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

[2] *Division 1, Institution 1, State 1, Country 1*

* Correspondence: Daniel Wolff, MD

Dept. of Internal Medicine III, University of Regensburg

F.J. Strauss Allee 11, 93053 Regensburg, Germany

phone: -49-941-944-15531, fax: -49-941-944-5543

e-mail: daniel.wolff@ukr.de

Short Title: Highly morbid forms of cGVHD

Keywords: Chronic graft-versus-host disease, allogeneic hematopoietic cell transplantation, consensus, lung, sclerosis, gastrointestinal tract, ocular

Word counts: abstract, xx; text, xx

Tables: x

Figures: x

References: x

Appendices: x

On-line Supplements: x

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, 2]. 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[3] 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[3, 4]. ScGVHD can manifest as localized disease (morphea-like), 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[5], 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[6-8 9]; they may represent bystanders or effectors depending on biopsy timing[6, 7]. In systemic sclerosis (SSc)[10] as well as cGVHD[11, 12] impaired function of regulatory T cells has been reported, and IL-2 treatment which expands regulatory T cells showed efficacy in advanced cGVHD[13]. Humoral immunopathology, such as stimulating PDGF-receptor antibodies, could have a role in severe fibrotic forms of cGVHD[14], 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[15, 16]. A possible mechanistic B cell role in ScGVHD has been suggested with improvement of sclerosis after B cell depletion[17]. Still, definitive evidence linking antibody-dependent mechanisms to human ScGVHD is lacking.

Recently, distinct dermal myeloid cell populations were identified in human skin[18]. 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[19, 20]. Relevant for ScGVHD, myeloid-sourced TGFβ[21, 22] promotes fibrosis through positive regulation of fibroblast proliferation and differentiation into myofibroblasts[23] and stimulation of extracellular matrix overproduction[24]. In addition, macrophage-derived TGFβ promotes epithelial mesenchymal transition (EMT) in models of lung fibrosis[25]. Partial EMT is involved in normal wound healing, though its disruption in the inflammatory environment can promote pathologic fibrosis in lung and skin[26]. 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[21, 22]. In patients, higher TGFβ levels are associated with adverse outcomes taking into account the challenges to correlating expression and activity[27, 28]. However, TGFβ is temporally restricted and has pleiotropic roles[22] 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[29, 30], 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[26, 31, 32]. 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_1R; profibrogenic) and cannabinoid receptor 2 (CB_2R; 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 TGFβ and TGFβ 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. belumosudil, 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[50], 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)[51]. Precision medicine with immune effector cell therapies targeting fibrosis have been explored in other diseases[52], and could be considered in ScGVHD. Multi-targeting approaches may be helpful to prevent evolution to sclerosis and to enhance safety without compromising efficacy[53].

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[54] 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)[55-57,58] , and 14%[58] 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[59]. Risk factors for onset include antecedent respiratory viral infections[60, 61] and impaired lung function early post-transplant[57,62.] 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[63] but 5-year survival remains low at approximately 50%, highlighting the need for novel prevention and treatment strategies[57].

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[64] and acquisition of a persistent inflammatory airway epithelial cell phenotype[65, 66]. 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[67]. In one murine model, alternatively activated macrophages drove BOS, supported clinically by evidence of leukotriene production, and polarized CD4 immune activation[19]. 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[68], reduced pulmonary dysfunction[69]. These mechanistic insights have not yet been confirmed in humans although biomarker studies support a prominent role of B cells with significantly elevated $CD21^{low}$ B cells and high sBAFF levels[70]. 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[71, 72, 73]. A concerning pattern is reduced FEV1 and FVC with normal FEV1/FVC ratio[71], likely reflecting "pseudorestriction" due to small airway obstruction. An open question remains whether lymphocytic bronchiolitis, which is responsive to anti-inflammatory agents[72], 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[58, 63], 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[74,75,76]. 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[77]. Restriction may be due to known interstitial lung disease entities including organizing pneumonia or extraparenchymal processes including truncal sclerosis[78], respiratory muscle weakness[73, 79], or pleural effusions. Nevertheless, histological studies of BOS in HCT demonstrate concomitant bronchiolar lesions and interstitial fibrosis[73], 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[80,81] accompanied by systemic corticosteroids taking into account a potential impaired graft-versus-leukemia effect associated with azithromycin as reported in a prophylaxis study[82]. However, a significant proportion of BOS patients continue to decline despite these treatments[83]. 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[84] and antiinflammatory and antifibrotic agents currently in use for pulmonary fibrosis[85]

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[86]. 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 [86, 87]), 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[88]. Incidence of esophageal, upper GI, and lower GI involvement is, respectively, 16%, 20%, and 13%, according to analysis from the cGVHD Consortium[89]. Most importantly, intestinal involvement is associated with greater risk of non-relapse mortality[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[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[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[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[96] indicating the need for further investigations on the association of dysbiosis, antibiotic strategies and GI-cGVHD[95].

Pathophysiology

Chronic GVHD is characterized by atrophy/destruction of tissues with subsequent fibrosis. However, intestinal fibrosis is rare in cGVHD[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[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[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[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[107-113] since current natural history trials as well as clinical trials revealed a significant number of wrongly labeled patients[97]. Electronic tools like the GVHD App may assist[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 manifestation with characteristic inflammatory, immune dysregulatory and fibrotic manifestations [23, 115-117]. OGVHD is usually diagnosed between 5-24 months after HCT[118-120], and it can severely impact quality of life and quality of vision [121, 122] due to severe symptoms such as burning, dryness [88, 123-125], and loss of visual function [126]. Preexisting dry-eye and Meibomian gland disease as a consequence of chemotherapies or possibly irradiation increases the risk for later oGVHD [127, 128]. 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 [118, 129-132]. In addition, a few reports have described intraocular involvement including choroid and retina [133]. 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 [117, 134-136]. 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 [137-140].

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 [124, 141] to previous NIH consensus criteria [142]. 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 [143], 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 [144 145], neutrophil extracellular traps (NETs)[116], extracellular DNA [146, 147] and decreased level of lactoferrin [148], DNAse [147], IL-7 and EGF [145] in the tear film. In lacrimal glands affected by oGVHD, early fibrosis and myxedematous tissue may herald a rapidly progressive fibrosis [117] 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[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[150, 151]. Activated macrophages and fibroblasts through both classical immunological pathway and sterile inflammatory pathway including presence of NETs[116] and extracellular DNA from the damaged tissue [147], activation of endoplasmic reticulum stress pathway [152] and tissue renin angiotensin system [153] synthesize an excessive amount of extracellular matrix, resulting in rapid interstitial inflammation and fibrosis[151, 154, 155].

Information from animal models and clinical analyses

Several animal models have been used to study biology, onset, time course, and therapies for oGvHD [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 [159 161] with subsequent fibrosis. Perez et al introduced a scoring system for murine models of oGVHD [156]. Several preclinical studies tested potential therapeutics such as siRNA [162], bromodomain inhibitors [163], Rebamipide [164] and VAP-1 [165] and a SYK inhibitor [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 [167, 168] as comparators in experimental studies to distinguish organ-specific cGVHD pathologies from secondary, damage-related disease.

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).

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[169], oral manifestations which impair QoL and may increase the risk for secondary malignancies[170], isolated fasciitis[171], 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[172], like polyserositis which is infrequent but difficult to treat[173], 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[174]. It remains to be established whether cognitive dysfunction is caused by cumulative neurotoxicity and acute GVHD, as demonstrated in experimental models and clinical investigations, [175, 176, 177] or whether cGVHD further contributes. Rare cases of cGVHD with acute disseminated encephalomyelitis (ADEM) have been reported [178, 179]. Similarly, peripheral nervous system dysfunction is prevalent in a high proportion of cGVHD patients [40, 41, 180] 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[181]. Endothelial dysfunction could be part of the pathophysiology of cGVHD in a variety of organs based on experimental [182, 183 184] and clinically evidence [185, 186]. It may contribute to long term cardiovascular morbidity and mortality[187, 188] 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 [189-192]. 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 [193] may identify a smaller but more informative study population where a drug effect can be observed [194].
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 [195]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[196-198], with the design being modified according to pre-specified rules during the conduct of the study to increase efficiency. For example, a Bayesian approach[199] 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

1. Wolff D, Hilgendorf I, Wagner-Drouet E, Jedlickova Z, Ayuk F, Zeiser R *et al.* Changes in Immunosuppressive Treatment of Chronic Graft-versus-Host Disease: Comparison of 2 Surveys within Allogeneic Hematopoietic Stem Cell Transplant Centers in Germany, Austria, and Switzerland. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2019; **25**(7)**:** 1450-1455. e-pub ahead of print 2019/03/17; doi: 10.1016/j.bbmt.2019.03.003

2. Wood WA, Chai X, Weisdorf D, Martin PJ, Cutler C, Inamoto Y *et al.* Comorbidity burden in patients with chronic GVHD. *Bone Marrow Transplant* 2013; **48**(11)**:** 1429-1436. e-pub ahead of print 2013/05/15; doi: 10.1038/bmt.2013.70

3. Inamoto Y, Storer BE, Petersdorf EW, Nelson JL, Lee SJ, Carpenter PA *et al.* Incidence, risk factors, and outcomes of sclerosis in patients with chronic graft-versus-host disease. *Blood* 2013; **121**(25)**:** 5098-5103. doi: 10.1182/blood-2012-10-464198

4. Martires KJ, Baird K, Steinberg SM, Grkovic L, Joe GO, Williams KM *et al.* Sclerotic-type chronic GVHD of the skin: clinical risk factors, laboratory markers, and burden of disease. *Blood* 2011; **118**(15)**:** 4250-4257. doi: 10.1182/blood-2011-04-350249

5. Inamoto Y, Martin PJ, Flowers MED, Lee SJ, Carpenter PA, Warren EH *et al.* Genetic risk factors for sclerotic graft-versus-host disease. *Blood* 2016; **128**(11)**:** 1516-1524. doi: 10.1182/blood-2016-05-715342

6. Berrie JL, Kmieciak M, Sabo RT, Roberts CH, Idowu MO, Mallory K *et al.* Distinct oligoclonal T cells are associated with graft versus host disease after stem-cell transplantation. *Transplantation* 2012; **93**(9)**:** 949-957. e-pub ahead of print 2012/03/02; doi: 10.1097/TP.0b013e3182497561

7. Yew PY, Alachkar H, Yamaguchi R, Kiyotani K, Fang H, Yap KL *et al.* Quantitative characterization of T-cell repertoire in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2015; **50**(9)**:** 1227-1234. e-pub ahead of print 2015/06/09; doi: 10.1038/bmt.2015.133

8. Bruggen MC, Klein I, Greinix H, Bauer W, Kuzmina Z, Rabitsch W *et al.* Diverse T-cell responses characterize the different manifestations of cutaneous graft-versus-host disease. *Blood* 2014; **123**(2)**:** 290-299. e-pub ahead of print 2013/11/21; doi: 10.1182/blood-2013-07-514372

9. Hill GR, Olver SD, Kuns RD, Varelias A, Raffelt NC, Don AL *et al.* Stem cell mobilization with G-CSF induces type 17 differentiation and promotes scleroderma. *Blood* 2010; **116**(5)**:** 819-828. e-pub ahead of print 2010/05/04; doi: 10.1182/blood-2009-11-256495

10. Ugor E, Simon D, Almanzar G, Pap R, Najbauer J, Nemeth P *et al.* Increased proportions of functionally impaired regulatory T cell subsets in systemic sclerosis. *Clin Immunol* 2017; **184:** 54-62. e-pub ahead of print 2017/05/20; doi: 10.1016/j.clim.2017.05.013

11. Koreth J, Kim HT, Jones KT, Lange PB, Reynolds CG, Chammas MJ *et al.* Efficacy, durability, and response predictors of low-dose interleukin-2 therapy for chronic graft-versus-host disease. *Blood* 2016; **128**(1)**:** 130-137. e-pub ahead of print 2016/04/14; doi: 10.1182/blood-2016-02-702852

