National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. The 2020 Highly morbid forms report

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Full Length Article
Report

National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. The 2020 Highly morbid forms report

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ABSTRACT

Chronic graft-versus-host disease (GVHD) can be associated with significant morbidity, in part because of nonreversible fibrosis, which impacts physical functioning (eye, skin, lung manifestations) and mortality (lung, gastrointestinal manifestations). Progress in preventing severe morbidity and mortality associated with chronic GVHD is limited by a complex and incompletely understood disease biology and a lack of prognostic biomarkers. Likewise, treatment advances for highly morbid manifestations remain hindered by the absence of effective organ-specific approaches targeting "irreversible" fibrotic sequelae and difficulties in conducting clinical trials in a heterogeneous disease with small patient numbers. The purpose of this document is to identify current gaps, to outline a roadmap of research goals for highly morbid forms of chronic GVHD including advanced skin sclerosis, fasciitis, lung, ocular and gastrointestinal involvement, and to propose strategies for effective trial design. The working group made the following recommendations: (1) Phenotype chronic GVHD clinically and biologically in future cohorts, to describe the incidence, prognostic factors, mechanisms of organ damage, and clinical evolution of highly morbid conditions including long-term effects in children; (2) Conduct longitudinal multicenter studies with common definitions and research sample collections; (3) Develop new approaches for early identification and treatment of highly morbid forms of chronic GVHD, especially biologically targeted treatments, with a special focus on fibrotic changes; and (4) Establish primary endpoints for clinical trials addressing each highly morbid manifestation in relationship to the time point of intervention (early versus late). Alternative endpoints, such as lack of progression and improvement in physical functioning or quality of life, may be suitable for clinical trials in patients with highly morbid manifestations. Finally, new approaches for objective response assessment and exploration of novel trial designs for small populations are required.

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Chronic graft-versus-host disease (GVHD) may be associated with significant morbidity, in part due to development of non-reversible fibrosis (eye, sclerosis, lung) with detrimental impact on physical functioning (sclerotic skin manifestations, lung) and survival (lung, gastrointestinal) [1,2]. Progress in prevention of long-term severe morbidity and mortality associated with chronic GVHD is limited by a complex and incompletely understood disease biology and lack of prognostic biomarkers associated with a highly morbid future course. Treatment advances for these highly morbid manifestations are limited because of both the difficulty in conducting clinical trials in a heterogeneous disease with small patient numbers and the absence of effective organ-specific approaches targeting "irreversible" fibrotic sequelae.

PURPOSE OF THIS DOCUMENT

The goal of this working group is to address gaps and outline a roadmap of research goals including suggestions on trial design for frequent, highly morbid forms of chronic GVHD, namely advanced skin sclerosis and fasciitis, and lung, ocular, and gastrointestinal (GI) involvement.

SUMMARY OF RECOMMENDATIONS

1. Future studies should phenotype chronic GVHD clinically and biologically, to describe the incidence, prognostic factors, mechanisms of organ damage, and clinical evolution of highly morbid manifestations including long term effects of morbid forms in children. Longitudinal multicenter studies with common definitions (diagnostic and inclusion criteria, documentation of organ involvement and endpoints with sufficient follow up) and research sample collections are needed (Figures 1 and 2).

2. Develop new approaches for early identification and treatment of highly morbid forms of chronic GVHD, especially biologically targeted treatments, with a special focus on prevention and treatment of fibrotic changes.

3. Establish primary endpoints for clinical trials addressing each highly morbid manifestation in relationship to the time point of intervention (early versus late). Alternative endpoints, such as lack of progression and improvement in physical functioning or quality of life, can provide compelling evidence of clinical benefit in clinical trials to evaluate treatment of highly morbid manifestations.

4. Explore novel trial designs for small populations, emphasizing the need for objective endpoints.

METHODS

Each working group was created to encourage global engagement in the topic [3]. Groups worked individually to review the relevant literature and create the initial draft of the paper, which was circulated for review and comment by the Steering Committee. Two iterative rounds of comments from the Steering Committee were collected before the November 2020 Consensus Conference with appropriate manuscript revisions. Based on additional comments from Conference participants and a 30-day public comment period, the paper was further revised for submission.

SCLEROSIS OF SKIN AND FASCIA
Current clinical knowledge

Skin is the organ most frequently affected by chronic GVHD. Although inflammatory disease manifestations characterized by erythematous or lichen planus-like clinical presentations are often responsive to therapy, management options for fibrotic disease remain limited although responses have been reported in superficial and deep sclerosis.
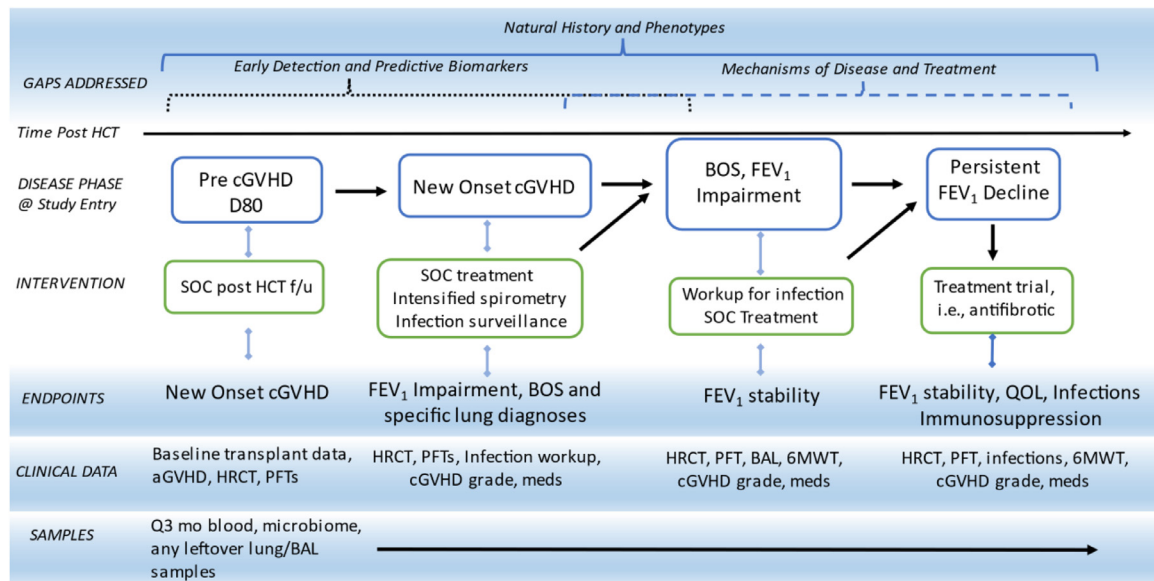
Figure 1. Potential Longitudinal Trial Design Proposal for Highly Morbid Manifestations of chronic GVHD using BOS as example. The proposed study approach aims to simultaneously address identified fundamental knowledge gaps in several domains, including (1) description of clinical evolution and clinical phenotypes, (2) early detection and predictive biomarker discovery, (3) mechanisms of disease through translational work, and (4) evaluation of novel treatments. High-risk patients are enrolled at a pre-diagnosis phase based on biomarker and clinical risk factors and followed over time through phases of chronic GVHD. Patients may also enter the longitudinal cohort at the time of chronic GVHD diagnosis, and if they develop a highly morbid manifestation, they are followed in that specific cohort category and may be enrolled on clinical trials. Longitudinal clinical data and serial tissue samples and specimens will be collected. In this Figure, lung disease is used as an example for the enrollment entry, interventions, endpoints, and data and samples to be collected. This schema can be easily expanded to reflect skin, GI, and other manifestations with relevant data collection and treatment agents. SOC indicates standard of care; f/u, follow-up; HRCT, high-resolution chest tomography; BAL, bronchoalveolar lavage; 6MWT, 6-minute walk test.

