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Current Definitions and Clinical Implications of Biomarkers in Graft Versus Host Disease

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Abstract

Hematopoietic cell transplantation (HCT) is a potentially curative treatment for many hematologic and non-hematologic disorders. Graft-versus-host-disease (GVHD) in its acute or chronic forms remains the most important non-relapse post-HCT complication. Biomarkers offer objective, unbiased information on systemic disorders, and significant focus has been placed on the discovery of biomarkers for GVHD. Ideally, a GVHD biomarker is actionable, utilizing the results of biomarker testing to guide clinical management of disease and clinical trial design. While many GVHD biomarkers have been identified, none have been properly qualified for clinical use. The National Institutes of Health (NIH) and Food and Drug Administration (FDA) provided biomarker subtype definitions; however, confusion remains about the proper definition and application of these subtypes in the HCT field. The 2014 NIH Consensus development project provided a framework for the development of biomarkers into clinical practice. This review aims to clarify the biomarker subtype definitions and re-emphasize the developmental framework. Armed with this knowledge, clinicians can properly translate GVHD biomarkers for clinical use.

Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) offers a curative option for many malignant and nonmalignant conditions. Graft-versus-host disease (GVHD) is a complex immunologic process that occurs on a pathobiological spectrum and manifests clinically

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as acute and chronic GVHD. Acute graft-versus-host disease (aGVHD) remains a major source of morbidity and mortality and chronic graft-versus-host disease (cGVHD) is the most common long-term complication of allo-HCT [1, 2]. For malignancies, allo-HCT is a successful immunotherapy in large part due to the graft-versus-tumor (GVT) effect. GVT is however tethered to GVHD and management decisions that balance the benefits of GVT with the risks of GVHD can be a challenge for clinicians.

Biomarkers offer objective, unbiased information on systemic disorders, and the need for actionable biomarkers has led to the discovery of multiple plasma biomarkers for GVHD. Both acute and cGVHD biomarkers have a significant number of potential clinical applications, however, while many biomarkers have been identified, none have been properly qualified for clinical use.

The National Institutes of Health (NIH) and Food and Drug Administration (FDA) provided biomarker subtype definitions [3], however confusion remains about the proper definition and application of these subtypes. For example, a risk biomarker and its defined threshold cannot be used as a predictive biomarker, which based on the current literature continues to occur. The 2014 NIH Consensus development project provided a 4-part framework for biomarkers to be implemented into clinical practice (Figure 1) [4]. Prior studies have not completed the proper development framework steps, therefore biomarkers with adequate statistical tests performance to reliably apply clinically are lacking. This review will re-emphasize the recommended workflow for proper translation of GVHD biomarkers. Accurate understanding of the biomarker definitions and appropriate use for specific outcomes would allow for proper biomarker guided-therapeutic trials.

1.0 FDA Biomarkers Definitions

The ‘Biomarkers, EndpointS and other Tools’ (BEST) Resource was established in 2016 by the FDA-NIH joint leadership council to define and outline biomarker roles in biomedical research, clinical practice and medical / pharmaceutical product development, and these guidelines have been regularly updated [3]. Five biomarker subtypes are typically used: diagnostic, predictive, response, prognostic, and risk. An overview of the biomarker subtype definitions per the NIH BEST Resource and applied to GVHD is found in Table 1.

A diagnostic biomarker is used to detect or confirm the presence of the disease [3]. For example, cytogenetic Philadelphia (Ph) translocation BCR-ABL1 t(9;22)(q34;q11) is a diagnostic biomarker in chronic myeloid leukemia (CML) [5]. Diagnostic biomarkers are most useful in a disease where treatment is indicated.

A predictive biomarker is used to identify a patient’s likelihood of response to or outcome of a particular treatment before the treatment is initiated [3]. While imperfect, PD-L1 expression in tumors detected using immunohistochemistry is a predictive biomarker of response to anti-PD-1/PD-L1 therapy [6]. Importantly, the FDA-NIH definition of a predictive biomarker requires assessment for each specific therapy, which differentiates prognostic and predictive biomarkers. Predictive biomarkers have become popular in hematology-oncology as they help optimize therapy decisions.

Response biomarkers show biologic response in patients after treatment is initiated [3]. These biomarkers are measured prior to therapy and a change represents an impact on a clinical endpoint. International normalized ratio (INR) is used as a response biomarker when evaluating a patient's response to anticoagulation with warfarin [7].

A prognostic biomarker helps determine the anticipated course for patients with clinically evident disease [3]. The T315I mutation in patients with Ph+ CML is a negative prognostic factor [5]. Another example is positive minimal residual disease following chemotherapy induction in acute leukemia.

A risk biomarker indicates potential for developing the disease in patients who do not have any clinical symptoms [3]. For instance, Factor V Leiden is a risk biomarker for an increased likelihood to develop a deep vein thrombosis [8]. The utility of a risk biomarker partially relies on whether interventions to reduce the risk of developing the disease exist and has low toxicity. The main distinction between risk and prognostic biomarkers is that a risk biomarker is obtained in patients who do not have the disease versus those who do.

