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Leiden
The Netherlands

Stem cell therapy for inflammatory bowel disease
Duijvestein, M.

CHAPTER 2

Long-term follow-up of autologous hematopoietic stem cell transplantation for severe refractory Crohn`s disease

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Daniel W. Hommes,[1] Marjolijn Duijvestein,[1] Zuzana Zelinkova,[2] Pieter C.F. Stokkers,[3] Maartje Holsbergen-De Ley,[1] Jaap Stoker,[4] Carlijn Voermans,[5] Marinus H.J. van Oers,[6] Marie José Kersten[6]

[1]Department of Gastroenterology and Hepatology, Leiden University Medical Center, Leiden, the Netherlands
[2]Department of Gastroenterology and Hepatology, Erasmus Medical Center, Rotterdam, the Netherlands
[3]Department of Gastroenterology and Hepatology, Academic Medical Center, Amsterdam, the Netherlands
[4]Department of Radiology, Academic Medical Center, Amsterdam, the Netherlands,
[5]Department of Experimental Immunohematology, Sanquin Research, Amsterdam and Landsteiner Laboratory, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands
[6]Department of Hematology, Academic Medical Center, Amsterdam, the Netherlands.

ABSTRACT

Although new therapeutic strategies have been developed to control Crohn`s disease, medical treatment for refractory cases is not able to prevent extensive and/or repeated surgery. Recently, several cases have been reported of successful remission induction in Crohn's disease (CD) patients by means of hematopoietic stem cell transplantation (HSCT). Here we report our long-term (4 to 6 years) outcome in three patients.

Patients

Three patients (two male, one female) with active severe CD were planned to undergo autologous HSCT. All patients were intolerant or refractory to conventional therapies, including anti-TNFα antibodies. Patients either refused surgery or surgery was considered not to be a feasible alternative due to the extensive disease involvement of the small intestine.

Methods

Peripheral blood stem cells were mobilized using a single infusion of cyclophosphamide 4g/m^2, followed on day 4 by subcutaneous injections with granulocyte colony-stimulating factor (G-CSF) 5μg/kg twice daily until leukapheresis. CD34+ cells were isolated after leukapheresis by magnetic cell sorting. In two of the patients a second round of stem cell mobilization using G-CSF only was required either because of low yield or because of insufficient recovery after CD34 selection. Prior to transplantation, immune ablation was achieved using cyclophosphamide 50 mg/kg/day (4 days), antithymocyte globulin 30 mg/kg/day (3 days) and prednisolone 500 mg (3 days). Endoscopy, barium small bowel enteroclysis and MRI enterography were performed.

Results

All three patients successfully completed stem cell mobilization and two of them subsequently underwent conditioning and autologous HSCT with CD34+ cell selection. Treatment was well tolerated, with acceptable toxicity. Now, 5 and 6 years post-transplantation, these patients are in remission under treatment. The third patient went into remission after mobilization and therefore she decided not to undergo conditioning and HSCT transplantation. After a successful pregnancy she relapsed two years later. Since then, she suffers from refractory CD for which we are now reconsidering conditioning and transplantation.

Conclusion

Autologous HSCT appears to be safe and can be an alternative strategy for CD patients with severe and therapy resistant disease.

INTRODUCTION

Crohn's disease is an inflammatory disorder of potentially any part of the gastrointestinal tract, leading to various intestinal but also extra-intestinal symptoms. It is thought that dysregulation of the normally controlled immune response to commensal bacteria in genetically susceptible patients drives the disease.[1] Although medical therapy of inflammatory bowel disease (IBD) has improved due to an extensive repertoire of immunosuppressive drugs and the introduction of anti-tumor necrosis factor-α (TNFα) compounds, the clinical course of the disease cannot be adequately controlled in a substantial group of patients. These patients may not respond or may become refractory to their medication, or they may develop treatment limiting toxicities.[2] The course of the disease is chronic and intermittent and the disease responds less well to medical therapy over time. Half of the patients require surgical resection during the course of the disease.[3] Unfortunately, surgery is accompanied by a high recurrence rate and many patients are at risk to develop short bowel syndrome. Therefore, there is an unmet need for more effective therapeutic strategies.

