Efficacy and safety of mini-extracorporeal photopheresis for the treatment of refractory graft-versus-host-disease in children and adolescents

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Table of contents

1 Introduction

1.1 Graft-versus-Host disease

Allogeneic stem-cell transplantation (allo-SCT) is the definitive treatment option of several malignant and non-malignant diseases. In Europe, more than 4500 allo-SCT are performed yearly in children.[108] Technical innovations, and better strategies focused on preventing infections and reducing toxicity, among others, have improved the outcome of allo-SCT in the last decades (50% reduction of non-relapse mortality (NRM) and better survival).[46,100] However, graft versus host disease (GVHD) remains the major cause of treatment failure and SCT related death.[49,6,134]

GVHD can be defined as a donor T-cell immune reaction after allo-SCT against genetically defined proteins in different organs in the immunosupressed recipient. It complies disturbances in pathways of immunological recognition, reconstitution and failure to acquire immunological tolerance, thereby resulting in both alloimmune and autoimmune attacks on multiple host tissues.[36,21] The need for increased and prolonged immunosuppression (IS) to treat GVHD, in addition to the immunosuppressive effects of the disease itself, increases the risk of infection, organ impairment, poor quality of life and ultimately, mortality.

GVHD has been classically classified according to its chronological pattern after allo-SCT, using day+100 as cut-off: acute GVHD (aGVHD) <100 days; chronic GVHD (cGVHD) >100 days. However, GVHD is now considered as a continuum and clinical manifestations, rather than time after SCT, should guide the difference between acute- and chronic-GVHD (**Table 1**).[37,61] Regarding this consideration, the following categories of GVHD are recognized: classical aGVHD occurring within 100 days after SCT or donor leukocyte infusion; persistent, recurrent or late-onset aGVHD (> day+100); classical cGVHD; overlap syndrome with concomitant acute- and chronic-GVHD signs.[110,61]

Table 1. Categories of acute and chronic GVHD *(adapted from Hart et al)*[61]

Acute GVHD*	
Classic aGVHD	Features that include maculopapular rash, nausea, vomiting, anorexia, profuse diarrhea, ileus or cholestatic hepatitis occurring within 100 days after SCT or DLI and without diagnostic or distinctive signs of cGVHD
Persistent, recurrent, late-onset aGVHD	Features of classic aGVHD without diagnostic or distinctive manifestations of cGVHD occurring beyond 100 days of SCT or DLI
Chronic GVHD	
Classic cGVHD	Without features of aGVHD
Overlap syndrome	Along with features of aGVHD

aGVHD: acute graft-versus-host disease; cGVHD: graft-versus-host disease; DLI: Donor lymphocyte infusion; GVHD: graft-versus-host disease; SCT: stem cell transplantation

** In the abscense of histologic or clinical signs or symptoms of cGVHD, the persistence, recurrence or new onset of characteristic skin, gastrointestinal or liver abnormalities should be classified as aGVHD regardless of the time after SCT*

Most accepted risk factors for the development of aGVHD and cGVHD are: unrelated donor, mismatched donor, older age of the donor, mutliparous female donor, older age of the recipient, stem cell source (risk: cord blood < bone marrow < peripheral blood stem cells), immunophenotypic makeup, genetic factors and certain conditoning regimens (myeloablative > reduded intensity).[138,27,77,38,60,81,28]

1.1.1 Acute GVHD

In pediatric patients receiving SCT from an unrelated donor, the incidence of grade II to IV acute GVHD ranges from 40% to 85% of recipients, depending on the degree of donor and stem cell mismatch, and is approximately 27% after HCT from an HLA-identical sibling.[43,31,24,35] Acute GVHD targets mostly three organs: skin (80%), gastrointestinal tract (>50%) and liver (50%).[94,18,149,95]

The pathophysiological aspects of aGVHD were described by Ferrara and Deeg.[33] There are increasing data regarding the effects of disrupting the intestinal microbiota diversity on the immune homeostasis and its relationship with the development of aGVHD.[56,102]

1.1.2 Chronic GVHD

Chronic GVHD affects up to 20-60% of allo-SCT recipients and remains the main cause of NRM in patients surviving longer than 2 years after SCT, negatively influencing both quality of life and long-term outcome.[49,6] Characteristic features include chronic inflammatory changes in almost every organ, typically involving ocular, oral, esophageal, skin, joint, fascia and genital tissues. Progression to fibrosis involving other organs occurs in severely affected pacients.[21]

The pathophysiology of cGVHD remains poorly understood. cGVHD involves multiple interactions among alloreactive and dysregulated T- and B-cells and innate immune populations, including macrophages, dendritic cells (DCs), and neutrophils, resulting in the activation of profibrotic pathways.[21] In contrast to aGVHD, B-cells seem to have an important role in cGVHD. B-cell activating factor (BAFF) has been identified as a key regulator of B-cell homeostasis associated to decreased apoptosis of activated B cells. BAFF levels are significantly elevated in active cGVHD patients promoting increased signaling through the ERK and AKT pathways. Patients with cGVHD also show reduced levels of circulating Bregs and impaired IL-10 production.[2,126]

1.1.3 Diagnosis and Staging of GVHD

Diagnosis of **aGVHD** is typically based on clinical symptoms in one or more of the main target organs (skin, liver, gastrointestinal tract) and, if posible, it should be confirmed by biopsy despite its low sensitivity (approximately 60%). The ultimate aGVHD diagnosis and decision to treat relies on careful integration of all available information.[143]

In 1974, Glucksberg published the first aGVHD classification with prognostic relevance.[44] The most commonly used grading system for aGVHD was revised in 1994 (Keystone Consensus 1994 criteria, **Table 2**) but some other classifications are also accepted.[119,123,129] Stage depends on the number and severity of organ involvement (I-IV). All systems are predictive for outcome showing that severe acute GVHD has poor prognosis, with near 25% survival at 5 years for grade III disease and 5% for grade IV.[14] The MAGIC criteria are considered the most current and detailed criteria to score the severity of aGvHD. However, there is an unmet need for developing pediatric population-adapted GvHD symptom scales and assessments.[129]

Currently, the only organs with specific pediatric modifications recommendations for GvHD assessment are: (1) adapted body surface area maps for skin involvement; (2) appropriate reference values for lung function; and (3) weight-adapted measures for diarrhea.[129]

Table 2. Clinical stage and grade of acute GVHD[34–36]

Stage	Skin	Liver	Intestinal tract
1	Maculopapular rash <25% of BS	Bili. 34–50 mmol/l	Adult: >500 ml diarrhea/day Child: 10–19.9 ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25–50% BS	Bili. 51–102 mmol/l	Adult: >1000 ml diarrhea/day Child: 20 – 30 ml/kg/day or 7–10 episodes/day
3	Generalized erythroderma	Bili. 103–225 mmol/l	Adult: >1500 ml diarrhea/day Child: > 30 ml/kg/day or >10 episodes/day
4	Generalized erythroderma with bullous formation and desquamation	Bili. > 255 mmol/l	Severe abdominal pain, with or without ileus

BS: body surface; Bili: bilirrubin

Grade	Degree of organ involvement
I	Stage 1–2 skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	Stage 1–3 skin rash; stage 1 gut involvement or stage 1 liver involvement (or both); mild decrease in clinical performance
III	Stage 2–3 skin rash; stage 2–3 gut involvement or 2–4 liver involvement (or both); marked decrease in clinical performance
IV	Similar to Grade III with stage 2–4 organ involvement and extreme decrease in clinical performance

Diagnosing and scoring the severity of **cGVHD** is challenging due to several reasons: pathophysiology is not fully understood, acute and chronic features often coexist, there are few validated biomarkers and less standarized measurement and scoring tools.[110] The 2014 National Institutes of Health (NIH) Chronic GVHD Diagnosis and Staging Consensus Recommendations are currently used.[70,129]

Thus, diagnosis of **cGVHD** is primarily clinical and requires at least one *diagnostic* sign in a target organ per NIH criteria[70] (ie, a sign found only in cGVHD) or at least one *distinctive* sign (ie, a sign highly suggestive of cGVHD) plus a pertinent biopsy, laboratory or other tests (e.g. Schirmer's test), evaluation by a specialist (ophthalmologist, gynecologist) or radiographic imaging showing cGVHD in the same or another organ, unless stated otherwise. Due to the frequent presence of typical clinical manifestations, biopsies are less commonly performed for cGVHD diagnosis and are more often used to rule out other diagnoses such as infection, drug reactions, or cancer.

1.1.4 Prognostic factors in GVHD.

The most established prognostic factors for poor survival and mortality in patients with **aGVHD** are the stage and the abscense of response to steroids.[142,90,95,109] The most validated serumbiomarker for aGVHD is ST2 (suppression of tumorigenicity 2) associated with significantly increased risk of aGvHD, treatment resistant aGvHD and transplant related mortality (TRM).[88]

Most consistently defined prognostic factors for **cGVHD** are thrombocytopenia (<100 × 10^9/L), progressive onset of cGVHD from aGVHD, performance status, NIH global severity stage (mild vs moderate vs severe), overlap syndrome, lung involvement and lymphopenia.[7,51,78,115] In the last years, many groups have focused on identifying biomarkers that can both predict the onset of GvHD as well as the prognosis and severity. Examples of biomarkers predicting prognosis include serum C reactive protein, serum albumin, IL-2 receptor, IL-6, HGF, microRNAs (miRNAs), regulatory T cells (Tregs), DC, monocytes, and γ-δ T, B cell–activating factor, CXCL9, ST2, matrix metalloproteinase-3, osteopontin, CXCL10, CXCL11, and CD163, among others.[84,146]

1.1.5 Prophylaxis and treatment of GVHD

Strategies to **prevent** GVHD onset are mandatory. Pharmacological interventions as the combination of cyclosporine A (CSA) or tacrolimus and a short course of methotrexate (MTX) or mycofenolatemofetil (MMF), posttransplant cyclophosphamide (Cy) and/or serotherapy with antithymocyteglobuline (ATG) are widely used.[137,89,9,12,34] Other approaches focus on ex-vivo graft manipulation to obtain a selection of non-alloreactive T-cells that preserve potent antileukemia and anti-infectious activities are also performed.[86,57]

Treatment of GVHD bases mostly on modulation of donor-alloreactive effector T cells. The need for increased and prolonged IS increases the risk of infection, relapse, organ impairment, poor quality of life and ultimately, mortality.

Rapidly progressive aGVHD manifestations and any proven intestinal or liver involvement require prompt treatment. Indolent progression of a skin rash without intestinal or liver involvement require more careful consideration of the benefits and risks of systemic IS treatment.[109]

First-line therapy for aGVHD grades II-IV consists on 2 mg/kg/day of methylprednisolone or a prednisone equivalent. By progression within 3 days or lack of improvement after 5-7 days, then the GVHD is considered to be steroid-refractory (SR) and a second-line approach needs to be started. Tapering of steroids should begin as soon as GVHD manifestations show major improvement.

Just around 50% of aGVHD patients respond to steroids and many responses are not durable.[116] For this reason prospective studies have evaluated the addition of other agents to steroids but have failed to show an advantage in survival but more toxicity.[95] Risk-based strategies are currently under investigation in an attempt to spare toxicity in steroid-responders and to identify patients who are less likely to respond and require aggressive upfront therapy.[90,83]

Currently, clinical trials using new aGVHD pharmacological strategies are ongoing, some of them with promising results.[3,64] First modest attempts to introduce extracorporeal photopheresis in the first-line setting are ongoing, also with preliminary remarkable results.[17,133]

Second-line strategies for SR-aGVHD are not well defined. They comprise significant toxicities, high failure rates, and 1-year survival rates of approximately 20-30%.[24] Very few prospective comparative studies have been carried out to assess the efficacy and safety of alternative approaches. Different agents as methotrexate, mycofenolate-mofetil, extraxorporeal photopheresis (ECP), IL-2R targeting (ie, basiliximab, daclizumab, denileukin and diftitox), alemtuzumab, horse ATG, etanercept, infliximab or sirolimus has been used in this setting and show an overall reponse rate (ORR) of approximately 50%.[95] In general, the median survival is approximately 6 months and there is no evidence that an specific agent is more effective than others.[95]

Recently, the FDA approved ruxolitinib for SR-aGVHD in adult and pediatric patients 12 years and older based on the REACH-1 study, an open-label, single-arm, multicenter trial that showed a Day-28 ORR of 57.1%.[69,120]

Patients with **cGVHD** require prolonged IS for an average of 2 to 3 years.[66] The goal is to prevent immune-mediated damage and alleviate symptoms, allowing and awaiting the development of tolerance. As a general rule, the intensity of treatment should be adapted to the extent and severity of disease. Patients with mild manifestations can often be managed with close observation or topical treatment. Systemic therapy is generally indicated for patients who meet criteria for moderate-to-severe disease according to the NIH consensus criteria.[70]

First-line therapy of cGVHD relies on systemic steroids. Standard is methylprednisolone or a prednisone equivalent at 0.5 to 1 mg/kg per day, followed by a taper to reach an alternate-day regimen, with or without associated calcineurin inhibitors (CNI). The prolonged treatment causes significant toxicity, including weight gain, osteopenia, myopathy, diabetes, hypertension, mood swings, cataracts, increased risk of opportunistic infections and growth impairment in children. Approximately 50% of patients with cGVHD fail to achieve control with first-line therapy.[147,110,40]

Mini-ECP

Generally accepted criteria for SR or steroid-dependent cGVHD include: 1) progression on prednisone at 1 mg/kg/day for 2 weeks, 2) stable disease on 0.5 mg/kg/day of prednisone for 4-8 weeks, and 3) inability to taper prednisone below 0.5 mg/kg/ day.[148,82]

There is no consensus regarding **second-line treatment** for cGVHD. Studies are heterogeneous and response rates reach 25%-80% with survival rates up to 70%.[148,66] The addition of an effective steroid-sparing agent as CNI, ECP, mTOR inhibitors, or mycophenolate mofetil is of crucial importance for long-term patient outcome.[106] Data regarding other drugs are sparse (thalidomide, hydroxychloroquine, pentostatin, rituximab, alemtuzumab, etanercept, etc). Tyrosine kinase inihibitors such as imatinib has been used for specific situations because of their ability to interfere with the platelet-derived growth factor (PDGF-R) pathway involved in fibrosis.[105]