2.0 Biomarker Development

Figure 1 provides 4 critical steps for the translation of GVHD biomarkers for clinical use, which consists of discovery of candidate proteins, validation of those with a unique assay, lockdown of this assay and finally qualification of the biomarker. The framework provides guidance to avoid previous biomarker development mistakes, including the absence of validation cohorts independent of the discovery or cases/controls cohorts, and strong reliance on retrospective rather than prospective evaluation [4]. It is important to highlight that the developmental process must be completed for each biomarker subtype. For example, an aGVHD risk biomarker cannot be used as an aGVHD predictive biomarker without repeating all 4 steps, and may not ultimately prove to be a biomarker for the new outcome. It is also critical that after the discovery step, analytic validity of an assay is established on a unique platform (Table 2). For instance, if an enzyme-linked immunosorbent assay (ELISA) is performed, the ELISA kit and technique must be the same at all steps. No previous biomarker studies have completed all of these steps; thus, no biomarkers qualify for clinical application yet.

During the developmental phases of GVHD biomarkers, special attention must be paid to statistical tests performance and establishing biomarker cut-points (Table 3). Receiver operating characteristic curve (ROC) is generally used to establish performance of an assay and is a good representation of sensitivity and specificity for all possible biomarker cut-points. Sensitivity indicates true positive rate (TP) while specificity indicates true negative rate (TN) for a specific cut-point. To establish the more granular positive predictive value (PPV) and negative predictive value (NPV), both a cut-point and GVHD incidence must be accounted for. Although prevalence is used for predictive values in the general population, in the case of HCT where all patients start at day of transplant, GVHD incidence, the number of individuals who develop GVHD during a particular period (such as a month), has been used. Indeed, if incidence increases, PPV increases while NPV decreases. Therefore, for rare GVHD subtypes such as bronchiolitis obliterans syndrome (BOS), using a biomarker's sensitivity and specificity provides a better picture to the clinician. For clinical applications,

several cut-point values for each biomarker should be tested to accommodate the best balance between efficacy and toxicity of an intervention. For example, when a drug is safe such as defibrotide for sinusoidal obstruction syndrome, lower cut-points of a high biomarker with more false positives will be acceptable. In contrast, for GVHD treatments such as corticosteroids that impact GVT, higher cut-points will ensure that most patients are truly GVHD positive and will receive the intervention exposing a minimum of false positive patients.

3.0 Acute GVHD Biomarkers

Acute GVHD remains the main cause of nonrelapse mortality (NRM) and a major hurdle for success of allo-HCT. A summary of plasma aGVHD biomarkers by subtype is found in Table 4.

3.1 Acute GVHD Biomarkers by Subtype

Diagnostic Biomarkers: Plasma biomarkers have been developed to confirm the presence of systemic or organ specific aGVHD. A biomarker panel of IL-2 Receptor-α (IL-2Rα), tumor necrosis factor receptor-1 (TNFR-1), interleukin-8 (IL-8) and hepatocyte growth factor (HGF) obtained at the onset of the clinical symptoms was able to confirm systemic aGVHD with high diagnostic accuracy [9].

Organ specific biomarkers may serve a more useful role in assisting in aGVHD diagnosis as they often represent proteins related to aGVHD damage from target tissues. Reg3α is a peptide primarily found in Paneth cells of the intestines and is released systemically as aGVHD damage occurs [10]. Reg3α has emerged as the most validated GI-aGVHD biomarker and concentrations at symptom onset were able to distinguish GI-aGVHD [11, 12]. The soluble form of T-cell immunoglobulin mucin protein-3 (TIM3), which may prevent immune suppression mediated via membrane-TIM3, was also associated with the GI-aGVHD [13]. Elafin, a serine protease inhibitor primarily made by keratinocytes in the skin [14], was found to be elevated at onset of skin aGVHD [15]. Elafin levels also correlated with higher incidence of stage III-IV skin aGVHD following haplo-Hct with post-transplant cyclophosphamide (PTCy) [16]. In a prospective study, plasma elafin was elevated in cutaneous GVHD but levels were unable to distinguish GVHD and other causes of rash [17].

Diagnostic biomarkers can help improve diagnostic accuracy. These biomarkers can also help distinguish aGVHD from other common post-HCT complications with overlapping symptoms, such as diarrhea from infectious colitis. In the BMTCTN 1202 study, diagnostic biopsies were obtained in 40% of suspected GVHD cases, but treatment initiation did not correspond with biopsy results and 10.5% of biopsies were equivocal [18]. Although a direct comparison of biopsies and diagnostic biomarkers is unlikely to be pursued, high biomarkers before or at treatment initiation (see predictive biomarkers) could help the decision making when biopsies are ambiguous.

Predictive Biomarkers: Per the FDA-NIH definition, a predictive biomarker must be assessed relative to each treatment. Stimulation-2 (ST2), the interleukin-33 (IL-33) decoy receptor involved in inflammatory signaling, is the most validated biomarker for aGVHD

and has been studied in a variety of clinical scenarios. When ST2 was measured at the start of corticosteroid treatment, patients with high ST2 were over twice as likely to have treatment-resistant aGVHD [19]. ST2 also emerged as a possible predictive biomarker for ruxolitinib for the treatment of steroid-refractory aGVHD. In the REACH1 clinical trial, significantly elevated ST2 levels were found in non-responders compared to responders [20]. Reg3α alone or in combination with ST2 has shown potential as a prognostic biomarker, however requires further evaluation relative to each specific therapy to be validated as a predictive biomarker [11, 21].

