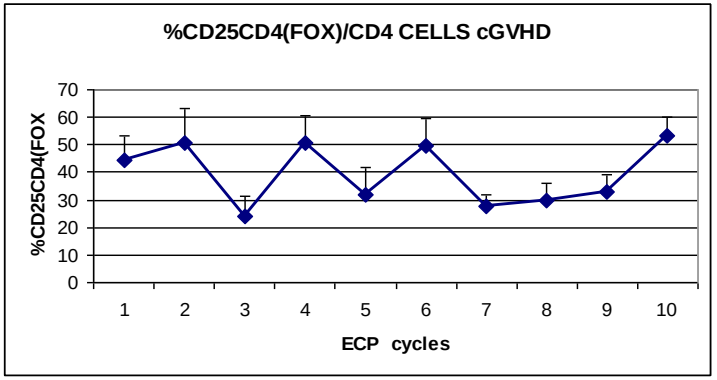


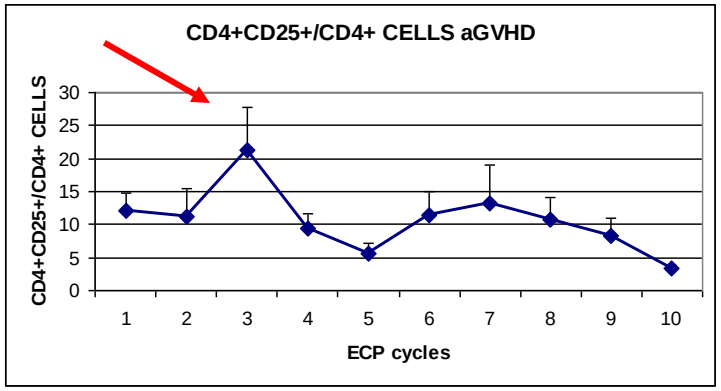
T-regs variations in ECP-responders with acute (left) and chronic (right) GvHD

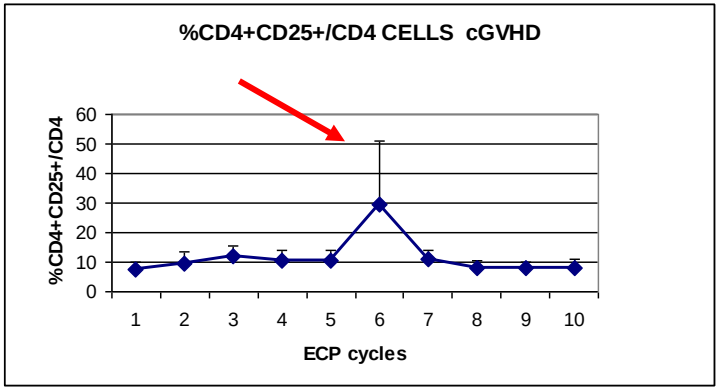






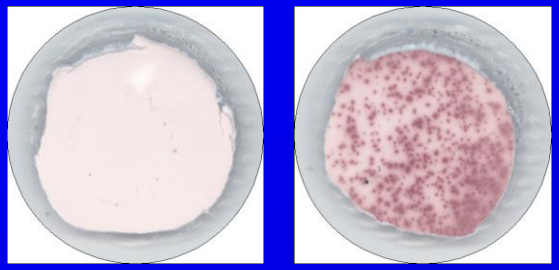






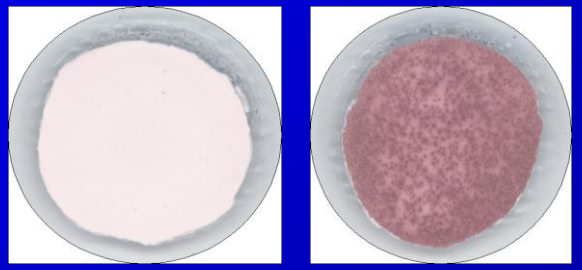
Th17-secreting cells (by Elispot), the principal responsible for GvHD, inversely correlate with % of circulating T cells