Recently, Ruxolitinib has been approved by the FDA for SR-cGVHD in adult and pediatric patients 12 years and older based on the study REACH 3, a phase 3, randomized, open-label, multicenter study of ruxolitinib in comparison to best available therapy for treatment of steroid-refractory chronic GVHD after allo-SCT. The ORR at week 24 was 49.7% for the study drug compared with 25.6% for other therapies (P <.0001).[152]

Treatment of cGVHD should be withdrawn gradually once the disease has resolved. As a general principle, withdrawal of systemic treatment should begin with the drug that is most likely to cause long-term toxicities. As in aGVHD, there is an urgent need for better standardized clinical trials of new agents to advance therapeutic success in cGVHD. Several clinical trials are now recruiting to evaluate the use of mesenchymal stem cells, IL-2 alone or in combination with Tregs or ECP, among others.[64]

1.2 Extracoprporeal Photopheresis

Extracorporeal photopheresis (extracorporeal photochemotherapy, extracorporeal photoimmunotherapy) is based on the biological effect of 8-methoxypsoralen (8-MOP) and ultraviolet light A (UVA) on mononuclear cells outside of the body after cell-collection by apheresis, which are then reinfused into the patient.[49]

1.2.1 Historical background

The ancient Egyptians already recognized that soon after eating a plant called *Ammi majus* people became unusually prone to sunburn. This propperties were employed to treat vitíligo. In the 1950s, a research group from the Michigan School of Medicine described that the active component of the plant, 8-methoxypsoralen (MOP), inhibits the S phase of the cell cycle through DNA cross-linking induction. In 1974, the efficacy of skin UVA irradiation following oral administration of psoralen, called PUVA (psoralen ultraviolet A), was reported for treating psoriasis.[107,104]

The first investigation of ECP for treatment of advanced cutaneous T-cell lymphoma (CTCL) was published by Edelson and colleages in 1983.[94] Some refractory patients reached a remission and others showed a significant skin response. Moreover, an increased survival was observed in ECP-treated patients compared to controls.[32]

The first ECP system (UVAR®; Therakos) was approved for CTCL (Sezary syndrome, mycosis fungoides) by the FDA in the United States in 1988. In the following years, ECP was adopted for the treatment of several autoimmune T-cell-mediated diseases: prevention of rejection in solid organ transplantation, Crohn´s disease, type 1 diabetes or atopic dermatitis, among others. Later-on, ECP was introduced in the treatment of both acute and chronic GVHD.

1.2.2 ECP for the treatment of GVHD

1.2.2.1 Mechanisms of action

ECP can rebalance the alloreactive immune system but the immunomodulatory mechanisms remain poorly understood. ECP acts through the photoactivated psoralens in ex-vivo lymphocytes inducing DNA damage and apoptosis of exposed cells, with activated T lymphocytes preferentially affected and release of immunomodulatory cytokines.[58] Additionally, a shift from an inflammatory state (TH1) to that of tolerance (TH2) has been described.[122] As only 5-10% of lymphocytes are exposed during the procedure (5 x10^9),[76] it is speculated that the immunoregulatory effects of ECP are more closely related to the induction of Tregs and promotion of DC differentiation. This process has been shown to reduce the production of inflammatory cytokines (IL-2, TNF-alpha, and IFN-gamma) while increasing production of anti-inflammatory cytokines (TGF-beta). Recent data point out to an immunogenic induced cell death that could explain both an antitumor immune response and immune tolerance.[42,91,8]

1.2.2.2 ECP Procedures

The ECP-procedure includes three subsequent steps: 1) collection of mononuclear cells (MNC); 2) irradiation of the MNC in the presence of 8-MOP by UVA at 320–400 nm wavelength; 3) re-infusion of the irradiated MNC to the patient.

Two different methods are used depending on centres preference and technical possibilities (**Table 3**):[75,132,29]

Table 3. Comparison between on-line and off-line methods *(adapted from Drexler et al)*[29]

	'One-step'/'on-line'	**'Two-step'/'off-line'**
Manufacturer	CELLEX® (Therakos) UVAR-XTS® (Therakos) AMICUS® ECP System (Fresenius Cabi)	Spectra Optia® (Terumo BCT) AMICUS® or COM.TEC ® (Fresenius Cabi) together with a UVA irradiator
Principle	Integrated device on a single instrument, all components have been validated together	Separate devides for each step.
Cell separator technology	Continuous or discontinuous	Continuous
Venous access	Single or double	Double
Antocoagulant	Heparin od citrate	Citrate
Quality control of cells	No	Yes
Duration	1.5-2h	2-4h
Weight limit	Yes, red blood cell priming if >15% extracorporeal volume	No

ACD: acid-citrate-dextrose; ECP: extracorporeal photopheresis; UVA: Ultraviolet light A

'**One-step'/'on-line' method.** In a closed ECP system, the cell separation, addition of psolaren, photoactivation and re-infusion stages are fully integrated and automated in one system. The technique requires one or two-needle (continuous flow). All components are validated, tested and approved. There is no risk of improper reinfusion and the risk of infection and contamination associated with the medical device is low. This method is currently FDA-approved as first line treatment for CTCL.

'Two-step'/'off-line' method. Open ECP systems use separate devices for leukapheresis and drug photoactivation. Although the components may be CE (Conformité Européenne) marked or have FDA approval, they are not specifically approved for photopheresis. Two needles or a double-lumen catheter are required. As several steps are involved in delivering therapy, there is a potential risk of infection and contamination, as well as a risk of cross-contamination and patient re-infusion error.

Mini-ECP

Both ECP techniques have demonstrated clinical efficacy, but almost all clinical studies have been performed using one specific ECP technique, and studies comparing both systems are almost completely lacking.[4,130,13]

The 'two-step' method became popular, especially in Europe, as the low extracorporeal volume, which is enabled by the use of continuous flow cell separators, allows to easily and safely adopt this procedure even in low-weight patients. Furthermore, it allows for high product purity in terms of MNC content and for a final hematocrit (Hct) below 2% which is particularly important given that higher Hct values may intercept UVA and thus compromise irradiation efficacy.

In general, open systems can only be used by certified centres for handling blood components separately, whereas the closed systems do not have this limitation.

1.2.2.3 Schedule of ECP

The treatment schedule of ECP for patients with SR-aGVHD reflects the schedules initially used for CTCL. ECP is usually administered on a weekly basis, with 2-3 treatments per week. There is currently no evidence that maintenance ECP is beneficial. Thus, as soon as patients achieve a complete remision, ECP might be discontinued. Response should be assessed weekly related to organ involvement according to published criteria.[119,71]

Treatment schedule of ECP for cGVHD is not well established. General recommendations consist of two ECP treatments on consecutive days (one cycle) every 2–4 weeks, usually for 12–24 weeks.[50,47,75,132,103] There is no evidence that a more intensive regime has an advantage.[41] Subsequent prolongation of the interval between ECP treatments is typically performed by many centres. Tapering is usually influenced by clinical response and the possibility to reduce concurrent IS.[82] In case of progression during tapering, a new intensification with subsequently slower weaning has been recommended.[127] In case of severe cGVHD forms some authors recommend to prolong ECP beyond 6 months.[5,11,49]

1.2.2.4 ECP for aGvHD and cGvHD in adults

Results of ECP for treatment of **SR-aGVHD** in adults are encouraging. The reported ORR of SR-aGVHD to ECP in adults range from 66% to greater than 80% in some studies. ORR for skin, liver and gastrointestinal SR-GVHD have been observed in a median of 75% (range 50–100%), 47% (range 0–100%) and 58% (range 0–100%) of patients, respectively.[95,1] Different studies show that responses to ECP are higher for skin aGVHD and single organ involvement compared to combined involvement. Response rates are better for early interventions and for milder grades of aGVHD at the start of treatment (CR rate 86% for grade II, 55% for grade III and 30% for grade IV aGVHD). [50,20,47] Best responses to ECP are usually observed after a median of 1-2 months of treatment and steroids can be tapered and discontinued in responders with a low risk of recurrence.[47,96,111]

First results of ECP for the treatment of **SR-cGVHD** in adults were also promising.[106] The first multicentre, randomized, controlled, prospective trial of ECP in 95 adult patients with steroid-refractory / -dependent / -intolerant cGVHD showed a significant improvement in skin involvement and a steroid-sparing effect after 3 months of treatment.[39] Best responses of cGVHD manifestations to ECP are reported in skin, mucous membrane and liver.[62] A review of individual studies regarding use of ECP in cGVHD summarized a mean response rate in cutaneous cGVHD of 68% (range 29–100%), including CRs in some patients, and the mean response rate in patients with hepatic or mucosal involvement of 63%. Other organ sites respond less frequently and there is currently insufficient published evidence to recommend ECP for cGvHD of the eyes, joints or lungs.[127]

Respose to ECP has been associated to survival. Greinix and colleagues reported that patients with GVHD reaching a CR with ECP had significantly improved OS of 59%, compared with 11% in non-responders ($P < 0.0001$).[50]

1.2.2.5 ECP for the treatment of GvHD in children

First studies in pediatric patients mostly used an off-line system and showed efficacy in SR acute and chronic GVHD.[1] Current recommendations are based on retrospective or observational studies.[144] The results can be summarized as follows: **1.** Significantly better overall survival (OS) in responders with acute and chronic GvHD; **2.** Improvement of clinical status; **3.** Feasibility in most pediatric patients if a proper CVC was available; **4.** Low frequency of side effects and infectious complications.[23,125,124,99,139] **5.** Possibility to reduce or withdraw other immunosupressants (steroid-sparing effect). Discontinuing immunosuppressive therapies, particularly corticosteroids, is a major advantage for ECP in preventing long-term complications in children.[99]

It is currently recommended that pediatric patients with **SR-aGVHD** should receive ECP as second-line therapy. [71,95,132] Some authors consider ECP even as first-line therapy for paediatric patients with grade IV aGVHD (in association with conventional IS approaches).[71]

Many authors report a response rate to ECP in **SR-aGVHD** ranging from 50% to 100% (depending on the organs involved).[136,135,99,19] A large, multicentre, retrospective study of 33 paediatric patients with SR-aGVHD showed 54% CR (skin 76%, GI 75%, liver 60%) and 21% PR.[99] The 5-year OS rate was significantly better for responders (69%) than non-responders (12%; $P = 0.001$). As a result of ECP, immunosuppressive therapy could be discontinued in 42% and reduced in 36% survivors. Together with the affected organ, the aGVHD stage has been correlated with response. In 15 paediatric patients with SR-aGVHD, the strongest predictor of response to treatment was disease stage: 100% response rate for stage II, 75% for stage III and 0% for stage IV, with stage of GVHD and response to ECP both being significant predictors of transplant-related mortality.[96]

In **SR-cGVHD** overall response responses rates from 33% to 93% has been reported, including up to 75%, 82% and 86% complete response in cutaneous, hepatic and mucosal GVHD respectively.[41,19,71] As in the acute setting, ECP seems to be particularly effective in SR-cGVHD when initiated early after steroid failure avoiding irreversible tissue damage, improving of quality of life and patient mortality.[71,75] A significantly higher 5-year overall survival rate was observed in ECP responders compared to nonresponders (96% vs. 58%, $P = 0.04$).[99]

1.2.2.6 Limitation of ECP for the application in children

Apheresis procedures in pediatric patients may be challenging due to several particularities including placement of an appropriately sized central venous catheter (CVC), fluid status, tolerance to extracorporeal volume, duration of procedure, anticoagulant selection and dosing.[112] Although ECP is in general well-tolerated with few acute side effects, benefits and risks must be carefully weighed and discussed.[71,80]

A. Weight

Data on the use of ECP is limited in pediatric patients. One of the more commonly used on-line system (Therakos CELLEX®) is approved only for patients weighting >40 kg. Of note, within published case series to date, there are very few patients weighting <15kg.[99,128] There is no clearly established weight limit for off-line devices.

B. Fluid status and transfusional support

Apheresis procedures on pediatric patients are often challenging because of the extracorporeal volume (EV). Complications attributed to EV include tachycardia, dizziness, nausea, and hypotension. Continuous flow cell separators offer lower extravascular volumes than discontinuous flow separators. Regarding "one-step" methods, the overall extracorporeal volume of the CELLEX® is significantly less than the UVAR-XTS® (216–266 mL for CELLEX® vs. 220–620 mL for UVAR-XTS®), and is therefore better suited for pediatric patients. In addition, for CELLEX®, the double-needle mode may be safer for low-weight patients since it reduces extracorporeal volume by 63% relative to the single-needle mode.[130,73]

The majority of pediatric series, however, report the necessary priming with red blood cells (RBC) for low-weight patients. The manufacturer of UVAR-XTS® and CELLEX® systems recommends a RBC prime for patients weighing < 35 kg. For both on-line systems, Hct must be > 27% to reach an effective buffy coat collection.[73] Electrolyte disturbances are not uncommon.[74]

According to the consensus recommendations published by the Italian Society of Hemapheresis and Cell Manipulation and the Italian Group for Bone Marrow Transplantation, all patients < 20kg should receive a RBC prime, irrespective of the pre-procedure.[117]

C. Venous access

The placement of an adequately sized, functional line is essential for successful completion of the procedure. In pediatric patients this usually means that a quite large CVC is needed to allow the necessary flow. Most centres use single- or double-lumen central catheters or Shaldon catheters. Limiting factors for CVCs are the necessity of surgery in an often critically ill child, size of CVCs, associated risks of CVC handling (infection, thrombosis, accidental explantation, etc.).

D. Anticoagulation

There are no evidence-based protocols for anticoagulant selection and dosing in pediatric patients undergoing ECP.[140] Heparin is the standard anticoagulant for on-line systems, and acid-citrate-dextrose (ACD) for off-line systems. However, both heparin or ACD can be used as anticoagulants for either system.[93] UVAR-XTS® and CELLEX® are officially approved for the use with heparin. Anticoagulant can be decided on the basis of the operating practices in individual centres and adjusted according to individual´s condition (in patients with low platelet count and/or gut bleeding, heparin should be avoided).

