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National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-Versus-Host Disease: III. The 2014 Biomarker Working Group Report

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Abstract

Biology-based markers to confirm or aid in the diagnosis or prognosis of chronic GVHD after allogeneic hematopoietic cell transplantation (HCT) or monitor its progression are critically needed to facilitate evaluation of new therapies. Biomarkers have been defined as any characteristic that is objectively measured and evaluated as an indicator of a normal biological or pathogenic process, a pharmacologic response to a therapeutic intervention. Applications of biomarkers in chronic GVHD clinical trials or patient management include: a) diagnosis and

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assessment of chronic GVHD disease activity, including distinguishing irreversible damage from continued disease activity, b) prognostic risk to develop chronic GVHD, and c) prediction of response to therapy. Sample collection for chronic GVHD biomarkers studies should be well-documented following established quality control guidelines for sample acquisition, processing, preservation and testing, at intervals that are both calendar- and event-driven. The consistent therapeutic treatment of subjects and standardized documentation needed to support biomarker studies are most likely to be provided in prospective clinical trials. To date, no chronic GVHD biomarkers have been qualified for utilization in clinical applications. Since our previous chronic GVHD Biomarkers Working Group report in 2005, an increasing number of chronic GVHD candidate biomarkers are available for further investigation. This paper provides a four-part framework for biomarker investigations: identification, verification, qualification, and application with terminology based on Food and Drug Administration and European Medicines Agency guidelines.

Keywords

Chronic graft-versus-host disease; biomarkers; NIH; consensus

BACKGROUND

Chronic GVHD is one of the most important long-term complications of allogeneic blood and marrow transplantation, resulting in significant morbidity and mortality [1]. Unlike acute GVHD, chronic GVHD is insidious in its onset and the diagnosis can be difficult. Moreover, chronic GVHD is a multifaceted disease that can affect almost all organs and tissues in the body. Thus, the identification and verification of biomarkers for chronic GVHD is more difficult than for acute GVHD. The scoring system proposed by the NIH Consensus Development Project on Criteria for Clinical Trials in chronic GVHD [2] has now been widely adopted by HCT centers. However, clinical characteristics are not fully informative in predicting the severity of the disease, response to therapy, or survival, and are not adequate to distinguish disease activity from irreversible tissue damage during treatment [3]. As an adjunct to clinical and histological criteria, the availability of biomarkers for chronic GVHD could potentially improve the classification of patients into risk groups and thereby refine chronic GVHD diagnosis, estimate the risk of developing chronic GVHD or predict response to therapy.

The pathogenesis of chronic GVHD involves a number of biological mechanisms potentially contributing to its development and evolution. Historically, chronic GVHD was believed to be a chronic continuation of the same effector mechanisms that cause acute GVHD, i.e., donor T cells having specificity for recipient-restricted histocompatibility antigens that produce cytokines. However, differences in the clinical presentations of acute and chronic GVHD suggest that the effector mechanisms might differ. Further, therapies that are clinically effective in treating acute GVHD are much less effective for chronic GVHD. Together, this suggests that both unique biomarkers and pathophysiologic mechanisms may be involved in chronic GVHD.

Candidate biomarkers may be identified from their correlations with certain chronic GVHD phenotypes. While some correlations might not reflect the underlying pathology, others will suggest new pathophysiologic pathways and potential therapeutic targets. Alternatively, potential biomarkers may be identified via biological hypotheses generated from pre-clinical or theoretical models of chronic GVHD pathophysiology. However, a biomarker does not necessarily directly represent an effector mechanism of chronic GVHD but may reflect a counter-response to control GVHD, or result from nonspecific inflammation or damage.

Purpose of this document

The purpose of this document is to facilitate the identification, verification, qualification and application of chronic GVHD biomarkers. In this document we discuss: a) a standardized nomenclature for biomarkers usage, b) a core set of potentially confounding factors that must be considered when measuring different types of chronic GVHD biomarkers, c) critical concepts and a recommended decision process specific to the selection and development of biomarkers in chronic GVHD, and d) considerations for a repository with the minimal essential clinical data to be provided with each sample.

Summary of 2014 changes

This document replaces the 2005 report [4] of the Biomarker Working Group of the NIH Consensus Development Project on Criteria for Clinical Trials in chronic GVHD.

SUMMARY OF RECOMMENDATIONS

The Biomarker Working Group makes the following recommendations:

1. Biomarker(s) of chronic GVHD should include all of the following components whenever possible:
    a. Marker should be identified as diagnostic, prognostic, or predictive of the potential to respond to a treatment, or correlate with response to a treatment that will lead to an important clinical outcome (see Table 1).
    b. Use of rigorous methodology for measuring the biomarker.
    c. Confirmation in at least 2 independent cohorts, each having sufficient power for statistical significance for a clinically relevant hypothesis.

