Monoclonal Antibodies to Treat Graft-Versus-Host Disease

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Abstract: To date, Graft-versus-host disease (GVHD) represents one of the most important complications of allogeneic hematopoietic stem cell transplantation (HSCT). GVHD is one of the major determinants of transplant-related mortality and it also may be an additional cause that affects patients late outcome. Despite of the development of new and advanced Human Leukocyte Antigens (HLA) matching techniques, this complication occurs in approximately 50-80% of patients who underwent allogeneic hematopoietic stem cell transplantation, and it is responsible for one-third of deaths after transplantation. Moreover, GVHD occurrence, if moderate, may strongly contribute to the eradication of residual malignant cells which survived after myeloablative conditioning regimen, allowing the patients to have a reduced risk of relapse so that the presence of this complication may have a determinant role for the allogeneic transplantation outcome through the so-called graft-versus-tumor (GVT) effect.

Keywords: GVHD, acute, chronic, therapy, alemtuzumab, rituximab, monoclonal antibodies.

INTRODUCTION

In the last two decades, transplantation with allogeneic marrow or peripheral blood hematopoietic stem cells has been increasingly used for a wide variety of hematologic diseases. Therefore, the number of HSCT continues to increase with more than 25,000 allogeneic transplantations performed annually. The GVT effect during allogeneic HCT effectively eradicates many haematological malignancies. The development of novel strategies that use donor leukocyte infusions, non-myeloablative conditioning, umbilical cord blood (UCB) and haploidentical transplantation have contributed to expand the indications for allogeneic HSCT [1-10]. Moreover, the increasing of alternative donor transplants enhances the number of patients at risk of developing GVHD [11]. Acute and chronic GVHD are common causes of significant complications of allogeneic hematopoietic stem cell transplantation and their severity is strongly related with post-transplant outcome [12]. GVHD may, contemporarily, enhance survival by decreasing the risk of disease relapse but increases non relapse mortality by causing organ failure or life-threatening infections. Their incidence and severity depend on several factors, such as donor/recipient HLA matching, recipient's age, intensity of the conditioning regimen, source of stem cells, and composition of the graft [13].

Acute GVHD is due to the presence of immunocompetent cells in the marrow inoculum, in particular of mature T lymphocytes that recognize major and minor histocompatibility antigens as "non self" and the tissue antigens as not belonging to the HLA system.

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The pathophysiology of acute GVHD is a three phase phenomenon. In the initial phase, the damage of host tissue, due to conditioning regimen, produces inflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ. Subsequently, donor T cells proliferate and differentiate in response to recipient antigen-presenting cells. Finally, activated donor T cells mediate cytotoxicity against target host cells through Fas-Fas ligand interactions, perforin-granzyme B, and cytokine production, including TNF-α [14,15,16].

In order to prevent GVHD, the primary pharmacologic strategy is based on the inhibition of the cytoplasmic enzyme, calcineurin, that is critical for the activation of T cells. The calcineurin inhibitors, cyclosporine and tacrolimus have similar mechanism of action. Calcineurin inhibitors are often administered in combination with other immunosuppressive agents, such as methotrexate. Anti T cell globulin (ATG) is also used for prophylaxis and therapy of GVHD through the elimination of either donor and recipient T-cells *in vivo* and is increasingly being used instead of *ex-vivo* T-cell depletion [17,18,19].

Bacigalupo *et al*. conduced a randomized trial to receive or not ATG in the conditioning regimen in 109 patients underwent unrelated donor bone marrow transplantation. They showed that the use of ATG to prevent aGVHD reduced the incidence of grade 3-4 aGVHD, with no improvement of survival result. However, the addition of ATG to cyclosporine and methotrexate provided significant protection against extensive chronic GVHD and chronic lung dysfunction, reduced late transplant mortality, and improved quality of life [17,18]. Recently, Finke *et al.* reported the findings of a phase 3 trial where patients undergoing unrelated HSCT were randomized to receive prophylaxis with or without ATG. This study demonstrated that the addition of ATG to GVHD prophylaxis with ciclosporin and methotrexate reduced the incidence of acute and chronic

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GVHD without increasing relapse or non-relapse mortality, and without effecting overall survival [19].

First-line treatment of GVHD is based on steroids and produces sustained responses in 50-80% of patients with acute GVHD and 40-50% of patients with chronic GVHD depending on the initial disease severity [20,21]. Non-responding patients are offered second-line therapy with combination of various agents, but currently available agents have not improved survival in these high risk populations.

Patients with steroid refractory acute GVHD receive a variety of second line therapies, with response rates around 40%. However, the outcome of these patients is poor, with a mortality rate approximately 70%. Therefore, the development of new strategies for effective intervention before the occurrence of steroid-refractory disease remains a high priority. In the past decades, several drugs and many supportive cares as ancillary therapies were tested in single-center studies for improving the management of acute GVHD [22].

ACUTE GVHD TREATMENT

Two therapeutic approaches to steroid refractory acute GVHD are generally available. The first one involves the use of cytotoxic agents directed towards effector T-cells, with agents such as ATG, OKT3 or mycophenolate mofetil (MMF). The second approach is based upon the blockage of cytokine pathways involved in the pathogenesis of acute GVHD, by using antibodies inhibiting especially TNF–α (etanercept, infliximab) and IL-2 (daclizumab, basiliximab) pathways. Recently, Monoclonal antibodies (MoAbs) have aroused considerable interest as effective tools to face acute or chronic GVHD [23]. The tested MoAbs act at different phases in order to contrast the cytochine release due to the tissue damage. In particular, in this review we will discuss about anti IL2, anti TNF, anti CD52 (Campath), and anti CD20 (Rituximab) MoAbs.

