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Clinical Applications for Biomarkers of Acute and Chronic Graft vs. Host Disease

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Abstract

Acute and chronic graft versus host disease (GVHD) are serious complications of allogeneic hematopoietic cell transplantation (HCT). The complex pathophysiology of these disease processes is associated with immune system activation, the release of cytokines and chemokines, and alterations in cell populations. The blood levels of specific protein and cellular levels in patients with GVHD have correlated with the development, diagnosis, and prognosis of GVHD. Here we review the most promising biomarkers for acute and chronic GVHD with clinical relevance. The utility of GVHD biomarkers in clinical care of allogeneic HCT recipients needs to be proven through clinical trials, and potential approaches to trial design are discussed.

Keywords

Acute GVHD; Chronic GVHD; Biomarkers; Allogeneic hematopoietic cell transplantation

INTRODUCTION

Graft versus host disease (GVHD) remains the most serious and challenging complication of allogeneic hematopoietic cell transplantation (HCT). Despite advances in treatment and prevention, concern over the morbidity and mortality of acute and chronic GVHD represents a barrier to greater utilization of allogeneic HCT as a potentially curative modality for patients with malignant and non-malignant diseases. New diagnostic and therapeutic tools are needed to customize the delivery of immunosuppressive drugs for optimal patient care. To that end, there has recently been considerable research effort devoted to the discovery and validation of GVHD relevant biomarkers. The paucity of validated biomarkers for acute GVHD is partly due to the complex pathology of GVHD that can be considered in a framework of three distinct sequential phases of immune system cellular activation and cytokine production, which would be expected to influence specific cellular and protein levels in the GVHD patients' blood [1]. GVHD is not only a systemic immunological disorder but also affects specific organ systems, including the skin, gastrointestinal (GI) tract, and liver. The clinical symptoms of the skin (maculopapular rash) and of the GI tract (nausea, diarrhea) caused by GVHD can be difficult to distinguish from other causes (e.g. infectious, drug-induced). Thus, biomarkers that are GVHD and target organ specific may improve the diagnosis, management, and prognosis of complications post-HCT. Potential applications

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include predicting response to treatment, defining new risk strata that incorporate biomarker values, and initiating pre-emptive therapy before onset of clinical symptoms. The latter is particularly relevant to chronic GVHD, where without objective biologically relevant measurements of disease, rigorous clinical trials remain difficult to conduct and interpret, and treatment remains palliative. Here we review the current state of the science of this rapidly evolving field.

IDENTIFICATION OF ACUTE GVHD BIOMARKERS

Advances in engineering have allowed for increased data throughput, enabling the study of complete sets of molecules (omics) with exponential speed, accuracy, and cost-effectiveness. Thus, analysis of the entire spectrum of molecular and cellular organization is now possible, enabling researchers to gain insight into the mechanism of diseases, with fewer *a priori* assumptions [2]. Proteomics has certain advantages in the study of acute GVHD. First, proteins are more proximate than other cellular metabolites to the ongoing pathophysiology of this disease. Indeed, studies using genomics, transcriptomics, and gene polymorphisms incompletely correlate with the expression of functionally-active proteins, which more accurately reflect cellular crosstalk, such that it is likely that proteins will provide the most ideal disease biomarkers [3,4]. Correlating the proteome with acute GVHD has been attempted by analysis of polypeptide fragments in the urine [5] and the measurement of single potentially informative proteins such as C-reactive protein[67] or cytokeratin-18[8]. A particularly successful strategy that we have used has been the analysis of plasma samples to identify multiple proteins differentially expressed in patients with acute GVHD. This technique, called the Intact Protein Analysis System (IPAS), matches the mass spectra in the plasma to a sequence database to identify proteins. Briefly, plasma from patients who never developed GVHD was pooled together (GVHD-negative) as was plasma from patients at the time that GVHD developed (GVHD-positive). The GVHD-negative and GVHD-positive pool were labeled with different carbon isotopes. The two pools were combined and specimens were subjected to a two-dimensional protein fractionation procedure. The individual fractions were then digested and analyzed on a new generation liquid chromatography tandem mass spectrometer (LC-MS/MS). Because protein digestion was performed in a top-down fashion prior to mass spectrometry, the term “intact” protein analysis is used [9]. The acquired spectra were automatically processed by the high-throughput Computational Proteomics Analysis System to identify proteins in the sample, with a false discovery rate of < 5% [10]. This resulted in the identification of proteins with a range of concentrations spanning seven logs [11]. This technique was therefore able to detect low abundance proteins, and is quantitative, as each GVHD pool was labeled with heavy and light stable isotopes. The list of proteins identified by MS/MS described above was then prioritized based on their degree of dysregulation, as indicated by at least a 1.5-fold increase in expression, the likelihood of involvement in GVHD pathways based on known pathways and uniqueness to the target organ that is associated with a given GVHD type. Finally, we prioritized proteins with available sandwich enzyme-linked immunosorbent assay (ELISA) antibodies in order to facilitate the development of a GVHD blood test. A list of candidate acute GVHD biomarkers with diagnostic or prognostic significance is shown in Table 1.

VALIDATION OF ACUTE GVHD BIOMARKERS

Validation of putative GVHD biomarkers is usually performed with immunoassays rather than mass spectrometry, and the sample set is created from a cases-controls repository involving large numbers of samples. This process should be done on a training set, followed by an independent validation set; validation using sets from multiple institutions is ideal. The final step of developing a clinical test uses the biomarkers in the clinic, typically on thousands of samples. For high-throughput purposes and standardization between

laboratories, only immunoassays are used at this step. Figure 1 describes the three steps of the process required to translate candidate biomarkers into a blood test.

