National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IIb. The 2020 Preemptive Therapy Working Group Report

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Abstract

Chronic graft vs. host disease (cGVHD) commonly occurs after allogeneic hematopoietic cell transplantation (HCT) despite standard prophylactic immune suppression. Intensified universal prophylaxis approaches have merit, but risk possible over-treatment and may interfere with the graft-vs-malignancy immune response. We summarize conceptual and practical considerations regarding preemptive therapy of cGVHD, namely therapeutic interventions delivered post-HCT to high-risk HCT recipients prior to overt cGVHD. This risk may be anticipated by clinical factors and/or risk assignment biomarkers or may be evidenced by early signs and symptoms of cGVHD that do not fully meet NIH diagnostic criteria. Truly preemptive, individualized, and targeted cGVHD therapies currently do not exist. The goals of this report are to: (1) review current knowledge regarding clinical risk factors for cGVHD; (2) review what is known about cGVHD risk assignment biomarkers; (3) examine how cGVHD pathogenesis intersects with available targeted therapeutic agents; (4) summarize considerations for preemptive therapy for cGVHD, emphasizing trial development (including trial design and statistical considerations). We conclude that robust risk assignment models that accurately predict impending cGVHD development after HCT along with early phase preemptive therapy trials represent the most urgent priorities to advance this area of research.

Introduction

Chronic graft-versus-host disease (cGVHD) is common after allogeneic hematopoietic cell transplantation (HCT) despite prophylaxis, and treatment of established cGVHD is unsatisfactory for most patients. Increasing peri-transplant GVHD prophylaxis risks over-treatment, increased infectious morbidity, and may compromise beneficial graft-vs-malignancy effects [See WG1 paper]. An alternative approach is to wait until post-HCT events (for example, acute GVHD) identify patients at a very high risk of cGVHD or early, subclinical indications of impending cGVHD are present, and then intervene. Subclinical features may include biomarkers with high positive predictive value (PPV) for cGVHD development, or early pre-diagnostic cGVHD signs and symptoms. This approach has the advantage of treating only those who likely need it, in contrast to prophylaxis which is given to all patients. Treating preemptively may allow more targeted and potentially less damaging therapy, as treatment is given before overt cGVHD is present. This paradigm has been successfully applied in cytomegalovirus PCR monitoring. However, developing a successful preemptive approach requires identifying subpopulations at very high risk or the earliest clinical symptoms and signs or biomarker profiles that have a high positive predictive value for developing overt disease. Preemptive therapies should mechanistically target essential cGVHD pathways to prevent the development of clinically important cGVHD and its associated burden of ineffective and prolonged immune suppressive therapy under current treatment standards. The design considerations and challenges for pre-emptive intervention trials more closely resemble those for prophylaxis trials than those for treatment trials of established moderate or severe chronic GVHD.

Summary of recommendations

1. Preemptive treatment may be the optimal approach because people who have a high risk of cGVHD development are treated early to prevent clinically evident cGVHD.
2. Risk assignment markers of impending cGVHD with high positive predictive value and moderately high negative predictive value are required for a preemptive approach. It is likely that a panel of markers (e.g. clinical, plasma, cellular, genomic, metabolomic) will be needed to identify appropriate candidates for preemptive trials. Studies to identify these markers should be multi-institutional, have accurate clinical diagnosis of cGVHD, and use testing that is readily translatable into practice.

3. More research is needed to identify pathologic processes that cause cGVHD. Preemptive therapies should correct these underlying mechanisms to promote tolerance and not simply improve symptoms, to prevent cGVHD from re-emerging when the preemptive treatment is discontinued.
4. A number of potential preemptive treatments could be tested, and trials will need academic and industry collaboration. As a key secondary outcome measure, relapse rates should be monitored to assure safety of preemptive cGVHD interventions without compromising graft vs. malignancy effect.
5. Initial preemptive therapy trials will need rigorous design with attention to eligibility criteria, interventions, clearly stated efficacy and safety measures, and benchmarks to determine whether there is sufficient promise for future study.

Methods

Each working group was created to encourage global engagement in the topic (see introduction to the series). Four groups worked individually since February 2020 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 were collected prior to the November, 2020 Consensus Conference with appropriate manuscript revisions. Based on additional comments from external reviewers, virtual Conference participants, and a 30-day public comment period, the paper was further revised for submission.

Gaps in knowledge and unmet need; highest priorities

Currently available tools (clinical signs and symptoms, risk assignment biomarkers) do not permit identification of a population at sufficiently high risk for subsequent cGVHD development to justify currently available preemptive interventions. The required positive predictive value (PPV) to warrant preemptive interventions is context-dependent and varies according to the risk of the intervention to be tested and the specific patient population. Eventually, personalized

preemptive therapy would be ideal because variation in causative pathways and heterogeneous clinical manifestations are expected among affected patients.

Many possible therapeutic agents used in therapy of advanced, established cGVHD could be tested as preemptive interventions. However, rational prioritization of such agents based on insight into cGVHD biology and careful clinical trial design to test safety and efficacy of these agents is needed. Initial studies may focus on prevention of the syndrome in total (e.g. any cGVHD, or specifically moderate/severe cGVHD), but organ-specific preemptive studies also have merit. Studies will need to clearly define short- and long-term treatment success metrics for interpretation and plan on subsequent larger, confirmatory multi-center studies to advance the field.

