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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-versus-host disease (GVHD) commonly occurs after allogeneic hematopoietic cell transplantation (HCT) despite standard prophylactic immune suppression. Intensified universal prophylaxis approaches are effective but risk possible overtreatment and may interfere with

the graft-versus-malignancy immune response. Here we summarize conceptual and practical considerations regarding preemptive therapy of chronic GVHD, namely interventions applied after HCT based on evidence that the risk of developing chronic GVHD is higher than previously appreciated. This risk may be anticipated by clinical factors or risk assignment biomarkers or may be indicated by early signs and symptoms of chronic GVHD that do not fully meet National Institutes of Health diagnostic criteria. However, truly preemptive, individualized, and targeted chronic GVHD therapies currently do not exist. In this report, we (1) review current knowledge regarding clinical risk factors for chronic GVHD, (2) review what is known about chronic GVHD risk assignment biomarkers, (3) examine how chronic GVHD pathogenesis intersects with available targeted therapeutic agents, and (4) summarize considerations for preemptive therapy for chronic GVHD, emphasizing trial development, including trial design and statistical considerations. We conclude that robust risk assignment models that accurately predict chronic GVHD after HCT and early-phase preemptive therapy trials represent the most urgent priorities for advancing this novel area of research.

Keywords

Chronic graft-versus-host disease; Allogeneic hematopoietic cell transplantation; Consensus; Risk assignment biomarkers; Preemptive therapy

INTRODUCTION

Chronic graft-versus-host disease (GVHD) is common after allogeneic hematopoietic cell transplantation (HCT) despite prophylaxis, and treatment of established chronic GVHD is unsatisfactory for most patients. Intensified chronic GVHD prevention can be effective, yet potentially risks overtreatment and increased infectious morbidity and may compromise beneficial graft-versus-malignancy effects [1]. An alternative approach is to wait until post-HCT events identify patients at very high risk of chronic GVHD or until early, subclinical indications of impending chronic GVHD are present and then intervene. This preemptive approach is the focus of this report.

Enrollment in chronic GVHD prevention trials is based on risk factors known before HCT, regardless of when the preventive intervention is delivered. In contrast, enrollment in preemption trials will be prompted by additional post-HCT events, signs, symptoms, or biomarkers indicating that chronic GVHD risk is higher than previously appreciated. This approach has the advantage of limiting treatment to those who are more likely to benefit. Preemptive treatment may allow more targeted and potentially less damaging therapy, as treatment is provided before overt chronic GVHD is present. This paradigm has been successfully applied in monitoring for cytomegalovirus (CMV) reactivation to prevent CMV disease; however, developing a successful preemptive approach requires the ability to identify subpopulations at very high risk of developing overt disease.

Subclinical features or biomarker algorithms that could prompt preemptive treatment include biomarkers with a high positive predictive value (PPV) for chronic GVHD development or early prediagnostic chronic GVHD signs and symptoms. PPVs and negative predictive values (NPVs) are highly dependent on the future prevalence of disease, which in the

chronic GVHD setting varies from a relatively high overall incidence to low prevalences of rarer subtypes or specific manifestations. When disease incidence is low, high PPV is achieved only when the biomarker has very high specificity.

Preemptive therapies should mechanistically target essential chronic GVHD pathways to prevent the development of clinically important chronic GVHD and its associated burden of immunosuppressive therapy under current treatment standards. Clinical trials are needed to determine whether such early intervention would lower the incidence of moderate to severe chronic GVHD and improve long-term outcomes. The design considerations and challenges for preemptive intervention trials more closely resemble those for prophylaxis trials than those for treatment trials of established moderate to severe chronic GVHD.

Purpose of this Report

The goals of this report are to (1) summarize current evidence about potential very early signs, symptoms, and biomarker patterns that indicate impending chronic GVHD; (2) address selection of preemptive therapy candidates; and (3) make recommendations about study design for preemptive treatment trials.

Summary of Recommendations

1. Preemptive treatment may be the optimal approach, because only people at high risk of chronic GVHD development are treated early to prevent clinically evident chronic GVHD. Rigorous study is needed to determine whether preemptive therapy improves outcomes.
2. Identifying risk assignment markers and clinical indicators with a high PPV after HCT is required for a successful preemptive approach. It is likely that a panel of markers (eg, plasma, serum or urine, cellular, genomic, transcriptomic, proteomic, metabolomic) will be needed to identify appropriate candidates for preemptive trials. Studies to identify these markers should be multi-institutional, ensure accurate clinical diagnosis of chronic GVHD, and use testing methods that are readily translatable into practice.
3. More research is needed to understand the evolution of biological processes after HCT that increase the risk of developing chronic GVHD, thereby improving the predictive capability to guide preemptive therapies.
4. Several potential preemptive treatments could be tested,and trials will need academic and industry collaboration. We recommend National Institutes of Health (NIH) moderate/severe chronic GVHD-free survival as a key primary endpoint in preemptive trials, but other endpoints would be appropriate when risk assignment markers predict specific manifestations of chronic GVHD, such as cutaneous sclerosis or bronchiolitis obliterans. As a key safety measure, relapse rates should be monitored to ensure that preemptive chronic GVHD interventions do not compromise graft-versus-malignancy effects. Infection rates should be monitored as well.