Evidence that hematopoietic stem cell transplantation (HSCT) would be an effective treatment for autoimmune diseases firstly came from several animal models[4, 5] and from incidental case reports on HSCT recipients with coexistent autoimmune disease.[6] Already more than 15 years ago a case report on the positive effect of HSCT on active IBD was published, followed by many others showing improvement of the clinical course of disease after HSCT indicated for other (malignant) diseases.[7] In the last couple of years, several cases have been reported of successful remission induction of patients with (therapy refractory) CD by means of an autologous HSCT. The most extensive experience on autologous HSCT applied specifically for CD has been reported by Oyama and colleagues.[8, 9] All twelve treated patients went into remission with a Crohn's disease activity index (CDAI) score of <150. There was no inpatient mortality and neutropenic fever was the most important complication. Recently published long-term follow-up data showed a clinical relapse-free survival of 63% at 3 years and 36% at 5 years. The percentage of patients in remission (CDAI < 150), steroid free, or medication free at any post transplant evaluation interval over 5 years post transplant was respectively 70%, 80% and 60%.[8]

Supported by these reports we have mobilized stem cells in three patients with severe refractory CD, for whom surgery was not a feasible option due to extensive disease activity, and performed autologous HSCT in two of these patients. Here, we report on the follow-up period of four to six years.

MATERIAL AND METHODS

Patient selection

Three patients with severe refractory CD were selected for an autologous HSCT procedure. The considerations for selection included longstanding refractory disease and exhaustion of the full range of therapeutic strategies such as anti-TNFα antibodies. In addition, surgery was unattractive because of unwillingness of the patients and the likelihood of either

developing short bowel syndrome (patient 1) or a definite ileostomy (patients 2 and 3). All cases were reviewed prior to selection by an independent gastroenterologist. The protocol was approved by the Medical Ethics Committee of the Academic Medical Center (AMC), Amsterdam, the Netherlands. All patients gave written informed consent.

HSCT protocol

Mobilization: Prior to cyclophosphamide treatment, semen was cryopreserved of the male patients. The female patient was offered treatment with the luteinizing hormone-releasing hormone analague gosereline, in an attempt to prevent chemotherapy-induced gonadal toxicity and premature ovarian failure.[10] Peripheral blood stem cells were mobilized using a regimen consisting of a single intravenous dose of cyclophosphamide 4g/m^2 followed on day 4 by granulocyte colony-stimulating factor (G-CSF) twice daily, 5μg/kg/day. Hyperhydration and mesna prophylaxis were given in order to prevent hemorrhagic cystitis. Partial bowel decontamination was obtained using oral ciprofloxacin and fluconazol. In addition, all patients received antibiotic prophylaxis consisting of clarithromycin. Peripheral blood stem cells (target >8 x10^6 CD34+ cells/kg body weight) were collected by large volume leukapheresis using a COBE Spectra leukapheresis machine (COBE BCT, Lakewood CO) as soon as the white blood cell count was >2x10^9/l and CD34+ cells were detectable in the peripheral blood. CD34+ cells were isolated within 48 hours after leukapheresis by magnetic cell sorting (CliniMACS CD34 selection procedure, Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. The CD34+ cells were cryopreserved in dimethyl sulfoxide (DMSO) and stored in the vapor phase of liquid nitrogen until transplantation. *Conditioning:* Prior to transplantation, immune ablation was achieved using an immunosuppressive conditioning regimen consisting of cyclophosphamide 50 mg/kg/day intravenously and equine antithymocyte globulin (Lymphoglobulin, hATG, Genzyme Corporation, Boston, MA, USA) at a dosage of 30 mg/kg/day (on days -4, -3, -2). Premedication with methylprednisolone at a dose of 500 mg/day was administered intravenously for three consecutive days from day 4 and prednisolone was subsequently tapered over a period of 14 days. Hematopoietic stem cells were thawed and infused intravenously on day 0. *Transplantation:* Patients were hospitalized and nursed in clean rooms. Following transplantation, partial bowel decontamination was obtained using oral ciprofloxacin and fluconazol, and patients received antibiotic prophylaxis consisting of metronidazol and intravenous penicillin starting on day 5 until recovery of neutropenia. Supportive care were given when indicated. The hemoglobin level was maintained above 9.7 g/dl and platelet counts above 10 x10^9/l. All blood products, excluding the peripheral blood progenitor cells, were irradiated prior to infusion.

Evaluation of clinical response

Clinical assessment during HSCT and follow-up included medical history, physical examination and standard biochemical tests (including CRP). Endoscopy and both barium small bowel enteroclysis and MRI enterography were performed at baseline and after 3 and 6 months where indicated.

