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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INTRODUCTION

Some forms of chronic graft-versus-host disease (cGVHD) are associated with significant morbidity in part due to their non-reversibility due to fibrosis and significant long term impact on quality of life (eye), physical functioning (sclerotic skin manifestations) and survival (lung, gastrointestinal)\(^1\). Progress in prevention of long term severe morbidity associated with cGVHD is limited by lack of biomarkers to predict a highly morbid course and absence of effective organ-specific approaches targeting “irreversible” sequelae. Moreover, treatment advances are limited by absence of effective and nontoxic therapy for highly morbid manifestations, and difficulty in conducting clinical trials due to disease heterogeneity and small patient numbers.

PURPOSE OF THIS DOCUMENT

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

SUMMARY OF RECOMMENDATIONS

1. Research should focus on phenotyping cGVHD clinically and biologically within cohort studies, in order to describe incidence, predictive factors, mechanisms of organ damage, and natural history of highly morbid conditions. Multicenter studies with common definitions and research sample collections are needed (Figure).
2. Develop new approaches for early identification and treatment of highly morbid forms of cGVHD, especially biologically targeted treatments, with a special focus on prevention and treatment of fibrotic changes.
3. Establish primary endpoints for clinical trials of each highly morbid manifestation in relationship to the time point of intervention (early versus late). Other endpoints, such as lack of progression and improvement in functioning or quality of life, may be realistic endpoints for clinical trials of highly morbid manifestations. Explore novel trial designs for small populations.

METHODS

Each working group was created to encourage global engagement in the topic. Groups worked individually to review the relevant literature and create the initial draft of the paper, which was reviewed and commented on by the Steering Committee. Two iterative rounds of comments from the Steering Committee were collected prior to 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 that is most frequently affected by cGVHD. While inflammatory disease manifestations characterized by superficial (erythematous or lichen planus-like) clinical presentations are often responsive to therapy, current management options for fibrotic disease remain limited.
Early sclerotic cGVHD (ScGVHD) is relatively rare but long-standing cGVHD is likely to advance to sclerosis, with 20% of patients having sclerosis after 3 years of cGVHD therapy with sclerosis prevalence exceeding 50% among those with severe cGVHD. ScGVHD can manifest as localized disease (morphelike), diffuse involvement, deep sclerosis, panniculitis, or fasciitis without additional epidermal manifestations. ScGVHD may cause joint contractures, vascular insufficiency, skin breakdown, neuropathy (including small fiber neuropathy, nerve compression syndrome and painful muscle cramping), myopathy via fascial compression and poor wound healing.

Pathophysiology:

Fibrosis represents the terminal step of an unchecked inflammatory alloreactivity cascade. The role of T cells in ScGVHD development is well defined and supported by defined genetic risk factors, but their role in an established sclerotic response is unknown. ScGVHD biopsy specimens demonstrate variable levels of CD4+ and CD8+ T cell infiltration with unknown clonal architecture; they may represent bystanders or effectors depending on biopsy timing. In systemic sclerosis (SSc), impaired function of regulatory T cells has been reported, and IL-2 treatment which expands regulatory T cells showed efficacy in advanced cGVHD. Humoral immunopathology, such as stimulating PDGF-receptor antibodies, could have a role in severe fibrotic forms of cGVHD, however, poor correlation of cGVHD severity, lack of damage of grafted donor skin, and limited response to PDGF-R inhibitors in patients with these antibodies argue against the broader relevance of this finding. A possible mechanistic B cell role in ScGVHD has been suggested with improvement of sclerosis after B cell depletion. Still, definitive evidence linking antibody-dependent mechanisms to human ScGVHD is lacking.

Recently, distinct dermal myeloid cell populations were identified in human skin. In animal models, macrophages contribute to development of fibrosis in both TGFβ-dependent and -independent fashion and their pathogenic role in cGVHD is increasingly recognized. Relevant for ScGVHD, myeloid-sourced TGFβ promotes fibrosis through positive regulation of fibroblast proliferation and differentiation into myofibroblasts and stimulation of extracellular matrix overproduction. In addition, macrophage-derived TGFβ promotes epithelial mesenchymal transition (EMT) in models of lung fibrosis. Partial EMT is involved in normal wound healing, though its disruption in the inflammatory environment can promote pathologic fibrosis in lung and skin. While fibroblasts represent critical mediators of fibrotic tissue injury, little is known about their homeostasis during cGVHD.

TGFβ is a keystone pathway in many fibrotic disorders, and has a documented role in preclinical ScGVHD. In patients, higher TGFβ levels are associated with adverse outcomes taking into account the challenges to correlating expression and activity. However, TGFβ is temporally restricted and has pleiotropic roles in different compartments and its use of distinct downstream signaling pathways makes it a challenging therapeutic target. Type I interferon (IFN) responses feature prominently in SSc skin fibrosis and ScGVHD as well, tightly linking adaptive and innate immune cross-talk in initiation and persistence of ScGVHD, with possible therapeutic implications.