In our initial validation studies, we used an antibody array approach to identify and sequential ELISA to validate four systemic biomarkers that, when combined into a GVHD biomarker panel, accurately discriminated GVHD-negative from GVHD-positive patients and carried prognostic significance[12]. Because biomarkers present at the time of GVHD diagnosis might be different between target organ-specific GVHD, we also sought to identify biomarkers that were specific for GVHD target organs to improve diagnostic and prognostic values of the systemic panel by comparing patients with skin-specific GVHD or GI-specific GVHD to patients without GVHD with IPAS. It is possible that it could be difficult to find proteins in the blood that are expressed in tissue, but since tissue proteins can leak into the blood stream, it might be a reasonable endeavor. These are proteins that normally function within cells but that can be released into plasma as a result of cell death or damage [13,14]. To assess the validity of this approach, we compared plasma pooled from ten patients with skin-specific GVHD to that from ten controls in the first IPAS run, and plasma pooled from ten patients with GI tract-specific GVHD to ten controls in a second IPAS run. Elafin emerged as the lead biomarker candidate of skin GVHD at the time of clinical diagnosis and we showed that plasma elafin concentrations have significant diagnostic and prognostic power, including long-term survival, as a biomarker of skin GVHD_ENREF_2 [15]. These data provide a proof-of-principle demonstration that biomarkers of disease-related tissue-specific changes can be detected in the plasma of patients. Using the same proteomics strategy, we discovered Regenerating-Islet-Derived-3-alpha (REG3α) as a biomarker of lower GI GVHD and subsequently validated it in two independent sets totaling 1014 patients from three different centers. This marker provides important prognostic information, including response to GVHD treatment and survival [16]. Physicians are interested in both low- and high-risk groups for predicting the development of GVHD and resulting clinical outcomes. Classical prognostic clinical outcomes in acute GVHD are maximum GVHD grade, non-relapse mortality (NRM), relapse mortality, and overall survival (OS). For example, we analyzed whether REG3α concentrations have prognostic significance for patients presenting with lower GI GVHD and hypothesized that the REG3α concentration at GVHD diagnosis would also correlate with NRM. We therefore divided the 162 patients into 2 equal groups based upon the median REG3α concentration: high (> 151ng/ml, n=81) and low (≤ 151 ng/ml, N=81). NRM was twice as high in patients with high REG3α concentrations, and this difference remained significant after adjusting for known risk factors of donor type, degree of HLA match, conditioning intensity, age and baseline disease severity. The incidence of relapse mortality was comparable for both groups, and thus patients with high REG3α concentrations at the time of GVHD diagnosis experienced significantly inferior OS [16] .

IDENTIFICATION AND VALIDATION OF CHRONIC GVHD BIOMARKERS

At the first meeting of the NIH biomarker consensus group in 2006, the ideal chronic GVHD (cGVHD) biomarker was formally defined[17,18]. Several inflammatory markers and cytokines like TGF-β1, TNF and IFN-γ, that are increased in acute GVHD, have been identified as candidate biomarkers for cGVHD, but none have been developed for clinical use. Genetic markers of cGVHD development have also been proposed, such as MHC class I chain-related protein A (MICA)-129 genotype and the negative regulator of T cell co-stimulation CTLA-4 +49 A/G*GG genotype, but the significance of these remains unknown [19,20]. Currently there are no validated biomarkers for cGVHD.

cGVHD pathophysiology remains inextricably linked to GVL in patients [21], thus further complicating efforts to define a predictive biomarker for this disease [21]. T cell responses

directed at minor histocompatibility antigens are vital to cGVHD pathogenesis. Recognition of B cell autoreactivity likely emanating from inciting T cell alloreactivity in cGVHD [22–25] sparks continued interest in antibody responses in patients [25]. When human B cell responses to alloantigens were characterized and correlated with cGVHD development, a resurgence of interest in B cell subsets and potential factors that drive B cells in this disease ensued [26–28]. Extensive reviews of candidate biomarkers have been recently published [29,30]. Thus, we will focus on newly elucidated BAFF and B cell pathophysiology that may inform larger scale efforts aimed at candidate biomarker validation in cGVHD.

B Cell Activating Factor (BAFF) and Human cGVHD

Characterization of the TNF family member BAFF changed the way we think about B cell autoreactivity [31] and led to the discovery of significantly elevated BAFF levels in patients with active cGVHD [32,33]. Ease of measurement, accessibility of plasma samples from patients and preliminary data suggesting that significant elevation of BAFF preceded cGVHD development [32] make soluble BAFF a tempting biomarker. In a prospective study of new onset cGVHD in 52 children and 28 control patients who never developed cGVHD, BAFF levels were elevated in patients with cGVHD that developed irrespective of time of onset post-HCT, and these levels decline in patients who show clinical response to treatment [33]. Anti-dsDNA antibodies were also elevated in patients who developed cGVHD, confirmatory of previous reports[25] and further implicating the importance of B-cells in cGVHD activity.

Measurement of soluble BAFF by itself is complicated by several factors: 1) BAFF is increased in setting of B lymphopenia [34,35] 2) BAFF levels are low in patients taking high dose steroids[32]; and 3) precise quantification of BAFF is challenging since BAFF may exist in an oligomeric form which is understimated using current ELISA[36]. Even without precise quantification, significantly increased BAFF levels are found in cGVHD patients as well as significantly higher BAFF/B cell ratios [37,38]. Further corroborating the relevance of BAFF in cGVHD pathophysiology and potentially pointing to novel genotypic predictive markers of disease is the reported increased frequency of BAFF polymorphisms in HCT recipients who developed cGVHD. Whether these genetic differences confer increased BAFF production remains to be determined[39].

Robust B cell Reconstitution: A Few Good B Cells in Human cGVHD

Poor B cell reconstitution in cGVHD linked to immune deficiency has been well described[40,41]. B cell numbers are not lower than normal in cGVHD, but they are lower compared to healthy post-HCT patients. That is, a well-described supranormal ‘surge’ in naïve B cell number found after lymphopenia induction in the healthy state is absent in cGVHD patients [42,43]. Transitional B-cells, which bridge newly formed B-cells in the bone marrow and peripheral maturation, circulate in the peripheral blood and have been defined in humans [44]. These human transitional subsets defined using IgD^{+} and $CD38^{Hi}$, $CD27^{-}$ populations are increased to supranormal numbers in patients who never develop cGVHD [38], suggesting return to B cell homeostasis is vital to a non-autoimmune phenotype in cGVHD [45].

Other B cell subsets identified using CD21 and additional markers of murine transitional B cells were found to be increased in cGVHD patients [46]. Interestingly, decreased proportions of $CD19^{+}CD21^{lo}$ cells prior to extracorporeal photopheresis (ECP) correlated with positive treatment outcome with ECP [47]. Subsequent analysis revealed a relative decrease in this cell population in those cGVHD patients with hypogammaglobulinemia [37].

The association of clinical decline in cGVHD patients who failed to reconstitute the naïve B cell compartment after rituximab [48] suggests that altered B cell homeostasis due to diminished bone marrow B cell production capacity is critical. To further examine this, Fedoriw et al. studied thirty patients who at a median post-HSCT follow-up time of two years, had developed cGVHD (n=15) or never developed (n=15) cGVHD. Bone marrow biopsies obtained approximately one month after HSCT revealed significantly fewer B cell precursors in the patients who later developed cGVHD (median = 2 vs. 44 cells/hpf; p=0.0007), and the difference was maintained after patients on high dose steroid therapy were excluded (median = 20 vs. 49 cells/hpf; p=0.0170) [49]. These data suggest that decreased pre-cursor B cells in bone marrow may be predictive of cGVHD development, while increased precursor B cells in the marrow, and/or increased transitional and naïve B cells in the blood may be predictors of a healthy post-HSCT outcome. Taken together, current evidence suggests examination of BAFF and B cells in blood and bone marrow may lead to testable biomarker candidates for good health after HSCT (Figure 2A).

