

## Novel Treatment for Graft-versus-Host Disease

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### Abstract

Allogeneic hematopoietic cell transplantation is a curative therapy for a variety of hematological diseases, but its success is hampered by acute and chronic graft-versus-host disease (GvHD). In the last five years, multiple novel therapeutic approaches for GvHD have entered the arena. The National Institutes of Health consensus criteria for chronic GvHD have set standards for designing and reporting clinical trials, and preclinical experiments of chronic GvHD have revealed the central roles of regulatory T cells, B-cell signaling, Th17 cells, Tc17 cells, follicular helper T cells, follicular regulatory T cells, and fibrosis-promoting factors. These scientific efforts and the resulting clinical studies led to the approval of ibrutinib, belumosudil and ruxolitinib for the treatment of refractory chronic GvHD. Recently, large randomized phase III trials showed that ruxolitinib was superior to the best available therapy for glucocorticoid-refractory acute GvHD (REACH2 trial) and glucocorticoid-refractory chronic GvHD (REACH3 trial). Furthermore, novel regenerative approaches, including IL-22, R-spondin, and glucagon-like peptide-2, and cellular therapies, such as the transfer of mesenchymal stem cells and regulatory T cells, are under intensive investigation. GvHD prevention using abatacept, dipeptidyl peptidase 4 inhibition, and post-transplant cyclophosphamide are also promising strategies that require further evaluation. In this article, we summarize the emerging knowledge of acute GvHD, chronic GvHD, and preclinical and clinical data of mesenchymal stem cells as GvHD therapy. In the next five years, basic and clinical studies will further advance the field, and dramatic changes in GvHD management will be encountered.

**Key words** graft-versus-host disease, mesenchymal stem cells, novel drugs, GVHD treatment, regenerative approaches

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### Introduction

Allogeneic hematopoietic cell transplantation (allo-HCT) is a curative therapy for a variety of conditions, including hematologic disorders, metabolic storage diseases, immune deficiencies, and hematological malignancies. Acute and chronic graft-versus-host diseases (GvHDs) are major barriers that need to be overcome to make allo-HCT safer<sup>1, 2</sup>. In the last five years, multiple novel therapeutic approaches for GvHD have been explored (**Table 1**). In this study, we summarize emerging knowledge of acute GvHD (aGvHD), chronic GvHD (cGvHD), and preclinical and clinical data on

using mesenchymal stem cells (MSCs) as GvHD therapy.

### Novel Drugs for GvHD

The conditioning regimen before allo-HCT causes tissue damage and activation of host antigen presenting cells (APCs) and neutrophils in the target organs of GvHD and secondary lymphoid organs.<sup>3</sup> Activation occurs via bacterial components and danger signals, such as ATP<sup>4</sup> and uric acid,<sup>5</sup> that can activate the NLRP3 inflammasome, which has been shown to play a role in aGvHD. Novel NLRP3 inhibitors that counteract in-

**Table 1. Novel therapeutic approaches for GvHD**

Targeted mechanism	Drug
Activation of host APC and neutrophils	NLRP3 inhibitors
Influx of bacteria	PNAG or vaccination against PNAG
Tissue regeneration	KGF, IL-7, R-spondin, GLP-2
JAK1/2	Ruxolitinib, Itacitinib, Baricitinib
TNF production in CNS GvHD	Takinib
DPP-4	Sitagliptin
Interaction of CD28 and CD80/CD8	Abatacept
B cell/BCR signaling	Ibrutinib, Acalabrutinib, Ofatumumab, Obinutuzumab
Proteasome	Ixazomib
Monocyte/macrophage	Axatilimab
Hedgehog	Glasdegib
ROCK2	Belumosudil
Pro-inflammatory immune cells	Mesenchymal stem cells

APC, antigen presenting cell; NLRP3, NLR family pyrin domain containing 3; PNAG, polyclonal antibodies to poly-N-acetylglucosamine; KGF, keratinocyte growth factor; GLP-2, glucagon-like peptide-2; TNF, tumor necrosis factor; CNS, central nervous system; DPP-4, dipeptidyl Peptidase-4; ROCK2, Rho-associated coiled-coil kinase 2.

