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# **PEDİYATRİK FOTOFEREZ**

## **TEKNİK ÖZELLİKLER**

### **VE**

## **YENİ UYGULAMALAR**

**DR. İKBAL OK BOZKAYA**  
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# FOTOFEREZ

## EKSTRAKORPOREAL FOTOFEREZ (EKF)

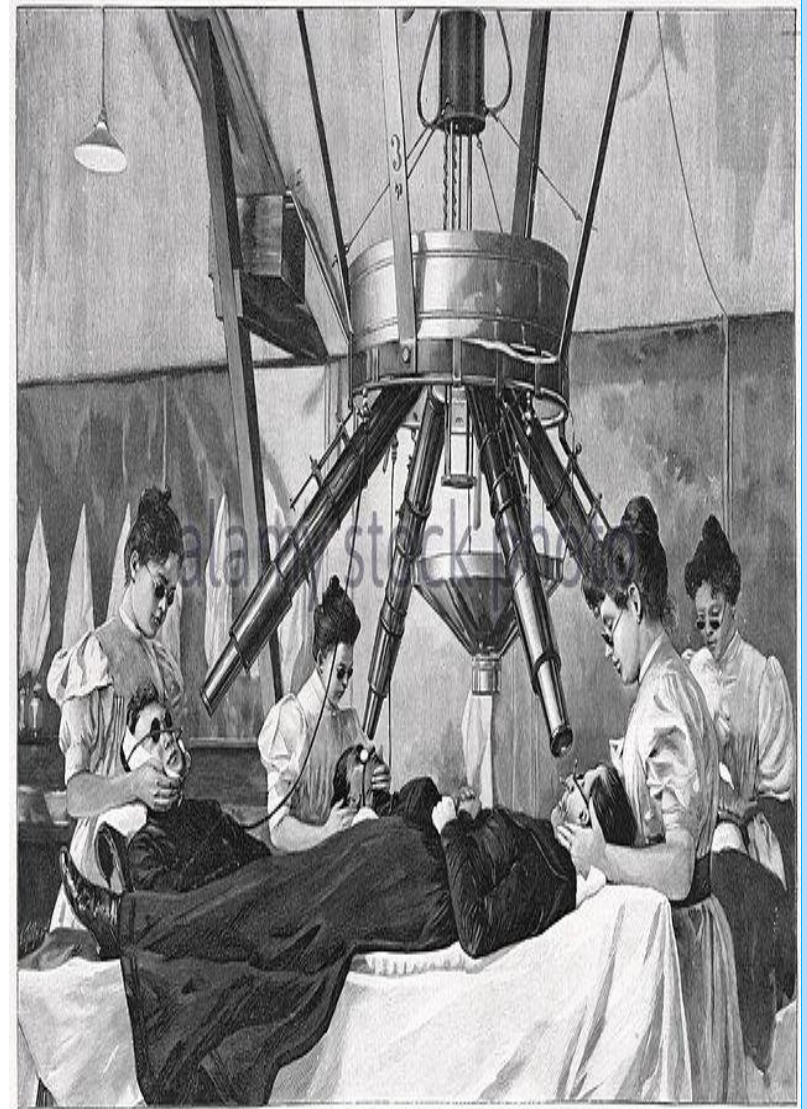
Periferik kandaki mononükleer hücrelerin aferez yöntemi ile ayrıştırılıp bir toplama torbası içinde ve psöralen bileşiği varlığında ultraviyole A (UVA) ışığına maruz bırakılıp ve sonrasında hastaya geri verilmesi



# FOTOFEREZ TARİHİNE BİR BAKIŞ

Heliyoterapi terimi ilk kez 3000 yıl önce Yunanlılar tarafından kullanılmıştır.

Fototerapiyi günümüzde ilk uygulayan kişi ise fototerapiyi karbon ışınlaması ile beraber kullanan Danimarkalı bilim adamı **Niels Finsen** dir. 1903 yılında Nöbel ödülünü kazanmıştır.



# TARİHÇE

- Psöralen ve güneş ışınları ilk kez yüzyıllar önce Mısırlılar ve Kızılderililer tarafından vitiligo hastalığının tedavisinde kullanılmıştır.
- 8 metoksipsoralen (8-MOP) ve UVA'nın vitiligo tedavisinde etkinliği ile ilgili ilk yayın 1953 yılında Lerner ve ark. tarafından yapılmıştır.
- 1974'de ise Parrish ve ark- 8-MOP ve UVA'yı kullanarak 'PUVA' terimini tanımlamışlardır.



ORIGINAL ARTICLE

ARCHIVE

# Treatment of Cutaneous T-Cell Lymphoma by Extracorporeal Photochemotherapy

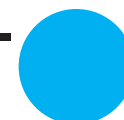
Richard Edelson, M.D., Carole Berger, Ph.D., Francis Gasparro, Ph.D., Brian Jegasothy, M.D., Peter Heald, M.D., Bruce Wintroub, M.D., Eric Vonderheid, M.D., Robert Knobler, M.D., Klaus Wolff, M.D., Gerhard Plewig, M.D., Glynis McKiernan, R.N., Inger Christiansen, R.N., Martin Oster, M.D., Hubert Honigsmann, M.D., Hubert Wilford, M.D., Eva Kokoschka, M.D., Thomas Rehle, M.D., Maritza Perez, M.D., George Stingl, M.D., and Liliane Laroche, M.D.

N Engl J Med 1987; 316:297-303 | [February 5, 1987](#) | DOI: 10.1056/NEJM198702053160603



# **Guidelines on the Use of Therapeutic Apheresis in Clinical Practice – Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Eighth Special Issue**

**Anand Padmanabhan<sup>1</sup> | Laura Connelly-Smith<sup>2</sup> | Nicole Aqui<sup>3</sup> | Rasheed A. Balogun<sup>4</sup> |  
Reinhard Klingel<sup>5</sup> | Erin Meyer<sup>6</sup> | Huy P. Pham<sup>7</sup> | Jennifer Schneiderman<sup>8</sup> |  
Volker Witt<sup>9</sup> | Yanyun Wu<sup>10</sup> | Nicole D. Zantek<sup>11</sup> | Nancy M. Dunbar<sup>12</sup> |**



Scleroderma (Systemic sclerosis)	TPE		III	2C	297
	ECP		III	2A	
Psoriasis	ECP	Disseminated pustular	III	2B	293
	Adsorptive cytaphe­resis	Disseminated pustular	III	2C	
	TPE	Disseminated pustular	IV	2C	
Atopic (neuro-) dermatitis (atopic eczema), recalcitrant	ECP		III	2A	199
	IA		III	2C	
	TPE/DFPP		III	2C	
Cutaneous T cell lymphoma (CTCL); Mycosis fungoides; Sézary syndrome	ECP	Erythrodermic	I	1B	221
	ECP	Non-erythrodermic	III	2C	
Graft-versus-host disease (GVHD)	ECP	Acute	II	1C	231
	ECP	Chronic	II	1B	
Inflammatory bowel disease	Adsorptive cytaphe­resis	Ulcerative colitis/Crohn's disease	III	1B	251
	ECP	Crohn's disease	III	2C	
Nephrogenic systemic fibrosis	ECP/TPE		III	2C	265
Pemphigus vulgaris	TPE	Severe	III	2B	279
	ECP/IA	Severe	III	2C	