E. Safety

Most commonly reported adverse events in children include hypotension (range 0–27%), catheter related infections (range 0–42%), and abdominal pain (range 0–20%). Low-weight patients (<20kg) seem to be at a greater risk of hemodynamic events. Other adverse events which should be taken into account include transient cytopenias, bleeding, symptomatic hypocalcemia (by ACD), hypothermia, mechanical hemolysis. There are no reported long-term side effects of this treatment.[26]

F. Duration and psychological impact

The psychological impact of repeated apheresis procedures in a usually sick child or adolescent should be taken into account. One-step systems are less time consuming.

2 Mini-Extracorporeal Phoropheresis (mini-ECP)[52]

In contrast to the well-documented safety and efficacy of classical ECP, clinical utilization of ECP in young children and critically ill patients is limited due to a number of technical and procedural difficulties. Major limitations for classical ECP procedures in these low body weight patient groups include the aforementioned extracorporeal volume, necessity of central venous access, frequent priming of apheresis machine with heterologous RBC concentrates, and a median ECP procedure time of 2-4 hours or longer, often requiring sedation of children.

Taking into account that no evidence of a clear correlation between the total number of reinfused WBCs and clinical efficacy of ECP has been reported, and to overcome the limitations of conventional ECP-systems in pediatric population, a mini buffy coat ECP technique (mini-ECP) has been developed at the Departments of Immunology and Transfusion Medicine and Pediatric Hematology and Oncology at the University Hospital Giessen and Marburg, Giessen.[52]

Only maximum 10% of the blood volume is drawn from the patient without the need of an apheresis. The technic includes preparation of the white blood cell (WBC)-rich buffy coat fraction in a functionally closed system under good manufacturing practice (GMP)-compliant conditions. After injection of 8-MOP into the buffy coat preparation, the cells are UVA irradiated (3 J/cm^2). After the process, irradiated cells are returned together with the autologous RBCs, platelets and plasma into the patient.[52]

The feasibility, preliminary safety and efficacy of the mini-ECP procedure in our centre were first reported in three children with acute SR-skin-GVHD (Grade 3) and classical ECP contraindications.[52]

Biological studies as WBC apoptosis and inhibition of lymphocyte proliferation were perfomed in parallel to the mini-ECP cycles for validation purposes (**Figure 1**).[52]

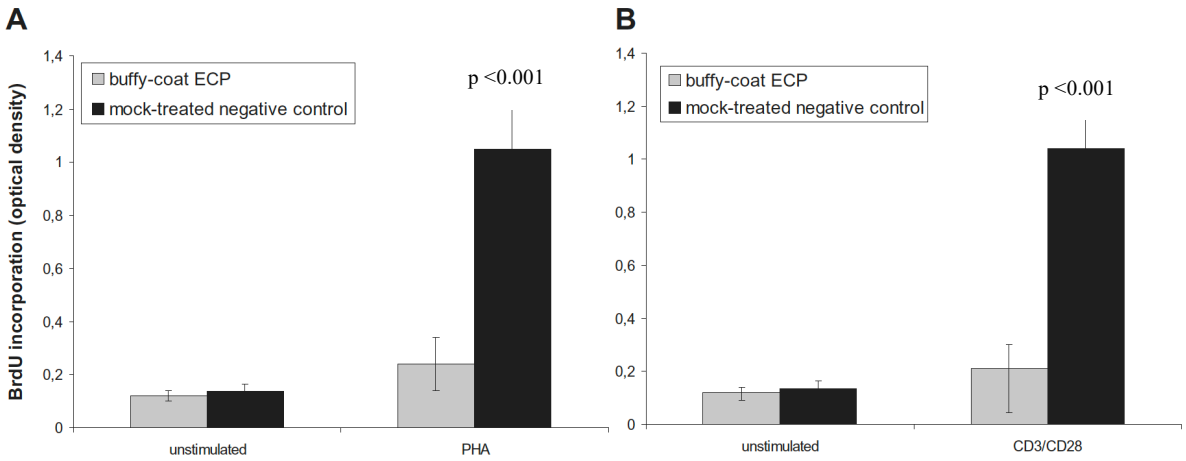


Figure 1. Mini buffy coat ECP inhibits lymophocyte proliferation.
Lymphocyte proliferation determined by quantitation of bromo-2-deoxyuridine incorporation after PHA (A) and CD3/CD28 stimulation (B) ***(adapted from Hackstein***

PHA: phytohemagglutinin; ECP: extracorporeal photopheresis

This satisfactory experience prompted the group to develop the present study focused on demonstrating the safety and efficacy of mini-ECP in small and/or critically ill children and adolescents.

3 Aim of the study

To retrospectively evaluate the efficacy and safety of mini-ECP for treatment of refractory graft-versus-host-disease in a large cohort of children and adolescents treated at the Institut for Immunology and Transfusion Medicine and Department of Pediatric Hematology and Oncology at the University Hospial Gießen and Marburg in Gießen.

4 Patients and methods

4.1 Patients

From October-2005 to May-2016 thirty infants, children and adolescents with therapy resistant GVHD after hematopoietic stem cell transplantation and contraindications for a classical ECP procedure were included in the study. Patients were classified in two cohorts according to accepted acute and chronic GVHD criteria (16 aGVHD and 14 cGVHD patients).[123,70] In our cohort, low weight was defined as less than 25 kg to consider mini-ECP as the only indication.

Patients were classified as having **SR-aGVHD** if they had no improvement in symptoms or progressed despite being on 1 mg/kg/day of prednisone equivalent for a minimum of five days.

SR-cGVHD was defined as: 1) progression on prednisone at 1 mg/kg/day for 2 weeks, 2) stable disease on 0.5 mg/kg/day of prednisone for 4-8 weeks, and 3) inability to taper prednisone below 0.5 mg/kg/ day.

The patients and/or their parents/legal representatives signed the informed consent of the study including data collection and data analysis. This retrospective study was approved by the Ethics Committee of the Medical Faculty of the Justus-Liebig University Giessen (#24/13).

4.2 Treatment schedule

Mini-ECP was performed on an outpatient or inpatient basis depending on the clinical status of the patients.

For patients affected by **aGVHD** the standard treatment schedule was two mini-ECP procedures on consecutive days once weekly until clinical improvement or up to 6 weeks, whichever occurred first. Mini- ECP was then tapered on an individual basis.

For **cGVHD** mini-ECP was initially started with two mini-ECP procedures on consecutive days per week. The frequency of mini-ECP treatments thereafter varied between 2×/2 weeks to 2×/4 weeks. Duration varied widely depending on the response and tolerability of the procedure. This schedule was modified individually depending on the response, concomitant illness, or adverse events.

The doses of all immunosuppressive drugs were documented at the beginning of ECP treatment and during therapy. Immunosuppression was tapered as tolerated and discontinued if it became dependent on the clinical response.

4.3 Mini-ECP technique

The mini-ECP was performed as reported previously.[52] The steps are summarized in **Figure 2**. In brief, 100 to 200 ml of citrate-anticoagulated whole blood was collected with an umbilical cord blood collection system (MQT2205PU, MacoPharma, Langen, Germany) containing 21 mL of citrate phosphate dextrose (CPD) as anticoagulant (capacity up to 200 mL of whole blood, without diversion pouches). The 12-gauge needle of the cord blood collection set was substituted by an infusion system with luer lock (Intrafix Primeline, Braun, Melsungen, Germany) which was connected to the umbilical cord blood collection system via sterile TSCD coupling (Terumo, Eschborn, Germany). The luer lock connection allowed direct connection of the blood collection system to both a central or peripheral intravenous line (**Figure 2A**).

The blood volume to be collected was determined according to the patient's body weight. As a reference, in patients more than 20 kg the blood volume to be collected was 200 mL and in patients less than 20 kg it was 100 to 150 mL after an individual assessment by the pediatrician. In no case more than 10% of the blood volume was drawn. Skin disinfection was performed by 2-propanol, 1-propanol, and bipenyl-2-ol (Kodan Tinktur Forte Farblos, Schülke & Mayr, Norderstedt, Germany).

After centrifugation (**Figure 2B**) of the collected blood at 380 × *g* for 15 minutes, plasma, RBCs, and buffy coat were separated with a separator device (Compomat G4, Fresenius, Bad Homburg, Germany) (**Figure 2C-D**). For whole blood collections of 100 to 200 mL, no CPD volume adjustment was performed because the autologous blood components (RBCs, plasma) were returned to the patient directly at the end of the procedure. The WBC-rich fraction was transferred into a UVA-permeable bag (MacoPharma) and diluted with 0.9% NaCl to a Hct of less than 3% (**Figure 2E)**. 8-Methoxypsoralen, prepared by our hospital's pharmacy, was added (final concentration, 300 ng/mL) and incubated (room temperature, 15 min) before UVA irradiation (3 J/cm^2; BS05 UV Chamber, Gröbel, Ettlingen, Germany) (**Figure 2F**). UVA-irradiated WBC and autologous residual blood were returned to the patients directly after irradiation of the product.

Figure 2. Mini-ECP steps

A) Modified umbilical cord blood collection system (MQT2205PU, MacoPharma, Langen, Germany).
B) Centrifugation.
C) Buffy coat obtention (Compomat G4, Fresenius, Bad Homburg, Germany).
D) product separation (plasma, red blood cells, buffy coat).
E) WBC-rich fraction transferred into a UVA-permeable bag (MacoPharma) and diluted with 0.9% NaCl to a Hct of less than 3% and 8 Methoxypsoralen addition.
F) UVA irradiation (3 J/cm^2; BS05 UV Chamber, Gröbel, Ettlingen, Germany)

4.4 Response assessment

The overall acute and chronic GVHD status and specific organ involvement were documented before start of the mini-ECP regimen.[119,123,70] Response was evaluated at the end of therapy or at the last follow-up (for patients who remained under treatment at time of analysis).[48,82]

The internationally accepted response categories for aGVHD and cGVHD used in this study are shown in **Table 4** and **Table 5** respectively. In short, the criteria were:

4.4.1 aGVHD

Complete response (CR) was defined as complete resolution of all signs of aGVHD. Partial response (PR) was defined as more than 50% improvement. Stable disease (SD) was used for "no clinical change" with the possibility of tapering the dose of immunosuppressive agents by at least 50%. Non-response (NR) was used for "no clinical change" without the possibility of effective tapering of immunosuppression. Progression (P) was defined as any progression of aGVHD symptoms or signs in at least one organ system.[71]

Table 4. aGVHD response criteria to mini-ECP *(adapted from Kanold et al)*[71]

Grade	Definition
CR	Complete resolution of all signs
PR	More than 50% improvement
SD	No clinical change AND tapering of IS ≥ 50%
NR	No clinical change AND tapering of IS < 50%
P	Progression of GVHD symptoms or signs in at least one organ/system

aGVHD: acute graft-versus-host-disease; CR: complete response; NR: non-response; P: progression; PR: partial response; SD: stable disease

4.4.2 cGVHD

CR was defined as resolution of all manifestations in each organ or site. PR was defined as improvement in at least one organ or site without progression in any other organ or site. SD means "no clinical change" with the possibility of tapering the dose of immunosuppressive agents by at least 50%. NR was used for "no clinical change" without the possibility of effective tapering of immunosuppression. Progression was defined as any progression of cGVHD symptoms or signs in at least one organ system (**Table 5**).The specific organ response was defined as CR, PR, NR, P.[82]

Table 5. cGVHD global response criteria to mini-ECP (*adapted from Lee et al*)[82]

Grade	Definition
CR	Complete resolution of all signs
PR	Improvement in ≥ one organ/site without progression in others
SD	No clinical change with the possibility of tapering the dose of IS agents ≥ 50%
NR	No clinical change AND tapering of IS < 50%
P	Progression of GVHD symptoms or signs in at least one organ/system

cGVHD: chronic graft-versus-host-disease; CR: complete response; NR: non-response; P: progression; PR: partial response; SD: stable disease

4.5 Safety

Adverse events were defined as any abnormal clinical finding temporally associated with the procedure and were graded according to the Common Terminology Criteria for Adverse Events (CTCAE) v4.0.[153]

4.6 Quality control

The recommended ECP quality control variable, inhibition of lymphocyte proliferation, was previously validated. [52,124] We evaluated the UVA irradiated white blood cell counts of the first 16 treated children (13 aGVHD and 3 cGVHD), and the Htc in every single procedure.

4.7 Statistics

Descriptive parameters such as median, range and rates have been used for the statistical analysis. Nonparametric tests were applied when necessary. All analyses were performed using SPSS (Version 25.0, SPSS, Inc., Chicago, IL).

5 Results

The clinical characteristics of the 30 patients before the start of mini-ECP are summarized in **Table 6** (16 patients: aGVHD) and **Table 7** (14 patients: cGVHD). In total, 1031 mini-ECP-procedures were performed in both cohorts (aGVHD: 328; cGVHD: 703). Patients were in median 5 years old (range, 0,5-20) and median body weight was 19 kg (range, 7-53). Processed blood volume was in median 180 ml (range, 90-400).

5.1 aGVHD

5.1.1 Characteristics of the aGVHD cohort

Thirteen patients were transplantated for a malignant disease and 3 patients for non-malignant diseases including primary immunodeficiencies or bone marrow failures. Patient *8a* and patient *16a* presented with classical clinical signs of aGVHD symptoms despite time of onset (>100 days).

The median age of the patients with aGVHD was 5 years (range, 0,5-20) and the median body weight was 19 kg (range, 7-50). The median interval between SCT and aGVHD-onset was 28 days (range, 14-161). The median interval between SCT and the start of mini-ECP was 47 days (range, 23-335). The median time interval between aGVHD-onset and start of mini-ECP was 15 days (range, 4-198). The processed blood volume per treatment ranged from 90 to 400 mL (median, 162 mL). Patients with aGVHD were treated for a median of 61 days (range, 17-560). In total, 328 mini-ECP-procedures were performed, in median, 15 (range, 8-52 procedures per patient). The median follow-up from start of mini-ECP was 329 days (range 33-3933).

The organ distribution of aGVHD among the 16 patients is summarized in **Figure 3**. Skin was affected in the majority of patients (15/16) followed by gut (4/16) and liver (2/16). Most patients (12/16) presented one organ-disease (**Figure 4**). Eleven out of 16 patients presented acute GVHD grade II, and 5 patients presented grade III-IV GVHD (**Figure 5**).

