

## Review Article

# Mesenchymal Stem Cell Benefits Observed in Bone Marrow Failure and Acquired Aplastic Anemia

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Acquired aplastic anemia (AA) is a type of bone marrow failure (BMF) syndrome characterized by partial or total bone marrow (BM) destruction resulting in peripheral blood (PB) pancytopenia, which is the reduction in the number of red blood cells (RBC) and white blood cells (WBC), as well as platelets (PLT). The first-line treatment option of AA is given by hematopoietic stem cell (HSCs) transplant and/or immunosuppressive (IS) drug administration. Some patients did not respond to the treatment and remain pancytopenic following IS drugs. The studies are in progress to test the efficacy of adoptive cellular therapies as mesenchymal stem cells (MSCs), which confer low immunogenicity and are reliable allogeneic transplants in refractory severe aplastic anemia (SAA) cases. Moreover, bone marrow stromal cells (BMSC) constitute an essential component of the hematopoietic niche, responsible for stimulating and enhancing the proliferation of HSCs by secreting regulatory molecules and cytokines, providing stimulus to natural BM microenvironment for hematopoiesis. This review summarizes scientific evidences of the hematopoiesis improvements after MSC transplant, observed in acquired AA/BMF animal models as well as in patients with acquired AA. Additionally, we discuss the direct and indirect contribution of MSCs to the pathogenesis of acquired AA.

## 1. Introduction

Red bone marrow (BM) is a gluey, complex, and heterogeneous tissue found in the medullary cavity of long bone and spongy bone cavities of the body. It is anatomically made up of the stromal cells (fibroblasts, adventitial reticular cells, adipocytes, and others) responsible for the tissue structure [1] and the parenchymal cells (hematopoietic cells—blood-producing cells) [2, 3]. To fabricate these blood-producing cells, BM contains a pool of hematopoietic stem cells (HSCs), which are self-renewing cells, differentiate into red (erythrocytes) and white (leukocytes) blood cells, and generate

megakaryocytes and these produce platelets (PLT) [2–4]. Only mature hematopoietic cells enter the bloodstream. With age, red BM tends to be substituted with yellow BM, which is mostly made up of fat cells [5, 6].

BM stroma is a key element of hematopoiesis that provides the structural and physiological support for blood cell production. It also consists of a heterogeneous population of different cell types among which is a rare population of nonhematopoietic skeletal progenitor cells named bone marrow stromal cells (BMSC) [7, 8]. Red BM (hematopoietic marrow) and stroma are crucial components of the hematopoietic microenvironment as they interact and produce

together—or individually—humoral growth and/or inhibitory factors necessary to maintain normal hematopoiesis, which is essential for life and human health.

BM can be susceptible to two types of failure syndromes: inherited or acquired. The inherited bone marrow failure (BMF) syndromes are a group of disorders usually diagnosed in childhood and passed down from parent to child through the association with some genetic abnormality [9], which may cause the aplastic anemia (AA) and cancer predisposition [10]. Young people and adults usually may develop the acquired BMF, which can be caused by different extrinsic and intrinsic factors including chemicals, irradiation, chemotherapy treatments, and immune system harms [11, 12].

Initially, BMF syndromes were denominated as “idiopathic AA” because at first, etiology was unknown. Nowadays, the term “AA” encompasses a heterogeneous BMF disorders which are characterized by BM cellular component ablation [13, 14].

Among BMF diseases, the acquired AA is more common. The treatment of acquired AA depends on the patient’s age, health, and the severity of the disease. Treatment of moderate cases of acquired AA is indicated blood transfusions and supportive care with an antibiotic. However, many moderate cases may progress to severe AA (SAA) [10]. Therefore, to treat acquired SAA, HSC transplant from matched sibling donor is a matter of choice, which in some cases is satisfactorily effective [15]. It can be used in combination or not with immunosuppressive (IS) therapies. However, most patients have no access to immediate HSC transplant due to the lack of a matched sibling donor. Frequently, extensive time is needed to find a suitable unrelated donor for HSC transplant in SAA patients [16, 17].

Allogeneic transplant of MSCs can be a potential supplementary alternative to treat refractory SAA, since these cells are hypoimmunogenic, thus displaying low expression levels of human leukocyte antigen (HLA) class I, no expression of HLA class II [18]. Potentially, these cells may also be an addition to IS therapies because they possess broad immunomodulatory properties, secreting several biological molecules that influence both adaptive and innate immune responses [19]. Some studies showed that MSCs can prevent graft-versus-host disease (GVHD) and improve hematopoiesis when coinjected with HSCs [20, 21]. Hence, animal models have been developed to assess the response of MSCs in acquired AA as well as the hematologic cell amelioration [22–24] to find conditions to improve HSC transplant regimens or even to evaluate its own effect to reverse BMF and consequently to enhance survival rates of the patients.