A Few Bad B cells in Human cGVHD

Improved understanding of B cell subsets in secondary lymphoid organs and in autoimmune diseases enabled identification of uniquely circulating B cell subsets in cGVHD [50]. Decreased numbers of naïve and transitional B cells result in a proportional increase in potentially autoreactive, antigen-experienced cells marked by cell surface CD27+ expression[38,51]. The CD27+ B cell population in autoimmune states is distinct from those anti-microbial ‘memory’ B cells typically found in healthy individuals. In HCT patients with cGVHD, we find that this population is activated and capable of *ex vivo* constitutive IgG secretion [38]. A subset of CD27+ B cells (pre-germinal center (GC) and plasmablast (PB)-like cells) uniquely circulate in diseased patients, including cGVHD patients [38,52]. The pre-GC population in cGVHD is of particular interest given the high expression of two important BAFF receptors found on these cells, further suggesting their potential pathologic role [38]. Prospective serial analysis of these B-cell receptor (BCR)-activated $CD27^{+}$ B cell subsets is warranted to determine whether their presence associates with cGVHD onset, severity or treatment response (Figure 2B).

INCORPORATING GVHD BIOMARKERS IN CLINICAL TRIALS

Given the progress being made in GVHD biomarker identification and validation it is not surprising that clinical trial design will begin incorporating biomarkers. As an example, TNF-receptor-1 (TNFR1) levels were shown to be elevated, relative to pre-HCT baseline, on day 7 post-HCT in patients who later went on to develop acute GVHD after myeloablative conditioning regimens[53]. In this study, the degree of change in TNFR1 levels strongly correlated with the timing and severity of acute GVHD as well as non-relapse mortality and overall survival. The outcome implications were particularly relevant in the 171 patients who underwent HCT from an unrelated donor, in that patients with high TNFR1 levels on day 7 post-HCT were much more likely to experience non-relapse death in the first post-HCT year (49% vs. 28%, p=0.01), translating into a significant difference in survival at one-year. These findings led to the development of a clinical trial that added the TNF-inhibitor etanercept to a standard tacrolimus/methotrexate GVHD prophylaxis regimen for recipients of myeloablative unrelated donor HCT. In this prospective clinical trial, etanercept effectively prevented the expected rise in TNFR1 levels in patients receiving non-TBI based conditioning regimens, but interestingly not in recipients of TBI-based conditioning. The patients who received non-TBI based conditioning unrelated donor HCT experienced attenuated forms of GVHD (primarily steroid-responsive skin GVHD), relatively low rates of 1-year NRM (16%) and high 1-year survival (69%)[54]. In light of the finding that a single biomarker, TNFR1, had predictive value for onset of acute GVHD, we tested whether other potentially informative biomarkers (IL2Rα and elafin) could be combined with TNFR1 into

a predictive GVHD biomarker panel. Levels of each biomarker was assessed at day 7 and day 14 post-HCT in 513 patients who had undergone unrelated HCT and had not yet developed GVHD[55]. Following its discovery as a GI-GVHD specific biomarker, reg3α was also assayed, and additional samples from day 21 and 28 in patients without GVHD were included. The endpoint was the development of grade II–IV acute GVHD by day 56 post-HCT. Day 56 was chosen under the assumption that the plasma proteome at a given time point would not reliably predict the occurrence of events many weeks or months later. After testing different biomarker combinations, a final panel consisting of IL2Rα, TNFR1, and reg3α was found to have strong predictive value. Patients can be categorized as at high risk on a weekly basis, up until day 28 for GVHD occurring within the first two months post-HCT. As with any screening test, improvements in sensitivity come at the expense of specificity and vice versa and which aspect to emphasize is a matter of clinical judgment. The experience with post-HCT CMV disease offers an instructive example in how the transplant community approached this sort of problem. Prior to the development of CMV predictive tests, the incidence of CMV disease was ~35%, with high mortality rates. The introduction of CMV pre-emptive strategies guided by polymerase chain reaction or antigenemia studies reduced CMV disease to ~5–15%[56]. Extrapolating from published data of the number of positive CMV screening tests compared to the expected number of cases of CMV disease, it appears that ~50% of positive CMV screens, if untreated, would not result in CMV disease[57,58]. The sensitivity of CMV screening tests is very high, in the range of 90%, meaning that relatively few cases of CMV disease develop in the absence of a positive screening test. Thus, it has become common practice to administer pre-emptive therapy to patients who were not likely to develop CMV disease in order to effectively prevent CMV disease cases. If we applied a similar standard to GVHD pre-emptive therapy (1:1 true positive to false positive), the sensitivity of the 3 biomarker GVHD prediction panel is 67%. While not yet as accurate as the gold standard, CMV screening, we believe that these results are sufficient to design a clinical trial to test whether a preemptive strategy would prevent GVHD. The toxicity of the intervention is an important consideration in trial design, as excess toxicity from preemption will dampen acceptance of the strategy. A short-course of corticosteroid therapy at the time that markers of alloreactivity are increasing may be a reasonable therapy to test. The success of preemption will need to include not only any reduction in the incidence of GVHD, but also any increase infectious complications and relapses that may occur. Ultimately, a randomized trial will be needed to assess the effectiveness of GVHD preemption. A possible randomized trial design is illustrated in Figure 3. The trial design assumes a GVHD grade II–IV incidence of 44% and that the intervention to preempt GVHD is successful 50% of the time. The biomarker panel to predict GVHD has a sensitivity of 66% and specificity of 50%. Given these parameters, 57% of patients will be categorized as high risk for GVHD. All patients receive a treatment, either placebo or intervention. Patients categorized as low risk for GVHD (43%), i.e. weekly biomarker panel results from day 7 to day 28 do not predict for GVHD, receive placebo alone. The expected GVHD incidence in the low risk patients is 36%. Patients categorized as high risk for GVHD based on a positive biomarker panel result are randomized to either placebo or the intervention. Patients randomized to placebo should be twice as likely to develop GVHD as the intervention group (54% vs 27%). The difference in GVHD rates between the two placebo groups (54% vs 36%) is due to overrepresentation of GVHD in the high risk, placebo-treated arm.