flammation<sup>6</sup> could become new therapeutic interventions for aGvHD. A second early step in the pathogenesis of aGvHD is the activation of monocytes<sup>7</sup> and neutrophil granulocytes<sup>3</sup> which cause tissue damage and antigen presentation<sup>8</sup>. Treatment with polyclonal antibodies targeting poly-N-acetylglucosamine (PNAG) or vaccination against PNAG reduced the influx of bacteria, decreased neutrophil recruitment, and improved aGvHD in mice<sup>9</sup>. Furthermore, APCs express major histocompatibility complex (MHC) and minor histocompatibility molecules that are recognized by the T-cell receptor of donor T-cells. The classical prevention and treatment of GvHD includes different immunosuppressive approaches, such as use of calcineurin inhibitors, antimetabolites, mTOR inhibitors, and corticosteroids; extracorporeal photopheresis (ECP), and others<sup>1, 2</sup>. These immunosuppressive mechanisms also suppress the beneficial anti-leukemic immune response and anti-infectious immunity.

Novel strategies to reduce immunosuppressive therapy after allo-HCT include regenerative approaches, such as KGF<sup>10</sup>, interleukin (IL)-7<sup>11</sup>, R-spondin<sup>12, 13</sup>, and GLP-2<sup>14</sup>. These tissue regenerative approaches act on the intestinal tract through their protective impact on Paneth cells and intestinal stem cells. Another important novel concept for aGvHD treatment is JAK1/2 inhibition with ruxolitinib, which was successfully translated from the mouse model<sup>15</sup> into clinical application<sup>16, 17</sup>. In a retrospective study, 95 patients received ruxolitinib as a salvage therapy for steroid-refractory GvHD<sup>18</sup>. The overall response rate (ORR) was 81.5%, and patients with severe intestinal or skin GvHD showed an impressive response to treatment. The reduction of inflammatory disease was linked to decreased serum proinflam-

matory cytokine levels and reduced numbers of activated T-cells. Although cytopenia was observed in some patients, ruxolitinib was found to be safe and well tolerated. The next step after the successful phase III trials may be a combination with other approaches; for instance, the combination of ruxolitinib with ECP was shown to have activity against cGvHD<sup>18</sup>. Novel approaches in GvHD could include kinase inhibitors for kinases relevant for the disease. GvHD caused activation of microglia cells as shown by morphological changes and differential gene expression. Selective genetic ablation of TGF- $\beta$ -activated kinase-1 (TAK1) or tumor necrosis factor (TNF) in microglia identified the TAK1/ TNF/MHC-II axis as a central mediator of CNS-inflammation<sup>19</sup>. The kinase inhibitor, Takinib, reduced TNF production and CNS GvHD.

Dipeptidyl peptidase-4 (DPP-4; also known as CD26) is a homodimeric type-II transmembrane receptor with serine peptidase activity in its extracellular domain. This molecule is expressed in hematopoietic cells, endothelial cells, and epithelial cells, and is soluble and enzymatically active in the blood. DPP-4 can be inhibited by the selective inhibitor sitagliptin. Prophylaxis treatment with sitagliptin showed a reduced incidence of aGvHD compared to historical controls (NCT 00862719).