Transplantation, cardiac	ECP	Cellular/recurrent rejection	II	1B	331
	ECP	Rejection prophylaxis	II	2A	
	TPE	Desensitization	II	1C	
	TPE	Antibody mediated rejection	III	2C	
Transplantation, liver	TPE	Desensitization, ABOi living donor	I	1C	337
	TPE	Desensitization, ABOi deceased donor/ Antibody mediated rejection	III	2C	
	ECP	Desensitization, ABOi	III	2C	
	ECP	Acute rejection/Immune suppression withdrawal	III	2B	
Transplantation, lung	ECP	Bronchiolitis obliterans syndrome	II	1C	339
	TPE	Antibody mediated rejection/desensitization	III	2C	



# EKF TEKNİK ÖZELLİKLER

- Bu işlem 3 aşamada gerçekleşir:
  - **Lökoferaz** (Buffy coat ayrıştırılması)
  - 8-metoksipsöralen (8-MOP) ile muamele edilip UVA ile **fotoaktivasyon**
  - Fotoaktive edilmiş lökositlerin **reinfüzyon**



# TEKNİK ÖZELLİKLER

"On-Line" Fotoferez

Tek sisteme entegre

Lökoferes (Buffy Coat toplanması)

↓  
Toplanan lenfositlerin 8-MOP tedavisi

↓  
Ultra-violet A ışınıyla irradasyon

↓  
Hastaya ürünün re-infüzyonu

"Off-Line" Fotoferez

Her bir basamak için  
ayrı ekipman

## Extracorporeal Photopheresis (ECP)

The photoactivated white blood cells are returned to the patient

The THERAKOS®UVAR® XTS® instrument draws blood from the patient

Blood is separated by centrifugation and red blood cells are returned

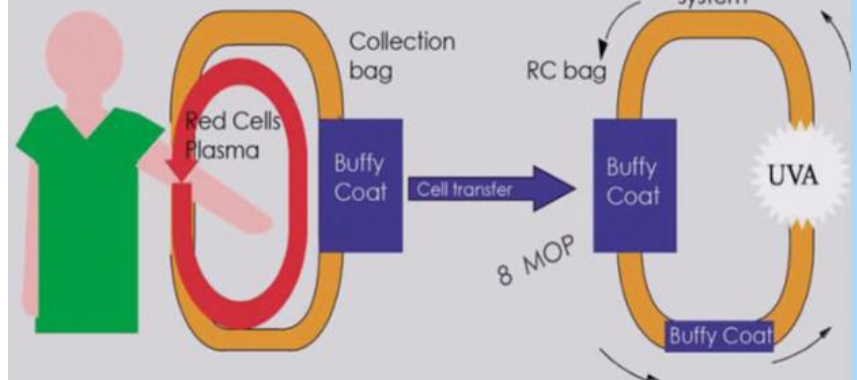
Photoactivation with UVA light

Methoxsalen

White blood cells are treated with Methoxsalen Sterile Solution and exposed to UVA light

## Photopheresis with the UVA PIT System

Collection with any System



# LÖKOFEREZ

*Question 10: What are the optimal venous accesses for performing ECP?*

**Recommendations.** The transfusion medicine specialist and/or the hematologist should preliminarily check if patients' venous access is adequate for the procedure. Peripheral venous needles (17-gauge inlet line and 17-/19-gauge return line) are most desirable to minimize any catheter-related infectious risk. In patients who have a long-term central venous access (CVC), this can be used for either inflow or outflow.

When peripheral venous access is not feasible or appropriate, after evaluation by the intensive care unit specialist, a double-lumen CVC must be inserted (7-10 Fr for children and 12-14 Fr for adults to provide adequate flow rates, i.e., 2-5 mL/kg/min) in subclavian or jugular veins to minimize the infectious risk. Depending on the expected duration and frequency of ECP treatment, a long- or short-term CVC should be positioned.

**Background.** The patient's venous access is usually checked at least 24 hours before the first ECP session. The choice of the best venous line is usually shared by the apheresis specialist and/or the attending hematologist and the intensive care physician. A CVC, usually double lumen, is sometimes inserted in selected patients who lack an appropriate peripheral venous access and in those with a body weight less than 20 kg. A standard port-a-cath CVC is not suitable for ECP while particular types of implantable ports (17 Fr) with double access may be used

## 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)*

- 17 G çekiş yapılan ven
- 17-19 G dönüş
- Santral venöz katater
  - Çocuklarda 7-10 Fr
  - Yetişkinlerde 12-14 Fr  
(2-5 mL/kg/dk)



# MONONÜKLEER HÜCRE TOPLANMASI

- Devamlı akım:

- COBE Spectra



COM.TEC

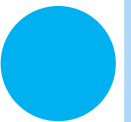


Amicus



- Devamlı olmayan akım: Haemonetics







# PSÖRALEN

## Hücrelerin UVA duyarlılığını artırır

- Otuzdan fazla bitkide (ıhlamur, kereviz, incir, karanfil, limon, maydonoz gibi) bulunan kimyasal bir maddedir
- Tedavi amacı ile genellikle 8-metoksipsöralen (8-MOP) kullanılır.
- Ticari ürün sıklıkla ‘Psoralea corylifolia’ isimli bitkiden elde edilir.



# PARENTERAL PSÖRALEN

## İV PSÖRALEN KULLANIMI:

Total ürün hacmi (ml) x 0.017 =

Eklenmesi gereken uvadex miktarıdır.

**(hct %3-5 arası dilüsyon ürün baz alınır)**



# ORAL PSÖRALEN

- İşlemden en az 2 saat önce 0.6 mg/kg şeklinde hastaya içirilir
- Toplam 50 mg üzerine çıkılmaması önerilir
- 24 saat içinde idrardan atılır