Initial preemptive therapy trials will need to be rigorously designed with attention to eligibility criteria, interventions, clearly stated efficacy and safety measures, and benchmarks to determine whether the results offer sufficient promise for future study.

METHODS

Each working group was created to encourage global engagement in the topic (see the introduction to this series [1]). Four groups worked individually since February 2020 to review the relevant literature and create the initial draft of the manuscript, which was reviewed and commented on by the Steering Committee. Two iterative rounds of comments were collected before 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 manuscript was further revised for submission.

Gaps in *K*nowledge and *Unmet Needs*

Currently available tools (clinical signs and symptoms, risk assignment biomarkers) do not permit identification of a population at sufficiently high risk for subsequent chronic GVHD development to justify currently available preemptive interventions. The required 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, as variations in causal pathways and heterogeneous clinical manifestations are expected among affected patients.

Many possible therapeutic agents used in the treatment of established chronic GVHD could be tested as preemptive interventions. However, a rational prioritization of such agents based on insight into chronic GVHD biology and careful clinical trial design to test the safety and efficacy of these agents are needed. Initial studies may focus on prevention of the syndrome in total (eg, moderate/severe chronic GVHD-free survival), but organ-specific preemptive studies have merit as well. Initial studies will need to clearly define short- and long-term treatment success metrics for interpretation and for planning subsequent larger, confirmatory multicenter studies to advance the field.

Clinical *Risk Factors* for Chronic GVHD

Individual variables known before HCT (eg, patient, donor, graft type) are used to determine prophylactic approaches for both acute GVHD and chronic GVHD and thus are most relevant to WG1 of this NIH Consensus Project. However, these same features may enrich for a patient population that will later manifest early prediagnostic features of chronic GVHD that could be targeted with preemptive interventions. In an analysis of 2941 HCT recipients, the profiles of risk factors for acute and for chronic GVHD were similar, but some notable differences were identified [2]. The use of mobilized peripheral blood stem cells as the graft source was strongly associated with chronic GVHD but not with acute GVHD, the use of female donors for male recipients had a greater effect on the risk of chronic GVHD than on the risk of acute GVHD, and older patient age was associated with chronic GVHD but had no effect on acute GVHD. Donor and recipient HLA mismatch and

the use of unrelated donors had a greater effect on the risk of acute GVHD compared with chronic GVHD, and the use of total body irradiation was strongly associated with acute GVHD but was not statistically significantly associated with chronic GVHD. Established clinical risk factors for the development of chronic GVHD include previous acute GVHD, use of mobilized peripheral blood stem cell grafts, nonuse of ex vivo or in vivo T cell depletion (ie, post-transplantation cyclophosphamide, antithymocyte globulin, and graft manipulation), female donor-male recipient combination, use of unrelated donors, and patient age (Table 1) [2–7]. Among these factors, acute GVHD is the sole post-HCT factor that may increase the risk for chronic GVHD above the baseline level of risk expected based on pre-HCT factors. Clinical risk factors for more severe forms of chronic GVHD, such as sclerosis of the skin and fascia, are listed in Table 2 [8–10].

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 acute GVHD biomarkers into clinical practice and research trials can inform similar efforts for the development of clinically useful chronic GVHD biomarkers. First, accurate clinical data are critical. Second, the collection of research samples should include both calendar-driven and event-driven samples [11–13]. Event-driven samples, obtained before or early during treatment, are particularly valuable, as they can provide crucial clues as to the most promising chronic GVHD biomarkers. Indeed, the algorithm that predicts the development of severe acute GVHD was based on a treatment-response and survival algorithm developed from acute GVHD onset samples [14]. Ultimately the clinical data, including transplantation characteristics, acute GVHD characteristics (eg, target organ severity), and outcomes (eg, response to treatment, survival), were linked to available samples to conduct biomarker validation studies. These strategies led to the development of multiple biomarkers for acute GVHD, including target organ-specific biomarkers, such as REG3α and elafin, and predictive algorithms that use concentrations of multiple biomarkers, such as the Mount Sinai Acute GVHD International Consortium (MAGIC) algorithm for predicting the risk of nonrelapse mortality in patients with acute GVHD [14–22].

Repeated validation of several biomarkers with prognostic significance at acute GVHD onset, as well as the establishment of Clinical Laboratory Improvement Amendments-certified laboratories that can provide rapid results, have allowed for the incorporation of acute GVHD biomarkers as inclusion criteria for clinical trials, enriching trial populations for desired risk factors. For example, ruxolitinib was approved by the Food and Drug Administration (FDA) to treat steroid-refractory (SR) acute GVHD owing in part to the favorable responses observed even in patients with high MAGIC risk scores [23,24]. One of the first trials to use biomarkers as an eligibility criteria was the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 1501 trial that defined acute GVHD 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 2 approaches in this low-risk population [25]. In an acute GVHD preemption study, the MAGIC algorithm was used to identify asymptomatic patients as early as 7 days after transplantation who were at high risk for SR acute GVHD and treat them with alpha-1-antitrypsin. Although this treatment did not reduce the incidence of SR GVHD compared with controls, real-time biomarker risk assignment for preemptive treatment proved feasible early after HCT in this proof-of-concept study [26]. Several other acute GVHD trials incorporating biomarkers (eg, NCT02133924, NCT02525029, NCT03846479) are in progress, illustrating the sustained traction of biomarkers in GVHD clinical research.

