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Perspective

Acute Graft-Versus-Host Disease, Prophylaxis and Therapy

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Abstract

Graft-versus host disease is one of the major causes of death in patients undergoing allogeneic hematopoietic stem cell transplantation. Major contributing factors to severity of graft-versus host disease include HLA mismatch, conditioning regimen intensity, age of donor and recipient, source of stem cell, and donor type. Strategies and clinical trials are ongoing to reduce the incidence of graft-versus host disease, and to improve on the survival outcome of patients with graft-versus host disease, Research efforts continue to develop new ways of identification, prevention, and treatment for graft versus host disease. In addition, efforts are also being made to incorporate biomarker for the early detection of graft versus host disease. In addition, strategies utilizing monoclonal antibodies and cytokines are being tested as potential therapeutic options. Here we provide evidence for recommended regimens for graft versus host disease, prophylaxis and treatment.

Keywords

Acute Graft-versus-host Disease; GVHD; hematopoietic stem cells; transplantation

1. Introduction

Allogenic Hematopoietic stem cells (allo-HSCT) has become the standard of care for many patients with hematologic cancers but also for many other disorders. Over the last two decades, HSCT has seen rapid expansion with a constant evolution in technology use. Bone marrow is supplemented as a stem cell source by peripheral blood or cord blood. The advent of novel conditioning regimens with lower intensity have expanded the use of HSCT to older patients or to those with co morbidities. However, its efficacy continued to be limited by the development of frequent and severe acute graft-versus-host disease (aGVHD).

The history of GVHD dates back to the 1910s, when an immunologic reaction was observed in chick embryos [1]. At that time, the concept of T cells was new and a link between GVHD and T cells did not exist [1, 2]. Since that time, many advances have been made throughout the decades with regard to our understanding of the disease and its treatment options. A pioneering discovery by Billingham in 1966, paved the way for a deeper understanding of the pathophysiology of GVHD in the field of allo-HSCT [3]. GVHD develops in 30-75% of recipients of allo-HSCT despite current GVHD prophylaxis. There are various risk factors for GVHD and few of the most significant factors are the degree of histocompatibility between the donor and the recipient, number of T-cells in the graft, recipient's age and GVHD prophylactic regimen used [4-6]. The interactions between the donor and host innate and adaptive immune responses leads to the development of aGVHD. The donor T cells are mainly responsible for aGVHD pathogenesis, while both the donor T cells and B cells are responsible for chronic GVHD (cGVHD)-mediated tissue injury. Despite remarkable clinical advances, GVHD remains the greatest obstacle in the success of allo-HSCT due to considerable morbidity and mortality. There are two main types of GVHD—acute and chronic—differing in the pathogenesis, time of onset, and clinical presentation. A-GVHD generally runs a limited course, whereas cGVHD may be active for years, requiring potentially years of immunosuppressive therapies and placing patients at risk for a number of late complications. Based on the timing of presentation of symptoms GVHD is classified as a) classic aGVHD (which is initial diagnosis within the first 100 days post allo-HSCT or DLI infusion), b) persistent, recurrent, late onset aGVHD (occurs beyond 100 days post-allo-HSCT or can be new onset of aGVHD with no prior history and with no cGVHD symptoms) [7, 8], c) classic cGVHD (may be present at any time post allo-HSCT with diagnostic and distinctive features of cGVHD), cGVHD can be de novo onset or quiescent d) Overalpap syndrome (may be present at any time post allo-HSCT with features of both cGVHD and aGVHD [9, 10].

Transplantation-related mortality ranges from 3% to more than 50%, depending on factors related to the patient (age, sex, comorbid diseases), the disease (stage, extent of involvement, and intrinsic disease characteristics), or the transplantation procedure (time from diagnosis to transplantation, type of graft, and human leukocyte antigen (HLA) compatibility of the donor) [11]. aGVHD, and often the treatment of aGVHD, results in organ toxicity, frequent infections, malnutrition and significant morbidity, thus delaying the recovery of patients from allo-HSCT. Although aGVHD is a substantial cause of morbidity and mortality after allo-HSCT, survival outcomes have however, improved over the last few decades because of improvements in non-relapse mortality rather than relapse incidence [12, 13]. This article aims to provide a perspective of the current prevention and management of aGVHD, highlighting both what is known and what is yet to be determined in this complex disease.

2. Acute GVHD

Acute GVHD is the major complication of allo-HSCT, developing in 30-50% of patients undergoing a matched-related or unrelated allo-HCT following standard aGVHD prophylaxis [14-16]. The pathophysiology of aGVHD is complex and the current dogma is that there is tissue damage due to conditioning that in turn activates the host antigen-presenting cells (APCs). The initial activation of host APCs by recipient conditioning in turn activates transplanted donor T lymphocytes that expand and differentiate into effector cells that mediate cytotoxicity against recipient tissues through Fas-Fas ligand interactions, perforin-granzyme B, and cytokine production [14, 16-18]. When severe, aGVHD carries a poor prognosis, with only 25% long term survival for grade III and 5% for grade IV [15].Recent data suggests that the majority of memory T cells reside in human peripheral tissues, skin, gut, liver, and lung [19, 20]. Clinical observations suggest that skin-resident T cells survive "lymphocyte-depleting" chemotherapy, as patients who are profoundly lymphopenic following chemotherapy can still develop T cell–mediated drug rashes despite the absence of circulating lymphocytes [21]. In agreement with these observations Divito et al. [22], showed that host T cells survive conditioning prior to allo-HSCT and are present in target tissues during aGVHD. The study showed a proinflammatory role for host T cells in peripheral tissues. In addition, a study showed selective depletion of naïve T cells from human allogeneic grafts reduced the incidence of cGVHD but did not affect aGVHD [23]. It is possible that both donor and host T cells may be required for severe acute GVHD, host T cells alone may be sufficient for mild GVHD. Hence, the assumption that donor T cells are important and that T cell depletion abrogates GVHD might change in the future and needs to be further studied.

