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Extracorporeal Photopheresis in Graft-Versus-Host Disease: Real-Life Experience Using a New In-Line Method

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Abstract: Background: Extracorporeal photopheresis (ECP) consists of the collection of a patient's peripheral blood mononuclear cells (MNCs) that, after incubation with a photosensitive molecule, are exposed to ultraviolet-A (UVA) and then reinfused into the patient. There are two methods for performing the ECP procedure: the "in-line" methods and the "off-line" methods. In the "in-line" method, all the phases of ECP (leukapheresis, photoactivation, and reinfusion) are achieved sequentially in extracorporeal circulation using a single instrument and a single sterile disposable kit without disconnection from the patient's blood circulation. In this paper, we report our real-life experience with a recently licensed in-line ECP system proposed by Fresenius-Kabi. Methods: The ECP procedures (n = 211) were performed using an Amicus cell separator and a Phelix UV irradiator with Amicus software 4.51 and Phelix software 1.0. A targeted 2000 mL of whole blood (WB) was processed, and 1.5 J/cm^2 of UVA light was delivered to the collected mononuclear cells (MNCs). Results: From May 2023 to April 2024, we performed 211 ECP procedures in 11 patients with graft-versus-host disease (GvHD). The processed blood volume was between 1992 and 2000 mL, and the blood flow speed during the procedures was highly variable (from 30 to 50 mL/min), so the total duration of the procedure was quite variable (from 92 to 118 min). The collection efficiency (CE2) for mononuclear cells was always satisfactory (from 55% to 73%), with a minimal presence of RBCs and PLTs. Conclusions: In our experience, the Amicus system-based ECP procedure is safe and well tolerated as we observed only one side effect. The duration of the procedure was always under two hours. The collection efficiency (CE2) for MNCs was satisfactory, with minimal platelet and RBC product contamination.

Keywords: ECP; GvHD; in-line methods

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1. Background

More than 50% of patients receiving a blood stem cell (BSC) allogeneic transplant develop acute and/or chronic graft-versus-host disease (GvHD), resulting in a significant rate of mortality. GvHD is responsible for one-third of transplant-related deaths and is also the cause of severe morbidities with a high impact on the quality of life of patients. In the last few years, GvHD has been observed more frequently because of higher patient age, increased use of unrelated and/or incompatible donors, reduced-intensity conditioning regimens, and increased use of peripheral blood stem cells (PBSCs). Corticosteroids are the first-line therapy in patients with GvHD; a complete response occurs only 25–40% of the time, and a partial response, with relevant clinical improvements, is achieved in 40–50% of cases. Around 40% of patients become steroid-resistant, so a large proportion of

patients with GvHD require second-line therapy. The available second-line therapies in patients with steroid-resistant GvHD include mycophenolate, tacrolimus, anti-thymocyte globulin, Janus kinase 1/2 (IAK 2) inhibitors such as ruxolitinib, interleukin-2 receptor (IL-2R) antibodies such as daclizumab, sirolimus, and everolimis that are the mammalian target of rapamycin (mTOR) inhibitors, tumor necrosis factor-alpha (TNF-α) inhibitors such as infliximab, anti-CD52 antibody (alemtuzumab), anti-α4β7 integrin antibody (vedolizumab), and extracorporeal photopheresis (ECP) used alone or in combination. However, the optimal second-line therapy is still not universally established [1–5].

The American Apheresis Society guidelines, the British Committee for Standards in Haematology and the British Bone Marrow Transplantation Society guidelines, and the Italian guidelines recently published by the Italian Society of Apheresis and the Italian Group for Bone Marrow transplant suggest the use of extracorporeal photopheresis (ECP) as a second-line therapy in corticosteroid-resistant graft-versus-host disease (GvHD) [6–8].

ECP consists of the collection of a patient's peripheral blood mononuclear cells (MNCs) that, after incubation with a photosensitive molecule (8-methoxypsoralen or 8-MOP), are exposed to ultraviolet-A and then reinfused into the patient [9]. There are two methods for performing the ECP procedure: the "off-line" and "in-line" methods [10–13].