This review aims to critically evaluate the potential of MSCs, focusing mainly of BMSC, on acquired BMF/AA in animal models and in recent AA reported clinical cases.

## 2. AA: Origin, Causes, Diagnostic, and Treatment

AA was first described in 1888 by Paul Erich merely as an “empty” BM with replacement by fat cells [25] and now is defined by decreased hematopoietic precursors in the BM,

resulting in BM hypoplasia, peripheral blood (PB) pancytopenia, and precocious fat replacement [26, 27].

The etiology of BM precursor destruction remained elusive for decades [13]. Currently, heterogeneous origin of this disease is accepted. Some inherited disorders can damage the BM cells and lead to AA, mostly as Fanconi anemia (FA), Shwachman-Diamond syndrome (SDS), and dyskeratosis congenita (DC) [9]. The acquired AA can be induced by many different factors such as antineoplastic drugs, antibiotics, nonsteroidal anti-inflammatory drugs, and pesticides, as well as active viral infections (Epstein Barr, hepatitis virus, human immunodeficiency virus, and parvovirus) and radiation exposure [17, 28].

Most of the acquired AA is the result of an immunomediated process that leads to apoptosis of BM cells triggered by cytotoxic T cells [17, 29]. This process occurs as the result of an imbalance between CD8<sup>+</sup> and CD4<sup>+</sup> T cells, including T helper (Th) type 1 (Th1), Th type 2 (Th2), regulatory T cells (Treg) and Th type 17 (Th17) cells, natural killer (NK) cells, and NK T cells. Besides that, there is abnormal production of cytokines including interferon- (IFN-)  $\gamma$ , tumor necrosis factor- (TNF-)  $\alpha$ , and transforming growth factor (TGF) [30–34].

For acquired AA diagnostic, the pancytopenia is evaluated using three main criteria: neutrophil count lower than  $0.5 \times 10^9$  cells/L, platelet count lower than  $20 \times 10^9$  cells/L, and reticulocyte count lower than 1% [35]. Patients with acquired AA often present symptoms of anemia purpura or hemorrhage, and, frequently, infection that may worsen the symptoms [35].

The treatment of acquired AA depends on the severity of the disease. As already mentioned, treatment for moderate cases is based on red blood cell (RBC) transfusions to treat anemia, on platelet transfusions to prevent bleeding, and on supportive care in association with antibiotics [36] aiming to reestablish blood cell volume and prevent secondary infections.

Many moderate cases may progress to severe pancytopenia [35]. Moreover, for severe cases, the first-line treatment to date is HSC transplants from matched sibling donor, more efficient in young patients [15] and IS therapies, most commonly used due to lack of histocompatible sibling donors (HLA) and indicated for older patients [17].

Nevertheless, the success of HSC transplant is limited due to late complications, such as graft rejection and relapse due to resurgent autoimmune attack, and more often due to development of GVHD [15, 37], whereas lack of response, relapse, and clonal evolution limit the success of IS drugs [38].

## 3. MSCs and Mechanisms of Action

BMSCs are a natural component of stromal BM cellular environment, which are found at low frequency (0.001–0.01%) [39]. When isolated in vitro culture, they show fibroblast-like cell morphology with capacity to form colonies and are able to differentiate mainly into mesoderm derivatives. Moreover, only BMSCs have been shown to self-renew *in vivo* [40, 41].

More recently, similar mesenchymal stem cells (MSC) to BMSC were found in umbilical cord (UC) blood and Wharton's jelly [42], in adipose tissue (AT) [43] in dental pulp (DP) tissue [44], and in amniotic fluid [45] and other fetal and postnatal tissues [46, 47]. According to the International Society of Cellular Therapy (ISCT), MSCs, firstly, must be plastic-adherent when maintained in standard culture conditions. Second, they are characterized by expression of cell surface antigens (CD105, CD73, and CD90), lack of expression of CD34, CD45, CD14 or CD11b, CD79 $\alpha$ , and HLA-DR surface molecules, and third, they showed the capacity to differentiate *in vitro* into adipocytes, osteoblasts, and chondroblasts [48]. However, MSCs derived from different sources have similar immune profile after *in vitro* culture expansion. On the other hand, it can possess a distinct differentiation potential and biological function, which depend on their embryonic and adult tissue origin [49, 50]. Moreover, profound differences in development potential between MSC sources were found, which are not dependent on donor age and may implicate with MSC clinical use [49, 50].