ECP is an immunomodulatory and immunosuppressive regimen. During ECP, PBMCs are collected by leukapheresis, treated with 8-methoxypsoralen (8MOP), exposed to UVA light, and re-infused into the patient. The application of ECP was shown to be a promising treatment strategy for aGvHD and cGvHD. Mechanistically, ECP increased FoxP3<sup>+</sup> Tregs and IL-10 production. Tregs depend on IL-2<sup>20</sup> and IL-2 treatment has

been shown to reduce cGvHD. GvHD or leukemia relapse<sup>21</sup> may change the metabolic activity of donor T-cells. Metabolic reprogramming of T-cells after allo-HCT from fatty acid oxidation to aerobic glycolysis has been reported<sup>22</sup>. The mTOR inhibitor rapamycin reduced the glycolytic activity of donor T-cells. The role of this pathway was confirmed using *Mtor*-deficient T-cells as donors, whereas further detailed analysis with *Raptor*-deficient T-cells revealed that T-cell pathogenicity is only dependent on mTORC1, but not mTORC2<sup>22</sup>. Abatacept, known as cytotoxic T-cell-lymphocyte-4 (CTLA4)-immunoglobulin, is a fusion protein between the extracellular domain of human CTLA4 and a modified Fc region of human IgG and blocks co-stimulation of T-cells. Therefore, blocking the interaction between CD28 and CD80/CD86 is a promising option for reducing aGvHD. In a clinical trial of abatacept for aGvHD prophylaxis (NCT01012492), patients received cyclosporine/methotrexate (StdRx cohort) or abatacept (ABA cohort) as GVHD prophylaxis. Compared to the StdRx cohort, the ABA cohort showed a significant inhibition of early CD4<sup>+</sup> T-cell proliferation and activation, mainly affecting effector memory T-cells. The treatment had minor effects on CD8<sup>+</sup> T-cells, suggesting that combination of therapies should be considered in future studies. GvHD analysis revealed only a low rate of aGvHD in the ABA cohort compared to the StdRx cohort, whereas all patients showed immune reconstitution. Furthermore, the investigators only reported two cases of grade II-IV aGvHD until day 100. The reconstitution of natural killer (NK) cells occurred relatively quickly upon abatacept treatment, while T-cell proliferation was reduced. Moreover, abatacept allowed long-lasting immune reconstitution. When interfering with aGvHD, it is important to consider the effect of graft-versus-leukemia (GVL). Recently, several measures have been studied to enhance the effects of GVL, such as kinase inhibition<sup>23</sup>, demethylation, and immune checkpoint inhibition<sup>24, 25</sup>.

### Update on the Treatments of cGvHD

Approximately 40% of patients who undergo allo-HCT develop cGvHD. It affects a variety of organs and causes significant morbidity and mortality. The manifestations of cGvHD resemble those of autoimmune diseases. Inflammation, cellular immunity, humoral immunity, and fibrosis have been implicated in the pathogenesis of cGvHD<sup>26</sup>. Characteristics of cGvHD may have ethnic differences. A recent study showed that the incidence of cGvHD according to the National Institutes of Health criteria is lower in Japanese patients than in white patients<sup>27</sup>. Moreover, Japanese patients have more frequent involvement with eyes and liver,

less frequent gastrointestinal involvement, and a greater number of involved sites. Despite the higher global score and a greater number of sites involved with cGvHD at onset in Japanese patients, the duration of immunosuppression and the probability of non-relapse mortality are similar between Japanese and white patients<sup>27</sup>. Knowledge of such ethnic differences in the characteristics of cGvHD is important for the management of patients with cGvHD in different regions.

The NIH consensus project for cGvHD has made major contributions in the advances of the field by setting standards for designing and reporting clinical trials<sup>28</sup>. Preclinical experiments of cGvHD have also revealed the central roles of regulatory T-cells, B-cell signaling, Th17 cells, Tc17 cells, follicular helper T cells, follicular regulatory T cells, and fibrosis-promoting factors<sup>26</sup>. All these efforts led to the first approval from the Food and Drug Administration (FDA) of ibrutinib, a BTK inhibitor, for the treatment of cGvHD after failure of one or more lines of systemic treatment<sup>29</sup>. The best response rate after ibrutinib treatment for patients with cGvHD, including erythematous skin or mouth, was 67%, and 71% of responders showed a sustained response for more than 20 weeks. Responses were observed across all involved organs evaluated, and most responders were able to reduce the dose of corticosteroids. Plasma levels of soluble factors associated with inflammation, fibrosis, and cGvHD significantly decreased over time with ibrutinib treatment. The most common grades 3-5 adverse events were pneumonia, fatigue, and diarrhea.