Etanercept is a fusion protein consisting of two identical chains of the human TNF-receptor p75 monomer fused with the Fc domain of human IgG1. It binds to soluble TNFα thus neutralising its activity.

Levine *et al*, used the association of steroids and etanercept as upfront GVHD therapy. They compared the outcome of a cohort of 61 patients affected by new–onset grade II-IV GVHD, enrolled in this trial, with a control group including 99 patients with aGVHD who received only steroids as initial therapy. Patients treated with etanercept were more likely to achieve CR than patients treated with steroids alone (69 vs 33%; P<0,001). The authors observed that plasma TNFR1 levels, a biomarker for GVHD activity, were high at GVHD onset and decreased significantly only in patient who achieved CR [24].

Alousi *et al*, evaluated in a prospective, multicenter trial, the etanercept used in association with the steroid therapy for the initial treatment of acute GVHD. One-hundred eighty patients were randomized to receive methylprednisolone (2 mg/kg/day) in association with etanercept, or mycophenolate mofetil (MMF), or denileukin diftitox (denileukin) or pentostatin. At randomization, 68% of patients had grade I to II a-GVHD and 32% had grade III to IV, out of which 53% had visceral organ involvement. Day 28 complete response rates were etanercept 26%, MMF 60%, denileukin 53%, and pentostatin 38%. Corresponding 9-month overall survival was 47%,64%,49%, and 47%, respectively. Cumulative incidences of severe infections were as follows: etanercept 48%, MMF 44%, denileukin 66%, and pentostatin 57% [25].

Kennedy *et al.* retrospectively reviewed the outcome of 16 patients with refractory acute predominantly visceral GVHD treated with a combination of ATG, tacrolimus and etanercept. The overall response rate (CR+PR) was 81%, in comparison to 60% of their previous experience that tested the use of ATG + tacrolimus as treatment for refractory visceral GVHD. In their study, the addition of etanercept to GVHD therapy seems to improve both the response rate and overall survival without increasing the occurrence of infectious events [26].

Infliximab is a chimeric anti-TNFα-receptor antibody, which binds with high affinity to both soluble and transmembrane forms of TNFα. Binding of infliximab to transmembrane TNFα results in cell lysis due to complement activation and antibody-mediated cellular toxicity. This drug has been used successfully for the treatment of a range of autoimmune and inflammatory disease, including Crohn disease, rheumatoid arthritis, psoriasis, and spondyloarthropathy.

Couriel *et al*, reported that a weekly dose of 10 mg/kg infliximab in patients with steroid refractory GVHD resulted in a 80% response rate in 37 patients with gastrointestinal involvement. Death occurred in 60% of the patients with acute GVHD and it was attributed to GVHD in the majority. Subsequently the same group has retrospectively evaluated 134 patients whit steroid refractory acute GVHD, out of which 21 received infliximab as a single agent. The subset of patients who received infliximab alone achieved CR in 62% of cases with an overall response rate of 67% (n=14). Only five patients (24%) did not responded, and 2 (10%) had progressive GVHD. Most of the patients (57%) had gastrointestinal (GI) involvement, and 19% had grade III-IV GVHD. Prognostic factor analyzed in this study, including age, sex, HLA compatibility and GVHD grading (grade II vs III-IV) did not seem to have impact on response or survival. The highest response rates were observed in GI and skin acute GVHD [27].

The gruppo italiano trapianto di midollo osseo (GITMO) confirmed these data in a multicenter retrospective study involving 32 patients with steroid-refractory grade II-IV acute GVHD. Infliximab was added as a second- line therapy in 14 patients and as third-line salvage treatment in 18 patients. This study reports the encouraging response rate result with 19% of CR and 40% of PR. Furthermore, in this study, infliximab is active in the treatment of severe steroid refractory acute GVHD, especially in case of GI involvement. Several infectious episodes due to different ethiological agents have been reported in some cases as fatal events [28]. Also in other series, infections remain the main issue related to the use of infliximab. The only controlled study on transplanted patients is the one carried out by Marty *et al*, who demonstrated that patients who received

infliximab to control severe GVHD had a significantly higher risk and a shorter time to diagnosis of non-Candida invasive fungal infections than did conventionally treated patients and that these differences were independent of the cumulative dose of steroids received [29].

Several monoclonal antibodies targeting IL-2 receptor have been used to treat acute GVHD: daclizumab is a humanized monoclonal IgG, which binds the α-chain of IL-2 receptor, denileukin diftiox is a recombinant protein composed of human IL-2 fused to diphtheria toxin, inolimomab is a murine anti IL-2R, basiliximab is a chimeric MoAb that binds to the α-chain of IL-2R on activated cytotoxic T-cells, inhibiting their proliferation .

Daclizumab is a 144-kDa humanized immunoglobulin G1 monoclonal antibody that binds specifically to the alpha subunit (p55 alpha, CD25 or TAC subunit) of the human high affinity interleukin-2 (IL-2) receptor, which is expressed on activated lymphocytes, and inhibits IL-2 binding. Basiliximab is a chimeric murine-human antibody, also selective for interleukin-2 receptor (IL-2R). Owing to its murine portion, basiliximab has less immunogenic properties when compared to daclizumab, allowing a low rate of anti-antibodies and increased half-life of about 7 days. Basiliximab has been used to prevent acute rejection in renal transplantation with great efficacy and with absence of severe adverse events. Lee *et al*, conducted a multicenter randomized study of corticosteroid with or without Daclizumab for up-front treatment of aGVHD. A high rate of 100 day-mortality and relapses were observed in the combination of corticosteroid and Daclizumab arm [30]. Willenbacher *et al*, treated 16 patients with steroid refractory GVHD who received 4 doses of daclizumab (1 mg/kg BW) on day 1, 7,14, and 21. Twelve patients suffered from grade III-IV acute GVHD and 4 patients from extensive chronic GVHD. Responses were observed in 9 patients (6 acute, 3 chronic) and 14 out of 16 patients developed infection during daclizumab treatment (3 fatal infectious related) [31]. Similar results have been reported by Przepiorka *et al*. They reported CR in 37% of cases with GI GVHD, and 17% of patients with hepatic GVHD. In addition, evaluable data concerning anti IL-2 receptor antibodies as basiliximab and daclizumab do not allow any recommendation for their use as refractory cGVHD treatment [32]. Finally, the anti CD3 antibody (Visilizumab) has been used to treat steroid-refractory aGVHD with encouraging results [33,34].