3. Grading of aGVHD

Acute GVHD primarily involved the skin (maculopapular erythematous skin rash), liver(hyperbilirubinemia and/or elevated liver enzymes), and gastrointestinal(GI) tract(upper and/or lower GI tract with nausea, vomiting, anorexia, weight loss, diarrhea, ileus, and/or GI bleed) and the severity is graded by evaluating these 3 target organs by pattern of their involvement, and symptoms [24-28]. The Glucksberg aGVHD classification was first proposed in the 1974. This classification staged skin, lower gastrointestinal tract and liver, each on a scale of 0 (absent) to 4 (severe) points (Table 1 and Table 2), to create a final overall grade of I (mild) to IV (life-threatening) [24, 29]. The Keystone aGVHD consensus panel reviewed the outcome of the Glucksberg classification in almost 6000 patients and confirmed the predictive value of maximum aGVHD grade for day 100 mortality [25] (Table 1 and Table 2). Three major recommendations resulted from the review (1) upper GI tract manifestations, in the presence of a positive biopsy, should be classified as overall grade II aGVHD; (2) GI stage 4 should be based on severe symptoms such as severe pain, bleeding and/or ileus and not diarrhea volume alone; and (3) functional status should be eliminated as an element of overall grade because it is non-specific and multiple factors play a role in functional status. The International Blood and Marrow Transplant research (IBMTR) aGVHD classification was also proposed by the Center for International Blood and Marrow Transplant Research (CIBMTR) which provided a more accurate prediction of Mortality [26] (Table 1 and Table 2). The Minnesota aGVHD grading is a further adaptation of the keystone concensus criteria. This criteria limited overall grade IV aGVHD to skin and gut stage four, instead of skin and liver stage four [28] (Table 1 and Table 2). The diagnosis should ideally be supported by histologic findings on biopsy, but this is

not strictly necessary. Since the diagnosis is based mostly on clinical presentations and symptoms, the diagnosis, grading and percentage of aGVHD among institutions can vary, making it challenging for data interpretation, clinical comparison, and consistency [29]. The Mount Sinai Acute GVHD International Consortium (MAGIC) has revisited these criteria based on a review of their extensive database containing detailed clinical information on aGVHD, and recommended more precise definitions for grade IV aGVHD [27] (Table 1 and Table 2).

Table 1 Comparison of staging of acute GVHD.

Organ Severity Stage	Original Glucksberg Critera[24]	Modified Glucksberg or Keystone Criteria[25, 26]	Minnesota Criteria[28]	MAGIC[27]
Skin				
0	No rash	No rash	No rash	No rash
1	Rash <25% of BSA	Rash <25% of BSA	Rash <25% of BSA	Rash <25% of BSA
2	Rash 25% to 50% of BSA	Rash 25% to 50% of BSA	Rash 25% to 50% of BSA	Rash 25% to 50% of BSA
3	Rash > 50% of BSA	Rash > 50% of BSA	Rash > 50% of BSA	Rash > 50% of BSA
4	Generalized erythroderma with bullous formation	Generalized erythroderma with bullous formation	Generalized erythroderma with bullous formation	Generalized erythroderma (> 50% BSA) plus bullous formation and desquamation >5% of BSA
Liver				
0	Total serum bilirubin <34umol (2mg/dl, or AST/SGOT 150-750IU	Total serum bilirubin < 2 mg/dL)	Total serum bilirubin <34umol (2mg/dl)	Total serum bilirubin <34umol (2mg/dl)
1	Total serum bilirubin 34–50 µmol/L (2 to 3 mg/dL)	Total serum bilirubin 34–50 µmol/L (2 to 3 mg/dL)	Total serum bilirubin 34–50 µmol/L (2 to 3 mg/dL)	Total serum bilirubin 34–50 µmol/L (2 to 3 mg/dL)
2	Total serum bilirubin 51–102µmol/L (3.1 to 6mg/dL)	Total serum bilirubin 51–102µmol/L (3.1 to 6 mg/dL)	Total serum bilirubin 51–102µmol/L (3.1 to 6mg/dL)	Total serum bilirubin 51–102µmol/L (3.1 to 6mg/dL)
3	Total serum bilirubin 103–255 µmol/L (6.1 to 15 mg/dL)	Total serum bilirubin 103–255 µmol/L (6.1 to 15 mg/dL)	Total serum bilirubin 103–255 µmol/L (6.1 to 15 mg/dL)	Total serum bilirubin 103–255 µmol/L (6.1 to 15 mg/dL)
4	Total serum bilirubin > 255 µmol/L (>15 mg/dL)	Total serum bilirubin > 255 µmol/L (> 15 mg/dL)	Total serum bilirubin > 255 µmol/L (>15 mg/dL)	Total serum bilirubin > 255 µmol/L (>15 mg/dL)

Upper GI				
0	NA	No persistent nausea and no histologic evidence of GVHD in the stomach or duodenum	No persistent nausea and no histologic evidence of GVHD in the stomach or duodenum	No or intermittent[a] a anorexia or nausea or vomiting
1	NA	Persistent nausea with histologic evidence of GVHD in the stomach or duodenum	Persistent nausea with histologic evidence of GVHD in the stomach or duodenum	Persistent[a] a anorexia or nausea or vomiting
Lower GI				
0	Diarrhea <500 ml/day	Diarrhea <500 ml/day	Diarrhea <500 ml/day	Diarrhea < 500 mL/day or <3 episodes/day for adults[b,c]
1	Diarrhea >500 ml/day	Diarrhea >500 ml/day	Diarrhea >500 ml/day	Diarrhea 500-999 mL/day or 3-4 episodes/day for adults[b,d]
2	Diarrhea >1000 ml/day	Diarrhea >1000 ml/day	Diarrhea >1000 ml/day	Diarrhea 1000-1500 mL/day or 5-7 episodes/day for adults[b,e]
3	Diarrhea >1500 ml/day	Diarrhea >1500 ml/day	Diarrhea >1500 ml/day	Diarrhea >1500 mL/day or >7 episodes/day for adults[b,f]
4	Diarrhea >2000 ml/day	Severe abdominal pain with or without ileus	Severe abdominal pain, bleed, with or without ileus	Severe abdominal pain with or without ileus or grossly bloody stools (regardless of stool volume)
Karnofsky Index score				
	>30%	NA	NA	NA
	<30%	NA	NA	NA

BSA: body surface area, AST aspartate transaminase, SGOT Serum glutamic oxaloacetic acid transaminase, PS performance status

[a]To be suggestive for GVHD: anorexia should be accompanied by weight loss, nausea should last at least 3 days, or be accompanied by at least 2 vomiting episodes per day for at least 2 days [18]

[b] One episode of diarrhea is considered to be about 200 ml for an adult and 3 ml/kg for a child (< 50 kg) [16]

[c] Diarrhea 30 mL/kg/day or >10 episodes/day for children

[d]Diarrhea 10–19.9 mL/kg/day or 4–6 episodes/day for children

[e] Diarrhea 20–30 mL/kg/day or 7–10 episodes/day for children

[f] Diarrhea > 30 mL/kg/day or >10 episodes/day for childre

Table 2 Comparison of grading of acute GVHD.

