REPORT

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Consensus Conference on Clinical Practice in Chronic GVHD: Second-Line Treatment of Chronic Graft-versus-Host Disease

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Steroid refractory chronic graft-versus-host disease (cGVHD) is associated with a significant morbidity and mortality. Although first-line treatment of cGVHD is based on controlled trials, second-line treatment is almost solely based on phase II trials or retrospective analyses. The consensus conference on clinical practice in cGVHD held in Regensburg aimed to achieve a consensus on the current evidence of treatment options as well as to provide guidelines for daily clinical practice. Treatment modalities are the use of steroids and calcineurin inhibitors as well as immunomodulating modalities (photopheresis, mTOR-inhibitors, thalidomide, hydroxychloroquine, vitamin A analogs, clofazimine), and cytostatic agents (mycophenolate mofetil, methotrexate, cyclophosphamide, pentostatin). Recent reports showed some efficacy of rituximab, alemtuzumab, and etanercept in selected patients. Moreover, tyrosine kinase inihibitors such as imatinib came into the field because of their ability to interfere with the platelet-derived growth factor (PDGF-R) pathway involved in fibrosis. An other treatment option is low-dose thoracoabdominal irradiation. Although different treatment options are available, the "trial-and-error system" remains the only way to identify the drug effective in the individual patient, and valid biomarkers are eagerly needed to identify the likelihood of response to a drug in advance. Moreover, the sparse evidence for most treatment entities indicates the urgent need for systematic evaluation of second-line treatment options in cGVHD.

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INTRODUCTION

Chronic graft-versus-host disease (cGVHD) remains the leading cause for late morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). Although half of the patients respond to first-line treatment, prognosis of steroid refractory cGVHD remains poor [1-3]. Primary treatment of cGVHD is based on controlled trials and consists of prednisone given with or without a calcineurin inhibitor (CNI). In contrast, evidence in steroid refractory cGVHD is limited almost

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exclusively to phase II trials or retrospective analyses. Until recently, no valid criteria for the diagnosis and staging of cGVHD severity were available, which limits the value of most reported trials on treatment of cGVHD. Moreover, most of the reported trials did not use uniform criteria for response and did not provide details on severity of cGVHD. An additional problem is the heterogeneity of the patients included in the analyses, because, for some treatment options, results in children differ substantially from results achieved in adults. Although not yet validated in a prospective fashion, the National Institutes of Health (NIH) consensus criteria on diagnosis and staging of cGVHD as well as on treatment response criteria, reported in 2005, now provide defined criteria that should improve the validity of future results on treatment of cGVHD [4-9].

The Consensus Conference on Clinical Practice in Chronic GVHD held in the fall of 2009 in Regensburg, Germany (complete program provided at www.gvhd.de), aimed to summarize the current available evidence for second-line treatment and to provide practical guidelines for the use of treatment modalities. The presented consensus was based on a review of published evidence and a survey on the current clinical practice in transplant centers from Germany, Austria, and Switzerland, with 31 of 37 centers responding to the survey. The results of the survey are shown in Table 1. Moreover, the consensus was circulated among all centers performing allogeneic HSCT in Germany, Austria, and Switzerland and was discussed during the Consensus Conference meetings. The Consensus Conference was organized under the auspices of the German Working Group on Bone Marrow and Blood Stem Cell Transplantation (DAG-KBT) and the German Society of Hematology and Oncology (DGHO), the Austrian Stem Cell Transplant Working Group of the Austrian Society of Hematology and Oncology, the Swiss Blood Stem Cell Transplantation Group (SBST), and the German-Austrian Paediatric Working Group on HSCT.

The evaluation of evidence and the subsequent recommendations were graded according to the system used by Couriel [10]. Because the evidence of the majority of treatment options in cGVHD is sparse and therefore the strength of recommendation falls into category C for most of the therapeutic options, category C and evidence III level were further specified as shown in Tables 2 and 3. Strength of recommendation and evidence levels were first rated by an expert panel and subsequently rated by all participants of the consensus process. Only evidence from the use in cGVHD was included in the evaluation. We mainly focus on reported clinical trials and retrospective analyses. The literature search was performed by the working group on second-line treatment within the Consensus conference using the Pubmed database. Only English literature was considered. Abstracts from the Bone Marrow Transplantation Tandem meetings, the European Bone Marrow Transplantation meetings, and the American Society of Hematology meetings were cited but were not included in the evidence rating.

PRINCIPLES OF SECOND-LINE TREATMENT OF CGVHD

Currently no uniformly accepted definition of steroid refractory cGVHD is available, and generally accepted criteria include (1) progression on prednisone at 1 mg/kg/day for 2 weeks, (2) stable disease on ≥0.5 mg/kg/day of prednisone for 4-8 weeks, and (3) inability to taper prednisone below 0.5 mg/kg/day. Treatment duration may vary depending on clinical manifestation (eg, sclerosis requires longer to respond) or toxicity of the agent (eg, shorter duration in the presence of significant toxicity) [3,7]. Although different treatment options are available for salvage therapy of steroid refractory cGVHD, the "trial-and-error system" remains to date the only way to identify the drug or drug combination effective in an individual patient. In principle, initial secondary treatment should include agents with an adequate safety profile and well-documented activity like CNI, extracorporeal photopheresis (ECP), mTOR inhibitors, or mycophenolate mofetil (MMF), whereas agents with significant side effects should be reserved to third- or fourth-line treatment. In addition, steroid sparing should be an important goal of salvage therapy of cGVHD. Because no predictors of response are yet available either for single immunosuppressive agents or combination therapies, most patients receive empirical treatment in daily clinical practice and changes of therapeutic components in case of lack of response are performed at the individual clinician's discretion. Nevertheless, at time of initiation of secondary or any further treatment, it is suggested not to change more than 1 drug at once, because adding several drugs at once may interfere with identification of the active component and might lead to prolonged use of inactive components. This does not apply to patients showing rapid progression of cGVHD, indicating complete failure of treatment, or the need to withdraw agents because of toxicity. In the presence of lack of response, continuation of at least 1 drug during the change period is suggested because there is a risk to end up with a new combination without individual efficacy, which would leave the patient without effective immunosuppression.

As in first-line treatment, response to salvage therapy should be assessed after 8-12 weeks. If patients have progression of cGVHD after 4 weeks, a new treatment option should be offered. However, patients should be exposed to therapeutic drug levels for an

Table 1. Results of the Survey on Second-Line Treatment of cGVHD (n = 30)

Agent	Frequently Used	Occasionally Used	Infrequently Used	Not Used but Regarded as Treatment Option	Not Regarded as Treatment Option	No Report on the Use
Steroids	30					
Cyclosporine	22	6		1	1	
Tacrolimus	9	8	7	5	1	
Photopheresis	13	9	5	1	1	1
Mycophenolat Mofetil	13	9	5	1	1	1
Mycophenolic acid	8	8	3	9		2
Sirolimus	6	6	7	9	1	1
Everolimus	2	9	3	10	2	4
Pentostatin			7	9	7	7
MTX		1	11	4	8	6
Imatinib		6	6	7	7	4
Rituximab	2	13	6	5	1	3
Hydroxychloroquine			3	9	9	9
Clofazimine			2	5	11	12
Thoracoabdominal irradiation		3		8	11	8
Pulse of steroids	5	11	6	2	5	1
Thalidomide		2	2	13	9	4
Azathioprine	1	1	3	10	9	6
Retinoids (Acitretin/Isotretinoine)		1 / 1	0 / 1	7 / 10	12 / 9	10 / 9
Alemtuzumab	1	8		7	9	5
Cyclophosphamide		3	1	9	10	7
Etanercept	2	3	5	10	6	4

MTX indicates methotrexate.