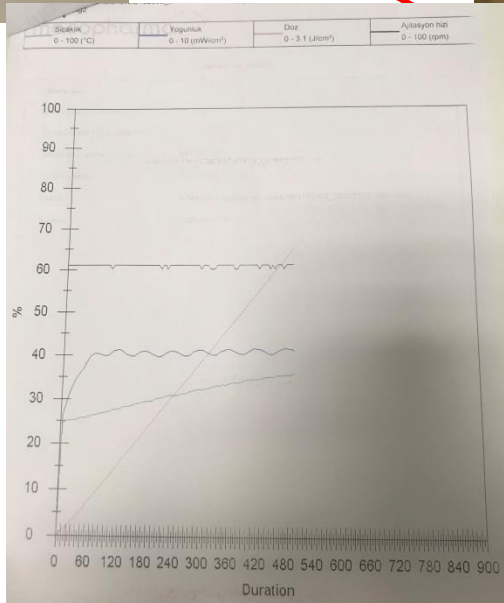
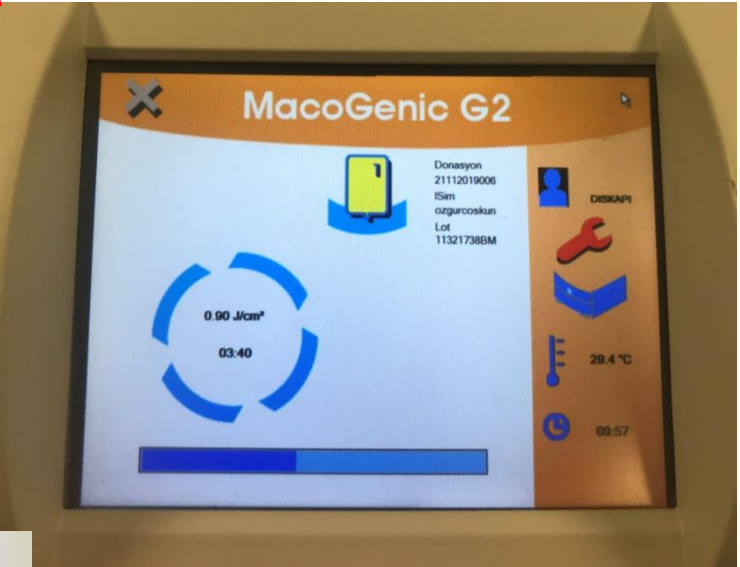
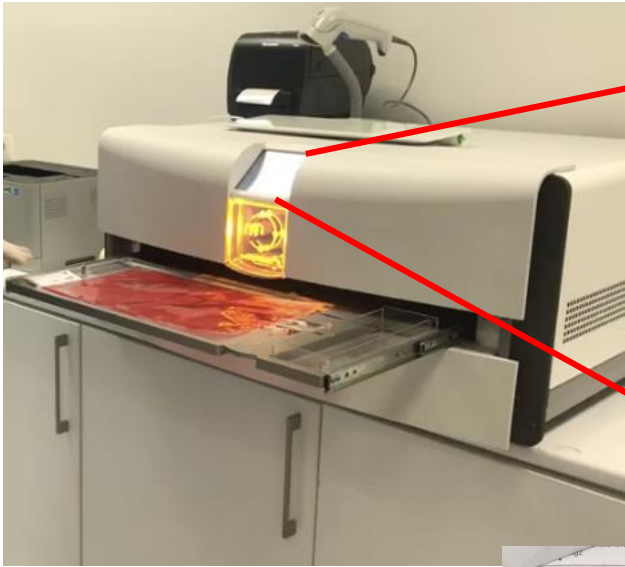


## PARENTERAL PSÖRALENİN AVANTAJLARI

- Bekleme süresi yok (Oral kullanım sonrası 2 saat)
- Doz standardize
- GIS yan etkiler daha az
- Deride fotoyaşlanma, kanser oluşumuna yatkınlık ve katarak oluşumu minimal



Hastanın yaklaşık 200 kat daha az 8-MOP maruziyeti ve sistemik 8- MOP ile ilişkili yan etkileri azaltılmış olur.





RESEARCH ARTICLE

# An automated mini buffy coat preparation method for use in mini extracorporeal photopheresis treatment of graft-vs-host-disease in a low body weight pediatric patient

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## Abstract

A mini extracorporeal photopheresis (mini-ECP) “off line” technique has been developed for use in the treatment of small children and patients with apheresis contraindications. Until now various methods have been used for buffy coat separation from whole blood. In this report we describe a protocol for mini buffy coat preparation using the automated Sepax laboratory separator for “off line” ECP treatment in a low body weight child with graft-vs-host-disease. According to our results this alternative method has been proven feasible and tolerable.

# MINI ECP

- 18 aylık E
- 8 kg
- Tüneli santral kataterden 10 mL/kg tam kan toplanmış( total kan volümünün  $<15\%$  )
- Eş zamanlı SF infüzyonu

Whole blood was processed using the Sepax cell laboratory separator on “UCB protocol” (Figure 1). This protocol allows processing of the initial blood volume from 35 mL to 290 mL, including the anticoagulant, and does not require the addition of a sedimentation agent. The final volume of buffy coat was set at 25 mL. The extracted buffy coat was transferred into the sing kit, and the buffy coat bag with irradiation bag. UV-A irradiated cells were pooled with the autologous red blood cells remaining after Sepax separation, and were infused back to the patient together. The plasma obtained after the buffy coat separation was not infused back, and was discarded because of its expected high cytokine content, as they are associated with the development of acute GVHD.<sup>8</sup>



FIGURE 1 Sepax cell laboratory separator (Biosafe, Switzerland) used for mini buffy coat preparation

Parametreler	<u>“On-line” Metod</u>		“Off-line” Metod
	UVAR-XTS	CELLEX	
	Tek makineye entegre sistem	Tek makineye entegre sistem	Her bir basamak için ayrı makine
UV-A Dozu	1.2 J/cm <sup>2</sup>	1.2 J/cm <sup>2</sup>	2 J/cm <sup>2</sup>
Aferez Tekniği	diskontinue	Diskontinue veya devamlı	Devamlı
Venöz Yol	Tek	Tek veya İki	İki
Antikoagülan	Heparin	Heparin	Asit Sitrat Dekstroz
Hücrelerin kalite kontrolü	Hayır	Hayır	Evet
Süre	1,5-2 saat	1,5-2 saat	3-4 saat
Pediyatrik kullanım	Hayır	Evet; >40 kg	Evet



## BRIEF REPORT

# Extracorporeal Photopheresis Performed on the CELLEX<sup>®</sup> Compared With the UVAR-XTS<sup>®</sup> Instrument Is More Efficient and Better Tolerated in Children With Steroid-Refractory Graft-Versus-Host Disease

Ekta Kapadia, MD,<sup>1\*</sup> Edward Wong, MD,<sup>2</sup> Evelio Perez-Albuerne, MD,<sup>1</sup> and David Jacobsohn, MD, ScM<sup>1</sup>

Extracorporeal photopheresis (ECP) is an effective therapy in children with refractory graft-versus-host disease (GVHD). The two most frequently used instruments are UVAR-XTS<sup>®</sup> and CELLEX<sup>®</sup>. We performed a retrospective chart review of ten patients who underwent ECP with both UVAR-XTS<sup>®</sup> and CELLEX<sup>®</sup> instruments for steroid-refractory acute or chronic GVHD to compare instrument run times,

percentages of cells treated, and complication rates. We found that compared to the UVAR-XTS<sup>®</sup> instrument, use of the CELLEX<sup>®</sup> instrument resulted in shorter run times, increased percentage of mononuclear cells treated, reduced incidence of line occlusions requiring TPA treatment, and decreased incidence of patient-related complications. *Pediatr Blood Cancer* 2015;62:1485–1488. © 2015 Wiley Periodicals, Inc.