Paracrine mechanism of BMSC action was first evidenced by their capacity to support HSC growth and differentiation *in vitro* [51]. Furthermore, MSCs derived from adipose tissue (AT) have also been demonstrated as being able to support hematopoietic niche *in vitro* and *in vivo* [52]. Many studies focused on BMSC's ability to secrete a series of bioactive molecules, as cytokines and growth factors in response to injury into BM microenvironment [53–56]. BMSCs interact with HSC niche secreting such bioactive molecules to support proliferation and long-term growth of HSCs, thus influencing hematopoiesis [57]. Therefore, C-X-C motif chemokine ligand 12 (CXCL12) is responsible for regulation of adhesion, expansion, migration, and homing of HSCs. The Flt-3 ligand (FLT3LG), interleukin-6 (IL-6), and thrombopoietin (TPO) influence HSC proliferation, differentiation, and self-renewal, while stromal cell-derived factor 1 (SDF-1) reduces the production of inflammatory cytokines and chemokines [58–61].

In addition to paracrine effect, general MSCs demonstrate immunomodulatory activity *in vitro* [62, 63]. MSCs interact with various immune cells and secrete soluble mediators [53]. They express several adhesion molecules, including vascular cell adhesion molecule- (VCAM-) 1, intercellular cell adhesion molecule- (ICAM-) 1, and lymphocyte function-associated antigen- (LFA-) 3 involved in T cell interactions, which provide signaling of immunomodulatory response. MSCs suppress T cell proliferation and activation and regulate the differentiation of Th cells [64]. MSCs are capable to inhibit B cell activation, as well as, dendritic cells (DCs) and their precursor proliferation, differentiation, and maturation [65]. Moreover, MSCs modulate the immune responses by generation of Tregs to prevent immune intolerance. It is an important mechanism which could prevent GVHD [66]. Therefore, MSCs have significant clinical implications in BMF, such as acquired AA and related disorders [63].

#### 4. BMSCs and AA

Although acquired AA is considered to affect mainly blood-producing cells, aplastic BM shows significant reduction in

endosteal cells, vascular cells, and perivascular cells—pericytes [67, 68]. There are also growing evidences in the scientific literature that MSCs, which showed pericyte-like properties [69, 70]. When isolated from the SAA, patients are affected by this disease. They may present aberrant morphology, impaired osteogenic potential, changes in gene expression, and reduced ability to support hematopoiesis *in vitro* [71–73]. The number of CD146<sup>+</sup> cells is reduced in aplastic BM [74]. This marker is expressed in bone marrow pericyte cells [70, 75–77] and BMSCs [76, 78–80] which can maintain the long-term repopulation potential of HSCs *in vitro* [81]. MSCs isolated from the patients with acquired AA patient were prone to differentiate into adipocytes rather than osteoblasts. These cells demonstrate downregulation of transcription factor (TF) GATA-2, which is expressed in hematopoietic progenitors, including early erythroid cells, mast cells, and megakaryocytes and overexpression of TF peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) [82], which has multiple roles in MSCs obtained from AT of the patients with acquired AA [83, 84]. These alterations contribute to the abnormal AT deposit, thus affecting BM tissue remodeling and repair. A low expression level of basic fibroblastic growth factor 2 (FGF2) gene in BMSCs of AA patients was also reported [85, 86]. It is well known that BMSCs are the genuine source of FGF2, which directly influences the HSCs and their precursors *in vitro* [87, 88]. Furthermore, BMSCs from AA patients were impaired in maintaining the immune homeostasis associated with CD4<sup>+</sup> T cells *in vitro*, which might cooperate with BMF [89]. In contrast to previous observations, one recent study shows that MSCs from patients diagnosed as moderate–severe AA did not present any alteration in morphology, osteogenic potential, gene expression, and ability to support hematopoiesis *in vitro* [90]. Figure 1 summarized recent studies that show the key role of MSCs in hematopoiesis and in AA pathogenesis, as well as demonstrate possible benefits from allogeneic MSC transplant in this disease.

#### 5. Animal Models for the Study of Acquired AA

Animal models have greatly contributed to elucidate different aspects of BMF and acquired AA. Initially, the attempt to mimic AA has used exposure to toxic/chemical agents and pharmacological drugs that result in BMF through a direct toxic effect [13, 91–97], which were then replaced by physical and biological agents, as irradiation [22, 23] and lymph node infusion [98–102]. The administration of toxic/chemical and pharmacological agents results in BMF in attempt to mimic AA [13, 103]. However, the use of toxic drugs did not provide the immune-mediated destruction of the animal BM, which is commonly observed in human AA disease [13].

In turn, the model which employs infusion of lymph node cells in preirradiated animal shares many pathophysiological features with human immune-mediated AA. These animals develop BM hypoplasia rapidly, which is followed by severe peripheral pancytopenia, adipose cell invasion in BM, and hematopoietic cell reduction [102]. The changes in T lymphocyte subsets and IFN- $\gamma$  ratio also occur [99].