Thirty of 37 transplant centers performing allogeneic HSCT within Germany (n = 34), Austria (n = 3), and Switzerland (n = 1) responded to the paper-based survey on second-line treatment sent via e-mail to representatives of the centers. (One center responded only for first-line treatment and was excluded from the analysis of second-line treatment.)

adequate length of time (at least 4 weeks) before concluding treatment failure. Patients with sclerotic skin lesions may require substantially longer for responses (up to 6 months) and treatment may be continued provided that the patient is closely monitored to recognize progression of cGVHD. In principle, less immunosuppressive therapy is preferable when treating cGVHD, and thus, agents being identified as ineffective should be discontinued to avoid side effects. In addition, immunosuppression should be reduced as soon as disease control has been achieved. Thus far, no controlled trial showed evidence for a beneficial impact of a 3-agent treatment in first-line therapy [11-13]. Moreover, a retrospective analysis performed by Mitchell et al. [14] demonstrated a decline of quality of life in the presence of multiagent (≥2) treatment independent of severity of cGVHD. These findings, however, do not necessarily imply that novel immunosuppressive agents when used in combination would have the same negative impact on patients' outcome, as data in this regard are lacking.

In pediatric patients, systemic steroid therapy can be deleterious on a growing child. Therefore, addition of an effective steroid-sparing agent is of crucial importance for long-term patient outcome. Moreover, topical therapy should be offered in mild cases both early in the course of cGVHD as well as at the end of systemic steroid taper. However, topical steroids or topical CNI may lead to significant systemic drug levels if applied to large areas in small infants, and thus, their use should be restricted to limited areas.

Although no predictors of response for a single agent are yet available, the side effects of specific agents may limit their use in individual situations. CNI may be used with caution in case of significant renal impairment. Thoracoabdominal irradiation as well as pentostatin may not be given to patients with altered marrow function [15-17]. mTOR inhibitors had a lower response rate in patients with low platelets, but it is unknown whether this is a drug specific effect or an indicator for cGVHD severity as suggested by the risk score developed by Akpek et al. and Couriel et al. [2,18].

From the efficacy standpoint, most of the immunosuppressive agents are used for treatment of a broad spectrum of symptoms of cGVHD. However, some agents may be more relevant in specific indications because of a specific mode of action. This is the case in retinoids, which have been solely applied to sclerotic skin lesions because of their interference with collagen synthesis [19]. On the other hand, rituximab may be considered in immune thrombocytopenia because of its directed efficacy on B cells [20-22].

Although currently no valid recommendation can be made for an individual patient, certain combination of drugs should be avoided because of overlapping toxicities. With regard to myelosuppressive capacity, caution is required when considering thoracoabdominal irradiation or pentostatin in combination with mTOR inhibitors [16-18,23]. Moreover, the combination of mTOR inhibitors with CNIs has been associated with a significant rate of transplantation-associated microangiopathia (TAM) [18,24,25].

Table 2. Strength of Recommendation of Treatment

Strength of Recommendation Level	Definition of Recommendation Level
A	Should always be offered
B	Should generally be offered
C	Evidence for efficacy is insufficient to support for or against, or evidence might not outweigh adverse consequences, or cost of the approach. Optional
C-1	Use in second-line treatment justified
C-2	Use in greater than second-line treatment justified
C-3	Use because of increased risk profile limited to specific circumstances
C-4	Experimental, use only in clinical trials or individual cases
D	Moderate evidence for lack of efficacy or for adverse outcome supports a recommendation against use. Should generally not be offered

During long-term immunosuppression adequate monitoring for infectious complications including screening for viral reactivation and fungal infections is recommended. Moreover, antifungal prophylaxis should be considered, especially in patients receiving a multiagent immunosuppressive regimen or with a history of invasive fungal infections. Steroids require monitoring for steroid-induced osteoporosis and diabetes mellitus. MTOR inhibitors require monitoring of drug levels, signs for TAM, hyperlipidemia, and blood counts. CNIs require monitoring of drug levels, arterial blood pressure, and renal function. Moreover, interactions of certain immunosuppressive agents with comedications such as azole derivates for antifungal prophylaxis need to be taken into account.

EVALUATING EFFICACY OF TREATMENT OF CGVHD

In the absence of a single approved immunosuppressive agent for salvage therapy of cGVHD clinicians must resort of trying "off label" drugs. To be confident about success or failure of each immunosuppressive agent applied, the Consensus Conference advised that a baseline NIH-style comprehensive organ assessment be obtained to serve as a comparison for follow-up evaluations. In addition, reasons for treatment changes including progression of symptoms, toxic side effects, or patient's request should be documented.

Table 3. Quality of Evidence Supporting the Recommendation

Strength of Evidence Level	Definition of Evidence Level
I	Evidence from ≥1 properly randomized, controlled trials
II	Evidence from >1 well-designed clinical trial without randomization, from cohort or case-controled analytic studies (preferable from >1 center) or from multiple time series, or dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities based on clinical experience, descriptive studies or reports from expert committies
III – 1	Several reports from retrospective evaluations or small uncontrolled clinical trials
III – 2	Only 1 report from small uncontrolled clinical trial or retrospective evaluations
III – 3	Only case reports available

The German version of the modified cGVHD staging form can be downloaded on www.gvhd.de or www.gvhd.eu. Although most of the organs like oral and ocular manifestations can be assessed easily and are frequently reported by the patients, it is of importance to ask for manifestations infrequently reported like vaginal manifestations, to prevent prolonged suffering and irreversible damage. The same applies for screening of lung manifestations, because mild involvement can be only detected by evaluation of lung function. Because moderate lung manifestations already interfere significantly with quality of life and physical activity, early intervention seems preferable to avoid progression to more severe stages taken into account, that prospective evaluation of this approach has not been performed yet [26].

SECOND-LINE TREATMENT OPTIONS IN CGVHD (TABLE 4)

Prednisone (B III-1)

Corticosteroids have traditionally been the backbone of cGVHD therapy. Although the use of steroids in first-line treatment is based on controlled trials, their role in second-line therapy remains less clear because of a lack of data. In many studies on second-line treatment of cGVHD drugs like MMF, sirolimus or ECP were combined with continuous steroid administration [18,23,27-30]. Thus, the contribution of steroids to the reported response rates in these studies remains uncertain. Because steroid-sparing is an important goal in cGVHD patients, their dose is usually reduced once symptoms of cGVHD are resolved and steroids may be stopped before dose reduction of other immunosuppressants. If cGVHD flares during steroid taper, increasing the dose by 1 or 2 taper steps may be enough to control symptoms. Considering the potential side effects of systemic steroids alone and even more so in combination with other immunosuppressive agents, regular monitoring for osteoporosis, arterial hypertension, and steroid induced diabetes mellitus is recommended.

