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# Use of mesenchymal stem cells as treatment for graft-versus-host disease: current knowledge and controversies

*“Overall, it is fair to admit there are uncertainties and unanswered questions regarding MSCs and their use in GvHD. However, it is also necessary to stress that a significant number of patients seem to have benefitted from this approach.”*

**KEYWORDS:** graft-versus-host disease ■ mesenchymal ■ regenerative medicine ■ stem cells ■ transplantation

In 2002, Bartholomew *et al.* reported the systemic administration of autologous or allogeneic mesenchymal stem cells (MSCs) increased the viability of allogeneic skin grafts in baboons [1]. MSCs certainly exerted a graft-supporting function and the observation of a reduction in T-cell proliferation in the animals suggested MSCs also induced immunotolerance.

Bartholomew's report, together with other evidence, indicate that MSCs have immunosuppressing activities with therapeutic potential in disorders such as graft-versus-host disease (GvHD) or autoimmune aplastic anemia, since immune reaction is in their pathogenesis. During the past decade, a number of clinical trials exploiting the immunomodulatory activity of MSCs led to different rates of success. In particular, the treatment of GvHD with MSCs showed encouraging results in the initial reports. Despite this, the number of ongoing trials on this cell therapy for GvHD to date registered at [clinicaltrials.gov](http://clinicaltrials.gov) is rather small, compared with the number of active studies using MSCs for regenerative medicine.

## Identity of MSCs

Mesenchymal stem cells are commonly defined as undifferentiated, multipotent cells, able to differentiate *in vitro* into mature cells of mesenchymal lineage such as bone, fat and cartilage [2]. Some of the beneficial effects that MSCs exert on hemopoiesis are presumably derived from their ability to differentiate into bone marrow stromal cells, which are normal constituents of the hematopoietic niche (HN) [3].

The reports on the ability of MSCs to differentiate into other cell lineages, in particular Purkinje, respiratory epithelium, cardiac and smooth muscle cells, neuroectodermal tissue and paraventricular astrocytes, put into

question what was understood by MSCs or, alternatively, the methodology for isolating them as a single cell type *in vitro*. The absence of specific markers for the identification of MSCs would favor this possibility.

In most cases, MSCs are purified using their ability to adhere to culture vessels in the presence of fetal calf serum. Adherent cells are subsequently characterized as MSCs by testing their differentiation properties into mesenchymal lineages, and via an extensive panel of monoclonal antibodies, the most widely accepted profile being the coexpression of CD105 (the TGF- $\beta$  receptor III), CD73 (epitopes SH3 and SH4 of ecto-5' nucleotidase), HLA class I and CD90, in the absence of hematopoietic cell markers (CD45, CD34 and HLA class II) [4]. Only a small percentage (15%) of MSC have stem cell-like properties, with a capacity for differentiation into all mesenchymal lineages [5]. Since testing for their ability to differentiate may take several weeks, cells are frequently infused to patients without the results of these tests, adding uncertainty to their ultimate identity.

## Physiological functions of MSCs: the hematopoietic niche

Mesenchymal stem cells are normal constituents of the HN, where they play a role in the support and maintenance of the hematopoietic stem cells (HSCs). In particular, *N*-cadherin positive osteoblast-differentiated cells appear to play a role in the maintenance of the undifferentiated state of HSCs [6]. The mechanism involved in this effect seems to be the suppression of HSC proliferation, maintaining their immature phenotype and supporting their long-term survival [7]. Certain agents such as tacrolimus could favor these properties *in vitro* by stimulating the osteogenic differentiation of MSCs [8].



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Mesenchymal stem cells have been used *in vivo* to replicate the physiological conditions of the HN to facilitate the engraftment. The infusion of autologous MSCs was first reported to accelerate the post-transplant hematopoietic recovery in women receiving autologous transplants for metastatic breast cancer [9]. With a similar objective, Le Blanc *et al.* demonstrated the coinfusion of MSCs and CD34<sup>+</sup> cells from haploidentical donors enabled engraftment in four patients with previous primary (non-immune) graft failure [10]. This experience suggested MSCs contributed to the ‘anatomical’ reconstitution of the HN. Subsequent reports using HSCs from haploidentical donors [11] also showed the coinfusion of *ex vivo*-expanded bone marrow MSCs facilitated engraftment in this immune-mediated graft rejection-prone clinical setting, suggesting MSC immunosuppressing activity may also have played a role in the engraftment.

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Although other less successful clinical applications of MSCs capitalized much attention, engraftment facilitation and the treatment of graft failure are areas for continuous clinical research (e.g., the study opened at the University of Liege, Belgium [101]), given the fact that patients are receiving progressively more incompatible grafts that jeopardize the hematopoietic recovery.

### **MSCs exert an immunosuppressing activity whose ultimate pathways remain unclear**

Mesenchymal stem cells have been found to render a global inhibitory effect on the immune system (IS), affecting antigen-presenting cells, NK cells, as well as B and T cells. The mechanisms mediating these effects remain unclear, although there is extensive *in vitro* work implicating cell-to-cell contacts and various soluble factors (e.g., TGF- $\beta$ 1, HGF, indoleamine 2,3-dioxygenase and prostaglandin E2, among others) [12]. The relevance of these mechanisms taken individually could vary depending of the origin of the MSCs (mouse vs human) and the culture conditions, being difficult to establish which ones are relevant to humans *in vivo*. However, it would appear that TGF- $\beta$ 1 and HGF are consistently involved in the inhibitory

effects of MSCs on T cells [13]. Defining the actual mechanisms responsible for these effects would be of particular interest, since isolating these individual factors could potentially lead to newer therapeutic approaches.