12. Alho AC, Kim HT, Chammas MJ, Reynolds CG, Matos TR, Forcade E *et al.* Unbalanced recovery of regulatory and effector T cells after allogeneic stem cell transplantation contributes to chronic GVHD. *Blood* 2016; **127**(5)**:** 646-657. e-pub ahead of print 2015/12/17; doi: 10.1182/blood-2015-10-672345

13. Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP, 3rd *et al.* Interleukin-2 and regulatory T cells in graft-versus-host disease. *N Engl J Med* 2011; **365**(22)**:** 2055-2066. e-pub ahead of print 2011/12/02; doi: 10.1056/NEJMoa1108188

14. Svegliati S, Olivieri A, Campelli N, Luchetti M, Poloni A, Trappolini S *et al.* Stimulatory autoantibodies to PDGF receptor in patients with extensive chronic graft-versus-host disease. *Blood* 2007; **110**(1)**:** 237-241. doi: 10.1182/blood-2007-01-071043

15. Chen GL, Arai S, Flowers MED, Otani JM, Qiu J, Cheng EC *et al.* A phase 1 study of imatinib for corticosteroid-dependent/refractory chronic graft-versus-host disease: response does not correlate with anti-PDGFRA antibodies. *Blood* 2011; **118**(15)**:** 4070-4078. doi: 10.1182/blood-2011-03-341693

16. Spies-Weisshart B, Schilling K, Böhmer F, Hochhaus A, Sayer HG, Scholl S. Lack of association of platelet-derived growth factor (PDGF) receptor autoantibodies and severity of chronic graft-versus-host disease (GvHD). *Journal of cancer research and clinical oncology* 2013; **139**(8)**:** 1397-1404. e-pub ahead of print 2013/06/04; doi: 10.1007/s00432-013-1451-z

17. Arai S, Pidala J, Pusic I, Chai X, Jaglowski S, Khera N *et al.* A Randomized Phase II Crossover Study of Imatinib or Rituximab for Cutaneous Sclerosis after Hematopoietic Cell Transplantation. *Clinical Cancer Research* 2016; **22**(2)**:** 319-327. doi: 10.1158/1078-0432.ccr-15-1443

18. Xue D, Tabib T, Morse C, Lafyatis R. Transcriptome landscape of myeloid cells in human skin reveals diversity, rare populations and putative DC progenitors. *J Dermatol Sci* 2020; **97**(1)**:** 41-49. e-pub ahead of print 2019/12/15; doi: 10.1016/j.jdermsci.2019.11.012

19. Alexander KA, Flynn R, Lineburg KE, Kuns RD, Teal BE, Olver SD *et al.* CSF-1-dependant donor-derived macrophages mediate chronic graft-versus-host disease. *J Clin Invest* 2014; **124**(10)**:** 4266-4280. e-pub ahead of print 2014/08/27; doi: 10.1172/JCI75935

20. Du J, Paz K, Flynn R, Vulic A, Robinson TM, Lineburg KE *et al.* Pirfenidone ameliorates murine chronic GVHD through inhibition of macrophage infiltration and TGF-beta production. *Blood* 2017; **129**(18)**:** 2570-2580. e-pub ahead of print 2017/03/04; doi: 10.1182/blood-2017-01-758854

21. McCormick LL, Zhang Y, Tootell E, Gilliam AC. Anti-TGF-beta treatment prevents skin and lung fibrosis in murine sclerodermatous graft-versus-host disease: a model for human scleroderma. *J Immunol* 1999; **163**(10)**:** 5693-5699. e-pub ahead of print 1999/11/24;

22. Banovic T, Macdonald KPA, Morris ES, Rowe V, Kuns R, Don A *et al.* TGF-β in allogeneic stem cell transplantation: friend or foe? *Blood* 2005; **106**(6)**:** 2206-2214. doi: 10.1182/blood-2005-01-0062

23. MacDonald KP, Blazar BR, Hill GR. Cytokine mediators of chronic graft-versus-host disease. *J Clin Invest* 2017; **127**(7)**:** 2452-2463. doi: 10.1172/JCI90593

24. Banovic T, MacDonald KP, Morris ES, Rowe V, Kuns R, Don A *et al.* TGF-beta in allogeneic stem cell transplantation: friend or foe? *Blood* 2005; **106**(6)**:** 2206-2214. e-pub ahead of print 2005/06/09; doi: 10.1182/blood-2005-01-0062

25. Zhu L, Fu X, Chen X, Han X, Dong P. M2 macrophages induce EMT through the TGF-β/Smad2 signaling pathway. *Cell Biology International* 2017; **41**(9)**:** 960-968. doi: 10.1002/cbin.10788

26. Distler JHW, Györfi A-H, Ramanujam M, Whitfield ML, Königshoff M, Lafyatis R. Shared and distinct mechanisms of fibrosis. *Nature Reviews Rheumatology* 2019; **15**(12)**:** 705-730. doi: 10.1038/s41584-019-0322-7

27. Wu JM, Thoburn CJ, Wisell J, Farmer ER, Hess AD. CD20, AIF-1, and TGF-beta in graft-versus-host disease: a study of mRNA expression in histologically matched skin biopsies. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc* 2010; **23**(5)**:** 720-728. e-pub ahead of print 2010/03/02; doi: 10.1038/modpathol.2010.48

28. Kyrcz-Krzemien S, Helbig G, Zielinska P, Markiewicz M. The kinetics of mRNA transforming growth factor beta1 expression and its serum concentration in graft-versus-host disease after allogeneic hemopoietic stem cell transplantation for myeloid leukemias. *Medical science monitor : international medical journal of experimental and clinical research* 2011; **17**(6)**:** CR322-328. e-pub ahead of print 2011/06/02; doi: 10.12659/msm.881804

29. Farina G, Lafyatis D, Lemaire R, Lafyatis R. A four-gene biomarker predicts skin disease in patients with diffuse cutaneous systemic sclerosis. *Arthritis Rheum* 2010; **62**(2)**:** 580-588. e-pub ahead of print 2010/01/30; doi: 10.1002/art.27220

30. Hakim FT, Memon S, Jin P, Imanguli MM, Wang H, Rehman N *et al.* Upregulation of IFN-Inducible and Damage-Response Pathways in Chronic Graft-versus-Host Disease. *J Immunol* 2016; **197**(9)**:** 3490-3503. e-pub ahead of print 2016/10/04; doi: 10.4049/jimmunol.1601054

31. Dees C, Tomcik M, Zerr P, Akhmetshina A, Horn A, Palumbo K *et al.* Notch signalling regulates fibroblast activation and collagen release in systemic sclerosis. *Ann Rheum Dis* 2011; **70**(7)**:** 1304-1310. e-pub ahead of print 2011/04/01; doi: 10.1136/ard.2010.134742

32. Dees C, Zerr P, Tomcik M, Beyer C, Horn A, Akhmetshina A *et al.* Inhibition of Notch signaling prevents experimental fibrosis and induces regression of established fibrosis. *Arthritis & Rheumatism* 2011; **63**(5)**:** 1396-1404. doi: 10.1002/art.30254

33. Zerr P, Palumbo-Zerr K, Distler A, Tomcik M, Vollath S, Munoz LE *et al.* Inhibition of hedgehog signaling for the treatment of murine sclerodermatous chronic graft-versus-host disease. *Blood* 2012; **120**(14)**:** 2909-2917. e-pub ahead of print 2012/08/24; doi: 10.1182/blood-2012-01-403428

34. Radojcic V, Lee C, Pletneva M, Hicks K, Sarantopoulos S, Couriel D. Hedgehog blockade in the treatment of steroid-refractory sclerodermatous chronic graft-versus-host disease. *Bone Marrow Transplantation* 2019; **54**(1)**:** 305-306. doi: 10.1038/s41409-019-0559-4

35. DeFilipp Z, Nazarian RM, El-Jawahri A, Li S, Brown J, Del Rio C *et al.* Phase 1 study of the Hedgehog pathway inhibitor sonidegib for steroid-refractory chronic graft-versus-host disease. *Blood advances* 2017; **1**(22)**:** 1919-1922. e-pub ahead of print 2018/01/04; doi: 10.1182/bloodadvances.2017011239

36. Radojcic V, Flynn RP, Chung J, Du J, Perkey E, Paz K *et al.* Notch Signaling Mediated By Dll1/4 Notch Ligands Controls the Pathogenesis of Both Multi-Organ System Non-Sclerodermatous and Sclerodermatous Chronic Graft-Versus-Host Disease. *Blood* 2016; **128**(22)**:** 805-805.

37. Spiera R, Hummers L, Chung L, Frech TM, Domsic R, Hsu V *et al.* Safety and Efficacy of Lenabasum in a Phase II, Randomized, Placebo-Controlled Trial in Adults With Systemic Sclerosis. *Arthritis Rheumatol* 2020. e-pub ahead of print 2020/04/27; doi: 10.1002/art.41294

38. Cinar R, Iyer MR, Kunos G. The therapeutic potential of second and third generation CB1R antagonists. *Pharmacol Ther* 2020; **208:** 107477. e-pub ahead of print 2020/01/12; doi: 10.1016/j.pharmthera.2020.107477

39. Yuan CY, Zhou V, Sauber G, Stollenwerk TM, Komorowski R, Lopez A *et al.* Signaling Through the Type 2 Cannabinoid Receptor Regulates the Severity of Acute and Chronic Graft versus Host Disease. *Blood* 2020. e-pub ahead of print 2020/10/08; doi: 10.1182/blood.2020004871

40. Bilic E, Delimar V, Desnica L, Pulanic D, Bilic E, Bakovic M *et al.* High prevalence of small- and large-fiber neuropathy in a prospective cohort of patients with moderate to severe chronic GvHD. *Bone Marrow Transplant* 2016; **51**(11)**:** 1513-1517. e-pub ahead of print 2016/11/03; doi: 10.1038/bmt.2016.158

41. Kraus PD, Wolff D, Grauer O, Angstwurm K, Jarius S, Wandinger KP *et al.* Muscle cramps and neuropathies in patients with allogeneic hematopoietic stem cell transplantation and graft-versus-host disease. *PLoS One* 2012; **7**(9)**:** e44922. e-pub ahead of print 2012/10/03; doi: 10.1371/journal.pone.0044922

42. Curtis LM, Ostojic A, Venzon D, Holtzman NG, Pirsl F, Kuzmina ZJ *et al.* A randomized phase-2 trial of pomalidomide in subjects failing prior therapy of chronic graft-versus-host disease. *Blood* 2020. e-pub ahead of print 2020/09/26; doi: 10.1182/blood.2020006892

43. Gottlöber P, Leiter U, Friedrich W, Bunjes D, Schulz A, Kerscher M *et al.* Chronic cutaneous sclerodermoid graft-versus-host disease: evaluation by 20-MHz sonography. *Journal of the European Academy of Dermatology and Venereology : JEADV* 2003; **17**(4)**:** 402-407. e-pub ahead of print 2003/07/02; doi: 10.1046/j.1468-3083.2003.00516.x

44. Clark J, Yao L, Pavletic SZ, Krumlauf M, Mitchell S, Turner ML *et al.* Magnetic resonance imaging in sclerotic-type chronic graft-vs-host disease. *Arch Dermatol* 2009; **145**(8)**:** 918-922. e-pub ahead of print 2009/08/19; doi: 10.1001/archdermatol.2009.78

45. Mantero JC, Kishore N, Ziemek J, Stifano G, Zammitti C, Khanna D *et al.* Randomised, double-blind, placebo-controlled trial of IL1-trap, rilonacept, in systemic sclerosis. A phase I/II biomarker trial. *Clin Exp Rheumatol* 2018; **36 Suppl 113**(4)**:** 146-149. e-pub ahead of print 2018/10/03;