Pulse of Steroids (C-2 III-2)

Currently, only 1 publication evaluated the efficacy of high-dose corticosteroids. Akpek et al. [1] reported

Table 4. Second-line Treatment Options in cGVHD

Agent	Recommendation	Evidence	Side Effects	Comments
Steroids	B	III-1	osteoporosis, avascular necrosis, diabetes	important but need to spare steroids because of side effect profile
Photopheresis	C-1	II	venous access required	spares steroids, excellent safety profile
mTOR inhibitors	C-1	III-1	TAM, hyperlipidemia, hematotoxicity	increased risk for TAM in combination with CNI, lower efficacy in thrombocytopenia, requires frequent monitoring
CNI	C-1	III-1	renal toxicity, hypertension	spares steroids, should be avoided in renal impairment
MMF	C-1	III-1	GI complaints, infectious and relapse risk	increased risk for viral reactivation, spares steroids, GI toxicity may mimic GVHD clinically and histologically
Pentostatin	C-2	II	Hematotoxicity, infectious risk	best results in children, caution in presence of impaired marrow function, long-term immunosuppression
MTX	C-2	III-1	Hematotoxicity	best response in mucocutaeous cGVHD, spares steroids
Imatinib	C-2	III-1	Fluid retention	best results in sclerotic skin lesions, potentially effective in mild and moderate BO
Rituximab	C-2	II	Infectious risk	effective in auto-antibody mediated manifestations as well as cutaneous and musculosceletal cGVHD
Hydroxychloroquine	C-2	III-2	GI complaints	best results in mucocutaneous and liver involvement
Clofazimine	C-2	III-2	GI complaints, skin hyperpigmentation	best results in mucocutaneous cGVHD
Thoracoabdominal irradiation	C-2	III-2	Hematotoxicity	best results in fasciitis or steroid dependent mucocutaneous cGVHD, caution in presence of impaired marrow function
Pulse of steroids	C-2	III-2	Infectious risk	rapid control of symptoms, identification of steroid resistance
Thalidomide	C-3	II	Neurotoxicity, sedation, constipation	may be used in concomitant relapse of MM
Azathioprine	C-3	III-1	Hematotoxicity, infectious risk	increased risk for oral malignancies
Retinoids	C-3	III-2	Skin toxicity, Hyperlipidemia	effective in sclerotic skin lesions
Alemtuzumab	C-4	III-3	Infectious risk	last resort
Alefacept	C-4	III-3	Infectious risk	last resort
Etanercept	C-4	III-3	Infectious risk	may be used in overlap syndrome with GI manifestations

TAM indicates transplantation-associated microangiopathia; CIN, calcineurin inhibitor; cGVHD, chronic graft-versus-host disease; BO, bronchiolitis obliterans.

results in 61 patients with severe refractory cGVHD, who were treated with methylprednisolone at 10 mg/kg/day for 4 consecutive days followed by stepwise dose reductions. After 4 days, all patients received a course of additional immunosuppressive therapy. Twenty-seven patients (48%) showed a major response with substantial improvement of cGVHD manifestations, including softening of the skin, increased range of motion, and improved performance status; 15 patients (27%) showed a minor response, defined as improvement in some but not all symptoms of cGVHD. The treatment was well tolerated with no serious adverse events. Although all patients received additional immunosuppressive agents through their later course interfering with the evaluation of the impact of high dose steroids on the extend of response, the results demonstrate that high-dose methylprednisolone allows rapid clinical response in patients with prior uncontrolled cGVHD, requiring rapid control of symptoms. An additional advantage of a pulse of high dose steroids is the immediate identification of steroid resistance especially in cutaneous manifestations of cGVHD.

Calcineurin Inhibitors (C-1 III-1)

As in clinical practice, CNIs (either cyclosporine [CsA] or tacrolimus) are frequently employed in addition to corticosteroids as the initial treatment of cGVHD, however, only limited experience exists on their use as salvage therapy. In 2 small studies investigating the effect of tacrolimus in patients with refractory cGvHD, overall response rates ranged between 35% and 46% [31,32]. In a study of 39 patients receiving CsA already as part of their first-line treatment, a change of CsA to tacrolimus offered some benefit only in a small subset of patients [33].

In all, CNIs may represent a reasonable option for patients with refractory or progressive cGVHD, provided they have not been part of the first-line therapeutic regimen or have shown prior therapeutic activity. Moreover, a subset of patients may remain CNI dependent by showing repeated flares of symptoms of cGVHD after withdrawal of CNI. Tacrolimus clearance is age dependant in pediatric patients, and especially children younger than 6 years of age have

a higher clearance [34]. In contrast, a change from 1 CNI to another is unlikely to improve efficacy, but may be justified for the presence of certain side effects (eg, hyperlipidemia, hirsuitism, neurotoxicity). In general, however, the toxicity profile of both available drugs is usually overlapping (eg, nephrotoxicity, risk of microangiopathy). If chosen, the mode of administration and plasma trough level targets of both CNIs in second-line treatment are usually similar to those employed in first-line treatment. Because long-term renal toxicity is of concern, both substances may be applied with plasma trough level targets at the lower therapeutic limit.

Extracorporeal Photopheresis (C-I II)

During the last years a substantial number of patients have been treated with ECP for steroid-dependent or steroid-refractory cGVHD [29,35-46]. The mechanisms of action are complex including induction of apoptosis in all leukocyte subsets, inhibition of proinflammatory cytokine production, increase in anti-inflammatory cytokine production, reduced stimulation of effector T cells, and induction of donor-derived regulatory T cells (Tregs) [45,47]. Most of the clinical experience in ECP treatment of steroid-refractory cGVHD patients is based on retrospective analyses with a limited number of patients [29,35-38,40,41,43,44,46-50] with consistently high complete responses in up to 80% of patients with cutaneous manifestations and significant improvement in sclerodermatous skin involvement [29,46]. Couriel et al. [38] reported in 71 patients with steroid-refractory severe cGVHD a response rate of 61%, with an inferior outcome in patients with thrombocytopenia and a trend toward a higher response rate in de novo cGVHD. Kanold et al. [44] achived an overall response rate of 63% in 63 children given ECP. Improvement in visceral and lung manifestations of cGVHD to ECP has been less consistent [29,35,37,38,40,43,46]. Two studies demonstrated, that earlier initiation of ECP (<1 year) revealed better response rates in skin, liver, and mucosal cGVHD [37,50]. The latter was not confirmed by Foss et al. [40] and Apisarnthanarax et al. [35]. So far, no treatment schedule (weekly versus 2 weekly) has reportedly revealed superior response rates. However, because of the variety of ECP schedules, the impact of dose intensity (number of cycles per month) and length of treatment (number of cycles) cannot be assessed accurately. Recently, Flowers et al. [28] reported results of a prospective randomized phase II study in 95 patients with steroid-refractory/dependent/intolerant cGVHD given ECP for 12 to 24 weeks in combination with conventional immunosuppressants achieving no significant difference in improvement of total skin score (TSS) at week 12, but a significantly higher rate of complete and partial responses of skin cGVHD as assessed by the nonblinded investigator in the ECP arm compared to the control arm. In addition, significantly more patients in the ECP arm had at least a 50% reduction of steroid dose and at least a 25% decrease of TSS at week 12. Of note, a steroid-sparing effect of ECP has also been reported by other investigators [29,38,40,43,49]. Significantly improved survival rates and improvementsin quality of life have been reported in ECP responders [28,29,50]. Therefore, ECP may be a reasonable first choice in certain clinical scenarios of steroid-refractory cGVHD. It requires a venous access that may be difficult in patients with sclerotic skin lesions and may occasionally require a central venous line associated with increased risk for infections and venous thrombosis.