The experience of biomarker discovery in acute GVHD can allow us to extrapolate the lessons learned to chronic GVHD, but we anticipate significant challenges [27]. The time course of chronic GVHD spans years, not weeks, and patients are seen primarily in the outpatient setting. The multitude of organ systems involved poses a significant challenge to accurate clinical data capture in the context of routine clinical visits not just for staging, but also for documentation of clinical response to standard-of-care therapy. As discussed in the WG2a report, provider education enhanced by health informatics, with joint ownership of health care teams and patients, will be required to ensure that clinical data are reliable for research studies.

Identifying *Generalizable Markers Useful* for *Preemptive Studies*

Experience has shown that treatment of established chronic GVHD is often unsatisfactory. Initiating therapies earlier in the natural history of the disorder (ie, at an asymptomatic or minimally symptomatic state) has the best chance of mitigating the impact of chronic GVHD once prophylaxis has failed. Validated models that predict chronic GVHD development in asymptomatic patients with sufficiently high certainty to prompt treatment are not yet available. Several chronic GVHD biomarkers have been tested and validated in the multicenter setting with large sample sizes. A variety of markers have been characterized for chronic GVHD diagnosis, but here we present those with application as risk assignment biomarkers (Table 3), which according to FDA BEST recommendations [28] are biomarkers associated with increased risk of developing a condition in an individual who does not yet have clinical evidence of that condition. Clinically actionable thresholds must be defined, however, especially in the absence of clinical manifestations. Changes in biomarker values from serial longitudinal samples are likely important and are underrepresented in the literature. Risk assignment chronic GVHD biomarkers may or may not have overlap with biomarkers relevant to chronic GVHD diagnosis or chronic GVHD treatment response. Thus, unsupervised discovery approaches are needed. Although most studies to date have focused on blood immune cell populations or cytokines/chemokines, informative markers also may arise from interrogation of the target tissues, metabolome, or microbiome. Biomarkers of organ-specific chronic GVHD development are also needed to refine personalized risk determination and targeted interventions. Biomarkers are not limited to biological samples, but also may include results from imaging or other testing.

Conceptually, the ideal risk assignment biomarker has high sensitivity and specificity but, more importantly, should have high PPV and NPV. A biomarker with these ideal

characteristics would give clinicians confidence in applying chronic GVHD preemptive therapies in the population of patients with the greatest potential for direct benefit.

Recommendations.—To achieve the goal of identifying markers for preemptive treatment, we suggest the following. Multi-institutional studies should include HCT centers that use different transplantation approaches to increase generalizability of findings by reflecting “real world” situations. Real-time comprehensive and accurate clinical evaluation and documentation of chronic GVHD manifestations according to the NIH consensus criteria are required, including at diagnosis, over time, and in response to therapy. Prioritization of disease markers that are readily translatable to clinical practice because they are more easily performed in Clinical Laboratory Improvement Amendments-certified laboratories at reasonable cost or are readily available using standard testing or evaluation. Prospective studies addressing these needs are underway (NCT04372524 and NCT04188912).

Targeting the Most Promising Pathways for Preemptive Therapy

Our understanding of the complex pathophysiology of chronic GVHD has improved dramatically over the last 5 years. It is now clear that the disease manifestations of chronic GVHD represent the accumulation of several aberrant immunologic 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 chronic GVHD risk) and preemptive approaches (subsequent interventions delivered based on subclinical risk assignment biomarkers or early prediagnostic signs and symptoms). Thus, preventing chronic GVHD (and particularly moderate to severe chronic GVHD) should always be the goal. To date, ex vivo strategies including naive T cell depletion and TCRα/β and $CD19^+$ B cell depletions, as well as in vivo depletion strategies including antithymocyte antibody, alemtuzumab, and post-transplantation cyclophosphamide, have proven to be the most effective prophylactic strategies [29,30]. The subsequently invoked immune pathways during chronic GVHD are likely to coexist in an individual, depending on the particular disease features (eg, sicca syndrome, sclerotic skin, bronchiolitis obliterans syndrome [BOS]) and concurrent immune suppression (reviewed in [31,32]). Although the immune pathways attributed to chronic GVHD were initially described in mice, these have been partially confirmed in patient peripheral blood samples (Table 4).

