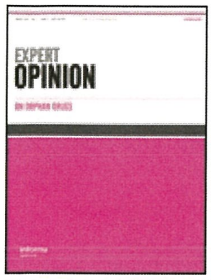

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REVIEW

Therapeutic targets and emerging treatment options in gastrointestinal acute graft-versus-host disease

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ABSTRACT

Introduction: Graft-versus-host disease (GVHD) continues to be the major lethal complication of allogeneic hematopoietic stem cell transplantation (HCT) but the standard of care, high dose steroids, has not changed in 40 years. Approximately 50% of GVHD patients will develop steroid refractory disease, typically involving the gastrointestinal (GI) tract, which has a very poor prognosis. Newly developed GVHD biomarker-based risk scores provide the first opportunity to treat patients at the onset of symptoms according to risk of steroid failure. Furthermore, improvements in our understanding of the pathobiology of GVHD, its different signaling pathways, involved cytokines, and the role of post-translational and epigenetic modifications, has identified new therapeutic targets for clinical trials.
Areas covered: This manuscript summarizes the pathophysiology, diagnosis, staging, current and new targeted therapies for GVHD, with an emphasis on GI GVHD. A literature search on PubMed was undertaken and the most relevant references included.
Expert Opinion: The standard treatment for GVHD, high dose steroids, offers less than optimal outcomes as well as significant toxicities. Better treatments, especially for GI GVHD, are needed to reduce non-relapse mortality after allogeneic HCT. The identification of high risk patients through a biomarker-defined scoring system offers a personalized approach to a disease that still requires significant research attention.

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Biomarkers; GVHD; graft-versus-host disease; microbiome; Paneth cells; steroid-refractory GVHD; treatment

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HCT) is increasingly used to cure malignant and benign hematologic diseases, with over 8000 transplants performed in the year 2013.[1] Transplanted T cells from the donor can recognize and eradicate hematologic malignancies through the immunologic graft-versus-leukemia (GVL) effect. Unfortunately, donor conventional T cells (Tcons) recognize normal recipient tissues and attack them, causing graft-versus-host disease (GVHD). The skin, liver, and gastrointestinal (GI) tracts are the primary targets of acute GVHD, which is the major cause of nonrelapse mortality (NRM) after HCT, [2] and develops in 40–60% of patients.[3]

GVHD in the skin is the most frequently involved target organ and presents as an erythematous maculopapular rash; liver GVHD, the least common, causes hyperbilirubinemia; GI GVHD involves the upper GI tract, causing nausea, vomiting, and anorexia and, more often, the lower GI tract, causing diarrhea and abdominal pain. Each GVHD target organ is staged on a 0–4 severity scale, and the individual stages are used to create a composite clinical severity grade (Table 1).[4–6] Onset clinical severity does not correlate as well with survival as does *maximal* clinical severity, which also reflects treatment response. Patients with significant (≥Grade 2) GVHD are all treated similarly with high-dose steroids, with intensification reserved for primary treatment failure. The high rate of treatment failure for lower GI GVHD accounts for the majority of NRM in the first 6 months after HCT.[7,8] Therefore, this manuscript will emphasize GI GVHD and the available and emerging therapies for its treatment.

2. Pathophysiology

The graft-versus-host (GVH) reaction is initiated when donor Tcons respond to genetically defined protein antigens expressed on host antigen-presenting cells (APCs).[2] Donor Tcons proliferate and differentiate during GVH, and the balance between effector and regulatory T cells (Tregs) plays an important role in its progression and resolution. This paradigm for GVHD pathophysiology involves three distinct phases. In the first phase, which commences weeks before the onset of symptoms, tissue damage from the radiation and/or chemotherapy given in the conditioning regimen initiates an inflammatory immunologic cascade involving both the innate and adaptive immune systems. The release of proinflammatory cytokines (e.g. TNFα, IL-1, and IL-6) promotes the activation of host APCs, which in turn drive donor Tcon proliferation, differentiation, and migration to target tissues.[10] Damage to the GI epithelium allows the translocation from the gut lumen of danger

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Article highlights

- Acute GVHD develops in 40–60% of allogeneic HCT recipients and is the major cause of NRM. The standard first-line therapy for acute GVHD is high-dose steroids, but 50% of cases are steroid refractory. GI GVHD accounts for the majority of NRM because of its high rate of treatment failure.
- New treatments for GVHD under study that exploit new targets involved in GVHD pathophysiology include blockade of leukocyte trafficking, JAK inhibition, histone deacetylase inhibitors, alpha-1 antitrypsin, induction of regulatory T cells, and restoration of GI barrier function through cytokines.
- A validated scoring system based on GVHD biomarkers (Ann Arbor risk scores) objectively stratifies patients according to risk of primary treatment failure and can identify patients for clinical trials before steroid refractory GVHD has developed.
- The loss of diversity in the GI microbiota is associated with increased GVHD mortality and relates to different factors, including exposure to antibiotics. Preservation or restoration of a healthy GI microbiome is an alternative strategy to treat GI GVHD.

This box summarizes key points contained in the article.

signals and pathogen-associated molecular patterns (PAMPs) such as bacterial cell wall components (e.g. lipopolysaccharide – LPS) and damage-associated molecular patterns (DAMPs) (e.g. ATP and extracellular matrix proteins) to amplify the cytokine cascade and trigger the development of chemokine gradients that attract donor Tcons.[11] Tregs are transcription factor forkhead box P3 (Foxp3+) CD4 + T cells that by suppressing alloreactive lymphocytes can dampen the effect of GVHD caused by Tcons [12] (Figure 1). In the second phase, also days to weeks before the onset of symptoms, T-cell traffic to target organs in a highly regulated process, during which interactions between adhesion molecules (e.g. MAdCAM-1) and integrins (e.g. α4β7) result in leukocyte adherence to the capillary endothelium and migration into the subendothelium [13] as shown in Figure 2. Chemokines regulate not only the trafficking of leukocytes, but also their activation and differentiation by binding to specific receptors, such as chemokine (C-C motif) receptor 5 (CCR5), CCR6, and CCR7.[14,15] In Phase 3, clinical symptoms commence when Tcons cause tissue destruction through direct cytotoxic activity, mostly through Fas ligand: Fas and perforin-granzyme pathways [16], as well as through cytokines such as TNFα (Figure 3).

3. Diagnosis

The diagnosis of acute GI GVHD is based on clinical signs and symptoms (which do not occur until Phase 3 of its pathophysiology).