Numerous investigators reported results on ECP for treatment of cGVHD in children and adolescents with high response rates in skin, liver, and oral manifestations of cGVHD and improved survival rates of steroid-refractory patients [39,48-54].

MMF (C-I III-1)

Since the first publication of a case series with 26 patients at Johns Hopkins, MMF is increasingly used in salvage therapy for refractory cGVHD [55,56]. Reported response rates in case series using different definitions range between 40% and 75%, and no randomized trial is available to prove the efficacy of second-line MMF in cGVHD alone or in combination with other immunosuppressive drugs. Most of the improvements have been observed in patients with limited disease [30,57-62] and steroid sparing was observed [59].

Nevertheless, some limitations for the use of MMF as salvage therapy have to be considered such as side effects, including gastrointestinal discomfort and diarrhea, which require dose reduction and may become a reason for drug discontinuation. In addition, MMF treatment can result in histopathologic changes of the gut mucosa, which may mimic intestinal GVHD [63]. Hematologic toxicity such as leukopenia and thrombocytopenia were observed especially in combination with herpes virus infections [64]. Grade II hematologic toxicity was reported for 6 of 21 pediatric patients and other reports showed an incidence of neutropenia or thrombocytopenia up to 10% [58,60,62]. Infectious complications were observed in several case series ranging from 10% to 50%. Baudard et al. [58] reported serious infectious complications such as aspergillosis, septicemia, and CMV reactivation in 6 of 15 patients including 3 deaths in patients given MMF either as a single agent or in combination. Krejci et al. [60,62] observed multiple serious infections in 14 of 21 pediatric patients, whereas others recently published serious infections in only 3 of 23 adult patients, respectively. Interestingly, in the latter study, 5 of 23 patients died

from noninfectious respiratory failure, a problem not mentioned in other studies [60]. One potential explanation for the different rates of infectious complications reported in association with MMF may be differences in severity of cGVHD, differences in the intensity of immunosuppression, as well as comorbidities and the use of prophylactic antifungal drugs.

Both in prophylaxis studies as well as the randomized trial mentioned, it became evident, that the use of MMF potentially increases the relapse risk in myelogenous malignancies if used as part of a triple agent regimen [13]. The published data on MMF as second-line therapy for cGVHD provide very little information in this respect. Baudard et al. [58] reported on 2 relapses in 20 patients with both acute GVHD (aGVHD) and cGVHD, and Furlong et al. [60] observed 2 relapses in 23 patients treated for cGVHD, respectively.

Given the information available, MMF represents a second-line treatment option. A patient's risk of relapse should be considered and may influence a decision to use MMF as part of a multiagent regimen.

Inhibitors of the Mammalian Target of Rapamycin (C-1 III-1)

Sirolimus and everolimus, inhibitors of the mammalian target of rapamycin (mTOR-I), combine immunosuppressive properties with antiproliferative effects on fibroblasts and smooth muscle cells [65]. mTOR-I exert their action by forming a complex with the mammalian target of rapamycin (mTOR). The generation of this complex results in cell cycle arrest in G1 via inhibition of DNA transcription, DNA translation, and protein synthesis. In contrast to CNIs, sirolimus may promote the generation of regulatory T cells [66]. The mTOR-I sirolimus and everolimus have been extensively studied as immunosuppressants in solid organ transplantation. Substituting CNIs by mTOR-I seems to overcome long-term threats, like chronic allograft dysfunction and vasculopathy as well as secondary skin cancer after solid organ transplantation [67].

Considerable toxicity like TAM has been observed when mTOR-I were used in combination with CNIs, which could be avoided in a CNI-free regimen [68]. Sirolimus has also been evaluated in second-line treatment of cGVHD in small phase II trials mostly in combination with CNIs achieving response rates between 56% and 81% [18,23,25,69]. Major adverse events observed were hyperlipidemia, renal dysfunction, cytopenias, and TAM, which lead to termination of therapy in up to one-third of treated patients. A CNI free treatment with mTOR-I in sclerodermatous manifestations of cGVHD resulted in a similar response rate of 76% and a low toxicity profile regarding nephropathy and TAM, which correlated with high trough levels of mTOR-I [70]. Importantly, no increased relapse rate has been observed, suggesting that the graft-versus-leukemia effect is not compromised by mTOR-I therapy [71]. Similar results were reported on use of everolimus in combination with steroids and in part with azathioprine [72].

Because mTOR-I possibly interfere with wound healing, they should be used with caution in patients with cutaneous or mucosal ulcers [73]. Hyperlipidemia is frequent, requires monitoring, and therapeutic intervention with drugs not interfering with the mTOR-I metabolism. In view of the reported side of effects of mTOR-I including TAM when combined with CNIs, cytopenias, and numerous interactions with drugs frequently used in patients with cGVHD (macrolides, antifungal azoles), close monitoring of blood counts, trough levels, and serum chemistry is advisable. In contrast to policy in prophylactic GVHD settings, a loading dose of mTOR-I should be avoided in salvage therapy of cGVHD patients, and initial dosing should be rather low in view of the long half-life of mTOR inhibitors (eg, 0.25-0.5 mg/day). In patients receiving concomitant voriconazole, the starting dose of sirolimus should be reduced by 90% (0.1 mg/day) [74].

Taken together, the mTOR-I sirolimus and everolimus appear to be an effective treatment option for cGVHD with an acceptable toxicity profile as long as low therapeutic drug trough levels are maintained (4-8 ng/mL) and combination treatment with CNIs is avoided.

