# Therapeutic apheresis in hematopoietic cell transplantation

*Hematopoetik kök hücre transplantasyonunda terapotik aferez*

Nina Worel[1], Gerda Leitner[1], Andrea Wagner[1], Hildegard T Greinix[2], Paul Höcker[1], Peter Kalhs[2]
[1]*Department for Blood Group Serology and Transfusion Medicine, Medical University of Vienna, Austria*
[2]*First Medical Department, Bone Marrow Transplantation, Medical University of Vienna, Austria*

## Abstract

With the introduction of hematopoietic stem cell transplant (HSCT), transplant-associated problems like graft-versus-host disease (GVHD) and transplant-associated microangiopathy (TAM) have occurred. In addition, approximately 40% of allogeneic HSCTs are performed across the ABO blood group barrier, bearing the risk for immunohematological complications like severe hemolysis and pure red cell aplasia (PRCA). All these problems can potentially require therapeutic apheresis. In this review, we address recent developments in therapeutic apheresis for patients undergoing allogeneic HSCT for prevention or treatment of hemolysis in minor ABO-incompatible transplantation, treatment of PRCA after major ABO-incompatible transplantation, and treatment of TAM and GVHD. *(Turk J Hematol 2008; 25: 164-71)*

**Key words:** Stem cell transplantation, therapeutic apheresis, hemolysis, pure red cell aplasia, thrombotic microangiopathy, graft-versus-host disease

*Received: January 18, 2008 Accepted: June 19, 2008*

## Özet

Hematopoetik kök hücre transplantasyonunun uygulanmasıyla Graft-versus-host hastalığı (GVHH) gibi transplanta bağlı problemler ve transplanta bağlı mikroanjiyopati (TAM) ortaya çıkmıştır. Ayrıca, allojenik HSCT'lerin yaklaşık %40'ı , şiddetli hemoliz ve saf kırmızı hücre aplazisi (PRCA) gibi immünohematolojik komplikasyon riski taşıyan ABO kan grubu karşın gerçekleştirilmiştir. Tüm bu problemler potansiyel olarak terapötik aferez gerektirebilir. Minör ABO uyumsuz transplantasyonda hemolizin tedavisi veya önlenmesi, majör ABO uyumsuz transplantasyon sonrası PRCA tedavisi ve TAM ve GVHH tedavisi için allojeneik HKHT uygulanan hastalara yönelik terapötik aferez ile ilgili en son gelişmeler bu değerlendirmede ele alınmaktadır. *(Turk J Hematol 2008; 25: 164-71)*

**Anahtar kelimeler:** Kök hücre transplantasyonu, terapötik aferez, hemoliz, saf kırmızı hücre aplazisi, trombotik mikroanjiyopati, graft-versus host hastalığı

*Geliş tarihi: 18 Ocak 2008 Kabul tarihi: 19 Haziran 2008*

**Address for Correspondence:** Prof. Nina Worel, Medical University of Vienna, Waehringer Guertel 18-20a-1090 A-1090 Vienna, Austria
Phone: +43-1-40 400-5302 E-mail: nina.worel@meduniwien.ac.at

## Introduction

In the 1970s and 1980s, clinical bone marrow transplantation (BMT) required HLA matching between the donor and recipient to be successful. Although earlier BMT procedures occurred between siblings, current molecular techniques have enabled the precise characterization of major histocompatibility class I and class II (MHC) genes, allowing the possibility of matching unrelated individuals. The first successful transplant from an unrelated donor for a patient with leukemia took place in 1979 at the Hutchinson Center in the United States [1].

In 2005, the European Group for Blood and Marrow Transplantation (EBMT) activity report documented 24,168 first hematopoietic stem cell transplantations (HSCTs) in Europe. There were 8,890 allogeneic (37%), 15,278 autologous (63%) and 3,773 additional re- or multiple transplants reported from 597 centers in 43 participating countries. A total of 671 planned allogeneic HSCTs after a first autologous transplant were reported. Compared to 2004, there was a 20% increase in allogeneic HSCT, whereas the numbers of autologous HSCT remained stable. The most noticeable increase was observed in unrelated HSCT, which comprised 41% of all allogeneic HSCTs [2].

With the introduction of allogeneic bone marrow (BM) or peripheral blood progenitor cell (PBPC) transplantation and the extension of this treatment to heavily pretreated or older patients and patients with higher co-morbidity, transplant-associated problems like severe infections, organ toxicity, transplant-associated microangiopathy (TAM) and graft-versus-host disease (GVHD) were observed more frequently. In addition, approximately 40% of allogeneic HSCTs are performed across the ABO blood group barrier, as the ABO system is inherited independently from the HLA system [3]. Three different groups of ABO incompatibility can be distinguished: minor, major and bidirectional ABO-incompatibility. Minor ABO-incompatible HSCT (e.g. 0 into A) is characterized by the presence of preformed anti-A/B antibodies of the donor directed against recipient red blood cell (RBC) antigens. In this setting, immediate or delayed hemolysis of patients' RBCs may occur. Recipients of major ABO-incompatible stem cell grafts possess isohemagglutinins directed against donor RBCs and are at risk for immediate or delayed hemolysis of donor RBCs and destruction of donor erythroid precursor cells, causing delayed erythroid engraftment and pure red cell aplasia (PRCA). Bidirectional incompatibility (e.g. A into B) represents a combination of major and minor ABO-incompatibility (Table 1). Thus, immunohematologic problems like immediate hemolysis during graft infusion and delayed hemolysis during donor cell engraftment or PRCA can occur. Recently, the impact of ABO incompatibility on the transplantation outcome was discussed in several studies. Resnick et al. [4] reported on 221 patients who underwent HSCT after reduced intensity conditioning (RIC) and found an increased incidence of non-relapse mortality (NRM) in the major and minor ABO incompatible groups. Seebach et al. [5] analyzed 3,103 patients, 995 of whom had an ABO incompatible donor. In contrast to Resnick et al., he found no evidence of a substantial effect of ABO blood group incompatibility on the outcome of conventional HSCT.

