Received: 20 December 2019 | Revised: 17 March 2020 | Accepted: 9 June 2020

DOI: 10.1002/jca.21804

RESEARCH ARTICLE

Journal of Clinical Apheresis ASFA WILEY

Pilot study of a new online extracorporeal photopheresis system in patients with steroid refractory or dependent chronic graft vs host disease

Katherine Radwanski[1] | **Edwin Burgstaler**[2] | **Jennifer Weitgenant**[1] | **Heather Dale**[2] | **Cheryl Heber**[1] | **Jeffrey Winters**[2]

[1]Fresenius Kabi USA, Lake Zurich, Illinois

[2]Division of Transfusion Medicine, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

Correspondence
Katherine Radwanski, Fresenius Kabi USA, 3 Corporate Drive, Lake Zurich, IL 60047.
Email: katherine.radwanski@fresenius-kabi.com

Funding information
Fresenius Kabi

Abstract

Background: A new protocol has been developed on the Amicus Separator that enables the device to perform online extracorporeal photopheresis (ECP) procedures when used in conjunction with the Phelix photoactivation device and associated disposable kit. The objective of this study was to evaluate the safety and performance of the Amicus ECP System in adult subjects with steroid-refractory or dependent chronic graft vs host disease (cGVHD).

Study Design and Methods: Eight subjects with mild to severe cGVHD underwent 31 procedures. Subject safety evaluations were performed pre and post procedure and adverse events (AEs) were recorded during treatment and 24 hours after the last procedure. In vitro evaluations of the treated cells included hematology counts and lymphocyte apoptosis, viability and proliferation as measures for ECP procedure validation.

Results: For n = 23 evaluable procedures, median (range) procedure time was 88 (78-110) minutes, during which 2.9 (0.6-4.7) $\times 10^9$ TNCs (approximately 90% MNCs) were treated and reinfused to the subjects. All subject safety evaluations (vitals, cell counts, plasma hemoglobin and bacterial and endotoxin testing) were within expected ranges. All device or procedure related AEs were mild in nature. After 24 hours in culture, 86 (52-98)% of treated lymphocytes were apoptotic compared to 27 (15-51)% in controls. Inhibition of lymphocyte proliferation was >91% in all procedures.

Conclusion: ECP procedures were safely completed in adult subjects with SR-cGVHD treated using the new online Amicus ECP system.

KEYWORDS

Amicus, cGVHD, ECP

1 | INTRODUCTION

Extracorporeal photopheresis (ECP) is a photoimmune therapy in which mononuclear cells (MNCs) are combined with a light sensitive drug, 8-methoxypsoralen (8-MOP), and photoactivated with UVA light ex vivo. Although originally developed for cutaneous T-cell lymphomas, ECP plays a wider role today in the treatment of acute and chronic graft vs host disease (GVHD) and in the management of heart and lung transplant rejection.[1] Other therapeutic indications currently under investigation for this therapy include GVHD prophylaxis,[2] kidney[3] and liver allograft rejection,[4] and bronchiolitis obliterans syndrome post stem cell transplantation.[5] This broad range in current and potential clinical applications is in part due to the excellent safety profile of ECP, which has minimal side-effects and no reported long-term complications, particularly in comparison with other immunosuppressive therapies.[6]

Two types of systems are currently in use for this therapy: (a) online systems, in which MNC collection, photoactivation and reinfusion are performed using a single, dedicated device in a closed system and (b) offline systems, which consist of two separate collection and photoactivation devices and multiple disposables that are used together in an open system. Advantages to the online system include shorter procedure time, option for single needle access, lower risk for intraprocedural contamination, and reduced risk of improper infusion.[7,8] On the other hand, offline systems benefit from procedural flexibility, including lower extracorporeal volume, wider range of whole blood (WB) processed and the ability to perform multiple therapeutic protocols on the separation device. However, users must follow guidelines for minimal cell manipulation, including performing multi-step procedures in a class A laminar airflow cabinet located in a class D laboratory as well as performing quality controls and sterility testing on the treated cell product.[7] These systems also require additional upfront validation since the protocol and materials are user defined and not validated for use together by the manufacturers.

A new type of online system has been developed to include benefits of both types of systems. The Amicus ECP system (Fresenius Kabi, Lake Zurich, IL) builds on the existing capabilities of the Amicus Separator (Fresenius Kabi, Lake Zurich, IL), which has established donor and therapeutic apheresis protocols, including MNC collection, and has been historically used in offline ECP systems.[9,10] A new photoactivation device, Phelix, was developed to work in conjunction with the Amicus Separator and a functionally closed disposable kit to provide ECP as a closed system. The aim of this pilot study was to evaluate the safety and performance of the investigational Amicus ECP System in adult subjects with steroid-refractory or -dependent chronic GVHD (cGVHD).

