



Published in final edited form as:

*Biomark Med.* 2014 January ; 8(1): 65–68. doi:10.2217/bmm.13.113.

## Biomarkers for fatal immune response to stem cell treatment could reduce mortality

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### Keywords

Graft-versus-Host Disease (GVHD); hematopoietic stem cell transplantation (HSCT); biomarkers; proteomics; personalized medicine; OMICS

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To improve the efficacy of allogeneic stem cell transplantation, a method is needed for identifying stem cell transplant recipients who are at highest risk for graft-versus-host disease (GVHD), a potentially fatal condition in which transplanted immune cells recognize and attack the recipient's tissues as foreign. Despite advances that have reduced the impact of GVHD, it remains a leading cause of death among patients who receive stem cells from another person, which is the reason such transplantations are often used as a last resort in treating blood and bone marrow cancers including leukemia and multiple myeloma. In this article, I discuss the discovery of proteomic GVHD biomarkers, their validation in large retrospective datasets, and relevant clinical applications within the context of stem cell transplantation.

In allogeneic stem cell transplantation, the immune and bone marrow systems of the recipient are replaced by those of the donor. The desired outcome is that the donor immune system will recognize residual tumor cells as foreign and eradicate them via the graft-versus-leukemia effect. However, an undesired outcome occurs when donor immune cells also attack normal host tissues, most commonly the skin, liver, and gastrointestinal tract, resulting in GVHD. Two classical forms of GVHD, acute and chronic GVHD, represent separate pathophysiological entities. Acute GVHD occurs within 100 days following transplantation, whereas chronic GVHD arises beyond 100 days from transplantation. Since 2005, the National Institutes of Health's classification system has recognized additional categories such as late-onset acute GVHD (after day 100) and an overlap syndrome presenting features of both acute and chronic GVHD [1]. The emergence of new forms of GVHD can be explained by wider application of allogeneic stem cell transplantation in older recipients who have undergone reduced-intensity conditioning before transplantation.

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### Financial disclosure

S.P. is an investigator of the Amy Strelzer Manasevit Research Program and the Lilly Physician Scientist Initiative Program. S.P. holds a patent on "Methods of detection of graft-versus-host disease" (U.S. Patent #13/573,766).

Unfortunately, neither the clinical characteristics of recipients and donors pre-transplantation nor the characteristics of the transplant can be used to reliably predict the risk and outcome of GVHD. Therefore, new diagnostic and predictive monitoring techniques are needed to optimize treatment plans for stem cell recipients and improve outcomes. Fortunately, explosive progress in “omics” technologies has occurred in recent decades, largely due to important advances in chemistry, biology, and engineering, specifically related to high-throughput technical devices and bioinformatics. These tools have enabled discoveries demonstrating the importance of epigenetics for DNA transcription, transcriptional regulation through microRNA or small interfering RNA, posttranslational modification, and regulation at the metabolic level. As omics technologies continue to improve our understanding of complex regulatory processes at both the transcription and translation levels, the classic paradigm that DNA determines the fate of the cell is being called into question.

### Omics tools for GVHD biomarker discovery

- Genomics
  - The severity of GVHD is linked directly to the degree of major and minor histocompatibility complex disparity between the donor and recipient. Therefore, extensive effort has been devoted to refining human leukocyte antigen (HLA) matching [2].
  - Non-HLA polymorphisms also have been linked to patients’ risk for GVHD [2].
- Transcriptomics
  - RNAseq is a promising new technology that can be used to assess the human immune system.
  - MicroRNAs may have specific roles in GVHD [3,4].
- Cellulomics or the study of blood cellular markers via multiparameter flow cytometry has identified several cellular subsets of interest in GVHD, particularly regulatory T cells [5-8].
- Proteomics continues to be an area of intense interest in GVHD research, because the proteome offers a better representation of the actual state of the disease at a specific time point.

Proteomics technologies are currently used to identify both biomarkers and novel therapeutic targets based on the elucidation of pathologic processes. The following approaches are currently applied to discovering candidate biomarkers:

- Antibody profiling

Antibody-based approaches employ immunoassays that identify proteins based on antibody–antigen interactions.
- Mass spectrometry

Most non-antibody proteomic strategies are based on mass spectrometry, which is a powerful tool for characterizing qualitative and quantitative changes in complex protein mixtures.

- High-throughput immunoassays for validation

All immunoassays for biomarker validation must produce a large amount of data rapidly. Immunoassays that analyze multiple quantities of a sample simultaneously are considered high-throughput. The ability to simultaneously analyze many different proteins, known as multiplexing, is another desired immunoassay property.