RESULTS

HSCT was offered to 3 patients (2 male, 1 female) with a median age of 28 (range 27-51). Patients were resistant or intolerant to conventional medication, including anti-TNFα therapy. All drugs, including steroids, were tapered after the mobilization procedure. Patient disease characteristics are described in table 1.

Safety and efficacy of stem cell mobilization

In general, the stem cell mobilization procedure with cyclophosphamide and G-CSF was safe. Besides minor expected side-effects such as nausea which were successfully managed, there were two major complications: in patient 1 a transient deterioration of kidney function was observed due to decreased oral intake because of nausea, which responded rapidly to intravenous fluids. Patient 2 had an episode of culture-negative neutropenic fever, which was empirically treated with broad-spectrum antibiotics. The blood cultures remained negative and the fever resolved promptly.

In patient 1, a sufficient number of stem cells was harvested (28.9×10^6 CD34+ cells/kg) in only one leukapheresis procedure. Despite a rather low recovery after CD34+ selection of 27%, a large enough amount of stem cells for reinfusion (7.87×10^6/kg) with a purity of 97% was obtained. In patient 2 and 3 however, a second stem cell mobilization procedure was required. In patient 2 the initial yield was sufficient (10.6×10^6 CD34+ cells/kg), but due to low recovery the yield after CD34 selection was borderline (2.5×10^6 CD34+ cells/kg). Therefore, a second mobilization procedure using only G-CSF 5 μg/kg twice daily for 5 days was performed two months later. The second mobilization yielded a total 2.1×10^6 CD34+ cells/kg, with 50% recovery after CD34 selection and a purity of 94%. In patient 3 the first attempt at stem cell mobilization with cyclophosphamide priming failed with a total yield after two leukapheresis procedures of 1.85×10^6 CD34+ cells/kg. Because of the low yield, CD34 selection was not performed. A second attempt at stem cell mobilization was done three months later using only G-CSF 5 μg/kg twice daily for 5 days. This resulted after two days in a yield of 5.84×10^6 CD34+ cells/kg, with a recovery of 53% and a purity of 89.7%. A third leukapheresis yielded 1.7×10^6 CD34+ cells/kg with a recovery of 66.5% and a purity of 79.7% after CD34 selection.

During the stem cell mobilization phase patient 1 required one transfusion of red blood cells (RBC) (3 units), patient 2 required two transfusions (in total 7 units of RBC) and patient 3 received one platelet transfusion (pooled platelets from five donors).

Safety of hematopoietic stem cell pretreatment and transfusion

During HSCT conditioning, patient 1 developed an allergic reaction (fever and hypotension) to antithymocyte globulin (ATG), that resolved with fluid challenge. Patient 2 had a quick and transient deterioration of kidney function due to hypotension after the first dose of ATG. In patient 1 and 2 respectively, a total of 5.9×10^6 and 3.5×10^6 CD34+ cells/kg were reinfused. The posttransplantation course was uneventful with an episode of rotavirus-associated diarrhea in both patients, clostridium difficile-

Patient	1	2	3
Basal demographic and clinical characteristics			
Sex	male	male	female
Year of birth	1953	1978	1978
Diagnosis	2003	2004	1999
Disease extension	stomach, duodenum, jejunum, ileum	colon including rectum	colon including rectum
Montreal			
Disease pattern	stricturing and penetrating	penetrating	penetrating
Extraintestinal disease manifestations	no	stomatitis	artralgia
Risk factors	none	previous smoker	previous smoker
Previous treatments			
5-asa	yes	yes	yes, intolerant
Corticosteroids	yes	yes	yes, intolerant
Antibiotics	yes	yes	yes
Azathioprine	yes	yes	yes, leucopenia
Infliximab	yes	yes	yes
Methotrexate	yes	yes	yes
visilizumab	no	yes	yes
Adalimumab	no	no	no
Surgery	no	no	no
HSCT			
Age at HSCT	51	27	28
Year of HSCT	2004	2005	2006
Crohn's disease medication			
At time of mobilization	prednisone 15 mg	prednisone 30 mg	none
At time of transplantation	prednisone 60 mg	prednisone 40 mg	not applicable

Table 1. Basal demographic, medical history, and clinical characteristics at time of HSCT.

associated diarrhea and an episode of fever caused by a central venous catheter-associated coagulase-negative staphylococci (CNS) bacteremia in patient 2. Recovery of neutrophils to >0.5x10^9/l was reached at day 12 and 14 in patient 1 and 2, and recovery of platelets >50x10^9/l was seen at day 14 and 9 respectively. Patient 1 required transfusion with six units of RBC and 3 platelet transfusions; patient 2 required six units of RBC and 1 platelet pool. Both patients discontinued their immunosuppressive therapies post HSCT and regained normal appetite and oral intake.