Unstimol PMA/Ionomycin



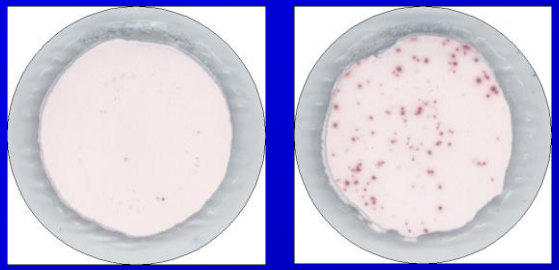
I° CYCLE ECP

%Foxp3/CD4+CD25+ cells = 0%



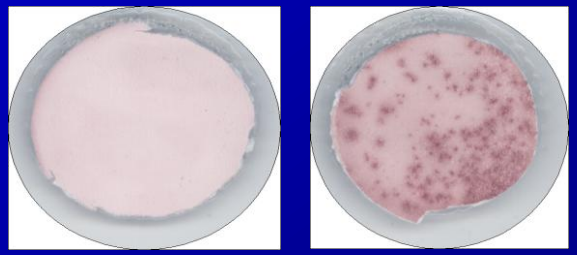
III° CYCLE ECP

%Foxp3/CD4+CD25+ cells = 0%



VII° CYCLE ECP

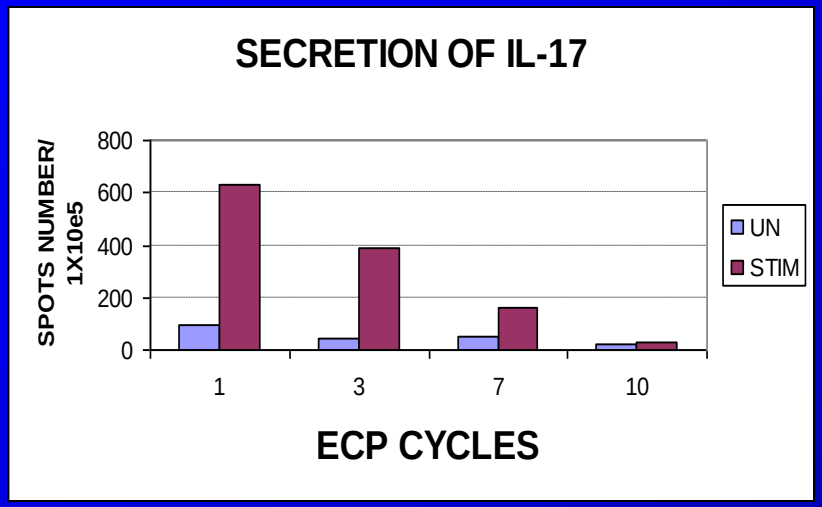
%Foxp3/CD4+CD25+ cells = 20%

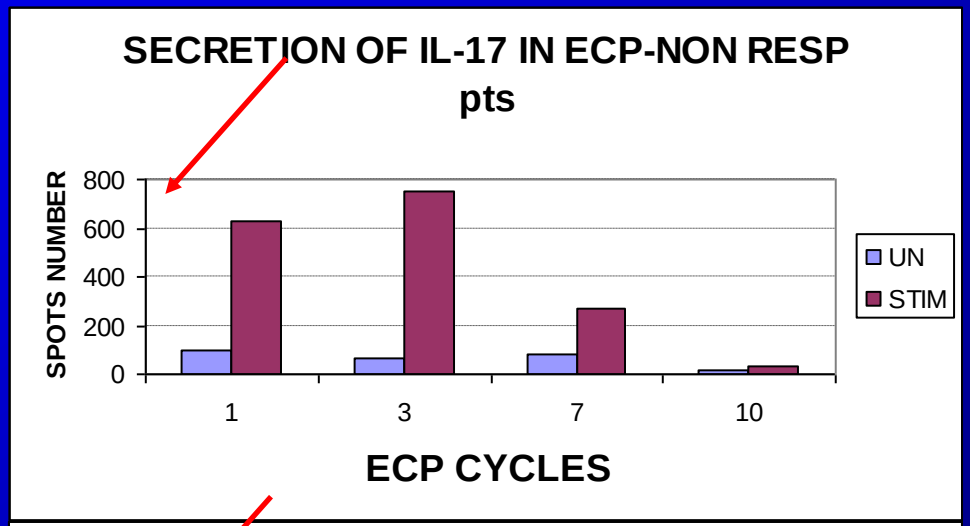


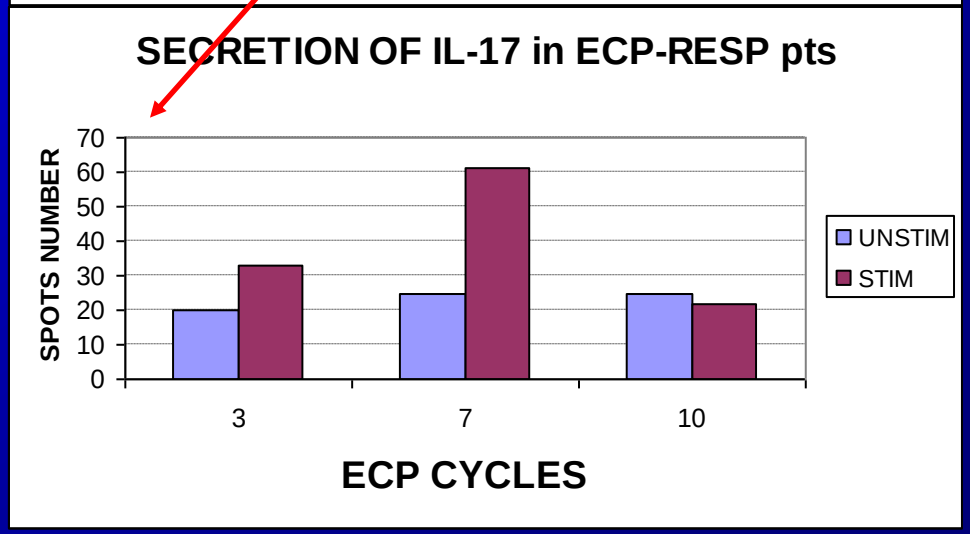
X° CYCLE ECP

%Foxp3/CD4+CD25+ cells = 0%

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







Interleukin-17–Producing T-Helper Cells as New Potential Player Mediating Graft-Versus-Host Disease in Patients Undergoing Allogeneic Stem-Cell Transplantation

Erica Dander,[1] Adriana Balduzzi,[2] Greta Zappa,[1] Giovanna Lucchini,[2] Paolo Perseghin,[3] Valentina Andrè,[1] Elisabetta Todisco,[4] Daoud Rahal,[5] Maddalena Migliavacca,[2] Daniela Longoni,[2] Graziella Solinas,[6] Antonello Villa,[7] Emilio Berti,[7] Pamela Della Mina,[7,8] Matteo Parma,[9] Paola Allavena,[6] Ettore Biagi,[1,2] Attilio Rovelli,[2] Andrea Biondi,[1,2] and Giovanna D'Amico[1]

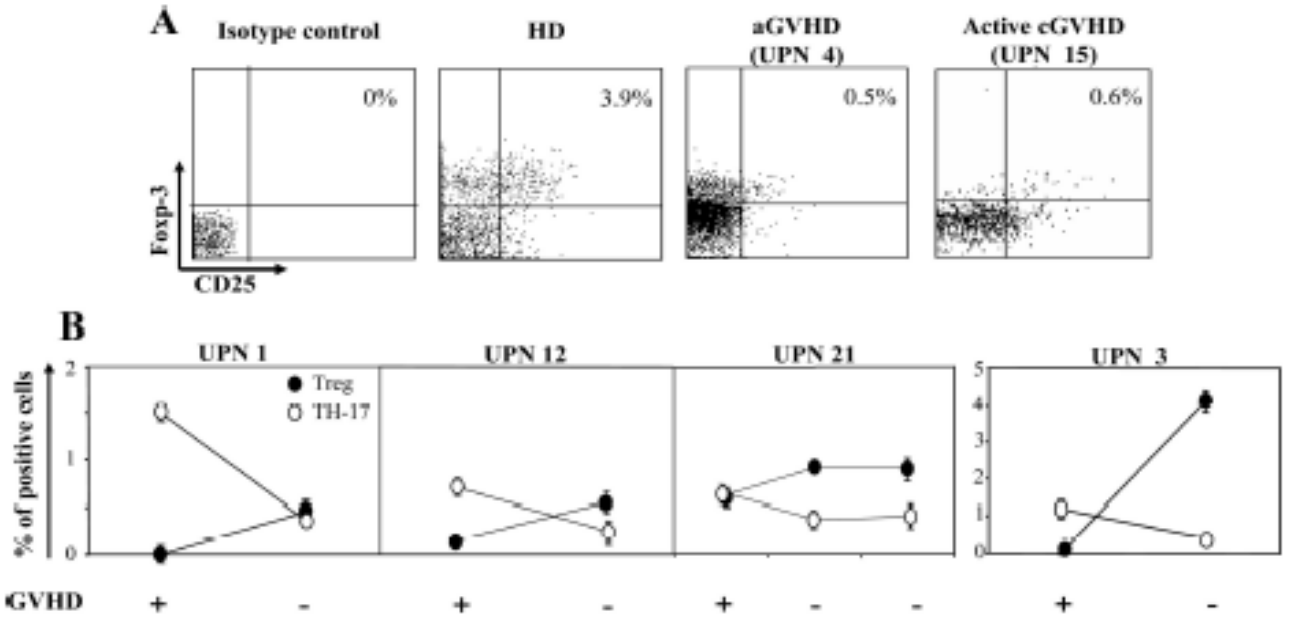
Transplantation • Volume 88, Number 11, December 15, 2009