    This rigor is required because the observation of a significant association in a single data set does not ensure that the findings can be generalized to other data sets or that the association is specific for the investigated condition. A biomarker confirmed to have a strong association with the investigated condition in at least two independent cohorts would support its use in chronic GVHD clinical trials or patient management. To identify such biomarkers, a coordinated approach to the identification, verification, qualification, and application of biomarkers should be implemented. Prior to clinical application, a higher degree of confidence may be required, such as confirmation of results by an independent group in a different environment and clinical setting.

2. Both hypothesis-driven studies and discovery-based approaches for identification of chronic GVHD biomarkers are likely to be successful in different circumstances.

3. Ideally, both chronic GVHD observational prospective studies and chronic GVHD clinical therapeutic trials should include correlative biological studies to allow the identification, verification, qualification and application of biomarkers whenever possible. The main advantages of observational prospective studies are: (1) the study population can be more heterogeneous and representative of the entire population; (2) generally longer longitudinal follow-up than clinical trials; and (3) more patients can be studied. Clinical trials have the advantages that: (1) the study population is often more homogeneous; (2) treatment is controlled; (3) clear outcome assessment; and (4) if randomized, potential sources of bias are minimized.

4. Samples from well-documented cases and controls should be stored using standardized protocols as proposed in this consensus paper, in order to create a resource for future biomarker studies. Samples types to be collected on cases and controls and minimal essential clinical data to be provided with each sample are detailed below.

DEFINITIONS OF BIOMARKERS AND THEIR APPLICATIONS

A biomarker has been defined by the NIH Biomarker Working Group as a characteristic that is objectively measured as an indicator of normal biological or pathogenic processes, or biological and clinical responses to a therapeutic intervention [5]. The Institute of Medicine has further defined “objectively” to mean “reliably and accurately” [6]. The term “biomarker” commonly refers to a biochemical variable (circulating protein or other biomolecule). Therefore, for the purposes of this document, certain evaluations that are routinely performed to determine the diagnosis of chronic GVHD or to assess the clinical severity of the disease are not discussed but may be considered as biomarkers in certain circumstances. Examples of such evaluations include pulmonary function testing, liver function testing, and radiographic assessment including computed tomography scans.

Biomarkers can be separated into distinct categories of diagnostic, prognostic, and predictive (including response biomarkers), as described in Table 1. Applications of chronic GVHD biomarkers critical to clinical care and research studies are summarized below and in Table 1. The definitions have been updated to reflect usage by the FDA and EMA [7, 8].

1. *Diagnose chronic GVHD*. For example, a biomarker could be used together with clinical criteria to determine eligibility for a clinical trial or clarify differential diagnosis (e.g., infection, drug reaction, other inflammatory disease vs. chronic GVHD). *Distinguish cumulative damage or irreversible tissue damage from current chronic GVHD activity*. Many of the organ systems involved in chronic GVHD develop cumulative tissue damage, and grading scales do not distinguish well between the extent of current areas of inflammatory activity (e.g., infiltrates of lymphocytes into tissue) and cumulative damage (sclerotic scarring, loss of lacrimal or salivary function due to loss of secretory acini). *Identify candidates for pre-emptive therapy*. For example, anti-HY titers increase weeks to months before

clinical manifestations and may identify candidates for pre-emptive therapy or suggest the type of treatment that should be used.

2. *Prognostic risk to develop chronic GVHD*. For example, gene polymorphisms in either the donor or recipient may be associated with risk of development of chronic GVHD. Identifying prognostic markers for development of severe forms of chronic GVHD, before onset or at the time of initial diagnosis chronic GVHD, is of particular interest. For example, a biomarker that could estimate the risk of severe GVHD leading to serious sequelae would be useful to help guide decisions about the need for and intensity of treatment.

3. *Predict potential for response to therapy*. For example, a biomarker may distinguish between different pathophysiologic processes that cause chronic GVHD and aid in determining treatments that are most likely to provide benefit for an individual patient.

4. *Serve as an intermediate marker of response to treatment, particularly a response related to a long-term outcome such as nonrelapse mortality*. For example, a biomarker could be used to monitor therapeutic response. This type of biomarker could also help guide decisions about treatment management by revealing that a treatment has not resulted in an adequate response and that a change in treatment is warranted.

Biomarkers that could be used to predict response to treatment, measure disease activity or distinguish reversible disease activity from irreversible damage would have very high clinical utility, since currently available clinical tools are not adequate for these purposes. In addition, biomarkers that are prognostic for the risk of developing severe chronic GVHD would also have high utility and could be used in pre-emptive trials.

Predictive biomarker studies will have to be annotated not only with demographic and GVHD-related data available at the time of collection, but also with GVHD data relevant to future outcomes. Ideally, samples stored in biorepositories will have outcome data as well, but many times are not set up this way. The CIBMTR TED forms have a lower level of detail regarding chronic GVHD than may be required for some predictive biomarker evaluations. If possible, samples should be de-identified to the repository, and the donating center should retain the identification links. When biomarker studies are performed, investigators could then contact the centers that donated the sample in order to update the data.

RISK FACTORS AND COVARIATES TO CONSIDER

While a biomarker may provide valuable assessment of chronic GVHD, other contributing, confounding conditions must also be considered. Some potential confounding factors are defined below and summarized in Table 2.

