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Chronic Graft-Versus-Host Disease
Pathogenesis and Clinical Management

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Abstract

Chronic graft-versus-host disease (cGVHD) is the most common and severe complication among patients surviving >100 days after allogeneic transplantation. It starts with the expansion of donor T cells in response to alloantigens or autoantigens that are unchecked by normal thymic or peripheral mechanisms of deletion. The T cells induce damage to target organs either directly through cytolytic attack, inflammatory cytokines and fibrosis, or by promoting B cell activation and production of autoantibodies.

HLA disparity, donor and patient age and sex, source of progenitor cells, graft composition and previous acute GVHD are the main factors that predict the risk of developing cGVHD. Once the diagnosis has been established, patients needing treatment (extensive cGVHD) must be identified. Poor prognostic factors such as extensive skin involvement, thrombocytopenia and progressive-type onset of cGVHD must be considered in order to define the immunosuppressive treatment requirements.

Prednisone, together with a calcineurin inhibitor such as ciclosporin or tacrolimus, can be considered the standard regimen as primary treatment for cGVHD. Using that approach, among high-risk patients (identified as those with extensive cGVHD plus thrombocytopenia) 3-year survival reached 52%. Concerning salvage regimens, to date there is no clear standard regimen for cGVHD treatment, the best choice being to enter the patient into a clinical trial. Immunosuppressive drugs that inhibit T cell activation, proliferation or survival, such as mycophenolate mofetil, the anti-interleukin-2α receptor antagonist daclizumab, sirolimus (rapamycin), extracorporeal photopheresis and pentostatin (deoxycoformycin), among other agents, have been used with a very wide range of complete responses ranging from 5% to 50%. In addition, anti-cytokine or B cell inhibitors such as etanercept or rituximab have also been evaluated.

The severe immunosuppression induced by those drugs increases the risk of infectious complications and may have a deleterious effect on the graft versus tumour effect after transplant so that newer strategies based on the selective depletion of alloreactive T cells and induction of more specific immunotolerance against host tissues are required.

Chronic graft-versus-host disease (cGVHD) is the most common complication after allogeneic stem cell transplantation (ASCT) and occurs in 20–77% of patients at long-term follow-up.[1-4] The

higher non-relapse mortality among patients who develop it, the higher incidence of secondary malignancies and the impairment of quality of life explains why cGVHD remains the most severe complication among patients surviving >100 days after ASCT.[5-7] Despite that, and contrary to acute graft-versus-host disease (aGVHD),[8-12] the pathophysiology of cGVHD is poorly understood. Although both entities overlap extensively in terms of pathogenesis, several lines of evidence suggest that cGVHD is not just a continuation of aGVHD, since the former is largely an inflammatory and fibrotic process, while the latter requires apoptosis and necrosis.[13] Moreover, 25–35% of cGVHD appears *de novo* without any prior aGVHD.[14]

Clinical management of cGVHD remains rather confusing and this is in part as a result of the fact that the clinical grading, and the subsequent therapy, is based on a classification described >2 decades ago (table I).[15] The increasing use of unrelated donors, peripheral blood as a source of haematopoietic stem cells and the progressive improvement in survival among older patients has highlighted the limitations of the classification system, which categorises patients into those requiring treatment (extensive cGVHD) or not (limited cGVHD) but does not stratify patients according to outcome.[16,17] The management of cGVHD is further complicated because it is, in many studies, a favourable prognostic factor because of a powerful graft-versus-leukaemia (GVL) effect, which contributes to the lower relapse rate observed among patients in whom it develops.[18-26] For that reason, immunosuppressive treatment must be administered carefully not only on the basis of the severity of cGVHD but also on the risk of relapse and the disease status at the time of treatment.[21]

Table I. Clinicopathological classification of chronic graft-versus-host disease (cGVHD)

Limited cGVHD
Either or both:
Localised skin involvement
Hepatic dysfunction as a result of cGVHD
Extensive cGVHD
Either:
Generalised skin involvement or
Localised skin involvement and/or hepatic dysfunction as a result of cGVHD
Plus:
Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis or
Involvement of eye (Schirmer tear test with <5mm wetting) or
Involvement of minor salivary glands or mucosa demonstrated on labial biopsy or
Involvement of any other target organ

The most common approach for the treatment of these patients has been the use of prednisone. When used as a single agent, survival at 3 years among high-risk patients reached 26%. In this subset of patients, the addition of ciclosporin increased survival to 52%.

As with aGVHD, to date there is no clear standard salvage regimen for cGVHD treatment. As mentioned previously, cGVHD starts with the expansion of donor T cells, so most treatment strategies have focused on the blockade of those cells. Among the strategies used, mycophenolate mofetil (MMF), daclizumab, sirolimus and pentostatin (deoxycoformycin) or extracorporeal photopheresis, have been used as rescue treatments. In addition, other strategies that focus of the blockade of B cells, such as rituximab, have also been reported.

Future strategies based on a selective depletion of alloreactive T cells or on the induction of specific tolerance against host antigens, such as expansion of regulatory T cells, the use of dendritic cells in different stages of maturation or the infusion of mesenchymal stem cells, are promising approaches.