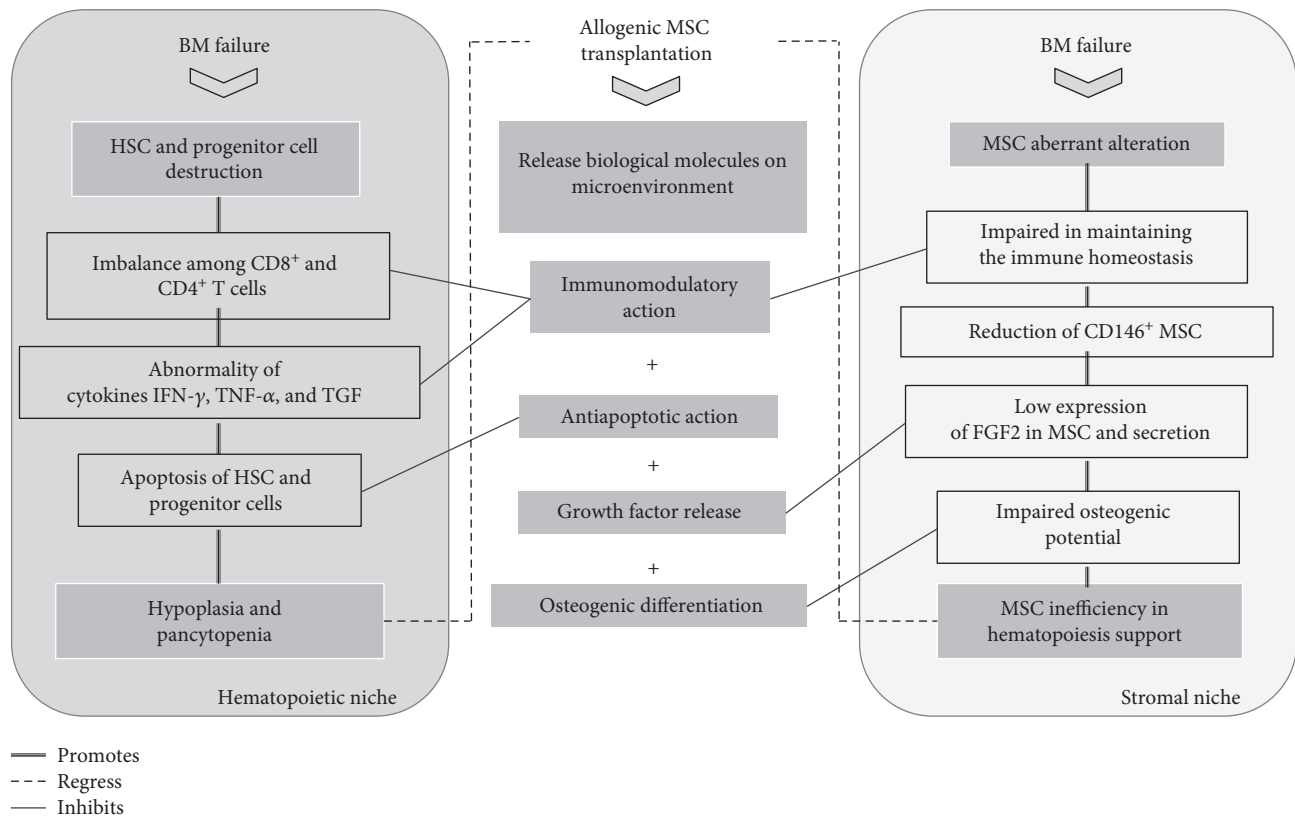


FIGURE 1: Effect of mesenchymal stem cell (MSC) transplant on bone marrow failure (BMF) etiology and progression. Acquired aplastic anemia (AA) is caused by destruction of hematopoietic stem cell (HSC) and progenitor cells associated with MSC abnormalities, caused by hematotoxic agents (drugs, chemical agents, radiation, and virus). These events lead to imbalance among CD8<sup>+</sup> and CD4<sup>+</sup> T cells and abnormal cytokine secretion, which correlates apoptosis of HSC and progenitor cells and consequently bone marrow (BM) aplasia and pancytopenia. Additionally, in turn of BM, imbalance aberrant alteration on MSC from BM niches arises. MSC aberrant alteration is observed by, impaired in maintaining the immune homeostasis, reduction of CD146<sup>+</sup> MSC and low expression of FGF2 in MSC and its secretion, which lead to MSC inefficiency in hematopoiesis support and collaborate to progress of disease. According to the literature for AA, MSCs improve engraftment of HSC and prevent apoptosis in BM failures. BMF improvements occur as a result of MSC transplant through very similar mechanisms, such as immunomodulation, release growth factors, and osteogenic support. Although in vivo improvement on hematopoiesis was not demonstrated, several properties of MSCs, as well as its association with AA, justify the use of MSC in BM failures.