Alemtuzumab is an unconjugated humanized IgG1 monoclonal antibody that targets CD52 on T and B lymphocytes, and NK cells, and has also demonstrated the activity on dendritic cells [35,36]. This MoAb is increasingly used to treat B-cell chronic lymphocytic leukemia and T-cell prolymphocytic leukemia. Alemtuzumab has shown to be effective in GVHD prophylaxis in conditioning regimens before allogeneic HSCT, reducing the incidence of acute and chronic GVHD; unlikely its use is related to higher risk of relapse and infection. [37-41]. Our transplant unit may be considered as pioneristic group for the use of alemtuzumab in the setting of steroid-refractory aGVHD: 3 patients affected by liver grade III acute GVHD have been successfully treated with alemtuzumab reporting a strong decrease of both bilirubin and transaminase levels [42].

Shub *et al*. reported the efficacy and safety of alemtuzumab to treat severe aGVHD in 18 consecutive patients refractory to standard high dose of steroid therapy. Initially, start doses of alemtuzumab 70–80 mg were administered and repeated after 3-4 weeks. In order to decrease the incidence and severity of infectious complications, the next 9 patients received a reduced starting dose of 20–33 mg and the last 6 patients received 3-13 mg repeated every 2–3 weeks. Seventeen of the 18 patients responded to the treatment with alemtuzumab and six patients remained alive with a median follow-up of 108 weeks [43].

Chakraverty *et al*, tested the feasibility of alemtuzumab dose escalation in the context of fludarabine-melphalan conditioning HLA identical sibling transplantation. Alemtuzumab was given 1-2 days before allogeneic transplantation and dose reduced from 60 to 20 mg in 4 sequential cohorts of 106 patients. They identified 30 mg of alemtuzumab as the dose related to low GVHD risk, no increase of non relapsed mortality, and improved lymphocyte recovery at 1 year [44]. Ruiz-Arguelles *et al*, reported the case of a patient with extensive cutaneous chronic GVHD affecting 100% of the body surface, with painful ulcerations involving 20% of it, and treated unsuccessfully during 9 months with steroids, cyclosporine-A, sirolimus, tacrolimus, mychophenolate mofetil, infliximab, and rituximab. Twenty-one months after the allograft the patient was given alemtuzumab, 10 mg/day subcutaneously, for 6 consecutive days every 4 months. Seven months after starting the treatment, 100% of the ulcers and pains disappeared. The reported data suggest that this agent may be useful in some patients with refractory forms of chronic GVHD [45].

Schnitzler *et al*. evaluated the efficacy and safety of alemtuzumab in 20 patients with steroid refractory grade III and IV GI GVHD after related and unrelated HSCT [46]. Overall response rate was 70%, with complete response in 35%. Despite the severe grade of GVHD, the median survival of 280 days and 1-year overall survival of 50% were higher or comparable to those associated with other treatment options. Cytomegalovirus reactivation, bacterial infection, and invasive aspergillosis were frequent complications; however, infection was not a significant predictor for survival. These data suggest that treatment with alemtuzumab has favorable activity in severe intestinal GVHD after allogeneic HCT, but emphasize the importance of careful monitoring and anti-infectious supportive care. [47].

The efficacy of alemtuzumab as treatment for steroid-refractory grade III-IV after HSCT was evaluated in a phase II trial. Ten adult patients (6 with acute GVHD grade III and 4 with acute GVHD grade IV) were treated [43]. Nine patients had gastrointestinal tract involvement, 7 had skin involvement, and 5 had liver involvement. Five patients responded to treatment, 2 with CR and 3 with PR. Eight infectious events (4 of grade 3-4) and 7 cytomegalovirus (CMV) reactivations were observed. Six patients had grade 3-4 cytopenia. All 10 patients died (7 resulting from acute GVHD progression, 2 from severe infection, and 1 from leukemia relapse), at a median of 40 days (range, 4 to 88

days) after alemtuzumab treatment. Overall, the available data suggest that steroid-refractory acute GVHD may be improved by treatment with alemtuzumab, but that this treatment does not overcome the dismal prognosis of patients with severe acute GVHD, demonstrating the need for alternative therapies to treat this complication [48].

Overall, the alemtuzumab trials have shown the achievement of good responses with lower doses of MoAb . Infection-related morbidity and mortality are mainly due to the cumulative dose of the agent and to the interval of its administration. The subcutaneous application is much better tolerated than the intravenous infusion, which requires premedication. Intense monitoring for signs of infection and adeguate anti-infectious prophylaxis are recommended.

CHRONIC GVHD TREATMENT

Chronic GVHD is the most common late complication following allogeneic HSCT, occurring in 25-80% of transplant recipient [48].

It is becoming a more frequent problem due to the older recipient age as well as the increasing in using alternative donors, peripheral blood stem cells, reduced-intensity conditioning regimen, and donor lymphocyte infusion. Standard primary treatment of chronic GVHD remains a combination of corticosteroids (CS) and calcineurin inhibitors. There is no standard therapy for those who fail to respond to resistant GVHD is associated with high morbidity.