**Key words:** allogeneic transplantation; extracorporeal photopheresis; graft-versus-host-disease




**TABLE II. Procedure Data, Technical, and Patient Related Complications in UVAR Versus CELLEX**

		UVAR	Cellex	<i>P</i> -value
	Number of Procedures	225	215	
	Average Run Time (minutes)	189.4	118	<0.0001
	Average Fraction of MNC Processed (%)	27	35	<0.0001
Technical Complications	Line Occlusions	19	4	0.0021
	Alarms	10	19	0.0828
Patient Related Complications	Hypotension (All patients < 35 kg)	18	0	<0.0001
	Hypertension	4	2	0.3728
	Blood product requirements	32	27	0.6753
	Citrate toxicity	5	1	0.2163



## Feasibility of extracorporeal photopheresis in pediatric patients with graft-versus-host disease after hematopoietic stem cell transplantation

Signe Winther-Jørgensen<sup>1</sup>  | Marietta Nygaard<sup>2,3</sup> | Carsten Heilmann<sup>1</sup> |  
Marianne Ifversen<sup>1</sup> | Kaspar Sørensen<sup>1</sup> | Klaus Müller<sup>1,4</sup> | Tania Masmus<sup>1</sup>

### 2.2.1 | ECP procedure and treatment regimen

ECP was performed with the Therakos™ CELLEX™ Photopheresis System (Mallinckrodt Pharmaceuticals, Therakos Ltd, Surrey, UK). One cycle of ECP consisted of treatment for 2 consecutive days. ECP was tapered and discontinued at the discretion of the treating physicians according to response and a UK consensus statement on the use of ECP.<sup>30</sup> The planned treatment regimen for patients with aGVHD were weekly cycles tapered to fortnightly cycles for 2–3 months, and subsequently discontinuation or, seldomly, tapering to monthly cycles for up to 3 months before discontinuation. Treatment regimen for patients with cGVHD was one cycle every second week for 3–6 months tapered to monthly cycles for 3–12 months before discontinuation.

### 2.2.4 | Transfusions and blood prime

If patients weighed less than 40 kg or were clinically unstable, blood prime was used. Patients received blood transfusion prior to ECP if their hematocrit was <0.27 when blood priming was not performed. Thrombocyte transfusion was administered if platelet count was <50 × 10<sup>9</sup>/L if heparin was used as anticoagulant or <25 × 10<sup>9</sup>/L if ACD-A was used as anticoagulant.





	Patient no.	ECP indication	Time from transplant to GVHD (d)	Time from GVHD to ECP (d)	Weight <sup>a</sup> (kg)	Vascular access	Co-responder catheter size	Anticoagulant	Blood prime (Y/N)	Duration of treatment (d/ no. of cycles)	No. of planned ECP treatments	Treatment with problems with vascular access/ canceled treatment <sup>b</sup> (%)	ECP-related complications
Acute GVHD	1	Prog	36	11	33	3 lumen Hickman	NA	Heparin→ACD→A	Y	15.6/15	30	90/0.0	-
	2	Contra (Viral reactivation)	21 <sup>c</sup>	5	16	2 lumen Cook/1 lumen Leader-cuff/1 lumen Leader-cuff/1 lumen Leader-cuff	Fr 7/Fr 4,5 Fr 5/Fr 6	ACD→A→Heparin	Y	46.4/19	38	5.3/5.3	-
	3	Prog	33	100	14	2 lumen Hickman/1 lumen Leader-cuff	Fr 7/Fr 5	ACD→A	Y	70/9	19	15.0/10.5	-
	4	Dep	11	41	25	1 lumen Leader-cuff/3 lumen Hickman	Fr 6/Fr 8	ACD→A	Y	62/7	14	0/0	-
	5	Dep	10	56	21	1 lumen Cook/1 lumen Cook	NA/NA	Heparin	Y	18.0/13	25	0/0	Fever
	6	Prog	110	73	15	1 lumen Leader-cuff/1 lumen Leader-cuff	Fr 6/Fr 7	Heparin	Y	119/1.0	22	22.7/13.6	Catheter-related sepsis
	7	Contra (side effects)	31	116	40	1 lumen Leader-cuff/1 lumen unperfused central line	Fr 5/Fr 6	Heparin	N/Y	169/13	26	0/0	Tiredness
Chronic GVHD	8	Dep	14 <sup>c</sup>	107	55	3 lumen Hickman	Fr 10	Heparin	N	113/11	22	10.2/0	Headache, mild hypotension
	9	Prog	46	12	56	3 lumen Hickman	Fr 10	ACD→A	Y/N	23/4	8	0/0	Tiredness
	10	Contra (side effects)	20/80	160	54	PVC/PVC	G 18/G 18	Heparin	N	70.4/0.6	66	7.6/7.6	Tiredness, Dizziness
	11	Contra (side effects)	14/66	658	17	1 lumen Leader-cuff/1 lumen Leader-cuff	Fr 6/Fr 6	Heparin	Y	12.0/8	20	0/0	Catheter-related sepsis
	12	Prog	15.6	73.4	40	1 lumen Leader-cuff/ PVC/1 lumen Leader-cuff/1 lumen Leader-cuff	Fr 6/18 G Fr 6/Fr 6	Heparin	N/Y	56.0/32	64	3.1/0	-
	13	Prog	12.7	324	43	1 lumen Leader-cuff	Fr 6	Heparin	N	69.4/29	60	10.0/3	-
	14	Dep	170	177	89	PVC/PVC	G 18/G 18	Heparin	N	456/29 <sup>d</sup>	55	0/0	-
	15	Contra (side effects)	36.5	40	45	PVC/PVC	G 18/G 18	Heparin	N	71/6 <sup>d</sup>	12	41.7/0	Tiredness

<sup>a</sup>Weight at first ECP cycle.

<sup>b</sup>Percent of planned ECP treatments with vascular access-related difficulties resulting in less whole blood processed/Percent of planned ECP treatments canceled due to vascular access-related difficulties.

<sup>c</sup>From last DU.

<sup>d</sup>ECP treatment ongoing, number of d/cycles as of March 2018.

## Patient evaluation and contraindications to ECP

*Question 7: Which information should be obtained to assess contraindications to ECP? Who should obtain such information?*

**Recommendations.** A strict collaboration between hematologist and transfusion medicine specialist is

advisable in evaluating candidates to ECP when ECP is performed by transfusion specialists. The transfusion medicine specialist and/or the hematologist responsible for ECP procedures should check patient eligibility to an ECP program; therefore, he or she should check patient clinical status (i.e., history of seizures or neurotoxicity due to calcineurin inhibitors, history of heparin-induced thrombocytopenia,<sup>1</sup> ongoing therapy with ACE inhibitors, aphakia, intolerance to citrate or psoralens), cardiac function (assessed by electrocardiogram, proBNP, echocardiogram when appropriate), liver and renal function tests, electrolytes, procalcitonin in febrile patients, coagulation variable. (The history of heparin-induced thrombocytopenia should be carefully investigated in patients eligible for ECP and ECP should not be performed until the

Kalsinörin inh nöbet,  
nörotoksisite,  
Heparin ilişkili trombositopeni  
ACE inhibitörü alıyor mu  
Afaki,  
Sitrat ve psöralen intoleransı

## 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)*

**Background.** ECP is a safe procedure, with a rate of adverse events similar to that of stem cell harvesting, namely, less than 5%.<sup>20,67</sup> Candidate patients should be screened according to AABB recommendations.<sup>66</sup> Children weighing less than 25 kg are no more susceptible to side effects due to their lower blood volume, provided that priming of the circuits is performed with leukoreduced, irradiated red blood cells (RBCs) before starting the procedure.<sup>30</sup> Adverse effects are usually mild and transient in patients reported in studies where a sterile solution of 8-MOP was directly added to cell suspensions after leukapheresis.