Imperfectly qualified predictive biomarkers have been used for aGVHD clinical trials. A biomarker score based on ST2 and Reg3α values created to estimate 6-month NRM was used to preemptively treat patients with alpha-1 antitrypsin (AAT) [22]. The study found no reduction of SR-aGVHD in the AAT group when compared to historical cohorts, however using imperfectly qualified biomarkers can skew study results.

Properly qualified predictive biomarkers can be used for more personalized aGVHD management. Predictive biomarkers would allow intensification of treatment for high risk GVHD patients and reduction of therapy for low or standard risk patients. There are multiple ongoing clinical trials for novel treatment agents for aGVHD, and predictive biomarkers could be used as an enrichment factor or trial eligibility for additional aGVHD therapies. Using these biomarkers, a subset of aGVHD patients who might benefit most from novel second-line agents can be enrolled.

Response Biomarkers: According to the FDA-NIH definition, a response biomarker is measured pre- and post-initiation of therapy to evaluate response to the treatment. A few of the aGVHD biomarkers have shown potential as response biomarkers for first-line aGVHD treatment. ST2 levels measured 14 days after starting systemic steroids was able to predict treatment failure by day 56 [23]. In the same study, the ability to predict therapy failure improved with the addition of TIM3 values [23].

Defining steroid-refractory aGVHD relies on a clinician's objective assessment and there is no standard of care on when to initiate second-line therapies. If improvement is noted, there is also no standard of care for the duration of therapy or taper rate of steroids. If first-line treatment fails, evidence supports initiating second-line therapy at the early stage may prevent advanced organ injury or development of severe aGVHD [24]. In practice, clinicians typically evaluate response at 1 week of treatment to decide on adjusting immunosuppressives. However, early clinical response has a low positive predictive value and does not correlate with long-term outcomes. Once qualified for clinical use, response biomarkers can assist in medication management decisions. For example, an early clinical responder with unchanged ST2 levels is not likely to require escalation of therapy. On the other hand, a patient with no clinical response and increasing ST2 levels will likely require additional therapy. For aGVHD treatment, clinical response at 28 days is currently the primary endpoint of many aGVHD clinical trials. Validated response biomarkers could provide earlier and more complete data for better evaluation of the effectiveness of novel aGVHD therapies.

Prognostic Biomarkers: Prognostic biomarkers for aGVHD are used to evaluate outcomes such as GVHD severity and NRM in patients who have GVHD. Elevated Reg3α at GVHD diagnosis was associated with grade 2–4 GI-GVHD and 1-yr NRM [11] and high levels in the first 21 days post-HCT correlated with NRM in both adult and pediatric patients [25]. ST2 values 14 days post-HCT was a better indicator for risk of death than other known risk factors [19], and levels 28 days post-HCT also correlated with 2-year NRM [26]. ST2 concentrations have also shown utility in alternative allo-HCT settings including cord blood transplant and haplo-HCT with PTCy [27, 28]. Like Reg3α, elevated ST2 was associated with increased NRM in both adult and pediatric cohorts [25]. Elafin levels was not prognostic of 6-month NRM in a contemporary cohort [29]. At 28 days post-HCT, TIM3 concentrations in addition to ST2, correlated with 2-year NRM [26].

As previously mentioned, the combined values of ST2 and Reg3α were used to create an algorithm as a prognostic biomarker to separate patients into groups with distinctly different 6-month NRM. The algorithm, known as the Magic algorithm probability (MAP), uses biomarker values with increased weight on ST2 at 7 days post-HCT [30]. This algorithm was tested and confirmed in a Japanese retrospective cohort of 112 patients [31]. The formula has also been applied in two studies at 7 and 28 days after corticosteroid treatment to estimate NRM, and showed patients with higher scores were more likely to die, independent of clinical response [32 33].

A note of caution however when applying an algorithm that utilizes multiple biomarkers and was generated for an alternative purpose. Change may occur in one biomarker while the other biomarker remains the same. This change will impact the algorithm score, so validation of the individual markers or development of a specific algorithm for that outcome is required. Also, it is important to highlight that several of the cited studies incorrectly categorized these biomarkers as risk or response. According to the BEST resource, a biomarker associated with outcomes is more accurately categorized as a prognostic biomarker.

Properly validated prognostic biomarkers can help anticipate the course of disease and assist in clinical management decisions. Acute GVHD grade at diagnosis does not correlate with outcomes. Currently patients receive first-line therapy with high dose corticosteroids which leads to a significant number of undertreated and overtreated patients. Undertreatment can lead to significant morbidity and mortality. Overtreatment can increase a patient's risk of infection and negatively impact the desirable GVT effect. Patients with GVHD who have lower prognostic biomarker scores may benefit from reduced immunosuppressive treatment.

Risk Biomarkers: According to the NIH-FDA definition, a risk biomarker for aGVHD indicates the risk to develop aGVHD. As discussed in the prognostic section, several studies previously incorrectly labeled biomarkers as risk. Currently, no biomarker that anticipates future aGVHD exists, which represents an important knowledge gap. TIM3 showed potential as a risk biomarker when levels 14 days post-HCT were associated with future grade 3–4 aGVHD with area under the ROC of 0.76, however, the PPV was only 16%, probably due to a grade 3–4 aGVHD incidence of 6.5% [23].