Overall Glucksberg MGIC Grade	Original Glucksberg Critera[24]	Modified Glucksberg or Keystone Criteria[25]	Minnesota Criteria[28]	MAGIC[27]	IBMTR Criteria[26]	Overall IBMTR Grade
0	no organ involvement (skin=0; and liver=0; and GI=0) corresponds to the absence of aGVHD	no organ involvement (skin=0; and liver=0; and GI=0) corresponds to the absence of aGVHD	no organ involvement (skin=0; and liver=0; and GI=0) corresponds to the absence of aGVHD	no organ involvement (skin=0; and liver=0; and GI=0) corresponds to the absence of aGVHD	no organ involvement (skin=0; and liver=0; and GI=0) corresponds to the absence of aGVHD	**0**
1	skin=1 or 2, without liver/GI involvement or decrease in performance status/fever	skin = 1 or 2, without liver/GI involvement	skin = 1 or 2, without liver/GI involvement	skin = 1 or 2, without liver/GI involvement	skin=1, without liver/GI involvement	**A**
II	skin=1 or 2 and (liver and/or GI involvement=1 or 2) with mild decrease in performance status	skin=3; and/or liver=1; and/or GI=1	skin=3; and/or liver=1; and/or GI=1	skin=3; and/or liver=1; and/or GI=1	skin=2; and/or liver =1 or 2; and/or GI=1 or 2	**B**
III	(skin and/or liver and/or GI=2, 3 or 4) with marked decrease in performance status	liver=2 or 3; and/or GI=2, 3 or 4	liver = 2, 3 or 4; and/or GI = 2 or 3	liver=2 or 3; and/or GI=2 or 3	skin=3; and/or liver=3; and/or GI=3	**C**
IV			skin = 4; and/or GI = 4			**D**

	(skin and/or liver and/or GI=2, 3 or 4) with Karnofsky <30%	skin=4; and/or liver=4	skin=4; and/or liver=4; and/or GI=4	skin=4; and/or liver=4; and/or GI=4

4. Acute GVHD and Biomarkers

The criteria of aGVHD are sometimes not adequate to guide treatment at symptom onset resulting in a delay in intensifying treatment. Thus, the need to develop a system to define risk across at the onset of clinical presentations is needed. Post- allo-HSCT changes in plasma proteins associated with markers of target organ damage was a valuable source to get an insight into the earliest stages of GVHD pathophysiology prior to clinical manifestations [30, 31]. The first validated test for aGVHD was measured using ELISA evaluating the levels of biomarkers in patients undergoing allo transplantation. Four biomarkers (IL-2Rα, TNFR1, IL-8, and HGF) effectively discriminated between patients with and without GVHD [32]. This biomarker panel was highly specific and predicted long-term survival independently of maximum GVHD grade. This biomarker panel provided a specific test for GVHD in the 85% of patients who do not have other major complications. Since resistance to GVHD therapy is associated with non-relapse mortality (NRM) and lower overall survival (OS). Thus, the need for biomarkers which would help with early assessment of treatment response. Also, this study lacked in comparing plasma concentrations of patients at GVHD onset with those of patients who had similar symptoms but which were from other causes. Hence, studies were done to determine organ-specific biomarkers to differentiate GVHD from other causes of similar symptoms [33]. Proteomics discovery and validation strategies were applied to identify the following biomarkers for GVHD. Tumor necrosis factor receptor-1 [TNFR1], IL-2 receptor-α [IL-2Rα], IL-8 for systemic GVHD; hepatocyte growth factor [HGF]) with diagnostic and prognostic value for acute GVHD; Elafin for skin GVHD, and REG3a-GI and ST2 for GI GVHD [32, 34-36]. No single biomarker predicted outcomes for GVHD and a panel was tested to see if it could overcome institutional effect and stratify patients for risk of NRM at 6 months. Every combination was tested and with competing regression model, biomarkers comprising of ST2, TNFR1 and REG3a was the best model and generated probability of NRM identified thresholds for Ann Arbor scores [32]. This panel possessed biological relevance to gastro intestinal GVHD which was of importance as most deaths of patients with GVHD - are due to poor response to treatment of GVHD in the gastro intestinal tract.

The Mount Sinai Acute GVHD International Consortium (MAGIC), a group of 25 HCT centers has since validated an algorithm that combines two GI biomarkers (ST2and REG3α) into a single value that estimates the probability of 6 month non-relapse mortality (NRM) for individual patients, known as the MAGIC algorithm probability (MAP). The MAP reflects GI crypt damage and serves as a 'liquid biopsy' of the lower GI tract; it also predicts response to treatment and maximum GVHD severity and is commercially available and used among centers in clinical practice [37]. This biomarker panels measured during the course of GVHD treatment provide valuable prognostic information, independent and additive to the observable clinical status of the patient at the time of measurement [38]. Limitation of MAP is that it is mostly focused on assessment of GI GVHD and not other organs such as the liver which can also be associated with high mortality. It is however being used as an affirmation of disease risk in conjunction with clinical staging to make decision on intensity of treatment. MAP was used in the BMT/CTN 1501 to confirm Minnesota low risk aGVHD in the randomized study between Prednisone and Sirolimus for low-risk aGVHD [39]. It is also been used in ongoing study for low risk aGVHD in which steroid-sparing drug such as Itacitinib, may be an optional treatment (NCT-03846479); and in ongoing study to predict patients for potential high risk GVHD and pre-emptively start them on treatment before onset of GVHD (Alpha-1-Antitrypsin - NCT03459040).

5. Prophylaxis for GVHD

Clinically significant grade II-IV aGVHD occurs in 34-40% of patients undergoing HLA-matched related HSCT, 47-52% of HLA-matched unrelated and is further increased in those lacking matched donors [40-42]. Prophylaxis after allo-HSCT is an ongoing effort due to the heterogeneity in the studies with respect to the donor type and graft source.

The GVHD pharmacological prophylaxis may include calcineurin inhibitors (CNIs); cyclosporine A (CSP) and tacrolimus (TAC), the antimetabolite methotrexate (MTX), the mTOR inhibitor sirolimus, the inosine monophosphate dehydrogenase inhibitor mycophenolate mofetil (MMF) as well as anti-thymocyte globulin (ATG) and corticosteroids, referred to methylprednisolone, prednisolone and/or prednisone. Guidelines for GVHD prophylaxis have been proposed by the European group for Bone and Marrow Transplantation and European LeukemiaNetT [43] however, the choice of GVHD prophylaxis generally varies by institution but is consistent with bone marrow transplant clinical trials network (BMT CTN) guidelines/protocol being proposed and takes into account the disease, degree of HLA disparity, conditioning regimen and patient characteristic (Table 3) [44-46].

Table 3 Prophylaxis of GVHD.