2 | MATERIALS AND METHODS

2.1 | Study design

This was a single-center, nonblinded, pilot study in adult patients previously started on and currently receiving ECP therapy for cGVHD. The Amicus ECP System was incorporated into the subject's existing ECP treatment regimen according to the physician's discretion. Eight subjects participated in the study under an Investigational Device Exemption. Each subject provided written informed consent, as approved by the Mayo Clinic Institutional Review Board, before participating in the study. An interim safety analysis was completed after treatment of the first three subjects prior to enrolling additional subjects.

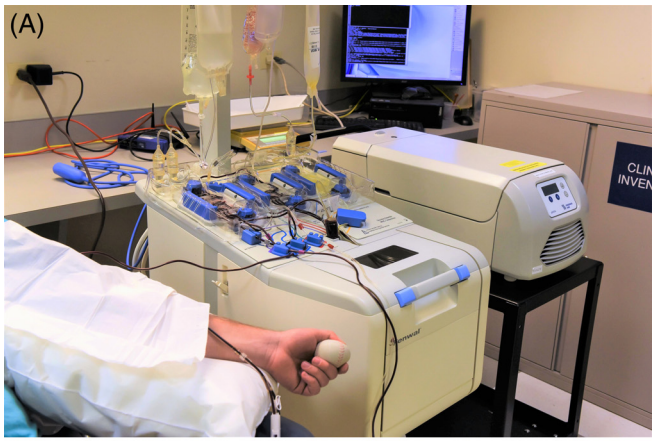
2.2 | Inclusion/exclusion criteria

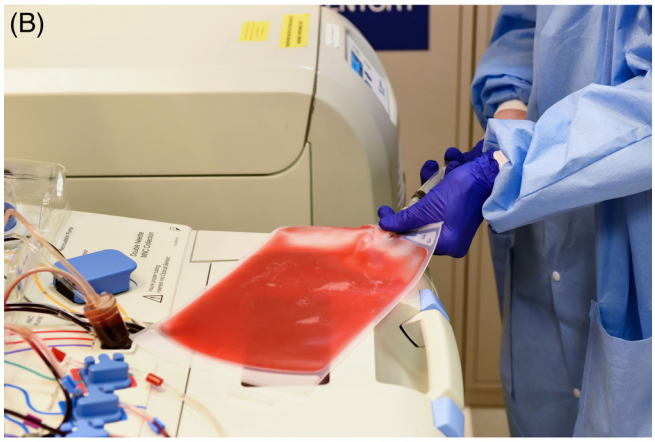
The following criteria were used to determine eligibility for enrollment into the study: adequate renal (GFR >30 mL/min/BSA), hepatic (AST between 10 and 120 unit/L), pulmonary and cardiac function (no cardiac disease or New York Heart Association Class I or II symptoms if cardiac disease was present) to ensure the subject could tolerate the extracorporeal volume shifts associated with ECP. WBC $\geq$ 1000/μL and platelet count $\geq$25 000/μL were required to ensure an adequate cell dose for treatment and to minimize bleeding risk. Women of childbearing potential had to agree to use a reliable method of birth control during the study and males were either surgically sterile or agreed to use an acceptable method of birth control during the study and for 30 days after last study treatment. The presence of any of the following criteria excluded the subject from participating in this study: hypersensitivity and/or allergy to psoralen or citrate; active gastrointestinal bleeding; overt signs of relapse of hematologic malignancy necessitating allogeneic stem cell transplant; uncontrolled viral, fungal or bacterial infections; total bilirubin value $\geq$15 mg/dL; veno-occlusive liver disease; life expectancy <8 weeks; HIV, hepatitis B, or C virus infection; and pregnant and/or lactating females. Subjects with aphakia, history of a light-sensitive disease, or receiving concomitant therapy with known photosensitizing agents were also excluded.

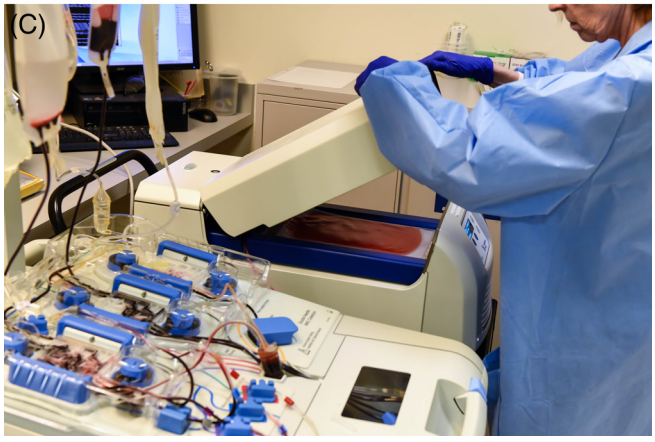
2.3 | ECP procedures

Double-needle access procedures (n = 31) were performed using Amicus software 4.51 and Phelix software

1.0 (Figure 1), which targeted 2000 mL of whole blood (WB) processed and 1.5 J/cm^2 of UVA light delivered to the collected mononuclear cells (MNCs). Anticoagulation consisted of acid citrate dextrose solution A (ACD-A). Heparin was not used. Additional settings included 12:1 WB to ACD-A ratio, maximum WB draw rate of 80 mL/min and 1.25 mg/kg/min citrate infusion rate. Supplemental calcium, either oral or intravenous, was not routinely administered. Unlike the MNC collection protocol on the Amicus Separator, the ECP protocol utilizes fixed, predefined offsets in order to minimize collected cell variability. In this software version, the corresponding MNC and RBC offsets were 1.5 and 6.8, respectively. After MNC harvest, 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-methoxypsoralen (20 μg/mL) and commenced photoactivation. After photoactivation, the Amicus Separator reinfused the treated cells and residual cells in the kit to the subject and the procedure was completed.