Sclerotic chronic GVHD (ScGVHD) at onset of disease occurs infrequently [4] but long-standing chronic GVHD is likely to advance to sclerosis, with 20% of patients having sclerosis after 3 years of chronic GVHD therapy. The prevalence of sclerosis exceeds 50% among those with severe disease [4-7]. ScGVHD can manifest as localized disease (morphea-like), diffuse involvement, deep sclerosis, panniculitis, or fasciitis without epidermal manifestations. ScGVHD may cause joint contractures, skin breakdown, neuropathy (including small fiber

neuropathy [8], nerve compression syndrome and painful muscle cramping [9]), myopathy as a consequence of fascial compression, and vascular insufficiency that contributes to poor wound healing.

Pathophysiology

Fibrosis represents the terminal step of an unchecked inflammatory alloreactivity cascade. The role of T cells in chronic skin GVHD development is well defined and supported
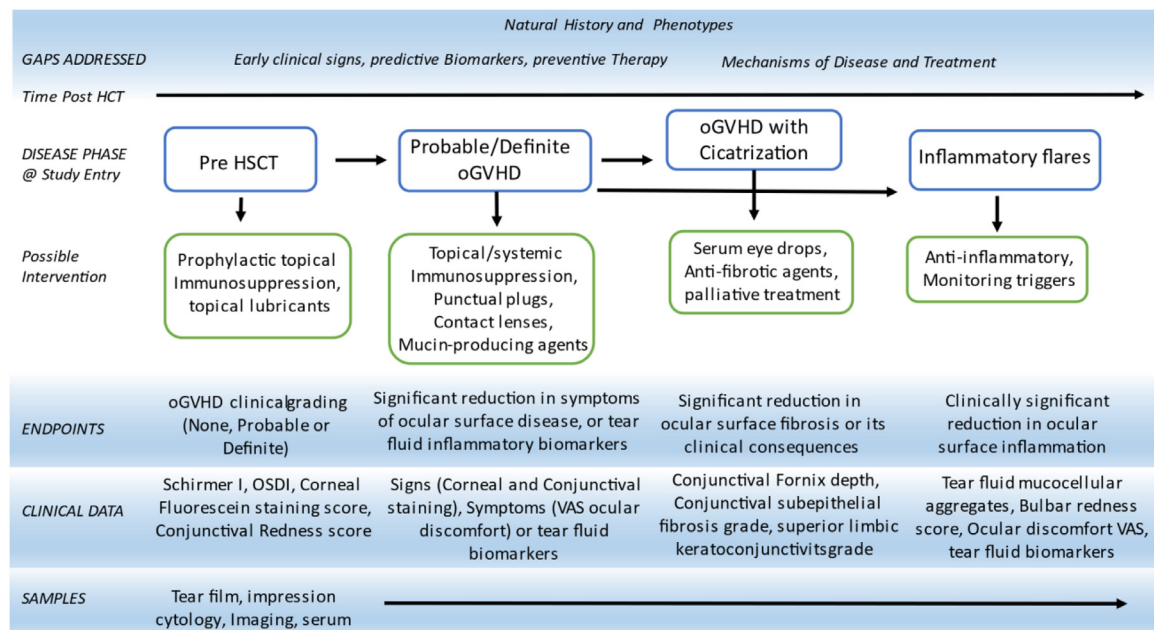

Figure 2. Potential longitudinal trial design proposal for oGVHD. OSDI indicates ocular surface disease index; VAS, visual analog scale.

by defined genetic risk factors [10], but their role in an established sclerotic response is unknown. ScGVHD biopsy specimens demonstrate variable degrees of CD4^{+} and CD8^{+} T cell infiltration with unknown clonal architecture [11-14]; and infiltrating T cells may represent bystanders or effectors depending on biopsy timing [11,12]. In systemic sclerosis (SSc) [15], as well as chronic GVHD [16,17] impaired function of regulatory T cells has been reported, and IL-2 treatment, which expands regulatory T cells, has shown efficacy in advanced chronic GVHD [18]. Agonist platelet−derived growth factor-receptor (PDGF-R) antibodies [19] or other antibodies targeting surface antigens [20] could have a role in severe fibrotic forms of chronic GVHD. Poor correlation of chronic GVHD severity, lack of damage in grafted donor skin indicating host specificity, and limited response to PDGF-R inhibitors in patients with these antibodies argue against the broader relevance of these findings [21,22]. A possible mechanistic role of B cells in ScGVHD has been suggested with improvement of sclerosis after B cell depletion [23]. Additional work is needed in chronic GVHD, to determine how B cells might promote fibrosis since definitive evidence linking antibody-dependent mechanisms to human ScGVHD is lacking [24].

Recently, distinct dermal myeloid cell populations were identified in human skin [25]. In animal models, macrophages contribute to development of fibrosis in both transforming growth factor beta (TGFβ)-dependent and -independent fashion and their pathogenic role in chronic GVHD is increasingly recognized [26-28]. Relevant for ScGVHD, myeloid-sourced TGFβ [29,30] promotes fibrosis through positive regulation of fibroblast proliferation and differentiation into myofibroblasts [31] and stimulation of extracellular matrix overproduction [30]. In addition, macrophage-derived TGFβ promotes epithelial mesenchymal transition in models of lung fibrosis [32]. Partial epithelial mesenchymal transition is involved in normal wound healing, although its disruption in the inflammatory environment can promote pathologic fibrosis in lung and skin [33]. Although fibroblasts represent critical mediators of fibrotic tissue injury, little is known about their homeostasis during chronic GVHD.

TGFβ is a keystone pathway in many fibrotic disorders and has a documented role in preclinical ScGVHD [29,30]. In patients, higher TGFβ levels are associated with adverse outcomes taking into account the challenges of correlating expression and activity [34,35]. However, TGFβ exerts distinct effects on post-transplantation complications early and late after transplantation [30], has pleiotropic roles in different compartments [36], and activates distinct downstream signaling pathways [36], making it challenging as a therapeutic target. Type I interferon responses feature prominently in SSc skin fibrosis and ScGVHD as well [37,38], tightly linking adaptive and innate immune crosstalk in initiation and persistence of ScGVHD, with possible therapeutic implications. The recent approval of tocilizumab in pulmonary manifestations of SSc highlights the importance of IL-6 in the pathophysiology of fibrosis and this cytokine may also play a role in ScGVHD [39-41].

Other interconnected pathways, commonly influenced by TGFβ, often create a feed-forward loop promoting aberrant tissue remodeling such as the developmental (morphogen) pathways, particularly Hedgehog, Wnt, and Notch, which are involved in fibrotic disorders [33,42-44]. Active Hedgehog signaling has been observed in the skin of patients with ScGVHD, and its targeting in preclinical models modulated collagen production by myofibroblasts and reduced fibrosis [45]. Hedgehog inhibitors have been tested in chronic GVHD with some efficacy, although their use is hindered by significant toxicities [46,47]. Recent data in ScGVHD suggested an immunomodulatory role of morphogen pathways with broad effects on adaptive immunity promoting chronic GVHD [46,48], thus providing an added impetus for clinical translation. The endocannabinoid system is involved in multiple inflammatory and fibrotic disorders, with opposing roles for signaling through cannabinoid receptor 1 (CB$_1$R; profibrogenic) and cannabinoid receptor 2 (CB$_2$R; antifibrotic/anti-inflammatory), and agents targeting these receptors (CB$_1$R antagonists, CB$_2$R agonists) are already in clinical trials [49-52]. Of the many immune mechanisms that contribute to development of chronic GvHD, it will be important to elucidate whether different immune pathways lead to specific clinical presentations.