This review addresses recent developments in therapeutic apheresis for patients undergoing allogeneic HSCT for the prevention or treatment of hemolysis in minor ABO-incompatible transplantation, treatment of PRCA after major ABO-incompatible transplantation, and treatment of TAM and GVHD.

### Red Blood Cell Exchange for Treatment or Prevention of Severe Hemolysis in Minor ABO-Incompatibility

Delayed immune hemolysis due to rapid isohemagglutinin production by donor-derived lymphocytes has been reported in transplants with a minor or bidirectional ABO-mismatched graft and is known as passenger lymphocyte syndrome [6-10].

Recent reports show controversial results regarding the effect of ABO incompatibility on the outcome of HSCT, ranging from adverse impact on the transplantation outcome [4] to no evidence of a substantial effect [5,11]. Special risk factors are: RIC due to lack of methotrexate (MTX), T-cell depletion, lack of anti-B-cell therapy in GVHD prophylaxis, and the use of PBPCs [6-8]. The incidence of hemolysis, which typically starts between 5 to 15 days after transplantation, is reported in up to 30% of patients at risk and can in certain cases be overcome by RBC exchange with group O RBCs or multiple RBC transfusions [6-10,12]. However, fatal hemolysis can occur resulting in multiorgan failure (MOF) and death [6-9]. One case, a 38-year-old man with blood group A who had been allotransplanted for multiple myeloma with PBPCs from his fully-matched sister with blood group O, developed severe immune hemolysis on day 9. Direct antiglobulin test (DAT) was positive (IgG and C3d) and elution showed an anti-A specificity. Hemolysis progressed rapidly, leading to MOF and death on day +20 post-transplant [13]. Bolan and colleagues [7] observed massive immune hemolysis in 3 out of 10 consecutive patients undergoing HLA-identical, related-donor PBPC transplants with minor ABO incompatibility. Nine of these patients underwent RIC with cyclosporin A (CsA) alone for GVHD prophylaxis. Catastrophic hemolysis of 78% of the circulating red cell mass led to anoxic death in the first case seen, but severe consequences were avoided by early, vigorous donor-compatible red cell transfusions in the subsequent two cases. Hemolysis occurred 7-11 days after transplantation and all patients with hemolysis had a positive DAT, with eluate reactivity against the relevant recipient antigen [7]. We observed severe hemolysis in 4 out of 25 (16%) patients with a minor or bidirectional ABO-mismatch [8]. Three of these patients underwent RIC and were given CsA with mycophenolate mofetil (MMF) for GVHD prophylaxis, and one patient underwent myeloablative conditioning and was given CsA alone. Hemolysis began 7 to 10 days after transplantation, and in all cases donor-type alloantibodies were detectable concomitantly with recipient-type RBCs. To overcome hemolysis, patients received RBC exchange with group 0 RBCs. Three patients recovered from hemolysis, one experienced acute renal failure and disseminated intravascular coagulation, required mechanical ventilation and subsequently died from MOF [8]. We then introduced a protocol with prophylactic RBC exchange in patients at risk (minor and/or bidirectional ABO-mismatched graft, PBPCs, RIC, lack of MTX) to avoid severe hemolysis; 20 consecutive patients were treated with prophylactic RBC exchange. The exchange procedure was accompanied by mild to moderate citrate reactions in three

patients, and hypotension in one patient. Eighteen of these 20 patients engrafted uneventfully, one rejected his graft, and another showed signs of mild hemolysis. In minor and/or bidirectional ABO-mismatched transplantation, patients with prophylactic RBC exchange had a significantly lower risk for transplant-related mortality (TRM; 16% vs. 53%) and significantly better overall survival (OS; 65% vs. 40%) at one year compared to the previously reported patients without prophylactic RBC exchange [12]. We observed that RBC exchange is a safe procedure, reducing the incidence of delayed severe immune hemolysis and thus reducing the risk of TRM in minor and/or bidirectional ABO-mismatched patient/donor pairs. Of course, RBC exchange is a cost-intensive and potentially risky procedure. Moreover, this is a small series of patients; comparative studies to evaluate the efficacy and safety of this procedure are needed. However, vigilant monitoring of patients at risk during the first two weeks posttransplant, including daily complete blood count and lactate dehydrogenase (LDH) and screening for donor isohemagglutinins, is recommended.

Case series of severe hemolysis in minor ABO-mismatched HSCT are given in Table 2.

### Plasma Exchange and Immunoadsorption for Treatment of PRCA in Major ABO-Incompatibility

The principles of the management of major ABO incompatibility are given in Table 1. Although ABO-incompatibility between donor and recipient does not appear to affect the outcome of HSCT in terms of incidence of graft rejection or delayed white blood cell or platelet engraftment, in a minority of major ABO-incompatible stem cell graft recipients, prolonged reticulocytopenia and an increased transfusion requirement have been observed [13-16]. Normally, donor-type erythropoiesis is established in the majority of patients undergoing HCT within the first three weeks. The occurrence of reticulocytes in the peripheral blood and the independence of further RBC transfusions indicate successful engraftment of donor RBCs. Nevertheless, up to 20% of patients given a major ABO-mismatched stem cell graft develop PRCA with the requirement of high numbers of post-transplant RBC transfusions. As erythroid precursor cells in the BM express ABO-antigens, they represent a target for anti-donor A/B antibodies produced by the remaining recipient B-lymphocytes and plasma cells [16-18]. Reasons for the apparent plasma cell persistence in patients with PRCA may be either an insufficient eradication during conditioning, especially with reduced-intensity protocols, or an inadequate post-transplant graft-versus-plasma cell effect [19]. Mielcarek and colleagues [20] observed a significantly earlier disappearance of isohemagglutinin titers in recipients of matched unrelated-donor grafts compared with recipients of matched related-donor grafts. Moreover, isohemagglutinin titers disappeared faster in patients with GVHD than in those without GVHD. Since the incidence of PRCA is relatively low and some patients are able to clear the hemagglutinins spontaneously, a prophylactic treatment is not recommended [3]. Aside from erythropoietin administration, depletion of isohemagglutinins by plasma exchange (PE) or immunoadsorption (IA) can be performed to overcome PRCA [16,21,22]. If this strategy is not successful, several other approaches have been proposed based on the pathophysiology of the disorder: enhancement of graft-versus-plasma cell reactivity by reduction of post-transplant immunosuppression or donor-lymphocyte infusion, rituximab and anti-thymocyte globulin [23-25].