Irradiation alone also causes BMF in animals. HSCs and committed BM progenitor cells present rapid cell turnover, thus being more sensitive to irradiation, when compared with other cell types [104–106]. The total body irradiation (TBI) using different doses of gamma irradiation ranging from two to eight grays (Gy) induces high expression of the apoptosis regulator gene (BAX gene) causing rapid lymphocyte death [107]. Low doses, around 2 Gy cobalt-60 gamma rays, result in decreased lymphocyte concentration and immune suppression in mouse. Medium and high doses of radiation (5 to 8 Gy) lead to BMF, neutropenia, thrombocytopenia, and anemia, as well as to low count of colony-forming unit granulocyte/macrophage (CFU-GM) and colony-forming unit fibroblast (CFU-f) [22]. Higher (above 8 Gy) doses of radiation may cause lethal hemorrhage or infections and death [108]. Therefore, care should be taken to choose the dose of radiation to induce BMF with minimized side effects and eventually low death incidence.

The murine models mimicking AA have improved over time. The development of an immune-mediated model in

the destruction of the BM was important, being more closely modeling human AA [13]. However, human AA is extremely heterogeneous disease. Therefore, different stem cell therapeutic strategies, as source of the stem cell, doses, concentration, and periodicity, as well as the administration route, should be considered to treat different AA pathologies [109, 110]. MSCs, in turn, although showed similar basic characteristics, have different embryonic origin that may reflect on their medicinal paracrine properties [111, 112].

## 6. Benefits of MSC Transplant in BMF and AA Animal Models

Despite the immune-mediated animal model being considered the closest to mimic the pathophysiology of human AA, most studies choose the BMF animal model, which has failure induction mainly from irradiation strategy, to assess the mechanism of MSCs. The term “bone marrow failure” encompasses any primary failure condition at the HSCs, resulting to the decrease of one or more circulating blood cell



lineage [113]. Table 1 summarizes the current knowledge regarding MSC transplantation into BMF and AA animal models. In the literature, the studies have used mouse irradiation doses ranging from 4 Gy to 8 Gy [22–24, 100, 114–116]. In immune-mediated AA animal model, the preirradiated (4 Gy) mouse received  $1 \times 10^6$  lymph node cells to induce acquired AA [98, 100]. MSCs from different sources were used in order to assess its possible therapeutic benefits, such as BMSC [22], umbilical cord-derived mesenchymal stem cells (UC-MSCs) [23, 24], adipose-derived mesenchymal stem cell (AD-MSCs) [115, 116], and multiparaplacenta-pooled cells, which contain MSCs derived from placenta, umbilical cord (UC), and UC blood [100]. Additionally, MSCs were infused in AA animal model in combination with HSCs [115] or extracellular superoxide dismutase (ECSOD), which is an extracellular scavenger of superoxide ( $O_2^{\cdot-}$ ) and the main regulator of nitric oxide (NO) in the blood vessel wall and other organs [23]. On the majority of published works, a single MSC transplant was used and doses ranged from  $1 \times 10^6$  to  $2.5 \times 10^7$  cells per mouse. The cells were mostly administered by endovenous (EV) [22, 23, 98, 116] and less by intraperitoneal (IP) route [100]. Only one study analyzed and showed engraftment of MSCs in BM after EV route [23]. Most in vivo studies did not use IS drugs before or during cell transplant [22, 24, 100, 114–116].

The preclinical studies report the increase of the levels of WBC [22, 24, 98], PTL [22, 23], and hemoglobin in PB [23, 100] after MSC transplant in comparison with the BMF control group that did not receive MSCs. Different studies show BM recovery and demonstrate an increased number of BM cells in vivo [22–24, 116] as well as an increased CFU-f [22, 116] and CFU-GM in vitro capacity [22, 98, 114]. Additionally, an increased megakaryocyte concentration was observed in BM after MSC transplantation [23, 116]. Besides, the increase of PB and BM cells, hematopoietic cytokines, as FLT3LG and TGF-beta1, was reported. These cytokines, which are secreted by MSCs, are important to HSC proliferation and differentiation process [24].

Although TBI led to HSCs and progenitor cells in BM apoptosis, few reports showed MSCs' antiapoptotic effect on HSC and hematopoietic progenitor cell preservation [22, 116]. The exact mechanism of MSCs' antiapoptotic effect is still under investigation. However, it has been shown that MSC transplant leads to a reduction of BAX gene expression in BM cells [116].