Factors affecting biomarkers directly and independent of onset of chronic GVHD

The conditions of a) immune reconstitution, b) concomitant acute GVHD, c) the type and intensity of current immunosuppressive therapy, d) presence of infections, and e) sample

processing and storage, may all affect expression of a chronic GVHD biomarker or its measurement and interpretation. In particular, the analysis of immune-related biomarker data must also account for time from transplant, since immune reconstitution occurs gradually. Organ involvement by chronic GVHD (types of tissues involved and NIH score) and the clinical presentation at onset reflect the chronic GVHD diagnostic phenotype, which may directly affect biomarker levels. Because of the heterogeneity and varied frequency of the clinical syndromes of chronic GVHD, it is unlikely that any given biomarkers will be applicable to all forms or presentations of this disorder.

Covariates and potential confounding factors

A variety of confounding factors may limit the ability to interpret results of chronic GVHD biomarker studies. Each of the following confounding factors may limit the scope and application of a particular biomarker or at least must be controlled for as possible confounding factors in any analysis: a) recipient characteristics such as age; b) donor characteristics including treatment of the donor with G-CSF or other agent (e.g., plerixafor) and graft manipulation (e.g., an HLA-haploidentical graft with T cell depletion or with administration of cyclophosphamide after transplantation); c) donor source (related versus unrelated) and the type of graft (peripheral blood, bone marrow, or umbilical cord blood); and d) recipient preparative conditioning regimen.

Donor versus Recipient Chimerism Criteria for evaluation of chronic GVHD Biomarkers

Significant recipient chimerism is associated with a higher frequency of donor tolerance [9, 10] and could affect the interpretation of chronic GVHD biomarkers when donor-derived hematopoiesis is not fully engrafted. In patients with questionable engraftment, chimerism should be examined, and the patient should be excluded from biomarker studies if results show < 90% donor chimerism, ideally in either lymphoid or myeloid populations.

CRITICAL FRAMEWORK FOR BIOMARKER STUDIES IN CHRONIC GVHD

So far, most potential chronic GVHD biomarkers have been identified based on evaluation at a single center or single laboratory, and have not been through all the steps of verification and qualification necessary to be approved for use in clinical trials, as discussed below and shown in Figure 1. Only a few studies have included patients derived from multiple centers or independent cohorts of patients. Thus, we propose a four-part framework for the development of chronic GVHD biomarkers (Figure 1). These recommendations are based on guidance for biomarker development from the Institute of Medicine [6] and the US Food and Drug Administration (FDA) in the Center for Drug Evaluation and Research (CDER) [11]. The HCT community will be able to move forward and translate biomarkers into the clinic only if these recommendations are carefully applied in order to avoid previous mistakes, such as 1) strong reliance on convenience samples rather than a prospectively defined population from which specimens are to be collected, 2) absence of a verification cohort that is independent of the discovery cohort, 3) improper statistical methods, for example, when deriving a multiple factor risk score, and 4) failure to consider commonly available clinical information before deducing the additive value of biomarkers. Avoidance of these mistakes will allow our HCT community to move forward in translating biomarkers

into the clinic [12, 13]. We have used the new recommended terminology in the headers below [5, 6] to avoid the term “validation”, whose different meanings have led to confusion. In Figure 1 we propose a workflow for biomarker discovery. Each step is explained below.

Step 1: Identification

The initial step is the identification of candidate biomarkers in a small experiment of well-matched cases and controls selected from the populations in which the biomarker is intended for use. At this initial step, it is important to define the clinical context of use and the clinician reported outcome (CRO) or patient reported outcome (PRO) data that will be captured to assess a clinical endpoint, for example, nonrelapse mortality (NRM) or relapse mortality (RM) or more chronic GVHD-specific scales such as the NIH chronic GVHD 0–3 organ score and the Lee chronic GVHD symptom scale. The most appropriate controls for the cases should be defined at this point. It should not be assumed that the same controls are appropriate for different clinical contexts. Factors that should be considered in the choice of controls are discussed in Table 2.

Step 2: Verification

This step confirms the analytical validity of a test. This includes, among other aspects, the test’s reproducibility and accuracy (% coefficient of variation, precision). Test practicality should also be considered: is the potential sample to be measured easy to obtain, is the sample stable until the test can be performed, and is it cost-effective? Of note, before the qualification step, parameters such as cutoff values and sample collection procedures are locked down (finalized) and cannot be changed without re-verification of the test under the revised conditions.

Step 3: Qualification

This step assesses the robustness of the test in all samples from the intended use population for a certain clinical outcome/CRO or PRO (i.e. correctness). Statistical considerations for this step are shown in Table 3. Other statistical analyses that have been proposed to estimate the performance of biomarkers are reviewed by Pepe et al. [14]. The qualification cohort for step 3 should be entirely distinct and separate from the identification cohort previously studied in step 1, including different center(s) and different demographics, so long as they are consistent with the intended use population. If, however, the demographics are too different from the population for which the biomarker’s use is intended, qualification testing could fail inappropriately.