Ideally, an aGVHD risk biomarker would provide information for future clinically significant aGVHD. Once risk biomarkers are discovered and validated, they offer a unique opportunity for aGVHD prevention. These biomarkers could prompt increased surveillance. The data provided by qualified risk biomarkers can also assist in the difficult balance of GVHD and GVT, delaying immunosuppressive weans for patients with high risk of subsequent aGVHD and accelerating weans for patients with high risk of relapse. Risk biomarkers could be utilized as inclusion criteria for preemptive clinical trials.

4.0 Chronic GVHD Biomarkers

Chronic GVHD remains the most important long-term complication of allo-HCT causing significant morbidity, mortality and impact on quality of life. Identification and validation of biomarkers in cGVHD have lagged compared to aGVHD for a variety of reasons including: (a) heterogenous impact on recipient organs, (b) increased time frame of onset and course of the disease and (c) lack of multicenter trials with sufficient number of patients' samples [34]. Additionally, age related differences in the biology of cGVHD may exist [35, 36]. A summary of plasma cGVHD biomarkers by subtype is found in Table 5.

4.1 Chronic GVHD Biomarkers by Subtype

Diagnostic Biomarkers: Potential biomarkers to assist in the diagnosis of cGVHD have been discovered. Soluble B-cell activating factor (sBAFF), which plays a role in immune reconstitution and B lymphocyte homeostasis [37, 38], was one of the first biomarkers correlated with cGVHD [39]. Multiple studies found sBAFF to be elevated in patients with both early and late onset cGVHD [39–43], and patients who subsequently developed cGVHD had significantly different BAFF/B cell ratios at 3 months post-HCT [38]. Of note, studies have shown that treatment with corticosteroids can impact sBAFF levels and total B cell number, questioning the diagnostic utility of sBAFF for some cGVHD patients [39, 43].

Chemokine (C-X-C motif) ligand 9 (CXCL9) and chemokine (C-X-C motif) ligand 10 (CXCL10) are inflammatory chemokines involved in the activation and recruitment of various immune cells. Increased CXCL9 concentrations were found in patients with new onset cGVHD [44] and in cGVHD patients at 6 and 9 months post-HCT [41]. A gene expression study also found upregulation of CXCL9 and CXCL10 genes along with elevated plasma levels at the onset of cGVHD [45]. A follow-up study showed CXCL9 and CXCL10 significantly correlated with cGVHD in one replication cohort, but only CXCL10 in the second [42]. It is important to highlight that a study found viral infections such as with cytomegalovirus could impact levels of pro-inflammatory biomarkers such as CXCL10 [46].

A 4-biomarker panel consisting of ST2, CXCL9, matrix metalloproteinase-3 (MMP-3), and osteopontin (OPN) had significant correlation with cGVHD diagnosis [47]. MMP3 plasma concentrations were individually analyzed and found to be significantly different in patients with and without BOS [48].

Dickkopf-related protein 3 (DKK3) is a modulator of Wnt signaling pathways and involved in pathologic fibrosis and autoimmunity [49]. DKK3 was first identified as a potential cGVHD diagnostic biomarker of sclerotic skin cGVHD by proteomics, but elevated levels were

associated with any subcategory of cGVHD, suggesting it might be more of a systemic biomarker [50]. Reg3α, the most validated GI-aGVHD biomarker, remains underexplored as a potential GI-cGVHD marker. Reg3α concentrations were found to be correlated with GI-cGVHD [51] and warrants further prospective evaluation.

The NIH cGVHD Consensus project in 2005 and 2014 provided standardization of cGVHD diagnosis, however implementation of the criteria continues to be a challenge [52, 53]. There is currently no standard of care for post-transplant visits, so the diagnostic evaluation in some locations may be performed by a clinician with limited transplant and GVHD experience. Additionally, early signs and symptoms may not be diagnostic for cGVHD and a patient could have irreversible organ damage prior to meeting the cGVHD diagnostic criteria [40]. Current evaluation tools are also unable to differentiate active cGVHD from cumulative cGVHD damage. Once qualified for clinical use, diagnostic biomarkers could help simplify and standardize cGVHD diagnosis. Diagnostic biomarkers could also lead to earlier diagnosis and treatment, which has been shown to reduce the impact of cGVHD [54].

Predictive Biomarkers: To date, no predictive biomarker to anticipate cGVHD treatment response exists representing a major unmet need. Currently, only about 50% of cGVHD patients respond to steroids and prognosis for steroid-refractory cGVHD (SR-cGVHD) remains very poor [55]. Per the FDA-NIH definition, a predictive biomarker is assessed specifically prior to each treatment. Priority should be placed on identifying predictive biomarkers for 1st line therapy. Once validated, these biomarkers will help identify patients at highest risk for SR-cGVHD and help select initially steroid dosing. Thankfully, newer agents for SR-cGVHD such as ruxolitinib and ROCK2 inhibitor belumosudil have been FDA-approved [55, 56], and predictive biomarkers for each of these treatments can be established. This would eventually allow a personalized treatment approach to cGVHD, which may offer an opportunity to reduce the impact of clinically significant cGVHD prior to irreversible organ damage.