**Table 1. ABO incompatible HSCT: mechanisms and risk factors and prevention of transplant-related problems**

| ABO incompatibility | Mechanism of action | Risk at graft infusion | Prevention of problems at graft infusion | Risk at engraftment |
|---|---|---|---|---|
| Minor | Donor isohemagglutinins directed against recipient RBCs | Hemolysis | 1. Plasma depletion of the graft | Hemolysis |
| Major | Recipient isohemagglutinins directed against donor RBCs | Hemolysis | 1. RBC depletion of the graft<br>2. PE to reduce isohemagglutinins<br>3. Transfusion of donor type RBCs | PRCA |
| Bidirectional | Combination of minor and major ABO incompatibility | Hemolysis | 1. RBC depletion of the graft<br>2. PE to reduce isohemagglutinins<br>3. Transfusion of donor type RBCs | Hemolysis |

**HSCT:** Hematopoietic stem cell transplantation. **RBCs:** Red blood cells. **PE:** Plasma exchange. **PRCA:** Pure red cell aplasia.

**Table 2. Reports of hemolysis after minor ABO-mismatched stem cell transplantation**

| Case series | # of patients | Donor | Risk factors | Day of hemolysis | Treatment | Outcome |
|---|---|---|---|---|---|---|
| Toren, 1996 | 1 | Sib | CsA alone | 8 | RBC exchange | Recovery on day 15 |
| Oziel-Taieb, 1997 | 1 | Sib | CsA + MP | 9 | RBC transfusion | Died of MOF on day 20 |
| Bornhauser, 1997 | 1 | URD | CsA, MTX day 1+3 | 9 | RBC transfusion | Recovered, died of cerebral hemorrhage on day 58 |
| Bolan, 2001 | 3 | Sib | RIC (n=2), CsA alone (n=3) | 7, 9, 11 | RBC transfusion | 1 patient died on day 9, 2 patients recovered |
| Worel, 2002 | 4 | Sib (n=1), URD (n=3) | RIC (n=3), CsA alone (n=1), CsA+MMF (n=3) | 7, 8, 9, 10 | RBC exchange | 1 patient died of MOF on day 35, 3 patients recovered |

**CsA:** Cyclosporin A. **MP:** Methylprednisolone. **MTX:** Methotrexate. **Sib:** Sibling. **URD:** Unrelated donor. **RIC:** Reduced intensity conditioning. **MMF:** Mycophenolate mofetil. **RBC:** Red blood cells. **MOF:** Multi-organ failure.

Some investigators reported a high efficacy of PE in the treatment of PRCA, and 3-5 procedures were usually sufficient to facilitate engraftment of donor erythrocytes. Gmür and colleagues [14] reported one patient with PRCA given PE five times between days 60 and 100 whose reticulocyte counts rose to normal by day 240. Among our seven patients with PRCA, four patients had PE starting on days 88, 139, 260 and 279 after transplantation [16]. We observed reticulocyte engraftment between 16 and 68 days after initiation of PE. Recently, Helbig and colleagues [26] published five patients with PRCA including two undergoing PE. One patient responded to nine treatments, whereas the other one was refractory to five PEs, a second transplant to reinforce graft-versus-plasma cell effect, additional donor-lymphocyte infusion, anti-CD52 antibody (MabCampath) therapy and a third transplant. Finally, he underwent another five procedures of PE with subsequent resolution of PRCA [26]. Thus, in some patients with PRCA, standard PE shows low efficacy in removing circulating antibodies. Therefore, IA, a novel method of removing persisting isohemagglutinins, has been used by some investigators. In contrast to standard PE, IA allows nearly a complete clearance of circulating immunoglobulins of all types and subtypes, without the substitution of protein solutions or even fresh-frozen plasma during treatment. We treated three patients with 18 to 36 cycles of IA and achieved an increase in reticulocytes between 119 and 204 days after initiation of treatment [16]. Rabitsch and colleagues [22] reported five patients responding to a median of 17 IA procedures (range: 9-25) starting between days 62 and 195 posttransplant. However, IA is a time-consuming and expensive treatment. Because of the small number of cases and the various treatment modalities described in the literature, it is not clear which treatment is optimal for PRCA patients.

The optimal way to remove residual isohemagglutinins is still unclear. By using apheresis techniques, PE compared to IA is a less cost-intensive method with the disadvantage of bearing a risk of disease transmission. Nevertheless, therapy of PRCA seems to be necessary to avoid transfusion of a high number of RBCs, which may lead to transfusion-associated iron overload and the possible development of hemosiderosis. Reports on treatment of PRCA after major ABO incompatible HSCT are given in Table 3.