Step 4: Application

In this final step, the biomarker is used in the clinic (e.g. to test all patients suspected of having chronic GVHD) or in a prospective randomized clinical trial, to test the potential to foretell the outcome. If the biomarker successfully qualified, the application step may test: (i) practicality of use in a consortium study, (ii) replacement of a clinical scoring system or invasive biopsies by a simple blood test, (iii) usefulness as an early surrogate indicator of response when testing a new drug as compared to the standard of care. Application testing may require Institutional Review Board (IRB) approval as well as Investigational Device

Exemption (IDE) or Investigational New Drug (IND) approval, if clinical management of patients in the study is based on the outcome of the test.

ENSURING ADEQUATE PATIENT CONSENT FOR CHRONIC GVHD BIOMARKER STUDIES

To allow chronic GVHD biomarkers studies, consenting at the time of sample collection is essential. Obtaining consent for both current and future research studies can be accomplished most easily by the appropriate provision at the time of enrollment, in advance of any sample collection followed by de-identifying the sample and providing the correlative clinical data. To obtain consent that allows for a broad variety of clinical studies and sharing with other investigators, a graded level of consent may need to be obtained in the initial consenting process, clarifying whether the participant will allow a) studies on the sample as per the currently outlined studies, b) storage for future studies in chronic GVHD, and c) storage for future studies that may involve disorders other than chronic GVHD. Due to societal sensitivities around “genetic testing” and the common requirement for deposition of genetic information into public databases, specialized consent should be obtained for gene-based studies. The consent process should strive to allow for testing or research exchange of coded samples, a process that protects the identity of the subjects but allows access to necessary clinical data through the original provider. A model consent form is attached in supplementary material.

SAMPLE REPOSITORY FOR INVESTIGATION OF CHRONIC GVHD BIOMARKERS

The largest barrier to new chronic GVHD biomarker development is the lack of good quality biological samples linked to detailed clinical data. Well-conducted large multicenter observation or interventional clinical trials represent excellent formats to provide the consistency of standardized documentation needed to support qualification studies correlating biomarkers with clinical endpoints of interest. However, single institution or observational studies with limited institutional participation in which standardized diagnostic criteria are employed may be sufficient for initial identification studies.

These sample repositories not only would support the exchange of chronic GVHD patient materials for verification of currently proposed biomarkers, but also provide a resource for biomarker discovery through implementation of new technologies. Multiple new technologies are becoming available for analysis of biological fluids, including serum, plasma, saliva, bronchoalveolar lavage fluid and urine). The proteome and metabolome [15, 16] can be analyzed by multiplex ELISA [17], and peptide arrays to identify autoantibody epitopes [18]. Preserving leukocytes supports future characterization of populations with flow cytometric analyses using as many as 20 fluorochrome channels or 40 channels with mass cytometry (CyTOF) [19]. Preserving cells also supports cell type-specific sorting for molecular analyses. Multiparameter cell characterization can also be done with paraffin embedded pathology specimens by fluorescent immunohistochemistry with imaging through confocal microscopes and multispectral fluorescent imaging analysis supported by

quantitative analysis software [20]. RNA and DNA can be recovered from preserved leukocytes, snap-frozen tissue biopsies, from paraffin sections, or even from specific tissue areas in sections through laser capture microdissection [21]. Analyses of RNA and miRNA transcriptomes have been used to characterize gene expression patterns in autoimmunity and inflammation [22, 23]. New bead arrays permit mapping of epigenetic DNA hypomethylation to specific gene loci [24]. Verification of identified genes individually by quantitative RT-PCR is being replaced by platforms utilizing direct multiplexed measurement with RNA and miRNA probe sets, incorporating hundreds of genes to identify multigene patterns [25]. High throughput exome sequencing can identify relevant gene polymorphisms in donors and recipients more rapidly and at lower cost than whole genome approaches, while RNAseq can identify both expression and altered sequences in expressed transcripts in sorted, well-defined cell subsets. Key to the integration of data from these methodologies is the rapidly maturing development of powerful tools for analysis and interpretation of results.

When future biomarker studies are designed, we propose the following considerations. The proposed collection and banking of specimens and data can be very expensive and will require support from research funding sources.

1. Prospective multicenter studies with collection and banking of samples with an accompanying patient data link, in a manner that complies with regulations for disclosure of protected health information of the country in which the trial is being performed. Assessors will require adequate training to collect the clinical data correctly. The chronic GVHD-focused clinical variables of interest are outlined in the companion NIH Consensus Development Project on Criteria for Clinical Trials in Chronic GVHD Working Group reports. Table 4 presents the minimal recommended data elements that should be linked with each stored sample.