Developmental (morphogen) pathways, particularly Hedgehog, Wnt, and Notch, are involved in fibrotic disorders. These pathways, commonly influenced by TGFβ and highly crosslinked, often create a feed-forward loop promoting aberrant tissue remodeling. 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.\(^{33}\) Hedgehog inhibitors, have been tested in cGVHD with some efficacy, though hindered by significant toxicities.\(^{34,35}\) Recent data in ScGVHD suggested the immunomodulatory role of morphogen pathways with broad effects on adaptive immunity promoting cGVHD\(^{34,36}\), thus providing an added impetus for clinical translation. The endocannabinoid system is involved in multiple inflammatory and fibrotic disorders, with opposing role for signaling through cannabinoid receptor 1 (CB\(_1\)R; profibrogenic) and cannabinoid receptor 2 (CB\(_2\)R; antifibrotic/anti-inflammatory) with agents already in clinical trials.\(^{37,38,39}\)

**Gaps in knowledge and unmet need; highest priorities**

The pivotal role of immune injury in the initial steps of fibrosis is well-accepted. However, the time when pathogenesis shifts 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 and minimal immunosuppression with all its associated risks. 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 findings. Optimized pre-clinical ex vivo approaches could be well suited for the latter (e.g. to evaluate the effect of TGF\(\beta\) and TGF\(\beta\) pathway inhibitors on sclerotic skin fibroblasts). Deeper interrogation should use -omics methods and novel tissue diagnosis 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. A clinical challenge is to separate direct immunological effects on skin, fascia, and nerves from indirect (compression) and other causative factors taking into account, that nerves may also be a potential target of cGVHD outside skin and fascial involvement (i.e. toxicity of the prior treatment, nutritional and electrolyte deficits among others).\(^{40,41}\)

Clinical trials need more robust and sensitive endpoints. It is particularly challenging to precisely quantify the evolution and the extent of deep-seated (subcutaneous/fascial) disease to assess disease response and the current organ-based grading system is poorly suited to detect responses in established sclerosis. Given this limitation, ScGVHD responses could be considered functional improvement (e.g. improved joint mobility documented by P-ROM and physician global and skin/joint tightening scale per the 2014 NIH Consensus), even if skin-specific scoring remains unchanged. Data supporting such an approach already emerged since the 2014 consensus\(^ {42}\) and the bedside validation in ScGVHD should be actively pursued. Imaging biomarkers that have been suggested include high-frequency ultrasound and magnetic resonance imaging, but rapid, safe, less costly and accessible clinical assessment tools are needed (Table 1).\(^ {43,44}\) 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.\(^ {45-48}\)

Translation of knowledge accrued from organ fibrosis (e.g. SSc and idiopathic pulmonary fibrosis) to ScGVHD should be accelerated. Some agents have already demonstrated promise in cGVHD (e.g. belenosudil, a ROCK2 inhibitor\(^ {49}\)), while many others remain unexplored (e.g. connective tissue growth factor (CTGF)- or cannabinoid receptor-directed therapies) (Table 2). Theoretically, avoiding unnecessary immunosuppression and side effects is possible with topical delivery methods, but most are
formulated for effectiveness against superficial skin conditions affecting the epidermis and papillary dermis and effective topical delivery in ScGVHD may be hampered by increased dermal thickness. Strategies to improve drug delivery include physical approaches (microneedles, laser, iontophoresis), particle-based drug carriers (lipid-based, nanoparticles) and chemical approaches (permeation modifiers, prodrugs). Precision medicine with immune effector cell therapies targeting fibrosis have been explored in other diseases, and could be considered in ScGVHD. Multi-targeting approaches may be helpful to prevent evolution to sclerosis and to enhance safety without compromising efficacy.

**Highest Priorities and Roadmap for progress for ScGVHD**

1. Longitudinal multicenter studies to test pathologic cell populations in lesional skin and peripheral blood, and cytokine and chemokine responses, to identify additional target pathways.

2. Capitalize on the enhanced resolution of next generation sequencing strategies, including single-cell RNA-, ATAC-, TCR-, and BCR-seq to query skin biopsies to provide biological insight into the individual mediators of ScGVHD, address the degree of temporal and clinical disease heterogeneity, and the origins (recipient versus donor) and phenotype of expanded and/or clonally expanded T cell and B cell populations. These investigations could be complemented by new techniques like MIBI-TOF combined with non-linear dimensionality reduction analysis approaches (tSNE/viSNE).

3. Efforts should center on molecular (transcriptional and epigenetic) definition of ScGVHD disease heterogeneity, where single-cell -omics offer promise of identifying potent prognostic and predictive biomarkers and therapeutic targets.

4. Analyze differences in mediators and targets (epidermal versus dermal structures, fascia, nerves) to permit personalized interventions.

5. 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.

6. Develop novel 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 cGVHD, with an incidence of 3-10% of allogeneic hematopoietic cell transplant recipients (HCT), and 14% in those with cGVHD. Although the histologic entity of obliterative bronchiolitis is the diagnostic lesion of lung GVHD, clinical diagnosis is largely based on pulmonary function studies which are difficult to perform in children under age 7. Risk factors for onset include antecedent respiratory viral infections and impaired lung function early post-transplant. Worse prognosis is associated with early onset after transplantation and severe FEV1 impairment at diagnosis. Contemporary series
show 2-year survival rate of 70% after BOS diagnosis but 5-year survival remains low at approximately 50%, highlighting the need for novel prevention and treatment strategies.

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 exact mechanisms by which lung epithelial cell injury alters immune and fibrotic responses to contribute to obliterative bronchiolitis after HCT. Mechanisms being explored in other disease contexts include airway stem cell depletion and acquisition of a persistent inflammatory airway epithelial cell phenotype. 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 interleukin-17 and interleukin-8. In one murine model, alternatively activated macrophages drove BOS, supported clinically by evidence of leukotriene production, and polarized CD4 immune activation. In another preclinical model, donor B-cells contribute to airway pathology through local alloantibody production. Disruption of germinal center formation, which is supported by T follicular helper cells, reduced pulmonary dysfunction. These mechanistic insights have not yet been confirmed in humans although biomarker studies support a prominent role of B cells with significantly elevated CD21low B cells and high sBAFF levels. The role of the microbiome, as suggested in other airway diseases needs to be investigated.