Another potential clinical application of GVHD biomarkers is to use them to risk-stratify patients at the time of GVHD onset. Gastrointestinal GVHD is considered a high risk feature in the GVHD grading system, but given the absence of further risk stratification, the standard of care for all patients with GI GVHD is prompt initiation of systemic steroid treatment, with the addition of second line agents reserved for patients who fail frontline therapy. Unfortunately, most patients who require second line therapy die, highlighting the

need for refinement of risk beyond what the current grading system provides. We have recently developed a risk stratification algorithm for patients with new onset GI GVHD that incorporates clinical stage, histologic grade, and plasma levels of the newly discovered GI GVHD biomarker, reg3α. This easy-to-use algorithm assigns one equal weight point to each of the three individual risk factors: clinical stage >1, histologic grade >3, and reg3α level > 151 ng/ml. Patients with 2 or more risk factors at onset were less likely to respond to treatment and this translated into highly significant differences in NRM. Patients with 2 or 3 risk factors (high risk) at the onset of clinical manifestations of GI GVHD experience 1y NRM rates of 71%, while patients who present with 0 or 1 risk factor (standard risk) experience 1y NRM rates of 30% ($p<0.0001$). Early identification of patients at high risk for treatment unresponsiveness may permit testing alternative therapies before refractory disease develops.

Pre-emptive strategies for chronic GVHD, similar to those discussed for acute GVHD above, are also being designed. Given the correlation between B-cell related biomarkers and the development of cGVHD, together with clinical data supporting the use of rituximab to prevent cGVHD[59], there is a Canadian trial under design that will administer rituximab to children identified as high risk for development of cGVHD on the basis of biomarker assays (personal communication, K. Schultz). The advent of the chronic GVHD consortium is likely to spur additional research endeavors along these lines[60].

References

1. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. Lancet. 2009; 373:1550–1561. [PubMed: 19282026]
2. Nicholson JK, Holmes E, Lindon JC, Wilson ID. The challenges of modeling mammalian biocomplexity. Nat Biotechnol. 2004; 22:1268–1274. [PubMed: 15470467]
3. Mann M, Jensen ON. Proteomic analysis of post-translational modifications. Nat Biotechnol. 2003; 21:255–261. [PubMed: 12610572]
4. Cristea IM, Gaskell SJ, Whetton AD. Proteomics techniques and their application to hematology. Blood. 2004; 103:3624–3634. [PubMed: 14726377]
5. Weissinger EM, Schiffer E, Hertenstein B, et al. Proteomic patterns predict acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Blood. 2007; 109:5511–5519. [PubMed: 17339419]
6. Pihusch M, Pihusch R, Fraunberger P, et al. Evaluation of C-reactive protein, interleukin-6, and procalcitonin levels in allogeneic hematopoietic stem cell recipients. Eur J Haematol. 2006; 76:93–101. [PubMed: 16405429]
7. Fuji S, Kim SW, Fukuda T, et al. Preengraftment serum C-reactive protein (CRP) value may predict acute graft-versus-host disease and nonrelapse mortality after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2008; 14:510–517. [PubMed: 18410893]
8. Luft T, Conzelmann M, Benner A, et al. Serum cytokeratin-18 fragments as quantitative markers of epithelial apoptosis in liver and intestinal graft-versus-host disease. Blood. 2007; 110:4535–4542. [PubMed: 17702900]
9. Faca V, Coram M, Phanstiel D, et al. Quantitative analysis of acrylamide labeled serum proteins by LC-MS/MS. J Proteome Res. 2006; 5:2009–2018. [PubMed: 16889424]
10. MacLean B, Eng JK, Beavis RC, McIntosh M. General framework for developing and evaluating database scoring algorithms using the TANDEM search engine. Bioinformatics. 2006; 22:2830–2832. [PubMed: 16877754]
11. Faca V, Pitteri SJ, Newcomb L, et al. Contribution of protein fractionation to depth of analysis of the serum and plasma proteomes. J Proteome Res. 2007; 6:3558–3565. [PubMed: 17696519]
12. Paczesny S, Krijanovski OI, Braun TM, et al. A biomarker panel for acute graft-versus-host disease. Blood. 2009; 113:273–278. [PubMed: 18832652]

13. Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. Mol Cell Proteomics. 2002; 1:845–867. [PubMed: 12488461]
14. Putnam, FW. The plasma proteins Structure, Function and Genetic control. New York: Academic Press; p. 1975-1987.
15. Paczesny S, Braun T, Levine JE, et al. Elafin is a biomarker of graft-versus-host disease of the skin. Sci Transl Med. 2010; 2:50–57.
16. Harris A, Ferrara J, Levine J, et al. Reg3a is a biomarker of graft versus host disease of the gastrointestinal tract (Abstract). Biology of Blood and Marrow Transplantation. 2011; 17:S163–S164.
17. Schultz KR, Miklos DB, Fowler D, et al. Toward biomarkers for chronic graft-versus-host disease: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: III. Biomarker Working Group Report. Biol Blood Marrow Transpl. 2006; 12:126–137.
18. Pavletic SZ, Lee SJ, Socie G, Vogelsang G. Chronic graft-versus-host disease: implications of the National Institutes of Health consensus development project on criteria for clinical trials. Bone Marrow Transplant. 2006; 38:645–651. [PubMed: 16980994]
19. Boukouaci W, Busson M, Peffault de Latour R, et al. MICA-129 genotype, soluble MICA, and anti-MICA antibodies as biomarkers of chronic graft-versus-host disease. Blood. 2009; 114:5216–5224. [PubMed: 19786616]
20. Azarian M, Busson M, Lepage V, et al. Donor CTLA-4 +49 A/G*GG genotype is associated with chronic GVHD after HLA-identical haematopoietic stem-cell transplantations. Blood. 2007; 110:4623–4624. [PubMed: 18056853]
21. Lee SJ, Klein JP, Barrett AJ, et al. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. Blood. 2002; 100:406–414. [PubMed: 12091329]
22. Schultz KR, Paquet J, Bader S, HayGlass KT. Requirement for B cells in T cell priming to minor histocompatibility antigens and development of graft-versus-host disease. Bone Marrow Transpl. 1995; 16:289–295.
23. Tivol E, Komorowski R, Drobyski WR. Emergent autoimmunity in graft-versus-host disease. Blood. 2005; 105:4885–4891. [PubMed: 15746077]
24. Zhang C, Todorov I, Zhang Z, et al. Donor CD4+ T and B cells in transplants induce chronic graft-versus-host disease with autoimmune manifestations. Blood. 2006; 107:2993–3001. [PubMed: 16352808]
25. Patriarca F, Skert C, Sperotto A, et al. The development of autoantibodies after allogeneic stem cell transplantation is related with chronic graft-vs-host disease and immune recovery. Exp Hematol. 2006; 34:389–396. [PubMed: 16543073]
26. Zorn E, Miklos DB, Floyd BH, et al. Minor histocompatibility antigen DBY elicits a coordinated B and T cell response after allogeneic stem cell transplantation. J Exp Med. 2004; 199:1133–1142. [PubMed: 15096539]
27. Miklos DB, Kim HT, Miller KH, et al. Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. Blood. 2005; 105:2973–2978. [PubMed: 15613541]
28. Small TN, Robinson WH, Miklos DB. B cells and transplantation: an educational resource. Biol Blood Marrow Transplant. 2009; 15:104–113. [PubMed: 19147088]
29. Holler, E.; Dickinson, A. Biomarkers in Chronic Graft versus Host Disease. In: Vogelsang, GB.; Pavletic, SZ., editors. Chronic Graft versus Host Disease. Cambridge: Cambridge University Press; 2009.
30. Rozmus J, Schultz KR. Biomarkers in chronic graft-versus-host disease. Expert Rev Hematol. 2011; 4:329–342. [PubMed: 21668397]
31. Brink R. Regulation of B cell self-tolerance by BAFF. Semin Immunol. 2006; 18:276–283. [PubMed: 16916609]
32. Sarantopoulos S, Stevenson KE, Kim HT, et al. High Levels of B-Cell Activating Factor in Patients with Active Chronic Graft-Versus-Host Disease. Clin Cancer Res. 2007; 13:6107–6114. [PubMed: 17947475]