Clinical risk factors for chronic GVHD

Individual variables known prior to HCT (e.g. patient, donor, graft type) are used to determine prophylactic approaches for both aGVHD and cGVHD, thus are most relevant to WG1 of this NIH Consensus Meeting. However, these same features may enrich for a patient population that will later manifest early pre-diagnostic features of cGVHD that could be targeted with preemptive interventions. Thus, we briefly review these here: In an analysis of 2,941 HCT recipients, the profiles of risk factors for acute and for cGVHD were similar, but some notable differences were identified.[1] Mobilized peripheral blood cells as the graft source was strongly associated with cGVHD but not with acute GVHD (aGVHD), the use of female donors for male recipients had a greater effect on the risk of chronic than on aGVHD and older patient age was associated with cGVHD, but had no effect on aGVHD. Whereas recipient human leukocyte antigen mismatching and the use of unrelated donors had a greater effect on the risk of acute than on cGVHD and total body irradiation was strongly associated with acute but not statistically significant associated with cGVHD. Established clinical risk factors for development of cGVHD include prior aGVHD, use of mobilized blood cell graft, non-use of ex-vivo or in-vivo T cell depletion (i.e., post transplant cyclophosphamide, ATG and graft manipulation), female donor into a male recipient, use of unrelated donors and patient age (**Table 1**).[1-6] Among these factors, aGVHD is the sole post-HCT factor that may enhance risk for cGVHD above the baseline level of risk expected according to pre-HCT factors. Clinical risk factors for more severe forms of cGVHD such as sclerosis of the skin and fascia are shown in **Table 2**.[7-9]

Lessons learned from acute GVHD biomarkers

The characteristics that define an optimal risk assignment GVHD biomarker include consistency in different clinical settings, additive value to readily available clinical information, and validation in large independent patient cohorts. The intensive effort that led to the development, validation, and incorporation of aGVHD biomarkers into clinical practice and research trials can inform similar efforts for the development of clinically useful cGVHD biomarkers. First, accurate clinical data is critical. Second, the collection of research samples should include both calendar-driven and event-driven samples.[10-12] Ultimately the clinical data, including transplant characteristics, aGVHD characteristics (e.g., target organ severity), and outcomes (e.g., response to treatment, survival) need to be linked to available samples to conduct biomarker validation studies. These strategies led to the development of multiple biomarkers for aGVHD, including target organ specific biomarkers, such as REG3α and elafin, and predictive algorithms that use concentrations of more than one biomarker, such as the MAGIC algorithm to predict the risk of non-relapse mortality in patients with aGVHD.[13-21]

Repeated validation of several biomarkers with prognostic significance at aGVHD onset as well as the establishment of CLIA certified labs that can provide rapid results has allowed for the incorporation of aGVHD biomarkers as inclusion criteria for clinical trials, enriching trial populations for desired risk factors. For example, ruxolitinib was approved by the FDA to treat steroid-refractory GVHD in part due to the favorable responses observed even in patients with high MAGIC risk scores.[22,23] One of the first trials to use biomarkers as an eligibility criteria was the BMT CTN 1501 trial that defined aGVHD as low risk based on biomarker and clinical parameters and randomized patients to either prednisone or sirolimus monotherapy as initial treatment. Despite the added complexity of a biomarker inclusion criterion, the study accrued rapidly, and demonstrated similar response rates between the two approaches in this low risk population.[24] Several other aGVHD trials incorporating biomarkers (e.g. NCT02133924, NCT02525029, NCT03846479, NCT03459040) are in progress, illustrating the sustained traction of biomarkers in GVHD clinical research.

The experience of biomarker discovery in aGVHD can allow us to extrapolate the lessons learned into cGVHD, but we anticipate significant challenges. The time course of cGVHD spans years, and not weeks, and patients are seen primarily in the outpatient setting. The multitude of organ systems involved poses a significant challenge in accurate clinical data capture in the context of routine clinical visits not just for staging, but for documentation of clinical response to

standard-of-care therapy. As discussed in the WG2a paper, provider education enhanced by health informatics, with joint ownership between health care teams and patients, will be required to make clinical data reliable to conduct research studies.

Identifying generalizable markers useful for preemptive studies

Experience has shown that treatment of established cGVHD is often unsatisfactory. Initiating therapies earlier in the natural history of the disorder (asymptomatic or minimally symptomatic state) has the best chance of mitigating the impact of cGVHD once prophylaxis has failed. Validated models that predict cGVHD development in asymptomatic patients with high enough certainty to prompt treatment are not available yet. Several cGVHD biomarkers have been tested and validated in the multicenter setting with large sample sizes (**Table 3**). Clinically actionable thresholds, however, especially in the absence of clinical manifestations, are not defined. While these represent potential risk assignment cGVHD biomarkers (biomarker associated with increased risk of developing a condition in an individual who does not yet have clinical evidence of that condition), we note the following: (1) Change in biomarker values from serial longitudinal samples is likely important and is under-represented in current published literature. (2) Risk assignment cGVHD biomarkers may or may not have overlap with biomarkers relevant to cGVHD diagnosis or cGVHD treatment response. (3) While most studies to date have focused on blood immune cell populations or cytokines/chemokines, informative markers may also arise from interrogation of the target tissues, metabolome, and/or microbiome. (4) Studied biomarkers to date are largely focused on systemic markers as correlates of the overall systemic syndrome of cGVHD, while organ-specific markers of organ-specific cGVHD development are also needed to refine personalized risk determination and targeted interventions.