Response Biomarkers: Currently, studies to identify biomarkers that show a biologic response to cGVHD are lacking. A limited sample size study measured ST2 levels prior to and at 2-month intervals of extracorporeal photopheresis (ECP), a second-line treatment modality for cGVHD. The study found ST2 levels declined over the course of treatment, however all patients had favorable response to ECP, so the study was unable to correlate ST2 with disease activity or outcomes [57]. Another study found sBAFF levels at 1 month after ECP predicted response of cutaneous cGVHD at 3 and 6-months [58].

A biomarker analysis of patients receiving Ibrutinib, a kinase inhibitor, found a reduction in multiple inflammatory biomarkers, including CXCL9 and CXCL10, after initiation of therapy and showed a continued downtrend at subsequent time points [59]. Once validated, response biomarkers could also serve as an early indicator of response and clinical efficacy endpoints for novel cGVHD treatment clinical trials.

The NIH cGVHD consensus response criteria require a subjective evaluation and has potential for bias. Response biomarkers could help standardize the disease response evaluation, which would be particularly useful in cGVHD clinical trials that rely on accurate

clinical data. Response biomarkers that precede clinical improvement could provide valuable information for management decisions. This would be especially helpful in therapies such as ECP where clinical improvement may not be evident for several weeks. Currently, there is no standard of care on timing to initiate second-line cGVHD treatment. Recognizing treatment failure early could help initiate alternative agents which may prevent progressive cGVHD damage.

Prognostic Biomarkers: Prognostic biomarkers to predict severe cGVHD and long-term outcomes have been identified. Elevated CXCL9 concentrations measured at onset of non-severe cGVHD symptoms were associated with subsequent development of severe cGVHD [60]. Increased DKK3 concentrations in newly diagnosed cGVHD patients were associated with increased NRM [50]. A study of a small cohort of BOS patients investigated matrix metalloproteinase-9 (MMP-9), a protein shown to contribute to neutrophil migration to the lungs, and found increased concentrations at diagnosis were associated with worse overall survival [61]. Another study found high Reg3α levels at time of onset of cGVHD were prognostic of increased NRM [51].

Distinguishing cGVHD patients who will have mild disease from those who will develop severe cGVHD would impact management decisions. Mild forms of cGVHD have been associated with increased overall survival due to an increase GVT effect [62], but severe forms can lead to significant morbidity and mortality [63]. Prognostic biomarkers could help identify patients at the highest risk for severe cGVHD, leading to more aggressive therapies upfront. These patients could potentially benefit from starting a second-line cGVHD therapy in addition to systemic steroids. Conversely, patients at low risk for severe disease may benefit from decreased initial treatment, which would limit the impact on the GVT effect [54].

Risk Biomarkers: Unlike for aGVHD, risk biomarkers that predict future cGVHD have been identified. The 4-biomarker panel consisting of ST2, CXCL9, MMP-3, and OPN when measured at 100 days post-HCT, at least 3 months before the clinical diagnosis of cGVHD, was able to stratify patients more likely to develop cGVHD [47]. A multicenter phase 3 trial of patients found CXCL9 levels at either 100 or 180 days post-HCT correlated with subsequent cGVHD development [26]. An additional study found CXCL9 concentrations and genetic polymorphisms in CXCR3 ligands as early as 28 days post-HCT were correlated with the risk of severe cGVHD [64]. CD163 is a scavenger receptor shed by activated monocytes/macrophages during times of oxidative stress [65]. CD163 concentrations at 80 days post-HCT were associated with subsequent *de novo-onset* cGVHD [61]. Of note, no association was found with subsequent quiescent-onset cGVHD, suggesting CD163 concentrations may be influenced by aGVHD and its treatment [66].

As with risk biomarkers for aGVHD, qualified risk biomarkers could offer an opportunity for cGVHD prevention. Currently, there is marked heterogeneity in discontinuation of immunosuppressants after HCT [67]. Data provided by risk biomarkers could help personalize strategies for adjusting these medications, such as early taper of prophylactic immunosuppression in low risk patients, allowing improved immune reconstitution. Similar to aGVHD, qualified cGVHD risk biomarkers could provide inclusion criteria for

preemptive clinical trials, identifying patients would potentially benefit most from these interventions.

5.0 Clinical implementation successes and challenges

An important achievement towards clinical application of GVHD biomarkers is the rapid turn-around for real time biomarker analysis by commercial laboratories for ST2 and REG3α. The next steps include incorporation of biomarkers into randomized clinical trials and finally clinical practice. Currently however, no biomarker for either acute or chronic GVHD have completed the qualification step. With that being said, BMTCTN 1501 (NCT02806947) a randomized phase II multicenter trial, was a positive study evaluating sirolimus and prednisone in patients with Minnesota standard-risk and low biomarker score aGVHD. The study aimed to create risk-adapted GVHD therapy at GVHD onset based on clinical parameters and prognostic biomarker values, and found that patients with 'standard risk aGVHD' who received sirolimus had similar outcomes to those who received corticosteroids [68]. This study highlights the potential for biomarkers to help accurately identify GVHD patients that can be treated with a steroid-free regimen. Two additional studies attempted to use risk/prognostic biomarkers early post-HCT, however these studies showed no impact on patient outcomes [22, 69]. The studies found no difference in the treatment vs control groups, which may be due to the poor sensitivity/specificity of the biomarker or from the treatment itself.