It is common practice to rule out infectious enteritis by testing the stool for adenovirus, rotavirus, or *Clostridium difficile*.[17] Diarrhea from pretransplant chemoradiotherapy-induced GI damage is common prior to engraftment and when GVHD symptoms occur; most centers perform diagnostic endoscopy to establish the diagnosis. However, endoscopic abnormalities are seen in less than one-third of GI GVHD cases and are nonspecific [18]; biopsies are usually performed to obtain histologic confirmation of the diagnosis. Flexible sigmoidoscopy is as sensitive as full colonoscopy.[19] Upper GI endoscopy is often performed, as the small bowel may be the major source of diarrhea, although this approach is not universally accepted.[20] Although GI GVHD symptoms may start as early as 9 days after HCT, they often do not start until patients have been discharged from the hospital, and accurate measurements of outpatient diarrheal output are not routinely available. The average volume per episode of diarrhea in an adult has been estimated to be ~200 ml, allowing for staging and grading of GI GVHD when only the number of diarrhea episodes is known.[9]

4. Histology

GI crypt dropout is characteristic of histologic GVHD, and intestinal stem cells (ISCs) are considered targets of the disease,[21,22] with crypt cell apoptosis a histologic hallmark. Nonetheless, GI GVHD histology is often not straightforward. Crypt damage from conditioning chemotherapy ± radiation can take weeks to heal, and persistent changes can overlap with the onset of GI GVHD symptoms. Other causes of crypt damage include infections and mycophenolate mofetil,[23] a commonly used immunosuppressant. The patchy nature of GI GVHD histology sometimes leads to false-negative biopsies through sampling error. Finally, the histologic grading system (Table 2) [24] has never been standardized for a number of key parameters, including the number of tissue sections or high-powered fields to be analyzed, or the number of apoptotic

Table 1. GVHD target organ staging.

Stage	Skin (active erythema only)	Liver (bilirubin)	Upper GI	Lower GI (stool output/day)*
0	No active (erythematous) GVHD rash	<2 mg/dl	No or intermittent nausea, vomiting, or anorexia	Adult: <500 ml/day or <3 episodes/day Child: <10 ml/kg/day or <4 episodes/day
1	Maculopapular rash <25% BSA	2–3 mg/dl	Persistent nausea, vomiting, or anorexia	Adult: 500–999 ml/day or 3–4 episodes/day Child: 10–19.9 ml/kg/day or 4–6 episodes/day
2	Maculopapular rash 25–50% BSA	3.1–6 mg/dl	-	Adult: 1000–1500 ml/day or 5–7 episodes/day Child: 20–30 ml/kg/day or 7–10 episodes/day
3	Maculopapular rash >50% BSA	6.1–15 mg/dl	-	Adult: >1500 ml/day or >7 episodes/day Child: >30 ml/kg/day or >10 episodes/day
4	Generalized erythroderma (>50% BSA) *plus* bullous formation and desquamation >5% BSA	>15 mg/dl	-	Severe abdominal pain with or without ileus or grossly bloody stool (regardless of stool volume)

*When stool volume is not quantified, a 200-ml/episode can be used as an estimate for adults [9].
Overall clinical grade (based on most severe target organ involvement):
Grade 0: No stage 1–4 of any organ.
Grade I: Stage 1–2 skin without liver, upper GI, or lower GI involvement.
Grade II: Stage 3 rash and/or Stage 1 liver and/or Stage 1 upper GI and/or Stage 1 lower GI.
Grade III: Stage 2–3 liver and/or Stage 2–3 lower GI, with Stage 0–3 skin and/or Stage 0–1 upper GI.
Grade IV: Stage 4 skin, liver, or lower GI involvement, with Stage 0–1 upper GI.

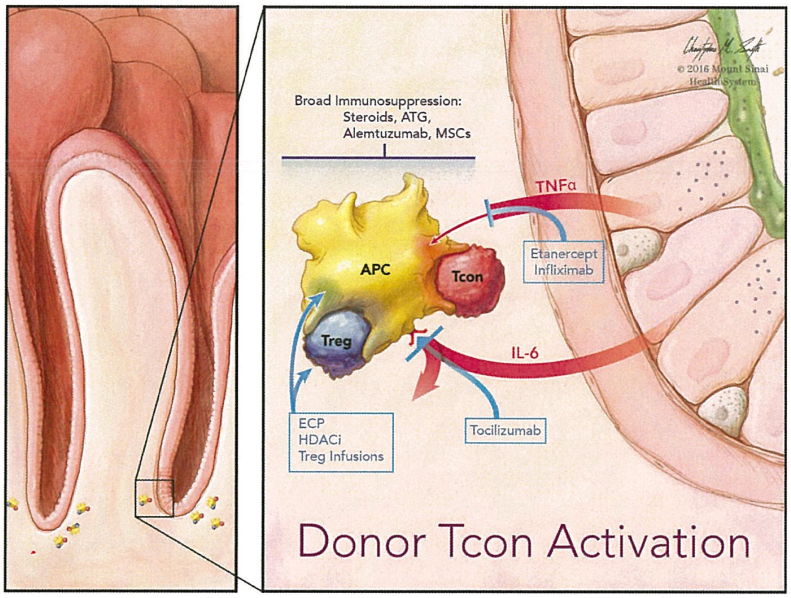

Figure 1. Blockade of donor Tcon activation (first phase of acute GVHD).
This schematic figure depicts key events in early acute GVHD pathophysiology in the GI tract, the most important GVHD target organ. A villus of the small intestine (left) is highly magnified (right).
The transplant conditioning regimen leads to generation of danger signals and pro-inflammatory cytokines (e.g. TNFα, IL-6) that activate APCs. For illustration purposes, one APC is shown interacting with one conventional T cell (Tcon) and one regulatory regulatory T cell (Treg). Physiologically, these cells are clustered beneath crypts, in the lamina propria. *Etanercept* and *infliximab*, two anti-TNFα antibodies, neutralize TNFα directly. *Tocilizumab*, prevents APC activation by inhibiting binding of IL-6 to the IL-6 receptor.
Steroids, ATG, alemtuzumab, and *MSCs* are broad immunosuppressors that act on multiple immunologic processes, including the activation and differentiation of Tcons through different mechanisms (see text). *ECP* and *vorinostat* suppress host APC and promote Treg expansion.
Abbreviations: APC, antigen-presenting cell; ATG, antithymocyte globulin; ECP, extracorporeal photopheresis; MSC, mesenchymal stromal cell.

cells required to diagnose Grade 1 GVHD. As a consequence, interobserver variability is high, histologic severity does not correlate well with clinical symptoms, and a negative biopsy does not necessarily rule out GVHD.[25] Paneth cells are primarily located in the crypts of the small intestine, and their number inversely correlates with high-risk disease.[26] Paneth cells are readily identified by their location in the GI tract and their histochemical staining with lysozyme. Their straightforward quantification can aid in establishing the diagnosis and in prognosticating its severity.