Therefore, older patients, treated with steroids and cyclosporine for a long period develop severe side effects, including hypertension, renal failure, glucose intolerance, sleep disturbances, cushingoid changes, osteoporosis and osteonecrosis. Various agents have been investigated as salvage therapy for chronic GVHD, although there is no standard approach that is uniformly accepted [49].

Anti-CD20 antibody (Rituximab) is a chimeric mouse-human immunoglobulin G antibody, mediating B-cell lysis by complement-dipendent cytotoxicity, antibody dependent cellular cytoxicity, and induction of apoptosis. In addition to the treatment of B-cell malignancies, Rituximab has been shown to be effective also in the treatment of several autoimmune diseases. Chronic GVHD is characterized not only by immunodeficiency but also by autoimmunity. Rituximab has been shown to be useful in combination with preparative regimen, for reducing incidence and severity of aGVHD [50,52].

Firstly, Ratanatharathorn reported a case of patient affected by thrombocytopenia with chronic GVHD treated with rituximab that showed apart from the recovery of thrombocytopenia, severe xerostomia of the patient also responded after 4 doses of rituximab at 375 mg/m^2 [53]. Based on this original positive experience, the same author reported an overall response rate of 50% in 8 patients treated for refractory chronic GVHD with rituximab 375mg/m^2 once a week for 4 doses, mainly in patients with skin involvement [54].

Subsequently, other cases of patients affected by chronic GVHD associated to immune phenomena like miastenia gravis, bullous pemphigoid, or autoimmune haemolytic anemia were treated with rituximab [55-57].

Similarly, Okamoto *et al.* reported encouraging results in treating 3 patients with sclerodermatous cGVHD [58].

Cutler *et al.* designed a phase I/II study with rituximab in steroid-refractory cGVHD in which 21 adult patients were treated with 38 cycles of rituximab. The response rate was 70%, including 2 patients with complete responses. Patients with cutaneous or musculoskeletal manifestations of chronic GVHD had the highest probability to respond to rituximab. Antibody titers against Y chromosome–encoded minor HLA antigens decreased, whereas titers against infectious antigens as EBV and tetanus remained stable. Toxicity was limited to infectious episodes [59]. GITMO performed a retrospective study concerning the use of Rituximab in 38 patients with refractory chronic GVHD. The median number of failed treatment lines in these patients was 3. The overall response rate was 65% with a significant improvement in the most cases with skin involvement (63%). An important response was also obtained in patients with mouth involvement (48% responses). For patients with skin and mouth chronic GVHD, the therapeutic effect of Rituximab allowed the reduction of baseline immunosuppressive therapy in responding patients [60].

Mohty *et al.* described 15 consecutive individual experiencing severe or refractory chronic GVHD who received and failed at least two lines of immunosuppressive therapy. Overall, 10 (66%) patients responded to Rituximab with 3 CR. Interestingly the authors reported 86% median dose reduction of coticosteroides in 11 of 15 cases treated with rituximab [61].

Interestingly, Von Bonin *et al.* reported an ORR of 69% with a lower dose of Rituximab at 50 mg/m^2 weekly in a group of 13 patients treated for corticosteroids-refractory cGVHD [62].

A systematic rewiew and meta-analysis carried out by Kharfan-Dabaja *et al.* reported a pooled of proportion of ORR and mortality of 66% and 15.8% respectively. The totality of evidence demonstrates that the skin is the most responsive site, particulary in cases of lichenoid or sclerodermatous chronic GVHD [63].

CONCLUSIONS

GVHD is one of the most important complications of allogeneic HSCT and one of the major determinants of transplant-related mortality and an additional cause affecting patient's late outcome. Despite a number of immunosuppressive agents have demonstrated therapeutic activity in GVHD, most of this treatment options have not been investigated in deep. The major obstacle in evaluating new drugs in treating acute and chronic GVHD is the lack of randomized trials, retrospective studies and methodological difficulties in defining resistant patients to first-line treatment and well established response criteria.

REFERENCES

[1] Kolb, H.J.; Schattenberg, A.; Goldman, J.M.; Hertenstein, B.; Jacobsen, N.; Arcese, W.; Ljungman, P.; Ferrant, A.; Verdonck, L.; Niederwieser, D.; van Rhee, F.; Mittermueller, J.; de Witte, T.;

Holler, E.; Ansari, H. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. *Blood*, **1995**, *86*, 2041-50

[2] Guglielmi, C.; Arcese, W.; Dazzi, F.; Brand, R.; Bunjes, D.; Verdonck, L.F.; Schattenberg, A.; Kolb, H.J.; Ljungman, P.; Devergie, A.; Bacigalupo, A.; Gomez, M.; Michallet, M.; Elmaagacli, A.; Gratwohl, A.; Apperley, J.; Niederwieser, D. Donor lymphocyte infusion for relapsed chronic myelogenous leukemia: prognostic relevance of the initial cell dose. *Blood*, **2002**, *100*, 397-405

[3] Cornetta, K.; Laughlin, M.; Carter, S.; Wall, D.; Weinthal, J.; Delaney, C.; Wagner, J.; Sweetman, R.; McCarthy, P.; Chao, N. Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT). *Biol. Blood Marrow Transplant.*, **2005**, *11*, 149-60.

[4] Wagner, J.E.; Barker, J.N.; DeFor, T.E.; Baker, K.S.; Blazar, B.R.; Eide, C.; Goldman, A.; Kersey, J.; Krivit, W.; MacMillan, M.L.; Orchard, P.J.; Peters, C.; Weisdorf, D.J.; Ramsay, N.K.; Davies, S.M. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. *Blood*, **2002**, *100*, 1611-1618.