2. Sample acquisition protocols should incorporate both calendar-driven time points and event-driven sample collection. Examples of event-driven sample collection include the point when the patient is first diagnosed with chronic GVHD (or within 2 months) or before start of systemic treatment or at the time of change in treatment. Thus, in the presence of chronic GVHD, we recommend that samples should be obtained within 2 months of onset or treatment change. Since the immune environment changes with post-transplant immune reconstitution, time-matched samples should also be obtained from patients who do not have chronic GVHD to serve as controls. In the absence of chronic GVHD, we recommend that samples should be obtained at 3, 6, and 12 months, with additional sampling considered at 9 months, and 18–24 months after transplantation. Ideally, additional samples should be collected yearly, if possible for 6–8 years especially if studies on long term changes associated with established cGVHD are being considered. This schedule will adequately provide samples during the period of greatest risk for development of chronic GVHD and will also allow long-term studies after chronic GVHD treatment.

3. A centralized repository at the National Marrow Donor Program (NMDP) or possibly a virtual repository with multiple sites collecting in a standardized manner

should be established. In the BMT CTN 1201 trial on acute GVHD, shipping of blood up to day +100 has provided materials for processing in a central site. Collection directly into EDTA, Cytochex and Paxgene tubes has provided material for studies of plasma antibodies and stable cytokines, flow cytometric phenotyping of lymphocytes and preservation of RNA for molecular analyses. This powerful approach avoids the need to have specialized local facilities for processing and preservation and standardizes processing and assays to ensure consistent results [26–28]. However, this approach is limited in three ways. First, plasma cytokines and chemokines are sensitive to both the choice of anticoagulant and the time interval from collection to processing and storage [27, 29]. At individual sites with dedicated core facilities, rapid separation and freezing of serum and plasma (both heparin and EDTA) are recommended for better preservation of cytokines and chemokines. Storing multiple small aliquots (0.5 – 1 mL/vial for plasma/serum and 2×10^6 for phenotyping studies and 5×10^6 cells for functional studies per vial with ideally 5 vials for each patient and time point) avoids refreezing. The volume of blood or tissue collected must be compliant with IRB maximum criteria. Unusual methods to obtain cells, such as leukapheresis, must be considered carefully regarding the risks to the subject. Collection in multiple anticoagulants is preferred, since anticoagulant choice affects measured ELISA cytokine levels and may limit metabolome recovery [27, 30–32]. Second, storing blood cells for later analysis – particularly in numbers adequate for flow cytometry (at least 5×10^6 cells) has the advantage of preserving valuable patient materials for flow cytometric, molecular and functional assays using rapidly developing new technologies. Finally, the calendar-driven sample approach is inflexible in timing and limited in scope of tissues examined. Collection of event-driven samples, such as on the day of diagnosis or of new organ involvement, may be critical to identifying transient biomarkers. Chronic GVHD is pleomorphic; biopsies of chronic GVHD target organs (skin, intestine, liver, and mucosa) or collection of local biofluids such as saliva or bronchial lavage fluid, can be more informative about infiltrating cells and local chemokines than systemic measurements of blood. Formalin-fixed, paraffin embedded diagnostic biopsies of chronic GVHD-affected tissues can be analyzed in more detail with multiparameter immunohistochemistry or serve as a source of securely preserved RNA and microRNA for multiplex RNA analyses [33, 34]. Even if resources to collect these sample types are not available at all sites, dedicated large cores/clinical centers should continue to collect and bank these quality samples locally or with the intent to ship cryopreserved materials to centralized repositories, as in the Chronic GVHD Consortium trials.

4. Subject permission to use banked samples in future research investigations and to exchange materials with other institutions should be incorporated into the informed consent documents. The use of new tools for genome sequencing raises ethical concerns relating to incidental findings and highlight the need for proper development of consent documents [35]. Genetic studies have the potential to identify incidental findings of potential health or reproductive importance that are outside the aims of the study but may have actionable results [36]. A recent study of exome sequencing suggested such actionable results might be found in 1% of

participants [37]. In the case of transplant recipients, incidental findings from testing blood cells could be relevant to the donor and their close relatives. A model template for consents for studies involving genomic sequencing has been developed at the NCI/CCR clinical research operations website (https://ccrod.cancer.gov/confluence/display/CCRCRO/Templates) to address these issues for patients and donors.

CANDIDATE BIOMARKERS IN CHRONIC GVHD

Potential chronic GVHD biomarkers have been evaluated in both hypothesis-driven and discovery-based testing for specific clinical applications. The data have come primarily from single centers or from a number of collaborating centers; in most cases, the findings have not been assessed as part of large multicenter trials. Despite promising prior investigation, few potential biomarkers have been verified in independent large cohorts of patients as recommended by this consensus document [38]. In Table 5 we present published candidate chronic GVHD biomarkers, organized by application (diagnostic, prognostic, risk stratification, predictive), and then in ascending strata based on the strength of the published evidence. This table illustrates how very few biomarkers have been identified from studies incorporating discovery and independent verification. In addition, studies of chronic GVHD therapeutic response are lacking. Among potential biomarker applications, we emphasize prognostic, risk stratification and predictive biomarkers as major priorities for future investigation. As a reminder, biomarkers are observational correlations and might not necessarily reflect the underlying chronic GVHD pathology. However, they often do, and the biology of the markers listed in Table 5 will be discussed elsewhere.