5. GVHD biomarkers

Despite a large number of plasma proteins, DNA single nucleotide polymorphisms, microRNA molecules, and peripheral blood cellular subsets with associations to GVHD,[27–29] only a small number of candidate biomarkers have been validated in multicenter patient cohorts. Of these validated biomarkers, those with greatest relevance to GI GVHD are tumor necrosis factor receptor-1 (TNFR1), regenerating islet-derived protein-3-α (REG3α), and suppression of tumorigenicity 2 (ST2). TNFR1, a membrane receptor for TNF-α that becomes soluble after binding its ligand, is not specific for GI GVHD but has been shown to strongly correlate with overall GVHD severity, response to treatment, NRM, and survival.[30,31] REG3α, which is secreted by Paneth cells, is an antimicrobial peptide and regulator of intestinal gram-positive bacteria. As already mentioned, Paneth cell loss correlates with GI GVHD severity and long-term outcomes.[26] As a biomarker specific for GI GVHD, REG3α discriminates between GVHD and non-GVHD causes of diarrhea.[32] ST2 is secreted in response to inflammatory stimuli, and it functions as a decoy receptor for interleukin-33 (IL-33), which drives Tcons toward a proinflammatory phenotype.[33] ST2 plasma concentrations at the initiation of GVHD treatment strongly correlates with eventual resistance to treatment and 6-month NRM, which is primarily driven by steroid-refractory (SR) GI GVHD.[34]

5.1. Biomarker-defined risk stratification

Individual GVHD biomarker concentrations vary widely among centers. To overcome this limitation, the Ann Arbor (AA) scoring system used several biomarkers from a multicenter cohort. An algorithm combining the plasma concentrations of TNFR1, REG3α, and ST2 at GVHD onset was developed to categorize patients according to risk of primary treatment failure and NRM.[35] Thresholds define three distinct scores: AA1, NRM ~10%; AA2, NRM ~25%; and AA3, NRM ~40%. Because relapse rates do not differ among the AA scores, these differences in NRM translate into significant differences in overall survival. It is important to note that AA scores identify patients who will later develop lower GI GVHD but who present with only a rash.

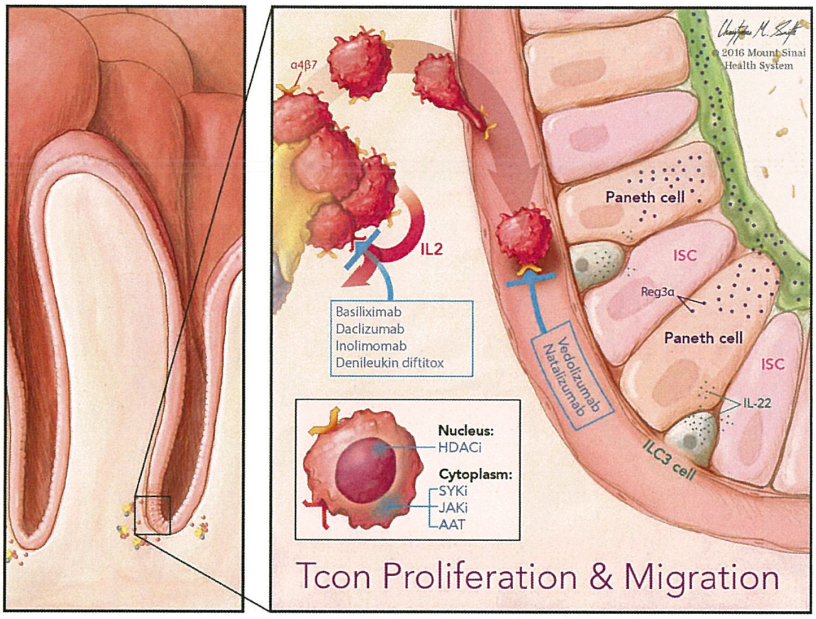

Figure 2. Blockade of Tcon proliferation and migration (second phase of acute GVHD).
Following activation by host APCs, donor Tcons proliferate and migrate from secondary lymphoid organs and tissues associated with the mucosa (e.g. Peyer's patches). Activated Tcons release cytokines (e.g. IL-2) that promote further proliferation and differentiation. *Basiliximab, daclizumab, inolimomab*, and *denileukin diftitox* all bind to IL-2 receptor (IL-2R). *HiDAC inhibitors* suppress APCs activity, enhance Treg activity, and reduce pro-inflammatory cytokines through DNA methylation (*azacitidine*) and histone acetylation (*vorinostat*), while *inhibitors of JAK1/2 (ruxolitinib), SYK (fostamatinib,* and *α-1-antitrypsin* (*AAT*) suppress cytokine production through cytoplasmic receptors. The migration of activated Tcons (T cell trafficking) into the GI subendothelium requires interaction between integrins (e.g. α4β7-integrins) expressed on Tcons and their receptors. *Natalizumab* and *vedolizumab* are antibodies that block α4 or α4β7, respectively. Intestinal stem cells (ISCs) are located at the base of the crypts, interspersed with Paneth cells, and are responsible for crypt regeneration. Type 3 innate lymphoid cells (ILC3s) produce IL-22 which is trophic for ISCs and which induces regenerating islet derived protein-3-α (REG3α), an antimicrobial peptide which destroys gram-positive bacteria. Abbreviations: APC, antigen-presenting cell; GI, gastrointestinal; GVHD, graft-versus-host disease.

Patients whose skin was the only target organ affected at the time of diagnosis and who were classified as AA3 by biomarkers were twice as likely to develop lower GI GVHD later as patients classified as AA1.[35] Biomarker-defined GVHD severity thus appears promising as an opportunity for early intensive intervention in patients diagnosed with high-risk GVHD.

6. Therapeutic options

6.1. Broad immunosuppression

Most therapeutic options for GVHD have been evaluated in the context of systemic disease where sometimes, only overall grades are reported. In this review, we highlight outcomes for GI GVHD wherever possible; otherwise, the results described apply to GVHD in general.

Intensified immunosuppression with systemic steroids is the only proven treatment for GVHD. Unfortunately, SR GVHD develops in ~50% of patients,[7,36,37] more often in patients with lower GI involvement,[7] and there is no established second-line therapy. The response rates to second-line treatment are typically low (20–40%), and survival is poor, highlighting the urgent need for better therapies.[36,38] Thus, SR GVHD remains a significant contributor to treatment-related mortality (TRM) and morbidity. As reviewed in the following section and summarized in Tables 3 and 4, several treatments have shown benefit in single-arm studies; however, that benefit has not been confirmed in the few that have been evaluated by more rigorous randomized trials.

6.2. Steroids

GVHD treatment usually begins with high doses of prednisone or, for patients unable to take oral steroids, methylprednisolone (MP) (Figure 1). A randomized, prospective clinical trial showed that a 50% reduction in the starting dose of steroids was effective, although patients with more severe GVHD were more likely to require addition of secondary immunosuppression.[39] Upper GI GVHD may also respond well to the combination of 1 mg/kg/d of prednisone and nonabsorbable steroid therapy.[40] Conversely, doses higher than 2 mg/kg/day do not improve outcomes.[41]

6.2.1. Nonabsorbable steroids

Oral nonabsorbable steroids, such as budesonide and beclomethasone, theoretically deliver high steroid doses to the GI tract without incurring the side effects of systemic steroid therapy. These agents have primarily been tested in combination with systemic steroids as part of first-line therapy for GI GVHD. It should be noted, however, that the term 'nonabsorbable' is a misnomer, because synthetic steroid screens often demonstrate significant absorption.[42] Results of this