Conceptually, the ideal risk assignment biomarker has high sensitivity and specificity, but more importantly, should have high positive and negative predictive value. A biomarker with these ideal characteristics would give clinicians confidence in applying cGVHD preemptive therapies in the population of patients with the highest potential for direct benefit.

Recommendation: To achieve the goal of identifying markers for preemptive treatment, future cGVHD risk assignment biomarker studies should be (1) ***Multi-institutional*** and include HCT

centers with volumes ranging from small to large, who practice in different ways, thus increasing generalizability of findings by reflecting “real world” situations. (2) Require comprehensive and accurate ***Clinical Evaluation and Documentation*** of cGVHD manifestations according to the NIH consensus criteria in real-time, including at diagnosis, over time, and in response to therapy. (3) ***Prospective***, in order to avoid recall bias about the manifestations and severity of cGVHD at onset. (4) ***Readily Translatable to Clinical Practice***, prioritizing markers that are more easily performed at CLIA certified labs at reasonable cost or are readily available using standard testing or evaluation.

Targeting the most promising pathways for preemptive therapy

Over the last five years our understanding of the complex pathophysiology of cGVHD has dramatically improved. It is now clear that the disease manifestations of cGVHD represent the accumulation of a number of aberrant immunological pathways, cascading from the initial response of transplanted naïve donor T cells. These insights may enable both more effective prophylactic approaches (delivered universally to subjects based on pre-HCT knowledge of cGVHD risk) and preemptive approaches (subsequent interventions delivered based on sub-clinical risk assignment biomarkers or early pre-diagnostic signs and symptoms). Preventing cGVHD (and particularly moderate/severe cGVHD) should thus always be the goal and naive T cell depletion and post-transplant cyclophosphamide represent the most effective prophylactic strategies at present.[25,26] The subsequently invoked immune pathways during cGVHD are likely to coexist in an individual, depending on the manifestation of particular disease features (e.g. sicca syndrome, sclerotic skin, bronchiolitis obliterans (BO)) and concurrent immune suppression (reviewed in[27,28]). While the described immune pathways attributed to cGVHD have been initially described in mice, these have been confirmed, at least in part (i.e. in peripheral blood) in patient samples and are listed in **Table 4**. The thymus is perhaps the organ most sensitive to GVHD, where both recipient thymic epithelium and donor dendritic cells are targets, resulting in impaired generation of tolerogenic FoxP3^{+} regulatory T cells (Treg)[29] and the failure to delete self-reactive T cell clones.[30] The effects of acute GVHD in this process are further exacerbated by myeloablative conditioning (MAC) and increasing recipient age.[28] These defects result in the expansion of autoreactive T cell clones and the failure to generate and maintain Treg homeostasis in the periphery that in preclinical systems, generate the full spectrum of cGVHD, including sicca and fibrosis.[30] Therapies aimed at improving Treg function have

focused on low dose IL-2 administration and/or Treg transfers.[31] The former has demonstrated important responses in a significant proportion of patients with refractory cGVHD but does require long-term systemic administration of the cytokine.[32,33]

The Th17 (CD4) and Tc17 (CD8) differentiation of donor T cells in the periphery is required for the generation of scleroderma and BO in most preclinical systems although these two pathologies seldom co-exist.[34,35] This pathway requires IL-6 signaling, which is dysregulated early after HCT, particularly after MAC, and is controlled by the transcription factor RORγt.[36,37] Both Th17 and Tc17 are polyfunctional, lineage promiscuous, and secrete large amounts of Th1 (e.g. IFNγ, TNF) and Th17 (IL-17A, GM-CSF) cytokines[37] that are important in the manifestations of late acute and cGVHD. Therapies that block IL-17A, RORgt[34,38] and broad cytokine signaling (e.g. STAT1/3 by ruxolitinib)[39] show efficacy in preclinical models. An overlapping lineage of T cells producing IL-22 but no IL-17A (Th22) are also present in patient skin lesions and induce skin cGVHD in mice.[40] Whether inhibition of cytokines directly responsible for the initiation (IL-6, IL-12), amplification (IL-21) and maintenance (IL-23) of these lineages are effective in cGVHD treatment remains to be formally tested and IL-12/23p40 would seem a particularly attractive initial target.