Thalidomide (C-3 II)

Although first-line treatment of cGVHD with thalidomide in combination with CsA and prednisone failed to result in improved response rates, thalidomide showed a therapeutic activity in second-line treatment of cGVHD [11]. The mechanisms of action are complex including inhibition of angiogenesis, expression of adhesion molecules, several cytokines (tumor necrosis factor [TNF]-alpha, interleukin [IL]6, IL12), and blockade of NF-kappaB activity [75,76]. Initially, Vogelsang et al. [77] reported results on treatment with thalidomide in 23 patients with refractory cGVHD and 21 patients with high-risk cGVHD. A complete response was observed in 14 patients, a partial in 12, and no response in 18 with acceptable toxicity consisting of sedation, constipation and neuropathy. These findings were confirmed by Browne et al. [78] reporting results on 37 patients with refractory extensive cGVHD given adjunct thalidomide with standard immunosuppressive therapy. Fourteen of 37 patients (38%) responded to thalidomide (1 complete, 13 partial) including 10 of 21 children (46%) and 4 of 16 adults (25%), respectively. Parker at al. [79] reported a response rate of 20% in 80 patients given thalidomide with better results in standard risk cGVHD and combined oral and skin manifestations (40%). Kulkarni et al. [80] observed comparable results in a cohort of 59 patients with a higher response

rate in children, which was confirmed by Rovelli et al. [81] achieving a response rate of (40%) in this population. The use of thalidomide is associated with significant adverse effects consisting of constipation and sleepiness in virtually all patients, neuropathy, skin erythema, neutropenia, and thrombocytopenia. Moreover, thalidomide requires anticoagulation because of an increased risk of venous thrombosis [12]. An unexplained feature remains the broad dose range tolerated throughout the trials on thalidomide ranging from 150 mg to 1600 mg/day. In summary, thalidomide remains a therapeutic option in second-line treatment of cGVHD, especially in mucocutaneous manifestations. The initial dose should be 100 mg given at night with subsequent dose escalation up to 400 mg/day. Thalidomide may be given with split doses with 3 to 4 doses per day.

Hydroxychloroquine (C-2 III-2)

Hydroxychloroquine is a 4-aminoquinoline antimalarial drug that displays therapeutic activity in a variety of autoimmune-mediated disorders, in particular, involving the mucocutaneous organ system. Moreover, hydroxychloroquine is associated with a steroid-sparing activity. Because of its ability to interfere with antigen processing and presentation, cytokine production, and cytotoxicity, as well as its synergistic immunosuppressive effects with CNIs in vitro, it was evaluated in a phase II trial in 40 patients with steroid-resistant or steroid-dependent cGVHD in combination with different immunosuppressive agents at 12 mg/kg (800 mg) per day [82]. Three complete responses and 14 partial responses were seen after a median of 8 (range: 4-24) weeks in 32 evaluable patients (53% response rate) including 20 children. All responders tolerated a >50% reduction in their steroid dose while receiving hydroxychloroquine. The highest response rates were observed in skin, oral, and liver manifestations, whereas efficacy in treatment of gastrointestinal manifestations was limited. Potential side effects of hydroxychloroquine are gastrointestinal symptoms like nausea, diarrhea, visual impairment because of retinal toxicity when given for >2 years, and neuropathy in patients with coexisting renal insufficiency [83]. Of note is the need for dose reduction in the presence of cholestasis [84,85]. The suggested dose of hydroxychloroquine (Quensyl®) is 800 mg/day (12 mg/kg/day) given orally in 2 fractions of 400 mg. A potential indication for the use of hydroxychloroquine may be steroid dependent skin or oral disease.

Azathioprine (C-3 III-1)

Azathioprine has been applied in treatment of cGVHD including topical treatment of oral manifestations [86,87]. In a double-blinded randomized trial in standard-risk cGVHD (platelets >100 × 10^9/L) patients, a significantly increased nonrelapse mortality (NRM) rate (40% versus 21%) and a significantly decreased overall survival (OS) (47% versus 61%) were observed in the combination arm of azathioprine + prednisone compared to prednisone + placebo [87]. Although the study was performed 25 years ago and supportive care since that time has improved considerably, the increased rate of oral malignancies, observed in association with azathioprine has to be of concern [88]. No standardized system for analysis of thiopurine S-methyltransferase gene polymorphism and the related thiopurine methyl transferase activity is available for hematopoietic donor chimera, and prospective typing may require the genetic analysis of the donor and recipient to predict the risk for toxic side effects related to azathioprine.

Etretinate (C-3 III-2)/Isotretinoin (C-3 III-3)

Retinoids are known to interfere with collagen synthesis in fibroblasts [89,90], promote the induction of regulatory T cells, and block the induction of Th17 cells [91,92]. Based on reports of the successful use of retinoids in systemic sclerosis, the efficacy of etretinate (a synthetic retinoid) was retrospectively evaluated in 32 patients with refractory sclerodermatous cGVHD by Marcellus et al. [19]. Etretinate was given in addition to standard immunosuppression within a dose escalation schedule starting with 0.25 mg/kg/day divided into 4 doses. Twenty of 27 evaluable patients showed improvement, including softening of the skin, flattening of cutaneous lesions, increased range of motion, and improved performance status. Four showed no response, and 3 had progression of their sclerosis. Overall, etretinate was well tolerated; however, skin breakdown and/or ulceration led to treatment discontinuation in 6 patients. Other frequent side effects are hyperlipidemia requiring monitoring of blood lipids, increase of transaminases requiring monitoring of liver function tests and teratogenicity. Because etretinate is no longer available in Germany, its active metabolites acitretin or isotretinoin may be suitable alternatives. Ghoreschi et al. [93] reported 5 patients receiving isotretinoin at a dose of 10 mg/day in combination with PUVA and observed a high response rate in sclerodermoid cGVHD. Isotretinoin has been shown to be active in systemic sclerosis, which shares common features with sclerodermoid cGVHD [94-96]. Isotretinoin is typically applied at 0.5 mg/kg/day in 2 fractions, and its cumulative dose should be limited to 120 mg/kg equaling 6 to 8 months of treatment. So far, reports on the efficacy of acitretin (Neotigason®) are lacking, but the close chemical relationship to its prodrug etretinate including comparable side effects suggest activity also in sclerodermoid manifestations of cGVHD. Acitretin may be given orally with an initial dose of 2 × 10 mg and subsequent dose escalation up to 30 mg/day.

Polyclonal Antibodies (ATG/ALG)

Polyclonal animal antihuman-lymphocyte antibodies have successfully been used for decades to prevent severe aGVHD and cGVHD after allogeneic HSCT. In addition, several reports on its activity in steroid-refractory aGVHD have been published. However, currently there is no evidence for safe and efficacious use of ATG/ALG as second-line treatment of cGVHD.

MONOCLONAL ANTIBODIES TARGETING T CELLS (BASILIXIMAB, DACLIZUMAB, OKT3, VISILIZUMAB)

For the directly T cell targeting agents Visilizumab and OKT3 no evidence for their clinical activity in refractory cGVHD is available in the literature. In addition, reported data on anti-IL2-receptor antibodies basiliximab and daclizumab do not allow any recommendation for their use in refractory cGVHD [97,98].