Gaps in knowledge and unmet needs; highest priorities

The pivotal role of immune injury in the initial steps of fibrosis is well accepted. However, the mechanisms responsible for the shift from active inflammation to feed-forward loops of dysregulated tissue remodeling remains unknown. Understanding this transition is essential to devise approaches with optimal therapeutic indices that minimize immunosuppression. Both skin and peripheral blood samples should be queried to identify abnormalities along the disease continuum to inform preclinical modeling with a goal of defining the mechanistic relevance of the observations. Optimized preclinical ex vivo approaches could be well suited for the latter (e.g., to evaluate the effect of TGFβ and TGFβ pathway inhibitors on sclerotic skin fibroblasts). Deeper interrogation should use -omics methods and novel tissue diagnostic approaches such as multiplex immunohistochemistry/immunofluorescence, which can be enhanced by artificial intelligence (machine and deep learning) to offer a spatial perspective into the disease process and facilitate the development of novel biomarker signatures.

Clinical trials need more robust and sensitive endpoints. It is particularly challenging to precisely quantify the evolution and the extent of deep-seated (subcutaneous and fascial) disease to assess response and the current organ-based grading system is poorly suited to detect responses in established sclerosis. Given this limitation, functional improvement (e.g., improved joint mobility documented by P-ROM and physician global and skin and joint tightening scale per the 2014 National Institutes of Health [NIH] Consensus) could be considered an ScGVHD response, even if skin-specific scoring remains unchanged. Data supporting such an approach have been published [53], and bedside validation in ScGVHD should be actively pursued. Imaging biomarkers that have been suggested include high-frequency ultrasonography and magnetic resonance imaging, but rapid, safe, less costly, and accessible clinical assessment tools are needed (Table 1) [54,55]. Gene expression biomarkers in SSc skin correlated highly with changes of the modified Rodnan skin score and have been utilized to support response assessment in several clinical trials in that disease [56-59], but its use in ScGVHD requires additional exploration.

Translation of knowledge accrued from organ fibrosis (e.g., SSc and idiopathic pulmonary fibrosis) to ScGVHD should be accelerated and is critical to improvement in patient care. Some agents have already demonstrated promise in chronic GVHD (e.g. belumosudil, a rho-associated coiled coil protein kinase-2 (ROCK2) inhibitor [60]), whereas many others have yet to be explored (e.g., connective tissue growth factor or cannabinoid receptor−directed therapies) (Table 2). Theoretically, avoiding unnecessary immunosuppression and side effects is possible with topical delivery methods [61], but most are

Table 1
Potential Objective Assessment Tools to Assess Skin Sclerosis in Chronic GVHD

Modality	Advantages	Disadvantages	Use in ScGVHD
High-frequency ultrasound, acoustic radiation force impulse, shear wave elasticity imaging, ultrasound surface wave elastography	Bedside use, easy to assess multiple sites, allows rapid comparability to previous images	Cost, requires training, requires marking of target area for repeat assessment; edema from active inflammation may confound imaging	CS [237] CS [54] CS [238] CS [239] CS [240]
Durometer	Bedside use, affordable, small, hand-held device, easy to use, provides numerical readout	"Anvil effect" from underlying bony structures, less sensitive for deep-seated disease; reproducibility requires consistent technique	CS [241]
MRI, MRI/positron emission tomography	Detection of deep-seated, sub-clinical involvement; useful for detecting active fascial inflammation; does not require marking of target area	Cost, inconvenient for patients, unclear if responsive to small improvements in fibrosis	CS [55] CS [242] CS [243]
Optical coherence tomography/ elastography	High-resolution imaging, including capability to assess local blood flow	Limited depth of penetration	CR [244]
Laser doppler flowmetry	2D flow map of skin perfusion; can assess dynamic changes; monitoring potential for compromised acral sites of sclerotic chronic GVHD	Affected by ambient temperature; movement, pressure or other contact with skin will influence perfusion	CR [245]
Suction probe (Cutometer, Dermaflex, Nimble)	Devices measure stiffness and elasticity; have been used in clinical assessment of morphea and systemic sclerosis	Affected by many variables, including sun damage, water balance, age, body location; does not capture changes in subcutaneous fat/fascia; remission may not result in return of elasticity	CS [237]
Myoton	Hand-held device, detects changes in tissue oscillation (skin stiffness and other properties) after a mechanical impulse	Requires adherence to measurement protocols and knowledge of muscular anatomy. Results depend on underlying muscle tone, patient positioning	CS [246] CS [247] CS [241]

CR indicates case report; CS, case series; MRI, magnetic resonance imaging; .

formulated to be effective only against superficial skin conditions affecting the epidermis and papillary dermis. Effective topical delivery in ScGVHD may be hampered by increased dermal thickness and possibly by lower permeability. Strategies to improve drug delivery include physical approaches (microneedles, laser, iontophoresis), particle-based drug carriers (lipid-based, nanoparticles) and chemical approaches (permeation modifiers, prodrugs) [62]. Precision medicine with engineered cell therapies targeting fibrosis have been explored in other diseases [63] and could be considered in ScGVHD. Multitargeting approaches may be helpful to prevent evolution to sclerosis and to enhance safety without compromising efficacy [64].

Highest Priorities and Roadmap for progress for ScGVHD

1. Longitudinal multicenter studies to evaluate pathologic cell populations in lesional and unaffected skin and peripheral blood, and cytokine and chemokine responses, should be done to identify early signatures for subsequent fibrosis and to identify additional target pathways.

2. Capitalize on the enhanced resolution of next generation sequencing strategies, including single-cell RNA-, assay for transposase-accessible chromatin (ATAC)-, TCR-, and BCR-sequencing to query skin biopsies to provide biological insight into the mediators of ScGVHD in individual patients, address the degree of temporal and clinical disease heterogeneity, and the origins (recipient versus donor) and phenotype of clonally expanded T- and B-cell populations. These investigations could be complemented by new proteomic technologies such as multiplexed ion beam imaging by time of flight (MIBI-TOF) [65] combined with nonlinear dimensionality reduction analysis approaches (visualization

of t-distributed stochastic neighbor embedding [tSNE]/ [viSNE]).

3. Analyze differences in expression and the spatial distribution of mediators and targets within the epidermis, dermis, subcutaneous fat, and fascia in an effort to understand differences in clinical presentations and identify interventions that could be personalized.

4. Test emerging therapies being developed for organ fibrosis and supported by biological insights in ScGVHD, focusing on early intervention. Promising candidates are listed in Table 2. Combination therapies targeting multiple pathways active in fibrosis should be considered to augment efficacy while minimizing toxicities aiming to stop or potentially reverse fibrotic changes.

5. Develop novel cost-effective tools for better measurement and documentation of change in skin sclerosis for clinical trials. Refinements of the current 2014 clinical response criteria are needed for skin sclerosis/fascia manifestations.