15 controlli

51 pazienti HSCT (full chimerism)

-19 pazienti: no GvHD

-32 pazienti: aGvHD (14), cGvHD (18)



Inverse Relationship Between IL-17$^+$ and Foxp3$^+$ T Cells in Patients Presenting GVHD

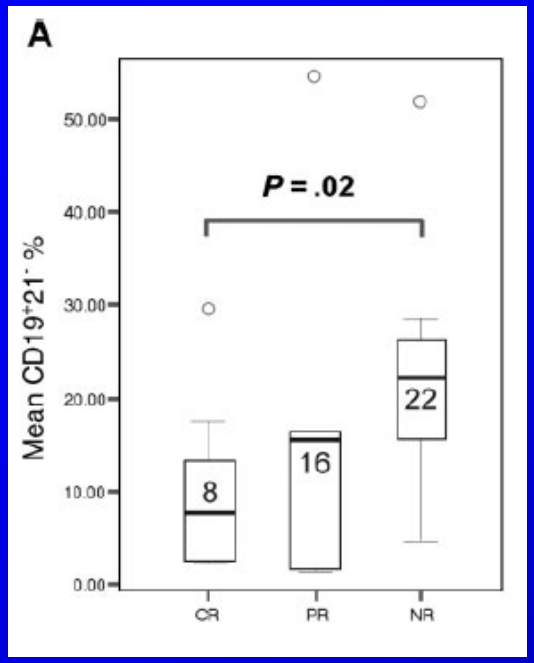
Dander et al, Transplantation,2009

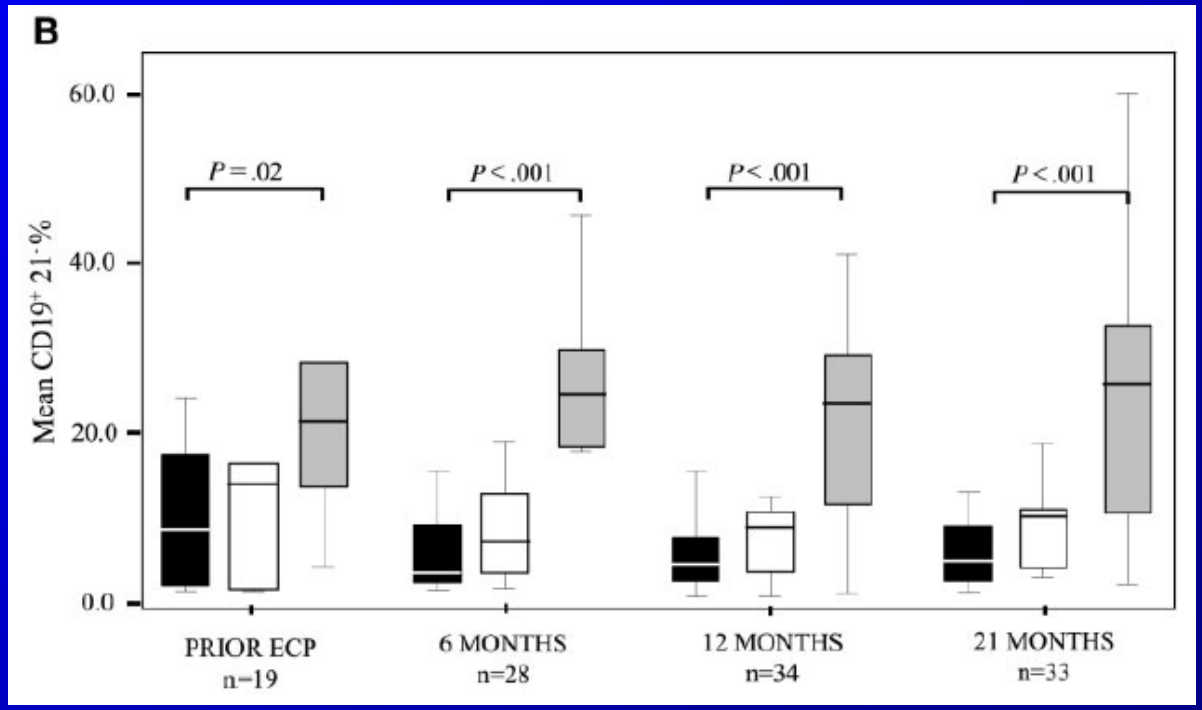
news

Proportions of immature CD19⁺CD21⁻ B lymphocytes predict the response to extracorporeal photopheresis in patients with chronic graft-versus-host disease

Kuzmina, Greinix et al: Blood 2009

Pre-ECP





Of note, CD21⁻ B lymphocytes are increased in proportion in autoimmune diseases such as systemic lupus erythematosus and active cGVHD.[1,10] Increased proportions of CD21⁻ B lymphocytes could be part of the autoimmune pathogenesis compatible with inefficient censoring of autoreactive B cells in cGVHD.[9] Disrupted