2.4 | Subject evaluations

Patient demographics, primary diagnosis, transplant source and date, cGVHD-severity, and steroid status were recorded at baseline as were concomitant medications taken on the day of the procedure and up to 7 days prior to the procedure. A pregnancy test was performed on all women with reproductive potential at the time of screening/baseline and prior to the start of any Amicus ECP procedure. Other eligibility parameters (eg, WBC and platelet count) were obtained within 7 days prior to starting an Amicus ECP procedure.

Subject blood samples were drawn pre-and post- procedure (inlet/return line of kit or directly from port if used) for hematology evaluation, including a complete blood count (CBC) with 5-part WBC differential and plasma hemoglobin.

Safety assessments included monitoring of subjects' vital signs pre- and post-procedure and adverse event (AE) reporting during the procedure. Subjects were also contacted approximately 24 hours post their last ECP procedure for an AE assessment. Procedure parameters, alarms, unanticipated adverse device effects (UADEs), and kit issues reported during the ECP procedure were recorded whereas AEs attributed to a subcutaneous port or peripheral vascular access, such as inflammation and complications due to access issues, were not.

2.5 | Collected and treated cell evaluations

Samples of the collected cells were obtained pre- and post-ECP treatment for hematology counts, plasma hemoglobin and lymphocyte viability, apoptosis, and proliferation assays. Samples were also drawn post ECP

treatment for endotoxin and 14-day bacterial aerobic and anaerobic culture analysis.

Samples for lymphocyte assays were shipped at room temperature overnight to the testing laboratory. Upon receipt, control and treated cells were purified using density gradient sedimentation (Ficoll-Paque PLUS, GE Healthcare Bio-Sciences, Piscataway, New Jersey). Cell samples for lymphocyte apoptosis/viability measurement were resuspended at a concentration of 1-2 $\times 10^6$/mL in RPMI 1640 tissue culture medium supplemented with 2 mM glutamine (Hyclone, Thermo Scientific, Waltham, Massachusetts) and 10% human serum (Sigma Aldrich, St. Louis, Missouri) and cultured in 25 cm^2 flasks (Falcon, Becton Dickinson, Franklin Lakes, New Jersey) at 37°C with 5% CO_2 in a humidified chamber for up to 3 days. Lymphocyte apoptosis and viability were measured using flow cytometry (FACSCanto II, BD Biosciences, San Jose, California), with antibodies directed at CD2/19 (total lymphocytes), Annexin-V FITC (apoptosis) and 7-AAD (viability) (BD Biosciences, San Jose, California).

For lymphocyte proliferation, treated and control samples were adjusted to approximately 5-10 $\times 10^6$/mL in RPMI 1640. Carboxyfluorescein succinimidyl ester (CFSE) (Life Technologies, Carlsbad, California) was dissolved in dimethyl sulfoxide (DMSO) to a concentration of 45 mM and stored frozen at −30°C until the time of use. CFSE stock solution was further diluted 1:100 in DMSO at the time of testing and the diluted CFSE solution was added to each cell sample (final CFSE concentration = 2 μM). Cells were incubated for 10 minutes in a 37°C water bath with periodic mixing to achieve even heating and CFSE uptake. After labeling, the cells were washed twice with RPMI 1640, resuspended and cultured as described above for apoptosis assays. Phytohemagglutinin (PHA, Life Technologies, Carlsbad, California), a plant lectin used as a mitogen to trigger lymphocyte proliferation, was added (2 μg/mL) to each of the culture flasks. After 3 days in culture, samples were assayed for lymphocyte proliferation using flow cytometry (FACSCanto II, BD Biosciences, San Jose, California), with CD2/19 antibodies for lymphocyte identification and CFSE fluorescence for cell division. Lymphocyte proliferation (%) was calculated by dividing the number of CFSE dim cells by the total number of CFSE bright plus dim cells. Inhibition of proliferation was calculated by dividing the difference between lymphocyte proliferation (%) in the control cells and ECP-treated cells by the lymphocyte proliferation (%) in the control cells.

2.6 | Calculations and statistical analysis

All statistical analyses were performed using standard statistical software (SAS version 9.4, SAS Institute, Cary, North Carolina). Mononuclear cell collection efficiency (CE) was determined by the formula: CE (%) = collected MNC yield/[Avg MNC count × (WB volume processed − AC volume)] × 100, where Avg MNC count = (subject pre-procedure MNC + subject post-procedure MNC)/2 and AC = anticoagulant volume.