CD52 Antibody Alemtuzumab (C-4 III-3)

Alemtuzumab is an unconjugated humanized IgG_1 monoclonal antibody that depletes T, B, and NK cells, and has also demonstrated activity on dendritic cells [99,100]. Alemtuzumab is licensed for treatment of B cell chronic lymphocytic leukemia and is the treatment of choice in T cell prolymphocytic leukemia [101]. Alemtuzumab (previously known as Campath-1H) as part of the conditioning regimen reduces the incidence of aGVHD and cGVHD, but may lead to higher risk of relapse and infection [101-103]. The use of alemtuzumab in cGVHD is very limited. Ruiz-Arguelles et al. [104] report a cGVHD patient with severe ulcerative colitis refractory to a variety of immunosuppressants who recovered completely 7 months after start of alemtuzumab initially given 10 mg for 6 days followed by monthly administration. Because several groups observed response of aGVHD to significantly lower doses of alemtuzumab, and infectious morbidity and mortality correlate with the cumulative dose of the drug, doses of 3 to 10 mg every 14 days analogous to the experience in aGVHD may also be considered in cGVHD patients [105,106]. The subcutaneous application is much better tolerated than the intravenous infusion, which requires premedication.

A pronounced suppression of the immune system leading to opportunistic infections such as CMV, adenovirus, and toxoplasma is the most important side effect of alemtuzumab [107,108]. Therefore, intense monitoring for signs of infections, and adequate anti-infectious prophylaxis are recommended. In patients with rheumatic diseases, severe bone marrow failure was observed during alemtuzumab therapy. In summary, further studies are needed to assess the efficacy and safety of alemtuzumab in treating cGVHD patients.

Rituximab (C-2 II)

Based on recent findings about the involvement of B cells in the pathogenesis of cGVHD therapeutic strategies specifically targeting B cells have emerged within the last years.

Rituximab is a monoclonal $IgG1_{kappa}$ chimeric mouse/human anti-CD20 antibody, which has been successfully used for treatment of large number of B cell malignancies and autoimmune diseases. Rituximab binds to the extracellular part of the CD20 molecule and induces apoptosis as well as cellular and complement mediated killing of normal and neoplastic B cells.

Ratanatharathorn et al. [109] reported the first patient with severe cGVHD and immune thrombocytopenia recovering completely after 4 doses of rituximab at 375 mg/m^2. Thereafter, several case reports described responses in patients with cGVHD-associated immune phenomena like myasthenia gravis, bullous pemphigoid, or autoimmune haemolytic anemia [22,110,111].

Two small studies on 8 and 6 patients published in 2003 and 2004 reported organ-specific response rates of 50% and 80%, mainly in patients with skin involvement [112,113]. Cutler et al. [114] conducted the first prospective phase I-II study reporting the efficacy of rituximab (375 mg/m^2) in 21 patients receiving a total of 38 cycles. Objective responses were seen in 70% of patients allowing a significant reduction of steroid doses. In 2 patients (10%) with complete clinical responses, all immunosuppressive therapy could be discontinued. Patients with cutaneous or musculoskeletal manifestations of cGVHD had the highest probability to respond to rituximab. Antibody titers against Y-chromosome encoded minor HLA-antigens decreased during the study period, whereas titers against EBV and tetanus remained stable. Side effects included mild infusion reactions and 9 CTC grade III-IV events that were predominantly infectious episodes [114].

Following this prospective study a number of retrospective studies were published covering more than 100 patients reporting good tolerability and a response rate of 50% to 80%. In the majority, investigators used the dose of 375 mg/m^2 once a week for 4 to 8 infusions [115-119].

In contrast, von Bonin et al. [120], using substantially lower doses of 50 mg/m^2/week for 4 weeks in 11 patients with steroid refractory cGVHD and 2 with posttransplant autoimmune disorders (glomerulonephritis and immune-thrombocytopenia), observed similar efficacy with an overall response rate of 69% including 3 patients (23%) with complete remission (CR).

In view of the reported activity and toxicity profile, rituximab can be recommended as a reasonable

second-line therapy of cGVHD, especially with sclerodermatous, lichenoid cutaneous disease, as well as in autoantibody-mediated cytopenias [21]. The active dose is still a matter of debate and should be further investigated in prospective studies.

Alefacept (C-4 III-3)

Alefacept is a dimeric Anti-CD2 LFA-3 fusion protein used for the treatment of psoriasis, and has been applied in the treatment of aGVHD and cGVHD [121-123]. Shapira et al. [121] reported 12 patients with cGVHD resistant to at least 2 lines of standard therapy who received alefacept at 30 mg once per week for a median of 8 (range: 1-25) weeks. Nine of 11 evaluable patients showed some response, which was marked in 3, moderate in 2, and minimal in 4. Six patients died because of GVHD progression and associated infections. Definitely, more studies are needed before alefacept can be generally recommended as a safe second-line treatment for cGVHD.

Etanercept (C-4 III-3) and Infliximab (C-4 III-3)

Data on the use of infliximab and etanercept for treatment of steroid-refractory cGVHD are limited to <10 patients for infliximab and <20 patients for etanercept [124-126]. Infliximab is a chimeric human anti-TNF-α-IgG1κ monoclonal antibody, which binds with high affinity to the soluble and transmembrane forms of TNF-α, hereby blocking their interactions with their cellular receptors and causing lysis of cells that produce TNF-α [127,128]. Etanercept is a fusion protein consisting of 2 identical chains of the human TNF-receptor p75 monomer fused with the Fc domain of human IgG1. Unlike infliximab, it does not eliminate TNF-positive cells via antibody-dependent cytotoxicity (ADCC) and induction of monocyte apoptosis. The elimination of TNF-positive cells has been associated with an increased rate of infectious mortality that applies to the use of infliximab. Busca et al. [124] reported a series of 8 patients with cGVHD treated with etanercept at 25 mg subcutaneously twice weekly for 4 weeks followed by 25 mg weekly for 4 weeks. Overall, 5 of 8 patients (52%) responded to the treatment (CR: n = 1, partial remission [PR]: n = 4) including 2 with gastrointestinal involvement. The results are in line with a report by Chiang et al. [129], who treated 10 patients with steroid-dependent cGVHD according to the same schedule. Seven of 8 patients finishing the 8-week treatment course without adverse side effects showed improvement. Although etanercept should be further investigated in cGVHD, it may be of use in selected patients with gastrointestinal or cutaneous manifestations of steroid-refractory cGVHD.

Imatinib (C-2 III-1)

Imatinib, a multikinase inhibitor successfully employed in *BCR-ABL*-positive malignancies, has recently been proposed as adjunctive treatment in cGVHD on the basis of its antifibrotic activity targeting the platelet-derived growth factor receptor (PDGFR) and transforming growth factor beta (TGF-β) pathways [130,131].