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 are unlikely to reflect the full spectrum of physiologic and histologic manifestations of BOS. A concerning pattern is reduced FEV1 and FVC with normal FEV1/FVC ratio, likely reflecting “pseudo-restriction” due to small airway obstruction. An open question remains whether lymphocytic bronchiolitis, which is responsive to anti-inflammatory agents, represents an early phase of disease or a distinct subtype of BOS. While some patients demonstrate stability of FEV1 after clinical recognition, this plateau could be due to treatment, a distinct biology, or the stage of the disease at diagnosis. More significantly, the clinical and biological risk factors for persistent refractory lung function decline are not known.

The association of cGVHD with restrictive lung impairment remains ill-defined for HCT survivors, and it is not currently recognized as a cGVHD manifestation. Restrictive allograft syndrome (RAS) is a phenotype of chronic lung allograft dysfunction (CLAD) in lung transplantation recipients, and is defined by a reduction in forced vital capacity or total lung capacity (TLC) with persistent lung infiltrates and carries a worse prognosis than classic BOS. While 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 cGVHD have been barriers to recognition. Restriction may be due to known interstitial lung disease entities including organizing pneumonia or extraparenchymal processes including trunical sclerosis, respiratory muscle weakness, or pleural effusions. Nevertheless, histological studies of BOS in HCT demonstrate concomitant bronchiolar lesions and interstitial fibrosis, suggesting that interstitial abnormalities, in addition to airway pathology, are part of the spectrum of lung cGVHD. Table
3 depicts the spectrum of lung abnormalities after HCT including diagnostic criteria and association with cGVHD.

**Treatment**

Treatment for BOS is aimed at stabilizing lung function, as there are no established therapies that reverse the underlying pathologic lesion of BOS. The combination of inhaled corticosteroids (fluticasone), azithromycin and montelukast (FAM), with or without a long-acting bronchodilator, has been established as organ-specific therapy for BOS\(^8^0,\)\(^8^1\) accompanied by systemic corticosteroids taking into account a potential impaired graft-versus-leukemia effect associated with azithromycin as reported in a prophylaxis study\(^8^2\). However, a significant proportion of BOS patients continue to decline despite these treatments\(^8^3\). Few effective options are available, and intensified immunosuppression contributes to lung infections, which in turn, worsen lung function. Agents that are under investigation or have shown utility in other chronic lung conditions including topical immunosuppressants\(^8^4\) and antiinflammatory and antifibrotic agents currently in use for pulmonary fibrosis\(^8^5\).

**Highest priorities and roadmap for progress in pulmonary cGVHD**

Our ability to prevent and treat lung manifestations of cGVHD remains hampered by an incomplete understanding of disease pathogenesis and natural history, owing in part to the relative rarity of BOS. Research priorities include the following:

1. **Pathogenesis.** The creation of a shared lung-specific biorepository to support biomarker discovery and mechanistic studies. Given the inherent challenges of procuring surgical lung tissue, universal protocols need to 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, or developing validated serum or plasma based assays should be utilized\(^8^6\). Coupling these samples with carefully annotated clinical databases will be critical. (Figure 2)

2. **Subtypes.** A longitudinal multicenter patient cohort followed from the time of cGVHD onset would allow for the comprehensive clinical phenotyping, classification and epidemiology of lung GVHD subtypes. Data to be collected include clinical disease history, pulmonary function tests, infections, chest computed tomography \(^8^6,\)\(^8^7\), and lung histology. Quantitative lung imaging techniques, i.e., parametric response mapping, may play an important role in delineating phenotypes.

3. **Treatment.** Targeted anti-inflammatory agents and antifibrotics are potential therapies and should be tested before severe BOS forms develop. Treatment trials must be informed by knowledge of natural progression and an understanding of pathogenesis and biomarkers of response. Clinically relevant endpoints include FEV1 stability (or lack of progression of FEV1 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 cGVHD. The 2014 NIH organ scoring of cGVHD does not distinguish between the site of gastrointestinal (GI) involvement (esophagus, upper GI, and lower GI). However, the NIH 2014 response criteria do distinguish between reported symptoms...
in these three areas\textsuperscript{88}. Incidence of esophageal, upper GI, and lower GI involvement is, respectively, 16%, 20%, and 13%, according to analysis from the cGVHD Consortium\textsuperscript{89}. Most importantly, intestinal involvement is associated with greater risk of non-relapse mortality\textsuperscript{88,90,91}.

Risks factors for intestinal involvement in cGVHD remain to be elucidated. Ethnicity, genetic diversity, environmental differences, diet, antibiotic use, supportive care or microbiota or microbe-derived metabolites may all influence GI-cGVHD\textsuperscript{92-96}. Age is a potential risk factor since children appear particularly susceptible to late GI-acute GVHD (aGVHD) affecting up to 24.7% of pediatric transplant recipients\textsuperscript{97} with subsequent GI overlap symptoms at time of cGVHD diagnosis. Loss of microbial diversity with predominant expansion of specific bacteria persisted for up to 1 year after HCT independent of onset of cGVHD\textsuperscript{95}. In contrast, a small study showed that increased relative abundance of butyrogenic bacteria after the onset of aGVHD was associated with subsequent steroid-refractory aGVHD or cGVHD\textsuperscript{96} indicating the need for further investigations on the association of dysbiosis, antibiotic strategies and GI-cGVHD\textsuperscript{95}.