In conclusion, although progress has been made, much work will be required to verify and qualify the candidate biomarkers identified in previous studies, and to implement high-throughput methods with appropriately collected specimens for future discovery-based approaches. Close coordination between multi-specialty clinical and laboratory-based groups, as well as regulatory agencies and industry partners, will be needed to pursue such studies successfully. We are confident that identification, verification and qualification of biomarkers will greatly assist the development and evaluation of new approaches for treating chronic GVHD.

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

Members of this committee included: Steven Pavletic, Georgia Vogelsang and Stephanie Lee (project chairs), Mary Flowers and Madan Jagasia (Diagnosis and Staging), David Kleiner and Howard Shulman (Histopathology), Kirk Schultz and Sophie Paczesny (Biomarkers), Stephanie Lee and Steven Pavletic (Response Criteria), Daniel Couriel and Paul Carpenter (Ancillary and Supportive Care), Paul Martin and Corey Cutler (Design of Clinical Trials), Kenneth Cooke and David Miklos (Chronic GVHD Biology), Roy Wu, William Merritt, Linda Griffith, Nancy DiFronzo, Myra Jacobs, Susan Stewart, and Meredith Cowden (members).

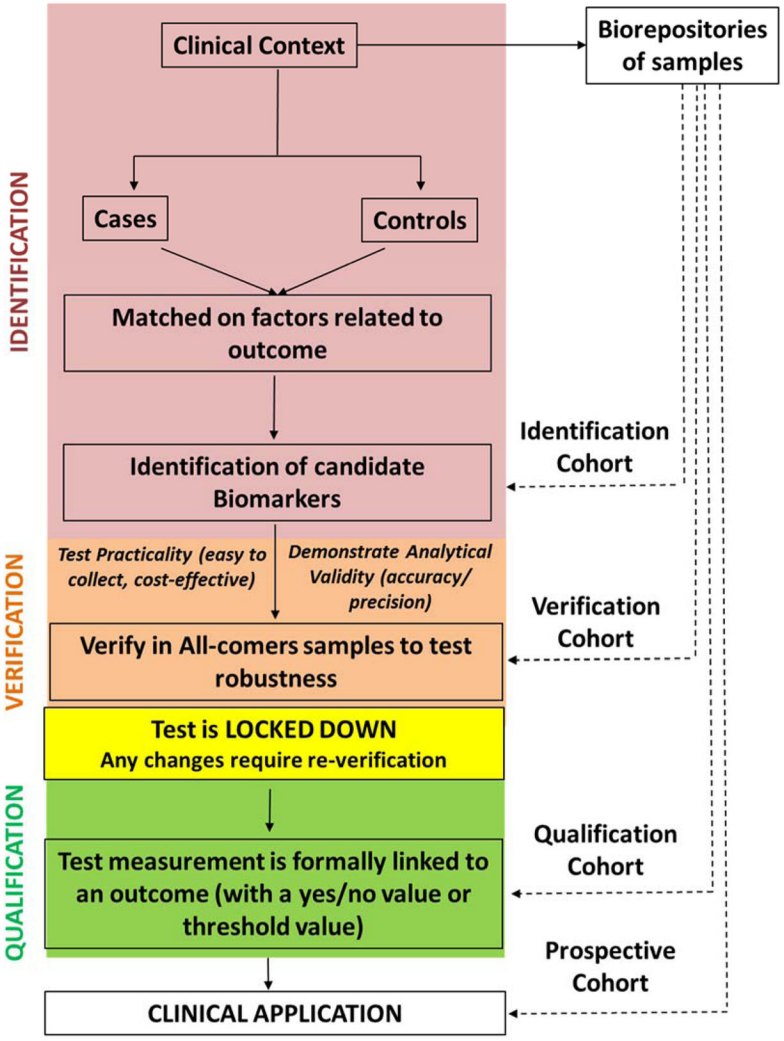

Figure 1.
Example of a predictive biomarker or response biomarker development project

Table 1

Revised Definitions of Biomarkers Biomarkers[a]