PULMONARY INVOLVEMENT
Current clinical knowledge

Bronchiolitis obliterans syndrome (BOS) is the only formally recognized manifestation of lung chronic GVHD, with an incidence of 3% to 10% of allogeneic hematopoietic cell transplant (HCT) recipients [66-68], and 14% [69] in those with chronic GVHD. Although the histologic entity of obliterative bronchiolitis is the diagnostic lesion of lung GVHD, clinical diagnosis is largely based on pulmonary function studies that cannot be performed in children under age 7 [70]. Risk factors for onset include antecedent respiratory viral infections [71,72] and impaired lung function early after transplantation [68,73]. Worse prognosis is associated with early onset after transplantation and severe impairment of forced expiratory volume first second (FEV_1) at diagnosis. Contemporary series

Table 2
Candidate Therapeutic Agents in Sclerotic Chronic GVHD

Target	Drug(s)	Target Cellular Subsets	Clinical Development Status	References
Connective tissue growth factor/cellular communication network factor 2	Pamrevlumab (FG-3019)	Fibroblasts	Phase 3—Idiopathic Pulmonary Fibrosis (NCT01890265)	[248, 249]
Autotaxin	Ziritaxestat (GLPG-1690)	Fibroblasts	Phase 3—Idiopathic Pulmonary Fibrosis (NCT03733444, NCT03711162)	[250]
Heatshock protein (HSP47)	ND-L02-s0201	Fibroblasts	Phase 2—Idiopathic Pulmonary Fibrosis (NCT03538301)	[61, 251, 252]
Pentraxin 2 (agonist)	PRM-151	Fibroblasts Macrophages	Phase 2—Idiopathic Pulmonary Fibrosis (NCT02550873) Phase 2—Myelofibrosis (NCT01981850)	[253, 254]
CB$_2$R (agonist)	Lenabasum (Ajulemic acid)	Fibroblasts T cells Macrophages	Phase 3—Systemic Sclerosis (NCT03398837)	[52]
CB$_2$R/Peroxisome proliferator-activated receptor (Dual Agonist)	EHP-101	Fibroblasts Endothelial cells Macrophages	Phase 2—Systemic Sclerosis (NCT04166552)	[255]
CB$_1$R/inducible nitric oxide synthase (dual antagonist)	MRI-1867	Fibroblasts T cells Macrophages	Phase 1	[49, 256, 257]
Oncostatin M (antagonist)	GSK2330811	Fibroblasts Endothelial cells T cells Macrophages	Phase 2—Systemic Sclerosis (NCT03041025)	[258, 259]
TGFβ	AVID200	Fibroblasts T cells Macrophages	Phase 1—Myelofibrosis (NCT03895112) Phase 1—Systemic Sclerosis (NCT03831438)	[260]
IL-6R	Tocilizumab	Fibroblasts T cells Macrophages	Phase 3—Systemic Sclerosis (NCT02453256) Phase 2—Steroid dependent immune related adverse events (NCT04375228)	[261-263]
CSF-1R	Axatilimab (SNDX-6352)	Macrophages	Phase 2—Chronic GVHD (NCT03604692)	[26]
ROCK2	Belumosudil (KD025)	T cells Macrophages	Phase 2—Chronic GVHD (NCT03640481, NCT02841995) Phase 2—Systemic Sclerosis (NCT03919799)	[264]
Interferon receptor type 1	Anifrolumab	T cells Macrophages	Phase 3—Systemic Lupus Erythematosus (NCT02446899) Phase 2—Rheumatoid Arthritis (NCT03435601)	[265]

CB$_2$R indicates cannabinoid receptor.

show a 2-year survival rate of 70% after BOS diagnosis [74], and 5-year survival remains low at approximately 50%, highlighting the need for novel prevention and treatment strategies [68].

Pathophysiology

The pathology of BOS is characterized by fibrotic narrowing and obstruction of small airways, likely the shared outcome of immune and non-immune mediated injury to the airway epithelium. A fundamental knowledge gap, however, lies in understanding the triggers of lung epithelial cell injury and subsequent mechanisms of altered immune and fibrotic responses that result in obliterative bronchiolitis. Mechanisms being explored in other disease contexts include airway stem cell depletion [75] and acquisition of a persistent inflammatory airway epithelial cell phenotype [76,77]. The immune dysregulation associated with BOS after lung allograft or HCT appears to involve oligoclonal expansion of CD4$^+$ T cells, reduced T regulatory cells, and higher levels of IL-17 and IL-8 [78]. In one murine model, alternatively activated macrophages drove BOS, supported clinically by evidence of leukotriene production, and polarized CD4$^+$ immune activation [26]. In another preclinical model, donor B cells contribute to airway pathology through local antibody production. In this model, genetic disruption of germinal center formation, which is supported by T follicular helper cells [79], reduced pulmonary dysfunction [80]. These mechanistic insights have not yet been confirmed

in humans, although biomarker studies support a prominent role of B cells with significantly elevated CD19$^+$CD21^{low} B cells and high soluble B-cell activating factor levels [81]. The role of the microbiome, which is influential in other airway diseases, should be investigated [82].

Physiological subtypes

Defining clinical phenotypes of BOS remains a significant knowledge gap that hampers our ability to identify patients at risk for morbidity and death from lung GVHD. Current NIH spirometric criteria used for BOS diagnosis defines this disorder based upon airflow obstruction. Although this definition encompasses most cases of BOS, it is unlikely to reflect the full spectrum of physiologic and histologic manifestations of BOS, nor does it facilitate identification of early disease [83-85]. A concerning pattern is reduced FEV$_1$ and forced vital capacity (FVC) with normal FEV$_1$/FVC ratio [83], potentially reflecting impaired exhalation caused by air trapping by small airways obstruction, resulting in a pattern that suggests restriction. This pattern underlines the need for a complete evaluation including lung volumes and a high-resolution chest computed tomography scanning in expiration. An open question remains whether lymphocytic bronchiolitis, which is responsive to anti-inflammatory agents [84], represents early phase of disease, a distinct subtype of BOS, or is a separate entity from BOS. Although some patients demonstrate stability of FEV$_1$ after clinical recognition, this plateau could be due to treatment, a distinct biology, or the

stage of the disease at diagnosis [69,74]. More significantly, the clinical and biological risk factors for progressive refractory lung function decline are not known.

The association of chronic GVHD with restrictive lung impairment remains ill-defined for HCT survivors and is not currently recognized as a chronic GVHD manifestation. Restrictive allograft syndrome is a phenotype of chronic lung allograft dysfunction (CLAD) in lung transplantation recipients, and is defined by a reduction in FVC or total lung capacity (TLC) with persistent lung infiltrates and carries a worse prognosis than classic BOS [86-89]. Although a similar entity is suspected to occur after HCT, confounding diagnoses for restrictive physiology and the lack of validated diagnostic criteria in the context of chronic GVHD have been barriers to recognition [89]. Although restriction may be due to known interstitial lung disease entities including organizing pneumonia, restrictive lung impairment in chronic GVHD can also be caused by extraparenchymal processes including truncal sclerosis [90], respiratory muscle weakness [85,91], or pleural effusions. In severe BOS, histological evidence of interstitial pathology is often associated with the bronchiolar lesions [85], suggesting that interstitial abnormalities, in addition to airway pathology, are part of the spectrum of lung chronic GVHD. Table 3 summarizes the spectrum of lung abnormalities, diagnostic criteria and association with chronic GVHD after HCT.

Treatment

Treatment for manifest BOS is aimed at stabilizing lung function, which reflects the sobering observations that diagnosis is usually made at a later stage of disease, and no therapies that reverse the end-stage lesion of obliterative bronchiolitis have been established. Nonetheless, efforts at early recognition and intervention may be able to reverse BOS [92]. The combination of inhaled corticosteroids (fluticasone), azithromycin, and montelukast, with or without a long-acting bronchodilator, has been established as organ-specific therapy for BOS [93,94]. Observations of potential impaired graft-versus-leukemia effects associated with prophylactic azithromycin given in the early posttransplant period raised concerns about its use to treat BOS [95]. A subsequent analysis of patients treated for manifest BOS did not show an increased risk for relapse [96]. Despite these treatments, lung function continues to decline in a significant proportion of BOS patients [97], and intensified immunosuppression contributes to lung infections, which in turn worsen lung function supporting the need for antimicrobial prophylaxis and pulmonary rehabilitation [98,99]. Agents that are currently under investigation have shown utility in other chronic lung conditions such as pulmonary fibrosis and include inhaled immunosuppressants [100] and anti-inflammatory or antifibrotic agents [101].