CR:nero

PR: bianco

NR:grigio

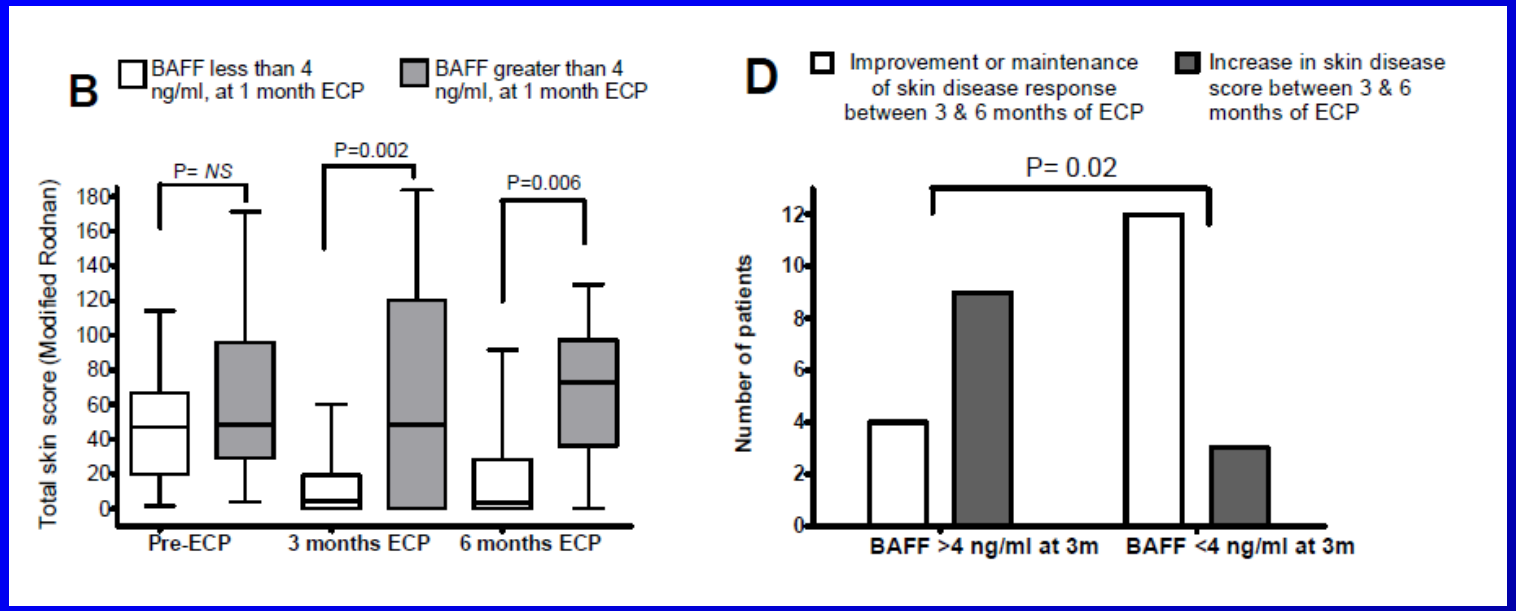
Circulating B-cell activating factor level predicts clinical response of cGvHD to ECP

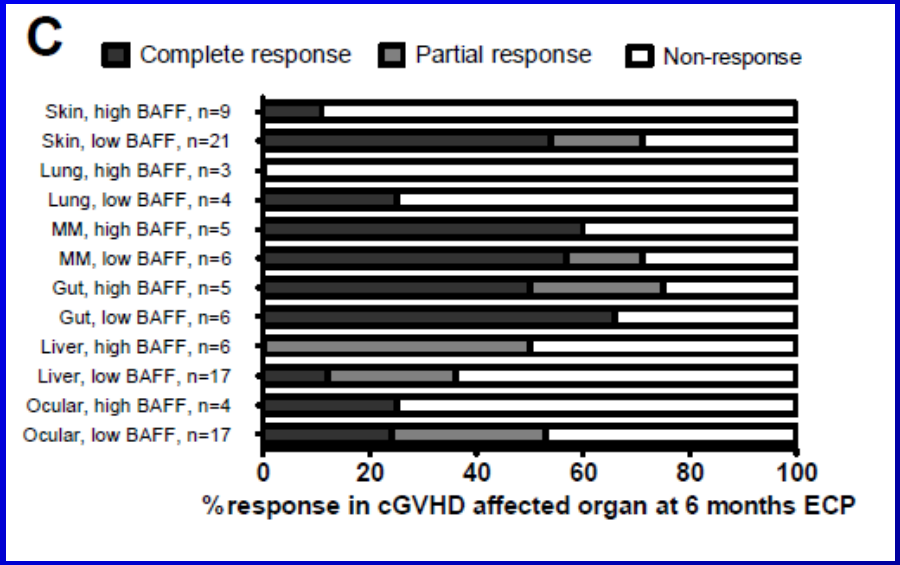
Whittle R and Taylor PC, Blood e-pub oct 20, 2011

Study performed in 46 pts

Table 1. Characteristics of chronic GVHD patients and response to ECP therapy

		Response following 3 months ECP, n=46 (%)			**Response following 6 months ECP, n=39 * (%)**		
Organ affected by cGVHD at ECP start	Pre-ECP, n=46	Complete response	Partial response	Non-response	Complete response	Partial response	Non-response
Skin, n (%)	35 (76)	12 (34)	13 (37)	10 (29)	12 (42)	3 (10)	14 (48)
Liver, n (%)	24 (52)	2 (8)	9 (38)	13 (54)	2 (10)	7 (36)	11 (55)
Ocular, n (%)	23 (50)	6 (26)	5 (22)	12 (52)	4 (21)	6 (32)	9 (47)
Gut, n (%)	12 (26)	5 (42)	5 (42)	2 (17)	6 (60)	1 (10)	3 (30)
Mucous membrane, n (%)	11 (23)	3 (27)	4 (36)	4 (36)	6 (60)	1 (10)	3 (30)
Lungs, n (%) †	10 (21)	1 (10)	1 (10)	6 (60)	1 (10)	0 (0)	6 (60)
Genital, n (%)	3 (6)	0 (0)	3 (100)	0 (0)	0 (0)	2 (100)	0 (0)





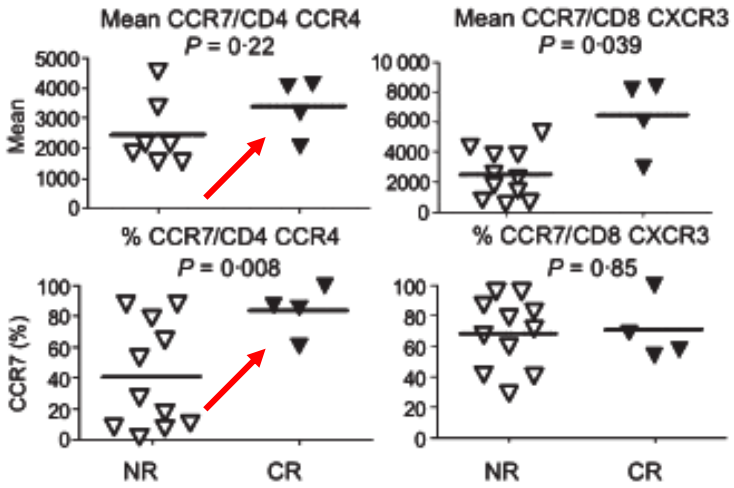
- Pre-ECP BAFF levels not correlate with severity in non-skin sites
- CR+PR > at 3-6 mts when BAFF< 4ng in eye, lung and mucosal inv.
- Pts with BAFF < 4 ng at 1 mt ahd > 50% steroid reduction than those with higher values (70 % vs 46%)