T follicular helper (Tfh) T cell differentiation after HCT is characterized by IL-21 secretion and controlled by the transcription factor bcl6 after HCT.[41] Tfh cells promote aberrant germinal center B (GCB) cell reactions. Associated with Tfh responses, B cells from patients with clinically active cGVHD were shown to have significantly increased survival rates along with constitutive activation and BAFF-associated signaling. [42] Aberrant germinal center B cell responses are associated with alloantibody generation and BO after HCT in mice.[41] Clinical correlation of these findings has been challenging in the absence of well annotated clinical cohorts with available samples for interrogation. Inhibitors of IL-21,[41] bcl6[43] and PI3K delta[44] have all shown efficacy in targeting this pathway in preclinical systems. Therapeutic agents such as anti-CD20 monoclonal antibody that can prevent the generation of memory B cells and plasma cells,[45] or delete the latter once formed (e.g. proteasome inhibitors)[46] have thus shown promise in preventing and treating cGVHD respectively. CD27+B cells from patients with cGVHD constitutively produce IgG[47] and are hyper-responsive to surrogate antigen.[42] Aberrant B cell receptor (BCR) signaling occurs in B cells from patients with active cGVHD. Inhibitors of the spleen tyrosine kinase (SYK) that is involved in donor B cell receptor signaling and antigen-responsiveness during cGVHD have also shown promise in preventing and treating BO in mice.[48]

It is thus clear that cGVHD involves a concerted B cell and T cell response. It is thus unsurprising that inhibitors of kinases involved in the pathogenic differentiation of both lineages have shown the greatest potential for the preemptive treatment of cGVHD to date. Ibrutinib, an inhibitor of bruton's tyrosine kinase was shown to be highly active in murine models of cGVHD[49] and has undergone successful phase II testing such that it is now approved for treatment of steroid refractory cGVHD.[50] This agent, originally developed for the treatment of CLL is not without toxicity and tolerance issues in this sensitive HCT population.[50] An inhibitor of Rho-associated kinase 2 (ROCK2) is a highly effective inhibitor of STAT3 phosphorylation-dependent Tfh and Th17 differentiation (and the subsequent GCB cell reaction), and is associated with significant efficacy in preclinical models.[51] Promising early evidence of clinical efficacy and tolerability is also emerging and the agent has been granted FDA break-through status.

The final common afferent arm of cGVHD appears to always involve the accumulation and alternative (M2) differentiation of tissue IL-17A[38] and CSF-1R-dependent macrophages[52] which secrete large amounts of fibrogenic factors (e.g. TGFβ, PDGF) that invoke collagen deposition in target tissue.[52] With this in mind, agents that inhibit CSF-1R or provide broad anti-inflammatory (including TGFβ) inhibition (e.g. pirfenidone) are highly active in preclinical models[52] and are currently undergoing clinical testing.

While all these pathways generate immunological defects that may be quantifiable clinically, to date the most robust biomarkers of cGVHD involve a composite panel including ST2, CXCL9, MMP3 and osteopontin.[53] These markers, however, do not permit recognition of a dominant immune pathway necessary to personalize drug selection for patients at high risk of cGVHD. Subsequently, the choice of a drug to prevent cGVHD will require an agent that is well tolerated and active across a number of the immune pathways known to be active in cGVHD. Considering this, ROCK2 and other JAK/STAT inhibitors would seem to be the most appropriate agents at present.

Recommendation: Continued research to identify specific pathways involved in both systemic and organ-specific cGVHD is needed to facilitate identification of candidates for preemptive intervention.

Challenges with a preemptive therapy approach

It is reasonable to hypothesize that treating cGVHD preemptively before the onset of advanced clinical manifestations would be more effective than treating after overt clinical symptoms. While broad-based prophylaxis strategies currently exist, truly preemptive, individualized, and appropriately targeted cGVHD therapies do not. Barriers to the rational choice and design of preemptive therapy include rudimentary understanding about pathogenesis of organ-specific cGVHD manifestations, the protean nature of cGVHD manifestations and variable time to cGVHD onset, and the need to correct the underlying dysfunction leading to cGVHD in order to prevent cGVHD recurrence upon withdrawal of therapy. Additional study is required to define the optimal starting point post-HCT for risk assignment and delivery of preemptive therapy. Ideally, the type and timing of intervention should be foundationally linked to mechanistic steps in cGVHD pathogenesis, and respect feasibility and safety considerations regarding expected post-HCT toxicity and recovery.

Another issue is that candidate indicators for initiating preemptive therapy have variable or unproven reliability. Pre-diagnostic, or "*forme fruste*" cGVHD manifestations are poorly established and cGVHD serum or plasma, urine and cellular biomarkers, have not passed the verification phase which requires real-time rather than just retrospective validation. Candidate cGVHD biomarkers include: CXCL9, CXCL10, ST2, MMP3, osteopontin, CD163, IL-17A, IL-21, soluble BAFF, as well as cellular populations such as CD4+CD45RA+, CD19+CD21^{low}, NK subsets, Tregs, and CD146/RORγCD4.[34,54] Such biomarkers have been associated with overall cGVHD rather than a specific phenotype and sensitivity and specificity may vary for different phenotypes. As well, most published cGVHD biomarkers have shown utility as diagnostic markers, rather than risk assignment markers most relevant to cGVHD preemptive therapy. Cofactors that might affect biomarker levels include pre-transplant characteristics such as donor source, total body irradiation, and chemotherapy conditioning agents, and post-HCT events such as acute GVHD, concurrent medications and/or infection, for example with steroids affecting sBAFF or CMV increasing CXCL10.[55,56] Autologous and time-matched allogeneic HCT controls without cGVHD could be helpful to adjust for some of these variables. In an imagined ideal state (**Table 5**), one might envisage a set of validated biomarkers tailored to certain applications relevant to personalized management of cGVHD. Among these, we focus here on risk assignment biomarkers to predict future development of cGVHD. In order to avoid inappropriately prolonged IST in people who were not destined to get cGVHD, risk assignment biomarkers need to have high positive predictive value (PPV). One would also like modest