**Plasma Exchange for Treatment of Transplant-Associated Microangiopathy (TAM)**

The occurrence of thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) was first reported to be associated with HSCT more than 25 years ago [27]. Recently, the term TAM has been agreed on by the transplant community to describe these complications after HCT. The incidence of TAM is reported to range from 0.5% to 76%, and cases of TAM vary widely in their clinical features, severity and response to therapy, with a mortality rate of up to 100% [28-32]. Endothelial damage caused by intensive conditioning chemotherapy, irradiation, CsA, GVHD and infection have been discussed as playing a role in the etiology of TAM [30,33-35]. In the last years, the occurrence of TAM has also been reported after RIC regimens [36-38]. George and colleagues [32] reviewed 35 articles reporting a total of 10,434 patients including 5,423 patients after allogeneic HSCT. Of these patients, 447 were diagnosed with TTP-HUS after allogeneic HSCT, but 28 different sets of diagnostic criteria were used in these reports. Therefore, TAM should be defined according to the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) Toxicity Committee of the American Society of Blood and Marrow Transplantation (ASBMT) as follows: (1) RBC fragmentation and ≥2 schistocytes per high-power field on peripheral blood smear, (2) concurrent increased serum LDH levels above institutional baseline, (3) concurrent renal (doubling of serum creatinine compared to the value before hydration and conditioning) and/or neurological dysfunction without other explanation, and (4) negative direct and indirect Coombs test results [39]. Treatment of TAM varies from center to center and consists of supportive care, withdrawal or reduction of calcineurin inhibitors and PE [28-38]. George and colleagues [32] reported that 334 of 447 patients with TTP-HUS received treatment consisting of PE in 184 (55%). In this systematic review of 35 articles, mortality rates in patients treated with PE ranged from 44% to 100% in single reports. In summary, the mortality was 82% in patients receiving PE compared to 50% in patients not treated with PE [32]. One problem was that insufficient data were available to assess the response to PE by conventional parameters such as recovery from thrombocytopenia, achievement of normal or near normal serum LDH levels, recovery from anemia, and survival for more than 30 days following completion of treatment. Moreover, results were biased by the fact that patients who were more critically ill were more likely to have been treated with PE [32]. We recently reported 11 patients with TAM after allogeneic HCT with RIC [40]. Treatment of TAM consisted of withdrawal of calcineurin inhibitors in the absence of GVHD or substitution with corticosteroids and initiation of PE therapy. PE was performed daily until clinical improvement, normalization of LDH levels and increase in platelet counts were seen. If LDH levels remained normal for at least 24 hours, PE was tapered and an additional three treatments were performed every other day. Seven patients (64%) responded to this treatment and recovered from TAM between 11 and 35 days after its occurrence. After a median follow-up of 30 months (range: 7-67) after clearance of TAM,

**Table 3. Reports on treatment of PRCA**

| Author | No. pts. | Pts. with PE (# PE) | Pts. with IA (# IA) | Start of treatment | Outcome |
|---|---|---|---|---|---|
| Gmür, 1990 | 1 | 5 | - | Day 60 | RBC engraftment on day 240 |
| Worel, 2000 | 7 | 4 (2-3) | 3 (18-36) | Day 80 - 279 | RBC engraftment between days 106 and 347 |
| Rabitsch, 2003 | 5 | - | 5 (9-25) | Day 62 - 195 | RBC engraftment in all patients observed |
| Helbig, 2007 | 6 | 2 (5, 9) | | | PRCA recovery after median of 13 months (range: 3-16) |

**PRCA:** Pure red cell aplasia. **No:** Numbers. **PTS:** Patients. **PE:** Plasma exchange. **IA:** Immunoadsorption. **RBC:** Red blood cells.

6 of 7 patients were still alive and in continuous complete remission. One patient died due to relapse 39 months after HSCT. In four patients (36%), no response of TAM could be observed and they died 7, 13, 16 and 69 days after diagnosis of TAM due to severe infection [38].

The prognosis of patients with TAM is reportedly poor, but there is extreme variation among case series. Recent reports suggest that PE does not affect survival of patients diagnosed with TAM and provide the basis for the current consensus statement that universal use of PE for TAM is not standard of care [32,39]. In view of the lack of other treatment modalities, however, and the observed benefit in some patients with TAM, PE should be considered as a possible treatment option for some of these patients [38,40,41].

**Extracorporeal Photoimmunotherapy for the Treatment of Severe Steroid-Refractory Chronic and Acute Graft-Versus-Host Disease**

Despite significant advances in stem cell manipulation and post-transplant immunosuppression, GVHD remains a major cause of long-term morbidity in survivors of allogeneic SCT. Improvements in immunosuppressive regimens have reduced the frequency and severity of acute GVHD, though the incidence of chronic GVHD has remained unchanged at 27-50% after matched related-donor transplants and 42-80% after unrelated-donor HSCTs [42]. Factors associated with GVHD have been well-described and include increased recipient and donor age, HLA-disparate and unrelated-donor transplants, prior acute GVHD, and the use of alloimmune female donors [43]. Extracorporeal photopheresis (ECP) is a novel immunotherapeutic modality initially developed by Edelson and colleagues [44] as a therapy for cutaneous T-cell lymphoma with circulating Sézary leukemia cells. Although the mechanisms of action, so far, are still unclear, ECP has demonstrated remarkable efficacy in a number of autoimmune disorders, including scleroderma, pemphigus vulgaris, rheumatoid arthritis, systemic lupus erythematosus, and solid organ allograft rejection [45,46]. ECP is based on the infusion of autologous blood mononuclear cells collected by apheresis, incubated with the DNA-intercalating agent 8-methoxypsoralen (8-MOP) and then irradiated with UVA at 2 $Jcm^2$/cell. Several retrospective and prospective studies have shown activity of ECP in controlling the manifestation of chronic GVHD with clinical responses in cutaneous and visceral GVHD in patients refractory to steroids and other lines of immunosuppressive therapy (Table 4) [47-52]. Complete responses of skin manifestations have been reported in up to 80% of steroid-refractory patients with improvement even in sclerodermatous skin [42,49,53].

In addition, a marked steroid-sparing effect was observed [42,49,50,53] and patients had no increased susceptibility to opportunistic infections. Based on these results and its excellent safety profile [53], ECP has become the standard second-line therapy for steroid-refractory and steroid-intolerant chronic GVHD patients in our center at the Medical University of Vienna [49-53]. In addition to CsA or tacrolimus plus steroids (1 mg/kg), ECP is given on two consecutive days every two weeks. As soon as GVHD responds, steroids are tapered and the interval between ECP cycles is increased to four weeks. Responses to ECP appear to be more frequent in patients treated earlier (<9 months) after diagnosis of chronic GVHD [51]. Moreover, Couriel and colleagues [50] observed a trend towards higher response rates in de novo chronic GVHD. A significantly longer survival in patients responding to ECP compared to those failing treatment has been reported [51]. Messina and colleagues [51] observed a five-year overall survival rate of 96% in ECP-responders compared to 58% in non-responders. As a surrogate marker for quality of life aspects, an improvement in Karnofsky performance scores from 50 or 60% before ECP to at least 90% after ECP has been reported [49,51].