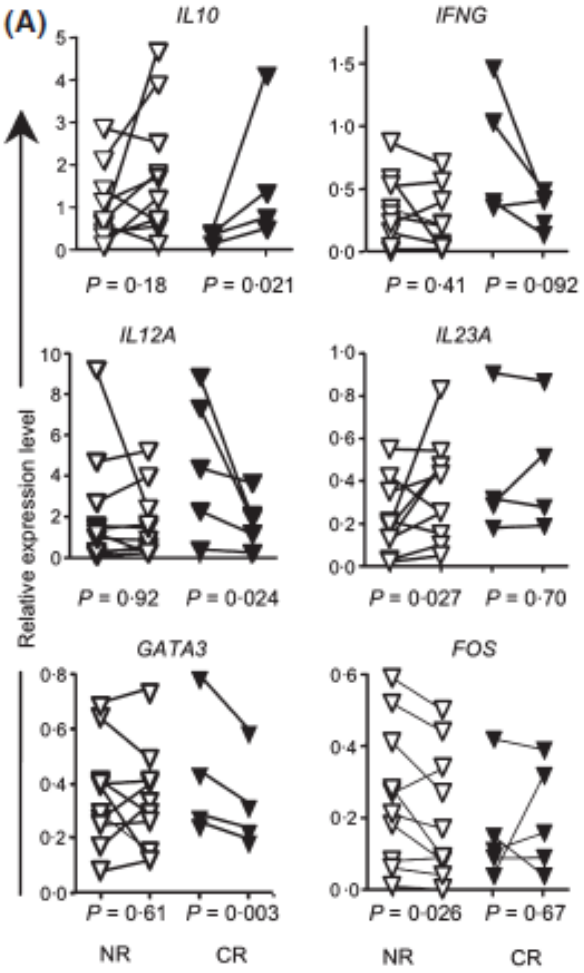
The AA postulates that “excess BAFF in ECP pts may perpetuate dysregulated B-cell homeostasis and augment T-cell associated inflammatory process..”

Decreased pro-inflammatory cytokines and increased CCR7 expression on T-lymphocyte subsets are predictive of response to extracorporeal photopheresis in patients with GvHD

Aoki et al, BJH 2011

- CCR7 receptor is implicated in naive, memory T-cell and mature DCs homing to lymph nodes
- 15 patients (1 BMT, 14 PBPCT), PDN-refractory cGvHD





CD4+CCR4+CCR7+Increase in CR to ECP

IL10 Increase in CR (anti-inflam)

IL12a and GATA3 transc. factor decreased (pro-inflam)

Plasma biomarkers in GVHD: a new era?

Sophie Paczesny, M.D., Ph.D[1], John E. Levine, M.D.[1,2], Thomas M. Braun, Ph.D[3], and James L.M. Ferrara, M.D.[1,2]

Biol Blood Marrow Transplant. 2008 January

Elafin

A TNF-α-induced epidermal proteinase inhibitor

Elevated only in pts with cutaneous GvHD,not in those with GI involvement or in those w/o GvHD

BRIEF REPORT

Biomarkers of immune activation to screen for severe, acute GVHD

August et al, BMT,2010

T-cell activation markers: sCD8,sIL-R, sCD40 ligand and sCD28

Inflammatory marker: sTNF-r1

Elafin is a biomarker of graft versus host disease of the skin

Sophie Paczesny[1], Thomas M Braun[2], John E Levine[1,3], Jason Hogan[4], Jeffrey Crawford[1], Bryan Coffing[5], Stephen Olsen[5], Sung W Choi[1], Hong Wang[4], Vitor Faca[4], Sharon Pitteri[4], Qing Zhang[4], Alice Chin[4], Carrie Kitko[1], Shin Mineishi[3], Gregory Yanik[1,3], Edward Peres[1,3], David Hanauer[1], Ying Wang[1], Pavan Reddy[3], Samir Hanash[4], and James LM Ferrara[1,3,*]

Sci Transl Med. 2010 January 6; 2(13):

Validated in 492 patients

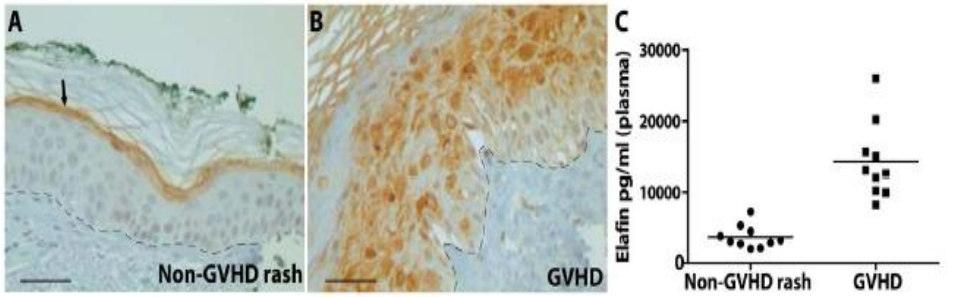
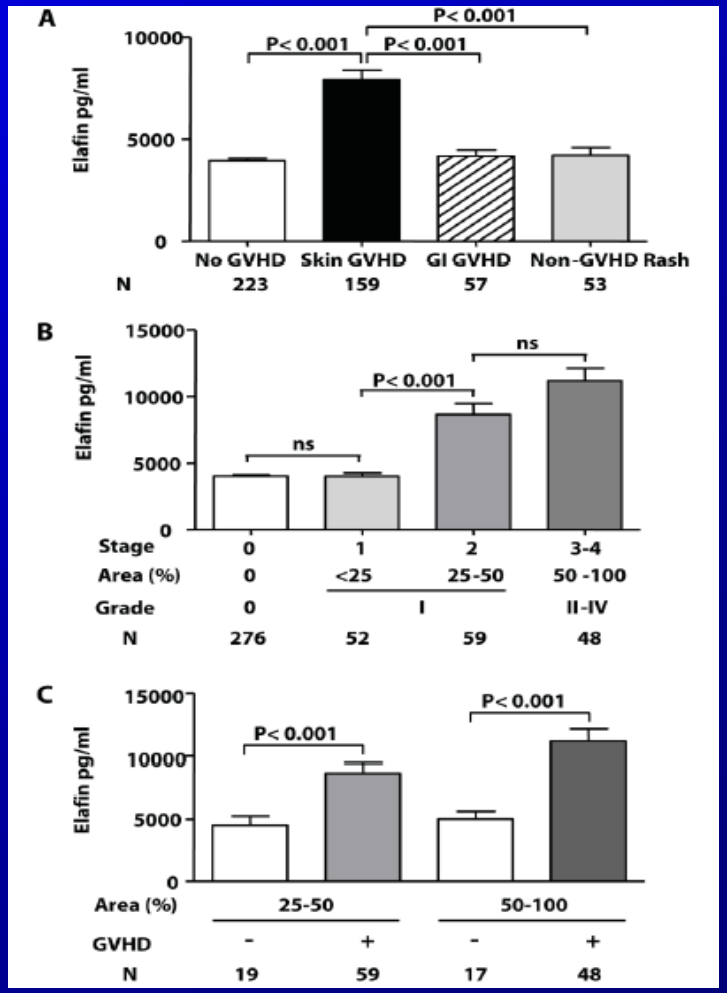