The thymus is perhaps the organ most sensitive to acute GVHD, in which both recipient thymic epithelium and donor dendritic cells are targets, resulting in impaired generation of tolerogenic $FoxP3^+$ regulatory T cells (Tregs) [33] and the failure to delete self-reactive T cell clones [34]. The effects of acute GVHD in this process are further exacerbated by myeloablative conditioning and increasing recipient age [32]. These defects result in the expansion of autoreactive T cell clones and a failure to generate and maintain Treg homeostasis in the periphery, generating the full spectrum of chronic GVHD, including sicca and fibrosis in preclinical systems [34]. Therapies aimed at improving Treg function have focused on low-dose IL-2 administration and/ or Treg transfers [35]. The former has

demonstrated important responses in a significant proportion of patients with refractory chronic GVHD but requires long-term systemic administration of the cytokine [36,37].

The Th17 (CD4) and Tc17 (CD8) differentiation of donor T cells in the periphery is required for the generation of scleroderma and BOS in most preclinical systems, although these 2 pathologies seldom coexist [38,39]. This pathway requires IL-6 signaling, which is dysregulated early after HCT, particularly after myeloablative conditioning, and is controlled by the transcription factor RORγt [40,41]. Both Th17 and Tc17 are polyfunctional and lineage-promiscuous and secrete large amounts of Th1 (eg, IFNγ, TNF) and Th17 (IL-17A, GM-CSF) cytokines [41], which are important in the manifestations of late acute and chronic GVHD. Therapies that block IL-17A, RORγt [38,40], and broad cytokine signaling (eg, STAT1/3 by ruxolitinib) [42] have shown efficacy in preclinical models. An overlapping lineage of T cells producing IL-22 but not IL-17A (Th22) is also present in patient skin lesions and was found to induce skin chronic GVHD in mice [43]. Whether inhibition of cytokines directly responsible for the initiation (IL-6 and IL-12), amplification (IL-21), and maintenance (IL-23) of these lineages are effective in chronic GVHD treatment remains to be formally tested, and IL-12/23p40 would seem a particularly attractive initial target.

Coordinated T and B cell responses are known to drive chronic GVHD. T follicular helper (Tfh) T cell differentiation after HCT is characterized by IL-21 secretion and controlled by the transcription factor bcl6 [44]. Tfh cells promote aberrant germinal center B (GCB) cell reactions. Associated with Tfh responses, B cells from patients with clinically active chronic GVHD have significantly increased survival rates along with constitutive activation and B cell activating factor (BAFF)-associated signaling [45]. Aberrant germinal center B cell responses are associated with alloantibody generation and BOS after HCT in mice [44]. 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 [44,46,47], bcl6 [48], and PI3K delta [49] 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 [50] or delete the latter once formed (eg, proteasome inhibitors) [51] have shown promise in preventing and treating chronic GVHD, respectively [52]. Targeting total $CD20^+$ B cells can result in excess levels of BAFF relative to the numbers of aberrantly activated B cells [53]. $CD27^+$ B cells from patients with chronic GVHD constitutively produce IgG [54] and are hyperresponsive to surrogate antigen and DAMPs [45]. Aberrant B cell receptor (BCR) signaling occurs in B cells from patients with active chronic GVHD. Inhibitors of the spleen tyrosine kinase (SYK), which is involved in donor B cell receptor signaling [55] and antigenresponsiveness during chronic GVHD, also have shown promise in preventing and treating BOS in mice [56,57]. In mice, BAFF promotes SYK protein and BCR activation [58]. Emerging evidence suggests that pathways that mediate chronic GVHD can be blocked without affecting antitumor responses.

Chronic GVHD involves a multitude of immune mechanisms, including aberrant B cell and T cell responses. Thus, it is unsurprising that to date, inhibitors of kinases involved in the pathogenic differentiation of both lineages have shown the greatest potential for the preemptive treatment of chronic GVHD. Ibrutinib, a dual inhibitor of Bruton’s tyrosine kinase and IL-2-inducible T cell kinase, was shown to be highly active in murine models

of chronic GVHD [59] and has undergone successful phase II testing, such that it is now approved for treatment of SR chronic GVHD [60]. This agent, originally developed for the treatment of chronic lymphocytic leukemia, has some toxicity and tolerability problems in this sensitive HCT recipient population [60]. An inhibitor of Rho-associated kinase 2 (ROCK2) is a highly effective inhibitor of STAT3 phosphorylation-dependent Tfh and Th17 differentiation (and the subsequent germinal center B cell reaction) [61] and an inducer of STAT5 phosphorylation (and the subsequent increase in Tregs), and is associated with significant efficacy in preclinical models [62]. Promising early evidence of clinical efficacy and tolerability is also emerging, and the agent has been granted FDA breakthrough status.

Chronic GVHD development in models of sclerodermatous disease relies on M2 macrophage differentiation, in which these tissue IL-17A- [40] and CSF-1R-dependent macrophages [63] secrete large amounts of fibrogenic factors (eg, TGFβ, PDGF) that cause collagen deposition in target tissue [63]. Agents that inhibit CSF-1R or provide broad anti-inflammatory (including TGFβ) inhibition (eg, pirfenidone) [64] are highly active in preclinical models [63] and are currently undergoing clinical testing.