[5] Barker, J.N.; Wagner, J.E. Umbilical cord blood transplantation: current practice and future innovations. *Crit. Rev. Oncol. Hematol*., **2003**, *48*, 35-43.

[6] Carella, A.M.; Giralt, S.; Slavin, S. Low intensity regimens with allogeneic hematopoietic stem cell transplantation as treatment of hematologic neoplasia. *Haematologica*, **2000,** *85*, 304-13

[7] Diaconescu, R.; Flowers, C.R.; Storer, B.; Sorror, M.L.; Maris, M.B.; Maloney, D.G.; Sandmaier, B.M.; Storb, R Morbidity and mortality with nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. *Blood*, **2004**, *104*, 1550-1558.

[8] Carella, A.M.; Beltrami, G.; Carella, M. Jr; Corsetti, M.T.; Scalzulli, R.P.; Greco, M. Immunosuppressive non-myeloablative allografting as salvage therapy in advanced Hodgkin's disease. *Haematologica*, **2001** , *86*, 1121-1123.

[9] Mielcarek, M.; Martin, P.J.; Leisenring, W.; Flowers, M.E.; Maloney, D.G.; Sandmaier, B.M.; Maris, M.B.; Storb, R. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood*, **2003**, *102*, 756-762.

[10] Carella, A.M.; Beltrami, G.; Corsetti, M.T.; Scalzulli, P.; Carella, A.M. Jr; Musto, P. A reduced intensity conditioning regimen for allografting following autografting is feasible and has strong anti-myeloma activity. *Haematologica*, **2004,** *89*, 1534-1536.

[11] Gratwohl, A.; Baldomero, H.; Schwendener, A.; Gratwhohl, M.; Apperley, J.; Frauendorfer, D.; Niederwieser, D. The EBMT activity survey 2008 impact of team size, density and new trends. *Bone Marrow Transpl*., **2010**, (29 March 2010) doi:10.1038/bmt.2010.69

[12] Ferrara, J.L.M.; Levine, J.E.; Reffy, P.; Holler, E. Graft-versus-host Disease. Lancet, **2009,** 373, 1550-1561.

[13] Loiseau, P.; Busson, M.; Balere, M.L.; Dormoy, A.; Bignon, J.D.; Gagne, K.; Gebuhrer, L.; Dubois, V.; Jollet, I.; Bois, M.; Perrier, P.; Masson, D.; Moine, A.; Absi, L.; Reviron, D.; Lepage, V.; Tamouza, R.; Toubert, A.; Marry, E.; Chir, Z.; Jouet, J.P.; Blaise, D.; Charron, D.; Raffoux, C. HLA Association with hematopoietic stem cell transplantation outcome: the number of mismatched at HLA-A,-B,-C,-DRB1,or DQB1 is strongly associated with overall survival. *Biol. Blood Marrow Transplant*., **2007**, *13*, 965-974

[14] Goker, H.; Haznedaroglu, IC.; Chao, NJ. Acute Graft-versus-host disease: pathobiology and management. *Exp. Hematol*., **2001**, *29*, 259-277

[15] Ferrara, J.L..; Levy, R.; Chao, N.J. Pathophysiologic mechanism of acute graft-versus-host disease. *Biol. Blood Marrow Transplant* ., **1999,** *5*, 347-356

[16] Ferrara, JL.; Deeg HJ. Graft-versus-host disease. *New Engl. J. Med*., **1991**, *324*, 667-674

[17] Bacigalupo, A.; Lamparelli, T.; Bruzzi, P.; Guidi, S.; Alessandrino, P.E.; di Bartolomeo, P.; Oneto, R.; Bruno, B.; Barbanti, M.; Sacchi, N.; Van Lint, M.T.; Bosi, A. Antithymocyte globulin for graft-versus-host disease prophylaxis in transplants from unrelated donors: 2 randomized studies from Gruppo Italiano Trapianti Midollo Osseo (GITMO). *Blood*, **2001**, *98*, 2492-2497.

[18] Bacigalupo, A.; Lamparelli, T.; Barisione, G.; Bruzzi, P.; Guidi, S.;, Alessandrino, P.E.; di Bartolomeo, P.; Oneto, R.; Bruno, B.; Sacchi, N.; van Lint, M.T.; Bosi, A. Gruppo Italiano Trapianti Midollo Osseo (GITMO).Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. *Biol. Blood Marrow Transplant*., **2006**, *12*, 560-565.

[19] Finke, J.; Bethge, W.A.; Schmoor, C.; Ottinger, H.D.; Stelljes, M.; Zander, A.R.; Volin, L.; Ruutu, T.; Heim, D.A.; Schwerdtfeger, R.; Kolbe, K.; Mayer, J.; Maertens, J.A.; Linkesch, W.; Holler, E.; Koza, V.; Bornhäuser, M.; Einsele, H.; Kolb, H.J.; Bertz, H.; Egger, M.; Grishina, O.; Socié, G. ATG-Fresenius Trial Group.Standard graft-versus-host disease prophylaxis with or without anti-T cell globulin in hematopoietic cell transplantation from matched unrelated donors a randomised, open-label, multicentre phase 3 trial. *Lancet Oncol*., **2009,** *10*, 855-64.

[20] MacMillan, M.L.; Weisdorf, D.J.; Wagner, J.E.; DeFor, T.E.; Burns, L.J.; Ramsay, N.K.; Davies, S.M.; Blazar, B.R.. Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems. *Biol. Blood Marrow Transplant*., **2002,** *8*, 387-394.