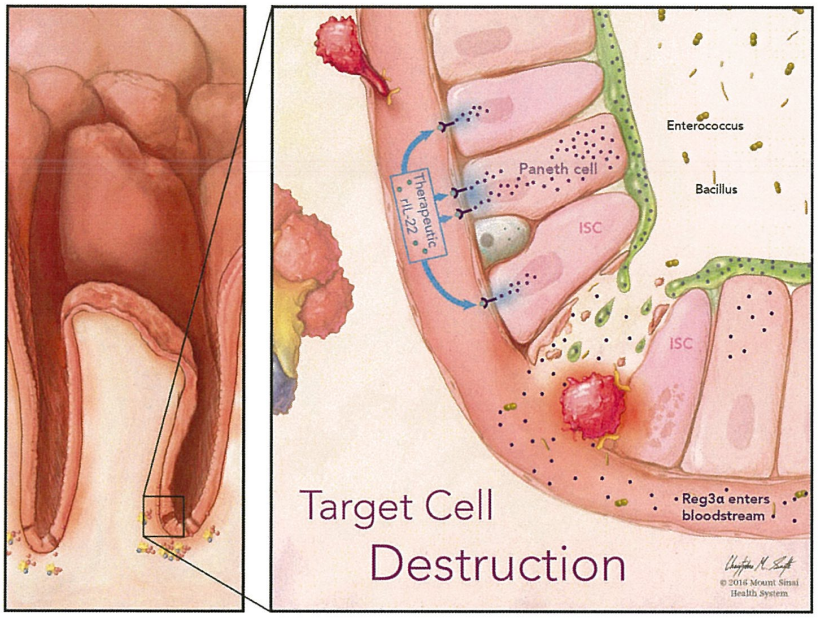

Figure 3. Prevention of target cell destruction (third phase of acute GVHD).
Activated Tcons that migrated to the GI epithelium secrete inflammatory cytokines and lyse ISCs and Paneth cells, disrupting the GI mucosal barrier. REG3α stored intracellularly and in the mucus leaks into the systemic circulation along with luminal contents, such as bacteria. Strategies to restore the integrity of the mucosal barrier are currently explored via clinical trial of *recombinant IL-22* (*rIL-22*) administration.
Abbreviations: GI, gastrointestinal; GVHD, graft-versus-host disease.

Table 2. Histologic grading for acute gastrointestinal graft-versus-host disease [24].

Grade	Histology
0	Normal mucosa
1	Isolated apoptotic epithelial cells without crypt loss
2	Loss of isolated crypts without loss of contiguous crypts
3	Loss of two or more contiguous crypts
4	Extensive crypt loss with mucosal denudation

approach have been mixed, [43,44] and the only Phase III trial involving budesonide did not meet its primary end point of time to treatment failure.[40] Steroid delivery through arteriography to the arterial blood supply to the gut has been explored in several small studies. In two prospective series, GI GVHD response rates of 70–85% were observed and appeared better than historical controls.[45–47] The need for trained and available interventional radiology specialists may explain the lack of larger and adequately powered randomized studies.

6.3. Sirolimus

Sirolimus, an mTOR inhibitor, blocks late G1 cell cycle progression, most prominently in T lymphocytes,[48] and also maintains Treg populations.[49] Sirolimus has proven efficacy in GVHD prophylaxis [50,51] and showed comparable activity to that of high-dose steroids as primary therapy for acute GVHD in one study.[52] Sirolimus is currently being compared to prednisone as a single agent for primary therapy of low-risk GVHD in a randomized trial conducted by the Blood and Marrow Transplant Clinical Trials Network. As a salvage therapy for SR GVHD, response rates of 57–76% have been reported for sirolimus.[48,53] Its side effect profile differs from other immunosuppressants and includes transplant-related microangiopathy (TMA), particularly when combined with calcineurin inhibitors, as well as hyperlipidemia.[53]

6.4. Purine synthesis inhibitors

Mycophenolate mofetil (MMF) blocks *de novo* purine synthesis through inhibition of inosine monophosphate dehydrogenase (IMPDH), which arrests lymphocytes, in S-phase.[33] Encouraging results of MMF as primary treatment for GVHD were observed in several small series, [54,55] including multicenter, randomized, Phase II trial.[56] However, a randomized, placebo-controlled trial was closed to accrual early for futility. [57] A significant drawback to MMF is its GI toxicity profile, which includes ulcerative esophagitis, reactive gastropathy, and pathologic changes very similar to GVHD that can cloud diagnostic biopsy interpretation.[58]

Pentostatin, a nucleoside analog, inhibits adenosine deaminase and induces cell death particularly in T and NK cells.[59] Pentostatin has shown mixed results for SR GVHD in small series, with survival ranging from 7 to 43%.[60–62]

6.5. Anti-T-cell serotherapy

Antithymocyte globulin (ATG) consists of polyclonal IgG antibodies against human T cells (Figure 1). The role of both

Table 3. Nonspecific T-cell targeting.

	Agent	Patient population	Study design	End points	Results	Author	Comments
LYMPHOCYTE-DEPLETING AGENTS	**Budesonide (BUD)**	22 pts ≥ Grade 2 GI GVHD vs. 19 historical control	Retrospective, systemic GC + BUD vs. GC only	Stool volume and frequency of bowel movement	ORR 77 vs. 32%, $p < 0.01$	*Bertz (1999) [43]*	Nonabsorbed is a misnomer, variable systemic absorption does occur with reports of adrenal suppression; pH milieu is important for effective action of the drug, and not always optimal in setting of GVHD and antacid therapy
	Oral beclomethasone diproprionate (BDP)	26 pts ≤ Grade 2 GI GVHD	Prospective, monotherapy BDP	Day 28 response	ORR 77% (CR 65.5%), 5 pts in CR relapsed; overall 50% pts required systemic steroids	*Castilla (2006) [44]*	
		62 vs. 67 pts, all with GI GVHD only	Phase III, biopsy proven, PRD + BDP vs. PRD 1 mg/kg/d + placebo, pts with > 1 l diarrhea excluded	Day 50 response; survival	Fewer treatment failure in BDP group through day 50 (31 vs. 48%, $p = 0.12$); 1 year OS 71 vs. 58%, $p = 0.04$	*Hockenbery (2007) [40]*	
	Intra-arterial steroids (IAS)	12 SR GI GVHD	Prospective, mean total dose MP 180 mg	Day 28 response	ORR 83%; 1 year OS 50%	*Bürgler (2014) [46]*	Safe procedure and no increase in infection rates. Pitfalls: trained and available interventional radiology service is needed
		11 SR GI GVHD	Pilot study, ≥ 2nd line, (MP 75 mg/m^2)	Response at discharge	ORR 72%; 1.5 year OS 27%	*Weintraub (2010) [45]*	
		19 SR GI GVHD vs. 14 historical controls	Phase II, mean total dose MP 180 mg	Day 28 response	ORR 79 vs. 42%, $p = 0.07$ (CR 63 vs. 21%, $p = 0.03$); 6 month OS 79 vs. 50% $p = 0.11$	*Nishimoto (2015) [47]*	
	Antithymocyte globulin (ATG)	79 SR GVHD; 52% lower GI. (horse ATG)	Retrospective; 2nd line; 1–5 courses ATG 15 mg/kg bid × 5 days + GC	Day 28 response	ORR 54% (CR 20%). Best response for skin GVHD and earlier. No impact on survival. 1 year OS 32%; one case of PTLD	*MacMillan (2002) [65]*	Commonly used agent for SR GVHD despite lack of proof of efficacy; no consensus on which brand to use; significant infectious complications, increased risk for PTLD
		211 pts; 61 with SR acute GVHD (rabbit ATG)	Phase III, multicenter; MP 5 mg/kg/d × 10 days ± ATG (1.25 mg/kg × 5 doses)	Day 30 response	ORR 55 vs. 48%, p = NS (CR 33 vs. 24%, p = NS); higher TRM with ATG; one case of PTLD	Van *Lint (2006) [67]*	
	Alemtuzumab (anti-CD52 antibody)	18 SR GVHD, (8 with GI GVHD)	Prospective, 2nd line	Day 28 response	ORR 83% (CR 33%)	*Gómez-Almaguer (2008) [71]*	Effective GVHD prophylaxis; less studied for GVHD treatment. Increased risk for CMV; less risk for EBV reactivation/PTLD due to B cell depletion
		24 SR GI GVHD	Retrospective	Survival	ORR 62% (CR 46%); 1 year OS 33%; one case of PTLD	*Meunier (2014) [72]*	
ANTIMETABOLITES	**Mycophenolate mofetil (MMF)** (inhibitor of purine synthesis)	19 (10 with GI) vs. 29 historical controls (11 with GI)	Phase II prospective with historical controls; pts NPO or ANC < 1500 excluded	Day 35 response	ORR 47 vs. 48% (CR 31 vs. 31%); MMF discontinued in 21% pts for toxicity; 1 year OS 16 vs. 52%	*Furlong (2009) [54]*	Usually well tolerated but can cause nausea and diarrhea; can induce GI pathology changes that mimic GVHD. Increased infections, myelosuppressive
		116 vs. 119 new onset GVHD	Phase III prospective, placebo controlled, multicenter; PRD + MMF 1 g Q8 h vs. placebo	Day 56 GVHD-free survival	Day 56 CR 60 vs. 54% ($p = 0.34$); 1 year OS 58% vs. 65% ($p = 0.34$); 1 year relapse 24 vs. 16% ($p = 0.08$); no benefit from MMF	*Bolaños-Meade (2014) [57]*	
		14 SR GVHD (12 with GI GVHD)	Retrospective, children only, high dose MMF	Day 28 and 56 responses	Day 28 ORR 83% (GI only); day 56 CR 100% (GI only); 3 year OS 86%	*Inagaki (2015) [55]*	
	Pentostatin (purine analog)	12 SR GI GVHD	Retrospective	Day 28 response	ORR 50% (CR 33%)	*Pidala (2010) [61]*	Limited activity. Variety of doses and schedules were used. High rate of infections
		24 SR GI GVHD	Retrospective	Day 28 response	ORR 38% (CR 21%); 2 year OS 17%	*Schmitt (2011) [62]*	
		15 SR GVHD (12 GI)	Retrospective	Day 28 response	ORR 33%; 53% received additional therapies	*Alam (2013) [60]*	