Recently, the results of three multicenter randomized trials for cGvHD treatment have been reported. The first international double-blind trial tested ibrutinib, in addition to standard corticosteroids, for the initial treatment of cGvHD in 193 patients (NCT02959944)<sup>30</sup>. The primary endpoint, complete, or partial response at 48 weeks did not differ statistically between the arms (prednisone + ibrutinib 41% versus prednisone + placebo 37%). Meanwhile, based on secondary endpoints, several numerical trends of improved outcomes were noted in the experimental arm without increasing toxicities. The second international trial compared ruxolitinib, a JAK1/2 inhibitor, with the best available therapy for glucocorticoid-refractory cGvHD among 329 patients (NCT03112603)<sup>31</sup>. The primary endpoint, the best overall response rate, was higher in ruxolitinib than the best available therapy (50% versus 26%), and failure-free survival was higher in ruxolitinib than the best available therapy. Anemia was a slightly frequent adverse event with ruxolitinib, while the frequencies of other adverse events were similar between the arms. Ruxolitinib was recently approved by the FDA for cGvHD after failure of one or two lines of systemic therapy in adult and pe-

diatric patients 12 years and older. The third U.S. trial was a pivotal phase II randomized trial of belumosudil, a ROCK2 inhibitor, for cGvHD after two or more lines of treatment in 132 patients (NCT03640481)<sup>32</sup>. Two different doses of belumosudil (200 mg QD or BID) were compared. The primary endpoint, the best overall response rate, was 73% in all patients, and the rates were similar across all subgroups. Belumosudil was well tolerated with manageable adverse events, consistent with the results of a previous phase IIa dose-finding study<sup>33</sup>. Belumosudil was recently approved by the FDA for cGvHD after failure of at least two prior lines of systemic therapy.

In addition to the encouraging results from multicenter randomized trials, an increasing number of investigational agents that target different biological pathways of cGvHD are under development in clinical trials. The targeted mechanisms include JAK1/2, B cell/B cell receptor signaling, proteasome, ROCK2, regulatory T cells, monocytes/macrophages, co-stimulatory molecules, Hedgehog, and others. To address the challenges in a rapidly changing field, a third NIH consensus project was held in November 2020. Investigators aimed to define basic and clinical research directions that may lead to significant changes in cGvHD management over the next five years<sup>34-38</sup>. Four working groups have discussed etiology/prevention, diagnosis/preemptive therapy, initial and subsequent lines of systemic treatment, and highly morbid forms of cGvHD, including sclerotic form, bronchiolitis obliterans, ocular GvHD, and gastrointestinal GvHD. Initial and second insults have been proposed as etiologies of cGvHD, and many emerging targets have been suggested for current and future preventive and therapeutic strategies<sup>34</sup>. Although further research on prognostic or predictive biomarkers of cGvHD is required, preemptive therapy might be possible in the near future<sup>35, 36</sup>. Glucocorticoid-free treatment has been proposed as a future study design for initial treatment<sup>37</sup>. Furthermore, researches highly prioritize the establishment of appropriate endpoints for each of the highly morbid form of cGvHD and designing novel trials using a small number of patients<sup>38</sup>.

### Mesenchymal Stem Cells (MSCs) as a Therapy for GvHD

MSCs are one of the most potent immunosuppressive cells in the immune system. Their immunosuppressive functions have been extensively investigated. The mechanisms involve activation of immunotolerance cells (i.e. Treg), suppression of pro-inflammatory immune cells via direct cellular contact (i.e. T lymphocytes, dendritic cells, and NK cells), and secretion of immunosuppressive soluble factors (i.e. IL-10). However,

the immunosuppressive function of MSCs can be altered in several situations, and MSCs may be converted as pro-inflammatory cells<sup>39</sup>. With such a wide spectrum of immunomodulatory capacity, it is natural to consider MSCs as a potential strategy for cellular therapy of autoimmune or immune-dysregulated diseases<sup>40, 41</sup>. GvHD is caused by immune dysregulation after allo-HCT<sup>42</sup>.