Recently, results of a multicenter, prospective, randomized, phase II study of ECP in steroid-intolerant, steroid-dependent, or steroid-refractory chronic GVHD have been reported [54]. Patients randomized to conventional treatment with CsA or tacrolimus plus steroids and ECP had similar improvement in total skin score compared to patients given conventional therapy alone. However, a significant steroid-sparing effect was observed in the ECP arm. In conclusion, ECP has objective activity in the treatment of chronic GVHD, is well tolerated and does not increase general immunosuppression. However, further evaluation of the efficacy of ECP in well-designed, prospective, controlled studies with homogeneous patient groups is warranted.

Acute GVHD is a major early complication of allogeneic HSCT that has a significant impact on transplant-related mortality [51,52]. Long-term survival in patients developing severe acute GVHD has generally been less than 30%. Durable complete responses to corticosteroids were reported in only 24% to 40% of patients with acute GVHD [55,56], and no standard effective treatments for steroid-refractory acute GVHD are available. So far, only a few patients with acute GVHD treated with ECP have been reported [57-60]. Our center performed a pilot study on ECP given to 21 patients with steroid-refractory acute GVHD grades II to IV and observed high response rates with favorable survival of ECP-responders [57]. In a subsequent prospective phase II study on acute steroid-refractory or steroid-dependent patients, complete resolution of GVHD was achieved in 82% of patients with cutaneous involvement, 61% with liver involvement, and 61% with gut involvement [58]. Using an intensified ECP schedule with two treatments on a weekly basis, response rates in grade IV and gut involvement could be improved significantly compared to the pilot study. Transplant-related mortality at four years was 14% in ECP-responders and 73% in non-responders ($p<0.0001$). The probability of survival was 59% among patients who responded completely to ECP compared to 11% in patients not responding completely. We observed an impressive steroid-sparing effect and durable responses to ECP without flair-ups of GVHD activity after discontinuation of immunosuppression. Moreover, duration of ECP was short since best responses were observed after a median of 1.2 months. Our current schedule of ECP for second-line therapy of acute GVHD consists of two consecutive days at weekly intervals until complete resolution of GVHD. Then, ECP is stopped immediately after achieving maximal response [58].

**Table 4. Studies of extracorporeal photopheresis in chronic GVHD**

| Author | No. patients | No. pts with overall response | Characteristics of response |
|---|---|---|---|
| Rossetti, 1996 | 9 | 4 | Response in skin in 3/5, lung in 2/4, GI |
| Miller, 1998 | 12 | | Response in skin, liver; no response in oral GVHD |
| Child, 1999 | 11 | 9 | Response in skin in 9/10, lung in 2/5, liver in 1/5; more improvement with twice monthly vs once monthly treatment |
| Bishop, 1999 | 33 | | Response in skin in 14/22, liver in 4/4, oral in 8/18, ocular in 4/12, pulmonary in 2/7, GI in 2/7 |
| Greinix, 1998 | 15 | 12 | Response in skin in 12/15, liver in 7/10, oral in 11/11, joints in 4/4, ocular in 5/6 |
| Messina, 2003 | 44 | 25 | Complete and partial response in pediatric patients |
| Apisarnthanarax, 2003 | 32 | 18 | Overall response in skin involvement 56% |
| Foss, 2002 | 25 | 16 | Response in skin in 15/25, liver in 4/4, oral in 5/15, joints in 4/9, pulmonary in 1/2, GI in 2/3 |
| Couriel, 2006 | 71 | 57 | Response in skin in 33/56, liver in 15/21, oral in 7/9, ocular in 4/6 |

**GVHD:** Graft-versus-host disease. **GI:** Gastrointestinal. **no:** Numbers. **pts:** Patients.

Garban and colleagues [60] also reported promising response rates to an intensified ECP schedule. In conclusion, use of ECP in acute GVHD seems promising. Since earlier start of ECP led to improved response rates, patients not responding to corticosteroids as first-line therapy should be referred to ECP promptly. In view of our high response rates and the excellent tolerance of ECP, this promising therapeutic modality should also be considered as upfront treatment in patients with severe acute GVHD.

## Conclusion

Therapeutic apheresis can be used successfully both for prevention as well as for treatment of selected severe side effects of HSCT. The different techniques now available allow a tailored approach to the different situations like ABO-incompatibility, TAM or GVHD. However, a profound understanding of the underlying pathophysiologic mechanisms involved is the prerequisite for selecting the appropriate technique. Further improvements in efficacy rates of therapeutic apheresis used for some life-threatening immunologic complications of allogeneic HCT are highly warranted to reduce transplant-related mortality and prolong patient survival.