Figure 2. Skin biopsies from BMT patients

Skin biopsies from BMT patients with rashes immunohistochemically stained for elafin. A) Biopsies histologically confirmed as drug hypersensitivity showed staining only in the granular cell layer (arrow). B) Biopsies histologically confirmed as GVHD showed a strong positive staining of at least 50% of the layers in epidermis. Scale bar = 50 μm, the dashed line represents the epidermal/dermal junction. C) Plasma elafin levels in biopsied patients (N=10 per group).



Decrease of $CD4^+CD25^+$ regulatory T cells and TGF-β at early immune reconstitution is associated to the onset and severity of graft-versus-host disease following allogeneic haematogenesis stem cell transplantation

Qing Li[a], Zhimin Zhai[b,*], Xiucai Xu[a], Yuanyuan Shen[b], Aimei Zhang[a], Zimin Sun[c], Huilan Liu[c], Liangquan Geng[c], Yiping Wang[d]

Leukemia Research, 2010

(b) **Expression of $CD4^+CD25^+$Treg cells at different groups ($\bar{x} \pm s$) %**

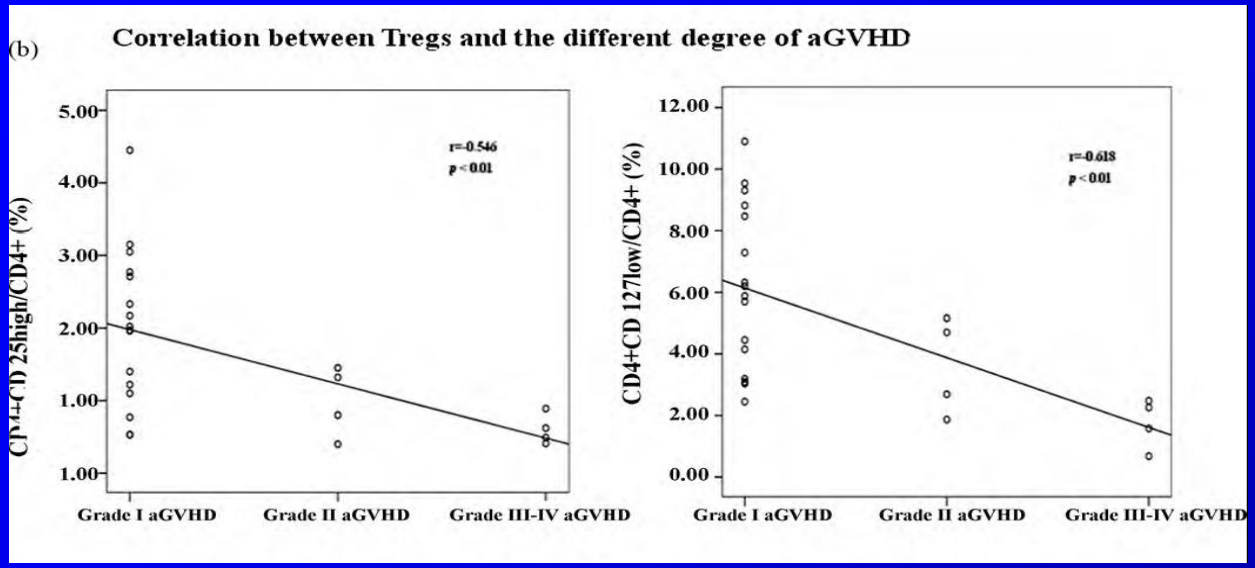
Group	Number	$CD4^+CD25^{high}/CD4^+$ (%)	$CD4^+CD25^+CD127^{low}/CD4^+$ (%)
Health control	24	2.07±0.59	6.52±1.25
non-GVHD	20	3.18±1.42*	8.13±2.12*
aGVHD	24	1.60±0.56*▲	5.01±2.16*▲
cGVHD	12	1.71±0.65▲	6.14±2.03▲

*$P<0.05$ vs. healthy controls, ▲$P<0.01$ vs. no-GVHD

(c) **Expression of adjusted $CD4^+CD25^+$Treg cells at different patient groups**

Group	Number	$CD4^+CD25^{high}/CD4^+$ (%)	$CD4^+CD25^+CD127^{low}/CD4^+$ (%)
non-GVHD	20	3.09 (0.23)	8.06 (0.48)
aGVHD	24	1.65 (0.20)*	5.12 (0.43)*
cGVHD	12	1.74 (0.29)*Δ	6.03 (0.62)#Δ