Although all these pathways generate immunologic defects that may be quantifiable clinically, currently the most robust biomarkers of chronic GVHD include a composite panel including ST2, CXCL9, MMP3, and osteopontin [65]. These markers do not permit recognition of a dominant immune pathway necessary to personalize drug selection for patients at high risk of chronic GVHD, however. Based on current knowledge, a fully personalized approach directing specific therapeutic agents to a dominant immune target or pathway within individual patients is not possible. Consequently, current efforts to preempt chronic GVHD will require an agent that is well tolerated and active across multiple immune pathways known to be involved in chronic GVHD. Considering this, ROCK2 and other JAK/STAT inhibitors would seem to be the most appropriate agents at present.

Recommendations.—Continuing research to identify specific pathways involved in both systemic and organ-specific chronic GVHD is needed to facilitate identification of candidates for preemptive intervention.

Challenges with a *Preemptive Therapy Approach*

Barriers to the rational selection of agents for preemptive trials include incomplete understanding of the pathogenesis of organ-specific chronic GVHD manifestations, the protean nature of chronic GVHD manifestations, the variable time to chronic GVHD onset, and the need to correct the underlying dysfunction leading to chronic GVHD to prevent chronic GVHD recurrence on 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 interventions should be foundationally linked to mechanistic steps in chronic GVHD pathogenesis and should 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. Prediagnostic, or “forme fruste,” chronic GVHD manifestations are not established, and serum, plasma, urine, and cellular biomarkers have not passed

the verification phase, which requires real-time rather than simply retrospective validation. Some candidate chronic GVHD biomarkers include CXCL9, CXCL10, ST2, MMP3, osteopontin, CD163, IL-17A, IL-21, soluble BAFF (sBAFF), and aminopeptidase N; cellular populations, such as $CD4^{+}CD45RA^{+}$, $CD19^{+}CD21^{low}$, natural killer (NK) and NK subsets [66], Tregs, and CD146/RORγCD4; and signaling pathways (T cell: phospho-SYK and phospho-STAT3; B cell: phosphorylated Bruton's tyrosine kinase and BCR hyperresponsiveness) [38,67]. Such biomarkers have been associated with overall chronic GVHD rather than with a specific phenotype, and sensitivity and specificity may vary for different phenotypes. Moreover, most published chronic GVHD biomarkers have shown utility as diagnostic markers rather than risk assignment markers, which are most relevant for chronic GVHD preemptive therapy. In addition, these considerations may differ among children, adolescents, and adults [68,69].

Cofactors that might affect biomarker levels include pretransplantation characteristics, such as donor source, total body irradiation, and chemotherapy conditioning agents; interval from HCT; and post-HCT events, such as acute GVHD, concurrent medications and/or infection, for example, with steroids affecting sBAFF or CMV increasing CXCL10 [70,71]. Autologous and time-matched allogeneic HCT controls without chronic GVHD 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 personalized management of chronic GVHD. To avoid inappropriate overtreatment in individuals who were not destined to develop chronic GVHD, risk assignment biomarkers must have high PPV. Moderately high NPV is also desirable so as not to miss individuals who could benefit from preemptive treatment, although lower NPV is less problematic because if untreated, these patients would simply receive the current standard of care to begin treatment if overt chronic GVHD develops.

No single recommended PPV or NPV can be endorsed for use in preemptive interventions. Several factors would need to be taken into consideration, including the clinical context (ie, patient, disease, and HCT variables), the trial type (focused on organ-specific versus systemic interventions and outcomes), and the risk profile of the intervention (with higher PPV needed for interventions of greater risk) [13]. Low specificity of a test has a major effect on the PPV for conditions of low prevalence, such as BOS, and low sensitivity has a major effect on the NPV for conditions of high prevalence, such as any chronic GVHD. It is unlikely that a single biomarker will be sufficient, and a biomarker panel (alone or with consideration of other clinical risk factors for chronic GVHD development) will be needed. Translation of the biomarker (or biomarker panel) performance into clinical trial eligibility criteria and study design will require careful consideration. Discovery through machine learning and modeling approaches may help identify a core set of clinical and biomarker variables that accurately predict chronic GVHD development for use as eligibility criteria in preemptive therapy trials. Multiple considerations are involved, and 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.—Multicenter studies with clinical and biomarker data collection before onset of chronic GVHD in accordance with NIH criteria are needed to identify and validate 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 chronic GVHD therapies that are affordable, nontoxic, and feasible to use is limited. The risk of overtreatment, increasing opportunistic infections, compromising graft-versus-malignancy effects, and drug-specific adverse events are key concerns with preemptive therapy. Moreover, agents used for chronic GVHD treatment might not have comparable risk/benefit profiles when repositioned in a preemptive intervention, highlighting the need for well-designed clinical trials in this area.