negative predictive value (NPV) so as not to miss people who could benefit from pre-emptive treatment, although lower NPV is less problematic because if untreated, these patients simply resort to current standard of care which is receiving treatment if overt cGVHD develops. We note that a single recommended PPV and/or NPV can't be endorsed here for use in preemptive interventions, and that several factors would need to be taken into consideration including the clinical context (i.e. patient, disease, HCT variables), trial type (focused on organ-specific vs. systemic interventions and outcomes), and the risk profile of the intervention (where more stringent PPV would be needed with interventions with greater risks).[12] It is unlikely that a single biomarker will be sufficient, and a biomarker panel (alone or with consideration of other clinical risk factors for cGVHD development) will be needed. Careful consideration will need to be given to the translation of biomarker (or biomarker panel) performance into clinical trial eligibility criteria and study design. Modeling approaches, including machine learning, may help in identifying a core set of clinical and biomarker variables that accurately predict cGVHD development, and thus could be translated into eligibility criteria for preemptive therapy trials. We note there are multiple considerations involved, and that selection of a machine learning approach requires careful consideration of hypotheses to be tested, model complexity, sample similarity, number of clusters, and thresholds for dichotomizing variables.

Recommendation: Multi-center studies with clinical and biomarker data collection before onset of cGVHD per NIH criteria are needed, in order to identify appropriate eligibility criteria triggers for preemptive clinical trials.

Choosing the most appropriate preemptive agents

Even if one assumes the existence of reliable predictors of future overt diagnostic GVHD manifestations, the portfolio of novel cGVHD therapies that are affordable, non-toxic, and feasible to use is limited. The risk for over-treatment, increasing opportunistic infections, compromising graft vs. malignancy effect, and drug-specific adverse events are key concerns with preemptive therapy. It should also be noted that ideal features of preemptive therapy are context-dependent. For example, in HCT for non-malignant disease, more potent interventions that completely prevent cGVHD morbidity are desirable. In the setting of HCT for malignant diseases, however, the desired end goal is more nuanced, most likely avoidance of more

severe cGVHD with little effect on graft vs. malignancy effects (assuming these can't be mechanistically separated based on current knowledge). In total, major goals are to first define who has impending cGVHD, second to select interventions with optimal safety and efficacy profiles, and third to define optimal trial design.

There is no precedent for selecting ideal interventions for preemptive therapy, as cGVHD trials to date have been prophylactic or for initial or subsequent treatment. A first step would be to align on "*forme fruste*" clinical signs, symptoms, or biomarkers that portend cGVHD phenotypes requiring treatment. Interventions must be rationally aligned with known pathogenesis, disrupt cGVHD natural history so that preemptive therapy may be eventually stopped, have a favorable risk-to-benefit ratio, and be cost-effective and convenient.

Key features of ideal preemptive interventions are presented (**Table 6**) to illustrate the considerations involved in selecting an agent for study. Given the current uncertainty in risk/benefit profile of preemptive interventions, a major consideration would be to prioritize those agents already tested in cGVHD therapy or similar human immune-mediated disorders. Some currently available therapeutic agents that fulfill some of these criteria include ibrutinib, KD025 (belumosudil), ruxolitinib, fostamatinib, SNDX-6352 (axatilimab), mTOR inhibitors (sirolimus, everolimus), AMG-592 (efavaleukin alfa), and methotrexate. Among these, many are orally deliverable. Low-dose weekly methotrexate is well-tolerated,[57] but published experience is very limited in early fasciitis/sclerosis.[58] The ROCK2 inhibitor (KD025) is mechanistically novel and targets anti-fibrotic pathways, is well-tolerated, and showed promising efficacy in moderate-to-severe steroid-refractory cGVHD making a potential good first candidate (NCT03640481).[51] The BTK-inhibitor, ibrutinib, garnered FDA approval for SR-CGVHD in 2017,[50] but given the non-trivial adverse events profile, acalabrutinib with less cardiac and coagulation concerns[59] might be a better choice if initial suggestions of efficacy with this class of agents are confirmed. Ruxolitinib was FDA approved for SR-AGVHD in 2019; the REACH3 CGVHD treatment trial has completed enrollment (NCT03112603) and the adverse event profile should be considered. Rates and severity of opportunistic infections and recurrent malignancy rates would certainly need to be evaluated whichever intervention is selected for study given risk-benefit considerations. Another agent might include the SYK inhibitor, fostamatinib (NCT02611063).