(*Continued*)

Table 3. (Continued).

	Agent	Patient population	Study design	End points	Results	Author	Comments
T-CELL APOPTOSIS	**Sirolimus** (Cell cycle arrest, prevention of dendritic cell Ag presentation and maturation; Treg expansion)	21 SR GVHD (10 with GI)	Pilot study	Day 14 response	ORR 57% (CR 24%). Sirolimus absorption confirmed in pts with GI GVHD	*Benito (2001) [48]*	Increased rates of thrombotic microangiopathy, myelosuppression; hypertriglyceridemia. As first-line therapy, sirolimus may be comparable to GC
		31 SR GVHD (27 with GI)	Retrospective	Best response	ORR 76% (CR 35%), (GI GVHD CR 44%); 1 year OS 44%	*Hoda (2010) [53]*	
		32 new onset GVHD (21 with GI) vs. 32 historical controls	Retrospective; Sirolimus vs. GC (historical control)	Day 28 response	CR 50 vs. 59% ($p = 0.47$); 1 year OS 56% (reported for sirolimus only); three cases of thrombotic microangiopathy	*Pidala (2011) [52]*	

Abbreviations: Ag: antigen; pts: patients; GC: glucocorticoids; SR: steroid-refractory; GVHD: graft-versus-host disease; GI: gastrointestinal; CR: complete remission; ORR: overall response rate; OS: overall survival; TRM: treatment-related mortality; MP: methylprednisolone; PRD: prednisone; NPO: nil per os; ANC: absolute neutrophil count; PTLD: post-transplant lymphoproliferative disease; CMV: cytomegalovirus; EBV: Epstein–Barr virus.

horse- and rabbit-derived formulations of ATG in GVHD prophylaxis is well established.[63,64] Rabbit-derived ATG induces more profound lymphopenia, but titers in the two brands vary widely and with no consensus as to which brand should be preferred. A retrospective series of 79 patients treated with horse ATG for SR GVHD showed a 54% CR/PR rate [65]; patients in that study were more likely to have skin involvement and less likely to have GI involvement compared to studies with less favorable results.[66] A large randomized study of rabbit ATG for SR GVHD did not improve outcomes, and its use was associated with a significantly increased risk of TRM.[67] Thus, improvements in response rates with ATG may not translate to survival benefit because of increased infectious complications and Epstein–Barr virus (EBV)-associated lymphoproliferative disease.[68]

Alemtuzumab, a humanized IgG monoclonal antibody to CD52 that is expressed on lymphocytes, monocytes, and APCs (Figure 1), is a more potent alternative to ATG,[69] and is effective as GVHD prophylaxis.[70] In small studies of SR GVHD patients, alemtuzumab provides overall response rates from 62 to 83%, with overall survival of 33–70%.[71,72] As expected, infectious complications frequently develop.

6.6. Targeted therapies

6.6.1. TNFα

6.6.1.1. Infliximab. Infliximab (Figure 1) is a chimeric antibody that neutralizes TNFα and lyses the cells that produce it by binding to its membrane-bound form.[73] In retrospective series of patients with SR GVHD, infliximab produced overall response rates ranging from 15 to 60%, but these were associated with high rates of fungal infections.[73–75] A randomized, Phase III trial showed no benefit from the addition of infliximab to steroids as primary GVHD therapy.[76]

6.6.1.2. Etanercept. Etanercept, which consists of two recombinant human TNFR (p75) monomers fused to the Fc portion of human IgG, neutralizes soluble TNFα (Figure 1), but does not lyse the cells producing it, and has a good safety profile.[77] In a prospective single-center study as primary GVHD therapy, the combination of etanercept and steroids was significantly superior to steroids alone (CR 69 vs. 33%); [30] however, these results were not reproduced in a randomized, Phase II study.[56] The drug is not as effective in the SR GVHD setting, with response rates of 40–50%, including for patients with severe GI GVHD.[78,79]

6.6.2. IL-6 receptor

Tocilizumab is a humanized anti-IL6 receptor antibody that blocks IL-6 signaling, which is pivotal in the differentiation of CD4+/IL-17-secreting T (Th17) cells from naïve T cells (Figure 1). In murine models, loss of IL-6 signaling reduced proinflammatory Th1 and Th17 cells, increased Tregs, and prevented GVHD.[80] In a prospective, 48-patient GVHD prophylaxis trial of tocilizumab plus cyclosporine and methotrexate plus tocilizumab, following HLA-matched allogeneic BMT, GVHD Grades II–IV developed in only 12% of patients, significantly better than the historical control rate of 60% ($p < 0.05$). [81] As a treatment for SR GVHD in two small retrospective

Table 4. Specific T-cell targeting.