Highest priorities and roadmap for progress in pulmonary chronic GVHD

1. Understand the onset and evolution of lung GVHD. A prospective longitudinal multicenter patient study cohort including adults and children followed up from the time of onset of chronic GVHD, is essential to comprehensively identify biologic triggers (e.g., viral infections), enable discovery of biomarkers for early diagnosis, provide biospecimens for translational mechanistic studies and microbiome analysis, and define lung GVHD subtypes (Figure 1).
   a. Serial pulmonary function tests (PFTs) and chest computed tomography [90,102] with quantitative lung imaging techniques (e.g., parametric response mapping) are clinical tools that could be implemented immediately as part of clinical care to delineate phenotypes and physiologic biomarkers that associate with BOS. Machine learning approaches that combine serial data from PFTs, imaging, and clinical risk factors might identify scenarios that predict high risk [103]. Additional modalities including hyperpolarized xenon-129 magnetic resonance imaging for the early detection of small airways disease in children should be further explored [104].
   b. The creation of a shared lung-specific biorepository will support biomarker discovery and mechanistic studies. Given the inherent challenges of procuring surgical lung tissue, universal protocols should be implemented to systematically collect excess bronchoalveolar lavage and lung biopsy specimens obtained during clinical care. Less invasive means of sampling airway epithelium, e.g., bronchial brushings, and development of validated serum or plasma-based assays should be explored [102]. Coupling these samples with carefully annotated clinical databases will be critical.
2. Test strategies for early diagnosis and novel treatments in clinical trials. Early diagnostic strategies coupled with preemptive treatment with targeted agents should be evaluated to avert severe BOS forms and potentially reverse obstruction before progression to advanced fibrosis. Novel therapies for established BOS need to be tested in clinical trials that are informed by knowledge of BOS evolution and an understanding of pathogenesis and biomarkers of response, which is possible only with a longitudinal prospective cohort. Clinically relevant endpoints include FEV_1 stability (or lack of progression of FEV_1 decline), infectious exacerbations, exercise tolerance, quality of life, reduction of systemic steroid use, and overall survival.

GASTROINTESTINAL INVOLVEMENT
Current clinical knowledge

Historically, the intestine has been less commonly affected by chronic GVHD, which may be partly explained by lack of documentation of GI involvement in the past. The 2005 NIH consensus (updated in 2014) provided for the first time a definition and severity grading for GI manifestations in the context of chronic GVHD [105]. The 2014 consensus requires the presence of other diagnostic or distinctive manifestations to distinguish chronic GVHD from acute GVHD diagnosis in patients with upper and lower GI symptoms (loss of appetite, diarrhea) that are typical of acute GVHD. However, the 2014 NIH organ scoring of chronic GVHD does not distinguish between the site of GI involvement (esophagus, upper GI, and lower GI), although the response criteria provide a more detailed framework for reporting and grading these manifestations [105,106]. Applying the NIH criteria, the respective incidence rates of esophageal, upper GI, and lower GI involvement are 16%, 20%, and 13%, according to a cross-sectional analysis from the Chronic GVHD Consortium [107]. Most importantly, intestinal involvement is associated with greater risk of non-relapse mortality and patients with histologically confirmed severe lower GI involvement as part of chronic GVHD are usually treated with regimens recommended for management of acute GVHD [108-110]. Of note, esophageal web or strictures or stenosis of the upper to mid third part are the only manifestations regarded as diagnostic signs of chronic GVHD [105]. Major limitations in diagnosis and management of GI symptoms such as nausea, loose stool and anorexia include multiple potential causes unrelated to GVHD [111] (i.e., maldigestion, toxic

Table 3
Pulmonary Syndromes After Allogeneic HCT

Entity	Established in definition of lung GVHD	PFT pattern	High Resolution Chest CT Findings	Lung Histology	Comment
Bronchiolitis obliterans syndrome	Yes	Fixed obstructive pattern: FEV_1 decline >10%, FEV_1/FVC < LLN. Elevated residual volume or residual volume/Total Lung Capacity. FEV_1/FVC > LLN with preserved TLC may be seen. DLCO may be normal or reduced.	Signs of air trapping (mosaic attenuation on expiratory phase) or bronchiolitis (centrilobular micronodules, bronchial wall thickening) or late sequalae (bronchiectasis)	Obliterative bronchiolitis: partial or complete fibroproliferative occlusion of terminal small bronchioles, lymphocytic bronchiolitis may also be seen	Subtypes of BOS have been defined based on timing of onset after HCT, initial tempo of onset, FEV_1 decline, histology, response to therapy, and prognosis.
Restrictive impairment caused by ILD entities[*]					
Organizing pneumonia [267]	No, but evidence supports association with acute and chronic GVHD.	Restrictive impairment with reduced TLC with FEV_1/FVC > LLN most common. Obstructive or mixed pattern may be seen. Reduced DLCO.	Patchy and peribronchial infiltrates or consolidation, and reticular ground glass opacities, often predominant in upper lobes and periphery [268]	Bronchiolar and alveolar granulation tissue	Bronchoscopy should be performed to rule out infection. Clinical diagnosis often made without lung histology and is empirically based on steroid-responsiveness
Nonspecific interstitial pneumonia [266]	No	Reduced TLC and DLCO	Confluent bilateral lower lobe ground glass opacities, bronchiectasis and lower lobe volumes loss, classically sparing the subpleural area	Diffuse alveolar wall thickening by uniform fibrosis; interstitial inflammation	Bronchoscopy should be performed to rule out infection
Pleuroparenchymal pulmonary fibroelastosis [266,269]	No	Reduced TLC and DLCO, occasionally obstructive and restrictive pattern. Progressive and severe impairment over time	Upper lobe fibrosis with subpleural and pleural thickening, loss of lung volume, and lower lobe traction bronchiectasis	Subpleural and pleural fibroelastic proliferation with minimal inflammation	Diagnosis is usually made by typical chest CT findings
Restrictive impairment not attributed to ILD[†]					
Truncal sclerosis	No. Sclerosis due to chronic GVHD is an indirect cause of ventilatory impairment	Reduced TLC; RV/TLC may be elevated but usually does not necessarily indicate small airways disease	No parenchymal infiltrates. Parametric response mapping shows low inspiratory volumes.	N/A.	
Respiratory muscle weakness	No. Weakness may result from chronic GVHD-related myositis or prolonged steroid use to treat chronic GVHD.	Concomitant reduction in FVC and FEV_1, reduced TLC with relative sparing of RV. Reduced supine FVC. Maximal inspiratory and expiratory pressures may be reduced.	Low lung volumes, normal parenchyma. If diaphragmatic weakness or paralysis is suspected, a fluorographic sniff test may show reduced diaphragmatic excursion	N/A. Evidence of myositis in a peripheral muscle.	Diagnosis of exclusion

RAS has been defined for lung transplantation [89] as a manifestation of chronic allograft dysfunction. BOS is the obstructive form of chronic lung allograft destruction after lung transplantation. RAS after lung transplantation is defined by restrictive physiology and persistent pulmonary infiltrates that represent heterogeneous histology. A similar syndrome of restrictive impairment as a manifestation of alloimmunity in the context of chronic GVHD may also exist; however, the epidemiologic associations and definitions remain to be determined. It is possible that ILD entities that occur in the context of chronic GVHD could be considered as an "RAS-like" condition, or "restrictive alloimmune syndrome" after HCT.

ILD indicates interstitial lung disease; RAS, restrictive allograft syndrome.