The ideal features of preemptive therapy are context-dependent. For example, in HCT for nonmalignant disease, more potent interventions that completely prevent chronic GVHD morbidity are desirable, although potential non-chronic GVHD effects, such as increased risk of infection or organ toxicity, also must be considered. In the setting of HCT for malignant diseases, however, the desired end goal is more nuanced, most likely avoidance of moderate/severe chronic GVHD, and with little effect on graft-versus-malignancy effects (assuming that these cannot be mechanistically separated, based on current knowledge). Major goals are (1) to define who has impending chronic GVHD, (2) to select interventions with optimal safety and efficacy profiles, and (3) to identify the optimal trial design.

There is no precedent for selecting ideal interventions for preemptive therapy, as chronic GVHD trials reported to date have been prophylactic in nature or for the initial or subsequent treatment of established chronic GVHD. A first step would be to align on forme fruste clinical signs, symptoms, or biomarkers that portend chronic GVHD phenotypes necessitating treatment. Interventions must be rationally aligned with known pathogenesis, disrupt chronic GVHD natural history so that preemptive therapy may be eventually stopped, have a favorable risk-to-benefit ratio, and be cost-effective and convenient. A risk assignment marker that is causally linked with subsequent chronic GVHD development, targetable through a therapeutic agent, and measurable for pharmacodynamic effect of the intervention would be ideal. Other markers may only portend subsequent risk without having a clear causal association with chronic GVHD.

Key features of ideal preemptive interventions are presented in Table 6 to illustrate the considerations involved in selecting an agent for study. Given the current uncertainty in risk/benefit profiles of preemptive interventions, a major consideration would be to prioritize those agents that have already been tested in chronic GVHD therapy or similar human immune-mediated disorders. Currently available therapeutic agents that fulfill some of these criteria include ibrutinib, KD025 (belumosudil), ruxolitinib, fostamatinib, SNDX-6352 (axatilimab), mTOR inhibitors (sirolimus, everolimus), IL-2 (proleukin; AMG-592 [efavaleukin alfa; IL-2 mutein]), proteosome inhibitors (ixazomib, carfilzomib), anti-CD20 (rituximab, ofatumumab), and methotrexate. Many of these agents are orally deliverable. Low-dose weekly methotrexate is well tolerated [72], but published experience in early fasciitis/sclerosis is very limited [73]. The ROCK2 inhibitor (KD025)

is mechanistically novel and targets antifibrotic pathways, is well-tolerated, and has shown promising efficacy in moderate-to-severe SR chronic GVHD making a potential good first candidate (NCT03640481) [62]. The BTK inhibitor ibrutinib garnered FDA approval for SR chronic GVHD in 2017 [60], but given the nontrivial adverse events profile, acalabrutinib, an agent with less cardiac and coagulation concerns [74], might be a better choice if initial suggestions of efficacy with this class of agents are confirmed. Ruxolitinib received FDA approval for SR acute GVHD in 2019; the REACH3 chronic GVHD treatment trial (NCT03112603) has completed enrollment. Rates and severity of opportunistic infections and recurrent malignancy would certainly need to be evaluated whichever intervention is selected for study, given risk-benefit considerations.

Recommendation.—Several agents that could be tested preemptively are available. Selection of agents for preemptive therapy will need to consider safety, biological 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 chronic GVHD, efficient early-phase trials should allow sequential testing of therapeutic agents. Topical or systemic interventions could be selected to target organ-specific chronic GVHD phenotypes, and systemic interventions could be selected to cover all manifestations of chronic GVHD. Overall, for trials focused on the prevention of chronic GVHD in total, we recommend NIH moderate/severe chronic GVHD-free survival as a primary endpoint of overall preemptive therapy trials, and also suggest that the full extent of outcomes (eg, chronic GVHD, relapse, death, infectious complications) should be transparently reported to ensure complete review of the effects of such interventions. Mild chronic GVHD or minimal nondiagnostic features of chronic GVHD would not be included in this recommended primary outcome measure but could be reported as secondary outcomes. Additional short-term endpoints could include the initial occurrence of any chronic GVHD, moderate/severe chronic GVHD, pharmacodynamic measures, and safety. Longerterm outcomes of interest will include prevention of chronic GVHD-associated disability, late morbidity and mortality, quality of life, and discontinuation of immune suppression. Separately, trials focused purely on organ-specific preemption will require specific protocol-defined organ-specific outcome measures. In total, any type of preemptive therapy trial would need to specify how to manage concurrent immunosuppressive medications and how to taper such medications when preemptive therapy is added.

Preemptive trials for specific chronic GVHD subgroups, including ocular sicca, BOS, and cutaneous sclerosis, have additional considerations. Prevention of ocular or localized sclerotic chronic GVHD provides opportunities to test topical interventions. To prevent severe ocular chronic GVHD, study subjects might be identified by a reliable risk assignment biomarker (high PPV, modest NPV), where a low risk-to-benefit intervention, such as autologous serum tears [75] or topical preparation of vitamin A-coupled liposomes with HSP467 siRNA [76] could be studied. A single-arm study with an historically benchmarked goal (primary endpoint) might show a reduced incidence of any, or only

moderate to severe, ocular chronic GVHD. The most rigorous design is the randomized placebo-controlled study in which topical ophthalmic (eg, cyclosporine) or cutaneous (eg, ruxolitinib) preparations could be tested to determine whether they prevent overt signs and symptoms. If sclerosis is targeted, then study candidates might have forme fruste sclerosis with edema plus a positive risk assignment biomarker for sclerosis or positive magnetic resonance imaging for sclerosis in the absence of symptoms (see WG2a).