It is known that the increased levels of IFN- $\gamma$  and TNF- $\alpha$  in irradiated mouse activate the Th1 and Th2 cells [24]. On the other hand, MSCs present immunoregulatory properties that could be used to attenuate the imbalance of immunologic system after radiation exposure [114, 117]. Hence, one study showed that MSCs reduce irradiation-induced hematopoietic toxicity. MSCs improved lymphocyte-mediated inhibition of CFU-GM and induced additional immunoprotective effects by expanding the Tregs, regulating chemokine receptor expression, and promoting the Th1/Th2 balance toward anti-inflammatory Th2 polarization [114].

Recent publications focused on preclinical studies demonstrated that MSCs could recover BMF by its antiapoptotic and immunoregulatory properties [22, 23, 114, 116].

However, all these studies evaluated MSC benefits after short-term experiments (from 24 hours to 30 days post-MSC injection); therefore, long-term benefits and stability on MSC transplant still need to be assessed.

## 7. Transplant of MSC in AA Patients

The clinical use of the MSCs in the hematological diseases has received special attention because of their inhibitory effects on the proliferation and cytotoxic activity of immune system cells in patients, which developed GVHD in response to allogeneic HSC transplantation [118, 119]. In AA patient transplant of allogeneic BM—or UC—MSCs were performed alone or in combination with HSCs [120–125] (Table 2). Some studies are registered at the National Institutes of Health (NIH) clinical trial database [126, 127]. The patients enrolled in these clinical trials presented severe stage of AA disease and did not respond to IS therapy (exhibit refractory stages). In addition, many patients prior to MSC transplant had already received treatment with HSC or BM cell transplant without clinical amelioration [121–123, 125]. In these studies, MSC doses ranged from  $1 \times 10^6$ /kg to  $1 \times 10^7$ /kg per transplant and the patients received one to two transplants per month.

Most of the studies did not evaluate whether MSCs engraft into host BM after EV transplant [121, 123–125]. Only one study showed MSC chimerism in a patient's BM microenvironment after MSC transplant. The chimerism study was performed by real-time PCR for the SRY gene for detection of male DNA in whole BM sample from a woman patient. This study showed improvement of BM stromal niche in a patient with SAA refractory to ATG and cyclosporine who was ineligible for allogeneic HSCT. After receiving two allogeneic transplants of MSCs, the biopsy demonstrated reduction of necrotic areas, but the BM improvement was not observed [120].

Cotransplant of MSC and HSC therapy also shows hematopoietic recovery in AA in humans [123, 128]. Six patients were treated, and two of them presented a hematopoietic recovery in both BM and PB three months after transplant [123].

All clinical studies used immunosuppression protocol [120–125]. In spite of this, some patients manifested adverse events such as mild self-limited febrile reactions, headaches, hypoxemia, mild dyspnea, and diarrhea after MSC transplant. All these adverse events were observed during or after MSC infusions and were mild and self-limited [123, 125]. Three studies reported a few deaths of SAA patients after the second or third MSC transplant alone [120, 125] or combined with HSCs [124]. However, these deaths occurred as a result of natural complications of AA disease [120, 124, 125]. Besides, no study reported occurrence of tumor after MSC transplant during the follow-up studies.

There are few clinical cases which use the therapy with MSCs on the AA disease, and then only in the most severe cases which did not respond to conventional treatment. And these studies show that the treatment was safe, but not enough to alone recover the BM. This observation can

TABLE 1: MSC transplantation in BMF and AA animal models.