In 2006, Majhail et al. [132] reported a patient with relapse of chronic myelogenous leukemia (CML) and concurrent bronchiolitis obliterans (BO) after HSCT, in whom imatinib at 400 mg daily not only resulted in molecular remission but also in amelioration of bronchiolitis. In 2008, Magro et al. [133] and Moreno-Romero et al. [134] contributed cases of imatinib-induced improvement of sclerodermatous cGVHD. Recently, a small retrospective series (n = 14) and 2 small prospective phase I-II studies (n = 19 and n = 9) on adjunct imatinib at 100 to 400 mg daily in refractory extensive cGVHD have been published [135-137]. Imatinib toxicity consisted of hematologic toxicity, fluid retention, and dyspnea, and was mostly mild, but precluded a dose increase and/or lead to drug discontinuation in 15% to 25% of patients. Of note, 1 CML patient developed secondary lymphoma. Responses to imatinib (about half partial, half complete) occurred within 6 months in 50% to 80% of patients with cutaneous, eye, and intestinal cGVHD. In pulmonary cGVHD best results were observed in mild BO, whereas in moderate and severe pulmonary cGVHD, only minor improvements were seen. Hence, imatinib seems to be safe and feasible but further prospective studies are warranted to confirm its role in therapy of cGVHD. Currently, an initial dose of 100-200 mg imatinib is suggested, with subsequent dose escalation up to 400 mg daily if tolerated. No data on the benefit of other tyrosine kinase inhibitors besides imatinib are currently available.

Pentostatin (C-2 II)

Pentostatin (deoxycoformicin; Nipent®) is a nucleoside analog that irreversibly inhibits adenosine deaminase, an enzyme expressed in lymphocytes that mediates recycling of purines [138,139]. Following the successful use of pentostatin in steroid-refractory aGVHD, and because of its low hematotoxic profile, the compound was subsequently investigated in refractory cGVHD [140].

Jacobsohn et al. [17] performed a phase II study in 58 patients with intensively pretreated refractory cGVHD given pentostatin at 4 mg/m^2 every second week for a median of 12 doses (range: 1-32 doses). The overall response rate was 55%, with major responses in 31 patients and improvement of lichenoid cutaneous manifestations in 69%. Toxicity was

minimal, with nausea as the most frequent adverse effect and severe infections grades III-IV in 20% of patients. Four patients discontinued therapy early. Mortality of 33% was mainly because of cGVHD with or without infection [17]. Encouraging results were also observed in 5 children with steroid-refractory cGVHD with significant improvement of lichenoid and sclerodermatous skin involvement [15]. Oral GVHD resolved completely in 2 patients and no severe infections were observed. Recently, a phase II trial in 51 children with steroid-refractory cGVHD has been published by the Pediatric Blood and Marrow Transplant Consortium. Application of pentostatin resulted in an overall response rate of 53%, including a 59% response rate in sclerosis. In 25% of patients, toxicity of the compound required discontinuation of treatment [141].

The moderate toxicity profile and the favorable response rate emphasize further evaluation of pentostatin in adults and children with cGVHD [17,141]. Application of a 4 mg/m^2 dose of pentostatin every second week for 3 months is recommended. In case of creatinine clearance <50 mL/min/1.73 m^2, the dose should be reduced by 50%; in case of clearance <30 mL/min/1.73 m^2, treatment should be interrupted. The dose should be reduced by 25% if grade III hematotoxicity occurs. Neutropenia, thrombocytopenia <20 × 10^9/L, or fever require dose reduction by 50%, whereas pentostatin should be completely withheld in case of severe infection [142]. As infections were reported as the most frequent complication of pentostatin application in cGVHD, the compound should not be given when severe immunodeficiency because of recurrent infections is assumed. Thus, pentostatin should not be used for pulmonary cGVHD.

Low-Dose Methotrexate (MTX) (C-2 III-1)

In view of the anti-inflammatory and antiproliferative properties and its successful use in patients with autoimmune disorders, for example, rheumatoid arthritis, several authors evaluated the use of low dose methotrexate (MTX) for cGVHD in limited case series. Observations that low doses of MTX can induce a sustained suppression of T cell activation and expression of adhesion molecules further support its use in cGVHD therapy [143].

The MTX dosage varied from 5 to 10 mg/m^2 weekly [144,145] to 5 to 10 mg/m^2 every 3 or 4 days in the different reports resulting in partial or complete remissions of steroid-refractory or severe cGVHD in >70% of adult patients [144,146,147]. Inagaki et al. [145] observed complete or partial remissions in 50% of 17 pediatric patients with steroid-refractory or steroid-dependent GVHD. Besides high response rates steroid-sparing effects were reported and in most cases, response was seen already after the first MTX dose [147].

Huang et al. [146] reported severe leukopenia (white blood cell count [WBC] <2 × 10^9/L) in 14% of patients with cGVHD that was reversible despite continuation of MTX therapy and did not lead to discontinuation of medication. Inagaki et al. [145] observed grade III-IV hematotoxicity in 15% and grade II hepatotoxicity in 7%, which improved after interruption of MTX. Mortality rates of ~5% because of pulmonary infections were reported [145,146]. In general, all studies reported good tolerance of low-dose MTX in adults as well as in children with moderate toxicity including easily manageable hematotoxicity.

These results support further evaluation of adjunct low-dose MTX as frontline therapy as reported by Wang et al. [148] in 86 patients with cGVHD. Grade III toxicity because of cytopenia or oral mucositis was only observed in 3% of patients. The highest response was seen in skin cGVHD with improvement in 90% of patients. Low-dose MTX does not seem to increase the risk of relapse of the underlying disease in cGVHD patients, and long-term use of weekly low-dose MTX seems feasible [145].

In conclusion, the aforementioned studies suggest that MTX is an option mainly for skin and oral manifestations of cGVHD. A dosage of 5 to 10 mg/m^2 weekly might be recommended; in the case of intestinal, involvement, intravenous (i.v.) application might be more suitable [144]. In the case of leukopenia <2 × 10^9/L or thrombocytopenia <50 × 10^9/L, dose reduction to 5 mg/m^2 seems to be more appropriate. In the case of renal insufficiency, dose reduction is also recommended. Because of the hepatotoxicity of the compound, hepatitis-like cGVHD seems to be a contraindication. Caution is advised in patients with preexisting renal insufficiency, pancytopenia, or recurring infections. Folate support should be performed in accordance with the experience of other investigators using MTX over long periods of time [149].

Cyclophosphamide (C-4 III-3)

Cyclophosphamide (Cy) is an established immunosuppressive and cytotoxic drug widely used as part of pretransplant conditioning regimens. In a few reports, high-dose Cy (200 mg/kg) followed by "pseudoautologous" stem cell rescue was applied in refractory cGVHD patients resulting in resolution of cGVHD, but also relapse of the underlying hematological malignancy [150].

Mayer et al. [151] evaluated pulsed treatment with Cy at a median of 1000 mg/m^2 in addition to steroids and MMF in 15 patients with steroid-refractory aGVHD or cGVHD (n = 3), resulting in complete resolution of liver GVHD in 2 patients and in partial remission of oral cavity GvHD in 1 case. Infectious

complications and severe transient pancytopenia were seen in 1 patient each [151].

Therefore, further evaluation of Cy in cGVHD appears justified. Hematopoietic impairment and a history of recurrent infections seem to represent contraindications and comedication with mesna (Uromitexan®) is recommended. The pulse schedule of Cy differs from doses used in patients with, for example, scleroderma, where long-term application of oral daily dose of ≤2 mg/kg body weight has been successfully applied [152]. Also, for other autoimmune disorders such as BOOP, lupus nephritis, or autoimmune vasculitis, Cy has been successfully used [153-155].