Study	Trial type	N	Prophylaxis treatment	Graft source	aGVHD incidence	cGVHD	OS/PFS
Hiraoka et al., 2001 [47]	Phase III Open label randomized	136	TAC vs CSP	BM	17.5% vs 48% (p<0.0001)	47.3% vs 47.8% (p=0.77)	62.9-OS 65.2% OS (p=0.93)
Nash et al., 2000 [5]	Phase III Open label randomized multicenter	180	TAC + MTX vs CSP +MTX		56% vs 74% (p=0.0002)	76% vs70% (p=0.88)	54 vs50 (p=0.46)
Kanda et al., 2016 [48]* *Similar efficacies	Open label Randomized controlled	107	CSP+MTX vs TAC+MTX	BM	39.6% vs 33.3% (p=0.41)	38.1% vs 46.9% (p=0.60)	66.3%-low risk,33.3%-high risk in CSP+MTX; 70.4%-low risk, 30.0%-high risk in the TAC+MTX (p=0.79)
Finke et al., 2009 [49]	Phase III Open label randomized multicenter	202	ATG-F vs no ATG-F	PB; BM	33%vs 51% (p=0.011)	12.2% vs 42.6% (p<0.0001)	59.2%vs 51.9%(p=0.47)
Finke et al., 2017 [50]	Phase III Open label randomized multicenter	202	ATG-F vs no ATG-F	PB; BM		14% vs 52% (p<0.0001)	49% vs 37% (p=0.31)
Walker et al., 2016 [51]	Phase III Open label randomized multicenter	203	rATG vs no ATG	PB; BM	50% vs 65% (p=0.12)	22% vs 33% (p=0.065)	25% vs 35% (p=0.24)

Bolaños-Meade et al., 2019 [52]	Phase II randomized		273	MTX +TAC (control gp); vs MMF+TAC +PTCy (gp1) MTX+TAC+bortezomib (gp2); MTX+TAC+Maraviroc (gp3)		30% control gp 27%-gp1 (p=0.0082) 26% -gp2 (p=0.14) 32% -gp3 (p=0.98)	38%- control gp; 28%-gp1 (p=0.069);39%-gp2 (p=0.72) and 43% -gp3 (p=0.36)	71% -gp1 (p=0.94), 68% -gp2 (p=0.49), 66%-gp 3 (p=0.28)
Cutler et al., 2004 [53]	Clinical trial		118	SIR/TAC/MTX in HLA match related (gp-1); HLA-matched, unrelated (gp-2); 1Antigen mismatch (gp-3)	BM; PB	26%-gp-1; 16%-gp2; 5%-gp-3	44% -gp-1, 31%-gp-2 and 48%- gp-3	51%-gp-1, 72%-gp-2 and 74%-gp-3
Cutler et al., 2014 [54]	Phase II multicenter		304	TAC/SIR vs TAC/MTX	PB	26% vs 34% (P=0.48)	53% vs 45% (p=0.06)	59% vs 63% (p=0.36)
Kennedy et al, 2021 [55]	Phase II randomized multicenter		145	CSP/MTX + Placebo vs. CSP/MTX + Tociluzumab 50 MRD 95 MUD	PB	40% placebo vs. 29% Toci (p=0.19) 48% vs.32% in MUD (p=0.13)		OS 79% vs 71% (p=0.27)
Watkins et all, 2021 [56]	Phase II randomized trial multicenter		312	CNI/MTX + Abatacept (116 pts) Vs. CNI/MTX+ Placebo control arm (196 pts) 8/8 and 7/8 HLA match Pediatric and adult Endpoint: severe aGVHD by Day +100	PB, BM	Severe aGVHD: 2.3% abatacept vs 30.2% placebo (P<0.001)	7/8 and 8/8 abatacept: 62% and 51.9%. Control 45.9% and 45.3% (p=-.74 and p=.55 respectively)	In the 7/8 HLA arms, abatacept improved 2 yr NRM vs. placebo (p=.01) and OS va. Placebo (p=.002) No difference in 8/8 HLA matched.

Abbreviations: TAC-tacrolimus; CSP-cyclosporine; MTX-methotrexate; MMF- mycophenolate mofetil; SIR-Sirolimus; PTCy-post-transplantation cyclophosphamide; ATG-F- Anti-T-lymphocyte globulin Fresenius; r-rabbit; gp-group.PB: peripheral Blood, BM, bone marrow, MRD matched related donor, MUD matched unrelated donor, Toci Tocilizumab.

5.1 Calcineurin Inhibitors (CNIs)

Calcineurin, is an enzyme that activates T-cells of the immune system which is the cause of GVHD. Calcineurin inhibitors help in the prevention of GVHD by inhibiting this action of calcineurin. The combination of MTX and CNIs, such as TAC or CSP is the most historical and widely used regimen used for GVHD prophylaxis [57]. Although grade 2-4 aGVHD was similar with either mycophenolate mofetil (MMF) or MTX, grade 3-4 aGVHD has been shown to be higher following MMF and CNI compared to MTX and CNI. Moreover, GVHD patients develop infectious complications, and toxicity [58]. Thus, prophylaxis regimen that would minimize GVHD and other adverse events translating into better outcomes were tested. Phase III trials comparing TAC/MTX versus CSP/MTX showed incidence of grade II-IV aGVHD being 56% in TAC/MTX versus 74% in CSP/MTX arm [5]. A meta-analysis of randomized trials showed that TAC/MTX was associated with lower aGVHD compared to CSA/MTX but there was no survival benefit of TAC/MTX, perhaps due to increased toxicity [5, 47, 59]. However, the combination of TAC/MTX remains a standard for GVHD prophylaxis with myeloablative (MA) conditioning in the U.S, despite its limited efficacy. The common practice is to use MMF in reduced intensity conditioning (RIC). In a prospective randomized phase 2 trial with RIC, patients were randomly assigned to one of the 3 arms: TAC/MTX/bortezomib , TAC/MTX/ maraviroc and TAC/MMF/cyclophosphamide(PT-CY) [52]. The purpose of this study was to find the best prophylaxis arm to compare with the most used standard prophylaxis TAC/MMF. TAC/MMF/PT-CY) yielded the best GVHD-free-relapse free survival (GRFS) outcome [52]. TAC/MMF/PT-CY is now being prospectively compared with TAC/MTX in a phase 3 randomized trial (BMT CTN 1703) (NCT03959241) for patients undergoing RIC allo-HSCT to determine the best GVHD prophylaxis regimen in this patient population. The combination of MTX/CNI has been widely used but despite this GVHD remains a significant barrier to allo-HSCT with mismatched unrelated donors (MMUD). A phase I/II trial evaluated a bortezomib based regimen for controlling GVHD after MMUD [60]. In this study bortezomib, administered once per day after peripheral blood (PB) grafts plus standard TAC/MTX. GVHD incidence was low and survival outcomes were comparable to those observed in HLA matched transplants. Adding a T-cell costimulation blockade abatacept to CNI/MTX was recently published in a phase II randomized trial [56], showing that abatacept improved severe aGVHD survival (sGFS) compared to placebo. S In 8/8s, grade 3-4 aGVHD was 6.8% (abatacept) versus 14.8% (placebo) (P = .13). SGFS was 93.2% (CNI/MTX plus abatacept) versus 82% (CNI/MTX plus placebo, P = .05). In the smaller 7/8 cohort, grade 3-4 AGVHD was 2.3% (CNI/MTX plus abatacept, intention-to-treat population), which compared favorably with a nonrandomized matched cohort of CNI/MTX (30.2%, P, .001), and the SGFS was better (97.7% v 58.7%, P, .001). Immunologic analysis revealed control of T-cell activation in abatacept treated patients (Table 3). A phase III double-blind trial with Tocilizumab (Toci) or placebo with MTX/CNI in 145 patients (50 matched related donor (MRD) and 95 matched unrelated donor (MUD)) was recently published [55]. The incidence of grade II-IV aGVHD at day 180 for the entire cohort was 40% placebo versus 29% Toci (p=0.19) and 48% versus 32% (HR 0.59; CI:0.30-1.16, p=0.13) for the MUD subgroup. Reductions in aGVHD were predominantly in grade II disease. A trend to improved aGVHD-free survival (aGVHD-FS) was noted in the Toci treated MUD group with 52% placebo 68% toci (p=0.13). -For entire cohort, overall-survival was 79% versus 71% (p=0.27) respectively. Hence, while Toci had a non-significant trend towards mostly grade II aGVHD and GVHD-FS, there was no improvement in overall survival [55](Table 3). CSA and TAC are considered equivalent and there has been discussions on the target serum concentrations. It has been shown that the target concentrations of TAC in the first 4 weeks

