Short Communication

Biomarkers of Allogeneic Cell Therapy in Acute Steroid-Refractory Graft-versus-Host Disease

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Abstract
Cell therapy requires precise screening and monitoring of patients to ensure that the transfer of either autologous or allogeneic cells to a patient results in a therapeutic effect to targeted organs or tissues. One well-established application of cell therapy is allogeneic hematopoietic stem cell transplantation (allo-HSCT) to treat hematologic conditions. A common complication following allo-HSCT, however, is the development of acute graft-versus-host disease (aGVHD), which leads to substantial morbidity and mortality. There is currently no widely effective treatment for aGVHD, but cell therapy using decidua stromal cells (DSCs) has shown success in academic-driven clinical studies. The introduction of selective biomarkers of cellular, immune, and disease response to DSCs can help select the right patient, the right treatment, and the right monitoring in the treatment of aGVHD. In this article, we discuss the relevance of precision medicine as an essential approach to leverage biomarkers as well as other clinical aspects that optimize safety and efficacy of cell therapy in aGVHD.

Keywords: Acute graft-versus-host disease; Allogeneic hematopoietic stem cell transplantation; Cell therapy; Decidua stromal cells
Abbreviations: aGVHD- acute graft-versus-host disease; allo-HSCT- allogeneic hematopoietic stem cell transplantation; BSA- body surface area; dd-cfDNA- donor-derived cell-free DNA; DSC- decidua stromal cell; IFN-γ-interferon gamma; IL-interleukin; MSC- mesenchymal stromal cell; REG3α- regenerating islet-derived protein 3-α; ST2- suppressor of tumorigenicity-2

1. Introduction

Allogeneic cell therapies are an important type of precision medicine—an approach to disease treatment and prevention offering tailored, individualized care that considers a patient’s genetics, lifestyle, and environment [1]. Allogeneic cell therapy involves the transfer of whole donor cells to a patient, with the aim of restoring or altering his or her own diseased or damaged cells, delivering treatment to a specific organ or tissue, or providing immunoregulatory functionality. Myriad cell types are available for cell therapies, with hematopoietic stem cell transplantation for the treatment of hematologic diseases being among the most common [2-4].

The transfer of new cells to a patient imposes toxicity and immune-related concerns, which are evidenced by the development of acute graft-versus-host disease (aGVHD) following allogeneic hematopoietic stem cell transplantation (allo-HSCT). Decidua stromal cells (DSCs) are used in cell therapy technologies that have shown promise in many diseases, including aGVHD; markers that describe how the transferred cells behave in the body, how the immune system responds to the new cells, and how the patient responds to the treatment.

2. Acute Graft-Versus-Host Disease and Decidua Stromal Cells

Allo-HSCT is the first-line treatment for several benign and malignant hematopoietic cancers and diseases [5]. Following allo-HSCT, aGVHD is the most frequent comorbidity [6, 7] and may cause considerable mortality [8-10]. Approximately half of patients who receive allo-HSCT develop aGVHD [9, 11, 12]; the disorder is fatal in up to 10% of these individuals [12], making it the second leading cause of death (after disease relapse) for allo-HSCT recipients [13]. Simply, in aGVHD, donor blood cells target the neoplastic cells, but they also mount an immune response against healthy cells and tissues in the host. This response usually appears within the first three months after allo-HSCT and primarily affects the skin, gastrointestinal tract, and liver with rash, secretory diarrhea, and abnormal cholestatic liver function, which present as the prominent signs of disease [9, 14].

Acute GVHD is staged according to the number of organs affected and the extent of involvement [9]. Treatment for aGVHD usually consists of steroids with or without calcineurin inhibitors, but only about half of patients respond to this treatment [7, 9]. Currently, only one second-line therapy is approved for treatment of steroid-refractory aGVHD: ruxolitinib, a selective inhibitor of members of the Janus tyrosine kinase family (JAK1 and JAK2), it has demonstrated improved overall response and failure-free survival compared with other therapies [15]. Simply, JAK proteins are important signal transducers and activators of transcription that impact the development, proliferation, and activation of immune cell types that are important in the progression of aGVHD [16]. Other treatment attempts with cytostatic agents, immunomodulatory agents, and biologic
therapies have demonstrated low response rates and only short-term survival, often only a few months [6, 7, 17, 18].