**Pathophysiology**

Chronic GVHD is characterized by atrophy/destruction of tissues with subsequent fibrosis. However, intestinal fibrosis is rare in cGVHD\textsuperscript{98,99}. Intestinal epithelium is the most rapidly self-renewing tissue in adults; intestinal epithelial cells are continuously regenerated from intestinal stem cells (ISCs), which are key to the regeneration of damaged intestinal epithelium\textsuperscript{100}. There are three types of epithelial cells: squamous, columnar, and cuboidal. It seems that tissues having squamous epithelium such as esophagus, mouth, and vagina, as well as those having cuboidal epithelium such as sweat glands and salivary glands are more prone to dysregulated fibrosis in cGVHD than those having columnar epithelium such as stomach, intestine, and trachea. Animal studies showed that both ISCs and their niche Paneth cells are targeted in aGVHD, resulting in impaired regeneration of the injured epithelium\textsuperscript{101-105}. The rapid and potent repair ability of the intestine may protect from early fibrotic processes that often accompany repair processes in other tissues. Profiling of immune cell populations and plasma markers at day 100 after HCT demonstrates biological differences between cGVHD and late-onset aGVHD\textsuperscript{106}.

**Highest priorities and roadmap for progress in gastrointestinal cGVHD**

1. Enforcement of the NIH 2014 terminology (acute versus chronic GVHD with overlap subtype of cGVHD) within and across studies\textsuperscript{107-113} since current natural history trials as well as clinical trials revealed a significant number of wrongly labeled patients\textsuperscript{97}. Electronic tools like the GVHD App may assist\textsuperscript{114}. The severity of individual GI manifestations should be recorded applying the response criteria not only at the time of diagnostic onset, but over time and in response to therapeutic strategies.

2. Generate experimental models able to address the role of dysbiosis, intestinal inflammation and subsequent cGVHD including other organ manifestations.

3. Collect blood and stool samples in either natural history cohorts or interventional clinical trials to allow study of human GI-cGVHD which includes metabolome and microbiome analyses including sufficient sampling and follow up of aGVHD trials.
OCULAR INVOLVEMENT

Current clinical knowledge

Ocular cGVHD (oGVHD) is one of the most frequent, rapidly-progressive organ manifestestion with characteristic inflammatory, immune dysregulatory and fibrotic manifestations. OGVHD is usually diagnosed between 5-24 months after HCT, and it can severely impact quality of life and quality of vision due to severe symptoms such as burning, dryness, and loss of visual function. Preexisting dry-eye and Meibomian gland disease as a consequence of chemotherapies or possibly irradiation increases the risk for later oGVHD. Early after transplantation, some patients already have a decrease of tear quantity and quality, yet eye involvement is only recognized once damage exceeds the eye’s ability to compensate. Most importantly, oGVHD is not another form of dry-eye disease (DED), and approaches and therapies for DED may fail in oGVHD. Table 4 summarizes the differences between DED and oGVHD.

OGVHD 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. In addition, a few reports have described intraocular involvement including choroid and retina. However, there are currently no specific signs that are diagnostic for oGVHD, although certain combinations of findings, such as conjunctival subepithelial scarring and superior bulbar and limbal keratoconjunctivitis are commonly seen. Without early diagnosis and appropriate treatment oGVHD progresses towards loss of visual function by complete loss of aqueous tear production 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 under these conditions of presumably increased rejection and impaired tear production, eventually resulting in loss of the eye.

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

Pathophysiology

Conditioning chemotherapy, radiation and infection precede the onset of oGVHD and may induce homing signals for mobilization and migration of circulating bone marrow cells including hematopoietic stem 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 mechanisms initiate oGVHD. Studies show increased levels of ICAM-1, IL-1β, IL-6, IL-8, neutrophil extracellular traps (NETs), extracellular DNA, and decreased level of lactoferrin, DNAse, IL-7 and EGF in the tear film. In lacrimal glands affected by oGVHD, early fibrosis and myxedematous tissue may herald a rapidly progressive fibrosis with activated fibroblasts already infiltrating into the lacrimal gland. Stromal fibroblasts in the lacrimal gland and conjunctiva
promote the functional interaction between pathogenic T cells and antigen presenting cells (APCs) including macrophages\textsuperscript{117, 149}. The functional interaction between CD4+ T cells and fibroblasts and senescent macrophages might result in the proliferation and activation of fibroblasts through cell–cell contact and T cell–derived soluble fibrogenic factors, such as IL-4, IL-6, and IL-17\textsuperscript{150, 151}. Activated macrophages and fibroblasts through both classical immunological pathway and sterile inflammatory pathway including presence of NETs\textsuperscript{116} and extracellular DNA from the damaged tissue\textsuperscript{147}, activation of endoplasmic reticulum stress pathway\textsuperscript{152} and tissue renin angiotensin system\textsuperscript{153} synthesize an excessive amount of extracellular matrix, resulting in rapid interstitial inflammation and fibrosis\textsuperscript{151, 154, 155}.

\textit{Information from animal models and clinical analyses}

Several animal models have been used to study biology, onset, time course, and therapies for oGVHD\textsuperscript{156-161 149, 156-161}. These models showed that T cells infiltrating the cornea and lacrimal glands derive from donor animals and lead to an oGVHD phenotype\textsuperscript{159 161} with subsequent fibrosis. Perez et al introduced a scoring system for murine models of oGVHD\textsuperscript{156}. Several preclinical studies tested potential therapeutics such as siRNA\textsuperscript{162}, bromodomain inhibitors\textsuperscript{163}, Rebamipide\textsuperscript{164} and VAP-1\textsuperscript{165} and a SYK inhibitor\textsuperscript{166}. As clinical signs in oGVHD are also present in isolated forms in other ocular disease, e.g. conjunctival fibrosis in ocular cicatricial pemphigoid (OCP) or chronic allergic keratoconjunctivitis, it may be necessary to use such models\textsuperscript{167, 168} as comparators in experimental studies to distinguish organ-specific cGVHD pathologies from secondary, damage-related disease.