## References

1. Hansen JA, Clift RA, Thomas ED, Buckner CD, Storb R, Giblett ER. Transplantation of marrow from an unrelated donor to a patient with acute leukemia. N Engl J Med 1980;303:565-7.
2. Gratwohl A, Baldomero H, Frauendorfer K, Urbano-Ispizua A, Niederwieser D; Joint Accreditation Committee of the International Society for Cellular Therapy ISCT; European Group for Blood and Marrow Transplantation EBMT. Results of the EBMT activity survey 2005 on haematopoietic stem cell transplantation: focus on increasing use of unrelated donors. Bone Marrow Transplant 2007;39:71-87.
3. Sniecinski IJ, Oien L, Petz LD, Blume KG. Immunohematologic consequences of major ABO-mismatched bone marrow. Transplantation 1988;45:530-3.
4. Resnick IB, Tsirigotis PD, Shapira MY, Aker M, Bitan M, Samuel S, Abdul-Hai A, Ackerstein A, Or R, Slavin S. ABO incompatibility is associated with increased non-relapse and GVHD related mortality in patients with malignancies treated with a reduced intensity regimen: a single center experience of 221 patients. Biol Blood Marrow Transplant 2008;14:409-17.
5. Seebach JD, Stussi G, Passweg JR, Loberiza FR Jr, Gajewski JL, Keating A, Goerner M, Rowlings PA, Tiberghien P, Elfenbein GJ, Gale RP, van Rood JJ, Reddy V, Gluckman E, Bolwell BJ, Klumpp TR, Horowitz MM, Ringdén O, Barrett AJ; GVHD Working Committee of Center for International Blood and Marrow Transplant Research. ABO blood group barrier in allogeneic bone marrow transplantation revisited. Biol Blood Marrow Transplant 2005;11:1006-13.
6. Bornhauser M, Ordemann R, Paaz U, Schuler U, Kömpf J, Hölig K, Ehninger G. Rapid engraftment after allogeneic ABO-incompatible peripheral blood progenitor cell transplantation complicated by severe hemolysis. Bone Marrow Transplant 1997;19:295-7.
7. Bolan CD, Childs RW, Procter JL, Barrett AJ, Leitman SF. Massive immune haemolysis after allogeneic peripheral blood stem cell transplantation with minor ABO-incompatibility. Br J Haematol 2001;112:787-95.
8. Worel N, Greinix HT, Keil F, Mitterbauer M, Lechner K, Fischer G, Mayr W, Höcker P, Kalhs P. Severe immune hemolysis after minor ABO-mismatched allogeneic peripheral blood progenitor cell transplantation occurs more frequently after nonmyeloablative than myeloablative conditioning. Transfusion 2002;42:1293-301.
9. Oziel-Taieb S, Faucher-Barbey C, Chabannon C, Ladaique P, Saux P, Gouin F, Gastaut JA, Maraninchi D, Blaise D. Early and fatal immune haemolysis after so-called 'minor' ABO-incompatible peripheral blood stem cell allotransplantation. Bone Marrow Transplant 1997;19:1155-6.
10. Toren A, Dacosta Y, Manny N, Varadi G, Or R, Nagler A. Passenger B- lymphocyte-induced severe hemolytic disease after allogeneic peripheral blood stem cell transplantation. Blood 1996;87:843-4.
11. Kim JG, Sohn SK, Kim DH, Baek JH, Lee KB, Min WS, Kim CC, Lee MH, Lee JJ, Chung IJ, Kim HJ, Lee JW. Impact of ABO incompatibility on outcome after allogeneic peripheral blood stem cell transplantation. Bone Marrow Transplant 2005;35:489-95.
12. Worel N, Greinix HT, Supper V, Leitner G, Mitterbauer M, Rabitsch W, Fischer G, Rosenmayr A, Höcker P, Kalhs P. Prophylactic red blood cell exchange for prevention of severe immune hemolysis in minor ABO-mismatched allogeneic peripheral blood progenitor cell transplantation after reduced-intensity conditioning. Transfusion 2007;47:1494-502.
13. Bensinger WI, Buckner CD, Thomas ED, Clift RA. ABO-incompatible marrow transplants. Transplantation 1982;33:427-9.