Agent		Patient population	Study design	End points	Results	Author	Comments
ANTI-TNFα BLOCKADE	**Infliximab**	52 SR GVHD (50 with GI GVHD)	Retrospective, 2nd line	Best response	CR 15%; median OS <2 months	*Pidala (2009) [75]*	Limited activity; increased infection rates. No benefit from infliximab as primary therapy
		29 vs. 28 pts new onset GVHD	Phase III, single center, open label; MP 2 mg/kg/d ± infliximab	Day 7 and 28 responses	Day 7 ORR 52 vs. 78% ($p = 0.03$); Day 28 ORR 62 vs. 58% ($p = 0.07$); 5 years OS 17 vs. 28% ($p = 0.4$) (infliximab worse except for day 28 ORR)	*Couriel (2009) [76]*	
		32 SR GVHD (29 with GI)	Retrospective, multicenter, most > 2nd line	Best response	ORR 59% (CR 19%). Most active in GI GVHD	*Patriarca (2004) [73]*	
	Etanercept	61 (37 with GI) vs. 99 pts (43 with GI) new onset GVHD	Phase II trial vs. historical control, single center; MP 2 mg/kg/d ± etanercept	Day 28 CR	Day 28 CR 69 vs. 33% ($p < 0.001$), GI 78 vs. 48%, $p = 0.005$; 6 months OS 69 vs. 55% ($p = 0.08$)	*Levine (2008) [30]*	Effective in primary therapy in single-center study (not reproduced in multicenter Phase II); some evidence for benefit in SR GI GVHD
		13 SR GVHD (11 with GI GVHD)	Retrospective; >2nd line	Best response	ORR 46% (CR 31%), GI GVHD 64%; 11 months OS 69%	*Busca (2007) [79]*	
ANTI-IL-2 RECEPTOR *Ab*	**Daclizumab**	18 SR GVHD	Retrospective	Day 28 response	ORR 50%; 2 years OS 28%	*Park (2014) [78]*	
		62 SR GVHD, (21 with GI)	Phase II prospective, 2nd line	Day 30 response	ORR 90% (CR 69%); 4 years EFS 55%	*Bordigoni (2006) [94]*	Increased rates of infections
		57 SR GVHD (11 with GI)	Retrospective, 2nd line	Day 43 response	ORR 54% (GI GVHD 46%); 8 years OS 25%	*Perales (2007) [95]*	
	Denileukin diftitox	22 SR GVHD (9 with GI)	Phase II prospective	Day 36 and 100 responses	Day 36 ORR 41% (2/9 w GI), Day 100 ORR 100%; 15 months OS 23%	*Shaughnessy (2005) [96]*	Increased rates of infections; lack of sustained response
		30 SR GVHD	Phase I prospective, ≥2nd line	Day 29 response	ORR 71% (CR 50%)	*Ho (2004) [97]*	
	Basiliximab	53 SR GVHD (18 with GI)	Retrospective	Best response	ORR 87% (CR 70%); 16 months OS 53%; 3 years EFS 48%	*Wang (2011) [93]*	Increased rates of infections
		23 SR GVHD (12 with GI)	Phase II prospective	Day 7 response	ORR 83% (CR 18%), one of 12 pts with GI obtained CR; 2 years OS 48%	*Schmidt-Hieber (2005) [92]*	
	Inolimomab	85 SR GVHD (59 with Grade III/IV)	Retrospective	Best response	ORR 63% (CR 29%). More efficacious for skin. 20 months OS 26%	*Bay (2005) [90]*	More antigenic than basiliximab and daclizumab. Poor long-term survival
		92 SR GVHD (82 with GI)	Retrospective, ≥2nd line	Day 30 response	ORR 42% (CR 14%); ORR GI 39%; 2 years OS 18%	*Garcia-Cadenas (2013) [91]*	
ANTI-IL-6R *Ab*	**Tocilizumab**	6 SR GVHD (5 with GI)	Retrospective, ≥2nd line	Day 56 response	ORR 67%	*Drobyski (2011) [82]*	Transaminase elevations; responses not durable
		9 SR GI GVHD	Retrospective, 3rd line	Day 7 response	ORR 44% (CR in 2/4 pts), not durable. median OS 26 days	*Roddy (2015) [83]*	
Treg MODULATION	**Extracorporeal photopheresis (ECP)**	9 SR GVHD	Retrospective	Day 90 response	ORR 100%	*Rubegni (2013) [86]*	Well tolerated, does not prevent development of chronic GVHD. No increase in infection or relapse rates seen
		249 SR GVHD	Review, 12 publications	Best response	Variable ORR: 65–100% (CR 62%, 66/106 pts). Pts initially treated 2 to 3 times per week. OS 42–87%	*Kitko (2015) [79]*	

Abbreviations: pts: patients; SR: steroid-refractory; GVHD: graft-versus-host disease; GI: gastrointestinal; CR: complete response; ORR: overall response rate; OS: overall survival; EFS: event-free survival; MP: methylprednisolone.

series, responses to tocilizumab were not durable, and most patients died.[82,83]

6.6.3. Extracorporeal photopheresis

Extracorporeal photopheresis (ECP) isolates peripheral white blood cells by leukopheresis, then incubates them with the 8-methoxypsoralen (DNA-crosslinking agent), photoactivated by ultraviolet A irradiation. The leukocytes are then reinfused. Experimental GVHD models suggest that ECP reduces GVHD by inducing Tregs, but this finding has not yet been confirmed in humans [84] (Figure 1). Several studies have investigated ECP as a treatment for SR GI acute GVHD, with an overall response rate of 60% in more than 100 patients.[85–89] The frequency of ECP sessions needed to obtain a response is not yet established, but it appears that two to three sessions per week until maximal response is achieved is desirable. ECP has not been found to increase the risk of disease relapse or infections and is considered at least comparatively safe.

6.6.4. IL-2 receptor

Activated T lymphocytes express the IL-2 receptor (IL2R, also known as CD25). Several antibodies against the IL-2 receptor (Figure 2) have been studied as treatment for GVHD: inolimomab (murine derived), basiliximab (chimeric mouse/human antibody), and daclizumab (humanized anti-IL2R antibody). Denileukin diftitox acts in a slightly different manner using a Trojan horse strategy (recombinant IL-2 linked to diphtheria toxin). Activated T cells lyse following endocytosis of their IL-2 receptor occupied by IL-2 linked to the denileukin diftitox. In both retrospective series and prospective Phase II studies, anti-IL2R antibody therapy induced responses in 40–85% of patients with SR GVHD.[90–97] Inolimomab appears to offer the least survival benefit, perhaps because of the increased immunogenicity of the murine antibody. Of particular note, a Phase III study of steroids ± daclizumab as primary GVHD therapy showed no differences in efficacy between the two arms, and daclizumab caused both more relapses and more GVHD-related complications resulting in significantly higher mortality.[98] This surprising effect may be because of the intense expression of IL-2R on Tregs that control GVHD.[99]

7. Emerging therapies

7.1. Histone deacetylase inhibition

Histone deacetylase (HDAC) inhibitors such as vorinostat suppress host APC functions, enhance Treg activity, and reduce proinflammatory cytokines (Figures 1 and 2). They attenuate acute GVHD and improve survival in several experimental models of GVHD.[100,101] In a Phase II clinical trial, vorinostat prevented acute GVHD in patients of reduced intensity conditioning allogeneic HCT,[102] and a Phase I/II trial of panobinostat in addition to systemic steroids as first-line treatment of acute GVHD demonstrated a high response rate (85%).[103] Current GVHD clinical trials with HDAC inhibitors include vorinostat to prevent GVHD after unrelated donor HCT (NCT01790568) and azacitidine to treat SR acute GVHD (NCT0145314).