\textit{Gaps, highest priorities and roadmap for progress in oGVHD}

Currently, there are no treatments specifically approved for oGVHD. This may be in part because the natural history of oGVHD is largely unknown and the innate and adaptive immune mechanisms that trigger and sustain oGVHD are incompletely understood. Furthermore, oGVHD clinical trials are challenging because of lack of well-defined and specific primary efficacy outcome measures, and small sample size. Gaps in clinical management include uncertainty whether to refer patients post-HCT ‘as-needed’ for eye care or have ‘pre-scheduled’ frequent follow ups, and whether to start treating oGVHD with aggressive anti-inflammatory and immunosuppressive topical therapy then taper based on reduction in signs (step-down treatment) or start treating with lubrication therapy and escalate treatment based on continued symptoms (step-up treatment).

\textit{Highest priorities and roadmap for progress in ocular cGVHD}

1. Establish early diagnostic criteria (clinical signs and/or biomarker) separating oGVHD from other forms of DED so that appropriate interventions can be promptly instituted. This revision requires a better understanding of the immunopathology using 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 for pre-clinical testing of promising drug candidates and studies of functional connections between organ-systems that are sequentially or simultaneously affected by cGVHD.

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

3. Develop efficacy outcome measures that can be used in oGVHD-specific clinical trials to assess response to specific interventions (punctal plugs, corneal lenses). Such measures need to distinguish ophthalmologist-driven tools from those assessments which can be done in the hematologist-oncologist office. Given the known divergence between signs and symptoms in oGVHD, validated patient-reported measures may also be appropriate primary endpoints.

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

5. Evaluate systemic treatment options with regard to 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 which are either part of NIH-defined cGVHD or occur in association with cGVHD require further research efforts. These include genital involvement which is significantly more common than reported in large registries due to the lack of routine screening, oral manifestations which impair QoL and may increase the risk for secondary malignancies, isolated fasciitis, 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 cGVHD, like polyserositis which is infrequent but difficult to treat, immune mediated cytopenias and renal complications (glomerulonephritis, nephrotic syndrome) require more study. All have in common the lack of knowledge of the incidence, their specific pathophysiology and relationship in the context of cGVHD.

In addition, other potential organs may also be targeted by cGVHD 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. It remains to be established whether cognitive dysfunction is caused by cumulative neurotoxicity and acute GVHD, as demonstrated in experimental models and clinical investigations, or whether cGVHD further contributes. Rare cases of cGVHD with acute disseminated encephalomyelitis (ADEM) have been reported. Similarly, peripheral nervous system dysfunction is prevalent in a high proportion of cGVHD patients but the 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 cGVHD. For example, impaired sensitivity of the ocular surface has been reported after HCT. Endothelial dysfunction could be part of the pathophysiology of cGVHD in a variety of organs based on experimental and clinically evidence. It may contribute to long term cardiovascular morbidity and mortality and additional study is warranted.
Study design considerations

Due to the rare incidence and prevalence of the highly morbid conditions, feasibility is a concern, and novel approaches to clinical investigation are needed. Careful selection of endpoints that can demonstrate benefit with a reasonable number of patients is critical since underpowered studies do not advance the field. Studies need to 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 the following recommendations are offered:

1. Careful consideration of eligibility criteria utilizing enrichment strategies may identify a smaller but more informative study population where a drug effect can be observed.

2. Some established cGVHD manifestations may be permanent and a worthy goal could be “stable disease/improved trajectory” or functional or symptom improvement instead of partial or complete remission. These endpoints require acceptance that lack of worsening and/or improved patient functioning/patient-reported outcomes are meaningful clinical benefits even if cGVHD organ function does not improve. Lack of worsening can be documented in comparison to concurrent or historical controls or the patient’s prior trajectory.

3. While a non-randomized single arm study, without concurrent controls, may seem attractive, this design is necessarily less precise, and outcomes less definitive. Alternatives to consider include use of historical controls or utilizing each patient as their own control. Single case experimental design (SCED) or N-of-1 trials may be the most feasible option for the very rare highly morbid forms of cGVHD. 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.

4. Efficiency of study design should be optimized. The more complex designs are adaptive, with the design being modified according to pre-specified rules during the conduct of the study to increase efficiency. For example, a Bayesian approach 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 criticism of the Bayesian approach is subjectivity.

5. Optimize data analysis strategies, for example, continuous outcomes are more efficient when the sample size is small; consider longer studies; and use covariate adjustment, such as statistical stratification. Consider if 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.

CONCLUSIONS

While cGVHD treatment in the past was applied in a one fits all fashion and initiated after moderate symptoms started, this approach does not recognize that some manifestations disproportionately cause morbidity and mortality. Prevention of the highly morbid manifestations has emerged as one of the...
most important goals for the next few years. 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 3-7 years, a better understanding of local tissue pathophysiology will lead to
therapeutic targets. Eventually, organ-specific therapeutic clinical studies will be necessary and choice of
endpoints and careful study design, recognizing the small eligible population, can increase the chance of
a successful trials.
REFERENCES


46. Lafyatis R, Mantero JC, Gordon J, Kishore N, Carns M, Dittrich H et al. Inhibition of beta-Catenin Signaling in the Skin Rescues Cutaneous Adipogenesis in Systemic Sclerosis: A Randomized,


940

944

948 10.1038/nature06196

949

954 10.1084/jem.20101559

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Poe JC, Jia W, Di Paolo JA, Reyes NJ, Kim JY, Su H et al. SYK inhibitor entospletinib prevents ocular and skin GVHD in mice. JCI Insight 2018; 3(19). e-pub ahead of print 2018/10/05; doi: 10.1172/jci.insight.122430