33. Fujii H, Cuvelier G, She K, et al. Biomarkers in newly diagnosed pediatric-extensive chronic graft-versus-host disease: a report from the Children's Oncology Group. Blood. 2008; 111:3276–3285. [PubMed: 17925486]
34. Gorelik L, Gilbride K, Dobles M, Kalled SL, Zandman D, Scott ML. Normal B cell homeostasis requires B cell activation factor production by radiation-resistant cells. J Exp Med. 2003; 198:937–945. [PubMed: 12975458]
35. Cambridge G, Isenberg DA, Edwards JC, et al. B cell depletion therapy in systemic lupus erythematosus: relationships among serum B lymphocyte stimulator levels, autoantibody profile and clinical response. Ann Rheum Dis. 2008; 67:1011–1016. [PubMed: 17962238]
36. Bossen C, Cachero TG, Tardivel A, et al. TACI, unlike BAFF-R, is solely activated by oligomeric BAFF and APRIL to support survival of activated B cells and plasmablasts. Blood. 2008; 111:1004–1012. [PubMed: 17942754]
37. Kuzmina Z, Greinix HT, Weigl R, et al. Significant differences in B-cell subpopulations characterize patients with chronic graft-versus-host disease-associated dysgammaglobulinemia. Blood. 2011; 117:2265–2274. [PubMed: 21063025]
38. Sarantopoulos S, Stevenson KE, Kim HT, et al. Altered B-cell homeostasis and excess BAFF in human chronic graft-versus-host disease. Blood. 2009; 113:3865–3874. [PubMed: 19168788]
39. Clark WB, Brown-Gentry KD, Crawford DC, et al. Genetic variation in recipient B-cell activating factor modulates phenotype of GVHD. Blood. 2011; 118:1140–1144. [PubMed: 21628416]
40. Corre E, Carmagnat M, Busson M, et al. Long-term immune deficiency after allogeneic stem cell transplantation: B-cell deficiency is associated with late infections. Haematologica. 2010; 95:1025–1029. [PubMed: 20133894]
41. Storek J, Espino G, Dawson MA, Storer B, Flowers ME, Maloney DG. Low B-cell and monocyte counts on day 80 are associated with high infection rates between days 100 and 365 after allogeneic marrow transplantation. Blood. 2000; 96:3290–3293. [PubMed: 11050018]
42. Storek J, Ferrara S, Ku N, Giorgi JV, Champlin RE, Saxon A. B cell reconstitution after human bone marrow transplantation: recapitulation of ontogeny? Bone Marrow Transplant. 1993; 12:387–398. [PubMed: 8275039]
43. Allman DM, Ferguson SE, Lentz VM, Cancro MP. Peripheral B cell maturation. II. Heat-stable antigen(hi) splenic B cells are an immature developmental intermediate in the production of long-lived marrow-derived B cells. J Immunol. 1993; 151:4431–4444. [PubMed: 8409411]
44. Sims GP, Ettinger R, Shirota Y, Yarboro CH, Illei GG, Lipsky PE. Identification and characterization of circulating human transitional B cells. Blood. 2005; 105:4390–4398. [PubMed: 15701725]
45. Miller JP, Stadanlick JE, Cancro MP. Space, selection, and surveillance: setting boundaries with BLyS. J Immunol. 2006; 176:6405–6410. [PubMed: 16709796]
46. Greinix HT, Pohlreich D, Kouba M, et al. Elevated numbers of immature/transitional CD21- B lymphocytes and deficiency of memory CD27+ B cells identify patients with active chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2008; 14:208–219. [PubMed: 18215781]
47. Kuzmina Z, Greinix HT, Knobler R, et al. Proportions of immature CD19+CD21- B lymphocytes predict the response to extracorporeal photopheresis in patients with chronic graft-versus-host disease. Blood. 2009; 114:744–746. [PubMed: 19608761]
48. Sarantopoulos S, Stevenson KE, Kim HT, et al. Recovery of B-cell homeostasis after rituximab in chronic graft-versus-host disease. Blood. 2011; 117:2275–2283. [PubMed: 21097674]
49. Fedoriw, Y.; Deal, AM.; Dunphy, CH., et al. Decreased TdT+ Bone Marrow B Cell Precursors after Allogeneic Stem Cell Transplantation is Associated with Development of Human Chronic Graft versus Host Disease (cGVHD). In: Rawlings, DJ.; Lund, FE.; Tangye, SG.; Monroe, JG., editors. B Cells: New Insights into Normal versus Dysregulated Function 2011. Whistler, British Columbia: Keystone Symposia; 2011.
50. Bohnhorst JO, Bjorgan MB, Thoen JE, Natvig JB, Thompson KM. Bm1-Bm5 classification of peripheral blood B cells reveals circulating germinal center founder cells in healthy individuals and disturbance in the B cell subpopulations in patients with primary Sjogren's syndrome. J Immunol. 2001; 167:3610–3618. [PubMed: 11564773]