#### Pre-clinical *in vitro* evidence

MSCs can be isolated from a variety of tissues, such as the bone marrow, skeletal muscles, dental pulp, bone, umbilical cord, and adipose tissue. MSCs have immune privilege status due to low expression of MHC-II and costimulatory molecules on their cell surface. However, this status may change once MSCs differentiate into more lineage-specific cells, or they are exposed under the influence of interferon or infection<sup>43, 44</sup>. MSCs can interact with a wide variety of immune cells through direct cell-to-cell interactions and secretion of soluble factors. MSCs inhibit the proliferation of most immune cells *in vitro*, including B cells, T cells, NK cells, and dendritic cells (DCs), producing a condition known as “division arrest anergy”<sup>45</sup>. In addition, MSCs can alter a variety of functions exerted by the immune cells, including cytokine release, cellular cytotoxicity by T- and NK cells, maturation and antibody secretion by B cells; and differentiation, maturation, and antigen presentation of DCs. It is thought that MSCs must be stimulated to execute their immunomodulatory function<sup>46</sup>. Among different stimulations, the release of pro-inflammatory cytokines, such as TNF- $\alpha$  and interferon-gamma may be the primary factors in the activation of MSCs. Furthermore, MSCs can recruit immune-tolerant Tregs to the lymphoid organs and graft tissues. Investigations are still ongoing to elucidate the exact mechanisms and molecules involved in the immunosuppressive effect of MSCs.

#### Pre-clinical *in vivo* evidence

In search of the possible immunomodulatory mechanisms *in vivo*, many molecules, such as prostaglandin E 2, TGF- $\beta$ , IL-6 and IL-10, matrix metalloproteinases, indoleamine-2,3-dioxygenase, and nitric oxide<sup>30</sup> have all been identified in different experimental models. *In vivo* studies have shown conflicting results regarding the immunomodulatory properties of MSCs in the HCT setting. However, studies have determined the efficacy of MSC therapy in different scenarios. Some studies have explored the therapeutic potential of MSCs in preventing or treating graft rejection<sup>47</sup>, while others have focused on the effects of MSCs in preventing or treating GvHD. Subsequent Phase I, II, and III clinical trials were conducted. Clinical conditions include a variety of



autoimmune diseases, such as inflammatory bowel diseases, lupus erythematosus, rheumatoid arthritis, and even type 1 diabetes mellitus<sup>48-51</sup>. In organ transplant settings, MSCs have been used to prevent rejection of solid organ allografts<sup>52</sup>.

Among all potential applications, GvHD in the HCT setting is the first to be evaluated. GvHD can be classified according to the temporal profile or underlying mechanisms of either aGvHD or cGvHD. The pathogenesis of aGvHD is related to alloreactive T-cells, especially cytotoxic T-cells. However, cGvHD is believed to be related with altered B cell subpopulations, aberrant B cell signaling pathways, T/B cell interactions, and production of autoantibodies<sup>53</sup>. The animal model for these two types of GvHD also varied. More pre-clinical data on the use of MSCs for aGvHD are available compared to cGvHD.

Together with another research group, we have found that systemic infusion of human MSCs can alleviate the severity of murine aGvHD manifestation and improve survival<sup>54</sup>. We utilized irradiated BALB/c host mice to receive C57BL/6 donor T-cell-depleted bone marrow with or without donor CD4+ T lymphocytes as controls. Human-derived MSCs were administered via tail vein injection. We found that both survival rate and GvHD score improved in the MSC-treated mice. The levels of pro-inflammatory cytokines/chemokines, including RANTES, CCL3, CXCL9, CCR5, and CXCR3, as well as donor T-cell alloreactivity, decreased after transplantation. Homing of MSCs into the lymphoid organs and target tissues was also observed.