[*] Multiple entities, as per the ATS/ERS classification of ILD may occur after HCT, beyond what is listed here [85,266]. If restrictive impairment is seen on PFT (i.e., reduced FVC with preserved FEV_1/FVC and reduced TLC), high resolution chest CT should be performed to evaluate for ILD and other entities.

[†] These entities are secondary to extrathoracic consequences of chronic GVHD.

effects of medication, autonomic nervous system dysfunction, bacterial overgrowth, endocrinological sequelae, etc.). Histopathology may not be able to completely resolve diagnostic uncertainty due to limited sampling, patchy involvement and nonspecific histological abnormalities in mild cases [112,113].

Risks factors for intestinal involvement in chronic GVHD remain to be elucidated. Ethnicity, genetic diversity, environmental differences, diet, antibiotic use, supportive care, and microbiota or microbe-derived metabolites may all influence GI GVHD [114-118]. Age is a potential risk factor because children appear to be particularly susceptible to late GI-acute GVHD which affects up to 25% of pediatric transplant recipients [119] and can persist to and beyond the diagnosis of chronic GVHD. A small study showed that increased relative abundance of butyrogenic bacteria after the onset of acute GVHD was associated with subsequent steroid-refractory acute GVHD or chronic GVHD [118] indicating the need for further investigations of dysbiosis and antibiotic strategies and their association with GI-chronic GVHD and other manifestations of the disease [117]. GI manifestations of chronic GVHD may have complex causes but are rarely directly fatal, and the mechanisms that increase the subsequent risk for nonrelapse mortality remain to be elucidated.

Pathophysiology

In many tissues chronic GVHD is characterized by atrophy and destruction with subsequent fibrosis, but intestinal fibrosis is a rare GI-manifestation of chronic GVHD [120,121].

Intestinal epithelium is the most rapidly self-renewing tissue in adults; intestinal epithelial cells are continuously regenerated from intestinal stem cells, which are key to the regeneration of damaged intestinal epithelium [122]. Tissues having squamous epithelium (e.g., skin, mouth, esophagus, and vagina) and tissues having cuboidal epithelium (e.g., sweat, lacrimal, and salivary glands) appear to be more prone to dysregulated fibrosis in chronic GVHD than those having columnar epithelium (e.g., stomach, intestine, and trachea). Animal studies have demonstrated that both intestinal stem cells and their Paneth cell niche are impacted in acute GVHD, with impaired regeneration of the injured epithelium [123-129]. The rapid and potent repair ability of the intestine may protect against early fibrotic processes that often accompany repair processes in other tissues. Profiling of immune cell populations and plasma markers at day 100 after HCT demonstrated biological differences between chronic GVHD and late-onset acute GVHD [130].

Highest priorities and roadmap for progress in gastrointestinal chronic GVHD

1. Enforcement of the NIH 2014 classification terminology distinguishing acute from chronic GVHD within and across studies [112,131-136] since current longitudinal observational and clinical trials revealed a significant number of incorrectly classified patients [119]. According to the NIH 2014 terminology any patient developing diagnostic or distinctive signs of chronic GVHD during treatment of acute GVHD should be classified as having chronic GVHD with documentation of all manifestations. The presence and severity of individual GI manifestations (esophagus, upper GI, lower GI) should be also recorded and the use of the 2014 response criteria form to document individual GI manifestations is necessary at the time of diagnosis and in response to therapeutic strategies. Electronic tools such as the GVHD App may assist [137]. Adhering to these terminology will allow more reliable future studies of GI GVHD.
2. Develop diagnostic tools (i.e., blood and/or histopathological biomarkers among others) to differentiate GVHD from other causes of GI-symptoms.
3. Generate experimental and clinical models able to address the role of dysbiosis and intestinal inflammation in chronic GVHD involving organ manifestations outside the GI tract.
4. Collect blood, stool and or other body sites samples (e.g., saliva) and GI biopsies in either longitudinal observational cohorts or interventional clinical trials to allow development of biomarkers [138-140], through metabolomic alterations and microbiome compositions with sufficient sampling. These studies should include follow-up of acute GVHD trials.

OCULAR INVOLVEMENT
Current clinical knowledge

Chronic ocular GVHD (oGVHD) is one of the most frequent, rapidly-progressive organ manifestations with characteristic inflammatory, immune dysregulatory and fibrotic pathophysiological mechanisms [31,141-143]. Ocular GVHD is usually diagnosed between 5-24 months after HCT [144-146], and it can severely impact quality of life and vision [147,148] because of severe symptoms such as burning, dryness [105,149-151], and loss of visual function [152]. Pre-existing dry-eye disease (DED) and Meibomian gland disease as a consequence of chemotherapies or possibly irradiation increases the risk for later

oGVHD [153,154]. Early after transplantation, some patients already have impaired tear quantity and quality [155], yet eye involvement is recognized only after damage exceeds the eye's ability to compensate. Most importantly, oGVHD is not another form of DED, and approaches and therapies for DED may fail in oGVHD. Table 4 summarizes the differences between DED and oGVHD.

Ocular GVHD mainly presents as ocular surface disease demonstrating features such as blepharitis, Meibomian gland disease, qualitative and quantitative alteration of tear film, loss of goblet cells, corneal and conjunctival epitheliopathy, corneal vascularization and fibrosis of ocular tissues including conjunctiva and lacrimal glands [144,156-159]. In addition, a few reports have described intraocular involvement including choroid and retina [160,161]. However, no specific signs that are currently diagnostic for oGVHD, although certain combinations of findings, such as conjunctival subepithelial scarring and superior bulbar and limbal keratoconjunctivitis are frequently present [143,162-164]. Without early diagnosis and appropriate treatment, oGVHD progresses towards loss of visual function by complete loss of aqueous tear production and tear film stability, and scarring of the cornea. The impaired epithelial barrier can lead to complications such as infection, corneal ulceration and melting, and endophthalmitis. High-risk corneal transplants fail frequently presumably due to immunological rejection and impaired tear production, eventually resulting in loss of the eye [165-168].

The 2013 International Chronic Ocular GVHD Consensus Group (ICOGVHD 2013) Diagnostic Criteria filled an existing gap by adding recommendations for specific examinations to be performed by eye care specialists [150,169] to previous NIH consensus criteria [170]. The 2013 classification facilitates diagnosis of oGVHD by providing a structured clinical approach for distinguishing definite oGVHD from probable or "none" categories. However, the 2013 classification is not designed to detect preclinical oGVHD or to assess severity, and it does not translate into the NIH 0-3 eye score. Other grading systems have been suggested and validated [171], but they have not yet been established internationally.