<25 kg lökosit azaltılmış ışıklı  
eritrosit süspansiyonu ile prime

# YASAL DURUM

- Bedelinin ödenebilmesi için üniversite, eğitim ve araştırma hastanelerinde sağlık kurulu raporu düzenlenmesi gerekiyor
- Ödemesine izin verilen hastalıklar:
  - Kutanöz T hücreli lenfoma
  - Sezary Sendromu
  - Graft versus host hastalığı
  - Pemfigus vulgaris
  - Psoriasis
  - Solid organ nakillerinde doku reddinin önlenmesi (Kalp, akciğer, böbrek nakillerinde)
- ‘Aferez-Fotoferez danışma kurulu’nun uygun görüşü alınmalıdır
- Kutanöz T hücreli lenfoma (MF, SS) için ilk 6 aylık tedavisinde onaya gerek yoktur



*Question 8: Which are the hematologic contraindications to ECP?*

**Recommendations.** Patients with severe anemia or thrombocytopenia should not be excluded from ECP, which should be delayed until anemia and thrombocytopenia have been corrected (hemoglobin [Hb]  $>8$  g/dL, platelets [PLTs]  $>20 \times 10^9/L$ ) by an adequate transfusion support (irradiated single-donor or irradiated and leukoreduced pooled PLTs and/or leukoreduced and irradiated RBCs as required). The procedure should be deferred in case of WBC count of less than  $1 \times 10^9/L$ . There is no evidence for a minimum MNC count allowing an efficient procedure.

**Background.** Cytopenia may limit the application of ECP treatment. In particular, children weighing less than 25 kg and with a predicted decrease of hematocrit to less than 20% during the procedure may require circuit priming with ABO-compatible filtered and irradiated RBC units. Even if no evidence directly supported a threshold for MNC count, it is common practice to delay ECP treatment until MNC count reaches at least  $200 \times 10^6$  cells/L.



ORIGINAL ARTICLE

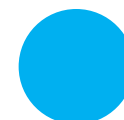
## Cryopreservation of mononuclear cells before extracorporeal photochemotherapy does not impair their anti-proliferative capabilities

ETIENNE MERLIN<sup>1,2</sup>, DALIL HANNANI<sup>3,4,6</sup>, RICHARD VEYRAT-MASSON<sup>1,5</sup>,  
JACQUES CHASSAGNE<sup>1</sup>, FRAÇOISE GABERT<sup>3,4,6</sup>, MARC BERGER<sup>1,5</sup>,  
FRANÇOIS DEMÉOCQ<sup>1,2,5</sup>, JOËL PLUMAS<sup>3,4,6</sup> & JUSTYNA KANOLD<sup>1,2,5</sup>

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### Abstract

**Background aims.** The clinical benefits of extracorporeal photochemotherapy (ECP) are well recognized, but its clinical use is limited by logistical difficulties, especially because of the need to perform repeated aphereses. The cryopreservation of mononuclear cells could allow maintenance of the ECP schedule while reducing the number of aphereses. The aim of this work was to assess whether previous cryopreservation impairs the immunomodulatory function of ECP-treated peripheral blood mononuclear cells (PBMC). **Methods.** Fresh or previously cryopreserved PBMC were exposed to ECP and added on day 0 into a mixed leukocyte reaction. Proliferation of alloreactive lymphocytes was measured by carboxyfluorescein succinimidyl ester (CFSE) dye dilution. Apoptosis was quantified by annexin-7AAD staining. **Results.** ECP-induced apoptosis was slightly increased in cryopreserved cells but the kinetics of apoptosis were similar to fresh cells. Lymphocytes stimulated in the presence of ECP-treated PBMC displayed a significant decrease in proliferation. The suppression was enforced when ECP-treated cells had been activated previously by allogeneic stimulation. Cryopreservation before ECP





## LETTER TO THE EDITOR

# Cryopreservation as a way to maintain extracorporeal photopheresis regimen for GvHD treatment while circumventing patient temporary inability to undergo apheresis

One fraction was treated, i.e., diluted with 0.9% NaCl to a hematocrit  $\leq 1\%$ , and incubated for 3 min with 0.06 mg of 8-methoxypsoralen (MOP) (Uvadex). Cells were transferred into an ultraviolet A (UVA)-permeable bag (Macopharma, Tourcoing, France) then exposed to UVA light (315–400 nm) at  $2 \text{ J/cm}^2$  for 15 min (Bio Genic Vilber Lourmat technology or Macogenic, Macopharma). The cellular product was immediately reinfused in to the patient.

The remaining one or two fractions collected were frozen, according the protocol used in each center for hematopoietic stem cells (HSC) cryopreservation in clinical routine: in Nancy, cells were frozen in a cryoprotective solution at a final concentration of 10% of DMSO (WAK-chemie Medical GmbH, Steinbach/TS, Germany) and 40% of human serum albumin (4%, HSA, LFB, Les Ulis, France), exposed to a controlled cooling temperature program up to  $-100^\circ\text{C}$  and then stored in nitrogen vapor at  $-150^\circ\text{C}$ . In CF, cells were frozen in a solution with final concentrations of 3.5% DMSO, 1% HSA and 2.5% hydroxyethylamidon (HES) (Voluven, Fresenius Kabi, Bad Homburg, Germany) for uncontrolled rate freezing and stored at  $-80^\circ\text{C}$  in mechanical freezers.

On a subsequent day, cells were thawed in a  $37^\circ\text{C}$  water bath, washed with a solution containing 50% of HSA (4%), 10% of A formula acid citrate dextrose (ACD) solution and 40% of 0.9% NaCl, with a cellular suspension rate of 33%, then centrifuged (400 g, for 15 min at  $10^\circ\text{C}$ ). The cells were suspended in 250 ml of 0.9% NaCl and UVA-irradiated in presence of 8-MOP, then immediately reinfused in to the patient.