*$P<0.01$ vs. no-GVHD, #$P<0.05$ vs. no-GVHD, Δ$P>0.05$ vs. aGVHD

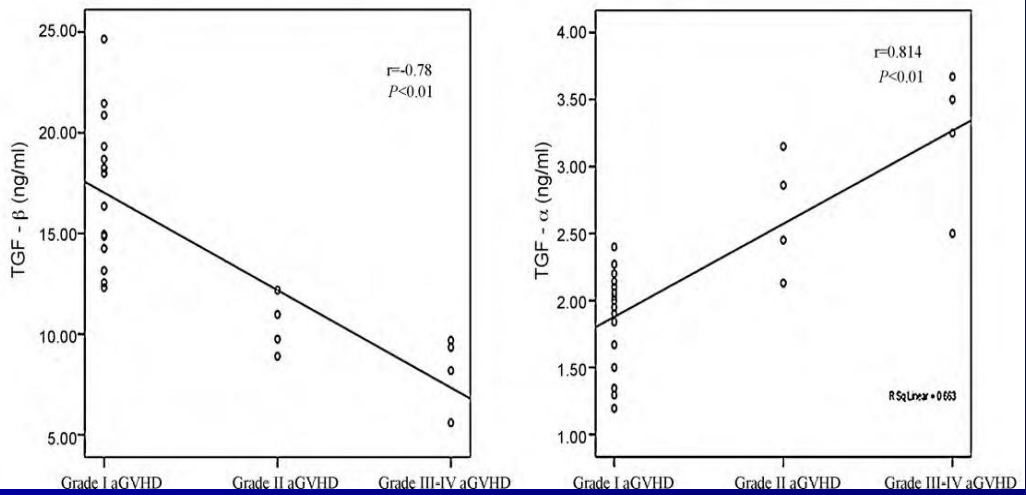


(a) The levels of serum TGF –β and TNF – α in aGVHD.

aGVHD	Number	TGF -β（ng/ml）	TNF – α （pg/ml）
Healthy control	24	27.31±8.04	1.68±0.52
Grade Ⅰ	16	18.02±3.63*	1.85±0.54
Grade Ⅱ	4	10.74±1.15*#	2.57±0.81*#
Grade Ⅲ-Ⅳ	4	8.31±1.29*#*	3.26±1.13*#*

*P <0.01vs. Healthy controls and non-GVHD group, #P <0.05 vs. Grade Ⅰ aGVH group, and *P >0.05 vs.Grade Ⅱ aGVHD group

(b) Correlation of TGF –β and TNF – α concentrations to the different degree of aGVHD.



TGF-beta

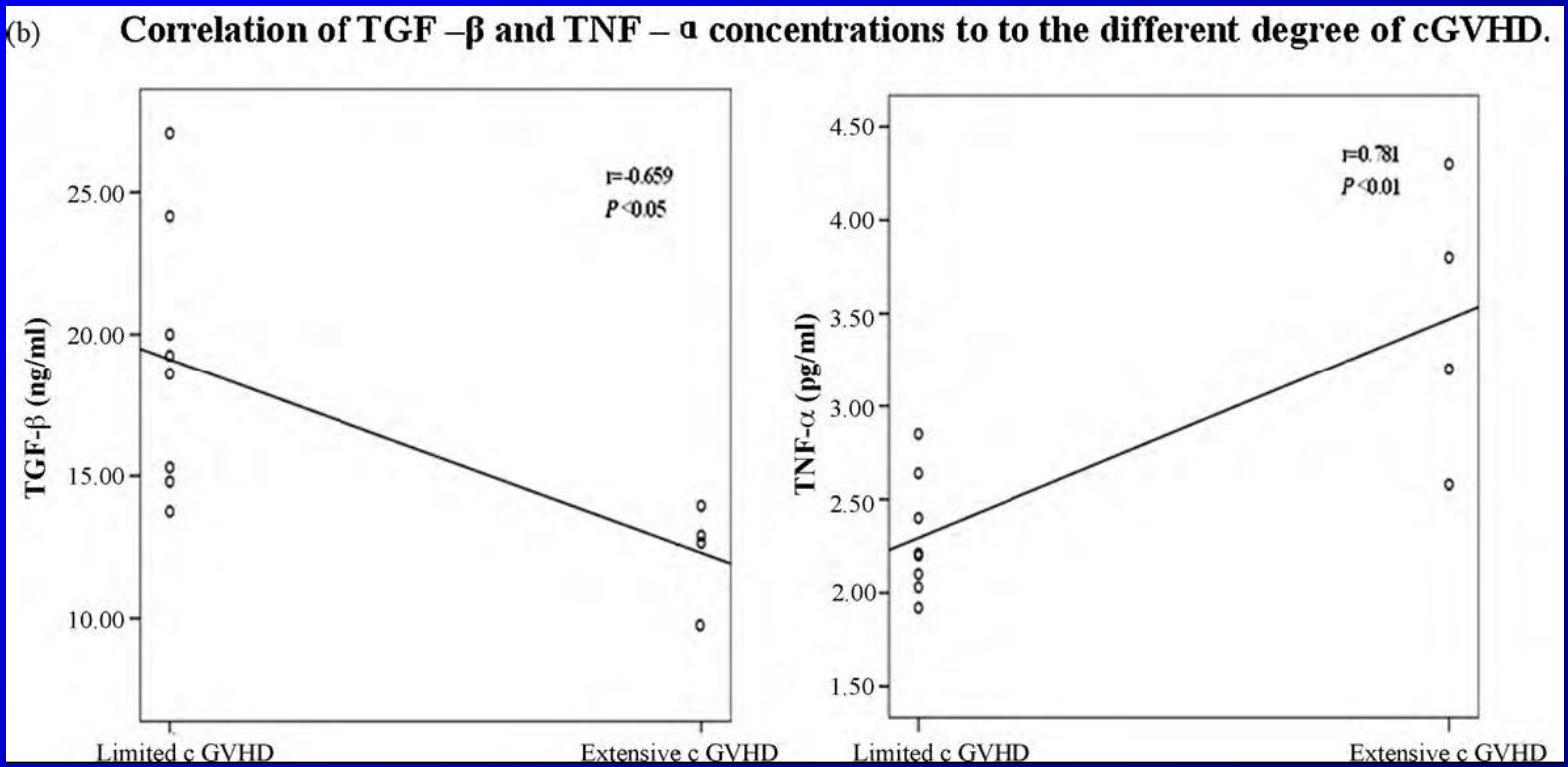
TNF-alpha

(a) The levels of serum TGF -β, TNF – α in cGVHD.