post-ASCT were associated with GVHD [48, 61]. For CSP-A, concentrations of 200–300 µg/L are usually recommended when the total daily dose is divided into 2 doses 12 hours apart f for preventing aGVHD [43]. Low levels of CSP-A early after allo-HSCT and TAC has been shown in acute myeloid leukemia patients [62-65]. On the other hand the target ranges of the blood concentration of TAC in the first 4 weeks post transplantation have varied significantly among studies [5, 59]. These studies have also emphasized the importance of adjusting dose levels to avoid toxicity [66, 67]. Przepiorka et al. [67] reported that mean TAC levels >20ng/ml is associated with increased risk of creatinine >2mg/dl. A study in the adult population reported that the early post-transplantation blood concentrations of TAC had a significant impact on the development of aGVHD with another reporting TAC level of <5ng/ml was associated with increased acute GVHD [68, 69]. In an attempt to understand the impact of early TAC levels on aGVHD incidence, Ganetsky et al. [61] showed that TAC concentration ≥12 ng/ml during the first week post-transplantation was associated with reduced risk of aGVHD. In our institutional study with approx. 700 patients, we found that week 1 TAC levels ≥ 10.15 ng/ml were associated with a lower risk of grade II-IV and III-IV aGVHD (manuscriptaccepted, Cancers, 2021). However, many factors need to be taken in consideration with adjusting CI dose based on drug levels including drug-drug interactions, serum creatinine, and appropriate logistics of the levels being drawn.

This also holds true for recipients lacking HLA-matched donors. A phase I/II trial evaluated a bortezomib based regimen for controlling GVHD after mismatched unrelated donor (MMUD) [60].

5.2 Sirolimus (SIR)

SIR is structurally similar to TAC but with different mechanism of action. It is a rapamycin inhibitor which binds to FK binding protein 12 complex with mammalian target of rapamycin (mTOR), a protein kinase that regulates mRNA translation and protein synthesis, an essential step in cell division and proliferation [70]. The FKBP12-mTOR complex inhibits IL-2 mediated proliferation signaling, with a reduction in DNA transcription, DNA translation, protein synthesis and cell cycling, ultimately leading to T-cell immunosuppression [53]. PTEN/PI3 kinase/Akt and the Janus kinase, are the upstream pathways which interact with mTOR and are important in mediating IL-2-driven signaling from the T-cell receptor [71]. SIR appears to exert some of its immunosuppressive properties via inhibition of dendritic cell activity through a reduction in antigen uptake [72], cellular maturation [73], intracellular signaling [74] and apoptosis induction in dendritic cells [75]. SIR and CNIs, they seem to work synergistically, because SIR does not interact with calcineurin or its downstream effectors [76].

The combination of SIR and TAC, when compared with combination of SIR and CSP, has demonstrated higher efficacy and lower toxicity profile [77-79]. In initial studies evaluating its role, as a GVHD prophylaxis, SIR was administered as a 12 mg oral loading dose on day –3, followed by a 4 mg/day single dose, with a target serum concentration of 3–12 ng/mL [6, 53]. In 3 clinical trials, the combination of SIR, TAC and MTX (5mg/m2 day +1, 3, 6 and 11) was used as GVHD prophylaxis. In the HLA matched, related donor setting, patients who underwent PB stem cell transplantation received SIR/TAC without MTX as GVHD prophylaxis with MA conditioning [80]. The hypothesis here was that omitting MTX will not increase the rate of GVHD and will reduce TRM. In the HLA matched, unrelated donor setting SIR/TAC and low dose of MTX was used with MA conditioning [6, 81, 82]. In the single antigen mismatch, SIR/TAC and low dose MTX was used with non-MA conditioning. In these studies, sirolimus was shown to have a reduced incidence of aGVHD and toxicity after match

related and match unrelated transplants. It was also shown to be effective with both non-MA and RIC regimens [53].