7.2. Regulatory T cells

Extensive preclinical data support the use of Tregs to control GVHD [104] (Figure 1). In humans, the onset of GVHD is associated with fewer Tregs, and there are lower mRNA levels of the Treg cell markers Foxp3 and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4).[12] A Phase I trial of Tregs expanded from umbilical cord blood reduced Grade II–IV acute GVHD compared to historical controls (60% vs. 43%, $p = 0.05$) and did not seem to increase relapses.[105] Strategies to expand Tregs *ex vivo* for use post-transplant are being explored, and infusion of Tregs after T-cell-depleted haploidentical HCT, with delayed infusion of Tcons, showed a low incidence of GHVD, despite lack of other GVHD prophylaxis.[106] A clinical trial of Treg infusions to prevent GVHD (NCT01634217) is in progress. The barriers to Treg expansion and preparation need to be addressed for the widespread use of this theoretically attractive strategy.

7.3. Mesenchymal stromal cells

Mesenchymal stromal cells (MSCs) are derived from a variety of tissues including fetal tissue, placenta, umbilical cord blood, bone marrow, and adipose tissue.[107–110] MSCs exert a wide range of immunomodulatory and anti-inflammatory effects, which include suppressing the functions of lymphocytes and APCs, but clinical trial results have not been consistent (Figure 1). Early responses of 50–70% in severe or SR GVHD [111,112] have not been reproduced in Phase III trials,[113,114] which may be because of differences among MSC preparations. A recent meta-analysis that included 241 patients from 12 clinical trials of treatment of SR GVHD with MSCs showed a promising overall response rate of 72%, [115] but because of considerable bias, the authors concluded randomized clinical trials are still needed. MSCs are approved for management of SR GVHD of the GI tract in children (Prochymal®, Osiris Therapeutics, Inc.) and are currently being evaluated in a Phase II/III trial for treatment of SR GVHD (NCT02241018).

7.4. Blockade of leukocyte trafficking

Blockade of leukocyte trafficking is a novel approach to manage GVHD of the GI tract (Figure 2). Pharmacologic agents for this strategy fall into two main categories: antibodies to endothelial receptor ligands on leukocytes and chemokine receptor antagonists. There are two lines of evidence to support antibody blockade of the α4β7 addressin as a means to control GI GVHD. First, increased numbers of memory Tcons expressing α4β7 correlate with the development of acute GI GVHD,[116,117] and decreased expression of α4β7 on Tregs also correlates with more GVHD.[118] Second, blockade of α4β7 effectively treats inflammatory bowel disease, an autoimmune disease mediated by Tcons, [119,120] and two antibodies have been approved for this indication. Natalizumab, a humanized monoclonal anti-α4 antibody blocks both α4β1 (central nervous system traffic) and α4β7 (GI traffic) and is used to treat both multiple sclerosis and Crohn's disease. A clinical trial of natalizumab for high-risk GI GVHD is currently underway (NCT02133924).

Vedolizumab, a humanized monoclonal α4β7 antibody that is more specific for leukocyte migration to the GI tract, is effective for both ulcerative colitis and Crohn's disease [120] and has recently been explored for GI GVHD (Floisand Y et al., ASH Meeting 2015). CCR5 is highly upregulated on activated Tcons [14] and mediates their migration to visceral tissues. [121] A clinical trial of the CCR5 antagonist maraviroc in combination with standard GVHD prophylaxis completely prevented GI GVHD in 35 patients.[122] Maraviroc prophylaxis is now being tested in an ongoing multicenter, Phase II study conducted by the BMT CTN (NCT02208037). It has not yet been studied as a treatment strategy, in part because of concerns of bioavailability of an oral drug.

7.5. SYK inhibition

Spleen tyrosine kinase (SYK) mediates multiple signaling events of the immune cells, and SYK inhibition reduces experimental acute GVHD [123] (Figure 2). This approach may also preserve antileukemia activity and antiviral immunity.[123] Fostamatinib, an oral prodrug that inhibits SYK, may also be active in rheumatoid arthritis and autoimmune thrombocytopenia. Unfortunately, common adverse effects include nausea and diarrhea, which may prove problematic for patients with GI GVHD.[124]

7.6. JAK inhibition

Activated Janus kinases (JAKs) are required for T-effector cell responses, and their blockade reduces acute GVHD in experimental models.[125] Inhibition of signaling pathways that are common to multiple cytokines might improve upon the incremental results from targeting single cytokines (Figure 2). Clinical responses obtained with JAK inhibitors in myelofibrosis (MF) are associated with suppression of several elevated proinflammatory cytokines (IL-1, IL-6, TNFα, and IFN-γ), all of which are involved in the pathophysiology of acute GVHD.[126] Ruxolitinib, a selective JAK 1/2 inhibitor approved for the treatment for MF, produced clinical responses in all six patients with SR GVHD that were reported, and responses correlated with reductions in proinflammatory cytokines.[125] A retrospective survey of 95 patients from multiple transplant centers treated with ruxolitinib for SR GVHD showed an 80% overall response rate and a 75% 6-month survival.[127] Major side effects of this approach are cytopenia and CMV reactivation.

7.7. Alpha-1 antitrypsin

Alpha-1-antitrypsin (AAT), a serine protease inhibitor with pleotropic effects that include suppression of cytokine production and cell-mediated immunity, suppresses GVHD in mouse models [128] (Figure 2). Current clinical trials investigate AAT in SR GVHD (NCT01523821 and NCT01700036).

7.8. Microbiome modulations

Recent research has shown that the gut microbiota is highly disturbed during GI GVHD.[129,130] The microbiota amplifies disease, because germ-free animals undergoing HCT have little or significant delayed GVHD.[131] The specific microbes responsible for GVHD are not yet identified, but an increase in *Enterococcus* and a loss of *Blautia* species are associated with severe GVHD.[132] Loss of bacterial diversity increases GVHD mortality and antibiotics reduce diversity, allowing *Enterococcus* to dominate the microbiota.[133] REG3γ (the murine equivalent of human REG3α) [134] is an antimicrobial peptide specific for gram-positive organisms and a biomarker that is specific for GI GVHD [35] (Figure 3). These results suggest that manipulation of the GI microbiome may become a novel strategy to prevent and/or treat GI GVHD similar to the ability of probiotics to control inflammatory bowel disease. [135] Such strategies currently under development include prophylactic administration of *Lactobacillus*spp. as a probiotic (NCT01010867 and NCT02144701), autologous fecal transplants to restore baseline commensal communities (NCT02269150).