[21] Martin, P.J.; Schoch, G.; Fisher, L.; Byers, V.; Appelbaum, F.R.; McDonald, G.B.; Storb, R.; Hansen, J.A. A retrospective analysis of therapy for acute graft-versus-host disease: secondary treatment. *Blood*, **1991,** *77*, 1821-1828.

[22] Picardi, A.; Lanti, A.; Cudillo, L.; Cerretti, R.; Dentamaro, T.; De Angelis, G.; Ferraro, A.; Di Veroli, A.; Adorno, G.; Arcese, W. Platelet gel for treatment of mucocutaneous lesions related to graft-versus-host disease after allogeneic hematopoietic stem cell transplant. *Transfusion*, **2010**, *50*, 501-506.

[23] Berger, M.; Biasin, E.; Saglio, F.; Fagioli, F. Innovative approaches to treat steroid-resistant or steroid refractory GVHD. *Bone Marrow Transpl*., **2008**, *42*, S101-S105

[24] Levine, J.E.; Paczesny, S.; Mineishi, S.; Braun, T.; Choi, S.W.; Hutchinson, R.J.; Jones, D.; Khaled, Y.; Kitko, C.L.; Bickley, D.; Krijanovski, O.; Reddy, P.; Yanik, G.; Ferrara, J.L.Etanercept plus methylprednisolone as initial therapy for acute graft-versus-host disease. *Blood*, **2008**, *111*, 2470-2475.

[25] Alousi, A.M.; Weisdorf, D.J.; Logan, B.R.; Bolaños-Meade, J.; Carter, S.; Difronzo, N.; Pasquini, M.; Goldstein, S.C.; Ho, V.T.; Hayes-Lattin, B.; Wingard, J.R.; Horowitz, M.M.; Levine, J.E. Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trial Network. *Blood*, **2009,** *114*, 511-517.

[26] Kennedy, G.A.; Bluter, J.; Westerrn, R.; Morton, J.; Durrant, S.; Hill, GR. Combination antithymocite globulin and soluble TNFα inhibitor (etanercept) +/- mycophenolate mofetil for treatment of steroid refractory acute graft-versus-host disease. *Bone Marrow Transplantation*, **2006,** *37*, 1143-1147.

[27] Couriel, D.; Saliba, R.; Hicks, K.; Ippoliti, C.; de Lima, M.; Hosing, C.; Khouri, I.; Andersson, B.; Gajewski, J.; Donato, M.; Anderlini, P.; Kontoyiannis, D.P.; Cohen, A.; Martin, T.; Giralt, S.; Champlin, R. Tumor Necrosis factor-α blockade for the treatment of acute GVHD. *Blood*, **2004**, *104*, 649-654.

[28] Patriarca, F.; Sperotto, A.; Damiani, D.; Morreale, G.; Bonifazi, F.; Olivieri, A.; Ciceri, F.; Milone, G.; Cesaro, S.; Bandini, G.; Dini, G.; Corradini, P.; Fanin, R. Infliximab treatment for steroid-refractory acute-graft-versus-host disease. *Haematologica*, **2004**, *89*, 1352-1359.

[29] Marty, F.M.; Lee, S.J.; Fahey, M.M.; Alyea, E.P.; Soiffer, R.J.; Antin, J.H.; Baden, L.R. Infliximab use in patients with severe graft-versus-host disease and other emerging risk factors of non-Candida invasive fungal infections in allogeneic hematopoietic stem cell transplant recipients: a cohort study. *Blood*, **2003**, *102*, 2768-2776.

[30] Lee, S.J.; Zahrieh, D.; Agura, E.; MacMillan, M.L.; Maziarz, R.T.; McCarthy, P.L. Jr; Ho, V.T.; Cutler, C.; Alyea, E.P.; Antin, J.H.; Soiffer, R.J. Effect of up-front daclizumab when combined with steroids for the treatment of acute graft-versus-host disease: results of a randomized trial. *Blood*, **2004,** *104*, 1559-1564.

[31] Willenbacher, W.; Basara, N.; Blau, I.W.; Fauser, A. A.; Kiehl, M.G. Treatment of steroid refractory acute and chronic graft-

versus-host disease with daclizumab. *Br. J. Haematol*., **2001**, *112*, 820-823.

[32] Przepiorka, D.; Kernan, N.A.; Ippoliti, C.; Papadopoulos, E.B.; Giralt, S.; Khouri, I.; Lu, J.G.; Gajewski, J.; Durett, A.; Cleary, K.; Champlin, R.; Andersson, B.S.; Light, S. Daclizumab, a humanized anti-interleukin-2 receptor alpha chain antibody, for treatment of acute graft-versus-host disease. *Blood*, **2000**, *100*, 83-89.

[33] Carpenter, P.A.; Appelbaum, F.R.; Corey, L.; Deeg, H.J.; Doney, K.; Gooley, T.; Krueger, J.; Martin, P.; Pavlovic, S.; Sanders, J.; Slattery, J.; Levitt, D.; Storb, R.; Woolfrey, A.; Anasetti, C. A humanized non-FcR-binding anti-CD3 antibody, visilizumab, for treatment of steroid-refractory acute graft-versus-host disease. *Blood*, **2002**, *99*, 2712-2719.

[34] Carpeneter, P.A.; Lowder, J.; Johnston, L.; Frangoul, H.; Khoury, H.; Parker, P.; Jerome, K.R.; McCune, J.S.; Storer, B.; Martin, P.; Appelbaum, F.; Abonour, R.; Westervelt, P.; Anasetti, C. A phase II multicenter study of visilizuma, humanized anti-CD3 antibody, to treat steroid-refractory acute graft-versus-host disease. *Biol. Blood Marrow Transplant*., **2005**, *11*, 465-471.