3 | RESULTS

3.1 | Patient characteristics

Patient demographics, underlying disease, transplant and cGVHD characteristics are presented in Table 1. Most patients had moderate, steroid dependent cGVHD post peripheral blood stem cell transplant from an unrelated donor for acute myeloid leukemia and were concurrently being treated with corticosteroids and a calcineurin inhibitor (tacrolimus).

3.2 | ECP procedures

Procedure parameters are summarized for n = 23 evaluable procedures in Table 2. Three procedures were not completed due to non-recoverable alarms that occurred during photoactivation, which were due to software issues that have been addressed in subsequent Phelix software versions. Five additional procedures were considered non-evaluable due to protocol/procedure deviations. Almost all Amicus ECP procedures (n = 29, 94%) were performed on the second day of consecutively scheduled treatments, with the Therakos CELLEX System (Mallinckrodt Pharmaceuticals, Hazelwood, Missouri), being used for the first day of treatment in all but one case. Vascular access was obtained using peripheral venous access (n = 27, 87%) or subcutaneous port (n = 4, 13%). Thirteen Amicus Separator alarms were noted in 10 procedures, the majority of which were access related (inlet/return line occlusion or low pressure, n = 7). All Amicus Separator alarms were recoverable, and procedures were resumed. There were no additional Phelix alarms other than those noted above.

3.3 | Subject safety evaluations

All pre-and post-procedure vital signs (including systolic and diastolic blood pressure, pulse, temperature, and respirations) were within expected ranges (data not shown). Subject hematology counts, pre- and post-ECP procedure, are shown in Table 3. Changes in these parameters pre-to post procedure were as expected due to dilutional effects of crystalloid (ACD-A, saline) infusion. There was an

TABLE 1 Subject demographics and baseline characteristics

	Median (range) or n (%)
Demographics	
Age (years)	62 (29-73)
White	8 (100)
Male	6 (75)
Primary diagnosis	
Acute myeloid leukemia	6 (75)
Myelodysplastic syndrome	2 (25)
Transplant characteristics	
Peripheral blood	8 (100)
Unrelated donor	5 (63)
10/10 match	8 (100)
Time from transplant (years)[a]	4.1 (0.7-7.0)
NIH severity[b]	
Mild	1 (13)
Moderate	5 (63)
Severe	2 (25)
Steroid status[b]	
Refractory	2 (25)
Dependent	6 (75)
Concomitant medications for cGVHD	
Corticosteroids	5 (63)
Tacrolimus	6 (75)
Ruxolitinib	2 (25)

[a]Time from transplant to first Amicus ECP procedure.
[b]Status at first Amicus ECP procedure.

TABLE 2 ECP procedure parameters

	Median (range), n = 23
Subject blood volume (mL)	5249 (3929-6120)
Total WB drawn (mL)[a]	2352 (2318-2376)
ACD-A used (mL)	191 (185-195)
Saline used (mL)	712 (643-1092)
WB flow rate (mL/min)	64 (49-74)
Photoactivation time (min)	18 (14-19)
Procedure time (min)	88 (78-110)

[a]Including anticoagulant volume.

insignificant decrease in peripheral plasma hemoglobin by a median of 1.9 mg/dL ($P = .519$), also likely due to dilutional effects. Plasma hemoglobin in the treated vs collected cells increased by a median of 1.4 mg/dL ($P < .001$). Endotoxin levels in treated cells were low, ≤0.500 EU/mL in all samples. All treated cells were also negative in 14-day aerobic and anaerobic bacterial cultures.

3.4 | Adverse events

There were six mild intra-procedure AEs that occurred during five Amicus ECP procedures. Two were citrate related reactions that occurred in a single procedure, one occurrence of muscle discomfort at the return site and three procedures terminated early due to Phelix alarms. All recovered without sequelae. In the 24-hour period following the Amicus ECP procedure, one subject reported moderate neck pain that was determined to be musculoskeletal in nature (CT scan negative for thrombosis). Throughout the course of the study, one subject reported four severe AEs: right arm swelling, lower extremity weakness, respiratory failure and acute multifocal respiratory failure, ultimately leading to the subject's death. Ultrasound and chest CT scans ruled out deep vein thrombosis and pulmonary embolism as causes and the subject's death was attributed to cGVHD disease progression. A second subject reported two severe AEs: hospitalized with sepsis after right hip replacement and hospitalization for pneumonia. These occurred 9 and 12 weeks, respectively, after the patient's last treatment with the experimental device. In total, three subjects reported a total of seven serious AEs (n = 1 moderate and n = 6 severe); all were deemed to be unrelated to the study device or procedure.

3.5 | Collected cells

Collected cell yields are presented in Table 4. Median (range) MNC purity of the collected cells was 93 (72-99)%. Median volume and hematocrit of the collected cells prior to 8-MOP addition was 215 (210-220) mL and 2.0 (1.5-2.7)%. Based on these values and the subjects' pre- and post-MNC counts, median MNC collection efficiency (CE1) was 56 (15-70)%. Hematology counts of the ECP-treated cells were slightly lower than collected cells (data not shown).