51. Jacobi AM, Odendahl M, Reiter K, et al. Correlation between circulating CD27 high plasma cells and disease activity in patients with systemic lupus erythematosus. Arthritis Rheum. 2003; 48:1332–1342. [PubMed: 12746906]
52. Arce E, Jackson DG, Gill MA, Bennett LB, Banchereau J, Pascual V. Increased frequency of pre-germinal center B cells and plasma cell precursors in the blood of children with systemic lupus erythematosus. J Immunol. 2001; 167:2361–2369. [PubMed: 11490026]
53. Choi SW, Kitko CL, Braun T, et al. Change in plasma tumor necrosis factor receptor 1 levels in the first week after myeloablative allogeneic transplantation correlates with severity and incidence of GVHD and survival. Blood. 2008; 112:1539–1542. [PubMed: 18502834]
54. Choi S, Stiff P, Braun T, et al. Standard GVHD prophylaxis augmented with TNF-Inhibition in alternative donor HCT: Lower TNFR1 levels correlate with better outcomes. ASH Annual Meeting Abstracts. 2009; 114:43.
55. Paczesny S, Braun T, Lugt MV, et al. Three Biomarker Panel at Day 7 and 14 Can Predict Development of Grade II–IV Acute Graft-Versus-Host Disease. ASH Annual Meeting Abstracts. 2010; 116 675-
56. Boeckh M. Current antiviral strategies for controlling cytomegalovirus in hematopoietic stem cell transplant recipients: prevention and therapy. Transpl Infect Dis. 1999; 1:165–178. [PubMed: 11428987]
57. Einsele H, Ehninger G, Hebart H, et al. Polymerase chain reaction monitoring reduces the incidence of cytomegalovirus disease and the duration and side effects of antiviral therapy after bone marrow transplantation. Blood. 1995; 86:2815–2820. [PubMed: 7670117]
58. Boeckh M, Gooley TA, Myerson D, Cunningham T, Schoch G, Bowden RA. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. Blood. 1996; 88:4063–4071. [PubMed: 8916975]
59. Kharfan-Dabaja MA, Cutler CS. Rituximab for prevention and treatment of graft-versus-host disease. Int J Hematol. 2011; 93:578–585. [PubMed: 21547615]
60. Lee SJ. Rationale and Design of the Chronic GVHD Cohort Study: Improving Outcomes Assessment in Chronic GVHD. Biology of Blood and Marrow Transplantation. 2011; 17:1114–1120. [PubMed: 21664473]
61. Nakamura H, Komatsu K, Ayaki M, et al. Serum levels of soluble IL-2 receptor, IL-12, IL-18, and IFN-gamma in patients with acute graft-versus-host disease after allogeneic bone marrow transplantation. J Allergy Clin Immunol. 2000; 106:S45–S50. [PubMed: 10887333]
62. Visentainer JEL, Lieber SR, Persoli LBL, et al. Serum cytokine levels and acute graft-versus-host disease after HLA-identical hematopoietic stem cell transplantation. Exp Hematol. 2003; 31:1044–1050. [PubMed: 14585368]
63. Shaiegan M, Iravani M, Babaee G, Ghavamzadeh A. Effect of IL-18 and sIL2R on aGVHD occurrence after hematopoietic stem cell transplantation in some Iranian patients. Transpl Immunol. 2006; 15:223–227. [PubMed: 16431290]
64. Malone FR, Leisenring WM, Storer BE, et al. Prolonged anorexia and elevated plasma cytokine levels following myeloablative allogeneic hematopoietic cell transplant. Bone Marrow Transplant. 2007; 40:765–772. [PubMed: 17700598]
65. Schots R, Kaufman L, Van Riet I, et al. Proinflammatory cytokines and their role in the development of major transplant-related complications in the early phase after allogeneic bone marrow transplantation. Leukemia. 2003; 17:1150–1156. [PubMed: 12764383]
66. Liem L, van Houwelingen H, Goulmy E. Serum Cytokine Levels after HLA-identical Bone Marrow Transplantation. Transplantation. 1998; 66:863–871. [PubMed: 9798695]
67. Mohty M, Blaise D, Faucher C, et al. Inflammatory cytokines and acute graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation. Blood. 2005; 106:4407–4411. [PubMed: 16141347]
68. Sakata N, Yasui M, Okamura T, Inoue M, Yumura-Yagi K, Kawa K. Kinetics of plasma cytokines after hematopoietic stem cell transplantation from unrelated donors: the ratio of plasma IL-10/sTNFR level as a potential prognostic marker in severe acute graft-versus-host disease. Bone Marrow Transplant. 2001; 27:1153–1161. [PubMed: 11551026]

69. Fujimori Y. Elevated interleukin (IL)-18 levels during acute graft-versus-host disease after allogeneic bone marrow transplantation. Br J Haematol. 2000; 109:652–657. [PubMed: 10886219]

70. Hori T, Naishiro Y, Sohma H, et al. CCL8 is a potential molecular candidate for the diagnosis of graft-versus-host disease. Blood. 2008; 111:4403–4412. [PubMed: 18256320]

71. Piper KP, Horlock C, Curnow SJ, et al. CXCL10-CXCR3 interactions play an important role in the pathogenesis of acute graft-versus-host disease in the skin following allogeneic stem-cell transplantation. Blood. 2007; 110:3827–3832. [PubMed: 17766680]

72. Holler E, Kolb HJ, Moller A, et al. Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation. Blood. 1990; 75:1011–1016. [PubMed: 2405918]

73. Symington FW, Pepe MS, Chen A, Deliganis A. Serum tumor necrosis factor alpha associated with acute graft-versus-host disease in humans. Transplant. 1990; 50:518.

74. Imamura M, Hashino S, Kobayashi H, Kubayashi S, Hirano S. Serum Cytokine Levels in Bone Marrow Transplantation: Synergistic Interaction of Interleukin-6, Interferon-γ, and Tumor Necrosis Factor-αin Graft-Versus-Host Disease. Bone Marrow Transpl. 1994; 13:745–751.