In the "off-line" method, all the phases of ECP (leukapheresis, photoactivation, and reinfusion) are achieved sequentially using separate equipment: A cell separator performs leukapheresis for mononuclear cell collection using a sterile disposable kit. Red blood cells and plasma are returned to the patient. Using a laminar flow cabinet, the MNC bag must be transferred to an appropriate special bag to which 8-MOP is added. After UVA irradiation, the collected cells are reinfused into the patient using a standard transfusion filter [10,11]. In the "in-line" method, all the phases of ECP (leukapheresis, photoactivation, and reinfusion) are performed sequentially in extracorporeal circulation using a single instrument and a single sterile disposable kit without disconnection from the patient's blood circulation. The cell separator collects the peripheral blood mononuclear cells, while the red blood cells and plasma are returned to the patient. The MNCs are collected in a bag, 8-MPO is added, and then it is exposed to UVA. After the irradiation treatment, the MNC concentrate is reinfused back into the patient [12,13]. A schematic pictorial comparison of the ECP methods performed with the off-line and in-line approaches is reported in Figure 1.

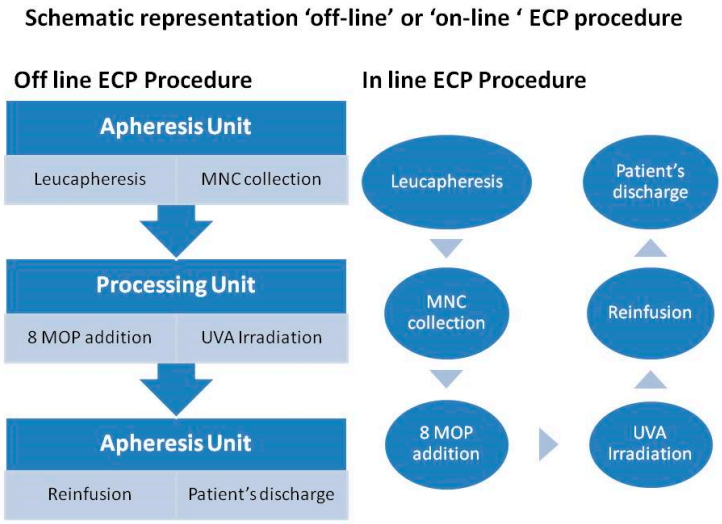

Figure 1. Schematic representation of the off-line and in-line ECP procedures.

In this paper, we report our real-life experience with a recently licensed in-line ECP system proposed by Fresenius-Kabi. Our study aimed to evaluate the following items:

the ease of introducing this in-line ECP method ex novo into an apheresis unit, the costs in light of the reimbursement regime practiced by the Italian national health system, the characteristics of the procedure with particular attention to the execution times and the efficacy of MNC collection, and the safety profile of the procedure with particular regard to the adverse events observed.

The graphical schematization reported in Figure 1 underlines how, in ECP performed using the off-line method, three separate and successive phases can be recognized that must be carried out in separate areas. The first phase consists of the collection of MNCs via leukapheresis, which must be carried out in the apheresis unit. The second phase consists of the addition of the photoactive drug and irradiation with UVA of the blood component obtained, which must be carried out in the processing laboratory. The third phase consists of the reinfusion of the treated blood component into the patient, which must be carried out in the apheresis unit.

In contrast, in ECP performed using the in-line method, we have a continuous process characterized by the succession of the phases described that occurs without interruption of continuity of the circuit and always inside the apheresis unit.

2. Materials and Methods

Study Location: This study was conducted in the Apheresis Unit of the Transfusion Medicine dell'Angelo Hospital, a large general hospital in northeast Italy. This was a single-center, non-blind, real-life study in adult patients. Each subject provided written informed consent before starting the ECP cycle. Additionally, each patient signed our standard consent form to use their clinical data for study purposes.

Patients: From May 2023 to April 2024, we performed 211 ECP procedures in 11 patients: 6 males and 5 females aged from 31 to 67 years. Personal and clinical data were obtained from the patients' clinical documentation. These data are reported in Table 1.