1. Pathogenesis

cGVHD starts with the expansion of donor T cells in response to alloantigens or autoantigens that are unchecked by normal thymic or peripheral mechanisms of deletion. The T cell precursors may undergo aberrant thymic education after ASCT that makes them autoreactive.[27] The role of alloreactivity versus autoreactivity in the pathogenesis of cGVHD remains an area of intense debate. Alloreactivity to minor histocompatibility antigens explains cGVHD as a late phase of aGVHD. By contrast, manifestations of cGVHD frequently mimic those of autoimmune diseases.[28,29] Thus, cGVHD is dependent on the continued presence of host reac-

tive donor T cells that, unlike those in aGVHD, fail to become tolerant to the host. Those T cells induce damage to target organs either directly through cytolytic attack, inflammatory cytokines and fibrosis, or by promoting B cell activation and production of autoantibodies.

In that sense, there is increasing evidence about B cell disregulation[30] that might contribute to the pathogenesis of cGVHD. Thus, in a murine model of cGVHD, the expansion of host B cells was thought to play a central role in the development of cGVHD with autoantibody production and glomerulonephritis.[31] In the clinical setting, occurrence of antinuclear, anti-double-stranded DNA and anti-smooth muscle autoantibodies ranges in frequency from 11% to 62%.[32,33] Nevertheless, the pathogenetic role of autoantibodies in cGVHD remains poorly defined.

The cytokine production pattern is clearly different between aGVHD and cGVHD.[34] Whereas the predominant cytokines produced in aGVHD are T helper (T_h)-1 cytokines, those in cGVHD are T_h2 cytokines. In a cGVHD mouse model, unstimulated splenocytes show increased interleukin (IL)-4 and IL-10 messenger RNA levels at day 7 of culture, which remain after 2–3 weeks.[35] Nevertheless, differences in the cytokine pattern between aGVHD and cGVHD are far more complex and, in that sense, the production of the T_h2-type cytokines IL-13 and, to a lesser extent, IL-5 has recently been identified as a strong predictor of aGVHD in an *in vitro* model.[36] Moreover, a mouse model showed that both forms of GVHD are initially characterised by increased T_h2 cytokine (IL-4 and IL-10) production.[37] Finally, several studies have shown decreased T_h2-type cytokine levels such as IL-4, IL-5 and IL-10 among patients who developed cGVHD compared with those who did not.[38,39]

In addition to an impaired thymic function after ASCT, which may be the primary reason for the abnormal T cell reconstitution, peripheral mechanisms of tolerance such as immature dendritic cells, which contribute to maintain immunological self-tolerance in the steady state,[40,41] are altered and play a crucial role not only in the development of aGVHD but also of cGVHD. In particular, both host and donor dendritic cells may contribute to the induction of CD4-mediated cGVHD in skin (mainly recipient) or gut (mainly donor dendritic cells).[42,43] Other mechanisms involved in maintaining self-tolerance, such as regulatory T cells, are reduced in frequency in patients diagnosed with cGVHD, although conflicting data have been reported in this regard.[44,45]

2. Clinical Management

2.1 Risk Factors

HLA disparity is a potent factor in predicting the risk of cGVHD, which occurs in approximately 40% of patients receiving HLA identical sibling unmanipulated transplants, 50% of those receiving HLA mismatched sibling transplants and 70% of those receiving matched unrelated transplants.[28,46] HLA matching between donor and recipient improves the success of unrelated haematopoietic cell transplantation, and several studies have suggested that it is advantageous to match at the allele level for HLA-A, -B, -C and DRB1. For patients with donors who are matched at the antigen level, Flomenberg et al.[47] reported that a single allele-level mismatch is associated with an 8–12% reduction in survival at 5 years. For patients who have multiple highly matched suitable donors, there might be an additional benefit from matching HLA-DPB1 and HLA-DQB1.[47-49] The age of the recipient is also an important factor. Thus, the incidence of cGVHD increases from 13% among patients aged >10 years but <20 years receiving a matched related donor to 46% for patients aged >20 years.[46] The donor's age may also influence the incidence of cGVHD, so age should be considered when selecting among comparably HLA-matched volunteer donors.[50] In addition, sex must be considered among the donor's characteristics in order to predict the risk of cGVHD, since sex mismatch, especially among male recipients, has been related to a higher incidence and/or more severe forms of cGVHD such as bronchiolitis obliterans.[51] This higher incidence has been attributed to the presence of antibodies

against minor histocompatibility antigens encoded in the Y chromosome.[52]

Stem cell source is also an important variable to be considered in terms of risk of cGVHD. Several studies, including a meta-analysis, have reported a higher incidence of extensive cGVHD when peripheral blood is used as a source of progenitor cells instead of bone marrow, although other randomised studies did not find significant differences.[53-60] In addition, cGVHD after peripheral blood stem cell transplantation (PBSCT) may be more protracted and less responsive to therapy compared with bone marrow transplantation (BMT). All these limitations must be considered in addition to the risk of relapse, since patients at high risk may benefit from receiving PBSCT, given the GVL effect associated with cGVHD. In addition, the use of PBSCT may decrease the risk of infections by shortening the period of neutropenia.[53-60] In addition to bone marrow and peripheral blood, cord blood has also been used as a source of progenitor cells. In a series of 682 adult patients undergoing unrelated cord blood or marrow transplantation, multivariate analysis showed that the incidence of cGVHD was not significantly different between patients receiving cord blood or those receiving bone marrow stem cells. Laughlin et al.[61] described a higher incidence of cGVHD among patients receiving HLA-mismatched cord blood compared with HLA-matched marrow from unrelated donors, although the incidence was similar when compared with those receiving mismatched marrow.[62] Interestingly, the outcome after cord blood transplantation was similar between patients receiving one versus two HLA-mismatched cord blood transplants, so the use of that source of progenitor cells allows a higher level of HLA disparity.