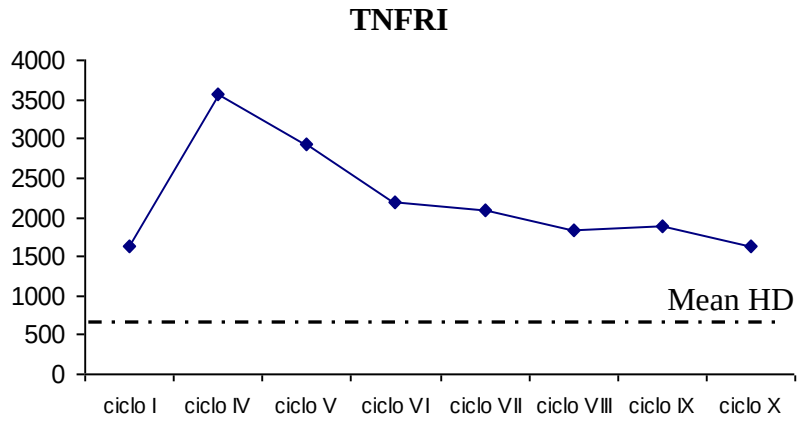
cGVHD group	Number	TGF -β（ng/ml）	TNF – α （pg/ml）
Healthy control	24	27.31±8.04	1.68±0.52
Llimited	8	18.67±4.12#	2.23±0.39#
Extensive	4	12.84±2.28*	3.17±1.03*

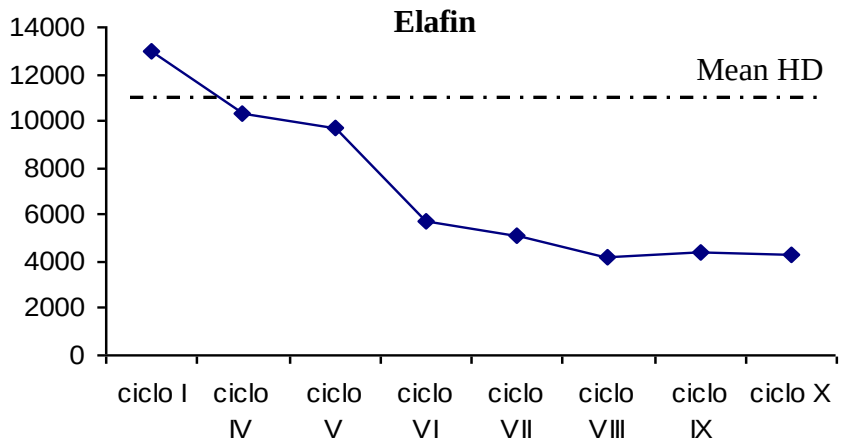
#*P* <0.05 vs. healthy controls and non-GVHD group, **P* <0.05 vs. healthy controls, non-GVHD, and limited cGVHD group.

(b) **Correlation of TGF –β and TNF – α concentrations to to the different degree of cGVHD.**



Patient 1 cGvHD

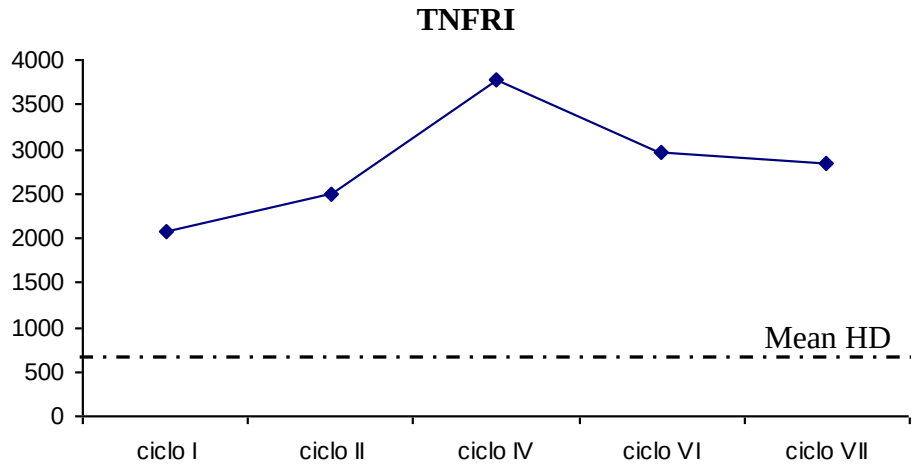




Active cutaneous cGvHD, Elafin levels decreased according to clinical improvement

TNFRI persisted elevated during ECP treatment (mild liver involvement)

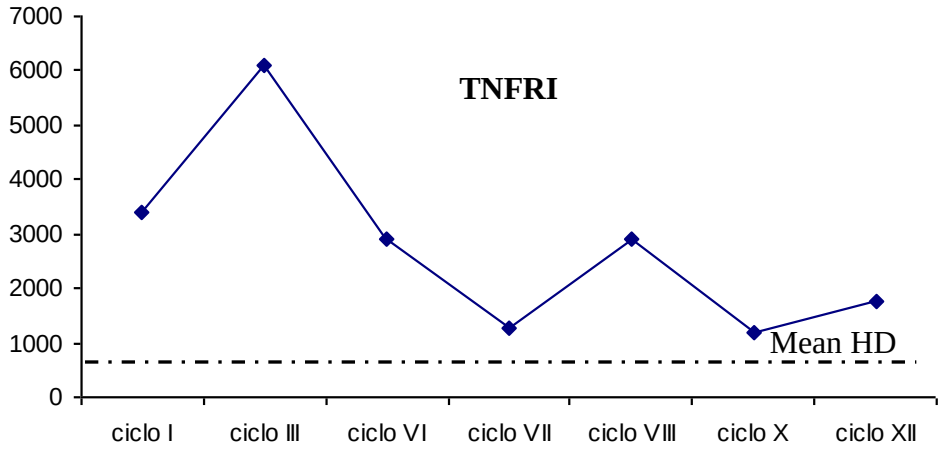
Patient 2 aGvHD



aGvHD w/o cutaneous involvement (mainly GI)

Increased levels of TNFRI during ECP treatment (liver involvement)

Patient 3 cGvHD



No active skin GvHD, Elafin levels always below mean HD levels.

sTNFRI decreased according to clinical improvement

- …..ECP is a powerful tool and that we are still learning the best ways 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.

ECP: 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

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G. Confalonieri, MD
E. Bruna, RN
L. Meroni, RN
E. Casarotto, LT
M. Pozzi, LT
V. Baldini, MD
M. Dassi, BSc
A. Incontri, BSc
P. Perseghin, MD

Dipartimento di medicina preventiva e tecnologie biomediche- Università di Milano-Bicocca
S. Galimberti, PhD

Thank you!