[35] Auffermann-Gretzinger, S.; Eger, L.; Schetelig, J.; Bornhauser, M.; Heidenreich, F.; Ehninger, G. Alemtuzumab depletes dendritic cells more effectively in blood than in skin: a pilot study in patients with chronic lymphocytic leukemia. *Transplantation*, **2007**, *83*, 1268-1272.

[36] Klangsinsirikul, P.; Carter, G.I.; Byrne, J.L.; Hale, G.; Russel, N.H. Campath-1G causes rapid depletion of circulating host dendritic cells (DCs) before allogeneic transplantation but does not delay donor DC reconstitution. *Blood*, **2002**, *99*, 2586-2591.

[37] Faulkner, R.D.; Craddock, C.; Byrne, J.L.; Mahendra, P.; Haynes, A.P.; Prentice, H.G.; Potter, M.; Pagliuca, A.; Ho, A.; Devereux, S.; McQuaker, G.; Mufti, G.; Yin, J.L.; Russell, N.H. BEAM-alemtuzumab reduced-intensity allogeneic stem cell transplantation for lymphoproliferative diseases: GVHD, toxicity, and survival in 65 patients. *Blood*, **2004**, *103*, 428-434.

[38] Kottaridis, P.D.; Milligan, D.W.; Chopra, R.; Chakraverty, R.K.; Chakrabarti, S.; Robinson, S.; Peggs, K.; Verfuerth, S.; Pettengell, R.; Marsh, J.C.; Schey, S.; Mahendra, P.; Morgan, G.J.; Hale, G.; Waldmann, H.; de Elvira, M.C.; Williams, C.D.; Devereux, S.; Linch, D.C.; Goldstone, A.H.; Mackinnon, S. *In vivo* CAMPATH-1H prevents graft-versus-host disease following nonmyeloablative stem cell transplantation. *Blood*, **2000,** *96*, 2419-2425.

[39] Kottaridis, P.D.; Milligan, D.W.; Chopra, R.; Chakraverty, R.K.; Chakrabarti, S.; Robinson, S.; Peggs, K.; Verfuerth, S.; Pettengell, R.; Marsh, J.C.; Schey, S.; Mahendra, P.; Morgan, G.J.; Hale, G.; Waldmann, H.; Ruiz de Elvira, M.C.; Williams, C.D.; Devereux, S.; Linch, D.C.; Goldstone, A.H.; MacKinnon, S. *In vivo* CAMPATH-1H prevents GvHD following nonmyeloablative stem-cell transplantation. *Cytotherapy*, **2001**, *3*, 197-201.

[40] Peggs, K.S. Role of MabCampath in allogeneic transplantation. *Ann. Hematol*., **2004**, *83*(Suppl 1), S66-S68.

[41] Pérez-Simón, J.A.; Kottaridis, P.D.; Martino, R.; Craddock, C.; Caballero, D.; Chopra, R.; García-Conde, J.; Milligan, D.W.; Schey, S.; Urbano-Ispizua, A.; Parker, A.; Leon, A.; Yong, K.; Sureda, A.; Hunter, A.; Sierra, J.; Goldstone, A.H.; Linch, D.C.; San Miguel, J.F.; Mackinnon, S. Nonmyeloablative transplantation with or without alemtuzumab: comparison between 2 prospective studies in patients with lymphoproliferative disorders. *Blood*, **2002**, *100*, 3121-3127.

[42] Carella, A.M.; Beltrami, G.; Scalzulli, P.R.; Carella, A.M. Jr; Corsetti, M.T. Alemtuzumab can successfully treat steroid-refractory acute graft-versus-host disease (aGVHD). *Bone Marrow Transplantation*, **2004,** *33*, 131-132.

[43] Schub, N.; Günther, A.; Schrauder, A.; Claviez, A.; Ehlert, C.; Gramatzki, M.; Repp, R. Therapy of steroid-refractory acute GVHD with CD52 antibody alemtuzumab is effective. *Bone Marrow Transplantation*, **2010**, In press.

[44] Chakraverty, R.; Orti, G.; Roughton, M.; Shen, J.; Fielding, A.; Kottaridis, P.; Milligan, D.; Collin, M.; Crawley, C.; Johnson, P.; Clark, A.; Parker, A.; Bloor, A.; Pettengell, R.; Snowden, J.; Pettitt, A.; Clark, R.; Hale, G.; Peggs, K.; Thomson, K.; Morris, E.; Mackinnon, S. Impact of *in vivo* alemtuzumab dose before reduced intensity conditioning and HLA-identical sibling stem cell transplantation: pharmacokinetics, GVHD, and immune reconstitution. *Blood*, **2010,** *116*, 3080-3088.

[45] Ruiz-Arguelles, G.J.; Gil-Beristain, J.; Magana, M.; Ruiz-Delgado, G.J. Alemtuzumab- induced resolution of refractory cutaneous chronic graft-versus-host disease. *Biol. Blood Marrow Transplant.*, **2008**, *14*, 7-9.

[46] Schnitzler, M.; Hasskarl, J.; Egger, M.; Bertz, H.; Finke, J. Successful treatment of severe acute intestinal graft-versus-host resistant to systemic and topical steroids with alemtuzumab. Biol. Blood Marrow Transplant., **2009**, 15, 910-918.

[47] Martinez, C.; Solano, C.; Ferra, C.; Sampol, A.; Valcarcel, D.; Perez-Simon, J.A. Alemtuzumab as treatment of steroid-refractory acte graft-versus-host disease: results of a phase II study. *Biol. Blood Marrow Transplant*., **2009**, *15*, 639-64.

[48] Farag, S.S. Chronic graft-versus-host disease. *Bone Marrow Transplantation*, **2004**, *33*, 569-577.