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,

690 Double-Blind, Placebo-Controlled Trial of C-82. *J Invest Dermatol* 2017; **137**(12)**:** 2473-2483. e-
691 pub ahead of print 2017/08/16; doi: 10.1016/j.jid.2017.06.032

692
693 47. Rice LM, Ziemek J, Stratton EA, McLaughlin SR, Padilla CM, Mathes AL *et al.* A longitudinal
694 biomarker for the extent of skin disease in patients with diffuse cutaneous systemic sclerosis.
695 *Arthritis Rheumatol* 2015; **67**(11)**:** 3004-3015. e-pub ahead of print 2015/08/05; doi:
696 10.1002/art.39287

697
698 48. Rice LM, Padilla CM, McLaughlin SR, Mathes A, Ziemek J, Goummih S *et al.* Fresolimumab
699 treatment decreases biomarkers and improves clinical symptoms in systemic sclerosis patients. *J*
700 *Clin Invest* 2015; **125**(7)**:** 2795-2807. e-pub ahead of print 2015/06/23; doi: 10.1172/JCI77958

701
702 49. Jagasia M, Salhotra A, Bachier CR, Zoghi M, PhD, FACP, Behyar, Lazaryan A, Weisdorf DJ *et al.*
703 KD025 for Patients with Chronic Graft-Versus-Host Disease (cGVHD) - Long-Term Follow-up of a
704 Phase 2a Study (KD025-208). *Blood* 2019; **134**(Supplement_1)**:** 872-872. doi: 10.1182/blood-
705 2019-125986

706
707 50. Yamakawa T, Ohigashi H, Hashimoto D, Hayase E, Takahashi S, Miyazaki M *et al.* Vitamin A–
708 coupled liposomes containing siRNA against HSP47 ameliorate skin fibrosis in chronic graft-
709 versus-host disease. *Blood* 2018; **131**(13)**:** 1476-1485. doi: 10.1182/blood-2017-04-779934

710
711 51. Chen Y, Feng X, Meng S. Site-specific drug delivery in the skin for the localized treatment of skin
712 diseases. *Expert Opin Drug Deliv* 2019; **16**(8)**:** 847-867. e-pub ahead of print 2019/07/18; doi:
713 10.1080/17425247.2019.1645119

714
715 52. Aghajanian H, Kimura T, Rurik JG, Hancock AS, Leibowitz MS, Li L *et al.* Targeting cardiac fibrosis
716 with engineered T cells. *Nature* 2019; **573**(7774)**:** 430-433. doi: 10.1038/s41586-019-1546-z

717
718 53. Distler A, Lang V, Del Vecchio T, Huang J, Zhang Y, Beyer C *et al.* Combined inhibition of
719 morphogen pathways demonstrates additive antifibrotic effects and improved tolerability.
720 *Annals of the Rheumatic Diseases* 2014; **73**(6)**:** 1264-1268. doi: 10.1136/annrheumdis-2013-
721 204221

722
723 54. Keren L, Bosse M, Thompson S, Risom T, Vijayaragavan K, McCaffrey E *et al.* MIBI-TOF: A
724 multiplexed imaging platform relates cellular phenotypes and tissue structure. *Sci Adv* 2019;
725 **5**(10)**:** eaax5851. e-pub ahead of print 2019/10/22; doi: 10.1126/sciadv.aax5851

726
727 55. Dudek AZ, Mahaseth H, DeFor TE, Weisdorf DJ. Bronchiolitis obliterans in chronic graft-versus-
728 host disease: analysis of risk factors and treatment outcomes. *Biol Blood Marrow Transplant*
729 2003; **9**(10)**:** 657-666. e-pub ahead of print 2003/10/22; doi: 10.1016/s1083-8791(03)00242-8

56. Arora M, Cutler CS, Jagasia MH, Pidala J, Chai X, Martin PJ *et al.* Late Acute and Chronic Graft-versus-Host Disease after Allogeneic Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant* 2016; **22**(3)**:** 449-455. e-pub ahead of print 2015/11/07; doi: 10.1016/j.bbmt.2015.10.018

57. Bergeron A, Chevret S, Peffault de Latour R, Chagnon K, de Margerie-Mellon C, Riviere F *et al.* Noninfectious lung complications after allogeneic haematopoietic stem cell transplantation. *Eur Respir J* 2018; **51**(5). e-pub ahead of print 2018/04/14; doi: 10.1183/13993003.02617-2017

58. Au BK, Au MA, Chien JW. Bronchiolitis obliterans syndrome epidemiology after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2011; **17**(7)**:** 1072-1078. e-pub ahead of print 2010/12/04; doi: 10.1016/j.bbmt.2010.11.018

59. Loeb JS, Blower WC, Feldstein JF, Koch BA, Munlin AL, Hardie WD. Acceptability and repeatability of spirometry in children using updated ATS/ERS criteria. *Pediatr Pulmonol* 2008; **43**(10)**:** 1020-1024. e-pub ahead of print 2008/09/12; doi: 10.1002/ppul.20908

60. Erard V, Chien JW, Kim HW, Nichols WG, Flowers ME, Martin PJ *et al.* Airflow decline after myeloablative allogeneic hematopoietic cell transplantation: the role of community respiratory viruses. *The Journal of infectious diseases* 2006; **193**(12)**:** 1619-1625. doi: 10.1086/504268

61. Sheshadri A, Chemaly RF, Alousi AM, Shah PK, Rondon G, Bashoura L *et al.* Pulmonary Impairment after Respiratory Viral Infections Is Associated with High Mortality in Allogeneic Hematopoietic Cell Transplant Recipients. *Biol Blood Marrow Transplant* 2019; **25**(4)**:** 800-809. e-pub ahead of print 2018/12/07; doi: 10.1016/j.bbmt.2018.11.022

62. Jamani K, He Q, Liu Y, Davis C, Hubbard J, Schoch G *et al.* Early Post-Transplantation Spirometry Is Associated with the Development of Bronchiolitis Obliterans Syndrome after Allogeneic Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant* 2019. e-pub ahead of print 2019/12/11; doi: 10.1016/j.bbmt.2019.12.002

63. Cheng GS, Storer B, Chien JW, Jagasia M, Hubbard JJ, Burns L *et al.* Lung Function Trajectory in Bronchiolitis Obliterans Syndrome after Allogeneic Hematopoietic Cell Transplant. *Ann Am Thorac Soc* 2016; **13**(11)**:** 1932-1939. e-pub ahead of print 2016/08/12; doi: 10.1513/AnnalsATS.201604-262OC

64. Swatek AM, Lynch TJ, Crooke AK, Anderson PJ, Tyler SR, Brooks L *et al.* Depletion of Airway Submucosal Glands and TP63(+)KRT5(+) Basal Cells in Obliterative Bronchiolitis. *Am J Respir Crit Care Med* 2018; **197**(8)**:** 1045-1057. e-pub ahead of print 2017/12/14; doi: 10.1164/rccm.201707-1368OC

65. Rao W, Wang S, Duleba M, Niroula S, Goller K, Xie J *et al.* Regenerative Metaplastic Clones in COPD Lung Drive Inflammation and Fibrosis. *Cell* 2020. e-pub ahead of print 2020/04/17; doi: 10.1016/j.cell.2020.03.047

66. Byers DE, Alexander-Brett J, Patel AC, Agapov E, Dang-Vu G, Jin X *et al.* Long-term IL-33-producing epithelial progenitor cells in chronic obstructive lung disease. *J Clin Invest* 2013; **123**(9)**:** 3967-3982. e-pub ahead of print 2013/08/16; doi: 10.1172/JCI65570

67. Vanaudenaerde BM, Wuyts WA, Geudens N, Dupont LJ, Schoofs K, Smeets S *et al.* Macrolides inhibit IL17-induced IL8 and 8-isoprostane release from human airway smooth muscle cells. *Am J Transplant* 2007; **7**(1)**:** 76-82. e-pub ahead of print 2006/10/26; doi: 10.1111/j.1600-6143.2006.01586.x

68. Flynn R, Du J, Veenstra RG, Reichenbach DK, Panoskaltsis-Mortari A, Taylor PA *et al.* Increased T follicular helper cells and germinal center B cells are required for cGVHD and bronchiolitis obliterans. *Blood* 2014; **123**(25)**:** 3988-3998. e-pub ahead of print 2014/05/14; doi: 10.1182/blood-2014-03-562231

69. Srinivasan M, Flynn R, Price A, Ranger A, Browning JL, Taylor PA *et al.* Donor B-cell alloantibody deposition and germinal center formation are required for the development of murine chronic GVHD and bronchiolitis obliterans. *Blood* 2012; **119**(6)**:** 1570-1580. e-pub ahead of print 2011/11/11; doi: 10.1182/blood-2011-07-364414

70. Kuzmina Z, Krenn K, Petkov V, Kormoczi U, Weigl R, Rottal A *et al.* CD19(+)CD21(low) B cells and patients at risk for NIH-defined chronic graft-versus-host disease with bronchiolitis obliterans syndrome. *Blood* 2013; **121**(10)**:** 1886-1895. e-pub ahead of print 2013/01/11; doi: 10.1182/blood-2012-06-435008

71. Bergeron A, Godet C, Chevret S, Lorillon G, Peffault de Latour R, de Revel T *et al.* Bronchiolitis obliterans syndrome after allogeneic hematopoietic SCT: phenotypes and prognosis. *Bone Marrow Transplant* 2013; **48**(6)**:** 819-824. e-pub ahead of print 2012/12/05; doi: 10.1038/bmt.2012.241

72. Holbro A, Lehmann T, Girsberger S, Stern M, Gambazzi F, Lardinois D *et al.* Lung histology predicts outcome of bronchiolitis obliterans syndrome after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2013; **19**(6)**:** 973-980. e-pub ahead of print 2013/04/09; doi: 10.1016/j.bbmt.2013.03.017

73. Meignin V, Thivolet-Bejui F, Kambouchner M, Hussenet C, Bondeelle L, Mitchell A *et al.* Lung histopathology of non-infectious pulmonary complications after allogeneic haematopoietic stem

cell transplantation. *Histopathology* 2018; **73**(5)**:** 832-842. e-pub ahead of print 2018/06/29; doi: 10.1111/his.13697

74. Belloli EA, Wang X, Murray S, Forrester G, Weyhing A, Lin J *et al.* Longitudinal Forced Vital Capacity Monitoring as a Prognostic Adjunct after Lung Transplantation. *Am J Respir Crit Care Med* 2015; **192**(2)**:** 209-218. e-pub ahead of print 2015/04/30; doi: 10.1164/rccm.201501-0174OC

75. Todd JL, Jain R, Pavlisko EN, Finlen Copeland CA, Reynolds JM, Snyder LD *et al.* Impact of forced vital capacity loss on survival after the onset of chronic lung allograft dysfunction. *Am J Respir Crit Care Med* 2014; **189**(2)**:** 159-166. e-pub ahead of print 2013/12/12; doi: 10.1164/rccm.201306-1155OC

76. Sato M, Waddell TK, Wagnetz U, Roberts HC, Hwang DM, Haroon A *et al.* Restrictive allograft syndrome (RAS): a novel form of chronic lung allograft dysfunction. *J Heart Lung Transplant* 2011; **30**(7)**:** 735-742. e-pub ahead of print 2011/03/23; doi: 10.1016/j.healun.2011.01.712

77. Glanville AR, Verleden GM, Todd JL, Benden C, Calabrese F, Gottlieb J *et al.* Chronic lung allograft dysfunction: Definition and update of restrictive allograft syndrome-A consensus report from the Pulmonary Council of the ISHLT. *J Heart Lung Transplant* 2019; **38**(5)**:** 483-492. e-pub ahead of print 2019/04/28; doi: 10.1016/j.healun.2019.03.008

78. Sharifi H, Lai YK, Guo H, Hoppenfeld M, Guenther ZD, Johnston L *et al.* Machine learning algorithms to differentiate among pulmonary complications after hematopoietic cell transplant. *Chest* 2020. e-pub ahead of print 2020/04/29; doi: 10.1016/j.chest.2020.02.076