Fourteen patients had steroid-refractory or steroid-dependent aGVHD. Two patients (*2a* and *4a*) received mini-ECP as first-line therapy without previous steroids due to the high relapse risk. All patients except the latter ones were receiving between one and three systemic immunosuppressive drugs before mini-ECP therapy was introduced. Two patients had even received mesenchymal stem cells before mini-ECP. The most frequent mini-ECP indication was the low weight of patients (12 patients). Other indications were low performance status (2 patients) and no central vascular access (1). Some patients had more than one indication for mini-ECP.

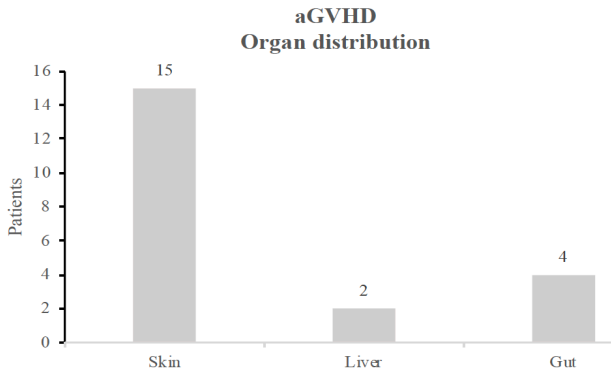


Figure 3. aGVHD. Organ

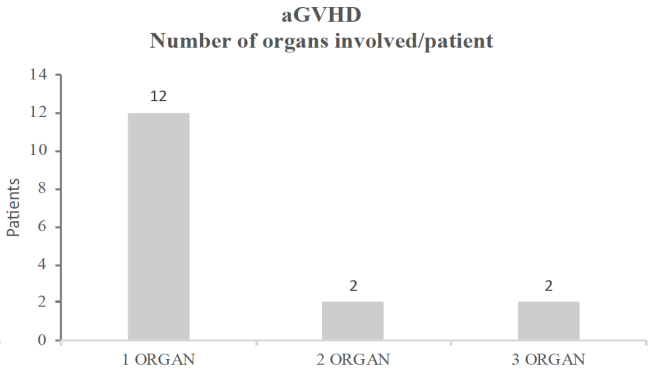


Figure 4. aGVHD. Individual organ involvement

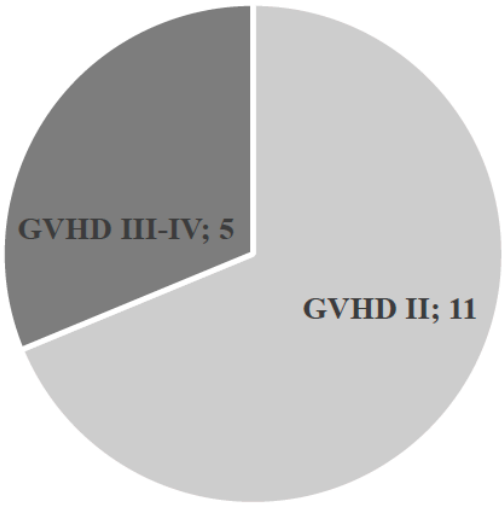


Figure 5. aGVHD. Severity grades

Table 6. Patient characteristics, adverse events and response to mini-ECP of the 16 patients with acute graft versus host disease

Pat.	Disease	GVHD Grade†	Organ Severity†	Treatment at start	GvHD onset (day)	Start ECP (day)	Age	BW	IND	Line	ml	Num ECP	AE (Grade)¶	OR	Steroids after ECP	Systemic IS after ECP	ECP duration (days)	Follow up (days)	Status last follow up
1a	ALL	II	Skin 3	Steroids MMF Sirolimus	+19	+118	7 Y	33kg	R	TL	170	20	No	CR	Tapered	Yes	225	267	Alive
2a	Infant ALL	II	Skin 3	None	+14	+25	0.5 Y	8.1kg	LW HRR	TL	90	8	No	CR	No	No	17	67	Dead ‖
3a§	sAML	II	Skin 3	Steroids	+26	+30	20 Y	50kg	R VA	PL	400	14	No	CR	No	Yes	34	127	Alive
4a	NB	II	Skin 3	None	+42	+50	5 Y	12 kg	LW HRR	TL	120	8	No	CR	No	No	34	209	Dead ‖
5a	MDS-AML	IV	Skin 2 Gut 4	Steroids	+28	+41	5 Y	19kg	LW R	TL	155	15	No	SD	No	Yes	57	191	Dead ‖
6a	sAML	II	Skin 3	Steroids	+18	+44	14 Y	33kg	R	TL	200	18	No	CR	No	Yes	82	3737	Alive
7a	Advanced MDS	II	Skin 3	Steroids	+31	+45	4 Y	15kg	LW R	TL	200	24	No	CR	No	No	228	3791	Alive
8a*	MDS	II	Skin 3	Steroids	+150	+189	6 Y	15kg	LW R	TL	150	15	No	CR	No	No	64	3220	Alive

Table 6 (continued). Patient characteristics, adverse events and response to mini-ECP of the 16 patients with acute graft versus host disease

Pat.	Disease	GVHD Grade†	Organ Severity †	Treatment at start	GvHD onset (day)	Start ECP (day)	Age	BW	IND	Line	ml	Num ECP	AE (Grade)¶	OR	Steroids after ECP	Systemic IS after ECP	ECP duration (days)	Follow up (days)	Status last follow up
9a	AML	III-IV	Gut 3-4	Steroids	+32	+48	5 Y	19kg	LW R	TL	120	35	No	CR	NA	NA	198	372	Dead ‖
10a	sAML	II	Skin 3	Steroids	+17	+42	5 Y	20kg	LW R	TL	200	18	No	CR	No	Yes	117	2563	Alive
11a	AML	II	Skin 3	Steroids	+28	+32	1 Y	11kg	LW R	TL	110	15	No	CR	No	NA	43	3933	Alive
12a	FA	IV	Skin 2 Liver 1 Gut 4	Steroids	+68	+77	14 Y	48kg	LPS R	TL	200	16	No	SD	No	Yes	50	797	Dead #
13a	SCID	IV	Skin 2 Liver 4 Gut 3	Steroids MMF MSCs	+19	+217	1 Y	7kg	LPS LW R VA	PS	100	10	No	PR	No	Yes	34	33	Alive
14a	ALCL	II	Skin 3	Steroids	+19	+23	5 Y	19kg	LW R	TL	200	52	No	CR	No	Yes	560	1868	Alive
15a	ALL	II	Skin 3	Steroids	+30	+99	8 Y	24kg	LW R VA	PS	190	47	No	CR	No	Yes	238	280	Alive
16a*	ALL	III	Skin 3 Gut 3	Steroids MMF Sirolimus MSCs	+161	+335	5 Y	17.8kg	LW R	TL	150	13	HD (G3)	PR	No	Yes	58	286	Dead #

† according to Rowlings et al[123]; *Late-onset acute GVHD; § mini ECP by peripheral venous catheter; ‖ Dead caused by relapse of malignant disease; # Dead caused by late onset infection; ¶ According to the Common Terminology Criteria for Adverse Events v4.0[153] a: related to acute GVHD; AE: adverse events; ALCL: anaplastic large cell lymphoma; ALL: acute lymphoid leukemia; AML: acute myeloid leukemia; BW: body weight; CR: complete response; FA: Fanconi anemia; HD: hemodynamic; HRR: high relapse risk; IND: Indication; IS: immunosuppression; L: low weight; LPS: low performance status; MDS: myelodysplastic syndrome; MMF: mycophenolate mofetil; MSC: mesenchymal stem cells; N: number; NA: Data not available; NB: Neuroblastoma; NR: non-response; OR: overall response; PL: peripheral line; PR: partial response; PS: port-sytem; R: refractory to previous treatment; sAML: secondary acute myeloid leukemia; SCID: severe combined immunodefficiency; SD: stable disease; TL: tunneled line; VA: vascular Access; Y: Years

5.1.2 Response of aGVHD to mini-ECP

Fourteen out of 16 patients (87.5%) with aGVHD responded to mini-ECP. Responses included 12 CR and 2 PR. Two patients remained stable (**Figure 6**). aGVHD Grade II resolved completely in all eleven patients (100% CR) including the two patients in whom ECP was used as first-line therapy. In contrast, GVHD Grade III-IV resolved completely only in one patient and partially in two children (PR). Two patients showed a stable disease at the end of treatment, both presenting a Grade IV aGVHD with at least skin and gut involvement (**Figure 7**). Photodocumentation of skin responses are represented in **Figure 8**.

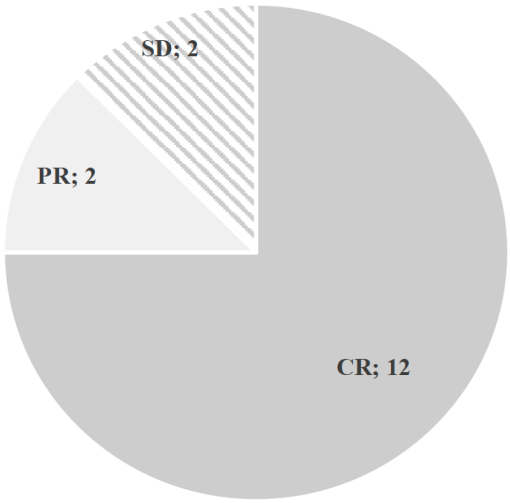


Figure 6. aGVHD. Overall response

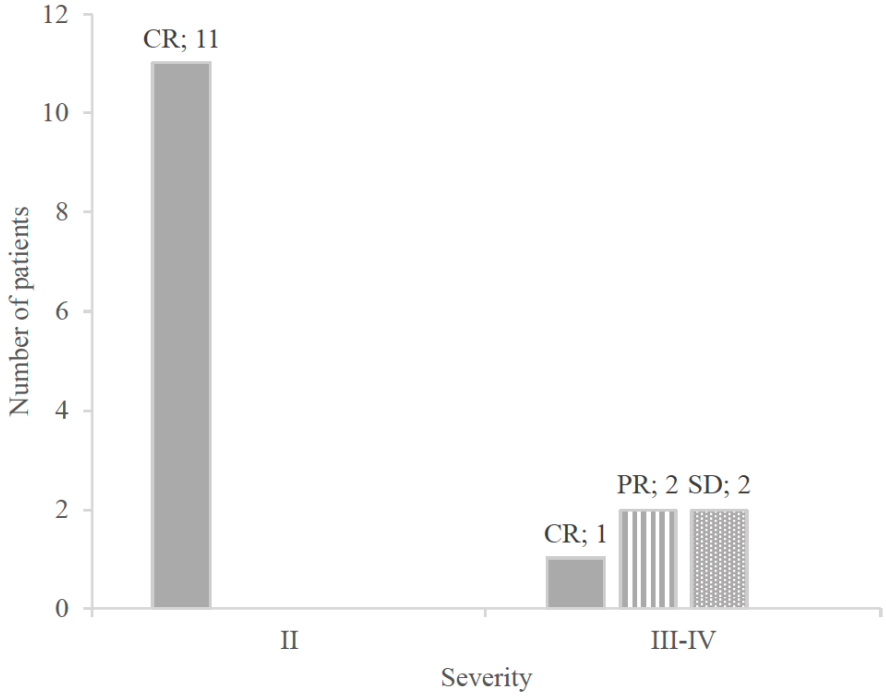


Figure 7. aGVHD. Response by severity (II vs III-IV)

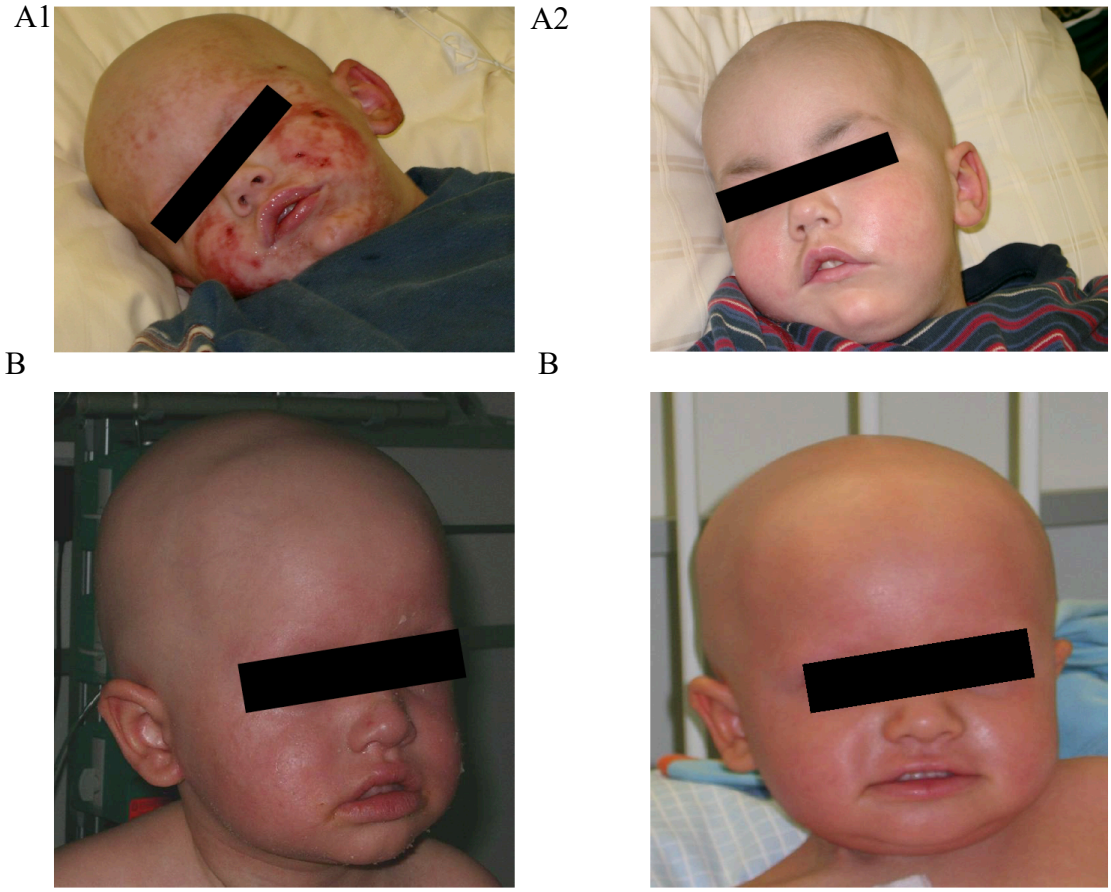


Figure 8. aGVHD. Skin response to mini-ECP
A: SR-aGVHD Grade IV. A1) At start of mini-ECP. A2) After 5 cycles.
B: SR-aGVHD Grade IV. B1) At start of mini-ECP. B2) After 6 cycles.