Clinical evaluation of HSCT treatment and follow-up

At a median follow-up of 62 (range 58-75) months all patients are alive. The two patients (Patient 1 and 2) who underwent the complete HSCT protocol are in clinical remission under immunosuppressive therapy. The patient (patient 3) who underwent stem cell mobilization only, remained in clinical remission and off of all immunosuppressive drugs for more than two years. A detailed description of the follow-up on these patients is described below:

Patient 1:

Patient 1 is a 51-year-old male with diagnosed CD of the stomach and almost the entire small bowel dependent on parenteral nutrition. This patient was offered autologous HSCT because he was unresponsive to immunosuppressive drugs (steroids in combination with azathioprine/methotrexate) and biologic therapy (infliximab). Surgery would have put him at great risk of developing a short bowel syndrome as the whole small intestine was affected by the disease. After mobilization, an initial clinical response was observed. However, the patient experienced a clinical and endoscopic relapse within ten weeks. After immune ablation a total of 5.9x10^6/kg CD34+ cells/kg was reinfused and the patient could be discharged on day 15 after HSCT. In week 8 following transplantation, the patient was clinically in a partial remission, gaining weight (4 kg) without need for supplementary parenteral nutrition.

Follow-up: At 8 weeks clear improvement of the inflammatory lesions was observed endoscopically (Figure 1A). Also, barium enteroclysis (Figure 1B) and MRI enterography (Figure 1C) showed regression of small bowel stenosis. A sharp drop in CRP levels was seen after the mobilization phase, which further decreased during follow-up (Figure 1D). Two months after transplant the patient unexpectedly developed leukopenia (1.6x10^9/l), which resolved spontaneously within 2 months. Six months post-transplant the patient developed abdominal pain and gastric ulcers were detected during gastroscopy. Biopsies did not reveal Helicobacter pylori, cytomegalovirus, Epstein–Barr virus or other infectious agents and the ulcers had vanished two months later. MRI performed at 10 weeks showed no improvement compared to the baseline MRI, which showed bowel wall thickening, stenosis and inflammation of the horizontal part of the duodenum and jejunum. At the MRI performed at 6 months persistence of stenotic segments of the duodenum was seen. However, inflammatory activity evidently decreased and the stenosis in the jejunum disappeared (Figure 1D). As expected, residual strictures in the duodenum persisted which were dilated three (1x), twelve (3x), and 36 (1x) months after transplantation.