Several other second-line cell therapies have been developed for treating steroid-refractory aGVHD, including mesenchymal stromal cells (MSCs) and DSCs [8]. MSCs, which are present in adult and fetal tissues, are multipotent, non-hematopoietic stem cells that can differentiate into various cell types. They are often isolated from bone marrow [19], but can also be found in adipose tissue, peripheral blood, dental pulp, endometrium, amniotic fluid, fetal membranes, the placenta, and the umbilical cord as well as other tissues and secretions [20-22]. MSCs possess immunomodulatory and anti-inflammatory processes and are able to avoid triggering an immune response. These characteristics make MSCs successful components of treatments for many diseases, but they have not been effective in preventing relapse or mortality in aGVHD [19, 23].

DSCs are similar to MSCs, but they are uniquely derived from fetal membranes of the maternal placenta [24]. Compared with MSCs, DSCs display more potent immunosuppressive properties and do not display any differentiation potential [8], which are key benefits in the treatment of aGVHD. Specifically, DSCs exhibit decreased production of interferon gamma (IFN-γ) and interleukin (IL)-17, increased secretion of anti-inflammatory IL-10, and higher expression of integrins [3, 4]. They suppress alloreactivity, enhance expression of programmed cell death ligands 1 and 2, and increase the frequency of regulatory T cells [23, 25, 26]. Furthermore, they do not upregulate human leukocyte antigen-II after IFN-γ stimulation [8]. Together, these features make DSCs ideal candidates for treating aGVHD (Figure 1).

Several studies have been conducted using DSCs in aGVHD and chronic GVHD (cGVHD), and promising results have been achieved (Table 1) [4, 8, 17, 27, 28]. Additionally, the studies have demonstrated that treatment with DSCs is safe and effective. During the long-term follow-up of an academic-driven study, patients receiving DSCs for steroid-refractory aGVHD achieved survival rates that were substantially greater than those achieved with traditional treatments, including MSCs, with a 1-year survival rate close to 80% and a 4-year survival rate near 60% [17].

<table>
<thead>
<tr>
<th>Author and year of study</th>
<th>Objective</th>
<th>Population</th>
<th>Dose</th>
<th>Results</th>
</tr>
</thead>
</table>
| Ringden, 2013 [4]        | Test initial efficacy of DSCs | 9 patients with acute GVHD | 2x10⁶ | Efficacy: 75% ORR  
Safety: DSCs were safe to infuse with no acute toxic effects |
<p>| Erkers, 2015 [8]         | Test efficacy and biodistribution of DSCs in humans | 3 patients with chronic GVHD | 1-2.8x10⁶ | Efficacy: 2 patients achieved PR, and 1 patient did not respond; DSCs were initially located in the lungs, followed by dissemination to the liver and spleen |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Objective</th>
<th>Participants</th>
<th>ORR/OS</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baygan, 2017 [27]</td>
<td>Test safety and adverse events of DSCs</td>
<td>44 patients with aGVHD in the DSC group and 40 with aGVHD in the control group</td>
<td>0.9-2.9 x10^6</td>
<td>Safety: No adverse events were reported</td>
</tr>
<tr>
<td>Ringden, 2018 [17]</td>
<td>Test efficacy of DSCs in different supplements</td>
<td>38 patients with aGVHD (24 with SR-aGVHD)</td>
<td>0.9-2.9 x10^6</td>
<td>Efficacy: ORR at Day 28 was 82% and 1-year OS was 63%</td>
</tr>
<tr>
<td>Sadeghi, 2019 [28]</td>
<td>Test long-term safety of DSCs</td>
<td>21 patients with aGVHD</td>
<td>0.9-2.9 x10^6</td>
<td>Safety: no major adverse events were reported</td>
</tr>
<tr>
<td>ASC930 in Patients</td>
<td>To evaluate efficacy of ASC930 in participants with SR-aGVHD</td>
<td>Planned for ~60 participants</td>
<td>Planned for 0.9-2.9 x10^6</td>
<td>Efficacy: DSCs are efficient in the long run with 66% 4-year OS</td>
</tr>
<tr>
<td>With Steroid-Refractory Acute</td>
<td>Graft Versus Host Disease (SR-aGVHD) [41]</td>
<td></td>
<td></td>
<td>Safety: no major adverse events or serious infections were reported</td>
</tr>
</tbody>
</table>
| aGVHD, acute graft-versus-host disease; DSCs, decidua stromal cells; ORR, overall response rate; OS, overall survival; PR, partial response; SR, steroid-refractory.

**Table 1:** Clinical studies conducted to date and planned [4, 8, 15, 27, 28].
Figure 1: Properties and mechanisms of action of DSCs [3, 4, 8, 23-26].