Pathophysiology

Conditioning chemotherapy, radiation and infection often precede the onset of oGVHD and may induce homing signals for mobilization and migration of circulating bone marrow derived hematopoietic cells and mesenchymal stromal/stem cells into the microenvironment of the ocular surface and lacrimal gland. However, it is not understood how innate and adaptive immune mechanisms are triggered and how these common mechanisms initiate oGVHD only in selected patients. Studies show increased concentrations of ICAM-1, IL-1β, IL-6, IL-8 [172,173], neutrophil extracellular traps (NETs) [142], extracellular DNA [174,175] and decreased concentrations of lactoferrin [176], DNAse [175], IL-7, and epidermal growth factor (EGF) [173] in the tear film. In lacrimal glands affected by oGVHD, early fibrosis and myxedematous tissue may herald a rapidly progressive fibrosis [143] with activated fibroblasts already infiltrating into the lacrimal gland. Stromal fibroblasts in the lacrimal gland and conjunctiva interact with pathogenic T cells and antigen-presenting cells including macrophages [143,177], resulting in the proliferation and activation of fibroblasts through cytokines, such as IL-4, IL-6, and IL-17 [178,179]. Macrophages and fibroblasts activated through both the classical immunological and sterile inflammatory

Table 4
Differences Between Chronic Ocular GVHD and Dry Eye Disease

		Dry-eye disease (DED)	Ocular GVHD (oGVHD)	Clinical trial endpoint consideration in oGVHD
Cause				
	Known immunological mechanisms	Autoimmune Th17, CD4+/CD8+ T-cell activation through extrinsic or intrinsic triggers, unknown antigen	Migration and activation of donor hematopoietic /mesenchymal stem cells	Inclusion of participants before onset of disease possible
	Meibomian gland dysfunction (MGD)	Caused by numerous factors (aging, rosacea, drugs) leading to evaporation caused by MGD	Caused by chemotherapy and oGVHD, leading to evaporation	MGD as secondary endpoint
	Fibrosis	Not typical for dry-eye disease (see below)	Early activation of fibroblasts and macrophages	Fibrosis as clinical endpoint feasible assessed by slit lamp microscopy of fornix and tarsal conjunctiva
	Other causes	Numerous: systemic drugs, contact lens wear, aging, etc.	Presumed: chemotherapy and/or conditioning procedures	Pre-treatments and underlying oncological disease, origin of donor cells, should be considered during stratification
Time course		Onset mostly unknown, slow progress in a majority of cases, over years to decades	Rapid manifestation after HCT, progresses within weeks to months	Preventive clinical trials vs. therapeutic clinical trials feasible
Impact on visual function		Mild to severe impact, blinding disease very rare	Mostly severe; if untreated, often blinding disease	Primary endpoint
Clinical findings (selection of typical findings)				
	Tear production	Reduced in aqueous deficient DED and in overlap (mostly slow onset)	Reduced (fast onset, rapid progression)	Secondary endpoint
	Blepharitis	Mostly mild/ moderate	Mostly severe	Secondary endpoint
	Meibomian gland dysfunction	Up to 80% in DED	Up to 100% in oGVHD	Unsuitable endpoint, as currently unclear mechanism
	Corneal and conjunctival intravital staining	Mild to severe	Mostly severe	Due to higher severity different grading systems needed to assess treatment success using staining as endpoint
	Conjunctival redness	Mild to severe	Mostly severe	Secondary endpoint, detection and grading systems should be validated
	Fibrosis	Rare finding, associated with severe rosacea, atopic keratoconjunctivitis or ocular cicatricial pemphigoid	Frequent finding	Primary or secondary endpoint, detection and grading systems need to be validated
	Filamentary keratitis	Rare finding, only in severe cases, mostly Sjögren Syndrome	Common finding, presumably related to activation of innate immune system	Primary or secondary endpoint
	Superior bulbar and limbal keratoconjunctivitis	Rare finding, distinct entity not typically related to DED	Frequent finding	Secondary clinical endpoint
	Intraocular involvement	Not related to DED	Intraocular involvement reported	Secondary endpoint in subgroup analysis is possible
	Correlation between signs and symptoms	Low correlation: strong symptoms, weak clinical signs	Low correlation: weak symptoms, strong clinical signs	Development of suitable symptom questionnaires for oGVHD is necessary

pathways involving NETs [142] and extracellular DNA from the damaged tissue [175], activation of the endoplasmic reticulum stress pathway [180] and the tissue renin angiotensin system [181] synthesize an excessive amount of extracellular matrix, resulting in rapid interstitial inflammation and fibrosis [179,182,183]. The limited knowledge about key pathological mechanisms translates into the current lack of biomarkers for early diagnosis of oGVHD and the absence of effective topical and systemic anti-inflammatory, antifibrotic, and possibly preventive therapies.

Information from animal models and clinical analyses

Several animal models have been used to study biology, onset, time course, and therapies for oGVHD [177,184-189]. These models showed that donor derived T cells infiltrate the cornea and lacrimal glands and lead to an oGVHD phenotype [187,189] with subsequent fibrosis. Perez et al introduced a scoring system for murine models of oGVHD [184]. Several preclinical studies tested potential therapeutics such as siRNA [190], bromodomain inhibitors [191], rebamipide [192], vascular adhesion protein-1 [193], and a spleen tyrosine kinase inhibitor [194]. Because clinical signs

in oGVHD are also present in isolated forms in other ocular disease (e.g., conjunctival fibrosis in ocular cicatricial pemphigoid or chronic allergic keratoconjunctivitis), it may be necessary to use such models [195,196] as comparators in experimental studies to distinguish organ-specific chronic GVHD pathologies from secondary, damage-related disease.

Gaps, highest priorities, and roadmap for progress in oGVHD

Currently, no treatments have been specifically approved for treatment of oGVHD, in part because the clinical evolution of oGVHD is largely undefined and the innate and adaptive immune mechanisms that trigger and sustain oGVHD are incompletely understood. Furthermore, oGVHD clinical trials are challenging because of the lack of well-defined and specific primary efficacy outcome measures, and small sample sizes. Clinical metrics, such as the Schirmer's test or intravital staining of the ocular surface, that are established for diagnosing generic DED, should be amended with specific oGVHD metrics and defined for better application as clinical endpoints. Gaps in clinical management include uncertainty regarding whether to perform baseline examinations before HCT and then refer patients for scheduled reevaluations or for evaluations only as needed after HCT. Another uncertainty is whether to start oGVHD treatment with aggressive anti-inflammatory and immunosuppressive topical therapy followed by tapering based on improvement (step-down treatment) or to start with lubrication therapy followed by escalation based on progression or lack of improvement (step-up treatment).

HIGHEST PRIORITIES AND ROADMAP FOR PROGRESS IN CHRONIC OCULAR GVHD

1. Establish early diagnostic criteria (clinical signs and biomarkers) distinguishing oGVHD from other forms of DED so that appropriate interventions can be promptly instituted. This revision requires a better understanding of the immunopathology derived from appropriate animal models for oGVHD that mimic the human situation as closely as possible. These animal models should also be used to identify therapeutic targets and to enable preclinical testing of promising drug candidates and identification of functional connections between organ systems that are sequentially or simultaneously affected by chronic GVHD.

2. Identify biomarkers associated with active oGVHD at the earliest possible time points. As the eye is easily accessible, tear film or impression cytology can be tested. Besides cytokines and genetic markers, optical biomarkers may be useful, including optical coherence tomography or confocal microscopy that can be used noninvasively.

3. Develop and validate efficacy outcome measures that are specific for oGVHD clinical trials. Preferential primary outcome measures are corneal fluorescein staining and ocular discomfort measured by visual analog scale or ocular surface disease index until more appropriate measures that assess specific interventions (e.g., punctal plugs, contact lenses, serum eye drops, amniotic membrane transplantation) are identified. Such measures should distinguish ophthalmologist-driven tools from assessments that can be done in the hematologist's office. Clinical trial designs that circumvent the challenges imposed by limitations of small sample size are also needed (Figure 2).

4. Conduct eye-targeted studies, for example, (a) punctal occlusion or not; (b) referral as-needed for eye care versus prescheduled frequent follow ups; (c) step down (start treating aggressively then taper) versus step up (escalate treatment based on response).

5. Evaluate systemic treatment options for efficacy in oGVHD. Currently oGVHD is treated with topical interventions independently of other organ manifestations despite obvious similarities in the pathophysiology. A systematic analysis of ocular effects of systemic immunosuppression is needed.