Fourteen patients started mini-ECP with ongoing steroid therapy. At the end of mini-ECP treatment, steroids could be withdrawn in 12 patients and significantly tapered (>50% dose reduction) in one patient. Four patients could be completely tapered from immunossupressive drugs at end of mini-ECP treatment. Immunossupression at end of ECP-treatment was not known from patient *9a* (**Figure 9**).

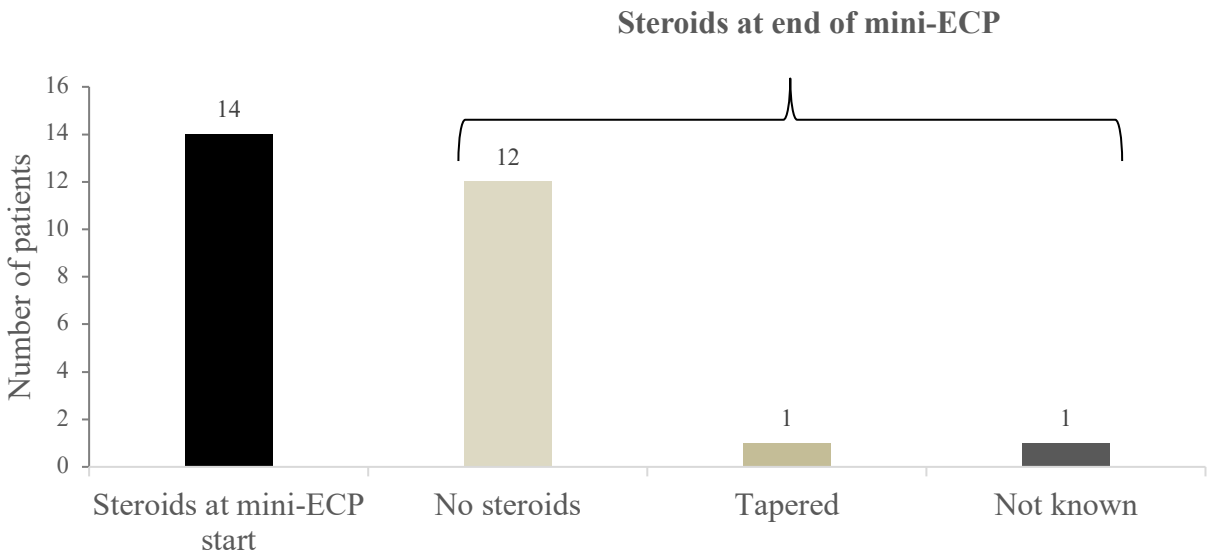


Figure 9. aGVHD. Sparing steroid effect of mini-ECP

5.2 cGVHD

5.2.1 Characteristics of the cGVHD cohort

Seven patients were transplantated for a malignant disease and 7 patients for non-malignant diseases including, five primary immunodeficiencies, one with haemoglobinopathy and a bone marrow failure.

Patients *3c*, *9c* and *13c* were first treated for a classical SR-aGVHD, and afterwards again with a cGVHD (one in the context of an ongoing process (patient *13c*), one for recurrence (patient *3c*) and one for an overlap syndrome (patient *9c*)). They were included in the cGVHD cohort. All children except one had active GVHD at the start of mini-ECP. The indication for ECP treatment in the latter patient (patient *8c*) was based on steroid dependence and cytomegalovirus (CMV) reactivation in an attempt to spare steroids. Patient *10c* was treated twice for extensive sclerodermiform cGVHD. After a very good partial response (PR) to first mini-ECP, treatment was tapered and stopped (*10c* A). She experienced reactivation of cGVHD 3 years later triggered by an infection, so a second mini- ECP treatment was started (*10c* B). Four patients were still on treatment at the time of data collection (*9c*, *10c* B, *12c*, *13c*).

Patients with cGVHD were in median 7 years old (range, 1-17) and the median body weight was 20 kg (range, 8-53). The median interval between SCT and GVHD-onset (including first aGVHD) was 44 days (range, 8-1232). The median interval between SCT and the start of mini-ECP was 546 days (range, 39-3521). The median time interval between GVHD-onset and start of mini-ECP was 144 days (range, 10-3495). Patients with cGVHD were trated for a median of 345 days (range, 43-855). In total, 703 mini-ECP-procedures have been carried out. In median, 35 (range, 8-129) mini-ECP procedures per patient were performed. The processed blood volume per treatment ranged from 100 to 400 mL (median, 200 mL). The median follow-up from start of mini-ECP was 779 days (range 43-2712).

The organ distribution of cGVHD among the 14 patients is summarized in **Figure 10**. Eight out of 16 patients showed an extensive cGVHD. Five of 12 patients with skin involvement showed chronic sclerodermoid GVHD, four of them with joint contractures. Five patients showed a moderate cGVHD **(Figure 11)**.

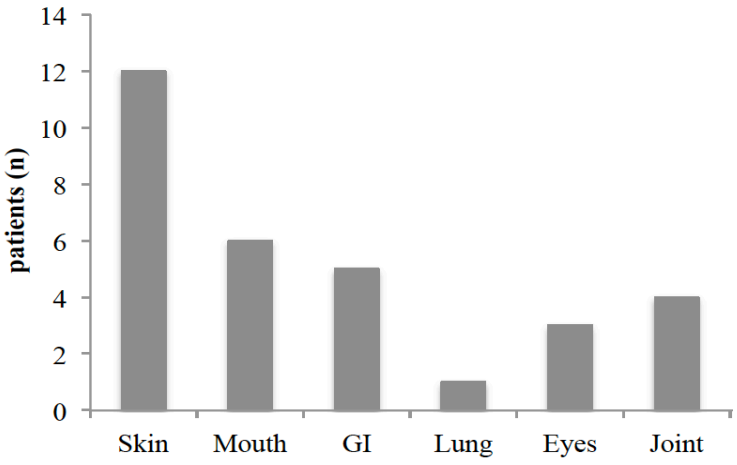


Figure 10. cGVHD. Organ distribution

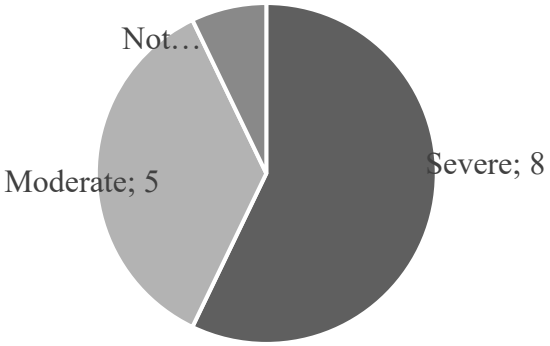


Figure 11. cGVHD. Severity grades

Mini-ECP was mostly indicated by low weight (9/14) and lack of an adequate vascular access (8/14). Three patients showed hemodynamic or respiratory restraints at base line that contraindicated the use of a classical procedure. In two patients a low performance status prohibited a classical ECP-procedure. Some patients presented more than one characteristic that made them suitable for mini-ECP.

Table 7. Patient characteristics, adverse events and response to mini-ECP of the 14 patients with chronic graft versus host disease

Pat.	Disease	GVHD Grade	Organ Severity †	Treatment at start	GvHD onset (day)	Start ECP (day)	Age	BW	IND	Line	ml	Num ECP	AE (Grade)¶	OR	Steroids after ECP	SOR	ECP duration (days)	Follow up (days)	Status last follow up
1c	Thal. major	Severe	Skin 3 (SCL) Joints 2	Steroids Imatinib Sirolimus	+590	+734	6 Y	27kg	VA	PS	200	62	No	PR	No	Skin PR Joints PR	458	1563	Alive
2c	AML	Moderate	Skin 2 GI 2	Tacrol. MMF	+17	+147	5 Y	36kg	VA	PS	200	29	No	CR	NA	Skin CR GI CR	218	1009	Alive
3c ¥	IBMF	Moderate	Skin 2 Mouth 1	CSA Steroids	+22	+41	1 Y	8kg	LW	TL	120	55	no	PR	Tapered	Skin PR Mouth PR	665	1281	Alive
4c	ALL	Severe	Skin 3 (SCL) Joints 3 GI 2 Mouth 2 Lung 2 Eyes 2	Steroids Imatinib	+8	+1246	7 Y	16kg	LW VA RI	PL	120	35	Fever (G2) Hypoxemia (G2)	SD	No	Skin NR GI NR Mouth NR Lung NR Eyes NR Joints NR	378	1219	Alive
5c	ALL	Moderate	Skin 2 GI 1	Steroids Sirolimus	+235	+336	16 Y	53kg	HD VA	PS	400	28	Fever (G2)	PR	No	Skin CR GI NR	294	888	Alive
6c	ALL	Moderate	Skin 2 (SCL) Mouth 1	Sirolimus Imatinib	+454	+1594	7 Y	17kg	LW VA	PS	130	29	No	CR	NA	Skin CR Mouth CR	345	755	Alive
7c	ALL	Severe	Skin 3	Steroids Sirolimus MMF	+200	+575	2 Y	10kg	LW	TL	100	34	No	CR	No	Skin CR	292	779	Alive
8c ‡	CGD	NA	NA	CSA Steroids	+111	+225	2 Y	13kg	LW VA	PS	120	18	No	NA	No	NA	127	674	Alive

Table 7 (continued). Patient characteristics, adverse events and response to mini-ECP of the 14 patients with chronic graft versus host disease

Pat.	Disease	GVHD Grade	Organ Severity †	Treatment at start	GvHD onset (day)	Start ECP (day)	Age	BW	IND	Line	ml	Num ECP	AE (Grade)¶	OR	Steroids after ECP	SOR	ECP Duration (days)	Follow up (days)	Status last follow up
9c ¥ ||	CGD	Severe	Skin 3 GI 3 Eyes 1	CsA Steroids	+29	+39	12 Y	30kg	LPS VA	PS	200	129	Sepsis (G4)	PR	Tapered	Skin PR GI CR Eyes CR	855	1026	Alive
10c A	SCID	Severe	Skin 3 (SCL) Mouth 2 Joints 3	Steroids Tacrol PUVA	+26	+1476	5 Y	13kg	LW VA	TL	130	72	No	PR	No	Skin CR Mouth CR Joints PR	834	2712	Alive
10c B ||		Severe	Skin 3 (SCL) Joints 3	Imatinib	-	+3521	10 Y	20kg	LW VA	PS	200	55	No	PR	NA	Skin PR Joints NR	622	622	Alive
11c	ALL	Moderate	Skin 2 Mouth 2 Eyes 2	Steroids MMF	+40	+115	7 Y	23kg	LW VA	TL	180	46	No	P	Yes	Skin NR Mouth P Eyes NR	324	450	Dead #
12c ||	SCID	Severe	Skin 3	Steroids Sirolimus	+1232	+1693	4 Y	18kg	LW VA	PS	200	8	No	SD	Yes	Skin NR	43	43	Alive
13c ¥ ||	HS	Severe	GI 3	CSA Steroids	+44	+57	17 Y	49kg	LPS HD	TL	400	25	No	PR	Tapered	GI PR	173	211	Alive
14c	CGD	Severe	Skin 3 (SCL) Mouth 2 Joints 2	Steroids Azathioprine	+67	+546	7 Y	28kg	VA	PS	200	78	No	NR	No	Skin NR Mouth NR Joints NR	586	586	Alive

¥ Prior aGVHD; † according the NIH revised criteria[70]; ‡ Not evaluable for response assessment; || Still on treatment; # Dead caused by late onset infection; ¶ According to the Common Terminology Criteria for Adverse Events v4.0[153]; AE: adverse events; ALL: acute lymphoid leukemia; AML: acute myeloid leukemia; BW: body weight; CGD: chronic granulomatous disease; CR: complete remission; CSA: ciclosporine A; GI: gastrointestinal; HD: haemodynamic; HS: histiocytic sarcoma; IBMF: inborn bone marrow failure; IND: Indication; LW: low weight; LPS: low performance status; MMF: mycophenolate mofetil; N: number; NA: non applicable; NR: non-response; OR: overall response; P: progression; PL: peripheral line; PR: partial remission; PS: port-sytem; RI: respiratory insufficiency; SCID: severe combined immunodefficiency; SCL: sclerodermic; SD: stable disease; SOR: specific organ response; TL: tunneled line; VA: vascular Access; Y: years

5.2.2 Response of cGVHD to mini-ECP

Response of cGVHD to mini-ECP was evaluable in 13 patients. The ORR and the specific organ response to mini-ECP are presented in **Figure 12** and **Figure 13**, respectively. Patient *8c* was treated without signs of cGVHD because of his steroid-dependence and CMV-reactivation with the goal to decrease the dose of steroids so that he was not evaluable for response. Patient *10c* was treated twice (**Table 7**). She responded with a PR of skin GVHD to the first ECP. The debilitating contractures resolved almost completely. The frequency of ECP was reduced after steroids were withdrawn and was finally stopped after 120 weeks. The cGVHD reactivated in skin triggered by an infection 3 years after cessation of the first mini-ECP. She responded again to the second attempt with a PR and remained on treatment at time of data collection.