All patients had GvHD that had become refractory/dependent on corticosteroid treatment, were recommended ECP treatment by the Hematology Department of the Ospedale dell'Angelo, and were evaluated collegially before enrollment. For patient enrollment, the following criteria were adopted: adequate kidney function (estimated glomerular filtration rate > 40 mL/min), no active liver disease (ALT $\leq$ 120 UI/L), adequate cardiac function (no cardiac disease or New York Heart Association Class $\leq$ II if cardiac disease was present), WBC $\geq$ 1000/μL, and platelet count $\geq$ 30,000/μL, Hb $\geq$ 100 g/L. A complete blood count was performed before each procedure. Any transfusion therapy to correct anemia or thrombocytopenia below the limits reported above was carried out in our apheresis unit before starting ECP. Subjects with hypersensitivity and/or allergy to psoralen or citrate were excluded. Patients with active uncontrolled viral, fungal, or bacterial infections were also excluded.

ECP procedures: The ECP procedures (n = 211) were performed using an Amicus cell separator and a Phelix UV irradiator with Amicus software 4.51 and Phelix software 1.0 (Fresenius-Kabi Italia, Isola dela Scala, Italy). A targeted 2000 mL of whole blood (WB) was processed, and 1.5 J/cm^2 of UVA light was delivered to the collected mononuclear cells. From May to August 2023, we adopted a double-needle-only kit (R6R2347C). From September 2023 to April 2024, we switched to a new single–double-needle kit (X7R2346C). Anticoagulation consisted of acid citrate dextrose solution A (ACD-A) (Fresenius-Kabi Italia, Isola dela Scala, Italy) with a 12:1 whole blood (WB)-to-ACD-A ratio; the maximum WB draw rate was 80 mL/min, with a 1.25 mg/kg/min citrate infusion rate. Supplemental calcium, either oral or intravenous, was not routinely administered. The Amicus ECP protocol utilized fixed, predefined offsets to minimize the collected cell variability. In this software version, the corresponding MNC and RBC offsets were 1.5 and 6.8, respectively.

After the MNC harvest (200 mL), the separator automatically added 170 mL of saline to the collected MNCs in the treatment container, after which the operator added 3.4 mL of 8-methoxy psoralen (20 µg/mL) (G.L. Pharma Italia srl. Milan, Italy) and started photoactivation. After photoactivation, the system automatically reinfused treated MNCs, and the procedure was completed [14–17].

Table 1. Patient series description and clinical data.

	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**
Gender	Female	Male	Female	Female	Male	Female	Male	Male	Male	Female	Male
Age *, years	66	67	64	59	58	65	31	59	30	57	36
Weight *, kg	85	72	67	61	60	68	43	71	64	40	49
Diagnosis	AML	AML	MDS	AML	MDS	AML	MDS	AML	ALL	ALL	AML
Donor type	MUD	MUD	MUD	FAM	MUD	MUD	MUD	FAM	FAM	MUD	FAM
Harvest	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC	PBSC
GVHD type	Chronic	Chronic	Acute	Chronic	Acute	Chronic	Acute	Acute	Acute	Chronic	Chronic
GVHD degree	Moderate	Moderate	Severe	Moderate	Severe	Severe	Moderate	Moderate	Moderate	Severe	Severe
Localization	Skin	Skin Eyes	Skin Liver	Skin Joints	Skin Lungs Mem-branes	Liver Gut	Skin	Skin Gut	Skin Mucous Mem-branes	Skin Lungs	Skin Gut Lungs
Hematological malignacy	REM.	REM	REM	REM	REM	REM	REM	Relapse	REM	REM	REM
Status	Alive	Alive	Alive	Alive	Alive	Alive	Alive	Deceased	Alive	Alive	Alive
Response to ECP	CR	PR	PR	CR	CR	PR	CR	NR	CR	PR	PR
Other therapies	Tacr	Ruxo	Tacr Pred	Ritux IG Pred	Ruxo	Benda Pred	Tacr Pred Ruxo	Pred	Pred	Cyclo Pred	Cyclo Pred

AML, acute myelogenous leukemia; MDS, myeloid dysplastic syndrome; ALL, acute lymphoblastic leukemia; PBSC, peripheral blood stem cells; MUD, marrow-unrelated donor; FAM, family-related donor; Weight * and Age * at first observation in our center; REM, remission of the hematologic disease; relapse, relapse of the hematologic disease; CR, complete response; PR, partial response; NR, no response; Tacr, tacrolimus; Ruxo, ruxolitinib; Pred, prednisone; Benda, bendamustine; Cyclo, cyclosporine. Patient 08 died despite being in relapse of hematological disease due to infectious complications.