However, murine MSCs are not exactly the same as human MSCs; murine MSCs have a unique marker, Sca-1, and can be isolated and quantified easily. Moreover, their immune pathways and expression of immune mediators are not the same as those in the human immune system<sup>55</sup>. Therefore, the direct translation of the data into a human setting must be interpreted with caution.

### Clinical evidence

MSCs have been extensively tested in steroid-resistant GvHD, a devastating condition that may occur in acute or chronic forms following allo-HCT. Results from an early single-center clinical trial showed that refractory aGvHD had a favorable response to allogeneic MSC treatment<sup>56</sup>. However, subsequent multi-center phase III trials failed to observe statistically significant effects of MSC treatment on patients with refractory aGvHD. Unfortunately, the results were only presented in international meetings without publication, so the results were not included in the subsequent meta-analysis<sup>57</sup>. Thus, the optimal method of administration of MSCs, such as the appropriate dosage and frequency

of administration, remains undetermined. In addition, due to the lack of specific biomarkers, tracking of MSC homing and engraftment *in vivo* is challenging. Other barriers include the sources and production of MSCs, which may have a significant impact on efficacy *in vivo*<sup>58</sup>. The heterogeneity of MSCs with different stromal differentiation potential and biological activities may also affect the outcome. Proper randomized clinical trials should be conducted when most of these variables are controlled.

To determine the efficacy of MSCs as a prophylaxis or treatment for GvHD after HCT, a Cochrane systematic review was performed, which included 12 completed randomized control trials with 879 participants. Seven trials used MSCs as prophylaxis, and five trials utilized MSCs as treatment. However, the major critique of the existing data was that the overall quality was low due to the suboptimal methodology adopted, including poor randomization. Therefore, the risks of performance and reporting biases were considered to be high. As mentioned in previously, the results from a large-scale multi-center randomized trial that was conducted more than a decade ago were not published, possibly because the trial failed to reach the expected outcome. Omitting such negative data may lead to a publication bias. The review also concluded that the mortality in both the prophylactic and therapeutic trials remained similar between the MSC-treated group and the control group. However, there were no significant side effects reported in the therapeutic trials, and the risk of malignant disease relapse did not increase. MSCs were well tolerated, but there was no health-related quality of life assessment found in these trials to support this observation. Among the prophylactic trials, it was shown that MSCs may reduce the risk of cGvHD (relative risk [RR]: 0.66, 95% Confidence Interval [CI]: 0.49-0.89), but not the risk of aGvHD. For the GvHD therapeutic trials, there was no evidence of any differences in the outcomes<sup>59</sup>.

Another recently published meta-analysis presented a different conclusion. This study included non-randomized trials and analyzed 16 prophylactic and 35 therapeutic studies. Six hundred fifty-four patients received MSCs for GvHD prophylaxis, and there was a 17% higher overall survival (95% CI: 1.02-1.33) with a lower incidence of grade 4 aGvHD (RR: 0.22; 95% CI: 0.06-0.81) and cGvHD (RR: 0.64; 95% CI: 0.47-0.88) than the controls. If both the prophylaxis and treatment group of >1,600 patients were observed, the overall survival rate of patients with aGvHD treated with MSCs was also significantly higher than that of the control group ( $p = 0.0214$ ), with a survival rate of approximately 50% (95% CI: 0.41-0.59). The response rate of patients with aGvHD to MSC treatment was approxi-

mately 67%, and 39% had a complete response. The skin had the best response rate among different organs affected by aGvHD. Twenty-two percent of patients with aGvHD infused with MSCs developed cGvHD, but 64% of this group survived. This meta-analysis suggests that allogeneic MSCs can serve as part of our armamentarium against GvHD, especially when GvHD is refractory to conventional therapies such as pulse steroids.

To resolve the conflicting results of these two meta-analyses, good quality clinical trials must be conducted<sup>60</sup>. In addition, a number of confounding factors must be properly standardized and controlled. We propose several novel solutions to standardize the production of clinical-grade MSCs.