3.6 | Lymphocyte assays

Lymphocyte apoptosis and viability results over 3 days of culture are shown in Figure 2. Levels of apoptotic and viable lymphocytes were initially similar between control and ECP-treated cells at $t = 0$ of culture. Over the next 24 hours, however, the impact of ECP treatment on lymphocyte apoptosis became apparent and this trend was maintained up to day 3 of culture, when nearly all ECP-treated lymphocytes were apoptotic and only approximately 30% were still viable. Median lymphocyte proliferation post-PHA stimulation was 51.9 (23.8-88.3)% in

TABLE 3 Subject hematology counts pre-to-post ECP procedure

Median (range), n = 23	Pre	Post	*P* value
WBC ($\times 10^3/\mu L$)	9.4 (6.0-17.0)	10.0 (5.6-15.9)	.013
Neutrophil ($\times 10^3/\mu L$)	6.34 (2.43-14.54)	6.18 (1.56-13.60)	.169
Lymphocyte ($\times 10^3/\mu L$)	1.23 (0.20-1.95)	1.09 (0.21-2.18)	.233
Monocyte ($\times 10^3/\mu L$)	1.13 (0.53-1.95)	0.99 (0.12-1.65)	.002
Basophil ($\times 10^3/\mu L$)	0.06 (0.03-0.10)	0.04 (0.01-0.08)	.022
Eosinophil ($\times 10^3/\mu L$)	0.17 (0.03-0.77)	0.07 (0.03-0.72)	<.001
Hematocrit (%)	36.4 (31.4-43.4)	34.3 (28.9-40.0)	<.001
Platelet ($\times 10^3/\mu L$)	344 (159-406)	320 (158-404)	<.001

TABLE 4 Collected cell yields

	n	Median (range)
WBC ($\times 10^9$)	22	2.9 (0.6-4.7)
MNC ($\times 10^9$)	19	2.7 (0.4-4.6)
Lymphocyte ($\times 10^9$)	19	1.3 (0.3-3.3)
Monocyte ($\times 10^9$)	19	1.1 (0.1-2.9)
Granulocyte ($\times 10^9$)	19	0.2 (0.0-1.0)
RBC (mL)	21	4.3 (3.3-5.9)
Platelet ($\times 10^9$)	22	40 (18-70)

control cells (n = 22) vs 1.4 (0.3-3.2)% in ECP-treated cells (n = 22). Median inhibition of lymphocyte proliferation due to ECP treatment was 97.1 (91.4-99.6)%.

4 | DISCUSSION

This first-ever pilot study of the Amicus ECP system evaluated safety and device performance in patients with steroid-refractory or -dependent cGVHD. Like previous reports for other ECP technologies, we found the procedure to be safe and well tolerated with a low incidence of device/procedure related AEs. The intraprocedural AEs that did occur were mainly related to citrate toxicity, which is common to apheresis procedures using ACD-A anticoagulation.[10] The low extracorporeal volume of the disposable set (163 mL), especially in comparison to the other online system (255-415 mL for Therakos CELLEX in double needle mode, depending on patient hematocrit[11]), may have contributed to the absence of any reported hypotensive reactions. Although the investigational system was limited to double-needle access, no major issues were encountered with obtaining or maintaining venous access during the procedures. The development of a single-needle version of the procedure is under way.

Ideal ECP system performance should include a short procedure duration, high MNC collection efficiency and purity with consistent photoactivation. In the Amicus ECP system, the procedure duration and collected cell yields were comparable to the online Therakos CELLEX system but with higher MNC purity, similar to offline ECP with the Spectra Optia (Table 5).[8] The median MNC collection efficiency (CE1) of the Amicus ECP system was 56%, which is higher than the 42% median MNC CE1 reported by Cid et al for cGVHD patients collected using the COBE Spectra/Spectra Optia leukapheresis devices for offline ECP and similar to the values reported by Brosig et al and Piccirillo et al for these same devices and the Therakos CELLEX in broader ECP patient populations (50-60%).[12-14]

The hematocrit and composition of the collected cells are important factors for consistent photoactivation in terms of UVA exposure and photoactivation time. In the Amicus ECP system, the median hematocrit of the collected cells was 2% (as targeted by the system) with a narrow hematocrit range even over a broad range of patient cell counts. The system collected MNCs in a concentrated volume with only 30 mL of residual plasma and used saline to dilute the cells prior to photoactivation. Utilizing this low percentage of plasma decreases the impact that the patient's plasma clarity may have on photoactivation time, which may be increased by conditions such as hyperlipidemia or hyperbilirubinemia.