Recommendation: Several agents that could be tested preemptively are available. Selection of agents for preemptive therapy will need to consider safety, biologic rationale, feasibility, cost

and logistical concerns in dissemination. Clinical studies will require academic and industry collaboration.

Potential study designs for preemptive trials

Because there is no precedent for preemptive therapy for cGVHD, efficient early phase trials should allow sequential testing of therapeutic agents. Topical or systemic interventions could be selected to target organ-specific cGVHD phenotypes or systemic interventions selected to cover all manifestations of cGVHD. Relevant short-term study endpoints could be to prevent any cGVHD (or specifically NIH moderate/severe cGVHD) or need for initiation of systemic therapy, or focus on preventing cGVHD disability, while avoiding non-relapse mortality and relapse. The primary endpoint for the first scenario would be cGVHD incidence (or moderate/severe cGVHD incidence) and/or need for initiation of systemic therapy. In the second scenario the incidence would include only severe forms of cGVHD, either individually (ocular, sclerosis, joint/fascia, BOS, esophageal stricture) or in any organ. Trial design should consider whether to focus on organ-specific outcomes and be powered accordingly.

Prevention of ocular or localized sclerotic cGVHD provides opportunities to test topical interventions. To prevent severe ocular cGVHD, study subjects might be identified by a reliable risk assignment biomarker (high PPV, modest NPV). A low risk-to-benefit intervention, like autologous serum tears,[60] or topical preparation of vitamin-A coupled liposomes with HSP467 siRNA,[61] could be studied. A single arm study with historically benchmarked goal (primary endpoint) might show a reduced incidence of any, or just moderate-to-severe, ocular CGVHD. A randomized placebo-controlled design, similar to the Restasis prophylaxis trial by Jagasia (NCT00755040) is an alternative. A second example could be to borrow the approach of a randomized placebo-controlled trial of topical ruxolitinib for cutaneous cGVHD (NCT03395340) but offer as a preemptive intervention; study candidates might have *forme fruste* sclerosis with edema plus a positive risk assignment sclerosis biomarker or positive MRI for sclerosis (see WG2a).

Clinicaltrials.gov currently shows that most lung cGVHD interventions address established BOS (NCT03674047),[62] whereas preemptive trials might test novel agents in subjects with earlier airflow obstruction (AFO) plus a positive risk assignment biomarker. Since not all AFO leads to BOS, biomarkers would once again need high PPV in particular. Generally well-tolerated

candidate systemic therapeutics might be KD025, or ruxolitinib. Controversy surrounding use of azithromycin in prevention of cGVHD remains, while at least newer published data suggests no evidence of increased relapse risk among patients with established BOS.[63] More targeted anti-neutrophil strategies such as orally administered neutrophil elastase inhibitor (NCT02669251) may hold promise for pre-emptive trials in BOS. Study designs could be modeled on the randomized double-blinded 6-month controlled trial of inhaled corticosteroid plus long-acting beta agonist.[62] This study, together with natural history study showing rapid FEV1 decline before a BOS diagnosis,[64] provides proof of concept that preemptive therapy might be efficacious for lung GVHD if administered early in the disease course.

An additional example of a potential systemic preemptive therapy is a study to prevent generalized sclerotic skin cGVHD with well-tolerated agents like low-dose methotrexate, KD025, CSF-1R targeting, or ruxolitinib. Eligibility would target a more homogeneous study cohort destined to develop morbid cGVHD-sclerosis/fasciitis, for example patients with: (a) positive risk assignment or predictive biomarkers for fasciitis or sclerosis and/or, (b) early stable decline in total P-ROM or, fluctuating P-ROM decline plus muscle cramping, arthralgias (*forme fruste*) or, (c) edema with positive sclerosis biomarker or positive Myoton or other test (see WG4). The study would be randomized and placebo controlled. The endpoint would be 3-year cumulative incidence of fasciitis/sclerosis benchmarked against a current expected cumulative incidence.[7,8] These studies would need to specify how to manage concomitant IST tapers when preemptive therapy is added. Development of biomarkers to predict and monitor therapy effects on the development of cGVHD would be essential component of such trials.

Analytic considerations in preemptive trials

Endpoints: The most obvious endpoint in the preemptive setting is the occurrence of overt cGVHD which meets NIH diagnostic criteria. While the development of cGVHD occurs most often within one to two years post HCT, the number of cases of cGVHD is influenced by the number of deaths without cGVHD that occur, as death without cGVHD is a competing-risk event for cGVHD. An increase in the risk of this competing risk can lower the observed cumulative incidence of cGVHD (if not the risk), and this needs to be carefully considered when examining the potential efficacy of an agent intended to prevent overt cGVHD, especially in the context of

single-arm trials. The timing of a preemptive intervention is important; it must be close enough to when cGVHD becomes clinically evident that few unrelated events intervene but early enough that the intervention can avert cGVHD. Biomarker levels may be useful for selecting trial candidates, but they are not good endpoints, because biomarkers per se do not indicate clinical benefit. Development of biomarkers to predict and monitor pre-emptive therapy effects on cGVHD should be essential component of such trials.