When compared to aGVHD, biomarkers for cGVHD are even less along the path to clinical application, although the NIH consensus criteria have helped provide a framework for a unified approach to cGVHD [4, 40, 54, 63, 70, 71]. Chronic GVHD biomarker levels are more difficult to validate due to overlap syndrome, infections, recipient chimerism and outpatient sample processing [4]. Unlike aGVHD, no comprehensive biorepository for cGVHD samples allowing for discovery of predictive, response and risk biomarkers exists. Many of the promising cGVHD biomarkers require validation in large independent cohorts.

Conclusion

Although a significant amount of research focus has been dedicated to GVHD biomarkers, no risk biomarker for aGVHD or predictive biomarker for cGVHD have been identified, representing major unmet needs. Both acute and chronic GVHD biomarkers have a significant number of potential clinical applications, however, there is much work left to be done before GVHD biomarkers can be incorporated in routine clinical practice. The FDA-NIH Biomarker Working Group provided updated definitions, however these biomarkers have not always been used in the proper clinical context according to the BEST subtype definitions. Clinical decision making for GVHD prophylaxis, preemption, treatment, and treatment monitoring requires a delicate balance between GVHD and the GVT effect. Once properly qualified, we believe biomarkers can provide an extra tool to assist with these decisions and will prove to positively impact patient outcomes.

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Highlights

- Plasma biomarkers represent potentially non-invasive, objective, and cost-efficient risk stratification of patients with GVHD
- Use of the correct biomarker NIH-FDA BEST terminology will forward the GVHD biomarker effort as it will enable physicians in our field and other fields and regulatory authorities to speak the same language
- Since no GVHD biomarkers have yet completed the FDA 4-step framework, additional studies are required before GVHD biomarkers qualify for clinical use
- No risk biomarker for aGVHD or predictive biomarker for cGVHD has been identified
- A randomized phase 2 clinical trial successfully used biomarkers to guide steroid-free GVHD treatment

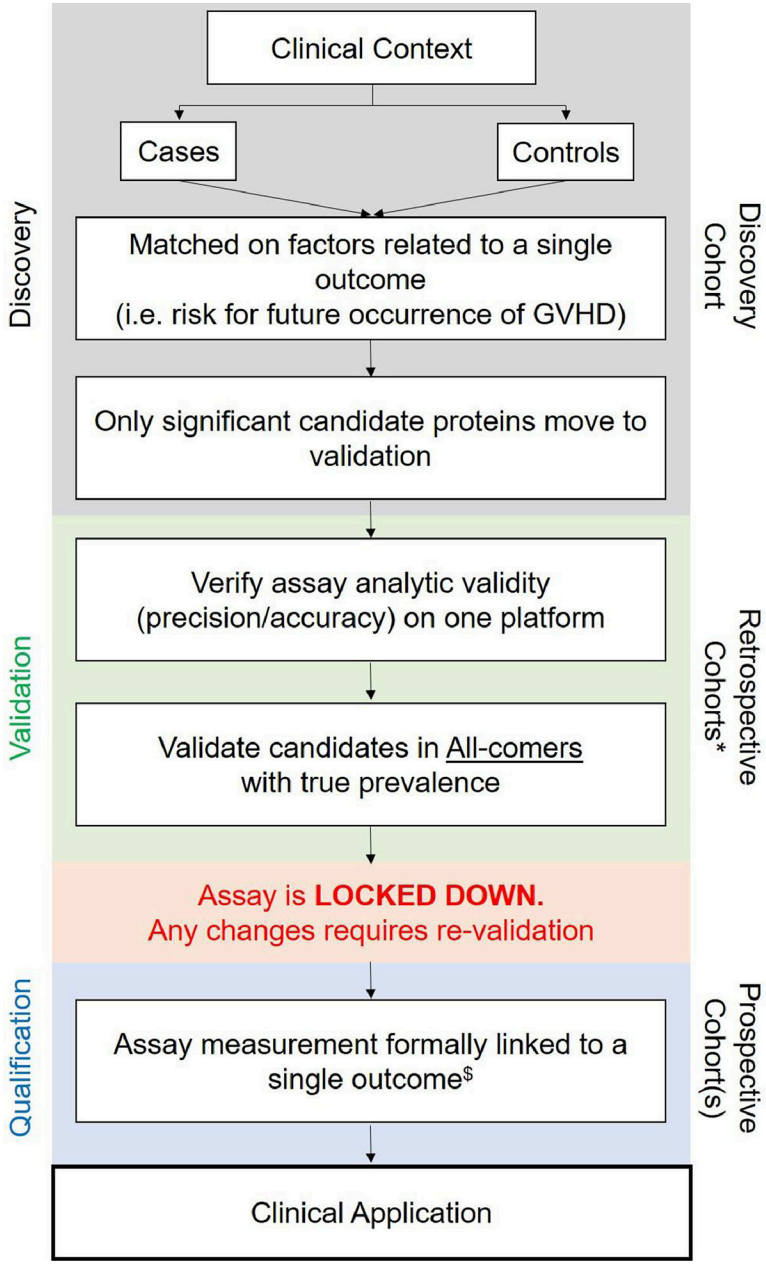

Figure 1.