To further evaluate the performance of the Amicus ECP system in this patient population, we also studied lymphocyte apoptosis and inhibition of lymphocyte proliferation as traditional ECP procedure validation markers. Although the mechanism of action of ECP has not been fully elucidated, apoptotic lymphocytes have been ascribed a central role in all theories to date, serving as the antigen source for downstream immunomodulatory effects.[15-17] The impact of ECP on lymphocyte proliferation is likely less relevant to the mechanism of action, mainly confirming the direct effects of ECP on treated lymphocytes. The trends we observed for both of these parameters in Amicus ECP-treated cells are in line with values reported for other online and offline systems[18-20]

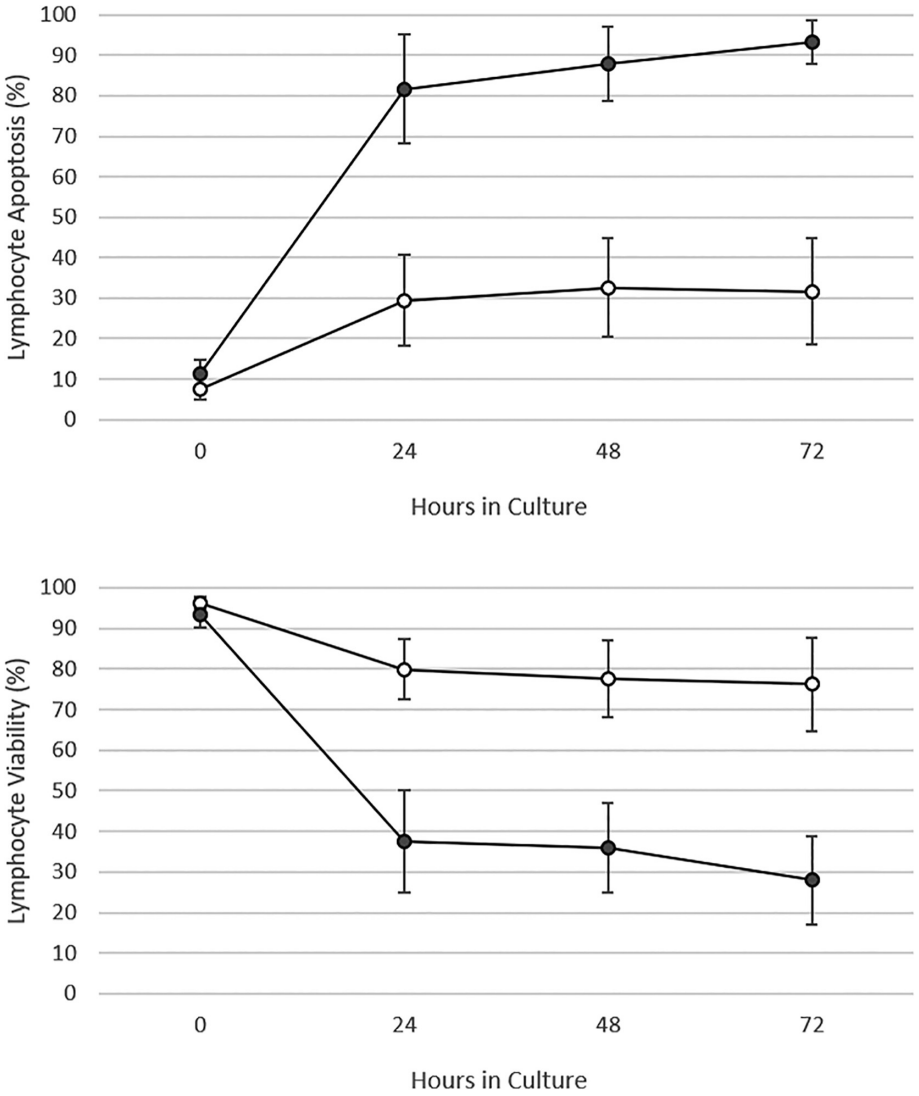


FIGURE 2 Lymphocyte apoptosis and viability in subject's control (○) or ECP-treated (●) cells over 3 days of culture. Although starting at similar levels at $t = 0$, control and ECP-treated cells quickly diverged with ECP treatment effects becoming apparent within 24 hours. Data are presented as mean ± SD. n = 10 at 0 hours; n = 21 at 24, 48, and 72 hours

TABLE 5 Comparison to offline and online ECP technologies (Bueno et al[8])

Mean ± SD	Amicus ECP	Optia[a]	Cellex[a,b]
Blood volume processed (mL)[c]	2160 ± 17	7504 ± 1114	1503 ± 70
Anticoagulant volume used (mL)	196 ± 18	699 ± 75	248 ± 12
Procedure time (min)	91 ± 7	272 ± 37	106 ± 40
Collected cell volume (mL)	216 ± 3	150 ± 0	173 ± 20
WBC ($\times 10^9$/L)	13.3 ± 5.3	62.1 ± 27.5	17.2 ± 8.5
MNC ($\times 10^9$/L)	12.7 ± 4.8	51.6 ± 23.1	11.0 ± 4.5
MNC purity (%)	90.9 ± 7.2	84.4 ± 15.9	63.8 ± 20.1
Hematocrit (%)	2.0 ± 0.4	2.1 ± 0.7	1.3 ± 0.6
Platelet ($\times 10^9$/L)	189 ± 75	1425 ± 530	432 ± 248