Calculations and statistical analysis: The data were analyzed using MedCalc Ver.8.0.0 (MedCalc SW bvda, Ostend, Belgium). First, using the D'Agostino–Pearson test, we evaluated the distribution of the data, and, having rejected the hypothesis of a "normal" distribution, we used a non-parametric statistical approach. The categorical data are presented as numbers (percentages) and the continuous data as medians (MED) and interquartile ranges (IQRs). For data comparisons, we adopted the Kruskal–Wallis test; an Alpha value defined as $p < 0.05$ was considered statistically significant. The collection efficiency, also defined as CE2, was calculated using the following formula: CE2 (%) = collected MNC yield × 100/pre-procedure WB MNC/blood volume processed (mL) subject post-procedure MNC)/2 and AC = anticoagulant volume [18,19].

Cost evaluation: The evaluation of the costs related to the introduction of the ECP procedure in our transfusion medicine center was performed using a health-activity-based costing approach (HABC) [20,21].

3. Results

Patient characteristics: From May 2023 to April 2024, we performed 211 ECP procedures in eleven patients who had received a PBSC transplant (four from a family donor and seven from an unrelated donor). The GvHD type (acute or chronic) and grading (mild, moderate, or severe) were classified according to international criteria [22,23]. The

patient demographics, underlying disease, transplant type, GvHD characteristics, and other immunosuppressive therapies are reported in Table 1.

Immediately before starting every single ECP procedure, a complete blood count was performed to verify alignment with the enrollment criteria; the administration of blood components, both packed red cells (PRCs) and/or platelet concentrates (PCs) for the correction of any anemia and/or thrombocytopenia, was always carried out in our apheresis unit before starting the ECP procedure. Transfusion before ECP was necessary in seventeen cases, PRC transfusion was necessary in eleven cases, and PC transfusion was necessary in six cases. The protocol in use at our organization provides for the use of irradiated blood components in these patients. No post-transfusion adverse events were observed.

ECP procedures: Those patients diagnosed with acute GvHD were treated with two ECP procedure cycles weekly for four weeks, followed by two consecutive ECP cycles every two weeks for at least another four weeks. ECP could be discontinued or continued if a response was achieved, depending on the hematologist's indications. Those patients diagnosed with chronic GvHD followed a different treatment schedule consisting of a weekly ECP procedure for four weeks, followed by a single procedure every two weeks for another four weeks, and then every four weeks until discontinuation depending on the response [24–28]. The procedure parameters are summarized in Table 2. Vascular access was obtained using a peripheral venous catheter in 92 (43.1%) procedures with wide inter-patient variability from 0% to 97.7%. The processed blood volume (from 1992 to 2000 mL) was always consistent with what was planned (2000 mL), with a treated blood volume fraction ranging from 42% to 61%. The blood flow speed during the procedures was highly variable (from 30 to 50 mL/min) depending on the conditions of the patient's peripheral veins, the type of venous access used, and the choice to use a one- or two-way procedure. Because of the variability in the blood flow maintained during the procedure, the total duration of the procedure fluctuated considerably (from 92 to 118 min). The duration of an ECP procedure performed using the double-needle method was 98 $\pm$ 18 min (median $\pm$ IQR), while the duration of an ECP procedure using the single-needle method was 109 $\pm$ 22 min (median $\pm$ IQR) ($p = 0.01$). The collection efficiency (CE2) for mononuclear cells was always satisfactory (from 55% to 73%), with an adequate positive selection compared to the collection efficiency (CE2) observed for total leukocytes (from 11% to 39%).

Quality evaluation: Table 2 reports the characteristics of the apheresis products. Platelet and erythrocyte contamination was always extremely limited, and mononuclear cells constituted the majority of the leukocytes collected (from 67.6% to 97.9%).