### **Ideal characteristics of a GVHD biomarker after stem cell transplantation**

A biomarker is defined as any characteristic that can be objectively measured and validated as an indicator of a biological, pathogenic, or pharmacological response process. The ideal biomarker for acute GVHD will:

- answer a clinically relevant question (e.g., indicate the GVHD risk after transplantation, prognosis after GVHD onset, and treatment responsiveness);
- be non-invasive (e.g., blood test rather than biopsy) and easily repeated at various time points;
- be reproducible, accurate, standardized, and cost-effective;
- offer high sensitivity and high specificity;
- be a potential therapeutic target.

### **Currently identified GVHD biomarkers**

Although GVHD is a systemic immunological disorder, it can affect specific organ systems (e.g., skin, gastrointestinal tract, and liver). Several biomarkers such as interleukin-2 receptor  $\alpha$  chain and tumor necrosis factor- $\alpha$  and its receptors have been identified based on GVHD pathology and are reviewed elsewhere [9]. More recently, biomarkers based on proteomics approaches have been reported [9]. The most recently reported biomarker, suppression of tumorigenicity 2 (ST2), was identified using a plasma proteomics approach comparing responders and non-responders as the most significant of 12 markers of non-response to GVHD therapy and subsequent nonrelapse mortality. Patients with high ST2 levels at therapy initiation were 2.3 times more likely to not respond to treatment and 3.7 times more likely to die within 6 months after therapy. Patients with low ST2 levels experienced less nonrelapse mortality than patients with high ST2 regardless of the severity of GVHD. In addition, 14 days after transplantation and before acute GVHD diagnosis, plasma ST2 levels were associated with nonrelapse mortality at 6 months after transplantation. Therefore, ST2 levels measured at initiation of GVHD therapy and early in the transplantation course improve risk stratification and nonrelapse mortality after transplantation. ST2 is thus considered a promising acute GVHD biomarker [10].

## GVHD biomarkers and personalized medicine

Due to recent progress in GVHD biomarker identification and validation, upcoming clinical trials will incorporate biomarkers.

- Biomarkers of GVHD at time of symptom presentation

Target-specific diagnostic biomarkers that can differentiate skin GVHD from other rashes and gastrointestinal GVHD from other forms of enteritis will eliminate the need for invasive biopsies. The most important role of biomarkers is risk stratification for risk-adapted clinical trials. The current standard therapy for all GVHD patients is immediate systemic steroid treatment, with second-line agents reserved for patients who fail to respond to initial therapy. Unfortunately, most patients who require second-line therapy die, emphasizing the need for improved risk stratification. Current biomarkers offer sufficient sensitivity and specificity for risk stratification in newly diagnosed GVHD patients, and early identification of patients at high risk for steroid unresponsiveness may permit alternative testing or additional therapies before refractory disease develops. Identifying patients who will respond well to treatment is of equal importance, because these patients may tolerate a more rapid tapering of steroid regimens, lowering the risks of long term toxicity, infection, and loss of the graft-versus-leukemia effect. A scheme to validate biomarkers in newly diagnosed GVHD could employ biomarker cutoff points to determine the patients' risk for treatment unresponsiveness. Low-risk patients would receive the standard GVHD treatment, whereas high-risk patients would be randomized to receive either the standard treatment or an intensified GVHD treatment. Comparison of outcomes between the randomized high-risk groups would show whether intensified treatment at GVHD onset improves response rates and lowers mortality in high-risk patients identified by biomarkers.

- Biomarkers of GVHD prior to clinical signs

Identification of patients at high risk for GVHD early in their transplantation and treatment course also has important therapeutic consequences, including the opportunity to apply preemptive interventions. Successful preemption must not only reduce the incidence of GVHD but also that of infectious complications and relapse. Ultimately, a randomized trial will be needed to assess the effectiveness of GVHD preemption, and an example of such a trial based on risk-stratification using biomarkers is as follows. Low-risk patients will receive no intervention, whereas high-risk patients will be randomized to receive either standard GVHD treatment or no treatment. Comparison of outcomes between the randomized high-risk groups will show whether the preemptive intervention lowers GVHD incidence in high-risk patients identified using biomarkers. The expectation is that subclinical GVHD can be treated in order to avert the full-scale graft-versus-host reaction.

### Future directions

- First, a blinded evaluation of these biomarkers is needed using samples collected in a multicenter prospective study to reduce center effects.

- Research should be focused on understanding novel pathological pathways in GVHD that could uncover new etiological mechanisms.
- Biomarkers may represent promising therapeutic targets, and the appropriate effector T-cell population should be identified for targeting to increase efficacy and decrease toxicity.
- Efforts to identify biomarkers post-transplantation have focused on acute GVHD. Currently, the clinical diagnosis and consensus criteria for chronic GVHD are labor-intensive. Thus, future discovery of biomarkers for chronic GVHD will be particularly valuable [11].

## Acknowledgments

Sophie Paczesny (S.P.) is supported by National Institute of Health grants R01-CA168814, R01-CA174667, and R01-HD074587.

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