Regarding graft composition, T cell depletion has been shown to be an efficient method for preventing aGVHD, and overall and extensive cGVHD. Accordingly, the use of antithymocyte globulin before transplantation decreased the incidence of extensive cGVHD from 62% to 39% among patients who did not or did receive T cell depletion.[63] Low incidences of cGVHD have also been reported using CD34+ cell selection.[64] Another antibody used against T cell antigens is alemtuzumab (Campath-1h), which is directed against a heterogeneous 23–30kd glycoprotein (CDw52) expressed on lymphocytes, monocytes and dendritic cells. In a comparative study in the reduced intensity conditioning allogeneic transplant (allo-RIC) setting, patients receiving alemtuzumab had an incidence of cGVHD of 5% compared with 66% among patients who received methotrexate ($p < 0.001$).[65] Interestingly, in a recent prospective, randomised trial on unrelated donor bone marrow transplants, *ex vivo* T cell depletion did not decrease the incidence of cGVHD (29% vs 34% among patients receiving unmanipulated grafts),[66] although the incidence of aGVHD was significantly reduced among patients receiving T cell depletion. It should be noted that, in this study, T cell depletion averaged 1 log, which is less intense than that obtained with many methodologies currently used, and that range may be enough to decrease aGVHD but not cGVHD.

In addition to T cell composition, CD34+ cells must be considered among the graft variables that may have influence on the risk of cGVHD. In that regard, the infusion of a high number of progenitor cells has been related to a higher incidence of extensive cGVHD among patients receiving PBSCT, both in the myeloablative or in the allo-RIC setting.[20,67] Those differences could be attributed, at least in part, to the faster donor T cell engraftment among patients receiving the higher doses of CD34+ cells.[20,68-70] By contrast, the dose of CD34+ cell infused did not modify the risk of cGVHD among patients undergoing BMT.[71,72]

A three-phase model has been developed in order to explain the pathophysiology of aGVHD.[9,10,73] According to the model, the conditioning regimen plays a key role in phase 1, because of the extensive damage induced in host tissues, including the intestinal mucosa. Cells from damaged tissues secrete many cytokines, which trigger the subsequent phases of aGVHD. Accordingly, the intensity of the conditioning regimen influences the incidence and characteristics of aGVHD, as it has been shown in non-myeloablative or RIC transplants.[74-76] Interestingly, the use of allo-RIC may also impact on the

incidence and characteristics of cGVHD.[75,76] Thus, in a retrospective comparative study, cumulative incidences of cGVHD were 63% and 71%, respectively, among patients receiving myeloablative and allo-RIC (p = 0.084), with the latter group displaying a higher incidence of limited cGVHD (hazard ratio for limited cGVHD among allo-RIC recipients = 3.3; 95% CI 1.42, 8.08; p = 0.0017).[75]

Finally, aGVHD is a major predictor of cGVHD and 70–80% of patients with grades II–IV aGVHD will develop cGVHD,[66,77] so some attempts to decrease aGVHD could also allow a decrease in cGVHD. Moreover, aGVHD has been identified as a risk factor not only for developing cGVHD but also GVHD-related mortality.[78]

2.2 Classification of Chronic Graft-Versus-Host Disease: When to Treat?

The median day of diagnosis of cGVHD is 201 days after stem cell transplantation (SCT) from an HLA identical sibling donor, 159 days after SCT from an HLA non-identical sibling, and 133 from an unrelated donor.[46] Once the diagnosis has been suspected clinically and confirmed histologically, the extent of involvement must be ascertained. Symptoms related to the different organs involved are summarised in table II.

The current dichotomous system of grading cGVHD (table I) divides patients into those needing treatment (extensive cGVHD) and those who do not (limited cGVHD).[15] Although highly reproducible among the transplant centres, the system does not stratify patients for outcome. Moreover, a majority of patients experience extensive cGVHD, thus making that group extremely heterogeneous. In an attempt to construct a better prognostic model, Akpek et al.[17] analysed a series of 151 patients who developed cGVHD after BMT. The probability of survival at 10 years after the diagnosis of cGVHD was 51%. Three variables were identified that predicted the outcome of the patients: extensive skin involvement, thrombocytopenia and progressive-type onset of cGVHD. At the time of primary treatment failure, the previously mentioned risk factors, in addition to a Karnovsky score <50%, were identified as independent predictors for a poor outcome. This model was validated in a multicentre study, which categorised the patients according to the following patient score (PS): PS = (1.949 × [skin extent]) + (1.293 × [platelets]) + (0.514 × [type of onset]). The following conditions applied: (i) if the extent of skin involvement is >50% of body surface area, put 1, otherwise put 0; (ii) if the platelet count is <100 000 cells/μL, put 1, otherwise put 0; and (iii) if the cGVHD is progressive-type onset, put 1, otherwise put 0. The probability of survival at 3 years for patients with PS 0 (favourable-risk group; 0 risk factor [RF]) was 92%, 71% for patients with PS between 0 and 2 (intermediate-risk group; 1 RF), and 9% for patients with PS ≥2 (high-risk group; >1 RF).[81]