Clinical response: As reported in Table 1, ECP could control the symptoms, achieving a complete or partial clinical response in 10/11 (91.9%) patients. In fact, we observed five complete remissions, five partial remissions, and one non-responder.

Safety profile: Among the two-hundred-eleven procedures, it was not possible to complete eleven (5.2%)—in one case due to the onset of shivering and hyperthermia, in nine cases due to problems in the management of venous access, and in a further case due to problems in the management of the circuit.

Cost analysis: The cost of the in-line ECP procedure performed in our transfusion medicine center using Fresenius-Kabi Amicus Blue equipment was EUR 985.

Table 2. Extracorporeal photopheresis procedures, characteristics of the apheresis products, and collection efficiency.

	01	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**
ECP procedures											
N° Proc.	31	19	11	16	18	16	35	5	19	14	17
Vol., mL	1995 (9)	1992 (15)	1997 (12)	1998 (5)	2000 (7)	2000 (11)	1999 (32)	2000 (11)	1998 (21)	2000 (17)	1999 (8)
PBV%	43 (4)	44 (9)	42 (9)	49 (2)	45 (5)	56 (2)	43 (2)	48 (6)	42 (3)	61 (8)	60 (7)
Flux., mL/m	50 (9)	50 (10)	45 (10)	40 (9)	40 (15)	50 (11)	30 (15)	45 (9)	40 (15)	15 (8)	40 (12)
Time, min	92 (7)	93 (11)	95 (14)	101 (19)	103 (19)	98 (13)	118 (19)	106 (20)	108 (18)	112 (6)	113 (21)
ACD, mL	170 (3)	170 (5)	170 (4)	170 (3)	169 (3)	171 (6)	169 (2)	170 (9)	169 (5)	170 (7)	169 (4)
Characterization of the apheresis products before adding 8-MOP											
WBC, 10^9/L	14,370 (9190)	10,630 (8659)	10,311 (4001)	8350 (4060)	5880 (1010)	10,140 (5830)	10,400 (6010)	10,865 (7894)	9720 (5937)	8990 (6260)	11,775 (4488)
RBC, 10^{12}/L	0.22 (0.06)	0.23 (0.05)	0.23 (0.06)	0.24 (0.04)	0.25 (0.04)	0.26 (0.03)	0.20 (0.04)	0.25 (0.05)	0.31 (0.04)	0.021 (0.06)	0.23 (0.04)
Hb, g/L	7 (2)	7 (1)	7 (1)	7 (2)	7 (1)	8 (2)	5 (1)	6 (1)	9 (1)	5 (1)	7 (2)
HCT, %	2.5 (0.6)	2.5 (0.5)	2.5 (0.8)	2.6 (0.7)	2.6 (0.8)	2.5 (0.6)	2.5 (0.5)	2.7 (0.8)	3.1 (0.9)	2.6 m (0.6)	2.5 (0.4)
PLT, 10^9/L	99 (57)	84 (43)	116 (63)	111 (67)	135 (59)	123 (18)	167 (48)	94 (37)	106 (40)	37 (14)	98 (33)
MNC, 10^9/L	13,777 (8995)	10,460 (7053)	9899 (7634)	7932 (4843)	5351 (3365)	8112 (4715)	9776 (3990)	7265 (3972)	9039 (4532)	7821 (3102)	11,186 (4160)
MNC%	95.8 (8.8)	97.9 (10.9)	95.6 (16.8)	95.3 (13.9)	91.2 (19.1)	79.6 (15.6)	94.3 (6.7)	67.6 (21.3)	92.9 (5.9)	87.2 (10.1)	95.2 (11.7)
Collection efficiency (CE2%) evaluated for mononuclear cells and total white blood cells											
MNC CE2%	64 (24)	65 (21)	66 (20)	63 (17)	73 (29)	67 (12)	55 (28)	59 (19)	55 (25)	72 (20)	69 (25)
WBC CE2%	27 (8)	28 (15)	29 (19)	22 (10)	28 (16)	24 (12)	11 (8)	19 (11)	27 (19)	39 (8)	37 (20)