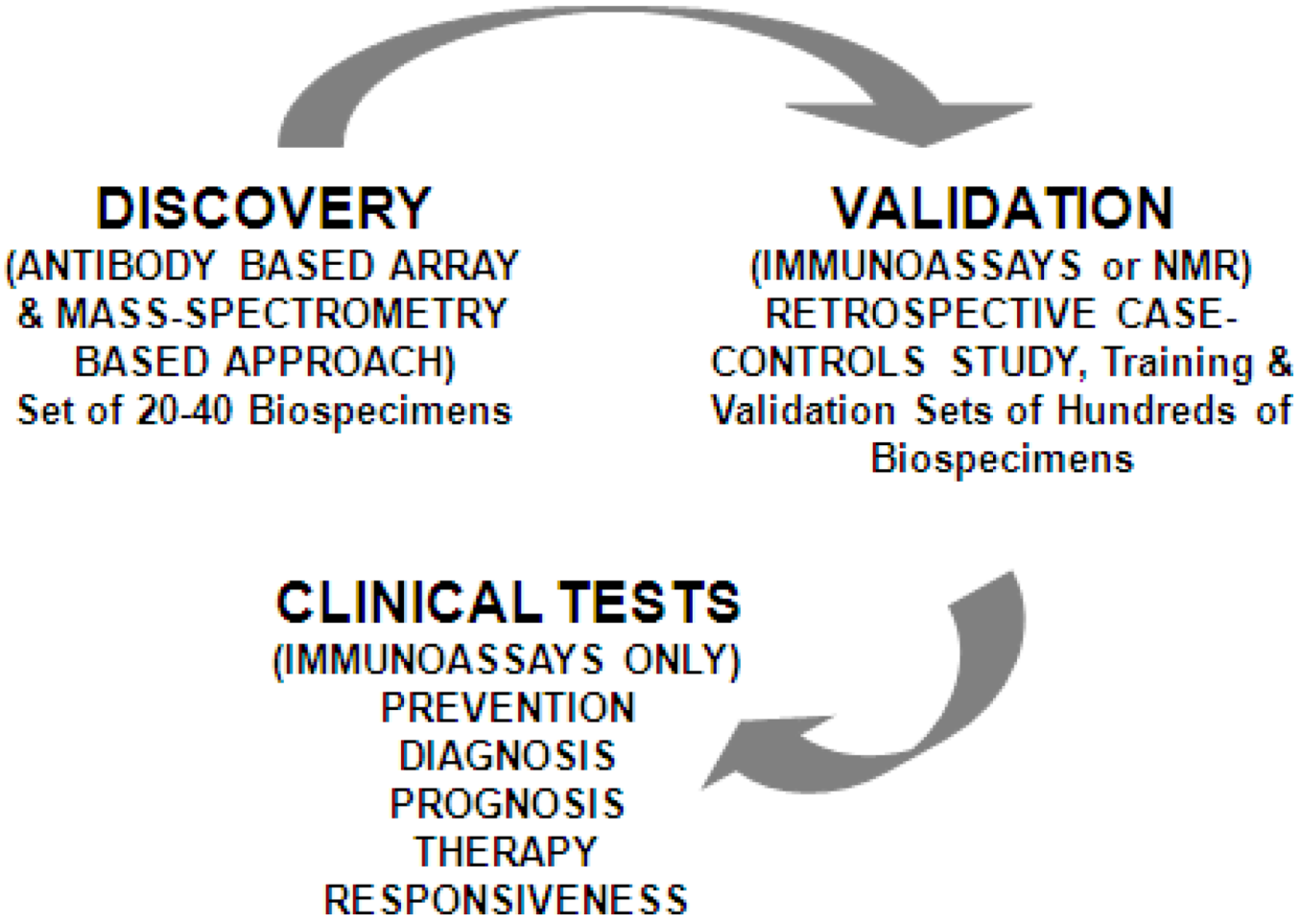

Figure 1. Biomarker Research Steps

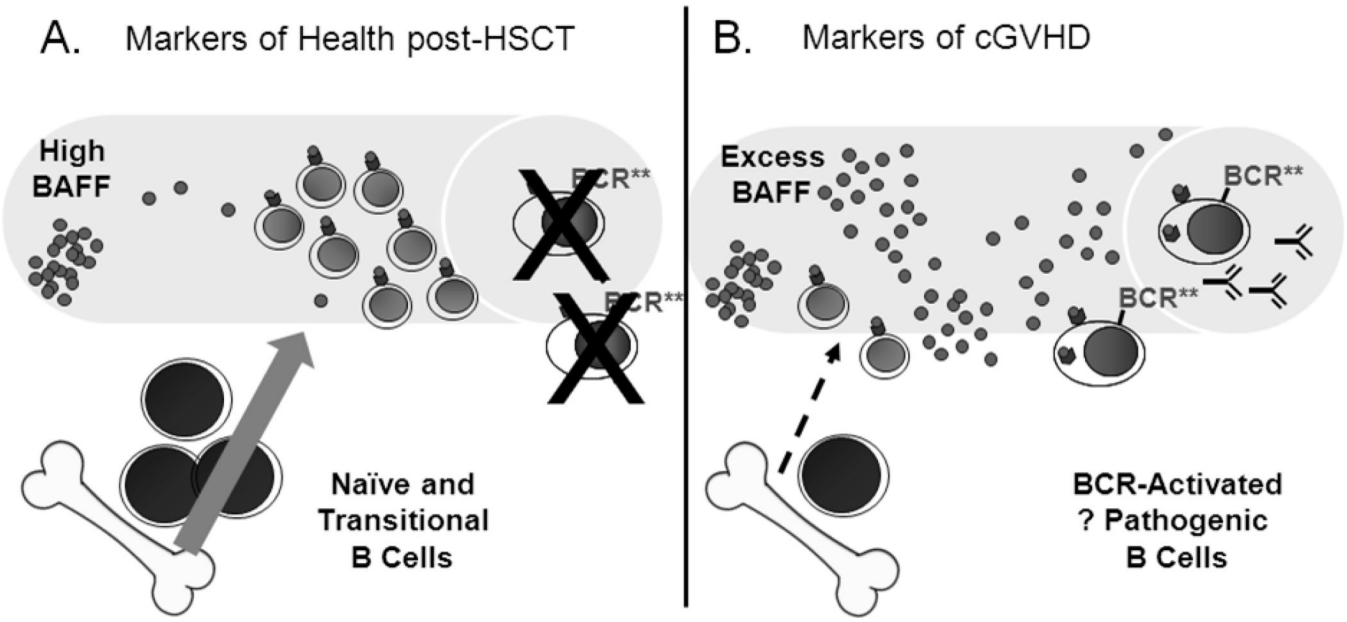

Figure 2. Biologically relevant BAFF and B cell markers in plasma, blood and bone marrow

A. Naïve and transitional B cells are increased in patients without cGVHD and these may serve as predictive markers of good post-HSCT health **B.** Low transitional and naïve B cell numbers and high BAFF/B cell ratios may serve as markers of cGVHD.