14. Gmür JP, Burger J, Schaffner A, Neftel K, Oelz O, Frey D, Metaxas M. Pure red cell aplasia of long duration complicating major ABO-incompatible bone marrow transplantation. Blood 1990;75:290-5.
15. Paltiel O, Cournoyer D, Rybka W. Pure red cell aplasia following ABO-incompatible bone marrow transplantation: response to erythropoietin. Transfusion 1993;33:418-21.
16. Worel N, Greinix HT, Schneider B, Kurz M, Rabitsch W, Knöbl P, Reiter E, Derfler K, Fischer G, Hinterberger W, Höcker P, Kalhs P. Regeneration of erythropoiesis after related- and unrelated-donor BMT or peripheral blood HPC transplantation: a major ABO mismatch means problems. Transfusion 2000;400:543-50.
17. Wada H, Suda T, Miura Y, Kajii E, Ikemoto S, Yawata Y. Expression of major blood group antigens on human erythroid cells in a two phase liquid culture system. Blood 1990;75:505-11.
18. Stussi G, Halter J, Schanz U, Seebach J. ABO-histo blood group incompatibility in hematopoietic stem cell and solid organ transplantation. Transfus Apher Sci 2006;35:59-69.
19. Bolan CD, Leitman SF, Griffith LM, Wesley RA, Procter JL, Stroncek DF, Barrett AJ, Childs RW. Delayed donor red cell chimerism and pure red cell aplasia following major ABO-incompatible nonmyeloablative hematopoietic stem cell transplantation. Blood 2001;98:1687-94.
20. Mielcarek M, Leisenring W, Torok-Storb B, Storb R. Graft-versus-host disease and donor-directed hemagglutinin titers after ABO-mismatched related and unrelated marrow allografts: evidence for a graft-versus-plasma cell effect. Blood 2000;96:1150-6.
21. Martelli M, Ponchio L, Beguin Y, Meloni G, Mandelli F, Cazzola M. Pure red cell aplasia following peripheral stem cell transplantation: complete response to a short course of high-dose recombinant human erythropoietin. Bone Marrow Transplant 1994;79:456-9.
22. Rabitsch W, Knöbl P, Prinz E, Keil F, Greinix H, Kalhs P, Worel N, Jansen M, Hörl WH, Derfler K. Prolonged red cell aplasia after major ABO-incompatible allogeneic hematopoietic stem cell transplantation: removal of persisting isohemagglutinins with Ig-Therasorb® immunoadsorption. Bone Marrow Transplant 2003;32:1015-9.
23. Verholen F, Stalder M, Helg C, Chalandon Y. Resistant pure red cell aplasia after allogeneic stem cell transplantation with major ABO mismatch treated by escalating dose donor leukocyte infusion. Eur J Haematol 2004;73:441-6.
24. Maschan AA, Skorobogatova EV, Balashov DN, Pashanov ED, Trakhtman PE, Schipitzina IP, Skvortsova YV, Rumiantzev AG. Successful treatment of pure red cell aplasia with a single dose of rituximab in a child after ABO incompatible peripheral blood allogeneic stem cell transplantation for acquired aplastic anemia. Bone Marrow Transplant 2002;30:405-7.
25. Bierman PJ, Warkentin P, Hutchins MR, Klassen LW. Pure red cell aplasia following ABO mismatched marrow transplantation for chronic lymphocytic leukaemia: response to antithymocyte globulin. Leuk Lymphoma 1993;9:169-71.
26. Helbig G, Stella-Holowiecka B, Wojnar J, Krawczyk M, Krzemien S, Wojciechowska-Sadus M, Markiewicz M, Wylezol I, Kopera M, Holowiecki J. Pure red-cell aplasia following major and bi-directional ABO-incompatible allogeneic stem-cell transplantation: recovery of donor-derived erythropoiesis after long-term treatment using different therapeutic strategies. Ann Hematol 2007;86:677-83.
27. Powles RL, Clink HM, Spence D, Morgenstern G, Watson JG, Selby PJ, Woods M, Barrett A, Jameson B, Sloane J, Lawler SD, Kay HE, Lawson D, McElwain TJ, Alexander P. Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone-marrow transplantation. Lancet 1980;16:327-9.
28. Pettitt AR, Clark RE. Thrombotic microangiopathy following bone marrow transplantation. Bone Marrow Transplant 1994;14:495-504.
29. Zeigler ZR, Shaduk RK, Nemunaitis J, Andrews DF, Rosenfeld CS. Bone marrow transplant-associated thrombotic microangiopathy: a case series. Bone Marrow Transplant 1995;15:247-53.
30. Fuge R, Bird JM, Fraser A, Hart D, Hunt L, Cornish JM, Goulden N, Oakhill A, Pamphilon DH, Steward CG, Marks DI. The clinical features, risk factors and outcome of thrombotic thrombocytopenic purpura occurring after bone marrow transplantation. Br J Haematol 2001;113:58-64.
31. Rutuu T, Hermans J, Niederwieser D, Gratwohl A, Kiehl M, Volin L, Bertz H, Ljungman P, Spence D, Verdonck LF, Prentice HG, Bosi A, Du Toit CE, Brinch L, Apperley JF. Thrombotic thrombocytopenic purpura occurring after allogeneic stem cell transplantation: a survey of the European Group for Blood and Marrow Transplantation (EBMT). Br J Haematol 2002;118:1112-9.
32. George JN, Li X, McMinn JR, Terrell DR, Vesely SK, Selby GB. Thrombotic thrombocytopenic purpura-hemolytic uremic syndrome following allogeneic HPC transplantation: a diagnostic dilemma. Transfusion 2004;44:294-304.
33. Chappell ME, Keeling DM, Prentice HG, Sweny P. Haemolytic uraemic syndrome after bone marrow transplantation: an adverse effect of total body irradiation? Bone Marrow Transplant 1988;3:339-47.
34. Holler E, Kolb HJ, Hiller E, Mraz W, Lehmacher W, Gleixner B, Seebacher C, Jehn U, Gerhartz HH, Brehm G. Microangiopathy in patients on cyclosporine prophylaxis who developed acute graft-versus-host disease after HLA-identical bone marrow transplantation. Blood 1989;73:2018-24.
35. Kalhs P, Brugger S, Schwarzinger I, Greinix HT, Keil F, Kyrle PA, Knobl P, Schneider B, Hocker P, Linkesch W. Microangiopathy following allogeneic marrow transplantation. Association with cyclosporine and methylprednisolone for graft-versus-host disease prophylaxis. Transplantation 1995;60:949-57.
36. Shimoni A, Yeshurun M, Hardan I, Avigdor A, Ben-Bassat I, Nagler A. Thrombotic microangiopathy after allogeneic stem cell transplantation in the era of reduced-intensity conditioning: the incidence is not reduced. Biol Blood Marrow Transplant 2004;10:484-93.
37. Nakamae H, Yamane T, Hasegawa T, Nakamae M, Terada Y, Hagihara K, Ohta K, Hino M. Risk factor analysis for thrombotic microangiopathy after reduced-intensity or myeloablative allogeneic hematopoietic stem cell transplantation. Am J Hematol 2006;81:525-31.
38. Worel N, Greinix HT, Leitner G, Mitterbauer M, Rabitsch W, Rosenmayr A, Höcker P, Kalhs P. ABO-incompatible allogeneic hematopoietic stem cell transplantation following reduced-intensity conditioning: close association with transplant-associated microangiopathy. Transfus Apher Sci 2007;36:297-304.
39. Ho VT, Cutler C, Carter S, Martin P, Adams R, Horowitz M, Ferrara J, Soiffer R, Giralt S. Blood and marrow transplant clinical network toxicity consensus summary: thrombotic microangiopathy after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2005;11:571-5.
40. Uderzo C, Fumagalli M, De Lorenzo P, Busca A, Vassallo E, Bonanomi S, Lanino E, Dini G, Varotto S, Messina C, Miniero R, Valsecchi MG, Balduzzi A. Impact of thrombotic thrombocytopenic purpura on leukemic children undergoing bone marrow transplantation. Bone Marrow Transplant 2000;26:1005-9.
41. Elliott MA, Nichols WL Jr, Plumhoff EA, Ansell SM, Dispenzieri A, Gastineau DA, Gertz MA, Inwards DJ, Lacy MQ, Micallef IN, Tefferi A, Litzow M. Posttransplantation thrombotic thrombocytopenic purpura: a single-center experience and a contemporary review. Mayo Clin Proc 2003;78:421-30.
42. Foss FM, Gorgun G, Miller KB. Extracorporeal photopheresis in chronic graft-versus-host disease. Bone Marrow Transplant 2002;29:719-25.