N° Proc, number of ECP procedures performed in each patient; Vol., processed blood; PBV%, fraction of total blood volume processed in each ECP procedure; Flux., flow velocity; Time, total time for ECP procedure completion; ACD, volume of ACD used in each ECP procedure; WBC, number of total WBCs in the apheresis products; RBC, number of total RBCs in the apheresis products; Hb, hemoglobin concentration in the apheresis products; HCT, hematocrit of the apheresis products; PLT, number of total PLTs in the apheresis products; MNC%, prevalence of mononuclear cells in the apheresis products; MNC CE2%, collection efficiency for mononuclear cells; WBC CE2%, collection efficiency for WBCs. Results are reported as the median value (interquartile ranges).

4. Discussion

To the best of our knowledge, this is one of the first evaluations conducted using the Amicus Blue (Fresenius-Kabi) in-line ECP system. Our aim was not to compare with other off-line or in-line ECP methods but simply to report the strengths and difficulties we encountered in introducing this system in apheresis units that did not previously perform ECP.

Feasibility of ECP introduction: When our transfusion medicine center received regional authorization to implement a clinic dedicated to ECP, our interest was immediately focused on acquiring an in-line system [24–28]. The following considerations supported this orientation. Our reference transplant program is authorized to treat adult patients only; therefore, the problems observed for in-line systems in the treatment of pediatric and/or very-low-weight patients did not seem to be relevant in our operative setting [29–31]. In our hospital, the apheresis unit is located inside the main body, while the processing unit is decentralized in a building approximately 600 m away. This would have made the transfer of the apheresis product from the collection unit to the processing unit and vice versa logistically complicated. By using an in-line system, patient/harvest continuity is never interrupted; therefore, an autologous blood component is not created, greatly simplifying the management process and significantly limiting the number of tests needed to qualify

it [32,33]. Furthermore, from the literature data, it is clear that the in-line method is much faster than the off-line method (around 120 min versus 240 min) [11,34].

Cost analysis: At the Italian national level, starting in January 2025, ECP will be included within the Minimum Assistance Levels (LEAs) with the code 99.83, with a reimbursement rate of EUR 900.60. This rate, although not able to cover the total costs of the procedure, is able to guarantee the coverage of the out-of-pocket costs deriving from the rental of the equipment and the purchase of disposable circuits and consumables [35–37].

Clinical response: In one patient, ECP treatment was suspended due to the recurrence of the underlying hematological disease (AML), and the patient died due to infectious complications. Indeed, infectious complications are seldom reported in patients with GvHD treated with ECP and are probably due to the immunosuppression that characterizes these subjects rather than to ECP itself [38,39]. Ten patients were evaluable and underwent several ECP procedures, ranging in number from 11 to 35, during the observation period. In our experience, the probability of obtaining a complete response was higher in patients with chronic moderate GvHD. In fact, among the four patients with acute GvHD, only one (25%) showed a complete response, compared to the four observed among the six patients with chronic GvHD (66%). Furthermore, among the five patients with moderate GvHD, we observed four (80%) complete responses and one (20%) partial response. On the contrary, among the five patients with severe GvHD, we observed a complete response in only one case (20%) and a partial response in four (80%). In any case, all the patients benefited from the ECP treatment. These data are in good agreement with the literature [40,41].