Reference	Mice model	Gender	Age (weeks)	BMF induction method	Cell source	MSC profile	Number of transplanted MSC	A.R.	Evidence of MSC efficacy
[22]	Balb/c	F	6-7	Irradiation (5.5 Gy)	BM-MSC	CD34 <sup>-</sup> , CD45 <sup>-</sup> , CD105 <sup>+</sup> , CD29 <sup>+</sup> , CD44 <sup>+</sup> , and Sca-1 <sup>+</sup>	2.5 × 10 <sup>7</sup>	EV	↑ WBC and PLT in PB, CFU-F, and CFU-GM ↑ BMC ↓ apoptotic cells in BM
[23]	Balb/c	M	6	Irradiation (5.8 Gy)	UC-MSC + ECSOD	CD14 <sup>-</sup> , CD73 <sup>+</sup> , CD90 <sup>+</sup> , CD105 <sup>+</sup> , CD44 <sup>+</sup> , CD29 <sup>+</sup> , CD34 <sup>-</sup> , CD45 <sup>-</sup> , CD19 <sup>-</sup> , and HLA-DR <sup>-</sup>	1 × 10 <sup>6</sup>	EV	Attenuate upregulation of apoptotic genes (p16, p21, p53, and NOX4) ↑ BM cells and megakaryocyte ↓ apoptotic cells
[24]	Balb/c	F	6	Irradiation (7 Gy)	hUC-MSC	CD105 <sup>+</sup> and CD34 <sup>-</sup>	NI	NI	↑ WBC in PB and BMC ↑ levels of hematopoietic cytokines (Flt3L and TGF-β1)
[100]	Balb/c	F	8	Irradiation (4 Gy) + lymph node cell infusion	Multiplicentas pooled cells	NI	1 × 10 <sup>7</sup>	IP	Higher survival ↑ HB
[114]	Balb/c	NI	NI	Irradiation (8 Gy)	CB-MSC	CD45 <sup>-</sup> , CD34 <sup>+</sup> , CD29 <sup>+</sup> , CD44 <sup>+</sup> , CD117 <sup>-</sup> , and Sca-1 <sup>+</sup>	NI	NI	↑ survival and gain body weight ↑ CFU-GM ↑ Treg cells ↓ suppressed Th1 immunity and regulation of T cell chemokine receptor expressions (CCR7 upregulation and CXCR3/CCR5 downregulation)
[115]	B6D2F1	NI	10-12	Irradiation (5-7 Gy)	HSC coinfusion of AD-MSC	CD29 <sup>+</sup> , CD44 <sup>+</sup> , CD73 <sup>+</sup> , CD90.2 <sup>+</sup> , CD105 <sup>+</sup> , CD106 <sup>+</sup> , CD144 <sup>+</sup> , CD166 <sup>+</sup> , CD34 <sup>-</sup> , CD45.1 <sup>-</sup> , CD80 <sup>-</sup> , and Sca-1 <sup>-</sup>	1 × 10 <sup>6</sup>	NI	BM reconstitution Facilitating and homing of HSC to recipient BM
[116]	Balb/c	M	6-8	Irradiation (4 Gy)	AD-MSC	CD29 <sup>+</sup> , CD31 <sup>-</sup> , CD34 <sup>-</sup> , CD45 <sup>-</sup> , and CD90 <sup>+</sup>	1 × 10 <sup>6</sup>	EV	↑ CFU-F, CFU-MK, and megakaryocytes in BM (cd41 <sup>+</sup> cells) Recovery of BM cells ↓ apoptotic cells

A.R.: administration route; NI: noninformed; F: female; M: male; BMF: bone marrow failure; MSC: mesenchymal stem cell; BMSC: bone marrow stromal cell; hUC-MSC: human umbilical cord-derived mesenchymal stem cell; UC-MSC: umbilical cord-derived mesenchymal stem cell; CB-MSC: compact bone-derived mesenchymal stem cell; ECSOD: extracellular superoxide dismutase; HSC: hematopoietic stem cell; AD-MSC: adipose-derived mesenchymal stem cell; CD: cluster differentiation; Sca-1: stem cell antigen-1; HLA-DR: human leucocyte antigen-D related; EV: endogenous infusion; IP: intraperitoneal infusion; WBC: white blood cell; PLT: platelet; PB: peripheral blood; CFU-F: colony-forming unit fibroblast; CFU-GM: colony-forming unit granulocyte-macrophage; BMC: bone marrow cell; BM: bone marrow; Flt3L: FMS-like tyrosine kinase 3 ligand; TGF-β: transforming growth factor beta; CCR7: C-C chemokine receptor type 7; CXCR3: C-X-C motif receptor 3; CCR5: C-C chemokine receptor type 5; RBC: red blood cell; HB: hemoglobin; NOX 4: nicotinamide adenine dinucleotide phosphate oxidase 4; CFU-MK: colony-forming unit megakaryocytes.

TABLE 2: MSC transplantation in reported clinical cases with SAA.