**Table 2.** ECP efficacy

No	Percentage of cryo-ECP sessions (%) <sup>a</sup>	ECP treatment duration (months)	1 month staging (aGvHD)/3 months staging (cGvHD)	Clinical response at 6 months	Immunosuppressive drugs on-going at 6 months	Steroid- sparing effect during ECP treatment (%) <sup>b</sup>	Outcome at the end of the study (follow-up in months)
1	51	14	CR	CR	None	-100	Alive, CR (38)
2	47	9	PR	CR	None	-100	Alive, CR (25)
3	23	2.5	CR	No R (chronic GvHD occurrence) Dead (No R)	New drugs: basiliximab	+ 100	Dead (GvHD and Infection) (13)
4	33	1	No R		CS, basiliximab	0	Dead (GvHD and infection) (1)
5	40	3	PR	Dead (PR)	CS	-50	Dead (infection) (3)
6	1	20	PR	CR	None	-100	Alive, CR (30)
7	4	8	CR	CR	None	-100	Alive, CR (25)
8	7	4	PR	No R	New drugs: methotrexate, rituximab and ruxolitinib	-50 and then +100 (flare up)	Dead (GvHD and infection) (12)
9	69	16	No R	No R	New drugs requirement	-60	Dead (GvHD) (23)
10	42	5	PR	CR	None	-100	Alive, CR (26)
11	17	1	CR	Dead (CR)	CS	-40	Dead (leukemia relapse) (1)
12	17	1	CR	CR	CS	0	Alive, CR (30)
13	27	21	PR	PR (CR on skin, gut and liver but pulmonary GvHD occurrence)	None	-100	Alive, pulmonary sequelae (34)
14	1	16	PR	PR	New drugs: methotrexate, rituximab and low-dose IL-2	-75	Alive, PR (44)
15	7	9	PR	PR	CS	-50	Alive, PR (30)
16	36	11	PR	CR	CS	-100	Alive, CR (13)
17	32	3	PR	PR	None	-100	Alive, GvHD relapse after ECP arrest (sclerodermic) (32)
18	33	15	PR	CR	None	-100	Alive, CR (52)
19	41	6	PR	No R	MD	No Steroid during ECP treatment	Alive, PR (10)
20	69	12	No R	No R	MD	-100	Alive, PR (54)

# VERİLERİMİZ

hasta no	ad soyadı	yaş	cinsiyet	tanı	verici tipi	HLA uyumu	ürün tipi	vücut ağırlığı	nakil tarihi	fotoferez k	sonuç
1	GT	15;	K	AML	KARDES	6/6.	P.K.H	49		30	EX
2	KK	16	K	APLASTİK	KARDES	10/10.	K.İ	38		48	HAYATTA
3	EY	8	E	HODGKIN	ANNE	10/10.	P.K.H	30	26.10.2010	16	EX
4	NA	15	E	ALL	KARDES	6/6.	P.K.H	56	15.04.2014	50	HAYATTA
5	MT	9	E	ALL	KARDES	6/6.	P.K.H	34	11.02.2014	30	HAYATTA
6	MŞ	11	E	ALL	KARDES	6/6.	K.İ	25	21.08.2013	20	HAYATTA
7	SD		K		AKRABA DIŞI		P.K.H	49		50	HAYATTA
8	RY	6	K	PRE B	KARDES	10/10.	K.İ	14,5	02.11.2015	29	HAYATTA
9	TT		E		AKRABA DIŞI		P.K.H	15		17	HAYATTA
10	GA	12	E		KARDES	6/6.	P.K.H	29	28.04.2014	38	HAYATTA
11	FA	11	K	PRE B	KARDES	10/10.	K.İ	35,5	07.01.2015	48	HAYATTA
12	AA	8	E	RELAPS A	ANNE	10/10.	K.İ	45	18.5.2016	40	HAYATTA
13	BF		E		AKRABA DIŞI		P.K.H	18		72	HAYATTA
14	YY	16	E	RELAPS A	ANNE	5/10.	P.K.H	53	04.07.2017	20	EX
15	MG		E		AKRABA DIŞI		P.K.H	24		12	EX
16	NBE	15	K	PRE B	AKRABA DIŞI	9/10.	P.K.H	60	25.04.2017	70	HAYATTA
17	MOB		E		AKRABA DIŞI		P.K.H	21		62	HAYATTA
18	EC	13	K	MDS	KARDES	10/10.	K.İ	45,5	24.02.2012	56	EX
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21	MAN	1	E	OSTEO	AKRABA DIŞI	10/10.	P.K.H	8	28.03.2019	12	EX
22	HS	3	E	JMML	ANNE	5/10.	P.K.H	19	15.02.2019	24	HAYATTA
23	MY	6	E	ALL	AKRABA DIŞI	9/10.	P.K.H	21	15.01.2019	4	HAYATTA
24	MKA	11	E	NHL	ANNE	5/10.	P.K.H+Kİ	28	29.01.2019	18	EX
25	BG	18	K	AML	KARDES	10/10.	P.K.H	55	16.08.2018	38	HAYATTA
26	ZT	8	K	ALL	KARDES	10/10.	P.K.H	25	26.09.2018	68	HAYATTA
28	MŞ							15		18	HAYATTA
29	MR	12	K	ALL	AKRABA DIŞI	10/10.	P.K.H	20	17.10.2018	9	EX
30	MEC	2	K	KGH	AKRABA DIŞI	9/10.	P.K.H	13	02.10.2019	6	HAYATTA
31	SİB	15	K	ALL	KARDES	10/10.	Kİ	60	23.05.2018	8	HAYATTA
32	TAB	15	K	MDS	AKRABA DIŞI	9/10.	Kİ	56	19.09.2019	6	HAYATTA
33	ÖC	9	E	ALL	ANNE	6/10.	P.K.H+Kİ	22	25.09.2019	2	HAYATTA





# VERİLERİMİZ

- 32 hasta
- E/K: 19/13
- Yaş (median): 11 (min-max: 1- 18y)
- Kilo (median): 28,5 (min-max: 8-76 kg)
- 13 MSD, 2 MRD, 12 MUD, 4 Haplo
- Median fotoferez seans: 29 (min-max: 2-72)