OTHER MORBID CONDITIONS

Other conditions that are either part of NIH-defined chronic GVHD or occur in association with chronic GVHD require further research efforts. These include genital involvement, which occurs more frequently than reported in large registries due to the lack of routine screening [197], oral manifestations that impair quality of life and may increase the risk for secondary malignancies [198], isolated fasciitis [199], and wasting syndrome not explained by GI manifestations. Although these are NIH consensus-defined conditions, limited understanding of organ-specific pathophysiology prevents the development of targeted treatment approaches. Moreover, associated syndromes seen with chronic GVHD [200,201], such as polyserositis, which occurs infrequently but is difficult to treat [202], immune mediated cytopenias and renal complications (e.g., glomerulonephritis, nephrotic syndrome) require more study. All have in common the lack of knowledge of their incidence, their specific pathophysiology and relationship in the context of chronic GVHD.

In addition, other potential organs may also be targeted by chronic GVHD, but the exact relationship has not been established. For example, central nervous system dysfunction is reported by a significant percentage of long-term survivors mainly as cognitive dysfunction [203]. It remains to be established whether cognitive dysfunction is caused by cumulative neurotoxicity and acute GVHD, as demonstrated in experimental models and clinical investigations [204-206], or whether chronic GVHD further contributes. Rare cases of chronic GVHD with acute disseminated encephalomyelitis have been reported [161,207]. Similarly, peripheral nervous system dysfunction is seen in a high proportion of chronic GVHD patients [8,9,208], but a relationship to alloimmunity has not been established. Autonomic nervous system dysfunction with dry mouth or eyes, dry skin, obstipation, diarrhea, and sweating disturbances are of interest due to overlap with symptoms of chronic GVHD. For example, impaired sensitivity of the ocular surface has been reported after HCT [209]. Endothelial dysfunction could be part of the pathophysiology of chronic GVHD in a variety of organs based on experimental [210-212] and clinical evidence [213,214] and may contribute to long term cardiovascular morbidity and mortality [215,216] Therefore additional study is warranted.

OTHER KNOWLEDGE GAPS

An additional gap is the limited knowledge of the age-dependent disease features and associated morbidity and mortality of chronic GVHD in children and individuals older than 70 years, 2 populations that are especially vulnerable to comorbidity induced by chronic GVHD. Although chronic GVHD in children may be difficult to diagnose, chronic GVHD manifestations involving the lungs and eyes [119] and other sequelae may have significant life-long consequences [217-219]. In older patients, chronic GVHD has been associated with decreased physical functioning [220], but the detailed contributions of chronic GVHD to mortality and potential insights for prevention and treatment of chronic GVHD in older patients are unknown. Moreover, while preliminary data

indicate that racial and ethnic background are associated with long term outcomes including GVHD, large studies are lacking [114,116,221]. Finally, clinical care of morbid forms of chronic GVHD requires long-term care engagement of a multidisciplinary team [222,223]. Development and evaluation of survivorship care structures to provide access to multidisciplinary subspecialty care taking into account the socioeconomic and travel situations of individual patients remain an urgent research need [224].

STUDY DESIGN CONSIDERATIONS

Because of the rare incidence and limited prevalence of the highly morbid conditions, feasibility is a concern in clinical trials, and novel approaches to clinical investigation are needed [225-228]. Careful selection of endpoints that can reliably demonstrate objective clinically significant benefit with a realistic number of patients is critical. Studies should be designed with attention to sample size, statistical power, and control of bias. A detailed discussion of innovative trial designs is beyond the scope of this paper, but we offer the following recommendations.

1. Careful consideration of eligibility criteria utilizing enrichment strategies based on diagnostic criteria, phenotype, or biomarkers [229] may identify a smaller but more informative study population where a drug effect can be observed [230]. Patients not meeting the eligibility criteria may be treated in observational cohorts.
2. Some established chronic GVHD manifestations may be permanent, and a worthy goal could be "stable disease or improved trajectory" (reflecting prevention of new damage) or functional or symptom improvement instead of partial or complete response. These endpoints require acceptance that lack of worsening or improved patient functioning or patient-reported outcomes are meaningful clinical benefits in a given patient even if chronic GVHD organ function does not measurably improve. Lack of worsening can be documented in comparison to concurrent or historical controls [231] or the patient's prior trajectory if well documented in real time.
3. Although a nonrandomized single arm study, without concurrent controls, may seem attractive, this design is necessarily less precise, and outcomes are less definitive. Alternatives to consider include use of historical controls or using each patient as their own control. Single case experimental design or N-of-1 trials may be the most feasible option for the very rare highly morbid forms of chronic GVHD. In such trials, each individual participant serves as their own control, and may receive multiple interventions in a crossover fashion. Multiple N-of-1 studies may then be combined in a meta-analysis. Of note, efforts should be made to document the course and response using objective response measures focused on clinically meaningful changes.
4. Efficiency of study design should be optimized. The more complex designs are adaptive [232-234], with the design being modified during the conduct of the study according to pre-specified rules to increase efficiency. For example, a Bayesian approach [235] is a statistical inference framework for leveraging existing data from different sources, synthesizing evidence of different types, including retrospective data and information gained during the conduct of the study. In particular, the data deficits of "small" clinical trials can be mitigated by incorporating past information. The combination of observed data and prior opinion is governed by Bayes' theorem and can result in smaller sample sizes needed to reach conclusions. The major criticisms of the Bayesian approach are the uncertainty regarding the prior probability and the subjective interpretation of results since formal significance testing is not required, although this problem could be addressed by using independent or blinded assessors.

5. Optimize data analysis strategies, for example, by using more efficient continuous outcomes when the sample size is small. Consider longer duration of studies and use covariate adjustment, such as statistical stratification. Consider whether the distribution is likely to be parametric (modeled by a probability distribution that has a fixed set of parameters) or non-parametric when designing the analysis plan.
6. When multiple agents are available, consider efficient study designs to rank the agents and eliminate less-effective ones through futility or selection designs.
7. Selection of the primary endpoint depends on the mechanism of action and targeted manifestations (e.g., if an antifibrotic agent is tested to target sclerotic lesions, response of inflammatory manifestations may be captured only as a secondary endpoint). However, all systemic and topical agents given and all changes in organs should be recorded. Evaluation of agents given systemically, even if targeted to a single specific organ manifestation, requires documentation of all other organ manifestations since broader effects cannot be excluded. The same documentation of systemic immunosuppression is needed in studies that evaluate topical agents. Protocols should specify how non-study systemic and topical agents are handled and how responses in nontargeted organs are interpreted. In addition, efficacy measures developed for studies of comparable diseases other than chronic GVHD may also be evaluated in chronic GVHD [236]. Last but not least, predictive biomarkers indicating response to specific treatments should be developed and validated.
8. Efforts should be made to enhance access of children, older patients and racial and ethnic minorities to clinical trials since these characteristics are relevant covariables. Inclusion of pediatric cohorts into adult trials should be considered if feasible.
9. Rare manifestations (e.g., glomerulonephritis, restrictive lung disease) mimicking well-characterized immune-mediated diseases outside the transplantation setting may be potentially included in basket trials that include nontransplant patients, acknowledging potential variations in pathophysiology.

CONCLUSIONS

The need to identify approaches for effective treatment and prevention of highly morbid manifestations has emerged as one of the most important future goals in the field. During the next 3 years, identification of new diagnostic tools including biomarkers of all types and clinical risk factors will be crucial to prevent highly morbid complications. In the next 3 to 7 years, we expect that a better understanding of local tissue pathophysiology will lead to identification of therapeutic targets. Eventually, organ-specific therapeutic clinical studies will be necessary. Careful study design recognizing the small size of the eligible population and designating appropriate endpoints will increase the likelihood of informative results.

Pharmaceutica. S.P. holds intellectual property on Biomarkers and assay to detect chronic graft versus host disease

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