[49] Wolff, D.; Schleuning, M.; von Harsdorf, S.; Bacher, U.; Gerbitz, A.; Stadler, M.; Ayuk, F.; Kiani, A.; Schwerdtfeger, R.; Vogelans, G.B.; Kobbe, G.; Gramatzki, M.; Lawitscha, A.; Mohty, M.; Pavletic, S.Z.; Greinix, H.; Holler, E. Consensus conference on clinical practice in chronic GVHD: second-line treatment of chronic Graft-versus-Host Disease. *Biol. Blood Marrow Transplant*., **2010**, *16*, 2054-2058.

[50] Khouri, I.F.; Saliba, R.M.; Giralt, S.A.; Lee, M.S.; Okoroji, G.J.; Hagemeister, F.B.; Korbling, M.; Younes, A.; Ippoliti, C.; Gajewski, J.L.; McLaughlin, P.; Anderlini, P.; Donato, M.L.; Cabanillas, F.F.; Champlin, R.E. Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. *Blood*, **2001**, *98*, 3595-3599.

[51] Khouri, I.F.; McLaughlin, P.; Saliba, R.M.; Hosing, C.; Korbling, M.; Lee, M.S.; Medeiros, L.J.; Fayad, L.; Samaniego, F.; Alousi, A.; Anderlini, P.; Couriel, D.; de Lima, M.; Giralt, S.; Neelapu, S.S.; Ueno, N.T.; Samuels, B.I.; Hagemeister, F.; Kwak, L.W.; Champlin, R.E. Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. *Blood*, **2008**, *111*, 5530-5536.

[52] Kharfan-Dabaja, M.A.; Bazarbachi, A. Emerging role of CD20 blockade in allogeneic hematopoietic cell transplantation. *Biol. Blood Marrow Transplant*., **2010**, *16*, 1347-1354.

[53] Ratanatharathorn, V.; Carson, E.; Reynolds, C.; Ayash, L.J.; Levine, J.; Yanik, G.; Silver, S.M.; Ferrara, J.L.; Uberti, J.P. Anti-CD20 chimeric monoclonal antibody treatment of refractory immune-mediated thrombocytopenia in a patient with chronic graft-versus-host disease. *Ann. Inter. Med*., **2000**, *133*, 275-279.

[54] Ratanatharathorn, V.; Ayash, L.; Reynolds, C.; Silver, S.; Reddy, P.; Becker, M.; Ferrara, J.L.; Uberti, J.P. Treatment of ghronic graft-versus-host disease with anti-CD20 chimeric monoclonal antibody. *Biol. Blood Marrow Transplant*., **2003**, *9*, 505-511.

[55] Zaja, F.; Russo, D.; Fuga, G.; Perella, G.; Baccarani, M. Rituximab for myastenia gravis developing after bone marrow transplant. *Neurology*, **2000**, *55*, 1062-1063.

[56] Ship, A.; May, W.; Lucas, K. Anti CD-20 monoclonal antibody therapy for autoimmune haemolytic anemia following T cell-depleted, haplo-identical stem cell transplantation. *Bone Marrow Transplant*., **2002**, *29*, 365-366.

[57] Szaboles, P.; Reese, M.; Yancey, K.B.; Hall, R.P.; Kurtzberg, J. Combination treatment of bullous pemphigoid with anti CD-20 and CD-25 antibodies in a patient with chronic graft-versus-host disease. *Bone Marrow Transplant*., **2002**, *30*, 327-329.

[58] Okamoto, M.; Okano, A.; Akamatsu, S.; Ashilara, E.; Inaba, T.; Takenaka, H.; Katoh, N.; Kishimoto, S.; Shimazaki, C. Rituximab is effective for steroid-refractory sclerodermatous chronic graft-versus-host disease. *Leukemia*, **2006,** *20*, 172-173.

[59] Cutler, C.; Miklos, D.; Kim, H.T.; Treister, N.; Woo, S.B.; Bienfang, D.; Klickstein, L.B.; Levin, J.; Miller, K.; Reynolds, C.; Macdonell, R.; Pasek, M.; Lee, S.J.; Ho, V.; Soiffer, R.; Antin, J.H.; Ritz, J.; Alyea, E. Rituximab for steroid-refractory chronic graft-versus-host disease. *Blood*, **2006,** *108*, 756-762.

[60] Zaja, F.; Bacigalupo, A.; Patriarca, F.; Stanzani, M.; Van Lint, M.T.; Filì, C.; Scimè, R.; Milone, G.; Falda, M.; Vener, C.; Laszlo, D.; Alessandrino, P.E.; Narni, F.; Sica, S.; Olivieri, A.; Sperotto, A.; Bosi, A.; Bonifazi, F.; Fanin, R. Treatment of refractory chronic GVHD with Rituximab: a GITMO study. *Bone Marrow Transplant*., **2007,** *40*, 273-277.

[61] Mohty, M.; Marchetti, N.; El-Cheick, J.; Faucher, C.; Furst, S.; Blaise, D. Rituximab as salvage therapy for refractory chronic GVHD. *Bone Marrow Transplant*, **2008**, *41*, 909-911.

[62] von Bonin, M.; Oelschlägel, U.; Radke, J.; Stewart, M.; Ehninger, G.; Bornhauser, M.; Platzbecker, U. Treatment of chronic steroid-refractory graft-versus-host disease with low-dose Rituximab. *Transplantation*, **2008**, *86*, 875-879.

[63] Kharfan-Dabaja, M.A.; Mhaskar, A.R.; Djulbegovic, B.; Cutler, C.; Mohty, M.; Kumar, A. Efficacy of rituximab in the setting of steroid refractory chronic graft-versus-host disease: a systematic review and meta-analysis. *Biol. Blood Marrow Transplant*, **2009**, 15, 1005-1013.

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