Nine of the 13 patients responded to mini-ECP with either CR or PR, resulting in an ORR of 69%. Three patients showed a complete resolution of GVHD signs, and six patients had a PR. Three patients did not show clinical improvement to mini-ECP and one patient progressed. Photodocumentacion of the skin response in patient 10c is presented in **Figure 14.**

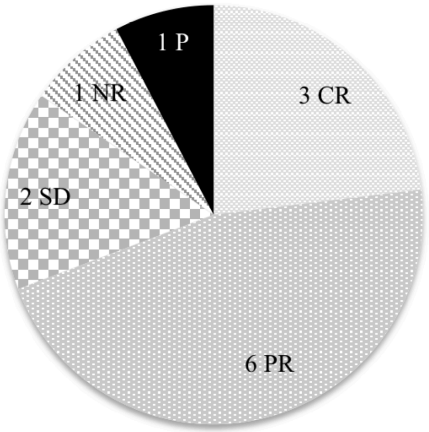


Figure 12. cGVHD. Overall response

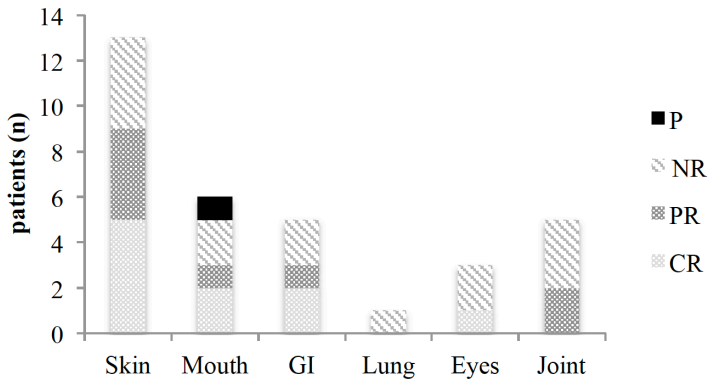
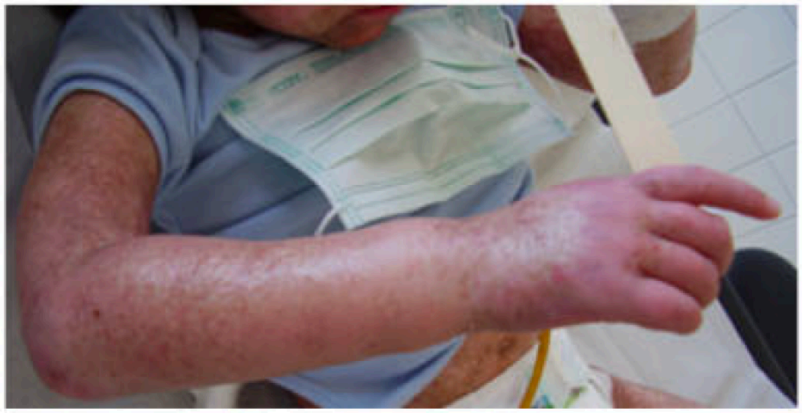
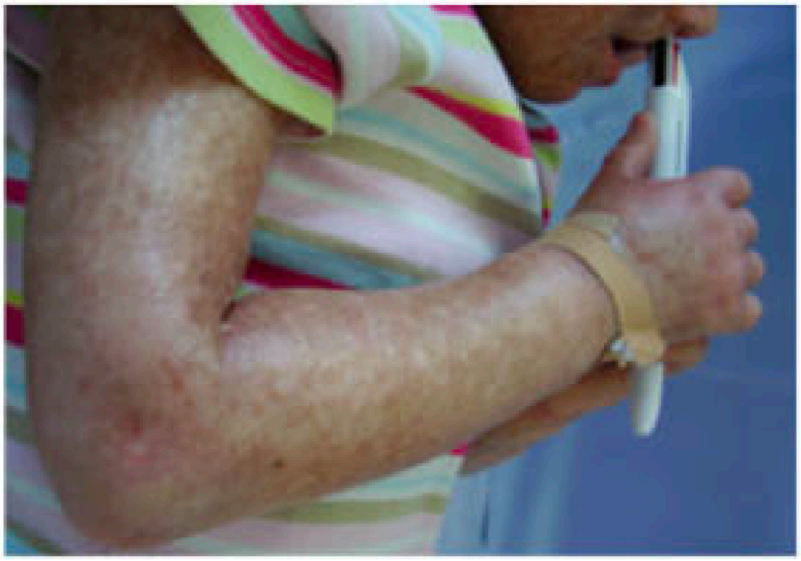


Figure 13. cGVHD. Single organ response

A



B



C

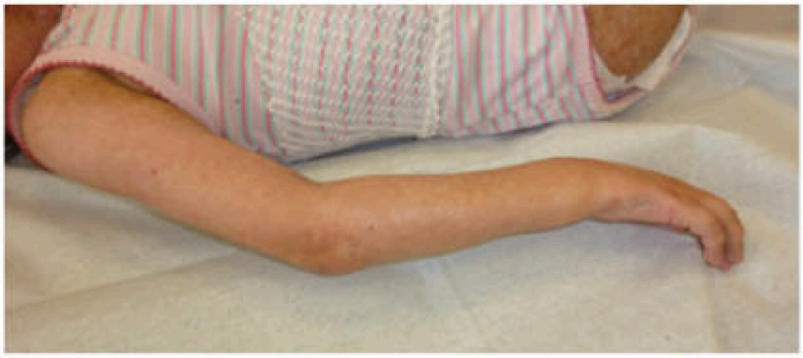


Figure 14. cGVHD. Skin response to mini-ECP
Patient 10c, affected by a scledodermic cGVHD.
A: At start of mini-ECP.
B: Day14 after start of mini-ECP
C: Day 128 after start of mini-ECP

Mini-ECP

All patients were receiving between one and three systemic immunosuppressive drugs before mini-ECP therapy was introduced. Twelve of the 14 patients were dependent on steroids. Corticosteroids were completely discontinued in seven patients, including patient *8c* (not evaluable for response assessment). Steroids were significantly tapered (>50% dose reduction) in three patients (**Figure 15**).

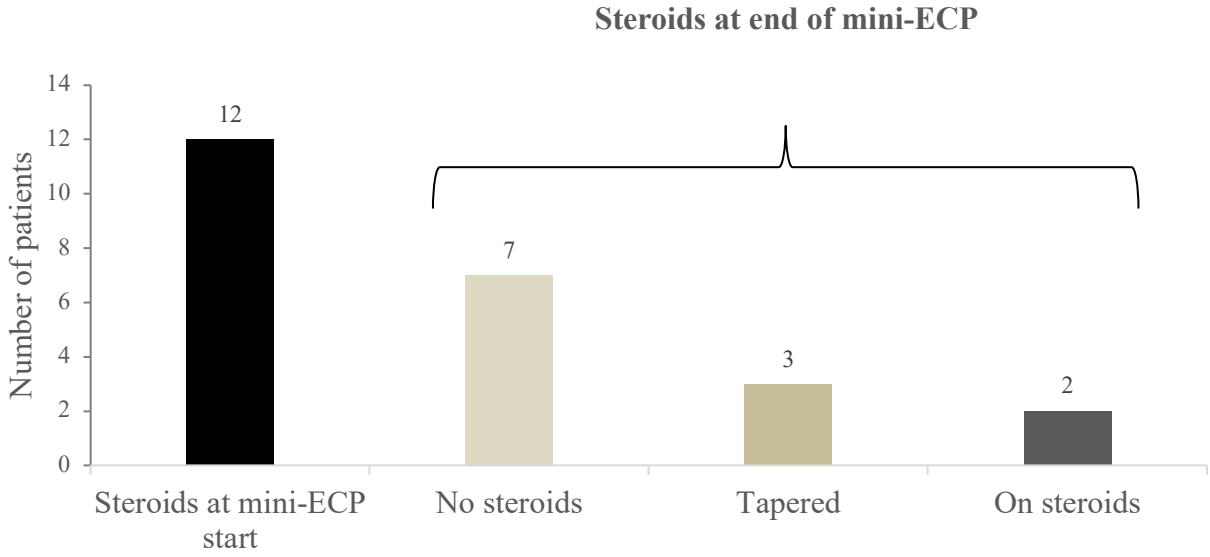


Figure 15. cGVHD. Sparing steroid effect of mini-ECP.

5.3 Quality control

For quality control purposes, the UVA-irradiated WBCs/kg body weight per procedure for the first 16 patients treated (13 aGVHD and 3 cGHVD) was calculated. Patients received a mean dose of 10.4×10^6 UVA-irradiated WBCs/kg body weight per procedure (range, $2.2 \times 10^6 – 24.56 \times 10^6$; **Figure 16**[54]) which is significantly lower compared with classical ECP.[83,85]

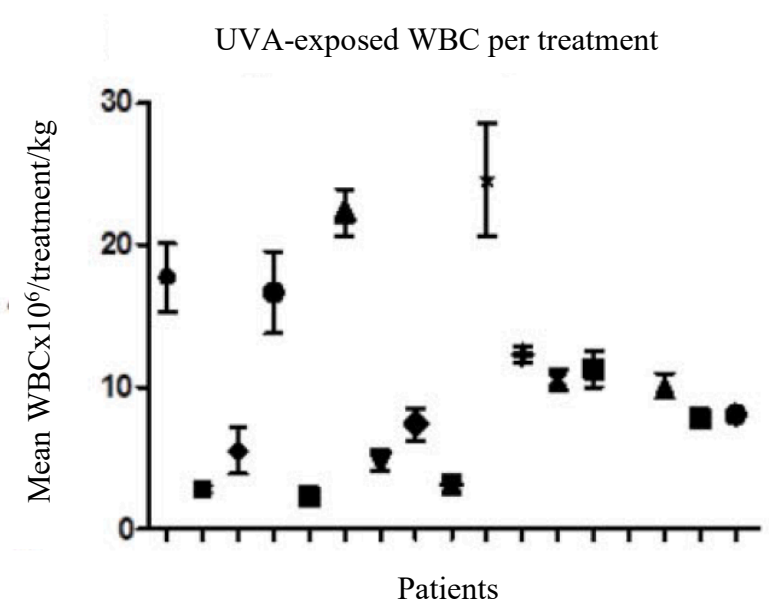


Figure 16. Mean dose of UVA-exposed WBC/treatment and body weight ***(adapted from Hackstein et al)***

In the first 13 patients treated for aGVHD, the number of UVA-irradiated WBC correlated with response. Patients responding to mini-ECP (CR plus PR) received significantly higher doses of UVA-irradiated WBCs than patients exhibiting no response to therapy (12.47 x 10^6 vs. 2.9 x 10^6 WBCs/kg/treatment; $p < 0.001$; two-tailed Mann-Whitney test; **Figure 17**[54]).

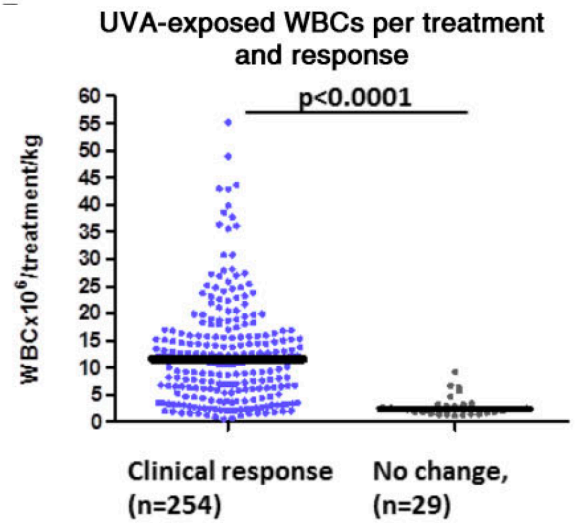


Figure 17. Clinical response according to number of irradiated WBC/kg/procedure ***(adapted from Hackstein et al)***

In the aGVHD cohort UVA irradiation of WBCs was performed at a mean Hct of 2.4% (range, 0.1%-11.2%) and in the cGVHD cohort UVA irradiation of WBCs was performed at a mean Hct of 2.9% (range, 0.4%-16.1%) which is similar to the range reported for classical ECP.[130]

6 Safety

All but two patients had a central venous catheter in place already before ECP. Patients *3a* and *4c* lacked a central venous line, so mini-ECP was performed through peripheral access that was removed after every session. The mini-ECP treatments were well tolerated.

Five adverse events (AE) were observed in four patients related to the procedure: 2 haemodynamic, 3 infectious.

Patient *16a* showed a low performance status when starting ECP and developed a syncope (CTCAE Grade 3) while blood extraction despite volume preload before the procedure. A recurrence of such an event prompted to interrump further mini-ECP attempts.

Patient *4c* developed fever immediately after a mini-ECP procedure without laboratory signs of infection. He was started on empirical antibiotics. The fever ceased 1 day later. The blood culture remained negative (CTCAE Grade 2). The same patient had chronic lymphedema related to massive sclerodermic changes of the skin, necessitating regular diuretics after reinfusion of the processed ECP product. After one procedure, supplemental oxygen was necessary for a few hours (CTCAE Grade 2) likely due to the reinfused volume.

Patient *5c* developed fever after mini-ECP during an immunoglobulin infusion. C-reactive protein was already elevated before the ECP procedure. He was started on antibiotics. Blood cultures remained negative (CTCAE Grade 2), and the fever ceased rapidly.

Patient *9c* presented with clinical sepsis due to Staphylococcus epidermidis 1 day after an ECP procedure. After antibiotic therapy and volume support the patient recovered without sequelae (CTCAE Grade 4). This complication was the only one likely related to a catheter infection.

No patient suffered viral reactivation during the procedure. Patient *8c* did not have any further CMV reactivation after starting Mini-ECP.

No bleeding episodes occurred in any of our patients. Transfusions related to the procedure were not required; signs of renal insufficiency or other complications related to mini-ECP were not observed.

No deaths related to mini-ECP occurred. Ten patients in the aGVHD cohort were alive at the last follow-up. Four patients died due to relapse of the malignant disease (*2a*, *4a*, *5a*, *9a*). Patient *12a* and *16a* died because of severe infections several years and months, respectively, after mini-ECP. All patients with cGVHD but patient *11c* were alive at the last follow-up. The latter died due to infectious complications under immunossupressive treatment for progressive cGVHD.

7 Discussion

Despite immense advances in the field, GVHD remains the major cause of TRM and long-term sequelae after SCT. Steroids still are the mainstay of GVHD therapy but are associated with significant toxicities and limited response.[49,6,134] Many currently available salvage therapies are associated with severe immunosuppression, high risk for infectious complications, and potential loss of the graft *versus* malignancy effect. Thus, there currently is no consensus regarding treatment of steroid-refractory aGVHD and cGVHD.