[a]Procedures performed in patients with GVHD or bronchiolitis obliterans post lung transplant.
[b]Double needle access in 13/17 (76.5%) procedures.
[c]Excluding anticoagulant volume.

and meet ECP verification standards proposed by an Italian consensus group[21] and the French regulatory body, ANSM.[22]

Given that most procedures in this study were performed on the second day of consecutive treatments, the low apoptosis levels at the start of culture and steady

levels in control (untreated) samples during culture suggest that few, if any, ECP-treated lymphocytes from the previous day's procedure are present in circulation after 24 hours. This may in part be due to the rapid kinetics of apoptosis in the ECP-treated cells from the previous day's procedure. Szczepiorkowski and colleagues recently reported that a median of 92% of ECP-treated cells are apoptotic after 24 hours of culture when using the Therakos CELLEX device, albeit in healthy subjects.[18] We observed a similarly high median of 86% apoptotic lymphocytes within the same time frame in our cGVHD patient population treated with the Amicus ECP system. Once apoptotic, these cells are likely immediately removed by the reticuloendothelial system and are therefore not collected and treated again in the following day's procedure. Although performing two consecutive days of treatment may have started as a conservative regimen for establishing safety in the early days of ECP,[23] it may be clinically equivalent to two procedures performed on non-consecutive days and still beneficial from a logistics perspective for some patients.

The primary limitation of our study was that no efficacy data could be gleaned due to intermixed ECP procedures performed using the Amicus ECP system and another device. Additionally, there were no consecutive days of Amicus ECP procedures completed in this study but as discussed above, the lymphocyte apoptosis data suggest that the treated cells are removed from the circulation quickly, such that each ECP procedure may be considered as essentially a standalone event from a safety perspective. Finally, since the mechanism of action is not fully known, there may be additional treated cell parameters of importance that were not measured in this study.

Lastly, it is worth noting that although the procedures performed in this study processed a fixed volume of approximately 2000 mL of WB, a subsequent version of the system (Amicus Separator software version 6.1) expands the programmable range of WB to process up to 4000 mL, thereby allowing the user to increase the cell yield treated per procedure, if desired. ECP practices today are widely varying in terms of cell dose treated, but with broadly similar clinical outcomes reported. In 2007, Perseghin et al first reported a cell dose effect in cGVHD patients treated with offline ECP procedures processing twice the patient's total blood volume.[24] Their analysis suggested that increased MNC dose/kg decreased the odds of treatment failure, and that, if the MNC dose infused was at least 100×10^6/kg per ECP treatment, a more positive and longer-lasting response was achieved. Since then, mini ECP procedures processing only 5-8 mL of WB/kg (roughly 1/20th of Perseghin et al) have also reported promising clinical outcomes.[25] Recently, Cid and colleagues have reported on performing one larger ECP procedure (processing one total blood volume, approximately 5 L of WB) vs two consecutive days of shorter procedures with seemingly equivalent clinical outcomes.[12] So, even though the optimum cell dose and treatment schedule is unknown, the ability to adjust the amount of whole blood processed during the procedure provides added flexibility that can aid in patient management and scheduling and in future studies in this area.

5 | CONCLUSION

Extracorporeal photopheresis procedures were safely completed in adult subjects with steroid-refractory or -dependent cGVHD using the Amicus ECP System. in vitro testing of the ECP-treated cells suggests comparability to other online and offline systems but with additional device flexibility in a closed system. The efficacy of the Amicus ECP system in this patient population and in others remains to be determined in future studies.

CONFLICT OF INTEREST

Katherine Radwanski, Jennifer Weitgenant, and Cheryl Heber are employees of Fresenius Kabi.

ORCID

Katherine Radwanski https://orcid.org/0000-0002-5680-6965
Edwin Burgstaler https://orcid.org/0000-0002-2578-1822

REFERENCES

1. Dunbar NM, Raval JS, Johnson A, et al. Extracorporeal photopheresis practice patterns: an international survey by the ASFA ECP subcommittee. *J Clin Apher*. 2017;32(4):215-223.
2. Michallet M, Sobh M, Garban F, et al. Extracorporeal photopheresis for GVHD prophylaxis after reduced intensity conditioning allogeneic hematopoietic stem cell transplantation: a prospective multicenter phase 2 study. *Leuk Lymphoma*. 2018; 59(2):372-380.
3. Del Fante C, Seghatchian J, Perotti C. Reflections on the usefulness of extracorporeal photopheresis in renal transplant rejection: a concise review of the involved mechanisms and therapeutic perspectives. *Transfus Apher Sci*. 2018;57(1): 115-117.
4. Mazzoni A, Giampietro C, Bianco I, et al. Extracorporeal photopheresis and liver transplantation: our experience and preliminary data. *Transfus Apher Sci*. 2017;56(4):515-519.
5. Mehrdad H, Langer KJ, Khera N, et al. Extracorporeal photopheresis improves survival in hematopoietic cell transplant patients with bronchiolitis obliterans syndrome without significantly impacting measured pulmonary functions. *Biol Blood Marrow Transplant*. 2018;24(9):1906-1913.
6. Alfred A, Taylor PC, Dignan F, et al. The role of extracorporeal photopheresis in the management of cutaneous T-cell