3. Biomarkers of Cell Therapy

A limitation of using DSCs in aGVHD is a lack of understanding of how the cells behave in the body and how they affect the immune system of the host. Biomarkers are an essential component to clarify the mechanisms of the treatment regimens. Infused DSCs can be radiolabeled to measure their presence in various organs over time. In a pilot study of three patients with severe cGVHD after stem cell transplantation, DSCs were labeled with $^{111}$indium and their distribution was tracked for 48 hours. DSCs traveled to the lungs, then to the spleen and liver [8]; they did not appear to travel to the organs typically affected by cGVHD such as the intestine, esophagus, or skin. This method of assessing the effect of DSCs might be applied to larger populations and used as a basis for further clinical study, but its invasive nature makes it cumbersome for routine use.

An alternative means of measuring DSCs in the body is the quantification of donor-derived cell-free DNA (dd-cfDNA), which is viable as a surveillance tool to describe the behavior of DSCs. After allo-HSCT, dd-cfDNA is detectable and quantifiable in the recipient’s blood. The ability to detect and differentiate donor and recipient DNA exploits differences between the genotypes of the donor and the recipient. This noninvasive test can detect precursors to organ injury following transplantation by measuring the progression of inflammation [29–31]. Quantification of dd-cfDNA can predict organ rejection as well as direct personalized immunotherapeutic treatment. Currently, dd-cfDNA is primarily used in solid-organ transplants, but work is underway to validate its application in allo-HSCT. There is no recognized threshold for the concentration of dd-cfDNA.
that indicates the onset of aGVHD, and more work is required to clarify the timing and measurement of dd-cfDNA relative to allo-HSCT [29].

4. Biomarkers of Immune Response

Immune response to DSCs can be measured with flow cytometry, which estimates immune response by simultaneously identifying and quantifying cellular systems and measuring the functional attributes of individual cells [32]. Mass cytometry pairs flow cytometry with mass spectrometry, offering high dimensional and unbiased examination of the immune system that is not limited by the number of parameters that can be analyzed at once [33]. Mass cytometry has been critical in elucidating how the immune system reconstitutes after allo-HSCT; it and allows individual cells to be described according to phenotype and function on the basis of cell-surface and intracellular proteins [5,34]. Patterns of immune reconstitution and post-transplant complications, including aGVHD, have been recognized using mass cytometry, leading to an appreciation of the complex, individualized biological processes that occur after allo-HSCT and the discovery of prognostic immune biomarkers [5]. Related technologies, including proteomics, multiomics, and single-cell “omics,” are also important to understand the effects of cell-therapy expression in individual cells [35-37], and these technologies could be applied in assessing immune response to DSC therapy.

5. Biomarkers of Disease Response

Disease response in aGVHD can be measured using surrogate safety and efficacy endpoints. Two biomarkers of endothelial dysfunction, which predict long-term outcomes, can be estimated from whole blood: suppressor of tumorigenicity-2 (ST2) and regenerating islet-derived protein 3-α (REG3α) [38, 39]. Both proteins have been identified in high concentrations in the blood of patients with aGVHD and are predictors of increased mortality. REG3α is produced in the pancreas and small intestine and displays enhanced expression during inflammation and is thought to be directly related to endothelial damage caused by aGVHD. REG3α is specifically useful in aGVHD that presents in the gastrointestinal system because this protein can distinguish between aGVHD-related and other causes of diarrhea. ST2 is part of the IL-1 family that is secreted by endothelial and epithelial cells as well as fibroblasts and has been associated with treatment-resistant aGVHD [40].

Both ST2 and REG3α are incorporated into the MAGIC (Mount Sinai Acute GVHD International Consortium) algorithm probability [39], which is a tool for assessing mortality after aGVHD treatment. The timing, methods, and cut-off values of laboratory measurement of these biomarkers need to be clarified and standardized to increase their application in aGVHD.

6. Conclusions

The prediction, diagnosis, and treatment of aGVHD after allo-HSCT is a clinical need that can be met with cell therapy that uses DSCs. To ensure the safe and effective use of cell therapies, though, sensitive, specific, and standardized biomarkers are needed to guide treatment and to assess response. We must understand how the cells behave in the body, how the immune system responds to the cells, and how the patient responds to the treatment. Finding the right treatment for the right patient and employing the right monitoring will ensure the success of cell therapy.
References


39. Srinagesh HK, Ferrera JLM. MAGIC biomarkers of acute graft-versus-host disease: Biology and