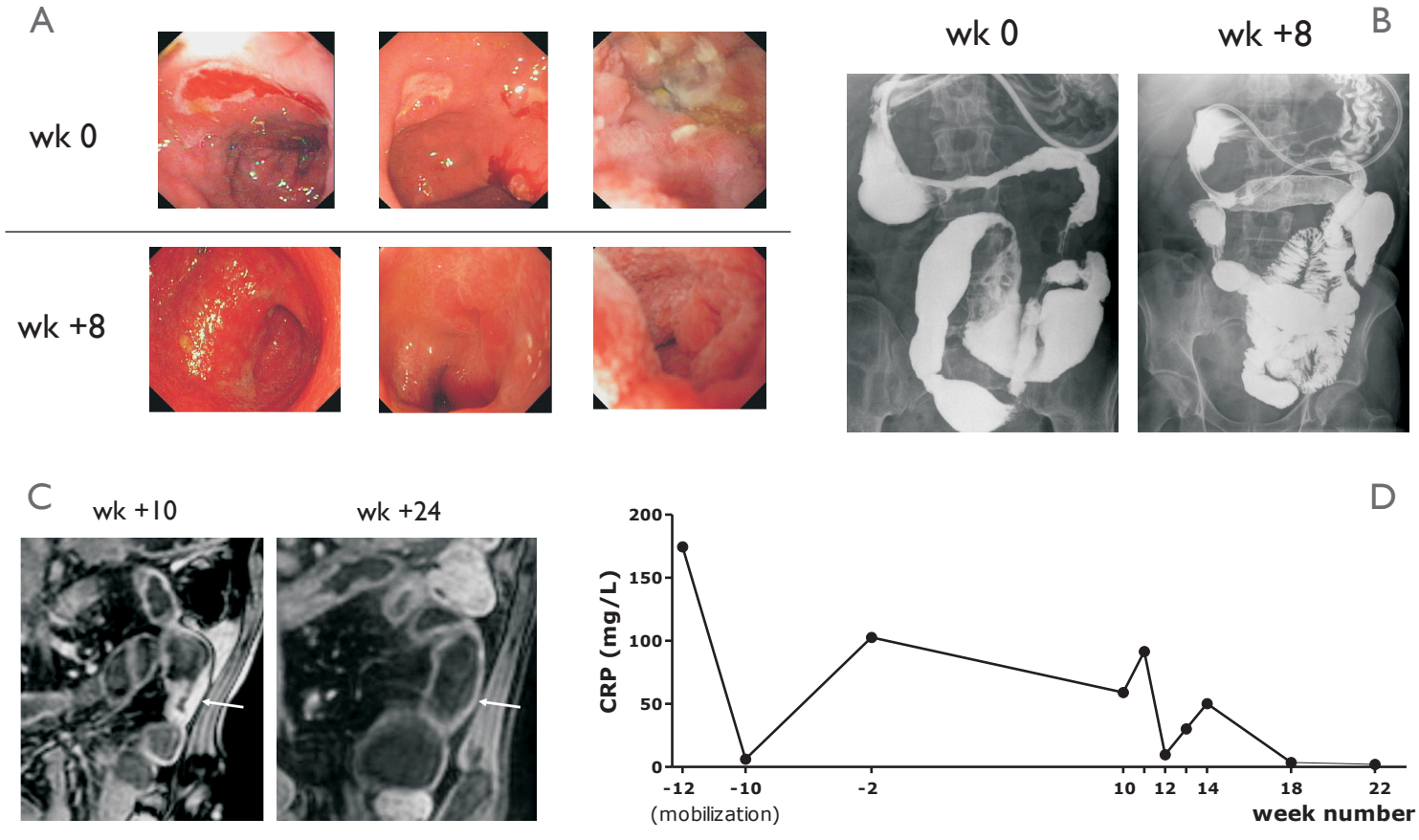

Figure 1. A Endoscopic changes after HSCT of patient 1. Duodenal ulcerations at the week 0 endoscopy (upper panels) and healing of duodenal mucosa at week 8 after HSCT (lower panels). B Barium small bowel enteroclysis of patient 1 at baseline and 8 weeks after HSCT shows regression of small bowel stenosis. C MRI enterography at week 10 and week 24 post HSCT. Coronal fat saturated T1-weighted sequence after intravenous contrast administration shows a reduction in the extension of a stenosis in the jenunum (arrow). D CRP levels during the mobilization phase and indicated number of weeks post HSCT.

Twelve months post-transplantation azathioprine was started (150 mg/day) and switched to certoluzimab two years post-transplantation because of leukopenia and thrombocytopenia. Repeat gastroscopy with enteral balloon dilatation of his proximal strictures was last performed in 2006. In 2007, he was admitted for unexplained ascites. Extensive work-up excluded common causes such as primary sclerosing cholangitis, portal hypertension, and hepatitis. The ascites resolved spontaneously without continued use of diuretics.

At present, the patient is doing relatively well. His weight has improved over the years and he now only sporadically vomits following meals. During his six years of follow up he did not have a luminal relapse of his CD and certolizumab treatment will be continued.

Patient 2

The next patient is a 27-year-old male patient with a short but complicated history of refractory CD of the colon unresponsive to immunosuppresive drugs (azathioprine and methotrexate), failing on infliximab and experimental anti-CD3 (visilizumab) therapy. In July of 2005 he underwent stem cell mobilization followed by HSCT in October of the same year. After HSCT, the stool frequency dropped from bloody and watery >10/day to semisolid without blood 6-7 times daily before hospital discharge. Dramatic improvement of the colonic mucosa was observed during colonoscopy at 10 weeks. The ulcerated areas had regained epithelium and no active inflammation was seen.

Follow-up: Six months after HSCT, 6-MP (mercaptopurine 100 mg) was started combined with infliximab. After having experienced no clinical complaints and having a high quality of life for five years, he relapsed with a mildly active distal colitis in the beginning of 2010. He was switched from infliximab to adalimumab, continuing 6-MP, and three months following his change of therapy he was again in complete clinical remission, with normalized CRP levels.