On the basis of the Karnofsky score, presence of chronic diarrhoea, weight loss and skin involvement, three subgroups of patients could be identified (low, intermediate and high risk) with different survivals in an International Bone Marrow Transplant Registry (IBMTR) study (figure 1).[78]

As mentioned in section 2.1, the use of PBSC instead of bone marrow as a source of progenitor cells modifies both the incidence and characteristics of cGVHD so that those differences may also modify prognostic factors. In a series of patients undergoing PBSCT, platelet count <100 000/mm^3 and prior liver aGVHD were identified as independent poor prognostic factors after cGVHD diagnosis.[80]

Other risk factors for non-relapse mortality among patients diagnosed with cGVHD have been identified, such as doses of prednisone >0.5 or >1 mg/kg bodyweight per day or serum total bilirubin >2 mg/dL at the time of cGVHD diagnosis, donor and patient age, and number of HLA mismatched loci.[82] Accordingly, in addition to the grade of cGVHD, all those variables should be taken into account before starting immunosuppressive treatment in patients diagnosed with cGVHD.

Finally, a consensus document[79,83] has recently been developed in an attempt to better define diagnostic criteria and clinical score systems. Biopsy is always encouraged but it is not mandatory if the patient has at least one diagnostic finding (sum-

Table II. Chronic graft-versus-host disease symptoms related to the different organs involved[79,80]

Organ	Clinical manifestation	Diagnosis
Skin	(40–76%) Poikiloderma Lichen planus-like features Sclerotic or morphea-like features	Clinical, biopsy
Hair	Scarring or non-scarring scalp alopecia	Clinical
Nails	Dystrophy, onycholysis Longitudinal ridging or splitting	Clinical, biopsy
Mouth	(25–55%) Lichen-type features Hyperkeratotic plaques Dryness, reduction in salivary flow	Clinical, biopsy
Eyes	Dryness, keratoconjunctivitis sicca Uveitis, punctate keratopathy	Clinical Schirmer tear test
Vagina	Lichen planus-like features Vaginal stenosis or scarring	Clinical, biopsy
Liver	(25–61%) Cholestasis	Liver function tests, biopsy
Gastrointestinal tract	(20–47%) Oesophageal web, stenosis, abnormal motility, malabsorption	Clinical, biopsy
Lung	(6–20%) Bronchiolitis obliterans	Biopsy, pulmonary function tests, computed tomography
Musculoskeletal system	(5–10%) Fasciitis, joint stiffness Polymyositis	Clinical, biopsy
Serous surfaces	(0–5%) Polyserositis	Clinical, biopsy
Haematopoietic system	Cytopenias Eosinophilia	Peripheral blood Bone marrow examination
Immune system	Profound immunodeficiency Functional asplenia Hypo/hypergammaglobulinaemia	Clinical Immunoglobulin quantification
Others	(0–10%) Autoimmune Disease Weight loss Fever Growth retardation Gonadal dysfunction Delayed pubertal development	As clinically indicated

marised in table II). Each organ involvement is scored from 0 to 3 (from none to severe organ involvement) so that cGVHD is classified into (i) mild cGVHD: one or two organs involved (except the lung) with no clinically significant impairment, i.e. maximum score 1 in all affected organs; (ii) moderate cGVHD: three or more organs involved without functional impairment (maximum score 1) or at least one organ with clinically significant involvement but no major disability (maximum score 2) or lung involvement with score 1; and (iii) severe cGVHD: indicates major disability in any organ (score of 3) or lung score 2. Although mild cGVHD may often be treated with topical therapies, moderate or severe cGVHD require systemic immunosuppressive treatment.

As well as non-relapse mortality, quality of life may also be heavily impaired by the occurrence of cGVHD. In this regard, previous cross-sectional studies have shown that quality of life may be impaired after allogeneic transplantation more significantly than after autologous transplantation, espe-

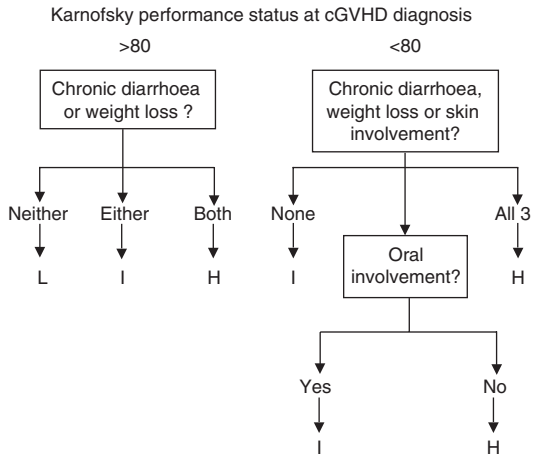

Fig. 1. Karnofsky performance status at chronic graft-versus-host disease (cGVHD) diagnosis (reproduced from Lee et al.,[78] with permission). **H** = high risk; **I** = intermediate risk; **L** = low risk, respectively. For oral involvement, **no** = intermediate risk; **yes** = high risk.