Clinicaltrials.gov currently shows that most lung chronic GVHD interventions address established BOS (ruxolitinib; NCT03674047) [77], whereas preemptive trials might test novel agents in subjects with earlier airflow obstruction plus a positive risk assignment biomarker. Because not all airflow obstruction leads to BOS, biomarkers would once again need high PPV. Controversy surrounding the use of azithromycin in the prevention of chronic GVHD remains [78], but recent published data suggest no evidence of increased relapse risk of recurrent malignancy among patients treated with azithromycin for established BOS, although an increased incidence of secondary neoplasms was observed [79]. More targeted antineutrophil strategies, such as orally administered neutrophil elastase inhibitor (NCT02669251), may hold promise for preemptive trials in BOS. Preemptive studies could be modeled on the randomized double-blinded 6-month controlled trial of inhaled corticosteroid plus a long-acting beta agonist for newly diagnosed BOS [77]. This study, together with a longitudinal study showing a rapid decline in forced expiratory volume before a diagnosis of BOS [80], 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 chronic GVHD 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 chronic GVHD sclerosis/fasciitis. This category could include patients with (1) positive risk assignment or predictive biomarkers for fasciitis or sclerosis, (2) early stable decline in total photographic range of motion score (P-ROM) or fluctuating P-ROM decline plus muscle cramping and arthralgias (forme fruste), or (3) edema with positive sclerosis biomarker or positive Myoton or another test (see WG4). The study design would need to consider the current expected incidence of cutaneous sclerosis under routine care [8,9].

Analytic *Considerations in Preemptive Trials*

Endpoints.—We currently recommend NIH moderate/severe chronic GVHD-free survival as the primary outcome for preemptive therapy trials. Importantly, we acknowledge the diversity of chronic GVHD manifestations and the varied significance of each. Thus, careful descriptions of the type and severity of chronic GVHD-associated considered failure in a preemptive therapy trial will be needed. Although the development of chronic GVHD occurs most often within 1 to 2 years post-HCT, the number of cases of chronic GVHD is influenced by the number of deaths without chronic GVHD that occur, given that death without chronic GVHD is a competing-risk event for chronic GVHD. An increase in the proportion who experience this competing risk can lower the observed cumulative incidence of chronic GVHD (if not the risk), and this question must be carefully considered when

examining the potential efficacy of an agent intended to prevent overt chronic GVHD, especially in the context of single-arm trials. The timing of a preemptive intervention is important; it must be close enough to when chronic GVHD becomes clinically evident such that few unrelated events intervene but early enough so that the intervention can avert chronic GVHD. Biomarker levels may be useful for selecting trial candidates, but they are not good endpoints, because biomarkers per se do not indicate clinical benefit. The development of biomarkers to predict and monitor preemptive therapy effects on chronic GVHD should be an essential component of such trials.

Composite endpoints may also be considered as secondary outcome measures in preemptive trials. One such endpoint is chronic GVHD-free, relapse-free survival (CRFS) which is relapse-free survival without moderate to severe chronic GVHD. Multiple other trial endpoints could be highly informative. For example, long-term success metrics (eg, durable freedom from chronic GVHD, ability to discontinue immunosuppressive therapy) will be needed to fully validate earlier success metrics in initial preemptive therapy trials. Patient-reported outcomes (eg, chronic GVHD symptom burden, quality of life, patient-reported function or disability) have great importance and should be included in preemptive trials.

Study Designs.—Preemptive trials are likely to be designed without much preliminary data. In addition to standard phase I, phase 2, randomized phase 2, and phase 2/3 study designs, novel approaches, such as basket, umbrella, and adaptive designs, could be considered [81]. 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 (eg, 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 outcomes between the experimental and placebo groups. The type 1 error in such trials is often chosen to be >5%. These master protocol types have not been used in chronic GVHD research to date. Another attractive trial design would be an adaptive platform that could provide flexibility to test multiple agents sequentially with decision rules to guide ongoing testing or rejection of given interventions. These trial designs would require extensive oversight and planning to execute, as well as collaboration with multiple sponsors.

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

CONCLUSIONS

We anticipate that within 3 years, risk assignment chronic GVHD biomarkers appropriate to guide preemptive interventions will be validated and modeling approaches will permit accurate identification of HCT recipients at high risk for chronic GVHD development. Within 3 to 7 years, early phase 2 trials will be conducted to test the efficacy and safety

of preemptive interventions. Larger phase 3 confirmatory studies with longer-term success endpoints will build from this foundation. Such approaches have the potential to have a meaningful impact on chronic GVHD development and improve disease management before damage becomes irreversible.