Ahadome SD, Abraham DJ, Rayapureddi S, Saw VP, Saban DR, Calder VL et al. Aldehyde dehydrogenase inhibition blocks mucosal fibrosis in human and mouse ocular scarring. JCI Insight 2016; 1(12): e87001. e-pub ahead of print 2016/10/05; doi: 10.1172/jci.insight.87001


1264 1265 1266 1267 1268 1269 1270 1271 1272 1273 1274 1275 1276 1277 1278 1279 1280 1281 1282 1283 1284 1285 1286 1287 1288 1289 1290 1291 1292 1293 1294 1295 1296 1297 1298 1299 1300 1301


203. Chen F, Wang L, Vain A, Ssempijja Y, Dellalana L, Zhang K et al. Interobserver Reproducibility of the Myoton and Durometer Devices to Measure Skin Stiffness and Hardness in Chronic


Table 1: Potential objective assessment tools to assess skin sclerosis (ScGVHD) in chronic GVHD

<table>
<thead>
<tr>
<th>Modality</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Use in ScGVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-frequency ultrasound, acoustic radiation force impulse (ARFI), shear wave elasticity imaging (SWEI), ultrasound surface wave elastography (USWE)</td>
<td>Bedside use, easy to assess multiple sites, allows rapid comparability to previous images</td>
<td>Cost, requires training, requires marking of target area for repeat assessment; edema from active inflammation may confound imaging</td>
<td>CS²⁰⁰ CS⁴⁴ CS²⁰¹ CS²⁰²</td>
</tr>
<tr>
<td>Durometer</td>
<td>Bedside use, affordable, small, hand-held device, easy to use, provides numerical readout</td>
<td>‘Anvil effect’ from underlying bony structures, less sensitive for deep-seated disease; reproducibility requires careful experimental technique</td>
<td>CS²⁰³</td>
</tr>
<tr>
<td>Magnetic resonance imaging (MRI), MRI/Positron emission tomography (PET)</td>
<td>Detection of deep-seated, sub-clinical involvement; useful for detecting active fascial inflammation; does not require marking of target area</td>
<td>Cost, inconvenient for patients, unclear if responsive to small improvements in fibrosis</td>
<td>CS⁴⁴ CS²⁰⁴ CS²⁰⁵</td>
</tr>
<tr>
<td>Optical coherence tomography/elastography</td>
<td>High-resolution imaging, including capability to assess local blood flow</td>
<td>Limited depth of penetration</td>
<td>CR²⁰⁶</td>
</tr>
<tr>
<td>Laser doppler flowmetry</td>
<td>2D flow map of skin perfusion; can assess dynamic changes; monitoring potential for compromised acral sites of ScGVHD</td>
<td>Affected by ambient temperature; movement, pressure or other contact with skin will influence perfusion</td>
<td>CR²⁰⁷</td>
</tr>
<tr>
<td>Suction probe (Cutometer®, Dermaflex®, Nimble)</td>
<td>Devices measure stiffness and elasticity; have been used in clinical assessment of morphea and systemic sclerosis</td>
<td>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</td>
<td>CS²⁰⁰</td>
</tr>
<tr>
<td>Myoton®</td>
<td>Hand-held device, detects changes in tissue oscillation (skin stiffness and other properties) after a mechanical impulse</td>
<td>Requires adherence to measurement protocols and knowledge of muscular anatomy. Results depend on underlying muscle tone, patient positioning</td>
<td>CS²⁰⁸ CS²⁰⁹ CS²⁰³</td>
</tr>
</tbody>
</table>

CR: case report, CS: case series
<table>
<thead>
<tr>
<th>Target</th>
<th>Drug(s)</th>
<th>Target cellular subsets</th>
<th>Clinical Development Status</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTGF/CTN2</td>
<td>Pamrevlumab (FG-3019)</td>
<td>Fibroblasts</td>
<td>Ph-3- IPF (NCT01890265)</td>
<td>210, 211</td>
</tr>
<tr>
<td>Autotaxin</td>
<td>Ziritaxestat (GLPG-1690)</td>
<td>Fibroblasts</td>
<td>Ph-3- IPF (NCT03733444, NCT03711162)</td>
<td>212</td>
</tr>
<tr>
<td>HSP47</td>
<td>ND-L02-s0201</td>
<td>Fibroblasts</td>
<td>Ph-2- IPF (NCT03538301)</td>
<td>50, 213, 214</td>
</tr>
<tr>
<td>Pentraxin 2 (agonist)</td>
<td>PRM-151</td>
<td>Fibroblasts Macrophages</td>
<td>Ph-2- IPF (NCT02550873)</td>
<td>215, 216</td>
</tr>
<tr>
<td>CB2R (agonist)</td>
<td>Lenabasum (Ajulemic acid)</td>
<td>Fibroblasts T cells Macrophages</td>
<td>Ph-3- Systemic Sclerosis (NCT03398837)</td>
<td>217</td>
</tr>
<tr>
<td>CB2R /PPARγ (Dual Agonist)</td>
<td>EHP-101</td>
<td>Fibroblasts Endothelial cells Macrophages</td>
<td>Ph-2- Systemic Sclerosis (NCT04166552)</td>
<td>218</td>
</tr>
<tr>
<td>CB2R /iNOS (dual antagonist)</td>
<td>MRI-1867</td>
<td>Fibroblasts T cells Macrophages</td>
<td>Ph-1</td>
<td>38, 219, 220</td>
</tr>
<tr>
<td>Oncostatin M (agonist)</td>
<td>GSK2330811</td>
<td>Fibroblasts Endothelial cells T cells Macrophages</td>
<td>Ph-2- Systemic Sclerosis (NCT03041025)</td>
<td>221, 222</td>
</tr>
<tr>
<td>TGFβ</td>
<td>AVID200</td>
<td>Fibroblasts T cells Macrophages</td>
<td>Ph-1- Myelofibrosis (NCT03895112) Ph-1- Systemic Sclerosis (NCT03831438)</td>
<td>223</td>
</tr>
<tr>
<td>IL-6R</td>
<td>Tocilizumab</td>
<td>Fibroblasts T cells Macrophages</td>
<td>Ph-3- Systemic Sclerosis (NCT02453256) Ph-2- Steroid dependent immune related adverse events (NCT04375228)</td>
<td>224-226</td>
</tr>
<tr>
<td>CSF-1R</td>
<td>Axatilimab (SNDX-6352)</td>
<td>Macrophages</td>
<td>Ph-2- cGVHD (NCT03604692)</td>
<td>19</td>
</tr>
<tr>
<td>ROCK2</td>
<td>Belumosudil (KD025)</td>
<td>T cells Macrophages</td>
<td>Ph-2- cGVHD (NCT03640481, NCT02841995) Ph-2- Systemic Sclerosis (NCT03919799)</td>
<td>227</td>
</tr>
<tr>
<td>Interferon receptor type 1</td>
<td>Anifrolumab</td>
<td>T cells Macrophages</td>
<td>Ph-3- Systemic Lupus Erythematosus (NCT02446899) Ph-2- Rheumatoid Arthritis (NCT03435601)</td>
<td>228</td>
</tr>
</tbody>
</table>
### Table 3: Pulmonary syndromes following allogeneic hematopoietic cell transplantation