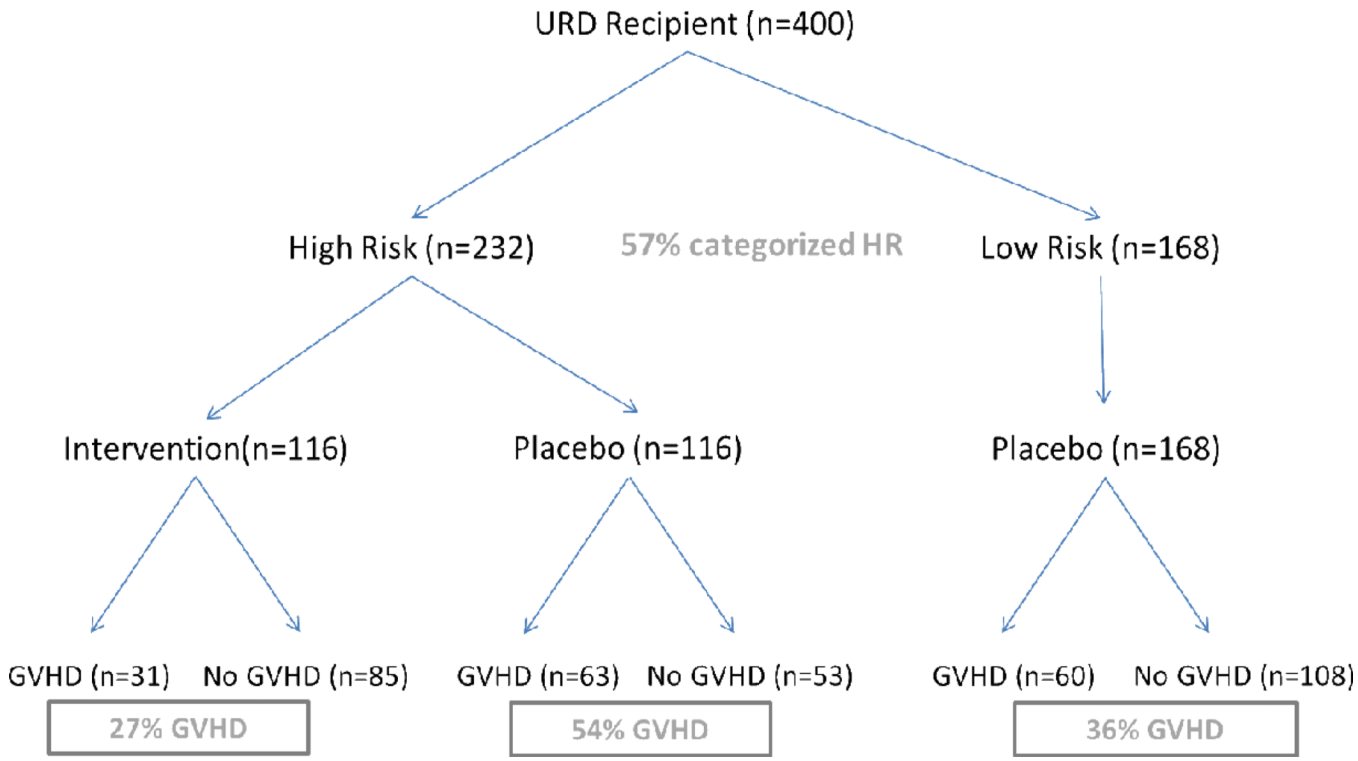

Figure 3. Possible Randomized, Double Blinded, Placebo-Controlled, Trial Design to Test GVHD Preemption

Table 1

Candidate Biomarkers of Acute GVHD with Diagnostic and Prognostic Significance

Protein	Name	Function	Target organ	Diagnosis	Prognosis
IL-2Rα	Interleukin-2 receptor α chain, CD25	Results from extracellular proteolyisis of the high affinity receptor of IL2, a key cytokine in the activation and proliferation of T cells.	Systemic	Nakamura 2000[61], Visentainer 2003[62], Shaiegan 2006[63], Paczesny 2009[12]	Paczesny 2009[12]
IL-6	Interleukin-6	Functions in inflammation and the maturation of B cells.	Systemic	Malone 2007[64]	
IL-8	Interleukin-8	Mediator of the inflammatory response.	Systemic	Paczesny 2009[12]	Schots 2003[65], Paczesny 2009[12]
IL-10	Interleukin-10	Pleiotropic effects in immunoregulation and inflammation; down-regulates expression of Th1 cytokines, MHC class II Ags, and costimulatory molecules on macrophages.	Systemic	Liem 1998[66]	
IL-12	Interleukin-12	Secreted by antigen presenting cells (particularly dendritic cells), required for the T-cell-independent induction of interferon (IFN)-γ, important for differentiation of Th1 and Th2 cells.	Systemic	Nakamura 2000[61], Mohty 2005[67]	
IL-15	Interleukin-15	Regulates T and natural killer cell activation and proliferation	Systemic	Sakata 2001[68]	
IL-18	Interleukin-18	Proinflammatory cytokine that augments natural killer cell activity, and stimulates IFN-γ production in Th1 cells.	Systemic	Nakamura 2000[61], Shaiegan 2006[63], Fujimori 2000[69]	
CCL8	Chemokine (C-C motif) ligand 8	Chemokine attracting monocytes, lymphocytes, basophils and eosinophils to inflamed sites.	Systemic	Hori 2008[70]	
CXCL10	Chemokine (C-X-C motif) ligand 10	Ligand for the receptor CXCR3, binding results in pleiotropic effects, including stimulation of monocytes, natural killer and T-cell migration, and modulation of adhesion molecule expression.	Systemic	Piper 2007[71]	
TNFα	Tumor necrosis factor (TNF)α	Key proinflammatory cytokine, secreted by macrophages	Systemic	Holler 1990[72], Symington 1990[73], Imamura 1994[74]	
TNFR1	Tumor necrosis factor Receptor-1	Expressed by all human tissues and is the major signaling receptor for TNFα	Systemic	Or 1996, Choi 2008, Paczesny 2009[12]	Paczesny 2009[12]
HGF	Hepatocyte growth factor	Regulator of cell growth, motility, and morphogenesis, secreted by mesenchymal cells.	Systemic/GI tract	Paczesny 2009[12], Sakata 2001[68]	Paczesny 2009[12]
KRT18	Cytokeratin-18 fragments	Induction of apoptosis results in early cleavage of KRT18 by caspases.	GI tract	Luft 2007[8]	
PI3	Elafin	Proteinase expressed by keratinocytes and involved in local innate immune defense.	Skin	Paczesny 2010[15]	Paczesny 2010[15]

Protein	Name	Function	Target organ	Diagnosis	Prognosis
REG3α	Regenerating islet-derived 3 alpha	Protein expressed by intestinal Paneth cells, direct antimicrobial activity.	GI tract	Harris 2011[16]	Harris 2011[16]