hasta no	ad soyad	yaş	cinsiyet	tanı	verici tipi	HLA uyumu	ürün tipi	vücut ağırlığı	nakil tarihi	fotoferez k	sonuç
1	GT	15;	K	AML	KARDES	6/6.	P.K.H	49		30	EX
2	KK	16	K	APLASTİK	KARDES	10/10.	K.İ	38		48	HAYATTA
3	EY	8	E	HODGKİ	ANNE	10/10.	P.K.H	30	26.10.2010	16	EX
4	NA	15	E	ALL	KARDES	6/6.	P.K.H	56	15.04.2014	50	HAYATTA
5	MT	9	E	ALL	KARDES	6/6.	P.K.H	34	11.02.2014	30	HAYATTA
6	MŞ	11	E	ALL	KARDES	6/6.	K.İ	25	21.08.2013	20	HAYATTA
7	SD		K		AKRABA DIŞI		P.K.H	49		50	HAYATTA
8	RY	6	K	PRE B	KARDES	10/10.	K.İ	14,5	02.11.2015	29	HAYATTA
9	TT		E		AKRABA DIŞI		P.K.H	15		17	HAYATTA
10	GA	12	E		KARDES	6/6.	P.K.H	29	28.04.2014	38	HAYATTA
11	FA	11	K	PRE B	KARDES	10/10.	K.İ	35,5	07.01.2015	48	HAYATTA
12	AA	8	E	RELAPS A	ANNE	10/10.	K.İ	45	18.5.2016	40	HAYATTA
13	BF		E		AKRABA DIŞI		P.K.H	18		72	HAYATTA
14	YY	16	E	RELAPS A	ANNE	5/10.	P.K.H	53	04.07.2017	20	EX
15	MG		E		AKRABA DIŞI		P.K.H	24		12	EX
16	NBE	15	K	PRE B	AKRABA DIŞI	9/10.	P.K.H	60	25.04.2017	70	HAYATTA
17	MOB		E		AKRABA DIŞI		P.K.H	21		62	HAYATTA
18	EC	13	K	MDS	KARDES	10/10.	K.İ	45,5	24.02.2012	56	EX
19	YE	17	E	KML BALI	KARDES	10/10.	K.İ	76	31.10.2016	44	HAYATTA
20	AAÖ	2	E	HLH	AKRABA DIŞI	9/10.	P.K.H	14	15.09.2017	50	EX
21	MAN	1	E	OSTEO	AKRABA DIŞI	10/10.	P.K.H	8	28.03.2019	12	EX
22	HS	3	E	JMML	ANNE	5/10.	P.K.H	19	15.02.2019	24	HAYATTA
23	MY	6	E	ALL	AKRABA DIŞI	9/10.	P.K.H	21	15.01.2019	4	HAYATTA
24	MKA	11	E	NHL	ANNE	5/10.	P.K.H+Kİ	28	29.01.2019	18	EX
25	BG	18	K	AML	KARDES	10/10.	P.K.H	55	16.08.2018	38	HAYATTA
26	ZT	8	K	ALL	KARDES	10/10.	P.K.H	25	26.09.2018	68	HAYATTA
28	MŞ							15		18	HAYATTA
29	MR	12	K	ALL	AKRABA DIŞI	10/10.	P.K.H	20	17.10.2018	9	EX
30	MEC	2	K	KGH	AKRABA DIŞI	9/10.	P.K.H	13	02.10.2019	6	HAYATTA
31	SİB	15	K	ALL	KARDES	10/10.	Kİ	60	23.05.2018	8	HAYATTA
32	TAB	15	K	MDS	AKRABA DIŞI	9/10.	Kİ	56	19.09.2019	6	HAYATTA
33	ÖC	9	E	ALL	ANNE	6/10.	P.K.H+Kİ	22	25.09.2019	2	HAYATTA



## FOTOFEREZ İŞLEMİNDE ÇÖZÜZEN ÜRÜNLERİN VİABİLİTESİ

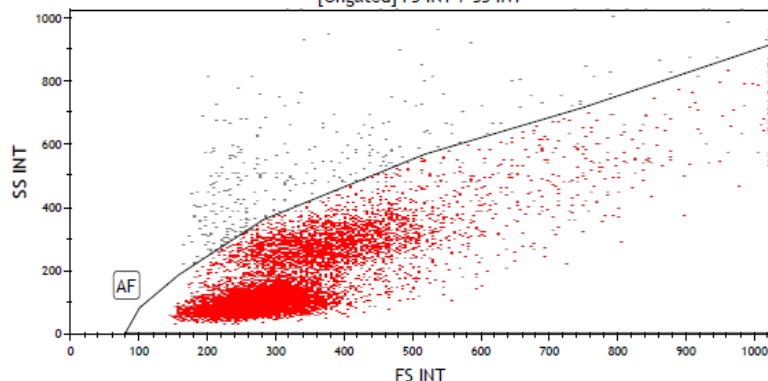
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4.ÜRÜN	92%	90%	90%	87%	89%	84%	89%	89%
5.ÜRÜN	96%	94%	88%	90%	96%	95%	92%	92%
6.ÜRÜN	90%	88%	91%	86%	91%	90%	90%	90%

# İŞLEM ÖNCESİ

# İŞLEM SONRASI

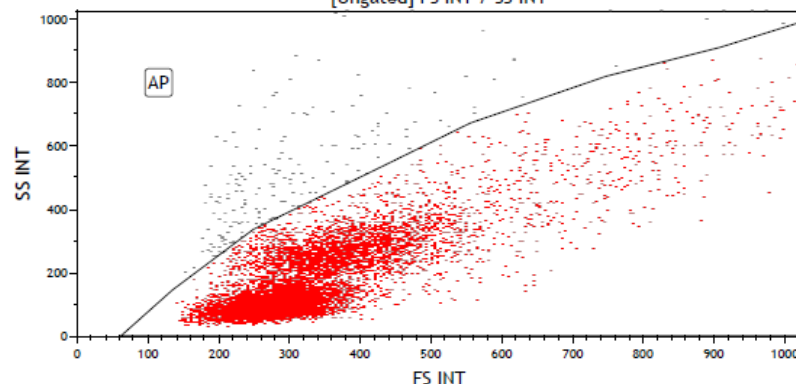
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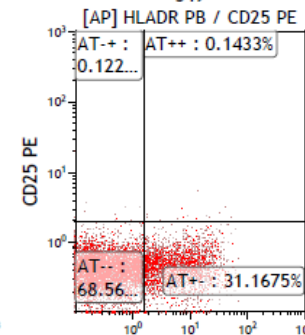
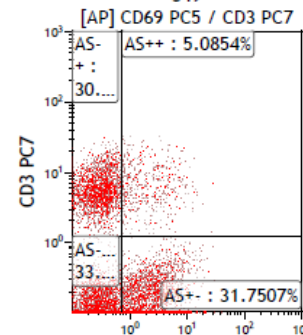
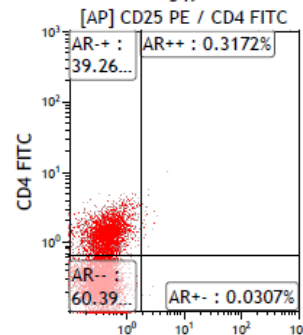
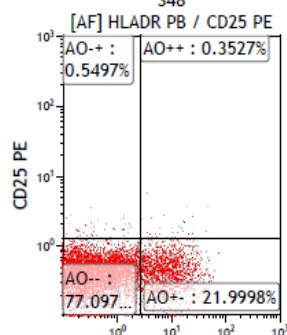
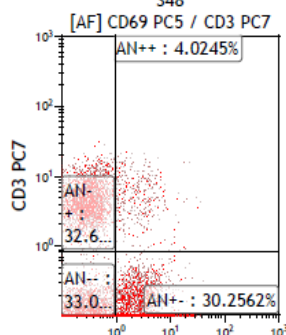
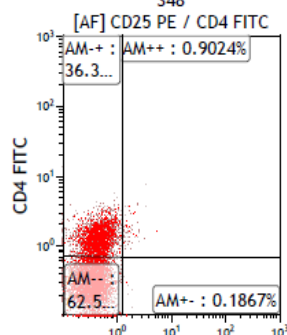
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Data Set 1:  
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Data Set 2:  
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349