### Possible causes of suboptimal clinical trial results and the possible solution to overcome these hindrances

We found that even for bone marrow-derived MSCs, several factors, such as the donor's health status and orders of aspiration, can affect the harvest yield. The optimal bone marrow samples for MSC collection should be obtained earlier in the process of harvesting<sup>61</sup>. We also noticed that MSCs from patients suffering from various diseases may not behave normally (e.g. MSCs from lupus patients)<sup>62</sup>. Interestingly, MSCs from patients with acute leukemia or immune thrombocytopenia purpura regain normal phenotype after several passages of *ex vivo* culture, suggesting that the influence of extrinsic factors on MSCs is transient and may not have a permanent impact.

It has been argued that even though MSCs are considered as "stem cells," they have finite expansion potential *in vitro* and will become senescent after a limited number of culture passages. This becomes a major quality control issue for clinical applications because one may encounter possible batch-by-batch variations. Thus, acquisition of accreditation from healthcare authorities will be a challenge. To overcome this issue of cell heterogeneity and the limited number of passage effects, we converted selected MSCs into induced pluripotent stem cells and then differentiated them back into MSCs. Such an approach can produce a relatively unlimited supply of homogeneous MSCs of the same origin. Quality control problems can then be overcome.

Several studies have proposed that MSCs from different tissues may differ in terms of their proliferative capacity and immunomodulatory function. However, none of these studies directly compared the three types of MSCs. We confirmed this observation by comparing MSCs from adipose tissue, umbilical cord, and bone marrow using *in vitro* and *in vivo* models. We found that adipose tissue-derived MSCs proliferated much bet-

ter than bone marrow-or umbilical cord-derived MSCs in both *in vitro* and *in vivo* models. We then explored possible mechanisms to elucidate such variation. By comparing the RNAseq profiles of MSCs derived from the three tissues, we identified potential unique immune molecules involved. Immunological tests confirmed that association with and blocking of this molecule would take away the proliferative and immunomodulatory advantages of MSCs. Furthermore, we discovered some existing drugs that can enhance the expression of this molecule and render MSCs more immunosuppressive. Thus, we can manipulate the immune potential of MSCs in the future by using extrinsic factors to maximize the clinical benefit of this approach.

We also explored the use of MSC-derived exosomes as a substitute for MSCs to improve product consistency<sup>63</sup>. Exosomes contain a wide spectrum of biologically active factors, such as cytokines, growth factors, and miRNAs. By conducting *in vitro* and *in vivo* experiments, we found that exosomes derived from MSCs are as effective as MSCs in treating lupus mice. We also identified some possible immunosuppressive molecules involved.

Lastly, to address the problem of product scale-up, in collaboration with the bioengineering team of Oxford University (UK), we invented an *ex vivo* automated oscillating cylinder culture system. This system utilizes microbeads as a culture-adherent surface that can improve the yield of MSCs. We found that it can generate much more cells than the microfiber culturing system since it provides a much larger surface area for attachment and allows the MSCs to cluster together even in the early culturing period. It is also easier to harvest from the microbeads because it tends to spread on the adherence surface once they are resting on a culture plate. All these quality improvement steps help to move MSC therapy back to the bedside again with much more consistency and efficiency.

### Conclusion

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New rational therapeutic concepts for GvHD should not only inhibit the inflammatory immune response, but also modulate the immune reaction to preserve graft-versus-tumor effects. Here, we have discussed recently developed concepts to modulate the immune response after allo-HCT to prevent life-threatening GvHD. In the next five years, basic and clinical research will further advance the field, and dramatic changes in GvHD management will be encountered.

### Author Contributions

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Y.I., R.Z., and G.C.F. C wrote the article.

## Conflicts of Interest

Y.I. received honoraria from Novartis, Janssen, and Meiji Seika Pharma; R.Z. received honoraria from Novartis, Incyte and Mallinckrodt, and G.C.F.C. declare no conflicts of interest.

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