cially in the case of severe cGVHD.[84-88] In contrast, in a comparison of quality of life between patients undergoing autologous or reduced intensity conditioning allogeneic transplantation, the former group had worse results in terms of quality of life during the first 6 months after transplant, but from day 180 and onwards, i.e. after the median day of cGVHD flare, the quality of life of patients undergoing allo-RIC worsened concomitantly with the appearance of symptoms such as itching and ocular or mouth disturbances.[7]

Nevertheless, as mentioned previously, cGVHD is associated with a GVL effect, which has been demonstrated among patients diagnosed with chronic myeloid leukaemia, multiple myeloma, acute myeloid leukaemia and chronic lymphocytic leukaemia, among other malignancies, both in the myeloablative and in the non-myeloablative conditioning setting.[18-26] Thus, at the time of starting immunosuppressive treatment, not only must the stage and adverse prognostic factors related to cGVHD be considered but also the risk of relapse of the primary haematological malignancy. Accordingly, minimal residual disease monitoring may contribute to more individualised treatment strategies.[21,89,90]

3. Treatment

3.1 Current Standard Treatment

Whenever possible, patients diagnosed with cGVHD should be entered on treatment protocols. Moreover, the complexity of the diagnosis and grading, as well as the impact of the cGVHD on survival and quality of life, requires a multidisciplinary approach for the management of this complication and its sequels. The most common approach for the treatment of these patients has been the use of prednisone. Survival at 3 years reported among high-risk patients (identified as those with extensive cGVHD plus thrombocytopenia)[91] reached 26% when prednisone was used as a single agent. In that subset of patients, the addition of ciclosporin increased survival to 52%.[92] By contrast, the combination therapy decreased the risk for steroid-related toxicity but did not improve the results of prednisone as a single agent among patients undergoing BMT who developed standard-risk cGVHD.[93] The Seattle group described the combination of daily prednisone at 1 mg/kg per day and daily ciclosporin at 10 mg/kg per day divided into two doses based on ideal or actual weight, whichever was lower. After 2 weeks, providing the disease had not progressed, prednisone was tapered by 25% per week on alternate days until prednisone was administered at 1 mg/kg every other day. Response to treatment was evaluated at 8, 20 and 40 weeks. In the case of a response at 20 weeks, prednisone could again be tapered by 25% per week for 2 weeks, in order to maintain 0.5 mg/kg on alternate days, followed by a reduction in the dose of ciclosporin to reach half of the initial daily doses until 40 weeks. Slow tapering of prednisone and ciclosporin was scheduled in the case of complete response after 40 weeks.[93]

The use of thalidomide as a primary treatment in high-risk patients has been studied in two controlled trials, and no clinical benefit for its addition to prednisone and ciclosporin was found. Unfortunately, the use of thalidomide was associated with neutropenia and neurological toxicity, and treatment

was stopped before resolution of cGVHD in 92% of patients.[94,95]

Thus, the use of prednisone together with a calcineurin inhibitor such as ciclosporin or tacrolimus can be considered the standard regimen as a primary treatment for cGVHD. Unfortunately, results of this strategy among patients receiving PBSCT are even poorer compared with those reported among patients undergoing BMT. Accordingly, in a comparative study, 25 of 39 patients who received peripheral blood as a source of progenitor cells required more than two treatment cycles compared with 14 of 32 patients receiving BMT.[60] In our own experience with patients undergoing myeloablative PBSCT, the complete remission rate of extensive cGVHD was 41% and the relapse rate reached 58%[75] after first-line treatment. Accordingly, a better approach remains to be defined for the treatment of extensive cGVHD in the PBSCT setting.

3.2 Current Rescue Therapy

As with aGVHD, to date there is no clear standard salvage regimen for cGVHD treatment. Again, the best choice is to enter the patient into a clinical trial (table III).

3.2.1 Mycophenolate Mofetil

MMF is an antimetabolite that results in noncompetitive, reversible inhibition of inosine monophosphate dehydrogenase. This leads to selective inhibition of lymphocyte purine synthesis and proliferation. As far as refractory cGVHD treatment is concerned, 12 (35%) patients had a complete remission (CR) and 15 (44%) a partial response (PR) in a series of 34 patients who received MMF as a salvage therapy. Of 30 patients who were receiving prednisone, 22 were able to decrease the doses.[96] Other authors have also reported a similar response rate of 67%.[97] MMF was well tolerated, although a few patients required discontinuation of MMF because of abdominal cramps. Opportunistic infections may also be of concern.[96,97]

3.2.2 Anti-Interleukin-2α Receptor Antagonists

As mentioned previously, cGVHD starts with the expansion of donor T cells, so most treatment strategies have focused on the blockade of these cells. When the T cells become activated, the IL-2 receptor is upregulated. Daclizumab, a monoclonal antibody directed against the α subunit of the high-affinity trimeric IL-2 receptor, has been used for the treatment of refractory aGVHD with a response rate as high as 100% in one study,[98] when used in combination with other immunosuppressive agents. Nevertheless, it should be mentioned that the use of daclizumab has been associated with infectious complications. In the cGVHD setting, daclizumab used at a dosage of 1 mg/kg on days 1 to 5 (or 1 and 2) and once weekly thereafter until day 28 showed efficacy in three of four patients, and two patients remained alive 13 and 21 months after treatment.[98-100]