Composite endpoints are another choice for a primary endpoint, and if appropriately constructed, they do not have competing risks. One such endpoint is cGVHD-free, relapse-free survival (CRFS) which is relapse-free survival without moderate-severe cGVHD. Composite endpoints have the advantage of more events occurring, thereby increasing statistical power to observe differences between groups, at least if the composite is a time-to-event endpoint. However, composite endpoints must be used with a degree of caution, as well. For example, group A in a trial might have a higher risk of death than group B, yet also a better CRFS than in Group B owing to more cGVHD and relapse events in group B. Each of the components that make up a composite endpoint, therefore, should be of similar clinical relevance to the other components.

Study Designs: Preemptive trials are likely to be designed without much preliminary data. Initial trials will likely be single-arm studies to obtain an estimate of effect (e.g., occurrence of cGVHD) or phase II studies with "success" defined quite simply as a lower observed rate of cGVHD than a historical benchmark.

Randomized Phase 2 trials might be appropriate in situations where multiple treatments are ready for testing in the preemptive setting. Such trials are not designed to find statistical differences between treatments, rather they generally intend to find one (or more) treatments that look the most promising to move forward to a definitive trial. This strategy is often referred to as "pick-the-winner". Placebo controls are often included, and the "winning" arm would be required to better than placebo by some pre-specified amount. A randomized Phase 2 trial might also be envisioned as part of a Phase 2/3 design, in which case the phase 2 can be expanded to a phase 3 if early results are promising.

The last broad category is master protocols, subsets of which are commonly referred to as basket or umbrella trials.[65] Basket trials are generally conducted with one treatment across a

variety of indications. Umbrella trials are typically conducted in a single disease "type" with treatments dictated by a characteristic or group of characteristics (e.g., a biomarker or group of biomarkers). Each group in an umbrella trial is randomized to an experimental treatment or a placebo/standard-of-care, and the number of patients is selected to have sufficient power to observe a statistically significant difference in outcome between experimental and placebo groups. The significance level in such trials is often chosen to be larger than 5%. These master protocol types have not been utilized in cGVHD research to date.

Recommendation: Initial preemptive therapy trials should have clear rationale for selection of the agent to be tested, clear eligibility criteria (that identify a population at high risk for cGVHD development), rigorous design with safety and efficacy endpoints, and have a justified benchmark for success to warrant additional study beyond initial phase II testing.

Conclusions

We anticipate that risk assignment cGVHD biomarkers appropriate to guide preemptive interventions could be validated within 3 years, and that modeling approaches will permit accurate identification of HCT recipients at high risk for cGVHD development. Within 3-7 years, early phase II trials will be conducted testing efficacy and safety of preemptive interventions. Larger phase, confirmatory studies with longer-term success endpoints will build from this foundation.

Table 1. Clinical risk factors for development of NIH chronic GVHD

Reference	Flowers et al., 2011	Afram et al., 2018	Lazaryan et al., 2016	Qayed et al., 2018	Watkins et al., 2017	Grube et al., 2016	Cuvelier et al., 2019
Study type	Single center	Multicenter	Single center	Multicenter Pediatric	Single center Pediatric	Single center	Multicenter, Prospective Pediatric
No. of patients	2941	820	469 (MSD)	476 (MSD-BMT)	442	390	302
History of aGVHD	increase	increase	No effect	NA	No effect	increase	Increase
Older patient age	increase	increase	increase	increase	increase	No effect	Increase
Older donor age	increase	No effect	NA	increase	increase	No effect	NA
Female donor to male recipients	increase	increase	No effect	No effect	No effect	No effect	No effect
Unrelated donor vs. MSD	increase	No effect	NA	NA	No effect	No effect	No effect
HLA-mismatch vs match	increase	NA	NA	NA	No effect	increase	No effect
PBSC vs. BM	increase	No effect	increased	NA	No effect	increase	Increase
ATG or campath	decrease	decrease	NA	NA	NA	NA	No effect

*****aGVHD – acute graft vs. host disease; MSD – matched sibling donor; PBSC – peripheral blood mobilized stem cells; BM – bone marrow; ATG – anti-thymocyte globulin

Table 2. Clinical risk factors for development of sclerotic manifestations in patients with chronic GVHD

Reference	Inamoto et al., 2013	Detrait et al., 2015	Martires et al., 2011
Study type	Single center	Multicenter	Single center
No. of patients	977	705	206
PBSC vs. BM	increase	increase	No effect
HLA-mismatch vs. match	decrease	No effect	No effect
TBI > 450 cGy	increase	NA	increase
Myeloma	NA	increase	NA
ATG	No effect	decrease	NA
Cord blood	No effect	decrease	No effect
Younger patient age	No effect	increase	No effect

*PBSC – peripheral blood mobilized stem cells; BM – bone marrow; TBI – total body irradiation; ATG – antithymocyte globulin