Data Set 2:  
BLAS I.SONR 16.01.2019  
349

Data Set 2:  
BLAS I.SONR 16.01.2019  
349



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AM++	3,503	35.0300	36.3344
AM--	18	0.1800	0.1867
AM++	87	0.8700	0.9024

CD69 PC5			
Gate	Number	%Total	%Gated
All	9,641	96.4100	100.0000
AN--	3,184	31.8400	33.0256
AN++	3,152	31.5200	32.6937
AN--	2,917	29.1700	30.2562
AN++	388	3.8800	4.0245

HLADR PB			
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All	9,641	96.4100	100.0000
AO--	7,433	74.3300	77.0978
AO++	53	0.5300	0.5497
AO--	2,121	21.2100	21.9998
AO++	34	0.3400	0.3527

CD25 PE			
Gate	Number	%Total	%Gated
All	9,773	97.7300	100.0000
AR--	5,902	59.0200	60.3909
AR++	3,837	38.3700	39.2612
AR--	3	0.0300	0.0307
AR++	31	0.3100	0.3172

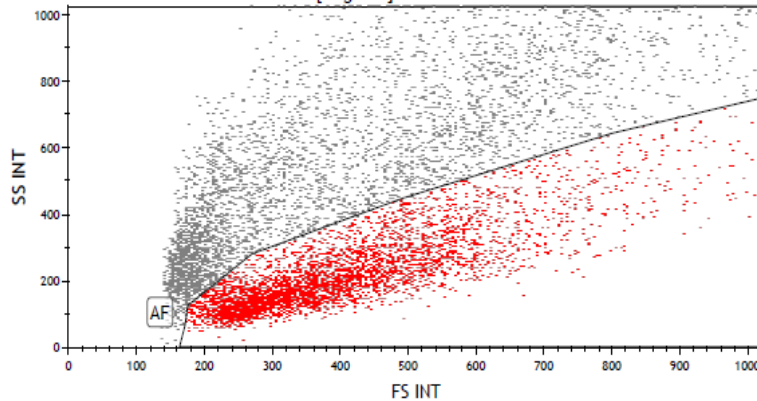
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AS--	3,103	31.0300	31.7507
AS++	497	4.9700	5.0854

HLADR PB			
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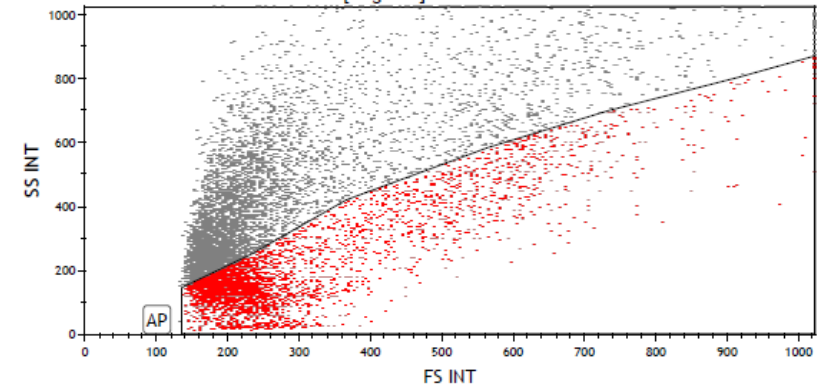
# İŞLEM ÖNCESİ

# İŞLEM SONRASI

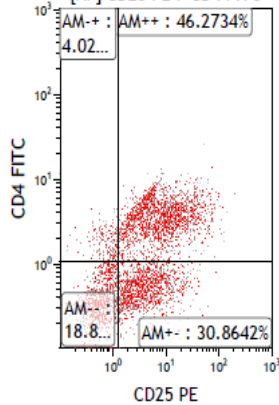
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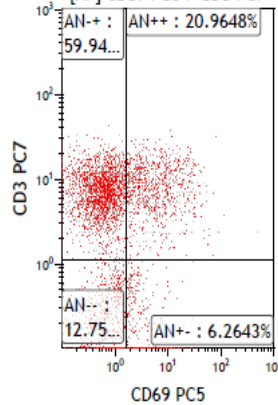


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[AF] CD25 PE / CD4 FITC



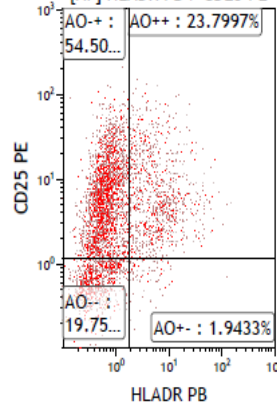
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AM++	2,024	20.2400 46.2734

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[AF] CD69 PC5 / CD3 PC7



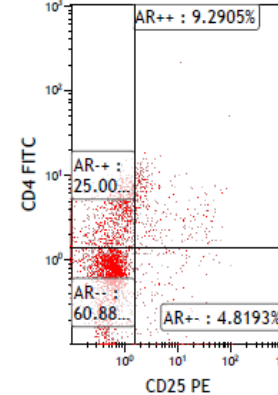
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[AF] HLADR PB / CD25 PE



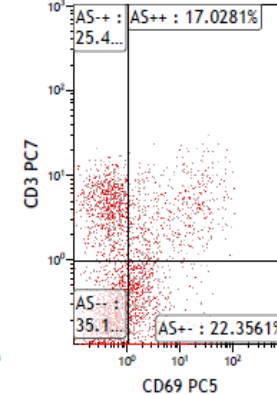
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AO-+	2,384	23.8400 54.5039
AO++	85	0.8500 1.9433
AO++	1,041	10.4100 23.7997

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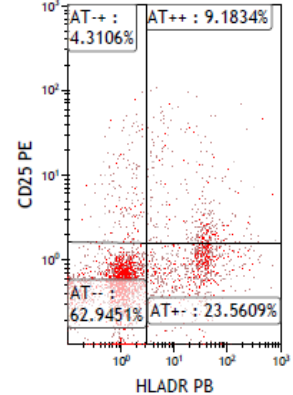
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AR-+	934	9.3400 25.0067
AR++	180	1.8000 4.8193
AR++	347	3.4700 9.2905

Data Set 2:  
ISLEMS 21.01.2019 380  
[AP] CD69 PC5 / CD3 PC7



Gate Number	%Total	%Gated
All	3,735	37.3500 100.0000
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AS++	835	8.3500 22.3561
AS++	636	6.3600 17.0281

Data Set 2:  
ISLEMS 21.01.2019 380  
[AP] HLADR PB / CD25 PE



Gate Number	%Total	%Gated
All	3,735	37.3500 100.0000
AT--	2,351	23.5100 62.9451
AT-+	161	1.6100 4.3106
AT++	880	8.8000 23.5609
AT++	343	3.4300 9.1834