79. Greer M, Riise GC, Hansson L, Perch M, Hammainen P, Roux A *et al.* Dichotomy in pulmonary graft-versus-host disease evident among allogeneic stem-cell transplant recipients undergoing lung transplantation. *Eur Respir J* 2016; **48**(6)**:** 1807-1810. e-pub ahead of print 2016/09/03; doi: 10.1183/13993003.01121-2016

80. Bergeron A, Chevret S, Chagnon K, Godet C, Bergot E, Peffault de Latour R *et al.* Budesonide/Formoterol for bronchiolitis obliterans after hematopoietic stem cell transplantation. *Am J Respir Crit Care Med* 2015; **191**(11)**:** 1242-1249. e-pub ahead of print 2015/04/04; doi: 10.1164/rccm.201410-1818OC

81. Williams KM, Cheng GS, Pusic I, Jagasia M, Burns L, Ho VT *et al.* Fluticasone, Azithromycin, and Montelukast Treatment for New-Onset Bronchiolitis Obliterans Syndrome after Hematopoietic Cell Transplantation. *Biol Blood Marrow Transplant* 2016; **22**(4)**:** 710-716. e-pub ahead of print 2015/10/18; doi: 10.1016/j.bbmt.2015.10.009

82. Bergeron A, Chevret S, Granata A, Chevallier P, Vincent L, Huynh A *et al.* Effect of Azithromycin on Airflow Decline-Free Survival After Allogeneic Hematopoietic Stem Cell Transplant: The ALLOZITHRO Randomized Clinical Trial. *JAMA* 2017; **318**(6)**:** 557-566. e-pub ahead of print 2017/08/09; doi: 10.1001/jama.2017.9938

83. Hefazi M, Langer KJ, Khera N, Adamski J, Roy V, Winters JL *et al.* Extracorporeal Photopheresis Improves Survival in Hematopoietic Cell Transplant Patients with Bronchiolitis Obliterans Syndrome without Significantly Impacting Measured Pulmonary Functions. *Biol Blood Marrow Transplant* 2018; **24**(9)**:** 1906-1913. e-pub ahead of print 2018/04/22; doi: 10.1016/j.bbmt.2018.04.012

84. Iacono A, Wijesinha M, Rajagopal K, Murdock N, Timofte I, Griffith B *et al.* A randomised single-centre trial of inhaled liposomal cyclosporine for bronchiolitis obliterans syndrome post-lung transplantation. *ERJ Open Res* 2019; **5**(4). e-pub ahead of print 2019/11/07; doi: 10.1183/23120541.00167-2019

85. Du J, Paz K, Thangavelu G, Schneidawind D, Baker J, Flynn R *et al.* Invariant natural killer T cells ameliorate murine chronic GVHD by expanding donor regulatory T cells. *Blood* 2017; **129**(23)**:** 3121-3125. e-pub ahead of print 2017/04/19; doi: 10.1182/blood-2016-11-752444

86. Cheng GS, Selwa KE, Hatt C, Ram S, Fortuna AB, Guerriero M *et al.* Multicenter evaluation of parametric response mapping as an indicator of bronchiolitis obliterans syndrome after hematopoietic stem cell transplantation. *Am J Transplant* 2020; **20**(8)**:** 2198-2205. e-pub ahead of print 2020/02/09; doi: 10.1111/ajt.15814

87. Sharifi H, Lai YK, Guo H, Hoppenfeld M, Guenther ZD, Johnston L *et al.* Machine Learning Algorithms to Differentiate Among Pulmonary Complications After Hematopoietic Cell Transplant. *Chest* 2020; **158**(3)**:** 1090-1103. e-pub ahead of print 2020/04/29; doi: 10.1016/j.chest.2020.02.076

88. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW *et al.* National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2015; **21**(3)**:** 389-401 e381. e-pub ahead of print 2014/12/23; doi: 10.1016/j.bbmt.2014.12.001

89. Pidala J, Chai X, Kurland BF, Inamoto Y, Flowers ME, Palmer J *et al.* Analysis of gastrointestinal and hepatic chronic graft-versus-host [corrected] disease manifestations on major outcomes: a chronic graft-versus-host [corrected] disease consortium study. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2013; **19**(5)**:** 784-791. e-pub ahead of print 2013/02/12; doi: 10.1016/j.bbmt.2013.02.001

90. Inamoto Y, Martin PJ, Storer BE, Palmer J, Weisdorf DJ, Pidala J *et al.* Association of severity of organ involvement with mortality and recurrent malignancy in patients with chronic graft-versus-host disease. *Haematologica* 2014; **99**(10)**:** 1618-1623. e-pub ahead of print 2014/07/06; doi: 10.3324/haematol.2014.109611

91. Pidala J, Vogelsang G, Martin P, Chai X, Storer B, Pavletic S *et al.* Overlap subtype of chronic graft-versus-host disease is associated with an adverse prognosis, functional impairment, and inferior patient-reported outcomes: a Chronic Graft-versus-Host Disease Consortium study. *Haematologica* 2012; **97**(3)**:** 451-458. e-pub ahead of print 2011/11/08; doi: 10.3324/haematol.2011.055186

92. Kanda J, Brazauskas R, Hu ZH, Kuwatsuka Y, Nagafuji K, Kanamori H *et al.* Graft-versus-Host Disease after HLA-Matched Sibling Bone Marrow or Peripheral Blood Stem Cell Transplantation: Comparison of North American Caucasian and Japanese Populations. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2016; **22**(4)**:** 744-751. doi: 10.1016/j.bbmt.2015.12.027

93. Inamoto Y, Kimura F, Kanda J, Sugita J, Ikegame K, Nakasone H *et al.* Comparison of graft-versus-host disease-free, relapse-free survival according to a variety of graft sources: antithymocyte globulin and single cord blood provide favorable outcomes in some subgroups. *Haematologica* 2016; **101**(12)**:** 1592-1602. e-pub ahead of print 2016/09/24; doi: 10.3324/haematol.2016.149427

94. Inamoto Y, White J, Ito R, Martin PJ, Fatobene G, Ito A *et al.* Comparison of characteristics and outcomes of late acute and NIH chronic GVHD between Japanese and white patients. *Blood Adv* 2019; **3**(18)**:** 2764-2777. e-pub ahead of print 2019/09/26; doi: 10.1182/bloodadvances.2019000386

95. Markey KA, Schluter J, Gomes AL, Littmann E, Pickard A, Taylor BP *et al.* Microbe-derived short chain fatty acids butyrate and propionate are associated with protection from chronic GVHD. *Blood* 2020. e-pub ahead of print 2020/05/21; doi: 10.1182/blood.2019003369

96. Golob JL, DeMeules MM, Loeffelholz T, Quinn ZZ, Dame MK, Silvestri SS *et al.* Butyrogenic bacteria after acute graft-versus-host disease (GVHD) are associated with the development of steroid-refractory GVHD. *Blood Adv* 2019; **3**(19)**:** 2866-2869. e-pub ahead of print 2019/10/06; doi: 10.1182/bloodadvances.2019000362

97. Cuvelier GDE, Nemecek ER, Wahlstrom JT, Kitko CL, Lewis VA, Schechter T *et al.* Benefits and challenges with diagnosing chronic and late acute GVHD in children using the NIH consensus criteria. *Blood* 2019; **134**(3)**:** 304-316. e-pub ahead of print 2019/05/03; doi: 10.1182/blood.2019000216

98. Spencer GD, Shulman HM, Myerson D, Thomas ED, McDonald GB. Diffuse intestinal ulceration after marrow transplantation: a clinicopathologic study of 13 patients. *Hum Pathol* 1986; **17**(6)**:** 621-633. doi: 10.1016/s0046-8177(86)80135-6

99. Tordjman M, Ouachee M, Bonnard A, Tilea B, Yakouben K, Viala J *et al.* Small bowel stenosis: a manifestation of chronic graft-versus-host disease in children? *Hum Pathol* 2018; **72:** 174-179. doi: 10.1016/j.humpath.2017.08.034

100. Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, Cozijnsen M *et al.* Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 2007; **449**(7165)**:** 1003-1007. e-pub ahead of print 2007/10/16; doi: nature06196 [pii]

10.1038/nature06196

101. Takashima S, Kadowaki M, Aoyama K, Koyama M, Oshima T, Tomizuka K *et al.* The Wnt agonist R-spondin1 regulates systemic graft-versus-host disease by protecting intestinal stem cells. *The Journal of experimental medicine* 2011; **208**(2)**:** 285-294. e-pub ahead of print 2011/02/02; doi: jem.20101559 [pii]

10.1084/jem.20101559

102. Hayase E, Hashimoto D, Nakamura K, Noizat C, Ogasawara R, Takahashi S *et al.* R-Spondin1 expands Paneth cells and prevents dysbiosis induced by graft-versus-host disease. *J Exp Med* 2017; **214**(12)**:** 3507-3518. doi: 10.1084/jem.20170418

103. Hanash AM, Dudakov JA, Hua G, O'Connor MH, Young LF, Singer NV *et al.* Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. *Immunity* 2012; **37**(2)**:** 339-350. e-pub ahead of print 2012/08/28; doi: 10.1016/j.immuni.2012.05.028

104. Lindemans CA, Calafiore M, Mertelsmann AM, O'Connor MH, Dudakov JA, Jenq RR *et al.* Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature* 2015; **528**(7583)**:** 560-564. doi: 10.1038/nature16460

105. Eriguchi Y, Takashima S, Oka H, Shimoji S, Nakamura K, Uryu H *et al.* Graft-versus-host disease disrupts intestinal microbial ecology by inhibiting Paneth cell production of alpha-defensins. *Blood* 2012; **120**(1)**:** 223-231. e-pub ahead of print 2012/04/27; doi: 10.1182/blood-2011-12-401166

106. Schultz KR, Kariminia A, Ng B, Abdossamadi S, Lauener M, Nemecek ER *et al.* Immune Profile Differences between Chronic GvHD and Late Acute GvHD: Results of the ABLE/PBMTC 1202 Studies. *Blood* 2020. doi: 10.1182/blood.2019003186

107. Sung AD, Hassan S, Cardona DM, Wild D, Nichols KR, Mehdikhani H *et al.* Late Gastrointestinal Complications of Allogeneic Hematopoietic Stem Cell Transplantation in Adults. *Biol Blood Marrow Transplant* 2018; **24**(4)**:** 734-740. e-pub ahead of print 2017/12/17; doi: 10.1016/j.bbmt.2017.12.772

108. Akpek G, Chinratanalab W, Lee LA, Torbenson M, Hallick JP, Anders V *et al.* Gastrointestinal involvement in chronic graft-versus-host disease: a clinicopathologic study. *Biol Blood Marrow Transplant* 2003; **9**(1)**:** 46-51. e-pub ahead of print 2003/01/21; doi: 10.1053/bbmt.2003.49999

109. Rieger K, Gunther U, Erben U, Kuhl A, Loddenkemper C, Pezzutto A *et al.* Confocal endomicroscopy in diagnosis of intestinal chronic graft-versus-host disease. *Hematol Oncol* 2018; **36**(1)**:** 291-298. e-pub ahead of print 2017/05/27; doi: 10.1002/hon.2446

110. Shulman HM, Cardona DM, Greenson JK, Hingorani S, Horn T, Huber E *et al.* NIH Consensus development project on criteria for clinical trials in chronic graft-versus-host disease: II. The 2014 Pathology Working Group Report. *Biol Blood Marrow Transplant* 2015; **21**(4)**:** 589-603. e-pub ahead of print 2015/02/03; doi: 10.1016/j.bbmt.2014.12.031

111. Salomao M, Dorritie K, Mapara MY, Sepulveda A. Histopathology of Graft-vs-Host Disease of Gastrointestinal Tract and Liver: An Update. *Am J Clin Pathol* 2016; **145**(5)**:** 591-603. e-pub ahead of print 2016/06/02; doi: 10.1093/ajcp/aqw050