<table>
<thead>
<tr>
<th>Entity</th>
<th>Established in definition of lung GVHD</th>
<th>PFT pattern</th>
<th>High Resolution Chest CT Findings</th>
<th>Lung Histology</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchiolitis obliterans Syndrome</td>
<td>Yes</td>
<td>Fixed obstructive pattern: FEV1 decline &gt;10%, FEV1/VC &lt; LLN. Elevated residual volume or Residual volume/Total Lung Capacity. FEV1/FVC &gt; LLN and preserved TLC may be seen. DLCO may be normal or reduced.</td>
<td>Signs of airtrapping (mosaic attenuation on expiratory phase) or bronchiolitis (centrilobular ground glass opacities or micronodules) and/or late sequelae (traction bronchiectasis, bronchial wall thickening)</td>
<td>Obliterative bronchiolitis (OB): partial or complete fibroproliferative occlusion of terminal small bronchioles, lymphocytic bronchiolitis may also be seen</td>
<td>There are subtypes of BOS based on timeframe after HCT, initial tempo of onset, FEV1 decline, histology, response to therapy, and prognosis.</td>
</tr>
</tbody>
</table>

**Restrictive impairment due to Interstitial Lung Disease (ILD) Entities:** Multiple entities, as per the ATS/ERS classification of ILD may occur after HCT, beyond what is listed here. If restrictive impairment is seen on PFT (ie reduced FVC with preserved FEV1/FVC and reduced TLC), high resolution chest CT should be performed to evaluate for ILD and other entities.

<table>
<thead>
<tr>
<th>Entity</th>
<th>Established in definition of lung GVHD</th>
<th>PFT pattern</th>
<th>High Resolution Chest CT Findings</th>
<th>Lung Histology</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organizing pneumonia</td>
<td>No, however there is evidence for association with aGVHD and cGVHD.</td>
<td>Restrictive impairment with reduced TLC with FEV1/FVC &gt; LLN most common. Obstructive or mixed pattern may be seen. Reduced DLCO.</td>
<td>Patchy and peribronchilar or consolidation, and reticular ground glass opacities, often predominant in upper lobes and periphery</td>
<td>Bronchiolar and alveolar granulation tissue</td>
<td>Bronchoscopy should be performed to rule out infection. Clinical diagnosis often made without lung histology and is empirically based on steroid-responsiveness</td>
</tr>
<tr>
<td>Non-specific interstitial pneumonia</td>
<td>No</td>
<td>Reduced TLC and DLCO</td>
<td>Confluent bilateral lower lobe ground glass opacities, bronchiectasis and lower lobe volumes loss, classically sparing the subpleural area</td>
<td>Diffuse alveolar wall thickening by uniform fibrosis; interstitial inflammation</td>
<td>Bronchoscopy should be performed to rule out infection</td>
</tr>
<tr>
<td>Pleuroparenchymal pulmonary fibroelastosis</td>
<td>No</td>
<td>Reduced TLC and DLCO, occasionally obstructive and restrictive pattern. Progressive and severe impairment over time</td>
<td>Upper lobe fibrosis with subpleural and pleural thickening, loss of lung volume, and lower lobe traction bronchiectasis</td>
<td>Subpleural and pleural fibroelastogenic proliferation with minimal inflammation</td>
<td>Diagnosis is usually made by typical chest CT findings</td>
</tr>
</tbody>
</table>

**Restrictive Impairment not Attributed to ILD:** These entities are secondary to extrathoracic consequences of cGVHD
<table>
<thead>
<tr>
<th>Truncal sclerosis</th>
<th>No. Sclerosis due to cGVHD is an indirect cause of ventilatory impairment</th>
<th>Reduced TLC; RV/TLC may be elevated but usually does not necessarily indicate small airways disease</th>
<th>No parenchymal infiltrates. Parametric response mapping shows low inspiratory volumes.</th>
<th>N/A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory muscle weakness</td>
<td>No. This may be the consequence of cGVHD-related myositis or prolonged steroid use to treat cGVHD.</td>
<td>Concomitant reduction in FVC and FEV1, reduced TLC with relative sparing of RV. Reduced supine FVC. Maximal inspiratory and expiratory pressures may be reduced.</td>
<td>Low lung volumes, normal parenchyma. If diaphragmatic weakness or paralysis is suspected, a fluorographic sniff test may show reduced diaphragmatic excursion</td>
<td>N/A. Evidence of myositis in a peripheral muscle.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Restrictive allograft syndrome (RAS) has been defined for lung transplantation (LT)\(^7\) as a manifestation of chronic allograft dysfunction. BOS is the obstructive form of CLAD in lung transplantation. RAS after LT 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 cGVHD 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 cGVHD could be considered as an “RAS-like” condition, or “restrictive alloimmune syndrome” after HCT.*
Table 4: Differences between ocular chronic graft-vs.-host disease (oGVHD) and dry eye disease