Table 3: Source and role of example chronic GVHD risk assignment biomarkers

Type	Source	Name	Role	References
Risk assignment, Diagnostic, Monitoring	Plasma	CXCL9	Also known as monokine induced by gamma interferon, chemoattractant for activated T cells, macrophages, and NK cells, binds CXCR3	50,53,66,67
Risk assignment, Diagnostic, Monitoring	Plasma	CXCL10	Also known as interferon gamma-induced protein 10, secreted by monocytes/endothelial cells/fibroblasts to attract macrophages, T cells, NK cells, binds CXCR3	50,66-68
Diagnostic, Risk assignment, Predictive	Plasma	sBAFF	B-cell activating factor, promotes survival and differentiation of B cells	55,66-71
Diagnostic, Risk assignment	Plasma	sST2	Soluble receptor that sequesters circulating interleukin-33	53,67
Risk assignment	Cells	CD19+CD21low B Cells	Memory B cells, chronic immune stimulation	72,73
Risk assignment	Cells	IFNγ+ CD4+ T cells	Pro-inflammatory helper T cells	74,75
Risk assignment	Cells	CD4+CD45RA+CD31+ T Cells	CD31+ naïve helper T-cells	72,73
Risk assignment / Differentiating	Cells and Plasma	CD3+CD69+ Activated T-cells; $CD56^{dim}$ NK cells; Naïve Th cells; ST2; sCD13	Chronic GvHD and late acute GvHD share some biomarkers, but cGvHD has a more complex immunopathology	76

Table 4: Chronic GVHD pathophysiology with application to biomarkers and therapeutic translation

Immune pathway perturbation	At risk population	cGVHD manifestation	Biomarkers	Prevention	Treatment
Thymic Dysfunction	MAC High dose TBI Acute GVHD Older age	Autoimmunity (e.g. cytopenias) Sclerosis	Treg deficiency Treg RTE	**PT-Cy** **T_N depletion** *IL-22*	**Low dose IL-2** *Treg transfers*
Th17/(Th1) differentiation	MAC/TBI (IL-6)	BOS Sclerosis	IL-17A **CD146/RORγ CD4 T**	ROCK2 inhibition STAT3 inhibition IL-6(R) inhibition *IL-17A inhibition* *IL-12/23 inhibition* *ROR γT inhibition*	**ROCK2 inhibition** **BTK inhibition** **STAT3 inhibition**
T_{FH} differentiation	not known	BOS	*IL-21* Activated T_{FH}	ROCK2 inhibition BTK inhibition *IL-21 inhibition* *Bcl6 inhibition*	**ROCK2 inhibition** **BTK inhibition**
Germinal Center B cell expansion	not known	BOS	**sBAFF** *GCB cells*	ROCK2 inhibition BTK inhibition aCD20 Ab *SYK inhibition* *PI3Kδ inhibition* *C5aR1 inhibition*	**ROCK2 inhibition** **BTK inhibition** *SYK inhibition*
Alloantibody generation (autoAb)	not known	BOS Scleroderma	**sBAFF** *AlloAb*	ROCK2 inhibition BTK inhibition aCD20 Ab *SYK inhibition* *PI3Kδ inhibition*	**ROCK2 inhibition** **BTK inhibition** *SYK inhibition*
Tissue macrophage (M2) accumulation	not known	BOS Scleroderma	*CSF-1, monocyte/M2 expansion*	*Inhibition of Th17 aCSF-1R*	*aCSF-1R*
Tissue fibrogenesis	not known	BOS Scleroderma	N/A	*Inhibition of Th17* *Pirfenidone*	*aTGFβ*

					Neutrophil elastase inhibitor
composite		All	**ST2, CXCL9, MMP3, osteopontin (6)**	ROCK2 inhibition BTK inhibition STAT3 inhibition	**ROCK2 inhibition** **BTK inhibition** **STAT3 inhibition**

*MAC – myeloablative conditioning; TBI – total body irradiation; Treg – regulatory T cells; BOS – bronchiolitis obliterans syndrome; RTE – recent thymic emigrants; PT-CY – post-transplant cyclophosphamide;

Table 5: Biomarker ideal state

Risk assignment Biomarker	Diagnostic Biomarker	Predictive Biomarker
- Should predict future development of cGVHD - High PPV needed to minimize prolonged inappropriate immune suppressive therapy - Moderate/High NPV	- Validation of "forme fruste", or early not fully diagnostic features of cGVHD: - Examples include: edema, early dry eyes, muscle cramps, arthralgias, early decline in P-ROM scores, early airflow obstruction, abnormal liver function tests	- Among those with established cGVHD, predict: - Future development of highly morbid forms of cGVHD - Response to therapy

*****cGVHD – chronic graft vs. host disease; PPV – positive predictive value; NPV – negative predictive value; P-ROM – photographic range of motion score

Table 6: Features of ideal preemptive therapeutic agents

Feature	Considerations
Biologic rationale	- Selection of interventions that target pathways implicated in cGVHD pathogenesis
Safety	- Low toxicity, limited interactions with concurrent post-HCT medications - Risk profile of intervention commensurate with severity of outcome to be prevented - When possible, minimize disruption of graft vs. malignancy effects
Tolerability/Cost	- Assure intervention adherence - Allow prolonged therapy to prevent late occurring cGVHD events - Patient and health care system able to afford treatment
Efficacy	- Prioritization of agents with demonstrated activity in cGVHD therapy or allied human immune mediated disorders
Transportability	- Logistics of delivering therapy permit dissemination - Orally available agents generally preferred

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