112. Milano F, Shulman HM, Guthrie KA, Riffkin I, McDonald GB, Delaney C. Late-onset colitis after cord blood transplantation is consistent with graft-versus-host disease: results of a blinded histopathological review. *Biol Blood Marrow Transplant* 2014; **20**(7)**:** 1008-1013. e-pub ahead of print 2014/04/08; doi: 10.1016/j.bbmt.2014.03.020

113. Shimoji S, Kato K, Eriguchi Y, Takenaka K, Iwasaki H, Miyamoto T *et al.* Evaluating the association between histological manifestations of cord colitis syndrome with GVHD. *Bone Marrow Transplant* 2013; **48**(9)**:** 1249-1252. e-pub ahead of print 2013/06/12; doi: 10.1038/bmt.2013.44

114. Schoemans HM, Goris K, Van Durm R, Vanbrabant K, De Geest S, Maertens J *et al.* Accuracy and usability of the eGVHD app in assessing the severity of graft-versus-host disease at the 2017 EBMT annual congress. *Bone Marrow Transplant* 2018; **53**(4)**:** 490-494. e-pub ahead of print 2018/01/14; doi: 10.1038/s41409-017-0017-0

115. Cooke KR, Luznik L, Sarantopoulos S, Hakim FT, Jagasia M, Fowler DH *et al.* The Biology of Chronic Graft-versus-Host Disease: A Task Force Report from the National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease. *Biol Blood Marrow Transplant* 2017; **23**(2)**:** 211-234. e-pub ahead of print 2016/10/08; doi: 10.1016/j.bbmt.2016.09.023

116. An S, Raju I, Surenkhuu B, Kwon JE, Gulati S, Karaman M *et al.* Neutrophil extracellular traps (NETs) contribute to pathological changes of ocular graft-vs.-host disease (oGVHD) dry eye: Implications for novel biomarkers and therapeutic strategies. *Ocul Surf* 2019; **17**(3)**:** 589-614. e-pub ahead of print 2019/04/10; doi: 10.1016/j.jtos.2019.03.010

117. Ogawa Y, Yamazaki K, Kuwana M, Mashima Y, Nakamura Y, Ishida S *et al.* A significant role of stromal fibroblasts in rapidly progressive dry eye in patients with chronic GVHD. *Invest Ophthalmol Vis Sci* 2001; **42**(1)**:** 111-119.

118. Ogawa Y, Okamoto S, Wakui M, Watanabe R, Yamada M, Yoshino M *et al.* Dry eye after haematopoietic stem cell transplantation. *Br J Ophthalmol* 1999; **83**(10)**:** 1125-1130. doi: 10.1136/bjo.83.10.1125

119. Na KS, Yoo YS, Mok JW, Lee JW, Joo CK. Incidence and risk factors for ocular GVHD after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2015; **50**(11)**:** 1459-1464. doi: 10.1038/bmt.2015.187

120. Shikari H, Amparo F, Saboo U, Dana R. Onset of ocular graft-versus-host disease symptoms after allogeneic hematopoietic stem cell transplantation. *Cornea* 2015; **34**(3)**:** 243-247. doi: 10.1097/ICO.0000000000000340

121. Sun YC, Chai X, Inamoto Y, Pidala J, Martin PJ, Flowers ME *et al.* Impact of Ocular Chronic Graft-versus-Host Disease on Quality of Life. *Biol Blood Marrow Transplant* 2015; **21**(9)**:** 1687-1691. e-pub ahead of print 2015/06/03; doi: 10.1016/j.bbmt.2015.05.020

122. Saboo US, Amparo F, Abud TB, Schaumberg DA, Dana R. Vision-Related Quality of Life in Patients with Ocular Graft-versus-Host Disease. *Ophthalmology* 2015; **122**(8)**:** 1669-1674. doi: 10.1016/j.ophtha.2015.04.011

123. Tung CI. Current Approaches to Treatment of Ocular Graft-Versus-Host Disease. *International ophthalmology clinics* 2017; **57**(2)**:** 65-88. doi: 10.1097/IIO.0000000000000167

124. Rapoport Y, Freeman T, Koyama T, Engelhardt BG, Jagasia M, Savani BN *et al.* Validation of International Chronic Ocular Graft-Versus-Host Disease (GVHD) Group Diagnostic Criteria as a Chronic Ocular GVHD-Specific Metric. *Cornea* 2017; **36**(2)**:** 258-263. doi: 10.1097/ICO.0000000000001109

125. Shikari H, Antin JH, Dana R. Ocular graft-versus-host disease: a review. *Survey of ophthalmology* 2013; **58**(3)**:** 233-251. doi: 10.1016/j.survophthal.2012.08.004

126. Shimizu E, Aketa N, Yazu H, Uchino M, Kamoi M, Sato Y *et al.* Corneal higher-order aberrations in eyes with chronic ocular graft-versus-host disease. *Ocul Surf* 2020; **18**(1)**:** 98-107. e-pub ahead of print 2019/10/13; doi: 10.1016/j.jtos.2019.10.005

127. Ban Y, Ogawa Y, Ibrahim OM, Tatematsu Y, Kamoi M, Uchino M *et al.* Morphologic evaluation of meibomian glands in chronic graft-versus-host disease using in vivo laser confocal microscopy. *Mol Vis* 2011; **17:** 2533-2543.

128. Engel LA, Wittig S, Bock F, Sauerbier L, Scheid C, Holtick U *et al.* Meibography and meibomian gland measurements in ocular graft-versus-host disease. *Bone Marrow Transplant* 2015; **50**(7)**:** 961-967. e-pub ahead of print 2015/04/22; doi: 10.1038/bmt.2015.72

129. Hessen M, Akpek EK. Ocular graft-versus-host disease. *Curr Opin Allergy Clin Immunol* 2012; **12**(5)**:** 540-547. e-pub ahead of print 2012/08/16; doi: 10.1097/ACI.0b013e328357b4b9

130. Kerty E, Vigander K, Flage T, Brinch L. Ocular findings in allogeneic stem cell transplantation without total body irradiation. *Ophthalmology* 1999; **106**(7)**:** 1334-1338. doi: 10.1016/S0161-6420(99)00720-4

131. Nassiri N, Eslani M, Panahi N, Mehravaran S, Ziaei A, Djalilian AR. Ocular graft versus host disease following allogeneic stem cell transplantation: a review of current knowledge and recommendations. *J Ophthalmic Vis Res* 2013; **8**(4)**:** 351-358.

132. Pathak M, Diep PP, Lai X, Brinch L, Ruud E, Drolsum L. Ocular findings and ocular graft-versus-host disease after allogeneic stem cell transplantation without total body irradiation. *Bone Marrow Transplant* 2018; **53**(7)**:** 863-872. doi: 10.1038/s41409-018-0090-z

133. Mirza N, Zierhut M, Korn A, Bornemann A, Vogel W, Schmid-Horch B *et al.* Graft versus self (GvS) against T-cell autoantigens is a mechanism of graft-host interaction. *Proc Natl Acad Sci U S A* 2016; **113**(48)**:** 13827-13832. doi: 10.1073/pnas.1609118113

134. Sivaraman KR, Jivrajka RV, Soin K, Bouchard CS, Movahedan A, Shorter E *et al.* Superior Limbic Keratoconjunctivitis-like Inflammation in Patients with Chronic Graft-Versus-Host Disease. *Ocul Surf* 2016; **14**(3)**:** 393-400. doi: 10.1016/j.jtos.2016.04.003

135. Kheirkhah A, Coco G, Satitpitakul V, Dana R. Subtarsal Fibrosis Is Associated With Ocular Surface Epitheliopathy in Graft-Versus-Host Disease. *Am J Ophthalmol* 2018; **189:** 102-110. doi: 10.1016/j.ajo.2018.02.020

136. Kusne Y, Temkit M, Khera N, Patel DR, Shen JF. Conjunctival subepithelial fibrosis and meibomian gland atrophy in ocular graft-versus-host disease. *Ocul Surf* 2017; **15**(4)**:** 784-788. doi: 10.1016/j.jtos.2017.08.002

137. Stevenson W, Shikari H, Saboo US, Amparo F, Dana R. Bilateral corneal ulceration in ocular graft-versus-host disease. *Clin Ophthalmol* 2013; **7:** 2153-2158. doi: 10.2147/OPTH.S51180

138. Tarnawska D, Wylegala E. Corneal grafting and aggressive medication for corneal defects in graft-versus-host disease following bone marrow transplantation. *Eye (Lond)* 2007; **21**(12)**:** 1493-1500. doi: 10.1038/sj.eye.6702589

139. Heath JD, Acheson JF, Schulenburg WE. Penetrating keratoplasty in severe ocular graft versus host disease. *Br J Ophthalmol* 1993; **77**(8)**:** 525-526. doi: 10.1136/bjo.77.8.525

140. Plattner K, Goldblum D, Halter J, Kunz C, Koeppl R, Gerber-Hollbach N. Osteo-Odonto-Keratoprosthesis in Severe Ocular Graft versus Host Disease. *Klin Monbl Augenheilkd* 2017; **234**(4)**:** 455-456. e-pub ahead of print 2017/03/23; doi: 10.1055/s-0042-123148

141. Ogawa Y, Kim SK, Dana R, Clayton J, Jain S, Rosenblatt MI *et al.* International Chronic Ocular Graft-vs-Host-Disease (GVHD) Consensus Group: proposed diagnostic criteria for chronic GVHD (Part I). *Sci Rep* 2013; **3:** 3419. doi: 10.1038/srep03419

142. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ *et al.* National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; **11**(12)**:** 945-956. e-pub ahead of print 2005/12/13; doi: 10.1016/j.bbmt.2005.09.004

143. Blecha C, Wolff D, Holler B, Holler E, Weber D, Vogt R *et al.* Verification of the new grading scale for ocular chronic graft-versus-host disease developed by the German-Austrian-Swiss consensus conference on chronic GVHD. *Ann Hematol* 2016; **95**(3)**:** 493-499. e-pub ahead of print 2015/12/15; doi: 10.1007/s00277-015-2575-9

144. Riemens A, Stoyanova E, Rothova A, Kuiper J. Cytokines in tear fluid of patients with ocular graft-versus-host disease after allogeneic stem cell transplantation. *Mol Vis* 2012; **18:** 797-802. e-pub ahead of print 2012/04/18;

145. Hu B, Qiu Y, Hong J. Tear cytokine levels in the diagnosis and severity assessment of ocular chronic graft-versus-host disease(GVHD). *Ocul Surf* 2020; **18**(2)**:** 298-304. e-pub ahead of print 2020/01/19; doi: 10.1016/j.jtos.2019.12.005

146. Tibrewal S, Sarkar J, Jassim SH, Gandhi S, Sonawane S, Chaudhary S *et al.* Tear fluid extracellular DNA: diagnostic and therapeutic implications in dry eye disease. *Investigative ophthalmology & visual science* 2013; **54**(13)**:** 8051-8061. doi: 10.1167/iovs.13-12844

147. Sonawane S, Khanolkar V, Namavari A, Chaudhary S, Gandhi S, Tibrewal S *et al.* Ocular surface extracellular DNA and nuclease activity imbalance: a new paradigm for inflammation in dry eye disease. *Invest Ophthalmol Vis Sci* 2012; **53**(13)**:** 8253-8263. e-pub ahead of print 2012/11/22; doi: 10.1167/iovs.12-10430

148. Sonobe H, Ogawa Y, Yamada K, Shimizu E, Uchino Y, Kamoi M *et al.* A novel and innovative paper-based analytical device for assessing tear lactoferrin of dry eye patients. *Ocul Surf* 2019; **17**(1)**:** 160-166. e-pub ahead of print 2018/11/07; doi: 10.1016/j.jtos.2018.11.001

149. Yamane M, Ogawa Y, Mukai S, Yaguchi S, Kamijuku H, Inaba T *et al.* Functional role of lacrimal gland fibroblasts in a mouse model of chronic graft-versus-host disease. *Cornea* 2017; **in press.**;

150. Yamane M, Sato S, Shimizu E, Shibata S, Hayano M, Yaguchi T *et al.* Senescence-associated secretory phenotype promotes chronic ocular graft-versus-host disease in mice and humans. *FASEB J* 2020; **in press**.