Recommended practices for biomarker development. *Validation requires evaluation in at least 2 independent cohorts.$Once a candidate protein reaches the qualification phase,it is called a biomarker of a specific type (see definitions).

Table 1.

Biomarker definitions as per NIH BEST Resource [3]

Biomarker Subtype	Definition
Diagnostic	An assay used to confirm the presence of the disease
Predictive	An assay used to identify individuals who are more likely than similar individuals without the biomarker to experience a favorable or unfavorable effect from exposure to a specific medical product (before treatment is received)
Response	An assay used to show that a biological response has occurred in an individual who has been exposed to a medical product (after treatment is received)
Prognostic	An assay used to identify likelihood of a clinical event, disease recurrence or progression in patients who have the disease
Risk	An assay that indicates the potential for developing the disease in individuals who do not have clinically apparent disease

Table 2.

Assay analytical parameters

1.	Precision: Repeatability and reproducibility of an assay
2.	Accuracy: Proximity of results to true value
3.	Sensitivity: Limit of detection of an assay
4.	Specificity: Interference and cross reactivity of an assay
5.	Robustness: Capacity of an assay to remain unaffected by small but deliberate variations in method parameters, and provides an indication of its reliability during normal usage

Table 3.

Statistical tests performance

1.	Sensitivity: Proportion of subjects who test positive for a specific condition among a group of people who have the condition; how well a test can detect a specific condition in people who actually have the condition
2.	Specificity: Proportion of subjects who test negative for a specific condition among a group of people who do not have the condition
3.	Receiver operator characteristic (ROC) curve: A plot of the true-positive rate versus the false-positive rate for all possible cut points of a biomarker
4.	Incidence: number of individuals who develop a specific disease during a particular time period
5.	*Positive predictive value (PPV): Likelihood that a person who has a positive test result does have the disease
6.	*Negative predictive value (NPV): Likelihood that a person who has a negative test result indeed does not have the disease

*Require biomarker cut-point values and depend on incidence of disease

Table 4.

Plasma Biomarkers for Acute GVHD

Name	Study	(n)	Associations/Timepoints in aGVHD *(D0 = HCT date)*	Framework steps completed	Potential clinical implementation	Ref
Diagnostic Biomarkers: Systemic						
Biomarker panel: IL-2-R-α, HGF, IL-8, TNFR-1	Paczesny 2009	424	Identified GVHD at onset of symptoms	Step 1: Discovery Step 2: 2 cohorts	• Improve diagnostic accuracy • Differentiate GVHD vs other complications	9
Diagnostic Biomarkers: Organ specific						
REG3α	Ferrara 2011	1014	Increased levels at onset of symptoms in GI-GVHD	Step 1: Discovery Step 2: 2 cohorts	• Improve diagnostic accuracy in specific organs (ie colitis/ dermatitis vs GI/skin GVHD)	11
TIM3	Hansen 2013	149	Increased levels associated with GI-GVHD	Step 1: Discovery Step 2: 2 cohorts		13
Elafin	Paczesny 2010	492	Increased levels at onset of skin GVHD	Step 1: Discovery		15
	Solán 2021	149	Increased levels in skin GVHD	Step 1: Previous discovery		16
Predictive Biomarkers						
ST2	Vander Lugt 2013	673	Increased levels at onset of treatment with corticosteroids associated with SR-aGVHD	Step 1: Discovery Step 2: 3 cohorts	• Intensify for high risk group • Reduce immunosuppression for low/standard risk group	19
Response Biomarkers						
ST2, TIM3	McDonald 2017	165	Increased levels after 14d of steroids predicts treatment failure	Step 1: Hypothesis	• Monitoring treatment response • Guide GVHD management • Future: Clinical efficacy endpoint	23
Prognostic Biomarkers						
REG3α	Ferrara 2011	1014	Increased levels at diagnosis associated with NRM	Step 1: Discovery Step 2: 2 cohorts	• Anticipate course of disease • Adjust immunosuppression	11
	Rowan 2020	415	Increased levels D+7, D+14 and D+21 associated with NRM	Step 1: Previous discovery Step 2: Multi-center cohorts		25
ST2	Vander Lugt 2013	673	Increased levels D+14 associated with NRM	Step 1: Discovery Step 2: 3 cohorts		19
	Ponce 2015	113	Increased levels D+28 associated with TRM in CBT	Step 1: Previous discovery		27
	Abu Zaid 2017	211	Increased levels D+28 associated with NRM	Step 1: Previous discovery Step 2: Clinical trial cohorts		26
	Kanakry 2017	58	Increased levels D+30 associated with NRM in haplo PTCy	Step 1: Previous discovery		28
	Rowan 2020	415	Increased levels D+7, D+14 and D+21 associated with NRM	Step 1: Previous discovery Step 2: Multi-center cohorts		25