Reference	Cell source	MSC profile	Number of patients	Age of patient (years)	Gender	Disease stage	Previous HSC or BM transplant	Number of transplants	A.R.	Number of MSC/kg	Immunosuppressive treatment	Adverse event	Follow-up (months)	Death (patients)	Evidence of MSC efficacy
[120]	BMSC	SH2 <sup>+</sup> , SH3 <sup>+</sup> , CD34 <sup>-</sup> , and CD45 <sup>-</sup>	1	68	F	Refractory SAA	-	2	EV	2 × 10 <sup>6</sup> and 6 × 10 <sup>6</sup>	CsA	NI	NI	1	MSC engraftment Partial recovery of BM stromal niche ↑ CFU-F (n = 1) Partial recovery of BM niche (n = 1)
[121]	BMSC	NI	1	26	M	SAA	+	2	EV	1 × 10 <sup>6</sup>	CTX, ATG, TBI, FAMP, and ALS	NI	NI	None	Enhance the HSC engraftment (n = 2)
[122]	UC-MSC coinjection HSC	CD13 <sup>+</sup> , CD29 <sup>+</sup> , CD44 <sup>+</sup> , CD73 <sup>+</sup> , CD90 <sup>+</sup> , CD105 <sup>+</sup> , CD14 <sup>-</sup> , and CD31 <sup>-</sup> , CD34 <sup>-</sup> , CD45 <sup>-</sup> , and HLA-DR <sup>-</sup>	2	11 and 13	F	SAA	+	1	NI	1 × 10 <sup>6</sup>	CsA, ATG, and Methylprednisolone	None	NI	None	Recovery of three hematopoietic cell line (n = 2) Recovery RBC and hemoglobin level (n = 2) Recovery PLT (n = 2)
[123]	BMSC	CD29 <sup>+</sup> , CD73 <sup>+</sup> , CD90 <sup>+</sup> , CD105 <sup>+</sup> , CD34 <sup>-</sup> , CD45 <sup>-</sup> , and CD14 <sup>-</sup>	14/4	16–56	M/F	Refractory SAA or NSAA	-	4–6	EV	6 × 10 <sup>5</sup>	CsA and ATG	Transient fever and headache (n = 2)	12	None	↑Neutrophil ↑PLT Enhance the HSC homing and engrafting
[124]	UC-MSC coinjection HSC	VEGFR2/Flk1 <sup>+</sup> , CD166 <sup>+</sup> , CD105 <sup>+</sup> , CD44 <sup>+</sup> , CD29 <sup>+</sup> , and HLA class I <sup>+</sup> CD34 <sup>-</sup> , CD45 <sup>-</sup> , CD14 <sup>-</sup> , and HLA class II <sup>-</sup>	14/23	15	F/M	SAA	+	1	EV	1 × 10 <sup>6</sup>	CTX, FAMP, and ATG	None	60	9	Partial hematologic response (n = 2)
[125]	BMSC	CD73 <sup>+</sup> , CD90 <sup>+</sup> , CD29 <sup>+</sup> , CD13 <sup>+</sup> , CD44 <sup>+</sup> , CD49e <sup>+</sup> , STRO1 <sup>+</sup> , HLA class I <sup>+</sup> , CD34 <sup>-</sup> , CD14 <sup>-</sup> , CD45 <sup>-</sup> , glycophorin A <sup>-</sup> , CD31 <sup>-</sup> , cadherin <sup>-</sup> , KDR <sup>-</sup> , and HLA class II <sup>-</sup>	9	19–50	F and M	Refractory SAA or NSAA	-	5	EV	2.7 × 10 <sup>6</sup>	CsA and ATG	Fever, hypoxemia, mild dyspnea, and diarrhea	20	4	Partial hematologic response (n = 2)

MSC: mesenchymal stem cells; BMSC: bone marrow stromal cell; hUC-MSC: human umbilical cord-derived mesenchymal stem cell; HSC: hematopoietic stem cells; CD: cluster differentiation; HLA-DR: human leucocyte antigen-D related; HLA: human leucocyte antigen; VEGFR2: vascular endothelial growth factor receptor 2; SAA: severe aplastic anemia; NSAA: nonsevere aplastic anemia; EV: endovenous infusion; CsA: cyclosporine A; ATG: antithymocyte serum; TBI: total body irradiation; FAMP: fludarabine; BM: bone marrow; CFU-F: colony-forming unit fibroblast; RBC: red blood cell; PLT: platelets; NI: noninformed; CTX: cyclophosphamide; SH2: Src homology 2; SH3: Src homology 3; VEGFR2/Flk1: vascular endothelial growth factor receptor 2.

suggest that therapy with MSCs is promising but still needs to be in combination with HSC transplant [120, 125].

## 8. Final Considerations

Regulatory agencies require that investigators provide robust data on in vivo efficiency of new biological products. They recommend the use of well-characterized animal models to predict the response in humans. In general, transgenic animals are more indicated for this purpose. However, as we mentioned above, in the case of acquired AA, an immune-mediated animal model is well accepted [102]. MSC therapeutic potential was assessed using two BMF models: immune-mediated and irradiation-induced model [22–24, 100, 114–116]. Although these studies helped to demonstrate the several benefits of MSCs on acquired AA, they present limitation—natural reversibility of AA pathogenesis following long periods of evaluation [129].

On the other hand, clinical studies which use MSCs, demonstrate that patients with a very severe form of AA were enrolled, as well as each study includes very limited number of patients. Another drawback of clinical studies includes the use of IS drugs [120–125] which hinder the interpretation of results, as these drugs may ameliorate and even recover BMF alone [130, 131]. In addition, IS drugs could negatively influence the therapeutic action of the MSCs [132].

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Vivian Fonseca Gonzaga, Cristiane ValverdeWenceslau, and Gustavo Sabino Lisboa contributed equally.

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