According to the consensus conference, it is difficult to draw any conclusions on use of CY in patients with cGVHD because the literature is very limited.

Clofazimine (C-2 III-2)

Clofazimine (Lamprene®) is an antimycobacterial drug that has anti-inflammatory activity and is thought to reflect functional inhibition of pathogenic T lymphocytes in various autoimmune skin disorders such as cutaneous lupus erythematodes, and has been extensively used for treatment of leprosy and *Mycobacterium avium* complex since the 1960s [156].

Based on its tissue distribution with secretion in sweat, tears, or skin, and its apparent immunomodulatory properties, this compound was explored in cGVHD. Lee et al. [156] reported 22 patients with cGVHD given 300 mg orally in a single daily dose for 90 days followed by dose reduction to 100 mg daily. Treatment lasted 7 to 835 days and was generally well tolerated besides mild gastrointestinal toxicity in 36% of patients or reddish-brown hyperpigmentation of the skin. Partial responses only were achieved in 55% of patients both with limited as well as extensive cGVHD. In 23% of patients, other immunosuppressive drugs could be reduced. The compound seemed most effective in skin, joint, or oral involvement. Another report from Rzepecki et al. [157] documented experience with clofazimine in 4 patients with cGVHD, who all achieved PR or CR of symptoms with good tolerance of the compound. One pediatric patient with cGVHD was reported to have an episode of methemoglobinemia under treatment with clofazimine that could be managed with methylene blue and ascorbic acid. However, it seems that this potentially dangerous adverse reaction is because of the known hemoglobin-oxidation potential of clofazimine [158].

Low-Dose Thoracoabdominal Irradiation (C-2 III-2)

The well-known immunosuppressive and immunomodulatory capacity of irradiation has been investigated by Robin et al. [16] in a retrospective analysis of low-dose thoracoabdominal irradiation (1 Gy) in 41 patients with refractory extensive cGVHD. Of note was a high response rate of 82% with best responses observed in fasciitis (79%) and oral GVHD lesions (73%). Two years after thoracoabdominal irradiation, a CR was achieved in 11 patients. Fifty-seven percent of patients had an at least 50% reduction of their corticosteroid dose by 6 months after treatment. Two-year cGVHD relapse incidence was 34% and patients with fasciitis, lymphocytes $>1.0 \times 10^9$/L, and platelets $>200 \times 10^9$/L had a better outcome. Side effects were a mild transient pancytopenia with a late nadir approximately 3 weeks after treatment. The high response rate was confirmed by Bullorsky et al. [159], demonstrating an improvement in 3 patients with cGVHD. In summary, low-dose thoracoabdominal irradiation is a safe and efficient option in patients with refractory cGVHD, allowing a significant tapering of systemic corticosteroids in most cases.

RESULTS OF THE SURVEY

Thirty of 37 transplant centers performing allo-HSCT within Germany (n = 34), Austria (n = 3), and Switzerland (n = 1) responded to the paper-based survey on second-line treatment sent via e-mail to representatives of the centers. One center responded only to specific question but did not report on the frequency of applied agents and was therefore excluded from the analysis presented in Table 1.

The first question involved a patient with de novo cGVHD of skin, oral mucosa, eyes, and liver 5 months after myeloablative conditioning for an allogeneic-related HSCT for AML in first CR not responding to initial treatment with prednisone at 1 mg/kg/day and asked about the first choice of an additional immunosuppressive agent. Two centers each (7%) preferred adding either MMF, tacrolimus, or pulse of steroids, whereas 11 centers (37%) mentioned CsA. One center (3%) preferred an mTOR inhibitor and 4 (13%) ECP. Several centers suggested using a triple agent combination consisting of CsA, a pulse of steroids, and ECP (n = 1), steroids, MMF, and CsA (n = 1), CsA or tacrolimus combined with MTX or sirolimus and ECP (n = 1), and steroids, CsA, and ECP (n = 3). One center suggested adding 3 agents consisting of CsA, ECP, and rituximab (n = 1).

Second, centers were asked whether mTOR inhibitors are combined with CNI in second-line treatment of cGVHD. Seventeen centers (57%) stated avoiding this combination completely, 7 each use either everolimus or sirolimus combined with tacrolimus, 5 each combine either everolimus or sirolimus with CsA.

The third question concerned a patient after allo-HSCT for standard-risk acute myelogenous leukemia (AML) in first CR with moderate cGVHD with skin, oral mucosa, and liver, involvement responding completely to first-line treatment with prednisone and

CsA in the skin and liver, but failing to respond with moderate oral involvement.

Twenty-eight centers (93%) stated not to change systemic immunosuppression but to add topical budesonide (n = 8), tacrolimus (n = 11), topical steroids not specified (n = 10), or topical dexamethasone (n = 3). Five other centers would add topical CsA. Three centers preferred a change of systemic immunosuppression adding MMF (n = 1), or ECP (n = 2) in combination with additional topical treatment with either tacrolimus (n = 1) or topical steroids (n = 2). Only 1 center reported not using topical immunosuppression and to start everolimus to improve oral cGVHD. (Several centers reported more than 1 approach.)

CONCLUSIONS

The continuing significant morbidity and mortality of cGVHD seen especially in patients with severe disease manifestations remains a therapeutic challenge [160]. Although a number of immunosuppressive agents have demonstrated therapeutic activity in cGVHD, most of these treatment options have not been investigated systematically. Moreover, evidence is sparse and limited to phase II trials or small case series with inhomogenous inclusion criteria, lack of documentation of severity of cGVHD, and insufficient response assessment.

To improve this situation, the Consensus conference on clinical practice in cGVHD proposed several goals to be achieved through the next decade for improvement of patient outcome. First of all, the currently available most frequently used treatment strategies should be evaluated in a controlled manner applying the NIH consensus criteria [5-9].

Because it will be difficult to assess the efficacy of the majority of substances in formal phase II/III trials, additional observational studies may provide useful information on their efficacy, but they do not replace the need for formal trials. In this context the Consensus conference on clinical practice in cGVHD achieved an agreement on the use of diagnostic criteria, severity staging, as well as response assessment of cGVHD in daily clinical routine as a prerequisite for performing observational studies within this clinical network of transplant facilities (Greinix et al., BBMT, submitted).

A second aim is to develop valid predictors of response to replace the "trial-and-error system" by an individualized approach taking into account the pathophysiologic heterogeneity of cGVHD. Thus, potential biomarkers including cytokines, proteomic patterns, and cellular subpopulations can be investigated within the Consensus consortium correlating laboratory parameters with observed responses according to the NIH staging and response assessment criteria. A third aim for improvement of patient outcome is defining more efficacious and safe treatment options for specified organ manifestations early in the course of cGVHD in clinical phase I/II trials to avoid development of irreversible organ damage and long-lasting immunodeficiency leading to severe infectious complications.

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