151. Ogawa Y, Morikawa S, Okano H, Mabuchi Y, Suzuki S, Yaguchi T *et al.* MHC-compatible bone marrow stromal/stem cells trigger fibrosis by activating host T cells in a scleroderma mouse model. *eLife* 2016; **5:** e09394. doi: 10.7554/eLife.09394

152. Mukai S, Ogawa Y, Urano F, Kudo-Saito C, Kawakami Y, Tsubota K. Novel Treatment of Chronic Graft-Versus-Host Disease in Mice Using the ER Stress Reducer 4-Phenylbutyric Acid. *Sci Rep* 2017; **7:** 41939. doi: 10.1038/srep41939

153. Yaguchi S, Ogawa Y, Shimmura S, Kawakita T, Hatou S, Satofuka S *et al.* Angiotensin II type 1 receptor antagonist attenuates lacrimal gland, lung, and liver fibrosis in a murine model of chronic graft-versus-host disease. *PloS one* 2013; **8**(6)**:** e64724. doi: 10.1371/journal.pone.0064724

154. Ogawa Y, Shimmura S, Kawakita T, Yoshida S, Kawakami Y, Tsubota K. Epithelial mesenchymal transition in human ocular chronic graft-versus-host disease. *The American journal of pathology* 2009; **175**(6)**:** 2372-2381. doi: 10.2353/ajpath.2009.090318

155. Ogawa Y, Kodama H, Kameyama K, Yamazaki K, Yasuoka H, Okamoto S *et al.* Donor fibroblast chimerism in the pathogenic fibrotic lesion of human chronic graft-versus-host disease. *Investigative ophthalmology & visual science* 2005; **46**(12)**:** 4519-4527. doi: 10.1167/iovs.05-0227

156. Perez VL, Barsam A, Duffort S, Urbieta M, Barreras H, Lightbourn C *et al.* Novel Scoring Criteria for the Evaluation of Ocular Graft-versus-Host Disease in a Preclinical Allogeneic Hematopoietic Stem Cell Transplantation Animal Model. *Biol Blood Marrow Transplant* 2016; **22**(10)**:** 1765-1772. e-pub ahead of print 2016/08/06; doi: 10.1016/j.bbmt.2016.07.012

157. Ogawa Y. Ocular Diseases and Epithelial Mesenchymal Transition. *Nippon Ganka Gakkai Zasshi* 2016; **120**(11)**:** 743-745. e-pub ahead of print 2016/11/01;

158. Martinez-Carrasco R, Sanchez-Abarca LI, Nieto-Gomez C, Garcia EM, Ramos TL, Velasco A *et al.* Assessment of dry eye in a GVHD murine model: Approximation through tear osmolarity measurement. *Exp Eye Res* 2017; **154:** 64-69. e-pub ahead of print 2016/11/08; doi: 10.1016/j.exer.2016.11.004

159. Herretes S, Ross DB, Duffort S, Barreras H, Yaohong T, Saeed AM *et al.* Recruitment of Donor T Cells to the Eyes During Ocular GVHD in Recipients of MHC-Matched Allogeneic Hematopoietic Stem Cell Transplants. *Invest Ophthalmol Vis Sci* 2015; **56**(4)**:** 2348-2357. e-pub ahead of print 2015/02/07; doi: 10.1167/iovs.14-15630

160. He J, Yamane M, Shibata S, Fukui M, Shimizu E, Yano T *et al.* Ocular Surface and Tear Film Characteristics in a Sclerodermatous Chronic Graft-Versus-Host Disease Mouse Model. *Cornea* 2018; **37**(4)**:** 486-494. e-pub ahead of print 2018/01/18; doi: 10.1097/ICO.0000000000001487

161. Hassan AS, Clouthier SG, Ferrara JL, Stepan A, Mian SI, Ahmad AZ *et al.* Lacrimal gland involvement in graft-versus-host disease: a murine model. *Invest Ophthalmol Vis Sci* 2005; **46**(8)**:** 2692-2697. e-pub ahead of print 2005/07/27; doi: 10.1167/iovs.05-0040

162. Ohigashi H, Hashimoto D, Hayase E, Takahashi S, Ara T, Yamakawa T *et al.* Ocular instillation of vitamin A-coupled liposomes containing HSP47 siRNA ameliorates dry eye syndrome in chronic GVHD. *Blood Adv* 2019; **3**(7)**:** 1003-1010. e-pub ahead of print 2019/04/04; doi: 10.1182/bloodadvances.2018028431

163. Copsel SN, Lightbourn CO, Barreras H, Lohse I, Wolf D, Bader CS *et al.* BET Bromodomain Inhibitors Which Permit Treg Function Enable a Combinatorial Strategy to Suppress GVHD in Pre-clinical Allogeneic HSCT. *Front Immunol* 2018; **9:** 3104. e-pub ahead of print 2019/02/09; doi: 10.3389/fimmu.2018.03104

164. Shamloo K, Barbarino A, Alfuraih S, Sharma A. Graft Versus Host Disease-Associated Dry Eye: Role of Ocular Surface Mucins and the Effect of Rebamipide, a Mucin Secretagogue. *Invest Ophthalmol Vis Sci* 2019; **60**(14)**:** 4511-4519. e-pub ahead of print 2019/11/02; doi: 10.1167/iovs.19-27843

165. Mukai S, Ogawa Y, Kawakami Y, Mashima Y, Tsubota K. Inhibition of Vascular Adhesion Protein-1 for Treatment of Graft-Versus-Host Disease in Mice. *FASEB J* 2018; **32**(8)**:** 4085-4095. e-pub ahead of print 2018/03/01; doi: 10.1096/fj.201700176R

166. 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

167. 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

168. Reyes NJ, Mathew R, Saban DR. Induction and Characterization of the Allergic Eye Disease Mouse Model. *Methods Mol Biol* 2018; **1799:** 49-57. e-pub ahead of print 2018/06/30; doi: 10.1007/978-1-4939-7896-0_5

169. Frey Tirri B, Hausermann P, Bertz H, Greinix H, Lawitschka A, Schwarze CP *et al.* Clinical guidelines for gynecologic care after hematopoietic SCT. Report from the international consensus project on clinical practice in chronic GVHD. *Bone marrow transplantation* 2015; **50**(1)**:** 3-9. e-pub ahead of print 2014/10/28; doi: 10.1038/bmt.2014.242

170. Treister N, Duncan C, Cutler C, Lehmann L. How we treat oral chronic graft-versus-host disease. *Blood* 2012; **120**(17)**:** 3407-3418. e-pub ahead of print 2012/08/18; doi: 10.1182/blood-2012-05-393389

171. Oda K, Nakaseko C, Ozawa S, Nishimura M, Saito Y, Yoshiba F *et al.* Fasciitis and myositis: an analysis of muscle-related complications caused by chronic GVHD after allo-SCT. *Bone Marrow Transplant* 2009; **43**(2)**:** 159-167. e-pub ahead of print 2008/09/03; doi: 10.1038/bmt.2008.297

172. Schoemans HM, Lee SJ, Ferrara JL, Wolff D, Levine JE, Schultz KR *et al.* EBMT-NIH-CIBMTR Task Force position statement on standardized terminology & guidance for graft-versus-host disease assessment. *Bone Marrow Transplant* 2018; **53**(11)**:** 1401-1415. e-pub ahead of print 2018/06/07; doi: 10.1038/s41409-018-0204-7

173. Leonard JT, Newell LF, Meyers G, Hayes-Lattin B, Gajewski J, Heitner S *et al.* Chronic GvHD-associated serositis and pericarditis. *Bone Marrow Transplant* 2015; **50**(8)**:** 1098-1104. e-pub ahead of print 2015/05/12; doi: 10.1038/bmt.2015.105

174. Scherwath A, Schirmer L, Kruse M, Ernst G, Eder M, Dinkel A *et al.* Cognitive functioning in allogeneic hematopoietic stem cell transplantation recipients and its medical correlates: a

prospective multicenter study. *Psychooncology* 2013; **22**(7)**:** 1509-1516. e-pub ahead of print 2012/09/05; doi: 10.1002/pon.3159

175. Hartrampf S, Dudakov JA, Johnson LK, Smith OM, Tsai J, Singer NV *et al.* The central nervous system is a target of acute graft versus host disease in mice. *Blood* 2013; **121**(10)**:** 1906-1910. e-pub ahead of print 2013/01/10; doi: 10.1182/blood-2012-09-456590

176. Mathew NR, Vinnakota JM, Apostolova P, Erny D, Hamarsheh S, Andrieux G *et al.* Graft-versus-host disease of the CNS is mediated by TNF upregulation in microglia. *J Clin Invest* 2020; **130**(3)**:** 1315-1329. e-pub ahead of print 2019/12/18; doi: 10.1172/JCI130272

177. Kaliyaperumal S, Watkins B, Sharma P, Furlan S, Ramakrishnan S, Giver C *et al.* CD8-predominant T-cell CNS infiltration accompanies GVHD in primates and is improved with immunoprophylaxis. *Blood* 2014; **123**(12)**:** 1967-1969. e-pub ahead of print 2014/03/22; doi: 10.1182/blood-2014-01-547612

178. Harvey CM, Gottipati R, Schwarz S, Auer D, O'Donoghue M, Russell NH *et al.* Acute disseminated encephalomyelitis following allo-SCT: central nervous system manifestation of GVHD. *Bone Marrow Transplant* 2014; **49**(6)**:** 854-856. e-pub ahead of print 2014/03/19; doi: 10.1038/bmt.2014.29

179. Blecha C, Angstwurm K, Wolff D, Holler E, Helbig H, Grassinger J *et al.* Retinal Involvement in a Patient with Cerebral Manifestation of Chronic Graft-Versus-Host-Disease. *Oncol Res Treat* 2015; **38**(10)**:** 532-534. e-pub ahead of print 2015/10/10; doi: 10.1159/000439490

180. Grauer O, Wolff D, Bertz H, Greinix H, Kuhl JS, Lawitschka A *et al.* Neurological manifestations of chronic graft-versus-host disease after allogeneic haematopoietic stem cell transplantation: report from the Consensus Conference on Clinical Practice in chronic graft-versus-host disease. *Brain* 2010; **133**(10)**:** 2852-2865. e-pub ahead of print 2010/09/18; doi: 10.1093/brain/awq245

181. Wang Y, Ogawa Y, Dogru M, Tatematsu Y, Uchino M, Kamoi M *et al.* Baseline profiles of ocular surface and tear dynamics after allogeneic hematopoietic stem cell transplantation in patients with or without chronic GVHD-related dry eye. *Bone Marrow Transplant* 2010; **45**(6)**:** 1077-1083. e-pub ahead of print 2009/11/10; doi: 10.1038/bmt.2009.312

182. Deschaumes C, Verneuil L, Ertault-Daneshpouy M, Adle-Biassette H, Galateau F, Ainoun F *et al.* CD95 ligand-dependant endothelial cell death initiates oral mucosa damage in a murine model of acute graft versus host disease. *Lab Invest* 2007; **87**(5)**:** 417-429. e-pub ahead of print 2007/03/06; doi: 3700541 [pii]

10.1038/labinvest.3700541

183. Janin A, Deschaumes C, Daneshpouy M, Estaquier J, Micic-Polianski J, Rajagopalan-Levasseur P *et al.* CD95 engagement induces disseminated endothelial cell apoptosis in vivo: immunopathologic implications. *Blood* 2002; **99**(8)**:** 2940-2947. e-pub ahead of print 2002/04/04;