Side effects: The data obtained in the present evaluation confirm how in-line ECP, although essentially remaining an invasive procedure, constitutes a therapeutic alternative not burdened by particularly relevant side effects. In our experience, among the 211 procedures, no serious adverse events were observed, and, in a single case (0.5%), we had to suspend the procedure because the patient experienced the onset of a chill and fever. The patient's blood culture was negative. In the literature, ECP therapy is characterized by an excellent safety profile with a low ratio of serious adverse events [42,43]. Due to increased photosensitivity, patients should be instructed to wear eye and skin protection for 24 h after ECP treatment. In our series, in 9/211 cases (5.2%), it was not possible to complete the ECP procedure due to problems related to venous access. To perform ECP procedures, there is the need for either repeating peripheral venous puncture or long-term venous access (peripheral or central), so, in almost all the series reported in the literature, prevalent side effects are related to problems with vascular access (the inability to find the vein, obstruction of access, local hematoma, arterial puncture, phlebitis, venous thromboembolism, catheter-related infection, etc.). In fact, finding and maintaining adequate venous access, in our experience, was the main problem despite using a trained and motivated nursing team dedicated only to therapeutic apheresis procedures. In 43.1% of the cases, it was necessary to place a peripheral venous catheter. We believe that the management of venous access in our therapeutic apheresis clinic could benefit from introducing an ultrasound scanner to guide the venipuncture maneuvers. Unfortunately, it has not yet been possible to acquire such equipment. It must be added that, during ECP procedures, having available trained nurses with good empathy to assist these patients constitutes a significant added value [44,45]. In one case, the ECP procedure was interrupted because of problems with kit management.

Collection efficiency: Evaluation of apheresis products: In our experience, the MNC median collection efficiency (CE2) was 64%; this value is satisfactory and comparable with the values reported for other in-line and off-line methods [10,11,13–15,18,43,44]. Some characteristics of the collected apheresis products, such as the presence of erythrocytes and platelets, can influence the effectiveness of photoactivation. In our series of proce-

dures, the median hemoglobin in the apheresis products was between 5 and 9 g/L, with a median hematocrit of between 2.2% and 3.1%. The median platelet count was between 68 and 135 10^9/L. These values are satisfactory and in line with what is reported in the literature [10–13]. Moreover, this in-line ECP system collects concentrated MNCs with only 30 mL of residual plasma and uses saline to dilute the cells prior to photoactivation. Utilizing this low percentage of patients' plasma decreases the impact of hyperlipemia and hyperbilirubinemia on photoactivation efficiency [45,46].

In our experience, we noticed that, at the end of the number of sessions foreseen by the protocol, it was very difficult to wean the patient from ECP. This is regardless of whether a partial or complete response had been obtained. This inability to discharge patients may, over time, lead to an overload of access, only partially appropriate, to our apheresis unit.

Study limitations: In our opinion, the main limitation of the present study lies in the difficulty in evaluating the degree of apoptosis of MNCs after photoinactivation. A further limitation is the small number of patients enrolled. It is important to evaluate this in-line ECP method in pathologies other than GvHS, such as in cutaneous T cell lymphoma.

5. Conclusions

This study reported a real-life, retrospective, monocentric study regarding the Fresenius-Kabi in-line Amicus ECP system. The safety and device performance were investigated. The ideal ECP system characteristics include safety, a short procedure time, and high MNC yields. In our experience, the ECP procedure performed using the Amicus system was safe and well tolerated because of the low extracorporeal volume of the disposable circuit. The most relevant problems relate to difficulty in the management of vascular access. The procedure duration was usually under two hours, even using single-vascular access. The blood volume processed in this study was 2000 mL, and the MNC collection efficiency (CE2) was satisfactory, with minimal platelet and RBC product contamination. Furthermore, the supplied circuit can add 8-MOP and carry out appropriate sampling using "doors" equipped with antibacterial filters, helping to maintain the sterility of the product. As a final consideration, the system enables the complete traceability of each procedure. Data should be printed locally, but we chose to transmit the procedure data to the information technology management informative system (EmoNet GPI).

Author Contributions: G.D.F. enrolled patients, obtained consents for data use, tabulated data, and wrote the manuscript. G.G. designed the study, performed statistical processing, and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The study did not receive funding and is based on the collection and processing of data from the daily clinical practice of our apheresis unit.

Institutional Review Board Statement: This is a retrospective study based on the analysis and processing of clinical data routinely collected during ECP which is a consolidated therapy in the second-line treatment of GvHD.

Informed Consent Statement: All patients gave informed consent before undergoing ECP-mediated therapy. In addition, each subject gave a written release for the authorization to use, even for research purposes, cellular products, clinical data and images (but not nucleic acids).

Data Availability Statement: Data are available after motivate request to Giulia De Fusco (giulia.defusco@auls3.veneto.it).

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Conflicts of Interest: The authors are employees of the Italian National Health Service and deny any conflict of interest.

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