3.2.3 Sirolimus

Sirolimus is a natural macrolide with immunosuppressive qualities that binds to the FK binding protein and inhibits cytokine-driven signalling pathways of the T cell via blockade of the mammalian target of rapamycin. It has been used as a rescue therapy in a series of 35 patients with severe steroid-resistant cGVHD at a loading dose of 6mg orally followed by a daily oral maintenance dosage of 2 mg/day. Thereafter, doses were adjusted to maintain blood concentrations between 7 and 12 ng/mL. Overall response rate reached 63%, including six complete and 16 partial responses.[101] Similar good results in terms of response have been reported in a series of 19 patients, with 15 of 16 evaluable patients having a clinical response.[102] Nevertheless, adverse effects are quite common and include renal function impairment, cytopenias, haemolytic uraemic syndrome, and hypertriglyceridaemia and hypercholesterolaemia. Some of those toxicities led to discontinuation of therapy in 5 of 16 patients. Moreover, only a minority of patients can finally discontinue all systemic immunosuppression.[101,102]

3.2.4 Pentostatin

Pentostatin is a nucleoside analogue that is a potent inhibitor of adenosine deaminase, blocking

Table III. Summary of chronic graft-versus-host disease (cGVHD) treatment

Treatment	Type of trial	Type of patients	Schedule	Main study results
Standard therapy				
Prednisone[83-85]	Randomised trial	Initial therapy	Alone +/− azathioprine 1.5 mg/kg/d	Prednisone alone: survival[a] at 3 years 26%, resulted in fewer infections and better survival in standard-risk cGVHD, but is less effective in high-risk patients
	Randomised trial	Initial therapy	+/− ciclosporin	Prednisone + ciclosporin improved survival[a] to 52% and reduced the risk of steroid-related toxicity
Thalidomide[86,87]	Randomised trial	Initial therapy	Thalidomide 200–800 mg/d + prednisone + ciclosporin	No clinical benefits
	Randomised trial	High-risk patients[a]	Thalidomide 200–800 mg/d +/− prednisone + ciclosporin	
Rescue therapy				
MMF[96,97]	Phase I/II trials	Primary and salvage therapy	MMF 1–2 g/d	35% CR, 44% PR
	Phase I/II trials	Refractory cGVHD	MMF 1–2g/d	67% overall RR
Daclizumab[98-100]	Phase I/II trials	cGVHD	Daclizumab 1 mg/kg/d days 1–5	75% overall RR
Sirolimus[101,102]	Phase I/II trials	Severe corticosteroid refractory cGVHD	Sirolimus 6 mg/d, followed 2 mg/d	63% overall RR
	Phase I/II trials	Severe refractory cGVHD	Sirolimus 10mg 1st day, then 5 mg/d	93% overall RR
Pentostatin[103]	Phase I/II trial	Corticosteroid refractory aGVHD	Pentostatin 1–2 mg/m^2/d, 3 days	76% overall RR, 63% CR
	Case reports	Refractory cGVHD patients		Significant improvement in symptoms
Extracorporeal photopheresis[104-109]	Phase I/II trials	Refractory cGVHD		56% overall RR in skin, 67% in liver, 71% in oral mucosa, 67% in eyes
CD20 antagonists[110-112]	Case reports/ phase I/II trial	Refractory cGVHD	Rituximab (375 mg/m^2 once a wk for 4 wks)	60% overall RR
TNFα antagonists[113]	Phase I/II trial	Corticosteroids refractory cGVHD	Etanercept (25mg, sc, twice wkly for 4 wks followed by once wkly for 4 more wks)	70% overall RR, 10% CR
Other strategies				
UDCA[114-116]	Phase I/II trials		10–15mg/kg/d	Decreases the risk of GVHD Improve liver function test
Beclometasone[117]	Phase I/II trials	Corticosteroid refractory aGVHD and cGVHD Severe gut GVHD	2mg/4×d in 28 days Enemas	60% overall RR 75% CR

a Among high-risk cGVHD patients, thrombocytopenia or cGVHD that evolved directly from acute GVHD as an indicator of a poor prognosis.

aGVHD = acute GVHD; **CR** = complete remission; **MMF** = mycophenolate mofetil; **PR** = partial response; **RR** = response rate; **sc =** subcutaneously; **UDCA** = ursodeoxycholic acid.

the metabolism of 2′-deoxyadenosine.[118] Several studies have been reported describing its efficacy in the management of aGVHD. A response rate of 76% (63% CR and 13% PR) at a dosage of 1–2 mg/m^2/day for 3 days has been described.[119] Experience in the treatment of cGVHD is more limited. In a small series of five paediatric patients treated with pentostatin at a dosage of 4 mg/m^2/day every other week for 24 weeks, a significant improvement in skin and oral symptoms was observed in every patient and the treatment was well tolerated.[103]

3.2.5 Extracorporeal Photopheresis

Extracorporeal photopheresis has been used in the cGVHD setting. Antigen-presenting cells and T cells are susceptible to photo-inactivation with either UVB or UVA irradiation in the presence of photosensitising agents such as 8-methoxypsoralen (8-MOP). UVA phototherapy may be administered to the skin surface as psoralen plus UVA irradiation or directly to peripheral blood leukocytes obtained from the patient by apheresis. Concentrated leukocytes are incubated with 8-MOP, irradiated and then re-infused, a technique known as extracorporeal photo-apheresis (ECP), usually starting with two to three weekly ECP treatments and then tapering according to response. Several studies have shown the efficacy of ECP on cGVHD.[104-109,120] Overall response rates up to 80% in skin (including both lichenoid changes and sclerodermal forms), 70% in liver, 71% in oral mucosa, 67% in eyes and 54% in lungs have been described, while the complete response rate is very wide ranging, from 5% to 50%.[119,120] Because of the absence of prospective, randomised trials, the role of ECP in the treatment of cGVHD is not well defined. However, the reported experience suggests that ECP may induce clinical improvement in patients with poor prognostic features, especially in those with cutaneous, oral, conjunctival or liver involvement, although lung or neuromuscular cGVHD is less sensitive to this therapy.