184. Sostak P, Reich P, Padovan CS, Gerbitz A, Holler E, Straube A. Cerebral endothelial expression of adhesion molecules in mice with chronic graft-versus-host disease. *Stroke* 2004; **35**(5)**:** 1158-1163. e-pub ahead of print 2004/04/10; doi: 10.1161/01.STR.0000125865.01546.bb

185. Biedermann BC, Sahner S, Gregor M, Tsakiris DA, Jeanneret C, Pober JS *et al.* Endothelial injury mediated by cytotoxic T lymphocytes and loss of microvessels in chronic graft versus host disease. *Lancet* 2002; **359**(9323)**:** 2078-2083. e-pub ahead of print 2002/06/28; doi: 10.1016/S0140-6736(02)08907-9

186. Tardieu M, Rybojad M, Peffault de Latour R, Robin M, de Masson A, Xhaard A *et al.* Localized edema with sclerodermatous evolution: a possible form of skin chronic graft-versus-host disease associated with endothelial activation. *Blood* 2013; **122**(3)**:** 463-465. e-pub ahead of print 2013/07/23; doi: 10.1182/blood-2013-03-488148

187. Tichelli A, Bucher C, Rovo A, Stussi G, Stern M, Paulussen M *et al.* Premature cardiovascular disease after allogeneic hematopoietic stem-cell transplantation. *Blood* 2007; **110**(9)**:** 3463-3471. e-pub ahead of print 2007/08/01; doi: 10.1182/blood-2006-10-054080

188. Clavert A, Peric Z, Brissot E, Malard F, Guillaume T, Delaunay J *et al.* Late Complications and Quality of Life after Reduced-Intensity Conditioning Allogeneic Stem Cell Transplantation. *Biol Blood Marrow Transplant* 2017; **23**(1)**:** 140-146. e-pub ahead of print 2016/10/19; doi: 10.1016/j.bbmt.2016.10.011

189. Lilford RJ, Thornton JG, Braunholtz D. Clinical trials and rare diseases: a way out of a conundrum. *BMJ* 1995; **311**(7020)**:** 1621-1625. e-pub ahead of print 1995/12/16; doi: 10.1136/bmj.311.7020.1621

190. Janosky JE. The ethics of underpowered clinical trials. *JAMA* 2002; **288**(17)**:** 2118; author reply 2119. e-pub ahead of print 2002/11/07;

191. Philippidis A. Orphan drugs, big pharma. *Hum Gene Ther* 2011; **22**(9)**:** 1035-1038. e-pub ahead of print 2011/09/22; doi: 10.1089/hum.2011.2515

192. Administration FaD. Rare Diseases: Common Issues in Drug Development. In: FDA, (ed), 2019.

193. Administration FaD. Enrichment Strategies for Clinical Trials to Support Approval of Human Drugs and Biological Products. In: FDA, (ed), 2019.

194. Administration FaD. BEST (Biomarker, Endpoints , and other Tools) Resource. In: FDA, (ed), 2020.

195. Administration FaD. Rare Diseases: Natural History Studies for Drug Development. In: FDA, (ed), 2019.

196. Administration FaD. Adaptive Designs for Clinical Trials of Drugs and Biologics. In: FDA, (ed), 2019.

197. Berry DA. Adaptive clinical trials in oncology. *Nat Rev Clin Oncol* 2011; **9**(4)**:** 199-207. e-pub ahead of print 2011/11/09; doi: 10.1038/nrclinonc.2011.165

198. Berry DA. The Brave New World of clinical cancer research: Adaptive biomarker-driven trials integrating clinical practice with clinical research. *Mol Oncol* 2015; **9**(5)**:** 951-959. e-pub ahead of print 2015/04/19; doi: 10.1016/j.molonc.2015.02.011

199. Administration FaD. Guidance for the Use of Bayesian Statistics in Medical Device Clinical Trials. In: FDA, (ed), 2010.

200. Osmola-Mańkowska A, Silny W, Dańczak-Pazdrowska A, Polańska A, Olek-Hrab K, Sadowska-Przytocka A *et al.* "Assessment of chronic sclerodermoid Graft-versus-Host Disease patients, using 20 MHz high-frequency ultrasonography and cutometer methods". *Skin research and technology : official journal of International Society for Bioengineering and the Skin (ISBS) [and] International Society for Digital Imaging of Skin (ISDIS) [and] International Society for Skin Imaging (ISSI)* 2013; **19**(1)**:** e417-422. e-pub ahead of print 2012/08/14; doi: 10.1111/j.1600-0846.2012.00659.x

201. Lee SY, Cardones AR, Doherty J, Nightingale K, Palmeri M. Preliminary Results on the Feasibility of Using ARFI/SWEI to Assess Cutaneous Sclerotic Diseases. *Ultrasound Med Biol* 2015; **41**(11)**:** 2806-2819. e-pub ahead of print 2015/08/12; doi: 10.1016/j.ultrasmedbio.2015.06.007

202. Zhang X, Zhou B, Osborn T. Ultrasound Surface Wave Elastography for Assessing Scleroderma. *Ultrasound in Medicine & Biology* 2020; **46**(5)**:** 1263-1269. doi: 10.1016/j.ultrasmedbio.2020.01.021

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

Cutaneous Graft-Versus-Host Disease Patients. *Blood* 2019; **134**(Supplement_1)**:** 4515-4515. doi: 10.1182/blood-2019-129125

204. Horger M, Bethge W, Boss A, Fenchel M, Claussen CD, Schmalzing M *et al.* Musculocutaneous chronic graft-versus-host disease: MRI follow-up of patients undergoing immunosuppressive therapy. *AJR Am J Roentgenol* 2009; **192**(5)**:** 1401-1406. e-pub ahead of print 2009/04/22; doi: 10.2214/ajr.08.1699

205. Sauter AW, Schmidt H, Mantlik F, Kolb A, Federmann B, Pfannenberg C *et al.* Imaging findings and therapy response monitoring in chronic sclerodermatous graft-versus-host disease: preliminary data of a simultaneous PET/MRI approach. *Clin Nucl Med* 2013; **38**(8)**:** e309-317. e-pub ahead of print 2013/03/05; doi: 10.1097/RLU.0b013e3182816559

206. Deegan AJ, Talebi-Liasi F, Song S, Li Y, Xu J, Men S *et al.* Optical coherence tomography angiography of normal skin and inflammatory dermatologic conditions. *Lasers in surgery and medicine* 2018; **50**(3)**:** 183-193. e-pub ahead of print 2018/01/23; doi: 10.1002/lsm.22788

207. Neid T, Danz B, Eismann R, Bramsiepe I, Wohlrab J, Marsch WC *et al.* [Sclerodermiform chronic graft-versus-host disease after allogenic peripheral blood stem-cell transplantation]. *Deutsche medizinische Wochenschrift (1946)* 2009; **134**(21)**:** 1106-1109. e-pub ahead of print 2009/05/14; doi: 10.1055/s-0029-1222575

208. Baker LX, Chen F, Ssempijja Y, Dellalana L, Vain A, Jagasia M *et al.* 867 Direct mechanical measurements of skin to quantify evolution of sclerotic disease. *Journal of Investigative Dermatology* 2020; **140**(7)**:** S113. doi: 10.1016/j.jid.2020.03.883

209. Chen F, Dellalana LE, Gandelman JS, Vain A, Jagasia MH, Tkaczyk ER. Non-invasive measurement of sclerosis in cutaneous cGVHD patients with the handheld device Myoton: a cross-sectional study. *Bone Marrow Transplant* 2019; **54**(4)**:** 616-619. e-pub ahead of print 2018/10/06; doi: 10.1038/s41409-018-0346-7

210. Richeldi L, Fernández Pérez ER, Costabel U, Albera C, Lederer DJ, Flaherty KR *et al.* Pamrevlumab, an anti-connective tissue growth factor therapy, for idiopathic pulmonary fibrosis (PRAISE): a phase 2, randomised, double-blind, placebo-controlled trial. *The Lancet Respiratory Medicine* 2020; **8**(1)**:** 25-33. doi: 10.1016/s2213-2600(19)30262-0

211. Makino K, Makino T, Stawski L, Lipson KE, Leask A, Trojanowska M. Anti-connective tissue growth factor (CTGF/CCN2) monoclonal antibody attenuates skin fibrosis in mice models of systemic sclerosis. *Arthritis Research & Therapy* 2017; **19**(1). doi: 10.1186/s13075-017-1356-3

212. Castelino FV, Bain G, Pace VA, Black KE, George L, Probst CK *et al.* An Autotaxin/Lysophosphatidic Acid/Interleukin-6 Amplification Loop Drives Scleroderma Fibrosis. *Arthritis & Rheumatology* 2016; **68**(12)**:** 2964-2974. doi: 10.1002/art.39797

213. Zabludoff S, Liu Y, Liu J, Zhang J, Xia F, Quimbo A *et al.* Late Breaking Abstract - ND-L02-s0201 treatment leads to efficacy in preclinical IPF models. *European Respiratory Journal* 2017; **50**(suppl 61)**:** PA881. doi: 10.1183/1393003.congress-2017.PA881

214. Ogawa Y, Razzaque MS, Kameyama K, Hasegawa G, Shimmura S, Kawai M *et al.* Role of Heat Shock Protein 47, a Collagen-Binding Chaperone, in Lacrimal Gland Pathology in Patients with cGVHD. 2007; **48**(3)**:** 1079. doi: 10.1167/iovs.06-0601

215. Pilling D, Gomer RH. The Development of Serum Amyloid P as a Possible Therapeutic. *Frontiers in immunology* 2018; **9**. doi: 10.3389/fimmu.2018.02328

216. Verstovsek S, Hasserjian RP, Pozdnyakova O, Veletic I, Mesa RA, Foltz L *et al.* PRM-151 in Myelofibrosis: Efficacy and Safety in an Open Label Extension Study. *Blood* 2018; **132**(Supplement 1)**:** 686-686. doi: 10.1182/blood-2018-99-115362

217. Spiera R, Hummers L, Chung L, Frech TM, Domsic R, Hsu V *et al.* Safety and efficacy of lenabasum in a phase 2 randomized, placebo‐controlled trial in adults with systemic sclerosis. *Arthritis & Rheumatology* 2020. doi: 10.1002/art.41294

218. Garcia-Martin A, Garrido-Rodriguez M, Navarrete C, Del Rio C, Bellido ML, Appendino G *et al.* EHP-101, an oral formulation of the cannabidiol aminoquinone VCE-004.8, alleviates bleomycin-induced skin and lung fibrosis. *Biochem Pharmacol* 2018; **157:** 304-313. e-pub ahead of print 2018/08/05; doi: 10.1016/j.bcp.2018.07.047

219. Cinar R, Gochuico BR, Iyer MR, Jourdan T, Yokoyama T, Park JK *et al.* Cannabinoid CB1 receptor overactivity contributes to the pathogenesis of idiopathic pulmonary fibrosis. *JCI Insight* 2017; **2(8)**(8)**:** e92281. doi: 10.1172/jci.insight.92281

220. Marquart S, Zerr P, Akhmetshina A, Palumbo K, Reich N, Tomcik M *et al.* Inactivation of the cannabinoid receptor CB1 prevents leukocyte infiltration and experimental fibrosis. *Arthritis Rheum* 2010; **62**(11)**:** 3467-3476. doi: 10.1002/art.27642

221. Reid J, Zamuner S, Edwards K, Rumley SA, Nevin K, Feeney M *et al.* In vivo affinity and target engagement in skin and blood in a first-time-in-human study of an anti-oncostatin M monoclonal antibody. *Br J Clin Pharmacol* 2018; **84**(10)**:** 2280-2291. e-pub ahead of print 2018/06/15; doi: 10.1111/bcp.13669

222. Stawski L, Trojanowska M. Oncostatin M and its role in fibrosis. *Connect Tissue Res* 2019; **60**(1)**:** 40-49. e-pub ahead of print 2018/07/31; doi: 10.1080/03008207.2018.1500558