TAC/MTX combination has been used widely but as MTX is known to have delayed neutrophil and platelet engraftment along with toxicity, it has been implicated in initiating toxicity. BMT-CTN 0402 was a phase III, multicenter trial which sought to determine if TAC/ SIR would lead to better GVHD and TRM outcomes compared to TAC/MTX. In this study, 114 days from the time of randomization, TAC/SIR prophylaxis provided equivalent GVHD-free survival when compared with TAC/MTX in MRD transplantation. However, TAC/ SIR was associated with more rapid engraftment and reduced oropharyngeal mucositis after MRD transplantation [54]. Thus, it was concluded that TAC/ SIR is an acceptable alternative to TAC/MTX after MRD HSCT and could be considered in patients at a high risk for oropharyngeal mucositis or higher risk of infection. SIR with MTX is associated with an increased incidence of VOD. Mortality after VOD diagnosis was unaffected, and overall treatment-related mortality was lowest when SIR was used without MTX. Similar findings were noted in MRD, and MUD as well as MMUD subgroups [83]. The Blood and Marrow Transplant Clinical Trials Network conducted a multicenter, randomized phase III trial comparing SIR/TAC vs MTX/TAC after HLA-matched, related (MRD) PB HSCT [54]. SIR/TAC showed more rapid engraftment and less oropharyngeal mucositis compared to MTX/TAC. SIR based prophylaxis regimens have also been shown for haploidentical, single HLA antigen mismatched related and matched unrelated transplantation (MUD). SIR has been shown to synergistically enhance immunosuppressive effects of CSP-A and TAC. SIR is not neurotoxic and seems to have minimal renal toxicity [84, 85], enhancing CNI-induced nephrotoxicity [86]. SIR may be implicated as a cause of thrombotic microangiopathy (TMA) [87]. Robson et al. [88] reported four renal transplant patients who developed TMA soon after the introduction of SIR into their immunosuppression protocol. In contrast to CNIs, no TMA was observed when SIR was combined with MMF for GVHD prophylaxis [70, 85, 86]. SIR combined with CNIs, increased incidences of TMA and possibly veno-occlusive disease (VOD) have been observed [89]. SIR may promote TMA via direct endothelial cell damage or potentiation of CNIs effects [88]. However, TMA related to SIR monotherapy has been less commonly reported [90].

6. T Cell Depletion

6.1 Post-Transplantation Cyclophosphamide (PTCy)

Initially developed for prophylaxis in the setting of a haploidentical transplant, the use of this regimen has gained widespread acceptance as an accepted approach in multiple types of allogeneic transplantation. It has been used for prevention of GVHD in HLA-matched and mismatched, as well as with both MA and non-MA regimens [91-94]. PTCy has been shown to have reduced incidences of lymphoproliferative disorders and lower grades 3-4 aGVHD and cGVHD by depleting alloreactive T cells in the host and/or support T cell mediated tolerance [95, 96]. Studies have shown that Cy as a single agent or together with MMF and TAC/CSP is safe and effective for lowering aGVHD and cGVHD with T cells replete grafts from partially HLA mismatched related donors [91, 94, 97, 98]. Recently, in a phase II randomized trial tested three approaches for GVHD prophylaxis (TAC, MMF, and PTCy; TAC, MTX, and bortezomib;and TAC, MTX, and maraviroc), comparing each to a nonrandomized contemporary cohort using a novel endpoint assessing GVHD relapse and survival. All patients received received RIC conditioning with MRD or MUD donors. High-dose PTCy-based regimen was shown to be effective versus MTX and CNI for GVHD prophylaxis [52].

6.2 Anti-T-Lymphocyte Globulin (ATG)

Allo-HSCT is increasingly used as a treatment for patients with life threatening blood diseases. The success of this is based on immune-based graft versus leukemia effect caused by donor T cells. But donor T-cells are also the cause of GVHD. Allo- HSCT are done using PB instead of bone marrow (BM) as the graft source. PB has the advantage of more hematopoietic stem cells but also it has more T cells. This causes higher incidences of both aGVHD and cGVHD. These observations have led to various studies aiming at assessing the impact of immunoregulation with ATG on transplantation outcomes. A better understanding of the pathobiology behind the GVHD process has thus led the way to novel approaches and medications. Adding ATG in one of its three commercially available preparations (Thymoglobulin, ATGAM, and ATG-Fresenius) for in vivo T- cell depletion has been shown to decrease the incidence of aGVHD and cGVHD, with mixed effects on disease relapse [49, 99-101] [102]. However, due to the differences in preparations and dose of ATG, it has been difficult to compare outcomes between them [101]. Initially, a high dose (10mg/kg) of thymoglobulin was utilized but it had a high incidence of relapse [103]. Remberger et al., evaluated four different doses of ATG to determine the optimal dose in patients who matched unrelated transplants [104]. The authors showed a dose dependent effect of ATG on aGVHD. However, higher doses also led to increased infection rate. In fact, the best results were seen with intermediate doses (6mg/kg, 8mg.kg) as reduced risk of GVHD was seen without a substantial risk of infections [104]. Recently, we reported aGVHD incidence to be higher at 4.5mg/kg versus 6mg/kg. There was a significant reduction in the cumulative incidence of aGVHD grade II-IV in the dose with 6 mg/kg ATG compared to 4.5mg/Kg [105]. There was no difference in relapse, progression free survival (PFS), non-relapse mortality (NRM), and overall survival (OS). But there was a significantly decreased risk in the incidence of cytomegalovirus (CMV) and epstein barr virus (EBV) reactivation at 180 days in the 4.5mg/kg group compared to the 6mg/kg group. ATG works through multiple mechanisms including T-cell depletion in the blood and lymphoid tissues by induction of apoptosis or complement-dependent lysis, apoptosis of naïve B cells, activated B-cells and plasma cells [106, 107], and by induction of regulatory T-cells, and natural killer (NK) cells [108] These effects could potentially lead to serious infections such as CMV and EBV, and possibly to disease relapse [109, 110]. The addition of ATG to other GVHD prophylaxis regimens decreases the cumulative incidence of cGVHD and allows more patients to discontinue pharmacologic prophylaxis for aGVHD, but has not demonstrated a survival benefit. The optimal indication, dosing regimen, time point of application is still a matter of debate. Given the heterogeneity of the patients undergoing allo-HSCT, there may not be a single, optimal dose. Instead, ATG-T dosing may depend on intensity of the preparative regimen, donor characteristics, and recipient lymphocyte counts.

7. Treatment for Acute GVHD

The mainstay of treatment of aGVHD is high-dose corticosteroids (methylprednisone or prednisone) [28, 111-114] (Table 2). One of the first studies assessing the efficacy of Steroids in treating aGVHD was a randomized study comparing methylprednisone (MP) 2mg/kg (n=47) versus 10 mg/kg (n=48), given for 5 days with subsequent tapering [111]. Primary endpoints were response to treatment and evolution of aG-VHD to grade III-IV. The study showed no difference in response (68% vs. 71%), evolution to aGVHD grade III-IV (17% vs.20%), CMV infections (55% vs. 60%), Treatment related mortality (TRM) (28% vs. 32%) and 3-yr OS (63% vs. 62%). Hence an initial dose

of MP 2mg/kg or the equivalent of Prednisone became the standard of care for first line therapy of aGVHD grade II-IV. The addition of Ruxolitinib to steroids for aGVHD in haploidentical transplant was a phase II study in 32 patients. The overall CR at day 56 was 96.8% [115]. We hope a phase III study is forthcoming. A phase III placebo controlled randomized trial comparing Steroids +/- Itacitinib has finished accrual in July 2020 and we await results (ClinicalTrials.gov Identifier: NCT03139604). The addition of other agents to steroids as first line treatment in aGVHD have been investigated, and none have shown a statistically significant outcome compared to steroids alone [114, 116, 117] (Table 4).