3.2.6 CD20 Antagonists

Although most therapeutic options regarding cGVHD have focused on T cell depletion, the increasing evidence about B cell disregulation, which might contribute to the pathogenesis of cGVHD, prompted different groups to evaluate the efficacy of rituximab in the management of patients with refractory cGVHD. Rituximab was administered at a dose of 375 mg/m^2 every week for 4 weeks, with retreatment planned within 12 weeks in case of incomplete response. Responses have been described in 9 of 15 patients,[110-112] and included resolution of thrombocytopenia related to presence of antiplatelet-IgG antibodies in the context of extensive cGVHD, severe skin involvement with sclerodermoid changes, membranous glomerulonephritis, lichenoid changes in the oral cavity and hair loss. The response to rituximab was observed after unsuccessful treatment with several immunosuppressive drugs.

3.2.7 Tumor Necrosis Factor-α Antagonists

Cytokines have been shown to be extremely important in the initiation and propagation of GVHD. Of note, IL-2 and tumor necrosis factor (TNF)-α lead to cellular activation as well as local tissue damage. Regarding the role of TNFα, experience in the cGVHD setting is quite limited. However, one study that included ten patients with steroid-refractory cGVHD reported the efficacy of etanercept, a recombinant soluble TNFα antagonist.[113] Etanercept 25mg (0.4 mg/kg for children) was administered subcutaneously twice weekly for 4 weeks, and then once weekly during 4 more weeks. Treatment was well tolerated and only one patient was withdrawn from the study. One patient reached CR, four reached PR, and one had improvement.

3.2.8 Other Strategies and Supportive Therapy

In order to manage cGVHD-induced specific organ damage it is important to take into account drugs with 'selective' organ activity. One of those drugs is ursodeoxycholic acid (UDCA), which decreases liver toxicity and may modulate the expression of HLA class I antigens in hepatocytes. The use of UDCA decreases the risk of GVHD. [114] In the cGVHD setting, UDCA 10–15 mg/kg bodyweight per day for 6 weeks has been shown to improve liver function tests, although those parameters may worsen again when the drug is stopped.[115,116] Furthermore, drugs with selective or predominant activity in the

gastrointestinal tract, such as beclometasone, are available. Although experience with beclomethasone in cGVHD is much more limited than in aGVHD, a response rate of 60% has been reported among patients receiving 2mg four times daily in 28-day courses.[117] Topical use of betamethasone enemas has also been reported in a series of eight patients with severe gastrointestinal GVHD, and diarrhoea or abdominal pain was resolved in six of them. Interestingly, the dosage of systemic corticosteroids could be reduced after the end of the treatment.[121]

Other strategies based on topical corticosteroid formulations are important for both treatment of organ-specific cGVHD and management of sequelae and symptoms. Accordingly, clobetasol, betamethasone dipropionate and hydrocortisone are available for topical use in patients with skin, vaginal or oral involvement, respectively. Also, topical preparations such as preservative-free artificial tears every 4 hours during the day and preservative-free ointment at night are important for the symptomatic management of ocular involvement. Finally, 0.1% tacrolimus ointment may be an effective option to treat cutaneous or mucosal GVHD when applied topically.[122,123]

Patients with cGVHD should receive prophylactic antibacterials for *Pneumocystis jiroveci* (previously *P. carinii*) and targeting encapsulated organisms for as long as the immunosuppressive therapy is administered. Some authors recommend antifungal prophylaxis in patients receiving long-term or high-dose corticosteroids for cGVHD.[124]

3.3 Potential Future Strategies

3.3.1 Anti-CD25 Immunotoxin

The therapeutic strategies previously reported are based on the nonspecific blockade of the immune system, which, apart from a variable efficacy in terms of GVHD response, translates into a higher risk of relapse and severe infections, as we have discussed. As an alternative, newer strategies should focus on the selective depletion of alloreactive T cells, allowing an appropriate immune response against other antigens. This strategy has been described using an anti-CD25 immunotoxin.[125] Accordingly, alloreactive T cells can be depleted specifically by using *ex vivo* purging. Using that approach, actuarial rates of aGVHD were 46% ± 13% for grades II–IV and 12% ± 8% for grades III–IV in a series of 16 elderly patients. Unfortunately, regulatory T cells also express the CD25 antigen, and some studies have suggested that CD25+ T cell depletion may increase GVHD incidence for that reason.[126]