### **MSCs to treat GvHD by taking advantage of their ability for inhibiting T-cell proliferation**

Among all the effects that MSCs exert on the components of the IS, their specific effects on T cells constitute the basis for the treatment of various autoimmune conditions, including GvHD. The report by Glennie *et al.* using murine MSCs [14] demonstrated that T-cell exposure to MSCs led to a state of ‘split anergy’, where T cells are arrested in G1 phase while their effector and cytokine-secretion functions remain unaffected. In the same report, a high number of MSCs appeared to be of critical importance to achieve these properties. This finding corroborated a previous report by Le Blanc *et al.* [15] where a high number of MSCs was found to be more important than the degree of histocompatibility to avoid T-cell stimulation in mixed lymphocyte cultures. Whether the effect of MSCs would be permanent remains controversial; while Glennie *et al.* demonstrated that the removal of MSCs or the addition of T-cell-stimulating factors such as IL-2 did not restore proliferation, other reports did not show sustained effects.

The observations by Glennie *et al.* may be of critical importance because, should this *in vitro* effect find parallel in the *in vivo* treatment of autoimmune conditions, MSCs would be an ideal therapy for such diseases. Most drugs and procedures used in these settings are aimed to reduce T-cell proliferation while suppressing their normal cytokine-secreting function, therefore impeding their normal reactivity against other stimuli. The result of these approaches is an increased rate of opportunistic infections or even secondary neoplasms that the inhibited IS is unable to attack. It could be hypothesized that stopping T-cell proliferation while maintaining other T-cell functions may allow a correct reaction against these complications.

The ability of MSCs to reduce T-cell proliferation in GvHD was first tested by Le Blanc *et al.* [16]. They infused haploidentical MSCs to a 9-year-old boy with steroid-refractory grade IV acute GvHD [17] and the authors observed an overall clinical improvement. This encouraging report led to the initiation of a multi-center trial where 55 patients suffering from

steroid-refractory, severe GvHD were included. The results of this trial were reported in 2008 [18] and demonstrated a complete response of acute GvHD in 30 patients and some degree of improvement in nine. Response to MSCs significantly improved the 2-year survival (53% in responders vs 16% in nonresponders;  $p = 0.018$ ). The Phase II design of the study, without a comparator arm, was a limitation; despite this, the results observed were remarkable since the prognosis of GvHD is largely determined by the response to steroids, and approximately 80% of patients with steroid-refractory acute GvHD [19] and a third of those with chronic GvHD [20], die of this complication. In addition, extensive chronic GvHD is associated with a prolonged impairment of quality of life [20]. Since other approaches for steroid-refractory acute GvHD usually induce responses in less than half of the patients, most of them transient [21], the favorable effect of MSC infusions deserve further investigation.

Patients with severe GvHD receive a number of immunosuppressive agents by the time the decision to administer MSCs is taken. Whether these drugs or antibodies may alter the response to MSCs remains mostly unknown. On the other hand, the combined immunosuppression secondary to pharmacological agents and MSCs could in theory increase the risk of infections. In this regard, Ringden and colleagues reported an increased incidence of opportunistic infections and virus-driven neoplasms in patients receiving MSCs and cyclosporin A simultaneously [22].

Mesenchymal stem cell infusions with prophylactic purposes do not seem to be efficacious to prevent GvHD. This was observed in a Phase I/II trial [23]. This could be justified by the fact that MSCs require the presence of IFN- $\gamma$  to exert their effects [24]. It would therefore appear that MSCs are only efficacious once the inflammatory reaction leading to GvHD has started.

### Long-term biological safety of MSCs remains unknown

To date, the long-term safety of administering MSCs remains unknown, mainly because of the limited follow-up of existing experiences. Areas of potential risk have been pointed out. Since most groups use the adherent properties to select MSCs, enzymes such as trypsin are required to detach cells from culture layers. Although exposure to trypsin is purposely reduced to a minimum to avoid the toxicity of this enzyme on MSCs, several passages are required to achieve a clinically effective cell dose. This multiplies the

exposure to trypsin, which can have mutagenic effects. Whether there is a real risk for developing sarcomas in MSC recipients remains to be determined. The clinical experience does not appear to support this possibility so far [18]. The introduction of 'cell factories' where MSCs are maintained in suspension by a continuous flow of culture media, overcomes this problem. On the other hand, the possibility of MSCs to undergo differentiation into bone or cartilage in abnormal sites is another concern that, to date, has not been reported in patients treated for GvHD. In addition, whether the infusion of MSCs may have an effect on the development of chronic GvHD, a somewhat mesenchymal disease, remains a matter of concern. Again, clinical experiences do not support this possibility. Still, even if any of these possibilities would become a real concern, the fact that patients had low or no possibilities of surviving to GvHD could counterbalance the drawbacks for using MSCs in this setting.

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Overall, it is fair to admit there are uncertainties and unanswered questions regarding MSCs and their use in GvHD. However, it is also necessary to stress that a significant number of patients seem to have benefitted from this approach. Research on the mechanisms involved in the immunomodulatory effects of MSCs has widened our knowledge on transplantation hematopoiesis and the immune pathways influencing the success of the procedure. It would be reasonable to believe that the next step in this area would be to investigate the effector mechanisms of MSCs in an isolated manner and, in the clinical setting, to extend the experiences to larger number of patients and also to carefully follow the already treated patients to rule out long-term complications.

### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

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#### ■ Website

- 101 ClinicalTrials.gov. Mesenchymal Stem Cell Infusion as Prevention for Graft Rejection and Graft-Versus-Host Disease <http://clinicaltrials.gov/ct2/show/NCT00504803>