Types of biomarkers	Recommended Change in Definition	Uses	Minimum matching criteria for control samples[b]	Modified from Biomarker Definition in Previous NIH chronic GVHD Consensus criteria (ref 2006)
Diagnostic	An assay that identifies patients at the onset of clinical disease • Different forms of chronic GVHD may have different markers • Different tissues may have different markers	To help in rapid diagnosis and initiation of therapy	• Time from transplant • Absence of relapse • Absence or presence of current or recent acute GVHD • Absence or presence of active infection • Absence of recent B cell depletion after BMT • Manipulation or treatment of the donor product (i.e., T cell depletion, G-CSF)	"Diagnose chronic GVHD."
Prognostic	An assay that categorizes patients by degree of risk for disease occurrence or progression or resolution	A prognostic biomarker provides information about the anticipated course of the disorder in that particular patient	• Time from transplant[c] • Prior acute GVHD • T or B cell depletion during conditioning	"Predict risk of developing chronic GVHD."
Predictive	An assay that categorizes patients by their likelihood of response or outcome to a particular treatment when measured prior to the treatment	A predictive biomarker provides information about whether a given patient is likely to respond to a treatment intervention in a particular way	• Time from transplant Current immune suppressive therapy (e.g. glucocorticosteroids, calcineurin inhibitors) • NIH Global severity score	"Predict response to therapy." "Assess prognosis or establish staging of chronic GVHD."
Response to treatment	An assay measured after initiation of therapy that is intended to substitute for a clinical efficacy endpoint (note: a pre therapy sample for comparison is required)	A response marker that can be utilized in place of an accepted clinical response endpoint (see the NIH chronic GVHD response criteria paper)	• Time from transplant • Absence of relapse • Absence or presence of current or recent acute GVHD • Absence or presence of active infection • Absence of recent B cell depletion after BMT • Manipulation or treatment of the donor product (i.e., T cell depletion, G-CSF)	"Measure disease activity."
Prior biomarker definition that was removed in revised NIH criteria				"Evaluate GVHD versus graft-versus-leukemia (GVL) or graft-versus-tumor (GVT) effect."

[a] The Predictive and prognostic definitions have been modified to be consistent with current FDA and EMA guidelines [7, 8]

[b] Factors listed in Table 2 should be considered in interpretation

[c] First 2 years: within 2 months, After 2 years: within 1 year. Appropriate time interval could be different in adult versus pediatric patients due to a faster immune reconstitution in pediatric patients

Table 2

Factors that must be considered for chronic GVHD studies

Factor	Impact on chronic GVHD biomarker
Factors affecting biomarkers directly	
Tissues involved and NIH chronic GVHD score	• Tissues involved • NIH chronic GVHD score
Immune reconstitution post HCT and time from transplant	• Some biomarkers vary with immune reconstitution post-HCT thus time and age matched (pediatric vs adult) controls are required.
Concomitant acute GVHD	• Concurrent acute GVHD may overlap with classic chronic GVHD manifestations • Biomarkers may represent late acute GVHD manifestations
Previous acute GVHD and treatment/ prophylaxis of acute GVHD	• The presence of previous acute GVHD and the therapy utilized to treat acute GVHD • Acute GVHD prophylaxis has the potential for long term impact on immune reconstitution (e.g. antibodies such as rituximab, alemtuzumab, and ATG)
Current immune suppression	• Many immunosuppressive treatments particularly steroids may affect concentrations affect concentration of biomarkers (e.g. sBAFF)
Current infection	• Active infections may change cytokine milieu and markers. • CMV reactivation, pulmonary infections.
Sample processing and storage	• Some B cell populations are lost when processed with Ficoll • Choice of serum or heparin, EDTA or citrate plasma alters analytes • Processing time after blood draw reduces some analytes • Collection of the samples may be specific for the type of assay and the type of tissue collected (i.e., serum, urine, saliva) These considerations apply both during the identification and verification of a biomarker as well as during its subsequent application.
Covariates and potential confounding factors	
Recipient characteristics	• Younger age associated with lower incidence of chronic GVHD • Non-malignant diagnoses may affect the incidence and type of chronic GVHD (particularly non-malignant disorders with marrow failure and chromosomal instability appear to have a higher rate of chronic GVHD). • Allo immunized patients may have a lower rate of engraftment resulting in split donor chimerism and affecting the incidence of chronic GVHD • Non-HLA polymorphisms may impact incidence or presentation (i.e., ABO incompatibility) • Biomarkers may be organ specific
Donor characteristics	• Unrelated versus related donor • HLA mismatched versus HLA matched • Female donor is associated with a higher incidence of chronic GVHD • UCB, Peripheral, peripheral blood, or marrow graft • Non-HLA polymorphisms may impact on incidence or presentation (i.e., ABO incompatibility) • Treatment of donor product (i.e., G-CSF, T cell or B cell depletion)

Factor	Impact on chronic GVHD biomarker
Preparative conditioning regimen	• MAC versus RIC • Use of T cell or B cell depletion (TCD, ATG, Campath 1H, Rituximab), all associated with a lower incidence of chronic GVHD • TBI associated with increased cutaneous sclerosis

Footnotes: UCB = umbilical cord blood; TCD = T cell depletion; ATG = Anti-thymocyte antibody; MAC = Myeloablative condition; RIC = Reduced Intensity Conditioning

Table 3

Statistical considerations

A. Analytical performance parameters

- Precision (repeatability and reproducibility of an assay)
- Accuracy
- Assay sensitivity (limit of detection)
- Assay specificity (interference, cross-reactivity)
- Sample type and matrix
- Sample preparation

B. Diagnostic accuracy

- Sensitivity: Proportion of subjects in a sample of patients with the target condition in whom the test is positive.
- Specificity: Proportion of subjects in a sample of patients without the target condition in whom the test is negative.
- Receiver operator characteristic (ROC): A plot of the true-positive rate versus the false-positive rate for all possible thresholds of a biomarker.
- Positive predictive value (PPV): Proportion of patients in the overall population with a positive test who have the target condition.
- Negative predictive value (NPV): Proportion of patients in the overall population with a negative test who do not have the target condition.