<table>
<thead>
<tr>
<th>Cause</th>
<th>Dry-eye disease (DED)</th>
<th>Ocular GVHD (oGVHD)</th>
<th>Clinical trial endpoint consideration in oGVHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known immunological mechanisms</td>
<td>Autoimmune Th17, CD4+/CD8+ T-cell activation through extrinsic or intrinsic triggers, unknown antigen</td>
<td>Migration and activation of donor hematopoietic/mesenchymal stem cells</td>
<td>Inclusion of participants before onset of disease possible</td>
</tr>
<tr>
<td>Meibomian gland dysfunction (MGD)</td>
<td>Caused by numerous factors (aging, rosacea, drugs) leading to evaporation caused by MGD</td>
<td>Caused by chemotherapy and oGVHD, leading to evaporation</td>
<td>MGD as secondary endpoint</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Not typical for dry-eye disease (see below)</td>
<td>Early activation of fibroblasts and macrophages</td>
<td>Fibrosis as clinical endpoint feasible</td>
</tr>
<tr>
<td>Other causes</td>
<td>Numerous: systemic drugs, contact lens wear, aging, etc.</td>
<td>Presumed: chemotherapy and/or conditioning procedures</td>
<td>Pre-treatments and underlying oncological disease, origin of donor cells, might need to be considered during stratification</td>
</tr>
</tbody>
</table>

Time course

| | Onset mostly unknown, slow progress in a majority of cases, over years to decades | Fast onset 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)

<table>
<thead>
<tr>
<th></th>
<th>Tear production</th>
<th>Blepharitis</th>
<th>Meibomian gland dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced in aqueous deficient DED and in overlap (mostly slow onset)</td>
<td>Mostly mild/moderate</td>
<td>Up to 80% in DED</td>
<td>Unsuitable endpoint, as currently unclear mechanism</td>
</tr>
<tr>
<td>Reduced (fast onset, rapid progression)</td>
<td>Mostly severe</td>
<td>Up to 100% in oGVHD</td>
<td></td>
</tr>
<tr>
<td>Condition</td>
<td>Severity</td>
<td>Grading System</td>
<td>Description</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>------------------------</td>
<td>---------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Corneal and conjunctival intravital staining</td>
<td>Mild to severe</td>
<td>Mostly severe</td>
<td>Due to higher severity different grading systems needed to allow measuring treatment success using staining as endpoint</td>
</tr>
<tr>
<td>Conjunctival redness</td>
<td>Mild to severe</td>
<td>Mostly severe</td>
<td>Secondary endpoint, detection and grading systems need to be validated</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>Rare finding, associated with severe rosacea, atopic keratoconjunctivitis or ocular cicatricial pemphigoid</td>
<td>Frequent finding</td>
<td>Primary or secondary endpoint, detection and grading systems need to be validated</td>
</tr>
<tr>
<td>Filamentary keratitis</td>
<td>Rare finding, only in severe cases, mostly Sjögren Syndrome</td>
<td>Common finding, presumably related to activation of innate immune system</td>
<td>Primary or secondary endpoint</td>
</tr>
<tr>
<td>Superior bulbar and limbal keratokonjunctivitis</td>
<td>Rare finding, own entity not typically related to DED</td>
<td>Frequent finding</td>
<td>Secondary clinical endpoint</td>
</tr>
<tr>
<td>Intraocular involvement</td>
<td>Not related to DED</td>
<td>Intraocular involvement reported</td>
<td>Secondary endpoint in subgroup analysis possible</td>
</tr>
<tr>
<td>Correlation between signs and symptoms</td>
<td>Low correlation: strong symptoms, weak clinical signs</td>
<td>Low correlation: weak symptoms, strong clinical signs</td>
<td>Development of suitable symptom questionnaires for oGVHD necessary</td>
</tr>
</tbody>
</table>

Abbreviations: DED, dry eye disease; oGVHD, ocular chronic graft-vs.-host disease; MGD, meibomium gland dysfunction; HCT, hematopoietic cell transplantation
Figure: Potential Longitudinal Trial Design Proposal for Highly Morbid Manifestations of Chronic GVHD. The proposed study approach aims to simultaneously address identified fundamental knowledge gaps in several domains, including 1) description of natural history 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 or/and clinical risk factors and followed over time through phases of cGVHD. Patients may also enter the longitudinal cohort at the time of cGVHD 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/specimens will be collected. In this Figure, lung disease is used as an example for the enrollment entry, interventions, endpoints, and data/samples to be collected. This schema can be easily expanded to reflect skin, GI, ocular, and other manifestations with relevant data collection and treatment agents.

Abbreviations: HCT, hematopoietic cell transplantation; cGVHD, chronic graft-vs.-host disease; BOS, bronchiolitis obliterans syndrome; SOC, standard of care; f/u, follow-up; FEV1, forced expiratory volume-first second; aGVHD, acute GVHD; HRCT, high resolution chest tomography; PFTs, pulmonary function tests, BAL, bronchoalveolar lavage; 6MWT, 6 minute walk test