Patient 3

The third case is a 28-year-old female patient with a similar history. She has a completely refractory (included failure of anti-TNFα therapy) CD of her colon complicated by arthritis and perianal abscesses, needing multiple hospital admissions yearly. Patient refused colectomy and was therefore planned to undergo HSCT. In this patient, complete clinical remission was achieved after the stem cell mobilization procedure. For this reason the stem cell transplantation itself was deferred.

Follow-up: The patient remained in complete clinical and endoscopic remission without any medication for two years and had an uncomplicated pregnancy 1.5 years after mobilization. In 2008, she relapsed with a severe active Crohn's colitis for which she was treated with infliximab and methotrexate. After three infusions she experienced an allergic response to infliximab and, despite proper anti-allergic medication, a loss of response. Transplantation of the mobilized and stored CD34+ cells was proposed to the patient, which she declined because of cyclophosphamide associated toxicity. Consequently, she was included in a phase I trial on the safety and feasibility of autologous mesenchymal stromal cell infusion in refractory CD, for which conditioning is not needed (see Chapter 3 of this thesis).[11] No response was achieved and currently she experiences

persistent but tolerable activity with weekly adalimumab treatment in combination with steroids. As no medical therapeutic options are left we are now reconsidering conditioning and transplantation.

DISCUSSION

Our first experience of autologous HSCT for CD, employing similar protocols for mobilization, conditioning and supportive care as used in other published articles on HSCT for CD[12] is encouraging, as our patients have, since the onset of their disease, never been in remission for as long as after HSCT. The effect of HSCT was only partially successful in resolving Crohn's lesions at endoscopy. There was no transplant-related mortality and adverse effects were manageable. In one patient, an unexplained prolonged leukopenia following transplantation was observed which occurred two months after initial neutrophil recovery and which was not associated with infectious complications.

We did observe some difficulties with stem cell mobilization and CD34+ selection. In two of the patients, the initial procedure yielded an insufficient amount of CD34+ cells either before or after selection. This may in part have been due to the prior use of methotrexate in both patients, combined with thalidomide in one of the patients, even though this medication was stopped two months before the cyclophosphamide mobilization treatment. A subsequent second attempt at stem cell mobilization using G-CSF only was successful, although two leukapheresis procedures were required for both patients. The recovery of CD34+ cells after selection using the CliniMACS selection procedure was rather low (median 48.5%, range 23-66.5%). Whether this is related to the underlying disease is unknown, but similar low recovery rates were not reported in the series published by Oyama et al.[9]

Recently, safety and efficacy of autologous HSCT with unselected peripheral blood stem cells was demonstrated in four previously refractory CD patients.[13] In our study, peripheral blood cells were used after CD34+ cell selection in order to deplete activated T-cells to reduce the likelihood of relapse related to the reinfusion of autoreactive T-cells.[14] The last case is a good example of the role of the immunosuppressive effects of the stem cell mobilization regime which includes a high dose of cyclophosphamide. In 390 patients undergoing autologous HSCT for various autoimmune diseases a mobilization associated mortality of 1.5% and an overall procedure related mortality of 9% were found.[15] To evaluate the efficacy of HSC mobilization followed by high dose immune ablation and autologous stem cell transplantation versus HSC mobilization only, a multicenter, prospective, randomized phase III study has been initiated by the European Crohn's and Colitis Organisation (ECCO) in collaboration with the European Group for Blood and Marrow Transplantation (EBMT).

Not only autologous but also allogeneic HSCT has been shown to maintain remission in CD patients.[16] Theoretically, due to the additional graft versus autoimmunity effect in allogeneic HSCT this approach could give a longer lasting effect than autologous HSCT in the treatment of CD, even after withdrawal of prophylactic immunosuppression.[17] Allogeneic HSCT is, however, associated with graft-versus-host disease and has a considerably higher morbidity and mortality than autologous HSCT.[18]

Therefore, allogeneic HSCT at this point does not seem to be a viable option for a nonmalignant disease with very low mortality rates.

In conclusion, autologous HSCT is a relatively well tolerated and safe procedure. Given the considerable mortality rate of HSCT for autoimmune diseases, this treatment should only be considered in highly selected patients with severe and therapy resistant disease, or for patients for whom surgery is not a treatment option. Larger clinical trials are and should be conducted as more information is warranted.

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