Available data regarding the efficacy and safety profile of ECP, including the lack of interactions with other agents, the low generalized immunosuppression, its impact on outcome and survival, supports its use as second-line therapy of SR-aGVHD and SR-cGVHD. [48,125,39,1] Althoug limited, available data on ECP for treating GVHD in children suggest similar efficacy compared to adults.[99,71,96,111,16]

Clinical utilization of ECP in small children and critically ill patients is limited due to a number of technical and procedural difficulties. Major limitations and contra-indications for classical ECP procedures in these patient groups include low body weight which limits extracorporeal volume, necessity of central venous access, frequent priming of apheresis machine with RBC concentrates, and long duration, sometimes requiring sedation in infants and young children.[71,25,112]

We previously described the development, quality control, and clinical application of a novel mini buffy coat ECP technique ("mini-ECP") facilitating treatment of small children and critically ill patients with contraindications for classical apheresis.[52]

The mini-ECP does not need an apheresis, it is rapid (<2 hours for the complete procedure), and less expensive (no need for expensive leukapheresis sets). It can be performed repeatedly in small or critically ill children and adolescents without the need of additional blood transfusions, central venous catheters, and medical sedation. The product manufacturing protocol warrants a limited plasma volume during the irradiation as the original whole blood product is separated in three components plasma, RBCs, and buffy coat directly after blood drawal. The UVA intensity used was about 3 J/cm^2 as per protocol. Moreover, the mini buffy coat ECP method is a functionally closed system since preparation of the WBC-rich fraction does not need density gradient centrifugations. 8-MOP is only added after separation to the WBC-fraction. Therefore, autologous RBCs,

plasma, and platelets can be returned to the patient.

After establishing the feasibility of mini-ECP for the treatment of GVHD in small children and those with contraindications for classical ECP-procedures[52] we here present data on the efficacy and safety of this novel ECP method to treat patients with SR-GVHD and contraindications for conventional ECP.

We report on 30 infants, children and adolescents affected by acute or chronic GVHD who received more than 1000 mini-ECP procedures at the University Hospital Giessen and Marburg. In our series, very low body weight, poor performance status, preexisting organ complications, difficulty to place an adequate CVC, or the lack of a central venous catheter prohibited performing the classical ECP procedure.

In the aGVHD cohort mini-ECP has shown to be effective, mostly for low-stage GVHD (Grade II / skin), being less useful in advanced stages (III-IV), which is in line with other reports.[99,71,111]

The steroid-sparing effect was quite remarkable and reaches the observed range observed with other ECP-methods.[71,111]

The use of mini-ECP as first-line approach in two children affected by aGVHD and high risk of relapse of their malignant disease was prompted by early reports with classical ECP.[17] Such approach should be prospectively validated as represents an attractive option regarding the prognostic impact of early interventions, without compromising anti-viral and anti-malignant effects.[133]

Among the 13 evaluable patients with **cGVHD**, the overall response rate of 69% was similar to the reported results for classical ECP in adults and children. [41,36,111] A steroid-sparing effect of classical ECP was observed with mini-ECP in the same order as compared to classical ECP.[71,111] The overall response rate of skin cGVHD was in line with previous observations of classical ECP.[99,111] Notably, three out of five children with sclerodermoid changes responded to mini-ECP. Joint impairment, as a sign of advanced stage chronic skin-joint GVHD, is difficult to treat with any ECP approach.[39]

Mini-ECP

The prognostic impact of an early intervention with ECP on SR-GVHD is well established and could also be documented in our series with mini-ECP. Therefore, attemps should be carried out to start ECP as soon as possible. [50,5,47] The patients in our cohorts who started mini-ECP sometimes very late in the evolution of the disease, mostly had cGVHD. This can be explained by the fact that some patients were refered from other centers for mini-ECP first after several lines of treatment due to the impossibility to be treated with conventional ECP locally. Due to to the retrospective nature of the study, it is not possible to establish the influence of mini-ECP in such patients that started very late. Although the 3 of the 5 patients with sclerodermoid that responded to mini-ECP started more than a year after GVHD onset, we propose an ealier start of ECP also for those children.

The experience with three patients treated twice with mini-ECP for both acute and chronic GVHD might give a hint that not only an early initiation of ECP is advisable, but longer treatment periods could be also recommended in some patients to facilitate immunomodulation and achieve immunotolerance. Our observation is also in line with the observation of other authors, leading to the recommendation to prolong ECP beyond 6 months in severe cGVHD cases.[5,11,49]

Table 8 shows a review of published data from pediatric studies with classical ECP for GVHD compared with mini-ECP .

Table 8: Review data of classical ECP in pediatric series *(adapted from DeSimone et al)*[25]

Reference	Pat.	Procedures	IND	Mean/Median weight (range)	Mean/Median age (range)	VA	Device	Blood prime	Median length hh:mm (range)	Complications (N,%)	CRR
Halle et al[55]	8	254	8c	37 (18–49)	10 (5–15)	CVC PL	COBE Spectra®	No	02:20 (01:00–04:00)	Catheter-related infections (n=2, 25%)	S = 3/8 L = 4/6 GI = 5/5
Salvaneschi et al[124]	23	NR	9a 14c	35 (17–89)	10 (5–18)	CVC PL	COBE Spectra®	No	NR	Hypotension (NR)	a = 5 (55%) c = 4 (28%)
Messina et al[99]	77	NR	33a 44c	a: 30 (10–85) c: 35 (15–68)	9 (0.3–21)	CVC PL	COBE Spectra® (n=44) UVAR XTS® (n=33)	No	03:30 (03:00-04:00)	Hypotension (n=21, 27%); Abdominal pain (n=8, 10%)	a = 23 (69%) c = 34 (77%)
Calore et al[15]	15	NR	15a	NR	10 (1–18)	NR	COBE Spectra®	Yes	03:30 (03:00-04:00)	Catheter-related infection (n=1, 7%)	a = 11/15 (73%)
Kanold et al[71]	23	750	9a 14c	a: 48 (13–68) c: 43 (13–80)	14 (4–18)	CVC PL	COBE Spectra®	No	a: 02:02 (01:01-04:10) c: 01:59 (01:12-03:40)	Overall (n=2, 8%)	a = 7 (58%) c = 4 (26%)
Berger et al[96]	25	NR	15a 10c	40 (17–72)	a: 11 (6–18) c: 12 (7–19)	NR	COBE Spectra® (<40kg) UVAR-XTS® (>40 kg)	No	03:30 (03:00-04:00)	Abdominal pain (20%); Catheter placement (n=2, 8%)	NA
Duzovali et al[30]	7	133	7c	32 (16–89)	10 (8–17)	CVC	UVAR-XTS®	Yes	03:30 (03:00-04:00)	Catheter-related infections (n=3, 42%) and placement (n=2, 29%)	S = 3/6 L = 1/5
Landolfo et al[72]	8	157	NR	19 (7–35)	NR	CVC PL	COBE Spectra®	Yes	2:03 (1:55-2:10)	NR	NR

Table 8 (continued): Review data of classical ECP in pediatric series *(adapted from DeSimone et al)*[25]

Reference	Pat.	Procedures	IND	Mean/Median weight (range)	Mean/Median age (range)	VA	Device	Blood prime	Median length hh:mm (range)	Complications (N,%)	CRR (N,%)
Schneiderman [128]	11	334	9c 2Px	29 (19–39)	NR	CVC PL	UVAR-XTS®	No	2:58 (1:30-5:03)	Overall in 31% of procedure days: tachycardia, dizziness, nausea, vomiting, hypotension, headache	NR
Perotti[111]	73	2360	50a 23c	a: 32 (StD 16) c: 39 (StD 17)	a: 10 (StD 5) c: 12 (StD 4)	CVC PL	COBE Spectra®	Yes	NR	Catheter-related infections (n=10, 14%); Chills (n=12, 0.5%) ; Abdominal pain (n=7, 0.2%); Headache (n=22, 0.9%); Fever (11, 0.4%)	a = 16/50 (32%) c = 5/23 (22%)
Merlin[98]	12	NR	12a	NR	11 (2–18)	CVC	COBE Spectra®	NR	NR	NR	a = 6 (50%)
Gonzalez-Vicent[45]	27	225	21a 6c	30 (9–77)	10 (1–17)	CVC	COBE Spectra®	Yes <15kg	03:00 (average)	Hypotension (n=3, 11%) Catheter-related infections (n=3, 11%)	a = 11 (52%) c = 3 (50%)
Rangarajan[121]	9	385	1a 8c	49 (19–86)	14 (4–24)	CVC	CELLEX®	Yes <35kg	1:46 (1:00–3:25)	Catheter-associated thrombosis (n=1, 0.2%); Delayed bleeding (n=1, 0.2%); Catheter-related infections (n=4, 1%); Procedures cancelled (n=15, 3.9%)	NR
Uygun[140]	12	194	6a 6c	28 (7–68)	12 (2–17)	CVC PL	CELLEX®	Yes <35kg	NR	Hypotension (n=7, 4%); Palpitation and tachycardia (n=6, 3%); Increase in purpuric lesions (n=4, 2%); Gastrointestinal bleeding (n=2, 1%); Pruritus (n=1, 0.5%); Catheter-related infection (n=1, 0.5%)	a = 7/10 (70%) c = 4/6 (66%)
Kapadia[73]	10	440	5a 5c	32 (22–65)	10 (8–27)	CVC PL	UVAR-XTS® (n=225) CELLEX® (n=215)	Yes <35kg	UVAR-XTS®: 3:09 (average) CELLEX®: 1:58 (average)	Line occlusions (n=23, 5.2%); Hypotension (n=18, 4%); Hypertension (n=6, 2%); Blood products required (n=59, 13%); Citrate toxicity (n=6, 1%)	a+c = 5 (50%)
Nelson[101]	30	NR	30a	26 (7–138)	29 (19–39)	NR	CELLEX®	NR	NR	None	a = 4 (13%)
Mini-ECP [54,141]	30	1031	16a 14c	a: 19 (7-50) c: 20 (8-53)	a: 5 (0,5-20) c: 7 (1-17)	CVC PL	Mini-ECP	No	1:00 – 2:00	Hypotension (n=1); Sepsis (n=1); Fever (N=2), Hypoxemia (n=1)	A = 12 (75%) C = 3 (23%)

a: acute GVHD; c: chronic GVHD; CRR: complete response rate; CVC: central venous catheter; d: days; GI: gastrointestinal; hh: hours; IND: Indication; kg: kilogram; L: liver; mm: minutes; NR: not reported; Pat: patients; PL: peripheral line; Px: prophylaxis; S: skin; StD: standard deviation; VA: Venous access

Mini-ECP

Mini-ECP collects and treats lower WBC numbers in comparison to conventional off-line ECP (highest cell number) and the in-line system (**Table 9**).[130,13] To date no evidence of a clear correlation between the total number of reinfused WBCs and clinical efficacy has been reported. Only few studies have shown the correlation of cell numbers treated with response to ECP until now, the majority performed in patients with chronic GVHD.[113,114,85,10,150]

Table 9. Collection data from different ECP procedures *(adapted from Brosig et al)* [13]

Procedure parameter	**Off-line**				**In-line**
	Amicus®	**Cobe Spectra®**	**Spectra Optia®**	**MINI-ECP**	**Therakos UVAR XTS®**
Total processed WBC (median)	73 x 10^8	66 x 10^8	63 x 10^8	3.4 x 10^8	30 x 10^8

WBC: white blood cells

Since composition of the ECP cell preparation varies across patients, it is difficult to accurately predict the efficacy and reproducibility of the cellular response. In an ECP animal model, antigen-specific immunosuppression has been achieved already with 0.2% of the blood volume.[67] Based on these data, Schreiner et al. reported clinical responses in three adult patients with cutaneous T-cell lymphoma with a small-scale ECP procedure using mononuclear WBCs after density gradient centrifugation from only 50 mL of blood.[131] Our results, and some previous data, may indicate that efficacy of ECP might be rather related to a minimal threshold cell number per kilogram of body weight. This threshold might even vary depending on the underlying disease and co-treatment of the patients.[113]

In our study, a subset of patients with aGVHD responding to mini-ECP (CR plus PR) received significantly higher doses of UVA-irradiated WBCs than patients exhibiting no response. However, cell counts also in responding patients were significantly lower compared with classical ECP. These results may indicate a possible WBC threshold for successful ECP in the lower range collected for min-ECP. Thus, a minimal number of UVA-irradiated WBCs may be required for a clinical response.

However, a direct correlation between the WBC dose and treatment response should be interpreted with caution because a lower WBC yield may just represent an epiphenomenon indicating disease severity.

Recently, the largest single-center study analyzing the impact of treated WBC on response in patients with SR-aGVHD showed a significant association between a higher mean number of lymphocytes and MNCs/kg body weight collected per single procedure and the response to ECP at 1 month (p=0.032 and p=0.028, respectively). Cutoff values of 8.4 x 10^6/kg body weight for lymphocytes and of 13.9 x 10^6/kg body weight for MNCs were associated with the OR to ECP at 1 month with a predicted sensitivity of 75% and 73%, respectively, and a predicted specificity of 56% and 52%, respectively.[150]

The influence of individual components of the ECP procedure on *in vitro* efficacy as measured by apoptosis induction has been quantified among patients receiving an off-line ECP-procedure. [150] A dose effect on apoptosis and percentage of proliferation inhibition was observed for three parameters: Hct, plasma ratio and UVA dose. Hct was the most stringent component, likely owing to its high absorbance of UVA and its shielding effect on other cells. Increasing the UVA dose could counteract the limiting effect of a high Hct concentration on the cellular response. A greater cellular response was observed when using a 0% plasma condition than with a 100% plasma matrix, suggesting that leukapheresis products should ideally be diluted in saline solution rather than plasma. Since our mini-ECP-approach included dilution by saline, this might have contributed to the observed efficacy in our cohort despite lower cell numbers treated. It is noteworthy that cell density did not correlate with the *in vitro* efficacy of ECP in the study.[79]

Despite the potential disadvantages of classical ECP devices in a subset of pediatric patients, alternative strategies are usually not locally available or recommended and medical teams should individualize the choice of the method. UVAR-XTS® and CELLEX® are the most used automated ECP systems in pediatric patients. Safe extracorporal volumes based on patient´s total blood volume and hematocrit have to be determined using tables provided in the procedure manual. Since these devices are approved for ECP, no further quality control procedures are recommended to assess the collected product for either device.