Table 4

Minimal essential clinical/routine laboratory data to be provided with each sample

Essential data	**Recommended data (will be marker specific)***
1 Clinical Phenotype • For diagnosis markers: NIH diagnosis and staging forms • For prediction of response: NIH response to treatment forms • This includes presence or not of concomitant features of acute GVHD Time after transplantation at chronic GVHD diagnosis or of time matched non-chronic GVHD patients **2** Current type of immunosuppression at the time of time of sample collection (if taking corticosteroids: add dose and weight of patient)	**1)** Prior acute GVHD **2)** MAC vs. RIC **3)** Prior chronic GVHD **4)** TCD vs. Not TCD **5)** PBSC vs. BM vs. UCB **6)** Recent B cell depletion **7)** Sex mismatch **8)** HLA mismatch **9)** Active uncontrolled infection (particularly CMV) **10)** Age of the patient and recipient **11)** WBC and ALC
	Although suggested they are not routinely captured at most centers **12)** Prior Immunosuppressive therapies failed **13)** Absolute T and B cells counts **14)** IgG levels

*Variables that could confound the analyses (Table 2) should be collected in minimal essential data

Table 5

Candidate chronic GVHD biomarkers

	Diagnostic			Prognostic/risk stratification			Predictive		
Category	**Cellular**	**Mediator**	**Antibodies**	**Cellular**	**Mediator**	**Gene polymorphism**	**Cellular**	**Mediator**	**Antibodies**
Evidence–based Category 1	B cells[39–44] Treg[45–47]	BAFF[17] CXCL9[17] elafin[17] Aminopeptidase N (sCD13)[17] sIL-2Rα[17] IL-4[48, 49] IL-6[50, 51] TNFα[50, 51]		Treg[45–47, 52, 53]	TNFα [52–54]	IL-10[55–59] IL-6[60–62] TNFα[56, 63, 64]	Treg[65–68][b]	sIL-2Rα[69, 70]	
Evidence-based Category 2	BAFF/B cell[43] CD3+ T cells[39] Effector memory (CD4+ and CD8+)[71, 72] Monocytes[73] Naïve CD8+ T cells[71] NK cells[39] Th17 cells[50] TLR-9 responsive B cells[44]	ADAMTS2[74] ADAMTS3[74] AREG[74] BCAT1[74] CPM[74] CXCL10[75] CXCR3[75] CXCR7[74] DAP2IP[74] haptoglobin[76] IFN-γ [48] IL-1β[51] IL-1Ra[77] IL-1R2[74] IL-2[48] IL-8[50] IL-10[77] IL-12[49] IRS2[74] SRGAP1[74] lactotransferin/ lactoperoxidase (salivary)[78] IL-1Ra + CTSB (salivary)[79]	ANA[80] Anti-dsDNA[69] Anti-PDGFR[81] H-Y antibodies[82]	B cells[43] DC2[83] NK[84, 85]	BAFF[86] BAFF/B-cell ratio[86] b-FGF[87] IFN-γ[53] IL-10[53] IL-15[88] TGFβ[52] VEGF[87]	BAFF[89] CCR6[90] CCR9[91] FAS[56] FCRL3[92] Haptoglobin[76] Heparanase[93] HMGB1[94] IFN-γ[95] IL-1[61] IL-1Ra[57] MadCAM-1[96] MICA[97] PARP1[98]	Immature/ memory B cell ratio[41] Th17 cells[50] TLR-9 responsive B cells[44]	BAFF[69]	Anti-PDGFR[99]

The table presents a synthesis of published candidates chronic GVHD biomarkers organized according to application. Importantly, these are candidates based on current knowledge. Additional replication of these findings is needed, and none of the summarized candidates meet criteria for qualification. The table presents general biomarker candidates, and does not specifically present data on association between biomarker candidates and chronic GVHD organ involvement or severity.

Definitions:

- Application: Diagnostic – distinguish chronic GVHD from non-GVHD controls; Risk stratification – determine risk for chronic GVHD development; Predictive – assess therapeutic response;
- Category: Cellular – immune cell populations; mediator – inflammatory or immune regulatory cytokines and other factors; antibodies – auto-antibodies detected in chronic GVHD; gene polymorphism – reported cytokine gene polymorphism associated with chronic GVHD.

- Strength of evidence: Category 1: Biomarkers supported by greater quality evidence, including consistent findings (e.g. candidate biomarker is elevated in chronic GVHD patients vs. controls) across ≥ 2 studies, irrespective of methodology used in each report, or (b) examined in 2 patient cohorts. – Category 2: Studies have demonstrated significant findings in a single cohort, or by a single group.

[a] Some of the markers have been evaluated in more than one independent patient cohort by either the original groups to identify the marker or by another laboratory. Moreover some markers have been identified by different methodologies when separate laboratories have evaluated the marker. We have not noted these differences in this table.

[b] This marker could be considered in the category response to therapy.

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