Table 4 aGVHD treatment: Randomized studies.

Study and trial type	aGVHD requirement	N	Steroid given and dose	Experimental agent	Prior GVHD prophylaxis	Response- standard vs. Experimental arm Day 28 or 56
Van Lint, 1998 [111] Randomized	Grade II-IV	95	MP 2 mg/kg (n=47)	MP 10 mg/kg (n=48)	CSA: 53% CSA/MTX 47%	ORR: 68% vs.71% aGVHD III-IV develop 17% vs.20% 3yr OS 63% vs.62% Overall no difference
Cragg 2000 [118] Randomized	Grade II-IV	96	Prednisone 60mg/m^2 x 7 d then taper (n=46)	Prednisone 20mg/m2 + ATG 15 mg/kg. Bid	MTX/CSA:53% MTX/ATG/Pred	ORR 78% both groups CMV infection 22% vs. 44% Pneumonitis 24% vs. 50% 2 yr OS similar. ATG added no benefit
Lee, 2004 [119] Randomized	Grade II-IV	102	MP 2mg/kg	MP 2 mg/kg +Daclizumab 1 mg/kg	NA	Study halted after a planned interim analysis showed worse 100d survival in daclizumab arm 77% vs. 94% 1-yr OS 29% vs. 60%. Daclizumab was detrimental
Couriel, 2009 [120] Randomized	Grade II-IV	63	MP 2 mg/kg	MP 2 mg/kg + Infliximab 10 mg/kg	Tacro/MTX 88%	ORR 58% vs. 62% OS 32% vs. 38% NRM 36% vs. 52% (p=0.3) No Benefit with adding Infliximab
Alousi, 2009 [114] Randomized 4 - arm	Grade II-IV	180	Historic Data with Steroid alone at 28day with CR 35%	MP 2mg/kg(or pred 2.5 mg/kg) + etanercept or MMF, or denileukin or Pentostatin	MMF based 24% (randomized to non-MMF arm)	CR: etabercept 26%, MMF 60%, denileukin 53%, penrostatin 38%. Severe infections: 48% vs. 44% vs. 62% vs.57%. Efficacy and toxicity data suggest use of MMF plus steroid to compare to steroid alone
Balanos-Meade [113], 2014. BMT/CTN 0802. Randomized	Any Grade requiring systemic treatment	235	Prednisone 2 mg/kg(or MP 1.6 mg/kg)	Steroid +MMF 1000 mg (or 20 mg/kg) every 8 hrs	No prior MMF allowed within 7 days	Day 56 GVHD-free survival grade III-IV 51.2% vs 54.1%; CR 53.8% vs. 60.3%. No diff CGVHD. Stopping rule for Futility triggered after 2nd interim analysis. MMF did not add ant benefit to steroid alone

Mielcarek, 2015 [121] Randomized	Grade II-IV	164	Cohort A: Prednisone 1 mg/kg vs 0.5 mg/kg for low grade IIa (n=102) Cohort B: Prednosone 2 mg/kg vs. 1 mg/kg for grade IIb or higher	NA	NA	Cohort A: the mean ±SD cumulative prednisone doses were 27.1±12.7 *versus* 22.2±13.7 mg/kg, respectively, at day 42 (18% reduction; *P*=0.08). cohort B: mean ±SD cumulative prednisone doses were 41.3±12.1 *versus* 38.4±14.1 mg/kg (7% reduction; *P*=0.4). OS and GVHD III-IV no difference
Pidala, 2020 [39] BMT/CTN 1501 randomized	standard risk (SR) aGVHD + Ann Arbor (AA1/2) biomarker status. Grade 1-II	122	Prednisone 2mg/kg (or equivalent MP)	Sirolimus (therapeutic level 10-14)	CNI-based 76.2% Post-Cy +others 23.8%	ORR 73% vs.64.8%. The day 28 rate of CR/PR with prednisone < 0.25 mg/kg/day was significantly higher for sirolimus than prednisone (66.7% vs 31.7%; P < .001). No diff in SR aGVHD, DFS, Relapse, NRM

MP stands for methylprednisone; CSA cyclosporine; MTX methotrexate; ORR overall response; OS overall survival; Pred prednisone; ATG antithymocyte globulin; CMV cytomegalovirus; Tacro tacrolimus; MMF mycophenolate Mofetil; NA not applicable/not available; CNI calcineurin inhibitor; Cy clclophosphamide; SR steroid refractory; DFS disease free survival; NRM non-relapse mortality

High dose steroids has toxicity risks such as infections, hyperglycemia, severe muscle weakness and deconditioning. Hence, the need for even lower doses of steroids less than 2 mg/kg is being sought. Unfortunately, there are paucity of Data as to the ideal dose, especially in patients with grade III-IV aGVHD. An area of consideration is to treat patients with less severe acute GVHD- grade II- with doses of MP or prednisone less than 1 mg/kg/day (0.5 mg/kg/day) while reserving the higher dose (2 mg/kg/day) in higher grades, thus reducing toxicity while maintaining efficacy. The study by Mielcarek et al tried to answer this [121]. Patients with acute GVHD grade II with symptoms that included the upper GI (nausea, vomiting, anorexia), or lower GI but stool volume less than 1 L per day, or skin rash < 50% of the body surface (acute GVHD grade IIa) were treated with either 0.5 mg/kg/day or 1 mg/kg/day. Patients with hepatic involvement (defined as total serum bilirubin > 2 mg/dl) or stool volume >1L/day or skin rash ≥ 50% of the body surface area (acute grades IIb–IV) received either 1 mg/kg/day or 2 mg/kg/day. In patients with acute GVHD grade IIa, no differences in OS or progression to grades III–IV acute GVHD were found. However, in patients with grades IIb–IV, patients receiving an initial treatment of 1 mg/ kg/day had a higher likelihood of requiring additional immunosuppression than those who received 2 mg/kg (41% vs 7%, p = 0.001). This supports the idea that patients with lower risk aGVHD may be safely treated with lower dose of steroids (MP 0.5-1.0 mg/kg or Prednisone equivalent) [121].