223. Lafyatis R, Spiera R, Domsic R, Papazoglou A, Ligon C, Zinger Morse CM *et al.* THU0329 SAFETY, TARGET ENGAGEMENT, AND INITIAL EFFICACY OF AVID200, A FIRST-IN-CLASS POTENT AND ISOFORM-SELECTIVE INHIBITOR OF TGF-BETA 1 AND 3, IN PATIENTS WITH DIFFUSE CUTANEOUS SYSTEMIC SCLEROSIS (DCSSC): A PHASE 1 DOSE ESCALATION STUDY. *Annals of the Rheumatic Diseases* 2020; **79**(Suppl 1)**:** 394-395. doi: 10.1136/annrheumdis-2020-eular.1753

224. Denton CP, Ong VH, Xu S, Chen-Harris H, Modrusan Z, Lafyatis R *et al.* Therapeutic interleukin-6 blockade reverses transforming growth factor-beta pathway activation in dermal fibroblasts: insights from the faSScinate clinical trial in systemic sclerosis. *Annals of the Rheumatic Diseases* 2018; **77**(9)**:** 1362-1371. doi: 10.1136/annrheumdis-2018-213031

225. Khan K, Xu S, Nihtyanova S, Derrett-Smith E, Abraham D, Denton CP *et al.* Clinical and pathological significance of interleukin 6 overexpression in systemic sclerosis. *Annals of the Rheumatic Diseases* 2012; **71**(7)**:** 1235-1242. doi: 10.1136/annrheumdis-2011-200955

226. Drobyski WR, Pasquini M, Kovatovic K, Palmer J, Douglas Rizzo J, Saad A *et al.* Tocilizumab for the Treatment of Steroid Refractory Graft-versus-Host Disease. *Biology of Blood and Marrow Transplantation* 2011; **17**(12)**:** 1862-1868. doi: 10.1016/j.bbmt.2011.07.001

227. Flynn R, Paz K, Du J, Reichenbach DK, Taylor PA, Panoskaltsis-Mortari A *et al.* Targeted Rho-associated kinase 2 inhibition suppresses murine and human chronic GVHD through a Stat3-dependent mechanism. *Blood* 2016; **127**(17)**:** 2144-2154. e-pub ahead of print 2016/03/18; doi: 10.1182/blood-2015-10-678706

228. Morand EF, Furie R, Tanaka Y, Bruce IN, Askanase AD, Richez C *et al.* Trial of Anifrolumab in Active Systemic Lupus Erythematosus. *N Engl J Med* 2020; **382**(3)**:** 211-221. e-pub ahead of print 2019/12/19; doi: 10.1056/NEJMoa1912196

229. Travis WD, Costabel U, Hansell DM, King TE, Jr., Lynch DA, Nicholson AG *et al.* An official American Thoracic Society/European Respiratory Society statement: Update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. *Am J Respir Crit Care Med* 2013; **188**(6)**:** 733-748. e-pub ahead of print 2013/09/17; doi: 10.1164/rccm.201308-1483ST

230. Freudenberger TD, Madtes DK, Curtis JR, Cummings P, Storer BE, Hackman RC. Association between acute and chronic graft-versus-host disease and bronchiolitis obliterans organizing pneumonia in recipients of hematopoietic stem cell transplants. *Blood* 2003; **102**(10)**:** 3822-3828. e-pub ahead of print 2003/07/19; doi: 10.1182/blood-2002-06-1813

231. Bondeelle L, Gras J, Michonneau D, Houdouin V, Hermet E, Blin N *et al.* Pleuroparenchymal fibroelastosis after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2020; **55**(5)**:** 982-986. e-pub ahead of print 2019/08/16; doi: 10.1038/s41409-019-0636-8

1511

1512 Table 1: Potential objective assessment tools to assess skin sclerosis (ScGVHD) in chronic GVHD

Modality	Advantages	Disadvantages	Use in ScGVHD
High-frequency ultrasound, acoustic radiation force impulse (ARFI), shear wave elasticity imaging (SWEI), ultrasound surface wave elastography (USWE)	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[200] CS[43] CS[201] CS[202]
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 careful experimental technique	CS[203]
Magnetic resonance imaging (MRI), MRI/Positron emission tomography (PET)	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[44] CS[204] CS[205]
Optical coherence tomography/elastography	High-resolution imaging, including capability to assess local blood flow	Limited depth of penetration	CR[206]
Laser doppler flowmetry	2D flow map of skin perfusion; can assess dynamic changes; monitoring potential for compromised acral sites of ScGVHD	Affected by ambient temperature; movement, pressure or other contact with skin will influence perfusion	CR[207]
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[200]
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[208] CS[209] CS[203]

1513 CR: case report, CS: case series

1514

1515

Table 2: Candidate therapeutic agents in ScGVHD.

Target	Drug(s)	Target cellular subsets	Clinical Development Status	References
CTGF/CTN2	Pamrevlumab (FG-3019)	Fibroblasts	Ph-3- IPF (NCT01890265)	210, 211
Autotaxin	Ziritaxestat (GLPG-1690)	Fibroblasts	Ph-3- IPF (NCT03733444, NCT03711162)	212
HSP47	ND-L02-s0201	Fibroblasts	Ph-2- IPF (NCT03538301)	50, 213, 214
Pentraxin 2 (agonist)	PRM-151	Fibroblasts Macrophages	Ph-2- IPF (NCT02550873) Ph-2- Myelofibrosis (NCT01981850)	215, 216
CB_2R (agonist)	Lenabasum (Ajulemic acid)	Fibroblasts T cells Macrophages	Ph-3- Systemic Sclerosis (NCT03398837)	217
CB_2R /PPARγ (Dual Agonist)	EHP-101	Fibroblasts Endothelial cells Macrophages	Ph-2- Systemic Sclerosis (NCT04166552)	218
CB_1R /iNOS (dual antagonist)	MRI-1867	Fibroblasts T cells Macrophages	Ph-1	38, 219, 220
Oncostatin M (antagonist)	GSK2330811	Fibroblasts Endothelial cells T cells Macrophages	Ph-2- Systemic Sclerosis (NCT03041025)	221, 222
TGFβ	AVID200	Fibroblasts T cells Macrophages	Ph-1- Myelofibrosis (NCT03895112) Ph-1- Systemic Sclerosis (NCT03831438)	223
IL-6R	Tocilizumab	Fibroblasts T cells Macrophages	Ph-3- Systemic Sclerosis (NCT02453256) Ph-2- Steroid dependent immune related adverse events (NCT04375228)	224-226
CSF-1R	Axatilimab (SNDX-6352)	Macrophages	Ph-2- cGVHD (NCT03604692)	19
ROCK2	Belumosudil (KD025)	T cells Macrophages	Ph-2- cGVHD (NCT03640481, NCT02841995) Ph-2- Systemic Sclerosis (NCT03919799)	227
Interferon receptor type 1	Anifrolumab	T cells Macrophages	Ph-3- Systemic Lupus Erythematosus (NCT02446899) Ph-2- Rheumatoid Arthritis (NCT03435601)	228

1518 **Table 3: Pulmonary syndromes following allogeneic hematopoietic cell transplantation**

1519

Entity	Established in definition of lung GVHD	PFT pattern	High Resolution Chest CT Findings	Lung Histology	Comment
Bronchiolitis obliterans Syndrome	Yes	Fixed obstructive pattern: FEV1 decline >10%, FEV1/VC < LLN. Elevated residual volume or Residual volume/Total Lung Capacity. FEV1/FVC > LLN and preserved TLC may be seen. DLCO may be normal or reduced.	Signs of airtrapping (mosaic attenuation on expiratory phase) or bronchiolitis (centrilobular ground glass opacities or micronodules) and/or late sequalae (traction bronchiectasis, bronchial wall thickening)	Obliterative bronchiolitis (OB): partial or complete fibroproliferative occlusion of terminal small bronchioles, lymphocytic bronchiolitis may also be seen	There are subtypes of BOS based on timeframe after HCT, initial tempo of onset, FEV1 decline, histology, response to therapy, and prognosis.
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.[73, 229] 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.					
Organizing pneumonia[230]	No, however there is evidence for association with aGVHD and cGVHD.	Restrictive impairment with reduced TLC with FEV1/FVC > LLN most common. Obstructive or mixed pattern may be seen. Reduced DLCO.	Patchy and peribronchilar or consolidation, and reticular ground glass opacities, often predominant in upper lobes and periphery	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
Non-specific interstitial pneumonia[229]	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[229, 231]	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: These entities are secondary to extrathoracic consequences of cGVHD					

Truncal sclerosis	No. Sclerosis due to cGVHD 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. This may be the consequence of cGVHD-related myositis or prolonged steroid use to treat cGVHD.	Concomitant reduction in FVC and FEV1, 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

*Restrictive allograft syndrome (RAS) has been defined for lung transplantation (LT)[77] 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.

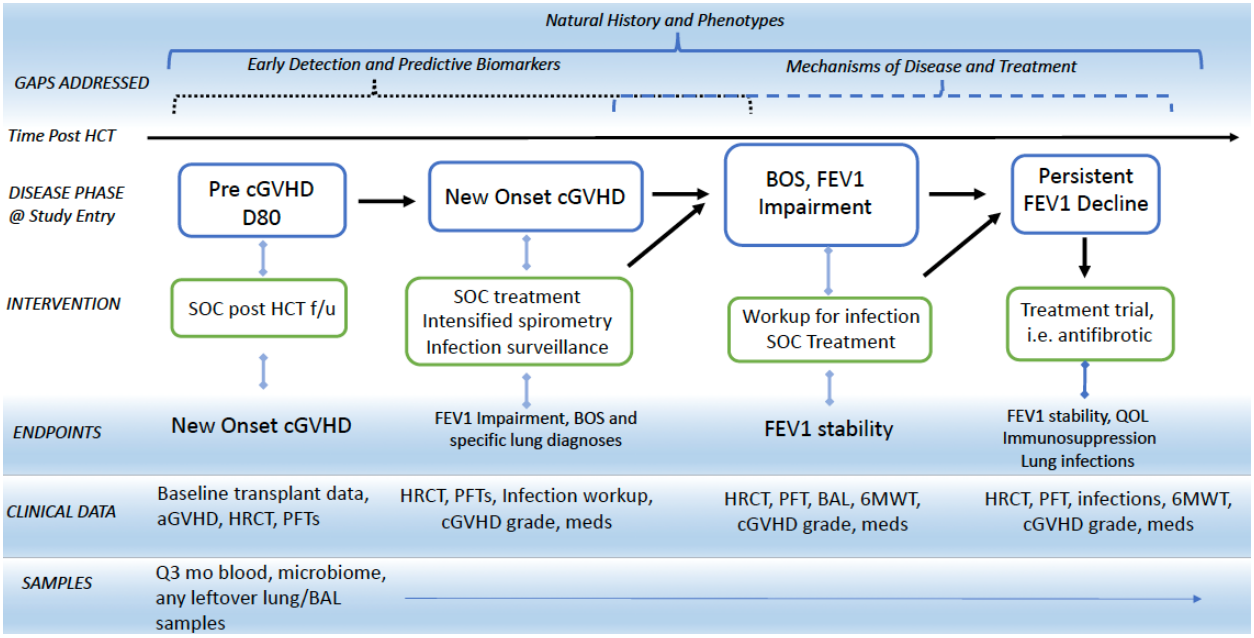
1534 **Table 4: Differences between ocular chronic graft-vs.-host disease (oGVHD) and dry eye disease**

		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
	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, might need to be considered during stratification
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)	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 allow measuring treatment success using staining as endpoint
	Conjunctival redness	Mild to severe	Mostly severe	Secondary endpoint, detection and grading systems need to 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 keratokonjunctivitis	Rare finding, own 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 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 necessary

1535 Abbreviations: DED, dry eye disease; oGVHD, ocular chronic graft-vs.-host disease; MGD, meibomium
1536 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