3.3.2 Inhibition of Nuclear Factor-κB

Another approach to selectively deplete alloreactive T cells would be to target transcription factors specifically expressed in activated T cells, such as nuclear factor (NF)-κB.[127] The NF-κB family has emerged as a key transducer of inflammatory signals involved in T cell activation. Accordingly, the diverse signalling pathways downstream of T cell receptor (TCR)/CD3 converge on several key transcription factors including nuclear factor of activated T cells, activator protein 1 and NF-κB. Activation of NF-κB is essential for T cell immunogenic responses and activates multiple target genes whose products can inhibit apoptosis. Bortezomib, a boronic acid dipeptide, is a potent, selective and reversible inhibitor of proteasome.[128] The proteasome is a multi-enzyme complex that is present in all cells. It degrades proteins that regulate cell-cycle progression and causes proteolysis of the ubiquitinated endogenous inhibitor of NF-κB, IκB. The latter blocks the nuclear translocation and transcriptional activity of NF-κB. In normal T cells, NF-κB translocation to the nucleus only occurs after TCR/CD3 and costimulatory molecule engagement, while it is not activated in resting T cells. That property allows specific apoptosis to be induced among activated T cells after a mixed lymphocyte culture, leading to a highly specific depletion of T cells alloreactive against primary donor antigens.

3.3.3 Regulatory T Cells

Other newer approaches for the treatment of cGVHD should be based on the induction of specific tolerance against non-tumour recipient antigens. In that sense, it is becoming increasingly clear that naturally arising CD4+/CD25+ regulatory T cells

can influence immune responses. The cells appear to be central to the control of autoimmunity and may regulate transplantation tolerance. The regulatory T cell family represents 5–10% of all peripheral freshly isolated CD4+ T cells. Regulatory T cells do not proliferate after allogeneic or polyclonal activation *in vitro*, and inhibit the activation and cytokine release of CD4+ and CD8+ T cells in an antigen non-specific manner. In addition to CD25, natural and induced regulatory T cells express high levels of the co-stimulatory molecule cytotoxic T lymphocyte antigen 4 (CTLA-4) and the transcription factor forkhead box P3 (FOXP3).[129-131] Abnormal numbers of regulatory T cells have been related to the development of cGVHD, although contradictory data have been reported.[44,45] Moreover, the infusion of regulatory T cells in an aGVHD mouse model inhibits GVHD lethality.[132] For that reason, different groups have tried to expand those regulatory T cells in order to reach sufficient doses to be used in the clinical setting and have shown that, after *in vitro* expansion, regulatory T cells maintain their functional characteristics.[133,134]

3.3.4 Dendritic Cells

Regulatory T cells can be generated by targeting antigens to immature dendritic cells.[135] There is growing evidence that dendritic cells, besides their well known T cell stimulatory functions, also maintain and regulate T cell tolerance in the periphery. That function is exerted by certain subsets of dendritic cells, and particularly those displaying an immature phenotype. The regulatory functions of dendritic cells include the induction of T cells with regulatory properties[136] and make dendritic cells a potential candidate for the induction of immunotolerance.[42,43,137] The active metabolite of vitamin D_3, 1,25 $(OH)_2D_3$, inhibits upregulation of the co-stimulatory molecules CD40, CD80 and CD86 and class II MHC molecules. In addition, 1,25 $(OH)_2D_3$ inhibits IL-12, while enhancing IL-10 production and promoting dendritic cell apoptosis.[138] Other immunosuppressive drugs, such as sirolimus, inhibit dendritic cell maturation. That effect is mediated through an intracellular receptor FKBP12 and affects IL-4 pathways of dendritic cell activation. Moreover, sirolimus promotes dendritic cell apoptosis[139] and may generate immunotolerance after transplantation.[140]

3.3.5 Mesenchymal Stem Cells

The generation of dendritic cells with an immature phenotype has also been obtained with the use of mesenchymal stem cells.[141,142] These are multipotential non-haematopoietic progenitor cells of the adult bone marrow. They have the capacity to differentiate *in vitro* and *in vivo* into several mesenchymal tissues, including bone, cartilage, muscle, adipose tissue and bone marrow stroma. They also have immunomodulatory effects and inhibit cell proliferation in mixed lymphocyte cultures,[143] or after stimulation with phytohaemagglutinin (unpublished data), which can also be attributed to their capability to induce differentiation of alloantigen-reactive CD4+CD25+ T cell subsets into a regulatory/suppressive phenotype.[144,145] In the clinical setting, preliminary experiences suggest that the infusion of mesenchymal stem cells may help to control refractory GVHD.[146]

4. Conclusions

cGVHD is a severe and common complication after allogeneic transplantation. Clinical management requires the development and standardisation of new grading systems that allow better classification and treatment of patients according to prognosis, and a comparison of the results of newer strategies.

The use of prednisone together with a calcineurin inhibitor, such as ciclosporin or tacrolimus, can be considered the standard regimen as primary treatment for cGVHD. To date, there is no standard salvage regimen for cGVHD and, accordingly, the best choice is to enter the patient into a clinical trial. Immunosuppressive drugs that decrease T cell activation or survival such as MMF, daclizumab, sirolimus and pentostatin have been used with promising results. In addition, the effect of anti-cytokine or anti-B cell agents such as etanercept or rituximab has also been explored.

Newer strategies based on the selective depletion of alloreactive T cells and the use of immature

dendritic cells and mesenchymal stem cells are being developed in order to generate immunotolerance.

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