The cytokine IL-22, which is produced especially by innate lymphoid cells (ILC3s) (Figure 2) and is important for ISCs recovery after transplantation, can protect and heal damaged intestinal epithelium (Figure 3). The absence of IL-22 is associated with worse acute GVHD,[21] and intraperitoneal IL-22 administration improved GI GVHD and survival in experimental models.[136] Although the exact mechanisms are not fully understood, IL-22 may mediate its beneficial effects through the induction of REG3γ,[134,137] which prevents pathogenicity from gram-positive bacteria and restores mucosal barrier function. A clinical trial is currently underway to test recombinant human IL-22 as first-line treatment of GI GVHD (NCT02406651).

7.9. TWEAK-Fn14 interaction inhibition

TNF-like weak inducer of apoptosis (TWEAK) binds the fibroblast growth factor-inducible 14 (Fn14) receptor, which is highly expressed in many cells of nonlymphoid lineage in contexts of tissue injury and regeneration. Chronic activation of the TWEAK-Fn14 pathway elicits GI tissue damage, and its blockade reduces intestinal cell death and prevents disease in experimental colitis.[138] Fn14 receptor expression was increased specifically on intestinal epithelial cells during GVHD and was observed only in GVHD samples.[139] An Fn14-specific antibody reduces experimental GVHD [139] through a mechanism specific to the target tissue and did not impair a GVL effect.

7.10. Bortezomib

Bortezomib is a proteasome inhibitor approved as a treatment for mantle cell lymphoma and multiple myeloma. Bortezomib inhibits APC function by reducing cytokine production and immunostimulatory activity,[140] and in the preclinical setting, it preferentially and specifically depleted alloreactive T lymphocytes.[141] Bortezomib was tested in combination with tacrolimus and methotrexate for GVHD prevention in a Phase 1 trial of 23 patients who received HCT from HLA-mismatched unrelated donors. The rate of Grade II–IV acute GVHD was low at 13%, and the 1-year NRM was zero.[142] A randomized, Phase II multicenter trial is trying to reproduce these results (NCT02208037). Although bortezomib was tested as a

treatment of acute GVHD in eight patients (NCT00408928), no results are currently available.

7.11. Aurora A kinase inhibition

A whole transcriptome analysis of donor T cells identified the aurora kinase A pathway as one of the most prominent in a nonhuman primate model of GVHD. These results were confirmed in human T cells from patients with GVHD. The aurora A kinase pathway encodes proteins controlling cell cycle progression, cell growth, differentiation, and survival. In experimental murine GVHD inhibition of the aurora A kinase pathway with a commercially available selective, reversible small-molecule inhibitor (MLN8237, alisertib) mitigated clinical severity and improved survival.[143] Human clinical trials have not yet been conducted.

7.12. Apoptotic cells

As noted previously, APC presentation of host antigen activates T cells. Dendritic cells (DCs), the most potent APC population, act in an immunogenic or a tolerogenic manner depending on their maturation state and the context in which the antigen is acquired.

During steady state, immature DC clearance of apoptotic cells from normal tissue turnover leads to a tolerogenic phenotype and induces Tregs.[144] ECP induces apoptosis, and this may be a mechanism by which ECP successfully treats established acute GVHD (refer to Section 6.6.3). The administration of apoptotic cells generated through ECP prevented GVHD and significantly prolonged survival in a murine model reducing DC activation and increasing Tregs.[145]

In a Phase I/II clinical trial, escalating doses of donor leukocytes in early apoptosis were infused into 13 recipients of HLA-matched related allogeneic HCT.[146] The incidence of Grade II–IV acute GVHD was 23%. None of the 6 patients who received higher doses of apoptotic cells developed acute GVHD. More data from this approach are expected (NCT00524784).

7.13. Rosiglitazone

Peroxisome proliferator-activated receptors (PPARs) are transducer proteins that regulate gene expression in response to ligand binding.

The PPARγ isotype receptors are expressed on inflammatory cells, and the PPARγ agonist rosiglitazone, clinically used as an insulin-sensitizing agent, has been shown to inhibit T-cell proliferative responses.[147]

Rosiglitazone was investigated in a murine GVHD model,[148] and when given orally for 15 days following GVHD induction, it improved liver, skin, spleen, and intestine GVHD. Rosiglitazone reversed GVHD-induced effects on serum cytokine levels to close to normal levels, consistent with previous results from a murine model of acute colitis.[149] Further studies will be needed in order to establish its safety and effects in humans.

8. Expert opinion

Despite decades of research and improvements in post-transplant immunosuppressive therapies, acute GVHD remains the major cause of NRM after allogeneic HCT, and its management presents significant challenges. The majority of treatment failure and early NRM occurs in patients with GI GVHD.[6,65] High-dose systemic steroids form the backbone of the first-line therapy, but less than 50% of patients achieve sustained remissions, and the toxicities are both significant and numerous.

Despite the critical need for new therapies for GVHD, and for GI GVHD in particular, no new treatment has been approved in 40 years. Factors contributing to this lack of progress include the heterogeneity and fragility of the patient population, numerous adverse events unrelated to disease or to investigational agents, as well as tremendous variations in trial end points such as degree of response (e.g. complete response or complete plus partial response), timing of primary end point assessment, and durability of response.[150] The summary treatment tables (Tables 3 and 4) in this paper illustrate the wide variety of end points used. Poor concordance in GVHD grading among centers, because of the absence of standardized disease staging guidance,[151] also contributes to the failure of single center strategies to reproduce in randomized, multicenter clinical trials.

Critical to the lack of progress in this area is the toxicity of intensified immunosuppression, especially the high rate of infectious complications, which offsets and even outweighs any clinical benefits. For these reasons, it has been impossible to define clearly the best therapeutic options to manage GI GVHD, and physicians rely mostly on their personal practical experience with most of the therapeutic options reviewed here. For high-risk patients, when participation in a clinical trial is not an option, we typically initiate anti-TNF therapy and then ECP if possible, which appear to facilitate steroid tapers in responding patients. We also use nonabsorbable steroids in patients with GI GVHD, but possess only anecdotal evidence for the effectiveness of these approaches.

Recent advances suggest a brighter future for this intractable problem. First, a large number of new and emerging therapeutic agents that target pathways involved in GVHD pathology are near or already in clinical testing. These new approaches may prove less toxic than less targeted intensification of immunosuppression. Next, recently published uniform GVHD staging guidelines that reduce grading variability among centers will facilitate comparisons of treatment responses across studies.[9] In addition, biomarker-defined GVHD scoring allows for early identification of high-risk patients. We hypothesize that earlier treatment of high-risk patients will improve TRM and overall survival before damage becomes firmly established. Clinical trials to test this hypothesis will begin enrolling patients in the year 2016. Furthermore, a more

dynamic understanding of the impact of the gut microenvironment and microbiome on acute GI GVHD supports highly novel strategies to re-establish a favorable gut milieu. Methods to restore the GI microbiota homeostasis through delivery of IL-22 or REG3α are particularly exciting. In the meantime, approaches that control the upstream inflammatory processes that cause or worsen GI GVHD, such as Treg infusions or blockade of T-cell traffic, are especially promising strategies. Further improvements in our understanding of GVHD pathophysiology will hopefully lead to combination therapies that target several pathways simultaneously; such combinations may well be required to control this major barrier to safer allogeneic HCT.

Declaration of interest

J Ferrara and J Levine both own a patent on GVHD biomarkers. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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