Name	Study	(n)	Associations/Timepoints in aGVHD *(D0 = HCT date)*	Framework steps completed	Potential clinical implementation	Ref
Biomarker Algorithm	Levine 2015	792	Score based on ST2 + Reg3a (+TNFR1) at GVHD categorizes into groups for NRM	Step 1: Previous discovery Step 2: 3 cohorts		21
	Hartwell 2017	1287	Score based on ST2 + Reg3a D+7 categorizes into groups for NRM	Step 1: Previous discovery Step 2: multi-center cohorts		30
	Hotta 2021	112	Score based on ST2 + Reg3a D+7 categorizes into groups for NRM	Step 1: Previous discovery Step 2: Multi-center cohorts		31
	Major-Monfried 2018	507	Score based on ST2 + Reg3a after 7d of steroids categorizes into groups for NRM in SR-aGVHD	Step 1: Previous discovery Step 2: 2 cohorts		32
	Srinagesh 2019	615	Score based on ST2 + Reg3a after 28d of steroids categorizes into groups for NRM	Step 1: Previous discovery		33
TIM3	Abu Zaid 2017	211	Increased levels D+28 associated with NRM	Step 1: Previous discovery Step 2: Clinical trial cohorts		26
Risk Biomarkers						
No validated aGVHD risk biomarker exists					• Implement preemptive strategies	

NRM, nonrelapse mortality, aGVHD, acute graft versus host disease, TRM, transplant related mortality, CBT, cord blood transplant, SR, steroid refractory, Haplo, haploidentical, PT Cy, post-transplant cyclophosphamide

Table 5.

Plasma Biomarkers for Chronic GVHD

Name	Study	(n)	Associations/ Timepoints in cGVHD *(D0 = HCT date)*	Framework steps completed	Potential clinical implementation	Ref
Diagnostic Biomarkers						
sBAFF	Sarantopoulos 2007	104	Increased levels in active cGVHD	Step 1: Discovery	• Improve diagnostic accuracy • Differentiate GVHD vs other complications	39
	Ahmed 2015	115	Increased levels at 6 and 12 months	Step 1: Previous discovery		41
	Kariminia 2016	283	Increased levels around time of diagnosis	Step 1: Previous discovery Step 2: 2 cohorts		42
	Rozmus 2019	107	Increased levels around onset of symptoms	Step 1: Previous discovery		43
CXCL9	Kitko 2014	320	Increased levels at diagnosis	Step 1: Discovery Step 2: 2 cohorts		44
	Kariminia 2016	85	Increased levels in 1 replication cohort	Step 1: Previous discovery Step 2: 2 cohorts		42
	Hakim 2016	95	Increased levels and upregulation of gene expression	Step 1: Previous discovery		45
CXCL10	Ahmed 2015	115	Increased levels at 6 and 12 months	Step 1: Hypothesis		41
	Kariminia 2016	283	Increased levels in both replication cohorts	Step 1: Previous discovery Step 2: 2 cohorts		43
	Hakim 2016	95	Increased levels and upregulation of gene expression	Step 1: Hypothesis		45
Biomarker panel: ST2, MMP3, CXCL9, OPN	Yu 2016	172	Increased levels at diagnosis	Step 1: Discovery Step 2: 2 cohorts		47
MMP3	Liu 2016	112	Increased levels in BOS patients	Step 1: Discovery		48
DKK3	Inamoto 2020	186	Increased levels at diagnosis	Step 1: Discovery		50
Reg3α	DePriest 2021	289	Increased levels associated with GI-cGVHD	Step 1: Previous discovery Step 2: 2 cohorts		51
Predictive Biomarkers						
No validated cGVHD predictive biomarker exists					• Intensify for high risk group • Reduce immunosuppression for low/standard risk group	
Response Biomarkers						
sBAFF	Whittle 2011	46	Increased levels 1 month after ECP predicted response of cutaneous cGVHD	Step 1: Previous discovery	• Monitoring treatment response • Guide GVHD management • Future: Clinical efficacy endpoint	58
ST2	Dunavin 2018	16	ST2 levels declined after 2-, 4- and 6-months of ECP	Step 1: Previous discovery		57

Name	Study	(n)	Associations/ Timepoints in cGVHD *(D0 = HCT date)*	Framework steps completed	Potential clinical implementation	Ref
Prognostic Biomarkers						
CXCL9	Giesen 2020	480	Increased levels at symptom onset associated with severe cGVHD	Step 1: Previous discovery	• Anticipate course of disease • Adjust immunosuppression	60
DKK3	Inamoto 2020	186	Increased levels at diagnosis associated with NRM	Step 1: Discovery		50
MMP-9	Inamoto 2021	33	Increased levels at BOS diagnosis associated with OS	Step 1: Discovery		61
Reg3α	DePriest 2021	289	Increased levels at GI-cGVHD diagnosis associated with nrm	Step 1: Previous discovery Step 2: 2 cohorts		51
Risk Biomarkers						
Biomarker panel: ST2, MMP3, CXCL9, OPN	Yu 2016	172	Levels D+100 associated with cGVHD development	Step 1: Discovery Step 2: 2 cohorts	• Implement preemptive strategies	47
CXCL9	Abu Zaid 2017	211	Increased levels D+100 or D+180 associated with cGVHD development	Step 1: Previous discovery Step 2: Clinical trial cohorts		26
	Dai 2021	287	Increased levels D+28 associated with severe cGVHD development	Step 1: Previous discovery		64
CD163	Inamoto 2017	167	Increased levels D+80 associated with de-novo cGVHD	Step 1: Previous discovery		66