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APPENDIX:

APPENDIX: NATIONAL INSTITUTES OF HEALTH CONSENSUS DEVELOPMENT PROJECT ON CRITERIA FOR CLINICAL TRIALS IN CHRONIC GVHD

Steering Committee

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Table 1

Clinical Risk Factors for the Development of NIH Chronic GVHD

Risk Factor	Study						
	Flowers et al., 2011 [2]	**Afram et al., 2018 [3]**	**Lazaryan et al., 2016 [4]**	**Qayed et al., 2018 [7]**	**Watkins et al., 2017 [5]**	**Grube et al., 2016 [6]**	**Cuvelier et al., 2020 [69]**
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 acute GVHD	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 versus MSD	Increase	No effect	NA	NA	No effect	No effect	No effect
HLA-mismatch versus match	Increase	NA	NA	NA	No effect	Increase	No effect
PBSCs versus BM	Increase	No effect	Increase	NA	No effect	Increase	Increase
ATG or campath	Decrease	Decrease	NA	NA	NA	NA	No effect

MSD indicates matched sibling donor; BMT, bon marrow transplantation; NA, not applicable; PBSCs, peripheral blood mobilized stem cells; BM, bone marrow; ATG, antithymocyte globulin.

Table 2

Clinical Risk Factors for Development of Sclerotic Manifestations in Patients with Chronic GVHD

Risk Factor	Study		
	Inamoto et al., 2013 [8]	**Detrait et al., 2015 [9]**	**Martires et al., 2011 [10]**
Study type	Single center	Multicenter	Single center
No. of patients	977	705	206
PBSCs versus BM	Increase	Increase	No effect
HLA-mismatch versus 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

TBI indicates total body irradiation.

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 IFNγ (MIG), chemoattractant for activated T cells, macrophages, and NK cells; binds CXCR3	[61,65,82,83]
Risk assignment, diagnostic, monitoring	Plasma	CXCL10	Also known as IFNγ-induced protein 10 (IP-10), secreted by monocytes/endothelial cells/fibroblasts to attract macrophages, T cells, and NK cells; binds CXCR3	[60,82–84]
Diagnostic, risk assignment, predictive	Plasma	sBAFF	B cell activating factor, promotes survival and differentiation of B cells	[70,82–87]
Diagnostic, risk assignment	Plasma	sST2	Soluble receptor that sequesters circulating IL-33	[65,83]
Risk assignment	Cells	$CD19^{+}CD21^{low}$ B cells	Memory B cells, chronic immune stimulation	[88,89]
Risk assignment	Cells	IFNγ^{+} $CD4^{+}$ T cells	Proinflammatory helper T cells	[90,91]
Risk assignment	Cells	$CD4^{+}CD45RA^{+}CD31^{+}$ T cells	$CD31^{+}$ naïve helper T cells	[88,89]
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 chronic GVHD has a more complex immunopathology	[68]

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Table 4

Chronic GVHD Pathophysiology with Application to Biomarkers and Therapeutic Translation

Immune pathway perturbation	At risk population	Chronic GVHD manifestation	Biomarkers	Prevention	Treatment
Thymic dysfunction	MAC; high-dose TBI; acute GVHD; older age	Autoimmunity (eg, 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/ ROR γ 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; PI3K δ 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; PI3K δ 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
Overall		All	ST2; CXCL9; MMP3; osteopontin (6)	ROCK2 inhibition; BTK inhibition; STAT3 inhibition	ROCK2 inhibition; BTK inhibition; STAT3 inhibition

MAC indicates myeloablative conditioning; TBI, total body irradiation; RTE, recent thymic emigrants; PT-CY, post-transplantation cyclophosphamide.

Table 5

Biomarker Ideal State

Risk Assignment Biomarker	Diagnostic Biomarker	Prognostic Biomarker	Predictive Biomarker
- Should predict future development of chronic GVHD -Accurate (both sensitive and specific) -high PPV desirable, especially if intervention is immunosuppressive -moderate/high NPV	- Validation of forme fruste, or early not fully diagnostic features of chronic GVHD -possible examples include edema, early dry eyes, muscle cramps, arthralgias, early decline in P-ROM scores, early airflow obstruction, abnormal liver function tests	- Predicts future development of highly morbid forms of chronic GVHD in those with established chronic GVHD	- Predicts response to therapy in established chronic GVHD treated with a therapeutic agent

Table 6

Features of Ideal Preemptive Therapeutic Agents

Feature	Considerations
Biological rationale	- Selection of interventions that target pathways implicated in chronic GVHD pathogenesis
Safety	- Low toxicity, limited interactions with medications given after HCT
	- Risk profile of intervention commensurate with severity of outcome to be prevented (and PPV of the assessment/ biomarker)
	- When possible, minimize disruption of graft-versus-malignancy effects
Tolerability/cost	- Ensure intervention adherence
	- Allow prolonged therapy to prevent late occurring chronic GVHD events
	- Patient and health care system able to afford treatment
Efficacy	- Prioritization of agents with demonstrated activity in chronic GVHD therapy or allied human immune-mediated disorders
Transportability	- Logistics of delivering therapy permit dissemination
	- Orally available agents generally preferred