In addition to lower dose steroids for Minnesota low grade aGVHD, a second strategy is a steroid-free approach, especially for these patients who also have low Ann Arbor risk score. The BMT/CTN 1501 tried to answer this using both staging system [39]. The Minnesota (MN) Risk Score uses organ involvement and severity to identify standard-risk (SR) versus high risk(HR) [10, 122, 123]. The Ann Arbor (AA) biomarker risk score has been validated in multicenter studies [38, 124]. BMT/CTN 1501 randomized patients with both Minnesota SR and AA score of 1-2 to receive either Prednisone (2 mg/kg) or Sirolimus (targeted level 10-14 ng/ml). The Primary endpoint was day-28 complete and partial response (CR and PR). The day 28 CR/PR rates were similar for sirolimus 64.8% vs 73% for prednisone. At 12 months, no differences were detected in steroid-refractory acute GVHD, disease-free survival, relapse, non-relapse mortality, or overall survival. Sirolimus was associated with reduced steroid exposure and hyperglycemia, reduced grade 2 to 3 infections, improvement in immune suppression discontinuation and patient-reported quality of life. Increased risk for thrombotic microangiopathy was higher with Sirolimus. Hence, the conclusion was for patients with clinical- and biomarker-based SR acute GVHD, sirolimus is a viable alternative to prednisone [39]. For now, there are no Clinical trials assessing steroid-free management for Minnesota and Ann Arbor high-risk aGVHD, and high dose steroids (1-2 mg/kg) remain first line treatment.

8. Treatment of Steroid-Refractory Acute GVHD

Despite aggressive treatment with high dose steroids, only 35-40% of patients will achieve durable responses. For patients who fail to respond or progress after initiation of corticosteroids, steroid-refractory aGVHD is associated with high rates of mortality, primarily from infections and/or multi-organ failure [15, 125]. Steroid-refractory aGVHD is characterized by progression after 3 days of high-dose steroids, involvement of new organ, no improvement after 7 days of high dose steroid for grade III aGVHD or persistent GVHD after 14 days of high-dose steroids [112, 126]. Until recently, there was no standard therapy for steroid refractory aGVHD or head-to-head clinical trials comparing agents. Off-label retrospective analysis and small prospective studies have included

treatments such as Etanercept, ATG, infliximab, basiliximab, alemtuzumab, mycophenolate mofetil(MMF), methotrexate, sirolimus, daclizumab, denileukin diftitox, mesenchymal stromal cells, and extracorporeal photophereis, all with variable responses [112]. Cytokines are among the critical effector molecules involved in GVHD pathogenesis and their kinetic pattern of expression is different in various affected tissues [127]. Newer agents that target GI GVHD such as tocilizumab, vedolizumab, alpha-1 antitrypsin and itacitinib are being explored with small prospective and retrospective studies showing varying responses based on organ involvement [128-132]. It remains to be seen whether these or other strategies will have a significant benefit on the long-term outcomes of GI GVHD. These agents were discussed in detail in a recent publication in pharmacotherapy [130].

The only approved drug for steroid-refractory aGVHD is Ruxolitinib based on the REACH 1 and REACH 2 trials [133, 134] In the phase 2 REACH 1 trial, patients aged at least 12 years with grades II to IV steroid-refractory aGVHD were eligible to receive ruxolitinib orally, starting at 5 mg twice daily (increase to 10 mg twice daily after 3 days in absence of cytopenia) plus corticosteroids (methylprednisone 2.0mg/kg/day or prednisone 2.5 mg/kg/day with tapering per institution guideline), until treatment failure, unacceptable toxicity, or death. The primary end point was overall response rate (ORR) at day 28; the key secondary end point was duration of response (DOR) at 6 months. Forty-eight of the 71 patients enrolled had grade III/IV aGVHD. At day 28, 39 patients (54.9%) had an overall response, including 19 (26.8%) with complete responses. Best ORR at any time was 73.2% (complete response, 56.3%). Responses were observed all across skin (61.1%), upper (45.5%) and lower (46.0%) gastrointestinal tract, and liver (26.7%). Median DOR was 345 days. Overall survival estimate at 6 months was 51.0%. At day 28, 24 (55.8%) of 43 patients receiving ruxolitinib and corticosteroids had a 50% or greater corticosteroid dose reduction from baseline. In a post hoc model-based analysis, grade II vs. grade III/IV aGVHD was significantly associated with day 28 response (P = .0042). The most common treatment-emergent adverse events were anemia (64.8%), thrombocytopenia (62.0%), hypokalemia (49.3%), neutropenia (47.9%), and peripheral edema (45.1%) [133]. The ORR was durable with encouraging survival compared with historical data in these patient population.

The REACH 2 trial randomized 309 patients with steroid-refractory grade II-IV aGVHD to ruxolitinib(10 mg twice a day) or investigators choice of control therapy [134]. The type of control therapy was chosen by the investigator at the time of randomization from the following options: antithymocyte globulin, extracorporeal photopheresis, mesenchymal stromal cells, low-dose methotrexate, mycophenolate mofetil, mammalian target of rapamycin (mTOR) inhibitor (everolimus or sirolimus), etanercept, or infliximab. Crossover from control therapy to ruxolitinib therapy was permitted if patients did not have a response at day 28 or if they had a loss of response thereafter and received additional systemic therapy and did not have signs of chronic GVHD. Standard supportive therapy (including growth factors, anti-infective medication, transfusion support, and other standard supportive care measures) was allowed in both treatment groups in addition to the continued use of calcineurin inhibitors and glucocorticoids. ORR at day 28 was higher in the ruxolitinib group than in the control group (62% vs. 39%, p<0.001). DOR at day 56 was higher in the ruxolitinib group than in the control group (40% vs. 22%, p<0.001). The estimated cumulative incidence of loss of response at 6 months was 10% in the ruxolitinib group and 39% in the control group. The median failure-free survival was longer with ruxolitinib than with control (5.0 months vs. 1.0 month). The median overall survival was 11.1 months in the ruxolitinib group and 6.5 months in

the control group (hazard ratio for death 0.83). Thrombocytopenia was more common with ruxolitinib 33% compared to control group 18%, otherwise toxicities and viral infections were similar.

9. Conclusion

Acute GVHD develops in 30-75% of recipients of allo-HSCT despite current GVHD prophylaxis. While majority is grade I-II, 15-20% are grade III-IV that carries a high morbidity and mortality rate. It is however very encouraging to witness the exciting work being done to prevent or reduce the severity of aGVHD with new prophylaxis combination such as post-HSCT cyclophosphamide. Biomarkers are emerging as added tools that can potentially detect early signs of aGVHD before onset of clinical symptoms, and also to stratify patients into risk groups that may or may not need high dose steroids, which can potentially reduce GVHD treatment-related mortality. As the field evolves, new agents are being evaluated for the prevention and treatment of aGVHD. However, more needs to be done to improve on the morbidity and mortality associated with aGVHD.

Author Contributions

Manuscript writing: N.S., and Y.E.; Literature review, scientific input and critical comments: N.S., and Y.E.

Competing Interests

The authors have declared that no competing interests exist.

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