43. Ratanatharathorn V, Ayash L, Lazarus HM, Fu J, Uberti JP. Chronic graft-versus-host disease: clinical manifestation and therapy. Bone Marrow Transplant 2001;28:121-9.
44. Edelson R, Berger C, Gasparro F, Jegasothy B, Heald P, Wintroub B, Vonderheid E, Knobler R, Wolff K, Plewig G, McKiernan G, Christiansen I, Oster M, Honigsmann H, Wilford H, Kokoschka E, Rehle M, Perez M, Stingl G, Laroche L. Treatment of cutaneous T-cell lymphoma by extracorporeal photochemotherapy. Preliminary results. N Engl J Med 1987;316:297-303.
45. Rook AH, Suchin KR, Kao DM, Yoo EK, Macey WH, DeNardo BJ, Bromely PG, Geng Y, Junkins-Hopkins JM, Lessin SR. Photopheresis: clinical applications and mechanism of action. J Investig Dermatol Symp Proc 1999;4:85-90.
46. Barr ML, Meiser BM, Eisen HJ, Roberts RF, Livi U, Dall'Amico R, Dorent R, Rogers JG, Radovanceviç B, Taylor DO, Jeevanandam V, Marboe CC. Photopheresis for the prevention of rejection in cardiac transplantation. Photopheresis Transplantation Study Group. N Engl J Med 1998;339:1744-51.
47. Rossetti F, Dall'Amico R, Crovetti G, Messina C, Montini G, Dini G, Locatelli F, Argiolu F, Miniero R, Zacchello G. Extracorporeal photochemotherapy for the treatment of graft-versus-host disease. Bone Marrow Transplant 1996;18(Suppl 2):175-81.
48. Child FJ, Ratnavel R, Watkins P, Samson D, Apperley J, Ball J, Taylor P, Russell-Jones R. Extracorporeal photopheresis (ECP) in the treatment of chronic graft-versus-host disease (GVHD). Bone Marrow Transplant 1999 May;23(9):881-7.
49. Greinix HT, Volc-Platzer B, Rabitsch W, Gmeinhart B, Guevara-Pineda C, Kalhs P, Krutmann J, Hönigsmann H, Ciovica M, Knobler RM. Successful use of extracorporeal photochemotherapy in the treatment of severe acute and chronic graft-versus-host disease. Blood 1998;92:3098-104.
50. Couriel DR, Hosing C, Saliba R, Shpall EJ, Anderlini P, Rhodes B, Smith V, Khouri I, Giralt S, de Lima M, Hsu Y, Ghosh S, Neumann J, Andersson B, Qazilbash M, Hymes S, Kim S, Champlin R, Donato M. Extracorporeal photochemotherapy for the treatment of steroid-resistant chronic GVHD. Blood 2006;107:3074-80.
51. Messina C, Locatelli F, Lanino E, Uderzo C, Zacchello G, Cesaro S, Pillon M, Perotti C, Del Fante C, Faraci M, Rivabella L, Calore E, De Stefano P, Zecca M, Giorgiani G, Brugiolo A, Balduzzi A, Dini G, Zanesco L, Dall'Amico R. Extracorporeal photochemotherapy for paediatric patients with graft-versus-host disease after haematopoietic stem cell transplantation. Br J Haematol 2003;122:118-27.
52. Apisarnthanarax N, Donato M, Körbling M, Couriel D, Gajewski J, Giralt S, Khouri I, Hosing C, Champlin R, Duvic M, Anderlini P. Extracorporeal photopheresis therapy in the management of steroid-refractory or steroid-dependent cutaneous chronic graft-versus-host disease after allogeneic stem cell transplantation: feasibility and results. Bone Marrow Transplant 2003;31:459-65.
53. Greinix HT, Socié G, Bacigalupo A, Holler E, Edinger MG, Apperley JF, Schwarz T, Ullrich SE, Albert ML, Knobler RM, Peritt D, Ferrara JL. Assessing the potential role of photopheresis in hematopoietic stem cell transplant. Bone Marrow Transplant 2006;38:265-73.
54. Flowers MED, Van Besien K, Apperley J, Bacigalupo A, Elmaagacli A, Grigg A, Kolb HJ, Bouzas LF, Michallet M, Reddy V, Wang J, Williamson P, Knobler R, Greinix H. A randomized single-blind study of extracorporeal photopheresis with UVADEX® plus conventional therapy (CT) compared to CT alone in chronic GVHD. Blood 2006; Suppl:758 (abstract).
55. Martin PJ, Schoch G, Fisher L, Byers V, Anasetti C, Appelbaum FR, Beatty PG, Doney K, McDonald GB, Sanders JE, Sullivan KM, Storb R, Thomas D, Witherspoon RP, Lomen P, Hannigan J, Hansen JA. A retrospective analysis of therapy for acute graft-versus-host disease: initial treatment. Blood 1990;76:1464-72.
56. Martin PJ, Schoch G, Fisher L, Byers V, Appelbaum FR, McDonald GB, Storb R, Hansen JA. A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. Blood 1991;77:1821-8.
57. Greinix HT, Volc-Platzer B, Kalhs P, Fischer G, Rosenmayr A, Keil F, Hönigsmann H, Knobler RM. Extracorporeal photochemotherapy in the treatment of severe steroid-refractory acute graft-versus-host disease: a pilot study. Blood 2000;96:2426-31.
58. Greinix HT, Knobler RM, Worel N, Schneider B, Schneeberger A, Hoecker P, Mitterbauer M, Rabitsch W, Schulenburg A, Kalhs P. The effect of intensified extracorporeal photochemotherapy on long-term survival in patients with severe acute graft-versus-host disease. Haematologica 2006;91:405-8.
59. Salvaneschi L, Perotti C, Zecca M, Bernuzzi S, Viarengo G, Giorgiani G, Del Fante C, Bergamaschi P, Maccario R, Pession A, Locatelli F. Extracorporeal photochemotherapy for treatment of acute and chronic GVHD in childhood. Transfusion 2001;41:1299-305.
60. Garban F, Drillat P, Makowski C, Jacob MC, Richard MJ, Favrot M, Sotto JJ, Bensa JC, Cahn JY. Extracorporeal chemophototherapy for the treatment of graft-versus-host disease: hematologic consequences of short-term, intensive courses. Haematologica 2005;90:1096-101.