lymphoma, graft-versus-host disease and organ transplant rejection: a consensus statement update from the UK Photopheresis Society. *Br J Haematol*. 2017;177(2):287-310.
7. Azar N, Leblond V, Ouzegdouh M, Button P. A transition from using multi-step procedures to a fully integrated system for performing extracorporeal photopheresis: a comparison of costs and efficiencies. *J Clin Apher*. 2017;32(6):474-478.
8. Bueno JL, Alonso R, Gonzalez-Santillana C, et al. A paired trial comparing mononuclear cell collection in two machines for further inactivation through an inline or offline extracorporeal photopheresis procedure. *Transfusion*. 2019;59(1):340-346.
9. Perseghin P, Incontri A. Mononuclear cell collection in patients treated with extracorporeal photochemotherapy by using the off-line method: a comparison between COBE Spectra AutoPbsc version 6.1 and Amicus cell separators. *J Clin Apher*. 2010;25(6):310-314.
10. Henriksson M, Newman E, Witt V, et al. Adverse events in apheresis: an update of the WAA registry data. *Transfus Apher Sci*. 2016;54(1):2-15.
11. Therakos Cellex Photopheresis System Operator's Manual. Rev 3.2-1460415.
12. Cid J, Carbassé G, Suárez-Lledó M, et al. Efficacy and safety of one-day offline extracorporeal photopheresis schedule processing one total blood volume for treating patients with graft-versus-host disease. *Transfusion*. 2019;59(8):2636-2642.
13. Brosig A, Hähnel V, Orsó E, Wolff D, Holler E, Ahrens N. Technical comparison of four different extracorporeal photopheresis systems. *Transfusion*. 2016;56(10):2510-2519.
14. Piccirillo N, Putzulu R, Massini G, et al. Inline extracorporeal photopheresis: evaluation of cell collection efficiency. *Transfusion*. 2019;59(12):3714-3720.
15. Edelson R. Mechanistic insights into extracorporeal photochemotherapy: efficient induction of monocyte-to-dendritic cell maturation. *Transfus Apher Sci*. 2014;50:322-329.
16. Lamioni A, Parisi F, Isacchi G, et al. The immunological effects of extracorporeal photopheresis unraveled: induction of tolerogenic dendritic cells in vitro and regulatory T cells in vivo. *Transplantation*. 2005;79:846-850.
17. Hannani D. Extracorporeal photopheresis: tolerogenic or immunogenic cell death? Beyond current dogma. *Front Immunol*. 2015;6:349.
18. Szczepiorkowski Z, Burnett C, Dumont L, Abhyankar S. Apheresis buffy coat collection without photoactivation has no effect on apoptosis, cell proliferation, and total viability of mononuclear cells collected using photopheresis systems. *Transfusion*. 2018;58(4):943-950.
19. Taverna F, Coluccia P, Arienti F, et al. Biological quality control for extracorporeal photochemotherapy: assessing mononuclear cell apoptosis levels in ECP bags of chronic GvHD patients. *J Clin Apher*. 2015;30(3):162-170.
20. Faivre L, Lecouflet L, Liu W, et al. Quality control of extracorporeal photochemotherapy: proliferation assay using CFSE validated according to ISO 15189:2007 standards. *Cytometry B Clin Cytom*. 2015;88(1):30-39.
21. Pierelli L, Perseghin P, Marchetti M, et al. 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. *Transfusion*. 2013;53(10):2340-2352.
22. AFSSAPS. *Report of the Commission of Gene Therapy and Cellular Meeting of April 7, 2011*. Rep. Department of Biological Products Evaluation Therapeutic Effects Unit, 7 Apr. 2011. Accessed August 15, 2019. http://ansm.sante.fr/var/ansm_site/storage/original/application/900fa3973c7580bf24eceb0b178efb4f.pdf.
23. Edelson R. Photopheresis: a new therapeutic concept. *Yale J Biol Med*. 1989;62(6):565-577.
24. Perseghin P, Galimberti S, Balduzzi A, et al. Extracorporeal photochemotherapy for the treatment of chronic graft-versus-host disease: trend for a possible cell dose-related effect? *Ther Apher Dial*. 2007;11(2):85-93.
25. Verdú-Amorós J, Woessmann W, Maecker-Kolhoff B, et al. Mini photopheresis for refractory chronic graft-versus-host disease in children and adolescents. *Transfusion*. 2018;58(11):2495-2500.

How to cite this article: Radwanski K, Burgstaler E, Weitgenant J, Dale H, Heber C, Winters J. Pilot study of a new online extracorporeal photopheresis system in patients with steroid refractory or dependent chronic graft vs host disease. *J Clin Apher*. 2020;35:342–350. https://doi.org/10.1002/jca.21804