Mini-ECP

A recent study compared both instruments in pediatric patients with SR-GVHD.[73] Median age and weight were 10.3 years (range, 7.5–26.9) and 31.7 kg (range, 21.8–65), respectively. All patients weighing less than 30 kg were primed with packed red blood cells. In total, 440 procedures were performed (225, UVAR-XTS®; 215, CELLEX®). The study showed that ECP performed with the CELLEX® instrument was better tolerated compared to UVAR-XTS® resulting in shorter run times (118 vs. 189.4 min, P<0.0001), increased percentage of mononuclear cells treated (27% vs. 35%, P<0.001), reduced incidence of line occlusions requiring TPA treatment, citrate toxicity, and decreased incidence of hypotensive episodes (0 vs 18, P<0.0001). All hypotensive periods were observed in patients weighing less than 35 kg, 15 procedures (3%) were terminated early secondary to patient hemodynamic instability, line occlusions, or alarms (UVAR-XTS®:8; CELLEX®:7). Twelve procedures were cancelled (9, UVAR-XTS®: hemodynamic instability, fever, line sepsis, or patient no show; 3, CELLEX®: bruising at port-a-cath site or low buffy coat). Data show relevant difficulties with both on-line devices. In our experience with more than 1000 mini-ECP procedures, with 73% of patients weighing less than 30 kg, no red blood priming was necessary, central venous line issues did not remain a major problem, and only one patient suffered hemodynamic complications (patient *16a*), reflecting the safety of the technic.

These observations suggest that for very low-weight children mini-ECP might be a more suitable approach than the approved devices and should be further developed to an even more automated procedure. However, one drawback of the mini-ECP procedure compared to other ECP-systems still is its dependence on an established manual buffy coat preparation and a separated UVA irradiation procedure (**Table 10**). [121,75,26]

Table 10. Differences among mini-ECP and most used automated (on-line) ECP methods in pediatric patients.[121,75,54,25,141]

Methodology	Automated	Line	Lower weight limit	ECV	Durantion
Therakos Cellex®	Yes	CVC	20kg (RBC prime if>15% ECV)	216-266 ml	90–180 min
Therakos UVAR-XTS®	Yes	CVC	40kg	220-620 ml	180–240 min
Mini-ECP	No	CVC / PVC	7kg (or lower)	100-200 ml	60-120 min

CVC: central venous catheter; ECV: extracorporeal volume; ml: milliliter; min: minutes; RBC: red blood cell

Some groups have developed different approaches to treat patients that are not good candidates to classical ECP:

Conventional ECP using the UVAR-XTS® machine in children weighing as little as 19 Kg using normal saline or 5% albumin boluses before initiation of the procedure has been reported.[128] The same closed system has been successfully performed in few children between 13-34 kg with periodical saline infusions or a system modification that allowed red blood priming during the procedure.[65] Matic et al have published on the feasibility and tolerability of a mini-buffy coat separation method using an automated Sepax system separator in one patient.[97]

One of the advantages of the mini-ECP is that it reduces overall buffy coat collection time from 240 minutes in conventional "off-line" apheresis ECP to often less than 30 minutes for whole blood collection, and so it interferes less with other therapies that need to be administered.

Recently, a modified classical approach (Low Volume - ECP) concerning the MNC collection and cell trasnformation has been approved by the ANSM (French security agency for food & drug) in order to render ECP easier and safer for children and adults, with shorter time (75 min) of procedure, lower final volume of cells (100 ml. Saline addition omitted) and lower injected 8-MOP.[63]

Altogether, the observation of only five complications among more than 1000 mini-ECP procedures suggest that the technique is safe, even in severely immunosuppressed children with GVHD. Although central venous access to draw the necessary blood volume is usually helpful and indicated for psychological reasons in children with refractory GVHD, the procedure can also be performed through a peripheral line and a port system with a small-caliber catheter.

Besides the retrospective analysis, the low number of patients (which, however, if rather large for children with GVHD and ECP), the known heterogeneity of signs, symptoms, and course of GVHD permit limited conclusions from our data. However, the observed safety, response, and steroid-sparing effect of the mini-ECP procedure in this study is completely in line with reports using classical ECP methods and, therefore, encourage the initiation of a prospective study of mini-ECP for both acute and chronic GVHD.

Ideally, ECP should be applied in the context of controlled trials. However, performing a randomized trial in this patient population will be challenging due to the limited number of patients, the variable disease presentation and the lack of well-defined response criteria. Multicentre collaboration and appropriate funding for such trials are needed but very difficult to receive.

In the current study, no parallel biomarker studies have been conducted. Robust biomarkers infomartion of GvHD would be highly useful in informing patient selection, intensity and duration of the ECP schedule, monitoring of response and other treatment decisions alongside the concurrent administration of other GvHD therapies.[92]

Further studies should carefully assess the quality of life, and investigate the correlation between the *in vitro* cellular response to ECP, validated and potential biomarkers and clinical outcome to both improve methodological aspects, and identify patients early on treatment who are responding to mini-ECP and exclude those who are unlikely to achieve clinical response.

In conclusion, our data suggest that mini-ECP is a feasible, safe and effective treatment option for children and adolescents with refractory GVHD for whom classical ECP cannot be technically performed. It represents a less invasive and faster procedure which may be particularly appealing for patients and families requiring long-term treatment by overcoming several difficulties associated to classical approaches. In general, ECP represents an attractive option to treat GVHD without compromising anti-viral and anti-malignant effects. Quality of life and efficacy of mini-ECP with its lower number of cells irradiated needs to be proven in prospective trials. Biomarker studies should be implemented to address a better patient selection, the intensity and duration of the ECP schedule, monitoring of response and decisions regarding combinations with other GvHD therapies.[151,22,87,118]

8 Abstract

Introduction. The success of allogeneic hematopoietic stem cell transplantation is limited by the emergence of graft-versus-host-disease (GVHD). Steroids are the treatment of choice for both acute and chronic GVHD, but less than 50% of patients respond. Extracorporeal photopheresis (ECP) is an effective second-line therapeutic option. Low body weight, extracorporeal volume, venous access and psychologic issues limit the use of classical ECP in young children and other critically ill patients. In 2005, we developed a mini-photopheresis-technique (mini-ECP) for the treatment of children and adolescents with GVHD and contraindications for classical ECP. Aim of the current study was to analyze the safety and efficacy of the mini-ECP for the treatment of children and adolescents with GVHD.

Patients and Methods. We retrospectively describe the clinical and laboratory characteristics of children and adolescents with GVHD treated with mini-ECP at the Department of Pediatric Hematology and Oncology and the Institute of Clinical Immunology and Transfusion Medicine of the University Hospital Giessen and Marburg, Giessen between 2005 and 2016, and analyze safety and efficacy of of the approach.

Results. Thirty patients with contraindications for classical ECP were treated with mini-ECP of whom 16 children had a steroid-refractory/dependent acute GVHD (5 Grade III-IV) and 14 patients a chronic GVHD (8 extensive). In total, 1031 procedures were performed. Patients were in median 5 years old (range, 0,5-20), the median body weight was 19 kg (range, 7-53). The overall response rate was 87.5% and 69% in the acute and chronic GVHD cohort, respectively. Steroids were withdrawn or significantly tapered in more than 80% of patients in both cohorts. Mini-ECP was well tolerated with only five adverse events observed in four patients, none of them fatal.

Conclusion. Mini-ECP represents a low invasive, safe and effective alternative ECP-technique for children and adolescents with acute or chronic GvHD and contraindications for classical ECP.

9 Zusammenfassung

Hintergrund. Eine Graft-versus-Host Erkrankung (GVHD) stellt eine schwere Komplikation der allogenen Blutstammzelltransplantation mit einer hohen Mortalität und Morbidität dar. Die Standard-Erstlinientherapie besteht in hochdosierten Steroiden, auf die jedoch weniger als 50% der Patienten ansprechen. Eine extrakorporale Photophorese (ECP) konnte als effektive Zweitlinientherapie etabliert werden. Die Anwendung der ECP bei kleinen oder kranken Kindern wird jedoch durch das geringe Körpergewicht, das Extrakorporalvolumen und die Notwendigigkeit eines zentralvernösen Zugangs sowie psychologische Beeinträchtigung begrenzt. Wir haben in 2005 eine Mini-ECP-Technik entwickelt, die die Behandlung von Kindern und Jugendlichen mit GVHD und Kontraindikationen für eine klassische ECP erlaubt. Ziel dieser retrospektiven Studie war es, Sicherheit und Effektivität der Mini-ECP zur Behandlung der GVHD zu analysieren.

Patienten und Methoden. Die Charakteristika aller Patienten, die aufgrund einer GVHD in der Abteilung für Pädiatrische Hämatologie und Onkologie und am Institut für Klinische Immunologie und Transfusionsmedizin zwischen 2005 und 2016 mit mini-ECP behandelt wurden, werden anhand einer Aktenanalyse und durch eigene klinische Untersuchung beschrieben. Sicherheit und Effektivität der mini-ECP werden analysiert.

Ergebnisse. Die Mini-ECP wurde bei 30 Patienten mit GVHD und Kontraindikationen für eine klassische ECP angewandt; 16 Kinder litten an einer steroidrefraktären akuten GVHD (5 Grad III/IV) und 14 Patienten an einer chronischen GVHD (8 extensive Erkrankung). Insgesamt wurden 1031 Mini-ECP-Anwendungen durchgeführt. Die Patienten waren im Median 5 Jahre (0,5-20) alt, und 19 kg (7-53) Körpergewicht. Das Gesamtansprechen (komplette Respons und partielle Respons) betrug 87.5% bzw. 69% für die Kinder mit acuter bzw. chronischer GVHD. Bei mehr als 80% der Patienten in beiden Kohorten konnte die Steroiddosis significant reduziert oder die Steroide abgesetzt werden. Es waren nur fünf Nebenwirkungen bei vier Kindern zu verzeichnen.

Schlussfolgerung. Die Mini-ECP bietet eine wenige invasive, sichere und effektive Alternative zur Behandlung der steroidrefraktären akuten und chronischen GVHD bei Kindern und Jugendlichen, die eine Kontraindikation für die Durchführung einer klassischen ECP haben.

10 Abbreviations

A	
a	Acute
ACD	Acid-citrate-dextrose
AE	Adverse event
aGVHD	Acute Graft-versus-host-disease
ALCL	Anaplastic large cell lymphoma
ALL	Acute lymphoid leukemia
allo-SCT	Allogeneic stem-cell transplantation
AML	Acute myeloid leukemia
APC	Antigen-presenting cells
B	
BAFF	B-cell activating factor
BW	Body weight
C	
c	Chronic
CD	Cluster of differentiation
CE	Conformité Européenne
CGD	Chronic granulomatous disease
CSA	Cyclosporine A
CPD	Citrate phosphate dextrose
CTCAE	Common Terminology Criteria for Adverse Events
CVC	central venous catheter
cGVHD	Chronic Graft-versus-host-disease
CNI	Calcineurin inhibitor
CR	Complete response
CRR	Complete response rate
CTCL	Cutaneous T-cell lymphoma
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T lymphocyte-associated antigen 4
CMV	Cytomegalovirus
Cy	Cyclophosphamide

D	
DAMPS	Danger associated molecular patterns
DC	Dendritic cells
DLI	Donor lymphocyte infusion
E	
ECP	Extracorporeal photopheresis
EV	Extracorporal volume
F	
FA	Fanconi anemia
FDA	Food and Drug Administration
G	
GI	Gastrointestinal
GVHD	Graft-versus-host-disease
GVL	Graft-versus-leukemia
GVT	Graft-versus-tumor
H	
Hct	Hematocrit
HD	Hemodynamic
HLA	Histocompatibility antigens
HRR	High relapse risk
HS	Histiocytic sarcoma
I	
IBMF	Inborn bone marrow failure
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IND	Indication
IS	Immunosuppression
J	
J	Joule
K	
kg	Kilogram

L	
L	Liver
LPS	Low performance status
LW	Low weight
M	
MA	Myeloablative conditioning
MDS	Myelodysplastic syndrome
MMF	Mycophenolate mofetil
mini-ECP	Mini-Extracorporeal photophoresis
miRNAs	microRNAs
ml	Milliliters
MMF	Mycophenolate mofetil
MNC	Mononuclear cells
Mo	Macrophages
MSC	Mesenchymal stem cells
MTX	Methotrexate
8-MOP	8-Methoxypsoralen
N	
N	Number
NaCl	Sodium Chloride
NB	Neuroblastoma
NIH	National Institutes of Health
NK	Natural Killer cells
NMC	Non-myeloablative conditioning
NR	Non-response
NRM	Non-relapse mortality
O	
ORR	Overall response rate
OS	Overall survival

P	
P	Progression
Pat	Patients
PAMPS	Pathogen associated molecular patterns
PDGF-R	Platelet-derived growth factor
PG	Prostaglandin
PHA	Phytohemagglutinin
PL	Peripheral line
PR	Partial response
PS	Port-sytem
PUVA	Psoralen ultraviolet A
Px	Prophylaxis
R	
RBC	Red blood cells
Reg3a	Regenerating Islet-derived 3-alpha
Regs	Regulatory cells
RI	Respiratory insufficiency
RIC	Reduced intensity conditioning
RNA	Ribonucleic acid
S	
S	Skin
sAML	Secondary acute myeloid leukemia
SCID	Severe combined immunodefficiency
SCL	Sclerodermic
SCT	Stem-cell transplant
SD	Stable disease
StD	Standard deviation
SOR	Specific organ response
SR	Steroid-refractory

T	
TCR	T cell receptor
TGF	Tumor growth factor
TH	T helper
TIM3	T-cell immunoglobulinmucin-3
TL	Tunneled line
TLR	Toll-like receptors
TNF	Tumor necrosis factor
Tregs	Regulatory T cells
TRM	Transplant related mortality
U	
UVA	Ultraviolet light A
V	
VA	Vascular Access
W	
WBC	White blood cells
Y	
Y	Years

11 List of tables

12 List of figures

13 Biblioghraphy

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Mit der Überprüfung meiner Arbeit durch eine Plagiatserkennungssoftware bzw. ein internetbasiertes Softwareprogramm erkläre ich mich einverstanden.“

Datum

Unterschrift

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